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ZOONOSIS

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PREFACE

The well-being of humans and animals is pretty much interdependent. It's impossible to ensure human health, without considering animal health and vice versa.

The need to enhance the collaboration between animal health workers and medical professionals, researchers and academicians has moved the editors to develop this publication. The book takes into account the major threats of animal and human health. This book provides the core concepts of Zoonosis with a critical focus on the key challenges and their effective management. The objective is to cover epidemiological interactions of various infectious diseases and their ecological implications as an emerging threat.

It is anticipated that this book would be of great use to a variety of readers. University students, graduates, practitioners, animal healthcare providers and health professionals would definitely find this book of great importance. The language of book has been intentionally kept easier for a non-technical person to grasp the concepts on interdependence of animal and human health. The editors wish to publish a series on the subject keeping in view the urgency to highlight these areas for awareness, research and development.

Editors

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Volume 4

Q-Fever and One Health: Integrating Human, Animal, and Environmental Health

01

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ABSTRACT

In the intricate tapestry of infectious diseases, Q fever emerges as a formidable challenge, transcending species boundaries and demanding a holistic approach. This zoonotic ailment, fueled by the Coxiella burnetii bacterium, underscores the interconnectedness of animal, human, and environmental health—a narrative that unfolds across continents from Australia to the Netherlands. This abstract navigates the historical contours of Q fever, delving into its etiology, transmission dynamics, impact on animals, and the ominous specter it casts on human health. It highlights the global prevalence, with outbreaks resonating from the Australian abattoirs to the extensive Q fever epidemic that gripped the Netherlands from 2007 to 2010.The intricate pathogenesis of C. burnetii, its diverse manifestations in both humans and animals, and the challenges in diagnosis and prevention set the stage for a comprehensive One Health approach. This collaborative strategy, weaving together insights from human health, veterinary science, and environmental studies, emerges as a beacon in the fight against Q fever. Case studies from different corners of the globe, including South Africa, Europe, Australia, the USA, and the Netherlands, showcase the diverse efforts and challenges in implementing the One Health paradigm. The abstract also navigates through direct and indirect diagnostic approaches, underlining the complexity of detecting and managing this elusive pathogen. In conclusion, Q fever serves as a poignant exemplar of the intricate web connecting animals, humans, and the environment. As the world grapples with emerging infectious threats, the One Health approach stands as a crucial strategy, uniting experts across disciplines to safeguard the collective well-being of our planet's inhabitants.

Keywords: Q fever, one health approach, diagnosis, Epidemiology, human, animal

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1. INTRODUCTION

Coxiella burnetii is an intracellular bacteria that triggers Q fever, a widespread communicable disease. While it may be a significant public health concern in specific regions, enhancing global awareness of this illness is crucial. Current knowledge about *C. burnetii* remains somewhat limited, particularly concerning its resilience (both intracellular and environmental) and infectious characteristics. Ruminants are identified as the primary reservoir for this bacterium, releasing the pathogen through various means such as milk, feces, urine, vaginal mucus, and, notably, birth products. Inhalation emerges as the principal mode of contagion. Despite recurrently showing no symptoms in humans and animals, Q fever can lead to acute or chronic diseases. Vaccines containing inactive whole-cell bacteria have been evaluated for both human and animal use, although some shortcomings exist in this approach. Experimental recombinant vaccines hold significant promise (Porter et al. 2011).

2. HISTORICAL OVERVIEW

In Australia, the disease's initial emergence occurred amongst abattoir workers in 1935 in Queensland amidst an outburst of an unexplained feverish illness (Query fever) (Derrick, 1937). This zoonotic bacterial disease affects various hosts, including humans, ruminants, small rodents, dogs, cats, birds, fish, reptiles, and arthropods. Notably, ruminants like cattle, sheep, and goats are assumed to be the primary source of the pathogen (EFSA 2010).

3. UNDERSTANDING THE ETIOLOGY

Q fever, a globally significant acute (sometimes chronic) zoonotic ailment, arises from an obligate intracellular Gram-negative bacterium of the Legionellales order. It was assumed to be a rickettsia-like organism in mouse spleen and liver after exposure to abattoir workers' urine (Mitscherlich and Marth, 1984). Belonging to the *Coxiella* genus in the gamma division of Proteobacteria, *Coxiella burnetii*, much like other members of Proteobacteria, exhibits exceptional resistance to harsh environmental conditions and chemical agents, allowing it to persist for extended periods, occasionally even years. It predominantly targets circulating monocytes and macrophages in body tissues (Maurin and Raoult, 1999).

4. TRANSMISSION DYNAMICS

Human infection can result from tick bites, the breathing of infection-inflicted airdrops, the utility of coarse dairy goods, direct contact with infected animals' milk, urine, excreta, semen, and other potential sources of contamination (Bernard et al., 2012). Clinical manifestations vary and can range from asymptomatic cases (around 60%) or self-restricting feverish infections characterized by exhaustion, nuisance, general discomfort, myalgia, and arthralgia to more severe pneumonia or hepatitis. While less common, complications such as endocarditis, osteomyelitis, and aseptic meningitis may arise. Roughly 1-2% of acute cases may progress to chronic disease (Schimmer et al., 2010). Chronic cases result in various additional symptoms, including hepatitis, pneumonia, heart involvement, neurologic signs, and even persistent fatigue (Morroy G, Keijmel SP, et al., 2016). It can also lead to long-lasting complications like endocarditis, hepatitis, or neurological symptoms (Tissot-Dupont H, Raoult D, 2007). Additionally, Q fever is associated with adverse outcomes in pregnancy, including abortion, neonatal death, preterm birth, and intrauterine growth retardation (Angelakis, 2010).

5. IMPACT ON ANIMALS

While Q fever is frequently asymptomatic in animals, cattle and camels are prone to infertility, metritis, and mastitis, while sheep and goats might experience abortion, stillbirth, and preterm birth (Angelakis et al. 2013).

6. UNDERSTANDING THE ZOONOTIC POTENTIAL

C. burnetii's zoonosis potency extends from the direct interaction between people and diseased animals, including wild and domesticated vertebrates and ticks capable of shedding the microorganism (Setiyono et al., 2005). Studies have demonstrated that improper effluent management and the shedding of vaginal mucus, feces, and urine are primary sources of environmental contamination (Beaudeau et al., 2006). Consequently, the environment becomes contaminated with traces of the pathogen found in dust, compost, fields, fleece, and windborne (Clark and Magalhaes 2018). Transmission amongst humans via close contact with small ruminants can lead to isolated infections or widespread outbreaks. Animal owners, families, employees, and veterinarians are at higher risk due to frequent exposure to small ruminants and contaminated materials (Plummer et al., 2018).

7. EFFORTS FOR PREVENTION

The World Organization for Animal Health (OIE) entitles Q fever as a disease affecting various animal species. To safeguard against Q fever, the OIE advocates for preventative measures, including standardized analytic testing and immunizations for small and large ruminants (OIE., 2019). Instances of localized and sporadic clusters are liable to be singled out in human and ruminant populace via a comprehensive analysis of conveyed cases (Bauer et al., 2020). However, accurately quantifying sporadic cases and minor outbreaks proves challenging, as these reporting systems rely on the vigilance of healthcare, veterinarians, and other pertinent stakeholders. It's reasonable to assume that under-reporting occurs because of these factors (Winter et al. 2021).

8. EPIDEMIOLOGY

The largest Q fever outbreak on record has emerged in a condensed populace where intensive farming sprouts exponentially. Reports of Q fever cases are also rising in countries such as France, Germany, and the USA (Dijkstra et al. 2012).

The case-control study unveiled that exposure to a slaughterhouse was the primary risk factor for *Coxiella burnetii* infection. A connection between slaughterhouse operations and the temporal distribution of cases was established. The initial peak of activity in 1996 occurred in week 9, with the first Q fever case identified four weeks later, aligning with the typical incubation period. The pandemic's zenith was in week 18, coinciding with heightened slaughtering activities in week 14. This suggests that the pathogen was harbored in a contaminated abattoir environment. The airborne transmission of the pathogen is also plausible (Carrieri et al., 2012).

Extensively, Q fever cases so far have been archived except in New Zealand. Perhaps upsurging figures of animals, including domestic mammals, marine creatures, reptiles, ticks, and birds, have been reported as bacterium shedders in recent years (Anderson et al. 2013). The recognition of Q fever as a reportable disease in the United States since 1999 led to a 250% upsurge in human cases between 2000 and 2004 due to enhanced case identification (McQuiston et al., 2006).

This pattern is notably prominent in countries like France, Spain, and the United States. Hyperendemic foci are also identifiable in some of these countries, such as Martigues in southeastern France, where the local mistral wind carries spores from sheep herds and raises Q fever incidence rates to 34.5/100,000 residents. Occasional outbreaks, often familial, may result from an acquaintance to a mutual basis, such as parturient pets like dogs or cats shedding *C. burnetii*(Eldin et al. 2017).

In Africa, the disease began to sprout in 1955 across nine countries, suggesting widespread infection all over the continent (Kaplan, 1955). Mali, Burkina Faso, Nigeria, and the Central African Republic, with high densities of native ruminants, have shown the highest Q fever seroprevalence rates (Dupont et al., 1995). Seroprevalence of Q fever varies greatly, i.e., it may be as low as 1%, as reported in Chad, and up to a prevalence of 16%, as Dupont reported in Egypt. Notably, because of limited analytical tools in many African countries, the factual potency of Q fever remains undervalued. In Tanzania, *C. burnetii* was suggested to be the causative agent in 5% of severe pneumonia cases (Prabhu et al., 2011). A survey in Tanzania revealed 26.2% of zoonotic infections among severely ill febrile patients, with 30% being attributed to Q fever (Crump et al. 2013).

Additionally, seroprevalence upsurges in domestic ruminants in most African countries. The catered cattle surveys range from 4% in Senegal to 55% in Nigeria. In Egypt, sheep herds exhibited a gigantic gain of 33% seropositivity. Goats and camels also showed significant seropositivity rates, with the latter suggested as a substantial reservoir. In rural regions, human homes are in close propinquity to domestic ruminants, facilitating zoonosis. Consequently, *C. burnetii*DNA was perceived in 2% to 22% of household samples in rural Senegal (Ratmanov et al., 2013). Specific *C. burnetii* genotypes have been identified in Africa, primarily in ticks, with only a few detected in humans (Sulyok et al., 2014 & Mediannikov et al., 2010).

9. THE OUTBURST IN NETHERLANDS

Amidst 2007 and 2010, the Netherlands grappled with its most substantial Q fever epidemic, tallying over 4,000 documented cases and a potentially even more significant estimated count exceeding 40,000 (Eldin et al. 2017). The outburst transpired in a populace with a historically low Q fever seroprevalence of 2.4% (Schimmer et al. 2012). Notably impacted were the Noord-Brabant province in the country's southern region, along with Gelderland and Limburg provinces (Roest et al. 2011).

The massive influx of animals, exemplified by a staggering 75% surge in the goat populace between 1985 and 2009, likely facilitated the entrance of *C. burnetii*-infected animals into the country (Delsing et al., 2010). Retrospective studies have revealed that the infection had already been initiated in 2005, marked by abortion cases exceeding 60% on some farms (Roest et al. 2011).

In response, Dutch authorities had to swiftly devise and execute a comprehensive public health strategy starting in 2008. Rampant abortion cases in goats and sheep prompted a nationwide vaccination program. Nonetheless, human cases persisted at alarming levels. Consequently, in 2009, a large-scale culling initiative was mandated to target over 50,000 goats and sheep, even those in gestation. The results were evident in 2010, as a decline in human cases became apparent (Eldin et al. 2017).

10. Q FEVER IN CAYENNE, FRENCH GUIANA

In Cayenne, the capital city of French Guiana, the bacterium is responsible for a staggering 24% of community-acquired pneumonia cases (CAP), marking the ever-peak incidence documented globally (Epelboin et al. 2012). The first case dates back to 1955, involving a slaughterhouse worker (Floch H. 1957). Subsequent sporadic cases surfaced over the next four decades. However, during the 1990s, a remarkable

Q fever incidence occurred. Within a cohort of febrile patients, seroprevalence rates surged from 2% in 1992 to 24% in 1996 (Eldin et al., 2014).

11. PATHOGENESIS

An intracellular pathogen solely responsible for unraveling acute and chronic phases, with a strict reliance on host cells. Inside eukaryotic host cells, it thrives within vacuoles that closely resemble phagolysosomes. This pathogen has a global presence maintains its stability in the environment through persistent infections in ruminant animals. In humans, aerosol-mediated infection leads to invading and controlling alveolar macrophages, a bacterial Type 4B secretion system and secreted effector proteins facilitate it (Shaw and Daniel, 2019).

From its initial discovery as a feverish illness among laborers in a meat processing plant in Brisbane, Australia, Q fever's association with animals has been established. However, the impact on domestic animals was initially considered minimal or absent. In humans, Q fever can manifest in various ways, including acute, chronic, asymptomatic, or mild forms (Maurin and Raoult 1999).

In animals, entry typically occurs through the oropharynx. This pathogen displays high infectivity and is capable of causing infection with exposure to just a single animal (McQuiston et al., 2002). As the chief replication in lymph nodes occurs, a subsequent phase of bacteremia persists for around 5 to 7 days. Following this, the microorganism localizes within the mammary glands and placenta of pregnant animals, a process observed in infected domestic animals (Woldehiwet 2004)

C. burnetii showcases distinct features that set it apart from other bacterial species. Notably, its capacity to bourgeon in lysosomal vacuoles within phagocytic cells and variations in the lipopolysaccharide (LPS) antigen during Phase I and II contribute to its distinctiveness. Moreover, *C. burnetii* can occur in two discrete physical forms: the metabolically latent SCV (minor cell variant), known for its resilience, and the metabolically vigorous LCV (large cell variant), residing within the host cell (Boden et al., 2014; Sireci et al.2021).

An intriguing aspect of this pathogen is its variable incubation period in humans, spanning from 2 to 4 weeks or even longer. This variability depends on factors such as the inoculation dose, infection route, and the antigenic phase of *C. burnetii*. Lipopolysaccharide (LPS) molecules in its cell wall, with unique structure and antigenicity, pose a significant feature (Abnave et al. 2017).

C. burnetii's antigenic diversity is paramount for serological diagnosis and vaccine development. Notably, acute Q fever is characterized by elevated concentrations of anti-phase II antibodies (IgG and IgM), whereas chronic infection exhibits high concentrations of anti-phase I antibodies (IgG and IgA) (Setiyono et al., 2005). The genetic diversity of *C. burnetii* is restricted, with roughly 30 diverse genotypes (M. Million, 2009).

Upon entering the body, the microorganism adheres to phagocytic cellular membranes, particularly monocytes/macrophages. avb3 integrin mediates adsorption of virulent bacterium, whereas avb3 and complement receptor CR3 mediate attachment of avirulent bacteria. Phase I bacteria continue within phagocytic cells, while Phase II bacteria are eradicated. Furthermore, bacteria belonging to Phase I are engulfed in much lower quantities than Phase II bacteria (Angelakis and Raoult, 2010).

Entering phagolysosomes, monocytes, and macrophages engulf small cell variants (SCVs). Within these compartments, SCVs fuse with lysosomal contents, transitioning into metabolically vigorous forms, undergoing progress, and eventually developing into large cell variants (LCVs). Both antigenic forms of *C. burnetii* typically coexist in the phagolysosomal niche. Nevertheless, Phase II bacteria are rapidly obliterated. The acidic habitat of phagolysosomes provides a favorable setting for *C. burnetii* growth. Notably, the bacteria can enormously replicate within this acidic environment, and its predisposition for

tenacious contamination stands out. The utterly evolving cycle of a metabolically active Phase I bacterium transpires inside this acidic niche (Ullah et al. 2022).

The average growth period for the acute phase is approximately 20 days. The severity of illness is determined by the bacterial strain's virulence and the infecting dose. For instance, the QPH1 plasmid-containing strain is more virulent than the QPRS plasmid strain (Patil and Raghunath 2022).

The immune regulation of *C. burnetii* involves T-cells, but its control doesn't lead to complete eradication. The presence of *C. burnetii* is perceived in individuals who sound to have recovered and in the dental pulp of guinea pigs that were experimentally infected and seemingly cured (Honstettre et al., 2004 & Aboudharam et al., 2004). Even months or years after infection, *C. burnetii* DNA may yet remain detectable in bloodstream monocytes or bone marrow (Capo et al., 2003). Within vertebrate hosts, the infection prompts the development of granulomas in affected organs. The formation of these granulomas is enabled by the movement of monocytes via the vascular endothelium. A central lipid vacuole is a defining feature of a distinctive Q fever granuloma encircled by a ring of fibrinoid material (Maurin and Raoult, 2004) .

During the acute phase, only a small number, if any, of individual bacteria can be identified within granulomas. The role of TLR4 becomes evident in the creation of granulomas; mice lacking this receptor show a reduction in granuloma numbers. In response to infection, specific immunoglobulins are produced. Phase II antigen mainly stimulates the production of IgG, while IgM targets both phase I and II cells. Convalescent patients' monocytes exhibit the ability to eliminate *C. burnetii*. TLR4 also affects the cytokine response (interferon and tumor necrosis factor) after acute infections (Honstettre et al., 2004 & Maurin and Raoult, 2004).

12. ONE HEALTH

Throughout history, the concept of One Health has united experts of all diversified categories, such as animal, human, and environmental Health, on a local and global scale, all working together to ensure the well-being of both people and organisms (CDC, 2018). Collaborative efforts include enhancing communication, equipping clinicians with better knowledge and attitudes towards Q fever management, reinforcing laboratory capabilities, improving veterinary parameters, environmental monitoring, human and animal sero-surveillance, and facilitating access to screening and vaccination. An essential aspect of this collaboration is establishing animal surveillance systems and promoting data sharing and intelligence exchange between public Health and veterinary agencies (Dorko et al., 2012).

To fortify individuals from the infection, it becomes essential for both human and veterinary health experts to possess comprehensive knowledge about Q fever's diagnosis, control, prevention and its potential as a zoonotic disease (Winter and Campe 2022).

The yearly rate of reported cases in the US varies from 0.28 to 2.40 per million people. Comparable rates are observed in England and Wales. On the other hand, Australia reports a higher annual incidence, ranging from 15 to 49 cases per million individuals (Mahumud et al. 2019). The urgency of disease control becomes evident due to its significant impact on human Health, the potential for transmission through animal movements, extensive involvement of both animals and humans, insufficient national readiness for outbreak management, and diagnostic challenges (Burke et al. 2012).

The economic repercussions of Q fever are substantial, involving diminished livestock production alongside costs incurred for medical consultations, laboratory tests, hospitalization, and reduced productivity. This collective impact necessitates international assistance and response to address Q fever effectively (Palmer et al., 2007). Recognizing the interconnectedness of communicable diseases between *Homosapiens* and animals, the One Health approach presents robust strategies for managing the financial burdens associated with Q fever (Dantas-Torres et al., 2012).

13. BRIDGING OF Q-FEVER AND ONE HEALTH

The recent outbreak of infections occurring beyond the traditional high-risk workplaces within the community has broadened the perspective of Q fever. It's no longer viewed solely as an industry-related ailment but recognized as a broader public health concern (Tan et al. 2022). The concept of One Health serves as a worldwide strategy, fostering collaborative efforts across human Health, animal health, and environmental sectors. This approach is essential in the realm of infectious diseases, where 75% of infectious diseases are zoonotic (Pearsall 2019).

Effective management of zoonotic diseases, including Q fever, might necessitate a deeper grasp of the interconnected factors that pave the way for emerging illnesses. Achieving this involves cross-sector communication, engagement of stakeholders, and data sharing via a unified platform (Rahaman et al., 2019 & Kahn LH, 2019).

In this context, the concept of One Health is a timely and pertinent reminder of the practical realities that demand multi-sector collaboration, mainly when catering to zoonotic ailments. In Australia and internationally, implementing the One Health approach has yielded success in managing Q fever eruptions (Bond et al., 2016; Biggs HM et al., 2014 & Vellema P et al., 2014). Reporting of Q fever instances may be inflicted by factors like the nearness of wildlife to human housings, extensive ecological contamination from livestock and wildlife, geographic remoteness of susceptible populations, and restricted access to medical care (Karki S et al., 2015). Furthermore, there's limited information about altogether dissemination among livestock and human (Alvarez J et al., 2018).

The coordination and collaboration process entails a spectrum of measures, ranging from enhancing human surveillance to instituting animal surveillance. It includes fostering data exchange and intelligence sharing between veterinary and public health entities, improving communication and equipping clinicians with enhanced Q fever management knowledge. Additionally, consolidating laboratory capabilities, refining veterinary control protocols, monitoring the environment, conducting human-animal sero-surveillance, and facilitating access to screening and immunization all form integral components of this collaborative approach (Dantas-Torres et al., 2012 & Dorko et al., 2012).

Table 1 enlists various studies related to One health approach of different locations in preventing and controlling Q Fever.

14. DIRECT DIAGNOSTIC APPROACHES

Such methods involve bacterial or its parts detection.

14.1. DIRECT VISUALIZATION AND STAINING

An alternative method is Stamp-Macchiavello staining, also known as Macc or conventional Giemsa stain. However, direct visualization through bacterioscopic examination offers limited sensitivity and specificity, as it might be mistaken for other infections like Brucella spp., Chlamydophila spp., or Chlamydia spp (Guatteo R et al., 2006).

14.2. IMMUNOHISTOCHEMISTRY (IHC)

In chronic cases, IHC is employed to diagnose Q fever. This technique helps locate *C. burnetii* in tissues preserved in acetone/paraffin (Angelakis and Raoult 2010). The avidin-biotin-peroxidase complex IHC staining method was given by
Dilbeck and McElwain (Dilbeck and McElwain 1994).

Table 1: Studies relating to One health approach of different locations in preventing and control of Q Fever

Location	Study Type	One Health Practiced	Observed and Expected Outcomes	Comments
South Africa (Simpson et al. 2018)	Cross-sectional	• Risk factor inspection amongst farmers, herders, and veterinary staff. • Human Serology Recommended • Arranging sessions to educate and train human and veterinary experts alike on zoonosis.	• Q fever included in the differential diagnosis of febrile illnesses • Positive Q fever serology demonstrated • Educated clients for better disease prevention	• Diagnostic challenges related to febrile illnesses identified. • The small sample size and non-random selection of participants limit the generalizability of the results.
Europe (Mori et al. 2018)	Systematic review	Practiced • Risk factors reviewed: • Occupational factors e.g., farmers, abattoir workers Husbandry factors e.g., goat farming Environmental factors e.g. infected livestock transportation. Recommended • Q fever observation in high occurrence countries Collaboration across disciplines	• One Health emerges as a paragon for Q fever control, addressing complex interactions between the reviewed factor. • Promote optimum Health of humans, animals, and the environment	• One Health focus was drawn from the Netherlands experience, which may fail to appreciate the subtleties of Q fever epidemiology that govern possible control options in other countries.
Australia (Bond et al. 2016)	Outbreak Investigation	Practiced • Multidisciplinary epidemiological investigation and animal serology • Skin and serological testing for workers, subsequent vaccination • PCR testing of aborted materials, vaginal swabs, environmental samples • General measures, e.g. biohazard sign erection • Site surveillance launched • Health education • Management of farm environment e.g. management Recommended • Mandatory vaccination for all occupational contacts • Further research to identify possible interstate introduction of Q fever • Livestock vaccination	• Comprehensive risk assessment techniques and consensus control measures developed • Workers protected by HEPA* filters • Goats identified as likely source of the outbreak • Controlled human cases without source control • Could not prevent infections in workers' family members • Ongoing farm environmental contamination due to intensive breeding and milking of goats demonstrated • Presumably, these public health measures controlled the outbreak • Prevent acute Q fever cases. • Traditionally held views that interstate importation of *C. burnetii* to Victoria may be established. • Livestock and wildlife prevalence of *C. burnetii* could be established. • Reduced environmental shedding	• Key similarities with the Dutch outbreak include outbreak source, both occurring at goat farms; use of human vaccination, and application of a One Health approach. Differences include magnitude of the outbreaks, livestock vaccination was not used in the Australian outbreak because of manufacture

USA (Dunne et al.,2009)	Review	Practiced • Multidisciplinary diagnostic facilities • Quick result production • Fewer communication pitfalls among stakeholders • Public-private partnerships Joint investigation of Q fever cases • Human and animal serology Recommended • Vector-borne disease control requires human, animal and' vector surveillance	• Sample testing from a range of source • Stewardship and collaborations • Coordinated local responses against diseases and threats • Positive Q fever serology demonstrated • Shared resources and expertise • Animals and humans are protected	• Local, state, and federal levels involving public and private partnerships that combine human, animal, and ecological sectors help minimize resource exhaustion in control of zoonotic diseases.
Netherlan d (Enserink 2010)	Review	Recommended • Resolution of conflict between human and animal health experts. • Improved analytic approach • Immunization	• Through improved parameters, controlling Q-fever. • Minimized human interaction with animals	• Whilst outburst, human-animal communicative interaction was found to be minimal, contrary to what was believed to have a strong network. In this scenario, One Health needs a practical approach to minimize the gap rather than a theoretical way of thinking.

14.3. BACTERIAL CULTURING

Cultivating *Coxiella burnetii* remains a complex task, and the diagnostic sensitivity of this method is low. However, advancements now permit the cultivation of *Coxiella burnetii* in a cell-free laboratory medium without a host cell (Kuley et al. 2015). As Coxiella is intracellular in vivo, this new medium accurately replicates the organism's metabolic requirements within the phagolysosome. This discovery significantly enhances the potential for *Coxiella burnetii* research. Bacterial isolation is rarely pursued because of its high infectivity, particularly in veterinary medicine (Müller et al. 2014).

14.4. PCR

DNA from *Coxiella burnetii* has been successfully identified in several materials, including cell cultures, biopsies, blood, arthropods, and serum samples (Bennett and Banazis 2014). While conventional PCR cannot enumerate the microorganisms present, the introduction of real-time quantitative PCR (RTq PCR) transforms this method into a rapid diagnostic tool that provides measurable data. RTq PCR can be automated, making it suitable for extensive research. Many primers are accessible for diagnosis, with a commonly used primer derived from the often-repeated DNA sequence IS1111 (present in 7 to 120 copies per genome) known for its high sensitivity (Mori et al. 2017).

15. INDIRECT DIAGNOSTIC APPROACHES

15.1. CFT (COMPLEMENT FIXATION TEST)

CFT was the traditional serological diagnostic method in veterinary medicine, as recognized by the OIE. Usually employing phase 2 antigens, CFT can detect around 65% of infections during the second week post the inception of clinical manifestations and up to 90% by the fourth week (Porter et al. 2011).

15.2. ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY)

ELISA serves as an alternative technique for diagnosing animals and humans alike. This method offers improved accuracy, simplicity, and standardization compared to CFT (van der Hoek et al. 2012). Furthermore, a significant correlation between highly positive ELISA results and the prevalence of goat abortions has been observed (Rousset et al. 2007).

16. CONCLUSION

Q-fever stands as an emerging zoonotic disease that prevails like wildfire. In the realm of infectious diseases, Q fever is a prominent example that caters to animal, environmental, and human health relationships, underscoring the significance of the One Health approach. As this zoonotic disease traverses the boundaries between species, it demonstrates the imperative need for collaborative efforts that bridge the expertise of human health professionals, veterinarians, and environmental experts. Whether they unfold within heavily populated regions or remote corners of the globe, Q fever outbreaks lay bare the intricate web that links animals, humans, and their shared environment.

A shining example emerges from the Netherlands, where a massive Q fever outbreak spurred the nation into action. The dynamic interplay of increased livestock populations and human interaction with the environment led to a pivotal realization – that the Health of animals, humans, and the atmosphere is inextricably linked. As the Dutch authorities grappled with the complexities of controlling the outbreak, they recognized the need for a comprehensive strategy that integrates human and animal health concerns while acknowledging the environmental factors at play.

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Mycoplasmosis: A Zoonotic Threat - Epidemiology, Pathogenesis and Economic Impact

02

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ABSTRACT

The world faces a growing threat from mycoplasmas, the smallest and most adaptable microorganisms that can infect a range of warm-blooded animals, birds, reptiles, insects, and plants. This chapter explores the epidemiology, pathogenesis, and economic impact of mycoplasmosis, emphasizing its zoonotic potential. Mycoplasmas, with their minimal genomes and lack of cell walls, navigate various host tissues, causing infections in the alimentary canal, respiratory and urogenital tracts, ocular region, mammary organs, and joints. Human infections involve species like M. pneumoniae and M. genitalium, responsible for respiratory diseases, joint infections, and reproductive issues. The chapter highlights the alarming rise of macrolide-resistant M. pneumoniae, impacting global health. Animal infections, such as contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP), result in painful symptoms and economic losses, with M. bovis being a significant pathogen in cattle.The zoonotic potential is evident as species primarily infecting animals, like M. ovis and M. suis, are found in humans. The economic impact spans various regions, with financial losses attributed to decreased productivity, embryonic mortality, and prevention efforts. Avian mycoplasmosis, affecting birds like chickens and turkeys, adds to economic burdens through decreased egg yield and hatchability. The chapter delves into the pathogenesis of mycoplasmas, highlighting their intracellular lifestyle and unique features such as variable surface proteins. The epidemiological landscape reveals their presence in chronic obstructive pulmonary disease (COPD) and sexually transmitted diseases. Vaccination efforts are explored, addressing diseases in various species, with emphasis on the challenges of developing cost-effective vaccines. Successful eradication programs for diseases like CBPP in China and M. hyo infections in Norway are discussed. In conclusion, mycoplasmosis poses a complex challenge globally, impacting both human and animal health. The chapter emphasizes the need for innovative strategies to address the limitations of existing control measures. Successful eradication programs offer hope, and a deeper understanding of mycoplasma biology is crucial for developing effective preventive and therapeutic interventions.

Keywords: Economic impact, Avian mycoplasmosis, Public health, Vaccination, Eradication

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1. INTRODUCTION

The smallest and easiest-to-replicate microorganisms are mycoplasmas. In warm-blooded animals, birds, reptiles, insects, and plants, various species occur as adept microbes. Owing to their trivial genome and walllessness, they are cautious, and some of them are difficult to develop due to their limited metabolism. Thus, they might work as extracellular and intracellular organisms whose endurance depends on their hosts' liberality. In vivo, mycoplasmas are pantropic. The predilection sites of mycoplasmas are the alimentary canal, the mucous surface of respiratory and urogenital tracts, the ocular region, mammary organs, and joints (Dawood et al. 2022).

Through their interaction with cell membranes of specific target cells, certain Mycoplasma species, such as Mycoplasma pneumoniae, M. hominis, and M. gallisepticum (MG), can confer to and enter these cells (Shibata et al. 2000; Vogl et al. 2008). Horizontal gene transfer (HGT) can occur when two Mycoplasma species coexist in a single habitat, leading to the emergence of pathogenic mycoplasmas and significantly affecting their disease-causing abilities (Bürki et al. 2015). The advent of drug resistance due to the exchange of resisting alleles among various microorganisms is becoming an alarming issue (Faucher et al. 2019).

In the context of human infections, research has demonstrated that six Mycoplasma species, namely M. pneumoniae, M. gentenitalum, Ureaplasma urealyticum, U. parvum (Lobão et al. 2017), M. hominis and M. penetrans, are responsible for various human illnesses, including acute respiratory diseases, joint infections, genital and urinary tract infections, and neurological disorders (Dawood, Ali et al. 2022). Conversely, species like M. ovis, M. suis, and M. haemofelis, which primarily infect animals, are noticed in humans and are considered communicable agents (Maggi et al. 2013). Additionally, severe circumstances, for instance, chronic obstructive pulmonary disease (COPD) and infertility, may arise owing to such infections (Feng et al. 2021; Kusanovic et al. 2020).

The unhindered routine utility of macrolides has been the global source of macrolide-resistant M. pneumoniae (MRMP). MRMP is believed to prevail from 15% to 30% in Taiwan (2010 to 2017) and less than 30% in Europe and America between 2008 and 2013. Conversely, MRMP prevalence has reached anywhere from 60% to 90% in China, Japan, and Korea (Waites et al. 2017; Yang et al. 2019). Clinical manifestations of infections vary from mild and self-restricting to potentially dreadfully lethal, as illustrated in Figure 1. For instance, it is a leading cause of community-acquired pneumonia (CAP), chiefly in infants and grown-ups (Li et al. 2019).

2. ANIMAL INFECTIONS

Numerous domestic and wild animals are susceptible to mycoplasmosis, with contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP) being notable diseases, particularly in low- and middle-income regions. These conditions are characterized by painful symptoms, reduced productivity, and fatalities, as depicted in Fig. 1 (Bolajoko et al. 2020).

2.1. BOVINE

Mycoplasma mycoides mainly prevails in cattle and water buffalo sprouting CBPP. WOAH alerts about this ailment as an alarming condition (OIE 2021). Amongst Other mycoplasmas, *M. bovis* is the most significant pathogen affecting cattle worldwide. Likely, it proliferates swiftly from the stage of infancy to old age. Calves under three months of age, mainly those weaned, exhibit the utmost incidence of *M. bovis* pneumonia, as indicated in a recent United Kingdom survey from 2006 to 2017 (Anne Ridley et al. 2018; Hazelton et al. 2020).

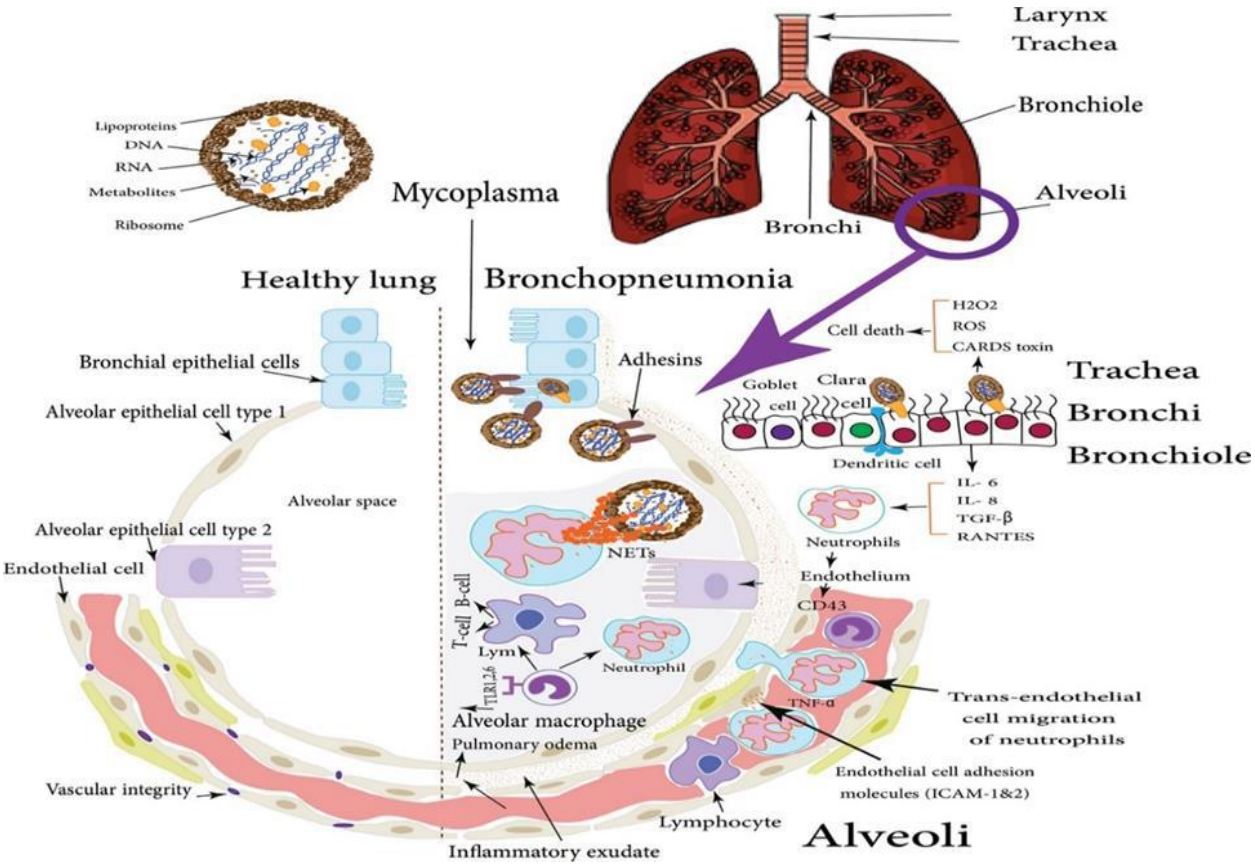

Fig. 1: the pivotal role of *M. pneumonae* In lung infection (Dawood Ali et al. 2022).

Notably, the agent that marauds the upper respiratory tract is *M. bovis,* in calves in the initial days of their infancy via infected dairy products utility and from already inflicted calves (Maunsell et al. 2009). Moreover, it ranks among the four vital bacterial agents allied with bovine respiratory disease (BRD), resulting in substantial fiscal deprivation due to increased morbidity and fatality rates, impaired growth, and elevated expenses related to prevention and panacea (Kudirkiene et al. 2021).

2.2. GOAT

Mycoplasma capricolum is a causative agent that inflicts CCPP, a dreadfully infectious ailment, in goats vitally proliferates in East Africa, specifically in Kenya, Ethiopia, and Tanzania (Falquet et al. 2014; Abd-Elrahman et al. 2020).

M. agalactiae triggers an OIE-notifiable ailment known as infectious agalactia (CA), which induces mastitis in dairy goats, resulting in substantial economic downgrading owing to arthritis, decreased or termination of milk production, cachexia, and corneal opacity leading to visual impairment (Santos et al. 2015).

Hemoplasmas, also known as pleomorphic minute bacteria, derive their name from their tendency to adhere to erythrocyte surfaces, potentially causing hemolytic anemia in several mammals. Two notable hemoplasmas, *M. ovis,* and *Candidatus M. haemovis*, are identified as tainting small ruminants, with increased mortality rates in young, elderly, and pregnant animals. In goats, *M.ovis* represents the obstinate infection. Surveys on the frequency of *M. ovis* contagion vary, as absentees in Australia and Tunisia exist in Hungary (20%) and in Malaysia (94%) (Dawood et al. 2022).

2.3. SHEEP

In the realm of ovine health, the first identification of *M. ovipneumoniae,* often referred to as "sheep atypical pneumonia," has been associated with infections in sheep and goats (Besser et al. 2013). This pathogen has triggered numerous global epidemics, which have been perilous to the lambing industry due to reduced ewe productivity and stunted lamb growth (Jaÿ et al. 2020). It's worth noting that Maggi and his research team predominantly identified species identical to M. ovis- in humans, indicating its potential for zoonotic transmission (Maggi et al. 2013).

2.4. SWINE

In the porcine world, *M. hyosynoviae* and *M. hyorhinis* are opportunistic microbes in the tonsils and upper respiratory tract. They are known to cause arthritis and polyserositis in piglets aged between 6 and 10 weeks, while older pigs above three months typically experience milder arthritis symptoms. Adult pigs with *M. hyosynoviae* arthritis are more susceptible, but the lesions primarily affect the synovial membranes and joints (Neto 2012; Gomes et al. 2015).

M. hyo pivotally intensifies the progression of the porcine respiratory disease complex (PRDC) infection, minimizing feed efficiency, decreasing animal growth performance, and lowering the typical routine improvement. This complex disease scenario often increases mortality, particularly when compounded with other pathogens (Olaniyi et al. 2020).

Additionally, the pig industry faces the threat of infectious anemia caused by *M. suis* hemoplasma species. Among others, *M. suis* is the primary pathogen responsible for pig hemoplasmosis. It adheres to the surface of RBCs, leading to their clearance by the spleen and resulting in reproductive failures, primarily stillbirths, as documented in Southern Brazil (Petri et al. 2020; Bordin et al. 2021).

3. AVIAN HEALTH

While more than 23 mycoplasma species are acknowledged in birds, four of them, namely *M. gallisepticum* (MG*), M. synoviae* (MS)*, M. meleagridis (MM), and M. iowae (MI)*—are responsible for avian mycoplasmosis. MG and MS are recognized as OIE-notified respiratory infectious agents that have inflicted substantial financial deprivations by causing a significant decrease in egg yield, hatchability, weight gain, and feed conversion efficiency. They also lead to increased embryonic mortality, condemnation of carcasses, and higher precaution and remedy expenses in layers, broilers, and breeder flocks (Yadav et al. 2021; Behboudi 2022).

These pathogens induce consistent respiratory issues in chickens, characterized by labored breathing, sinusitis, airsaculitis, and reduced carcass quality in broilers (Jelani, Ghulam. 2023; Michiels et al. 2016). Free-flying avian species like house finches are highly susceptible to wild MG transmission (Luttrell et al. 2001). Additionally, MI occasionally infects turkeys and, less commonly, chickens. In turkeys, MI infections result in delayed embryonic fatality, reduced hatchability, and limb deformities in young chicks. *MM* primarily causes air sac disease, musculoskeletal problems, and reproductive issues in turkeys and has been sporadically isolated from chickens (Pritchard and Béjaoui Khiari et al. 2011). Fig. 2 illustrates mycoplasmas' broad-ranging invasion into target tissues and their effective interactions with immune cells.

3.1. CANINE

Exceeding fifteen discrete mycoplasma species are so far documented, particularly for dogs. They typically coexist harmlessly, with only a few posing potential health concerns. One such concern is *M. cynos*,

primarily accompanied by a dog's lower respiratory tract (LRT) infections. *M. cynos* primarily leads to upper respiratory ailments in canines and enjoins the amplified severity of the canine respiratory disease complex (CRDC). Clinical manifestations involve coughing, mucus production, and the accumulation of exudate (Jambhekar et al. 2019; Chalker 2005). In addition, two hemoplasma species, *M. hemocanis,* and *M. haematoparvum*, have been observed in dogs (Rosanna et al. 2020).

3.2. FELINE

Focusing on domestic cats, four types of hemoplasmas are commonly encountered: *M. haemofelis, Candidatus M. haematoparvum*-like*, Candidatus M. haemominutum (CMhm), and Candidatus M. turicensis (CMt)* (Zhang et al. 2021). Amongst all, *CMhm* seems to be wildly contagious and causes hemolytic anemia. *M. haemofelis*, on the other hand, causes an extraordinarily appalling and potentially perilous anemia in cats, while others exhibit lesser severity and, nonetheless, can lead to grave illness in immunosuppressed felines (Willi et al. 2006). Non-hemotropic Mycoplasma (*M. felis*) can induce conjunctivitis, respiratory symptoms, and polyarthritis in cats (Greene and Chalker 2012).
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4. PATHOGENESIS

Mycoplasmas, in contrast to other extracellular bacteria, undergo a process of development and adaptation to their parasitic intracellular lifestyle after invading host cells. This adaptation leads to slower intracellular growth rates (Rüger et al. 2021). Their ability to conceal themselves and elude the host's imperishable defensive mechanism is partly due to this reduced intracellular growth rate.
M. bovis invades various cell types. Such foray serves mycoplasma by triggering inflammation, limiting immune cell responses, facilitating movement in the entire respiratory tract, and additional dissemination to multiple tissues from the lungs with the assistance of enormous invading enzymes (Van der Merwe et al. 2010).
M. bovis persists in necrotic lung lesions (Khodakaram-Tafti and Lopez 2004). They produce assaulting enzymes, including proteases, nucleases, sialidases, antioxidants, and hyaluronidases. Nucleases are critical in degrading host nucleic acids, influencing development, endurance, perseverance, and pathogenicity (Yiwen et al. 2021). Proteases can degrade IgG antibodies, as seen in the MIB-MIP system

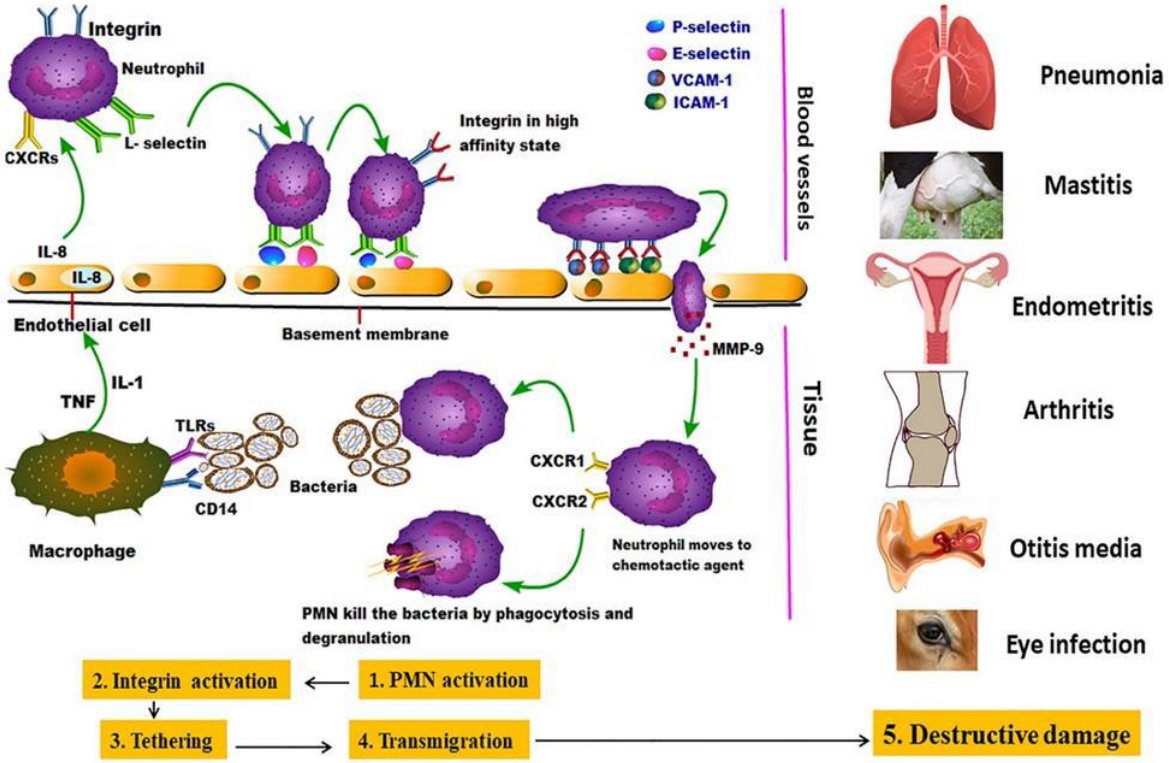

Fig. 2 illustrates the broad-ranging invasion of mycoplasmas into target tissues and their effective interactions with immune cells. This process begins with activating the first line of immune cells, neutrophils. In response to the presence of mycoplasma, neutrophils release various danger signals, setting off a cascade of events that lead to the activation of PMNCs (polymorphonuclear cells). This activation process involves Integrin activation, Tethering, and Transmigration and ultimately results in the deterioration of multiple body parts, giving rise to various inflammatory lesions (Dawood Ali et al. 2022).

(Nottelet et al. 2021). Sialidase and neuraminidase are infective biocatalysts involved in the hydrolysis of sialate, extracellular matrix (ECM) degradation, tissue invasion, and apoptosis (Robinson et al. 2017). MG exhibits a tropism for ciliated respiratory epithelium, allowing it to evade mucociliary clearance and invade host cells (Matyushkina et al. 2016).

Variable surface proteins (VSPs) appear to be strongly immunogenic lipoproteins that can be selectively expressed or silenced in response to environmental changes, resulting in alterations in surface antigenic phenotypes. For instance, in the genome of the *M. bovis* type strain PG45 (American strain), the vsp gene family contains 13 alleles, but only two are expressed, while the others become dormant. Additionally, the size of these proteins is tightly regulated (Clampitt 2021; Lysnyansky et al. 1999; Qi et al. 2012).

Tracking Mycoplasmas throughout their entire parasitic intracellular lifecycle is challenging owing to their small size and the absence of a cell wall, which sets them apart from other microorganisms. Consequently, their discreet intracellular existence significantly impacts cell metabolism, physiology, and immunity (Benedetti et al. 2020).

5. EPIDEMIOLOGY

COPD stands as a prominent cause of mortality in the USA, and the death toll rises to 130,000 individuals annually. Globally, over 3 million people succumb to COPD-related complications each

year. Low and middle-income countries bear a heavier burden of this disease. Notably, M. pneumonia has been found in higher concentrations within the lung microbiota of COPD patients (Marciniuk and Schraufnagel 2017).

In females, *M. genitalium* transfers via intercourse and is accompanied by various health issues such as cervicitis, pelvic inflammatory disease (PID), spontaneous abortion, premature birth, and infertility. It has been detected in 4% to 22% of women with PID and 10% to 30% of women experiencing clinical cervicitis (Gaydos et al. 2009). Conversely, *M. genitalium* is responsible for nearly 15%–20% of cases of Nongonococcal Urethritis (NGU) and persistent or recurrent Urethritis in men, both symptomatic and asymptomatic (Bachmann et al. 2020).

Mycoplasma capricolum, shortened as Mccp, accounts for CCPP. They are previously acknowledged as Mycoplasma biotype F38. These microorganisms belong to the Mollicutes class, with a unique characterization of lack of cell wall but possessing galactan and small genomes (0.58-1.35 Mb). They cause various diseases in animals and have limited biosynthetic capabilities. Numerous studies have explored the taxonomic associations within the F38 group of caprine mycoplasmas (Yatoo et al. 2019).

OIE proclaims that CCPP prevails wildly in around 40 countries, particularly Africa and the Middle East, with an enormous goat population. The ailment originated in Algeria in 1873 and has been documented in various countries, including Turkey, Iran, Oman, and Yemen. In Egypt, where the caprines are substantial, *Mccp* was recently separated and identified in sheep and goats in Giza in 2015 and Matrouh during 2017-2018 (Selim et al. 2021).

Mycoplasma gallisepticum, a member of the Mollicutes class, possesses a small genome size (996,422 bp for the Rlow strain) (Papazisi et al. 2003). Mycoplasmas primarily inhabit mucosal layers in the respiratory and urogenital tracts, eyes, mammary glands, and joints. One species of mycoplasma, MG, is capable of causing both acute and chronic diseases at various locations. However, it is commonly recognized as an airborne agent in diseased bird species. When it proliferates unchecked in susceptible birds, the organism spreads to the lungs and air sacs, causing severe inflammation of the sinus mucosa and trachea (Levisohn and Browning et al. 2010).

In poultry, proliferation from hens to chicks via egg becomes a vital way of dissemination. Therefore, outsourcing chicks or poults from MG free breeding is hindered somehow. Older birds, particularly those purchased from markets or mixed sources, can introduce MG into a flock, as seemingly healthy but infected birds can begin shedding the organism under stress, including social stress among other birds. Spread from bird to bird can occur through respiratory or contaminated fomites. However, within a flock, the spread is typically gradual and takes 6 to 21 days. The infection is believed to survive outside its host for 18 months or longer under farm conditions. MG holds between 30 and 70 variation vlhA genes, mostly translationally equipped, leading to genetic variation despite its small genome. Only a single gene seems to be transliterated at any given time, and these genes were acquired through lateral gene transfer between Mycoplasma species, resulting in the expression of a single variant of this lipoprotein on the cell surface. Several isolates have been obtained using various molecular epidemiological typing methods (Behboudi 2022).

6. ECONOMIC IMPACT

OIE has now announced that South Africa, Australia, Europe, and the United States are free of CBPP. In Asia, China and India appear free, yet the status of ailment remains blurred in the rest of Asia (OIE 2019). During the 45-week laying cycle, it has been documented that chickens lose approximately 16 eggs. Fiscal mishaps incited are because of diminished efficiency (around 10-20%), early embryonic mortality (roughly 5%-10%), and expenses of prevention and control of infection (Behboudi 2022).

Table 1: Illustration of Financial Loss of Different Mycoplasma Species in Different Regions.

Species	Disease	Regions	Financial Loss	References
Bovine	CBPP	Global	Sub-Saharan Africa 2 billion US$	(Anonymous 2018)
Avian	*Avain Mycoplasmosis*	Global USA	US $780 million every year $150 million annually	(Behboudi 2022)
Caprine	CCPP	Endemic Areas	US$507 million in endemic areas	(Yatoo Mohd Iqbal et al. 2019)
Ovine	CCPP	Tanzania	2,273,281TZS loss per household annually	(George 2017)

7. VACCINATION

Betlach et al. (2021) delved into the possible effect of various immunizations in reducing *M. hyo* spread. Their findings revealed that a three-dose regimen of commercial bacterin vaccination vitally abolished lung lesions at 28 days post-infection in tested gilts, along with an overall decrease in bacterial load in vaccinated gilts. In another study focusing on the practicality and monetary advantages of immunizing piglets against the bacteria at different ages, it was established that immunization at three days of age conferred a substantial advantage over-vaccination at 7 or 14 days of age (Vangroenweghe, 2021).

Recent investigations into *M. hyo* vaccinations have explored the effectiveness of novel bivalent and trivalent vaccines. These studies have concluded that such immunizations offer high fortification against the infection (Yang et al. 2021).

M. genitalium exhibits zoonotic characteristics, facilitating the transmission of sexually transmitted diseases between humans and animals (Nogueira et al. 2021).

Regarding *M. bovis*, which poses a significant threat to dairy cattle in many countries, vaccination is a central focus for disease control due to the growing antimicrobial resistance. However, commercially economical vaccines for the market are lacking. Based on our previous research, the efficacy of the attenuated P150 *M. bovis* strain was 87.7%, making it a potent adjuvant for a live vaccine (Dawood et al. 2022).

The existing CCPP vaccine is a bacterin with a saponin adjuvant, recommended for administration to kids at 4 months of age and then every 6 months. Its production is relatively expensive, following the meticulous cultivation of the causative agent and the substantial protein requirement for each vaccine dose (Dawood et al. 2022).

For the prevention of MG and MS, there are commercially available live attenuated and recombinant live poxvirus vaccines. Additionally, virulent MG live strains (F, ts-11, and 6/85 strains) can be safely employed (Yadav et al. 2021). Recent studies have shown that a regimen of three consecutive MG vaccinations, consisting of one live vaccine followed by two inactivated vaccine doses, provides an excellent shield in poultry (Kiers 2020). Temperature-sensitive strains such as MS-H and ts-11 exhibit remarkable efficacy when administered as eye drops in chickens and turkeys. These strains are readily available commercially and have significantly reduced the macrolides' utility in poultry and lowered disease prevalence in chickens, as demonstrated in Australia (Purswell et al., 2012)

8. ERADICATION OF MYCOPLASMA

Contagious bovine pleuropneumonia (CBPP) has been successfully eradicated from many regions worldwide. Catering China, where the disease erupted into exponential fiscal deprivation in the cattle industry from 1950-70. An adequate immunization was inoculated from rabbits, such as a virulent strain of Mmm (Ben-1). This vaccine exhibited high immunogenicity and practical efficacy (95-100%) in cattle for

28 months. Ultimately, the last reported CBPP case was observed in 1989, and in 2008, China was declared CBPP-free by the OIE. Initially, in the 20th century in Europe, vaccine administration was abandoned. Slaughtering was the only practical approach to confine the contamination. This approach remained hugely successful, and CBPP was obliterated by the mid-1960s (Dawood et al. 2022).

In Australia, attenuated vaccine strains (KH3J and T1/44) significantly abridged the cases. However, 1973 marks the year of thorough obliteration, primarily through minimized animal movement and a comprehensive elimination policy. In New Zealand, *M. bovis* appeared for the first time in 2017. The Ministry for Primary Industries (MPI) took bold steps to eliminate *M. bovis* from New Zealand despite the challenges posed by identifying and confining the movement of infected livestock. On 19 August 2021, the Chair of the Technical Advisory Group to MPI for the *M. bovis* program announced that *M. bovis* currently has only three active properties. Eradication may be within reach shortly (Dawood et al. 2022).

More recently, Gulliksen and colleagues have reported successfully eradicating *M. hyo* infections from the Norwegian pig population (Gulliksen et al. 2021).

9. CONCLUSION

In recent years, widespread Mycoplasma pathogens have raised significant concerns. This chapter summarizes the epidemiology, pathogenesis, economic impact, vaccination, and eradication programs of mycoplasma species globally in humans and different animals. Moreover, the immune response of Mycoplasma microorganisms as exceptional antigens with restricted metabolic limits poses a conspicuous impact. Accordingly, embracing innovative strategies to check the component of mycoplasmas contamination is fundamental. Lastly, several scenarios for eliminating mycoplasmas in many regions of the world have been successful and can be followed by others.

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Animals to Human Transmission of Intestinal Diseases: A Review of the Mechanism and Factors Involved

03

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ABSTRACT

Many intestinal diseases in animals either in pets or livestock animals have the potential to transmit to humans and cause disease. These diseases include bacterial (campylobacteriosis, salmonellosis, yersiniosis, plesiomonas and Aeromonas, clostridial disease, shigellosis, colibacillosis), protozoal (coccidiosis, giardiasis, amoebiasis, balantidiasis, trichomonas), helminthic (strongyloidiasis, echinococcosis, echinococcus multilocularis, taeniasis, coenurosis, dipylidium caninum, cutaneous larva migrans) and even viral (parvovirus infection). The mechanism of transmission, clinical features and intermediate vectors vary with disease to disease. The most common route for transmission of these diseases is the fecal-oral route. Many of the causative agents for these diseases are the normal inhabitants of the intestinal tract of the animals. The widespread presence of these reservoir hosts determines the prevalence of these diseases. Other factors such as the persistence of causative agent in the environment, effective fecal shedding and efficient use of transmission vectors also determine the prevalence of a zoonotic disease. Transmission of campylobacter to humans principally occurs through contaminated animal-origin food, water and direct contact with infected animals specially pets. Salmonella transmission primarily occurs through close contact with the infected animals, contaminated food, contaminated raw poultry and meat, aerosols and oropharyngeal secretions. The primary source of Yersinia serotypes 0.3 and 0.9 are swine. Cryptosporidial transmission happen in two ways: direct or indirect transmission. Direct transmission occurs through oral exposure to oocytes and indirect transmission occurs through cross-contamination. Transmission of giardia occurs through cysts that are very resistant to harsh environmental conditions. Helminthic diseases are transmitted by ingestion of either larvae (Strongyloidiasis), eggs (Echinococcosis) or meat of infected animal (Taeniasis).

Keywords: Zoonosis, Intestinal diseases, Bacterial zoonosis, Protozoal zoonosis, Helminthic zoonosis, Viral zoonosis

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1. INTRODUCTION

Humans have long been prone to intestinal diseases transmitted from animals. These intestinal diseases include bacterial, protozoal, helminthic, or viral infections. In recent times, certain factors, such as the use of immunosuppressive drugs (Bulbuloglu et al. 2022) and the prevalence of acquired immunodeficiency syndrome (AIDS) (Kelly et al. 2009) have heightened the importance of these conditions in the human population. The presence of many pathogens in the stool has made it complicated for researchers to discover the disease's actual cause. Therefore, many of these diseases lack comprehensive understanding. Pathogens can often exist in many species, indicating a potential absence of host specificity. This phenomenon then raises concerns about the possibility of transmission between different species. Despite knowledge about the host specificity of a pathogen, understanding its zoonotic risk is often impeded by challenges posed by its transmission mode. This raises concerns about humans health, which can only be prevented when we have accurate information about the host specificity and mode of transmission of the pathogen from one species to another.

Both pet animals and livestock species are essential for their zoonotic risks. In a pet-centric society, the involvement of dogs and cats in transmitting these diseases gains importance, while in rural areas, livestock animals are mainly involved in the incidence of zoonotic diseases.

2. BACTERIAL DISEASES

2.1. CAMPYLOBACTERIOSIS

Campylobacter is widely recognized as the primary cause behind bacterial foodborne diarrheal disease globally. Symptoms vary from mild to severe infections, particularly in children and the elderly, with potential permanent neurological effects. Lastovica et al. (2014) described 30 species and sub-species of Campylobacter genera, 17 of *Arcobacter*, and 7 of *Sulfurospirillum.* This microorganism, characterized by its cytochrome oxidase positivity, curved Gram-negative, non-spore-forming (Garénaux et al. 2008), rod shape, and unique corkscrew motility, thrive in low oxygen environments and is commonly found in the intestinal tracts of numerous wild and domestic animals, avian species notably poultry, are among the primary carriers of this organism (Silva et al. 2011; Davis and DiRita 2008; Ragimbeau et al. 2014). In 1906, two British veterinarians documented *Campylobacter* for the first time. They reported the discovery of a peculiar organism in large quantities in the uterine mucus of a pregnant sheep (Skirrow 2006). Subsequently, *Campylobacter* species were also isolated from the fecal samples of cattle and pigs experiencing diarrhea (Klein-Jöbstl et al. 2016; Debruyne et al. 2008; Epps et al. 2013). The studies raised increased interest in *Campylobacter* as the researchers observed a high occurrence of these bacteria in human diarrhea cases (On 2001; Huang et al. 2009).

Amongst the various *Campylobacter* spp., *Campylobacter jejuni* and *Campylobacter coli* are widely acknowledged as the most important enteropathogens. These two species are particularly noteworthy for their impact on causing gastrointestinal infections in humans. During the early 20th century, *Campylobacter* was identified as a pathogen primarily associated with animals. Extensive research during this period shed light on its prevalence and impact as a pathogen affecting various animal species (Acheson and Allos 2001). In the late 1970s, *Campylobacter* was recognized as an essential human

pathogen (Rautelin and Hanninen 2000). Before that, its role in causing human infections and its impact on human health was not fully understood.

2.1.1. TRANSMISSION TO HUMANS

Campylobacter is a part of the normal intestinal flora in a wide range of domesticated and wild animals (Horrocks et al. 2009). Moreover, *Campylobacter* can also be carried by pet animals, including dogs, cats, and birds (Pintar et al. 2015). Given the widespread presence of its reservoirs, it has the potential to contaminate surface water and soil. Transmission to humans principally occurs through ingesting contaminated animal-origin food, consuming contaminated water, and direct contact with infected animals, particularly pets (Luangtongkum et al. 2009; Fernandes et al. 2015). Nichols and Shane have described the transmission of *Campylobacter* by flies. Their findings have highlighted the role of flies as potential vectors for *Campylobacter* transmission (Shane et al. 1985; Nichols 2005).

2.1.2. CLINICAL FEATURES

Campylobacter infection usually manifests as an acute gastrointestinal illness that presents symptoms resembling those caused by *salmonella* or s*higella*. It is necessary to detect *Campylobacter* in the patient's stool because of its similarity with other diarrheal disorders. This ensures the definitive diagnosis of the specific pathogen responsible for the disease. In the outbreak investigations, the usual incubation period after the ingestion of *Campylobacter* has been observed to be four days or less. However, incubation periods of one week or longer have also been observed in other instances, and these may be more common when low levels of pathogens are ingested (Horn and Lake 2013). In addition to affecting the incubation period, the infective dose, the virulence of the strain, and the host's susceptibility to infection also affect clinical symptoms. Symptoms usually start with abdominal cramps, watery diarrhea, and fever. More than eight bowel movements are in the severe form of illness, followed by bloody diarrhea (Acheson and Allos 2001) in almost 1/3 of the patients, indicating that infection has spread to the large intestine and rectum. Other non-specific symptoms include headache, vomiting, rigor, and myalgia (Lastovica et al. 2014).
However, the most critical aspect of *campylobacter* infection is the cramping of the abdomen, which may be so severe that it imitates appendicitis. The disease may last around four days. However, the patient continues to shed the pathogen in stool for up to 69 days (Black et al. 1988). Shedding may last up to 54 days in poultry (van Gerwe et al. 2009), so poultry litter plays a significant role in transmitting *Campylobacter* infection to humans. Nosocomial infections in neonatal intensive care units have also been reported (Wagenaar et al. 2014).

2.2.3. TREATMENT

Infection is usually self-limiting for mild infections; however, erythromycin is attempted as a drug of choice in people with compromised immune systems or with severe symptoms like bloody diarrhea and fever. Alternatively, tetracyclines and fluoroquinolones may also be used as effective antibiotics. The increasing use of these antibiotics in humans and food animals has raised the frequency of resistant campylobacteriosis (Aarestrup and Engberg 2001; Wieczorek and Osek 2013).

2.2. SALMONELLOSIS

The genus Salmonella causes salmonellosis. Two species, *S. enterica* and *S. bengari,* are responsible for the disease. They are motile, gram-negative, rod-shaped, and facultative anaerobic bacteria. There are

more than 2400 serotypes (serovars) of *salmonella* (Brenner et al. 2000). They are present in the environment and produce illness only in people with compromised immune systems. They have a broad host range, from humans to domestic animals and avian species. Salmonella can be isolated and identified in almost all vertebrate species, but many salmonella serotypes have immensely evolved to be associated with a single host; examples include *S. Typhi* (primarily in humans), *S. Dublin* (in bovines), *S. Gallinarum* (specific to poultry), *S. Choleraesuis* (in swine) (Griffith et al. 2019). In the case of horses, salmonellosis is non-specific and can be caused by many of the serotypes.

In most cases, salmonellosis in humans is caused by four serotypes of *S. enterica* (*S. enteritidis, S. Typhimurium, S. Newport, S. Heidelberg*). They can survive for long periods in the environment because of their resilient nature, up to 54 days in water reservoirs and more than a year in cattle feces (McGuirk and Peek 2003; Moore et al. 2003). They can proliferate in a temperature range of 7°C to 45°C and a broad pH range of 6.5 to 7.5. They are sensitive to sunlight exposure and many commonly employed disinfectants, including phenolic compounds, iodophors, and sodium hypochlorite (Joseph et al. 2001). The source of infection for animals may vary but typically is from contaminated feed, water, and contaminated environment or by excreta from infected animals. Outbreaks may be a result of fertilizers and feed supplements that contain meat meal, bone meal, fish meal, and other by-products or plant products that may be contaminated. Water can also serve as a source of infection only when surface water is used for consumption. Tap water is unlikely a source of infection.

Centers for Disease Control and Prevention (CDC) reported that four serotypes of *salmonella enterica* were responsible for most of the *salmonella* cases (almost 60%), *S. enteritidis* (24.7%), *S. typhimurium* (23.5%), *S. newport* (6.2%), *S. heidelberg* (5.1%). S. typhi, which is restricted to humans only, other host-specific salmonella serotypes such as *S. Dublin* in bovines, *S. gallinarum* in poultry, and *S. choleraesuis* in swine can also be transmitted to humans. Moreover, non-host-specific serotypes can also be transmitted to humans and cause disease.

2.2.1. TRANSMISSION TO HUMANS

Salmonella has all the necessary characteristics that enable them to have a wide distribution and a wide range of reservoir hosts, their persistence in the environment for long periods, effective fecal shedding, and efficient use of transmission vectors. Its serotypes can be found and isolated from the feces of almost all cold-blooded and warm-blooded animals. Transmission to humans primarily occurs through the fecal-oral route. Transmission can occur by close contact with the infected animal or human by contaminated environment and food. Direct contact with the infected animal, clinically or sub-clinically, is an essential source of human infection. However, contaminated food remains the most significant mode of transmission for humans. Contaminated raw poultry and meat are the primary culprits of transmitting *salmonella* infection. In addition, aerosol transmission (for short distances) and transmission through oropharyngeal secretions because tonsils become contaminated following illness are also modes of transmission. *Salmonella* spp. are widespread in the environment and the reservoir hosts. When examined for salmonella in their feces, non-diarrhoeic dogs showed a 9.47% prevalence in Xuzhou and 3.6 percent in Trinidad and are carriers of a maximum of 28 salmonella serotypes. Multiple serotypes may be present simultaneously (Seepersadsingh et al. 2004; Wei et al. 2020). They shed them in their feces and may serve as a potential source of infection for humans. Dogs usually shed *salmonella* in their feces for 3 to 4 weeks and rarely as long as 100 days following infection (Bagcigil et al. 2007). In the case of cats, the isolation rate from feces was 1.77 percent (Wei et al. 2020). It suggests that pet lovers may have higher chances of acquiring the pathogen and developing the disease than others. Salmonellosis in humans is more common in fall and winter (Oloya et al. 2007).

2.2.2. CLINICAL FEATURES

Clinical features of the disease vary with the virulence of serotypes, host defenses, and the level of initial inocula. Non-typhoidal salmonellosis, along with other foodborne illnesses, is a worldwide health problem. Clinical symptoms for non-typhoidal salmonellosis begin to appear after 12 to 796 hours but sometimes may extend to a week or longer after entry of the bacteria into the body and vanish in 5-7 days (Eikmeier et al. 2018). Clinical manifestations of non-typhoidal salmonellosis are usually self-limiting diarrhea, fever, abdominal cramps, and vomiting (Onwuezobe et al. 2012). A non-typhoidal salmonellosis investigation in an outbreak found that all the cases had diarrhea that was followed by fever in 96.2%, headache in 84.9%, abdominal pain in 50.1%, nausea and vomiting in 49.1% and body ache in 39.6% (Singh et al. 2013). Septicemia may also occur in some instances (mainly in elderly >50) that may lead to pneumonia, osteomyelitis, or, in some cases, meningitis (Chen et al. 2012). Unlike other non-typhoidal pathogens, *S. Choleraesuis* produces severe septicemia (Griffith et al. 2019). The mortality rate is more in immuno-compromised people and infants.

2.2.3. TREATMENT

The disease is usually self-limiting. In severe cases, supportive therapy should be recommended. Antibiotic therapy should be avoided as it may prolong the carrier state of the pathogen. *In-vitro* resistance pattern for non-typhoidal salmonella infections was 79% for ampicillin, 72% for co-trimoxazole, 55% for gentamicin, and far less for chloramphenicol (0.3%). Thus, chloramphenicol may be considered the drug of choice for non-typhoidal salmonellosis (Graham et al. 2000). However, samples should be tested for antimicrobial sensitivity before antibiotic therapy because *salmonella spp.* show variable patterns of resistance. In septicemic patients, fluid therapy, intravenous steroids, and sometimes plasma transfusion may also be recommended.

2.3. YERSINIOSIS

Yersiniosis is generally a self-limiting gastrointestinal disease of worldwide concern. It is caused by 3 of the 11 species of the genus *Yersinia* of the family *Enterobacteriaceae*. *Y. pestis, Y. pseudotuberculosis,* and *Y. enterocolitica* are the three most important species because of their disease association with animals and humans (Duan et al. 2014). *Y. kristensenii, Y. intermedia, Y. aldovae, Y. fredericksenii, Y. bercovieri, Y. mollaretii, Y. ruckeri, and Y. rohdei* are widespread in the environment, but they are not associated with the disease (Sulakvelidze 2000). These are gram-negative bacilli with a facultative anaerobic nature. *Yersinia* colonies are lactose-negative. These bacteria can be isolated by 'cold enrichment' (the capability of bacteria to grow at 4°C) (Jiang et al. 2000). Virulence of the *Yersinia* species depends on the presence of 70-75 kb plasmid. *Y. enterocolitica* (YE) is the primary causative agent behind human yersiniosis. The related *Y. pseudotuberculosis* (YPT) can also cause the disease, but human infections are less common than YE. *Y. pestis* is associated with the respiratory system. There are 70 serovars of *Y. enterocolitica* and 21 of *Y. pseudotuberculosis* (Kenyon et al. 2017; Nieckarz et al. 2020).

2.3.1. TRANSMISSION TO HUMANS

YE infections are typically transmitted by fecal-oral route. Yersiniosis is found worldwide but is most commonly observed in Europe (Galindo et al. 2011). *Yersinia* is prevalent in food animals, particularly

pigs (Fredriksson-Ahomaa et al. 2006). Serovars 0:3 and 0:9 of YE are primarily found in swine and are the chronic carriers of these strains. However, these are very rarely isolated from the environment. They carry 0:3 and 0:9 serovars of YE in their feces and throat. *Yersinia* species were also isolated from domestic dogs in China (Wang et al. 2010) and bats in Germany (Mühldorfer et al. 2010). In a recent European study, wild rodents were found to carry YE, which suggested that they might facilitate reservoir transmission (Backhans et al. 2011). YE has also been isolated from flies (Rahuma et al. 2005), further complicating human disease transmission. Swine represents the primary source of yersiniosis. However, recent reports have also indicated the presence of *Yersinia* in contaminated chicken, milk, tofu, and water (Lynch et al. 2006; Bonardi et al. 2010). Serovar 0:8 of YE is much more prevalent in the environment than the 0:3 and 0:9 serovars. Sources of contamination of 0:8 serovar mainly include drinking water from wells or streams (Terech-Majewska et al. 2016), food washed from water (tofu, bean sprouts), and milk products (Longenberger et al. 2014). It was found in Germany that most infection cases due to pathogenic serotypes (0:3, 0:9) in humans were due to the ingestion of raw pork in the country (Bucher et al. 2008). Infection can also be transmitted from person to person involving the fecal-oral route. Healthy individuals may be asymptomatic carriers of yersiniosis and may be a source of infection for others. This problem becomes particularly concerning in the case of blood transfusion. *Y. enterocolitica*, present in blood products stored at 4°C, may proliferate and produce a septic shock upon transfusion. This condition is fatal in up to 54.5% of the cases (Guinet et al. 2011). Person-to-person transmission of serovar 0:8 has not been evidenced. Also, humans are not chronic carriers of this serovar. Transmission of *Y. pestis* mainly occurs through vectors; the most common is flea bites. Fleas ingest the bacteria along with blood and transfer it to the next host when they bite the infected host. The source of *Y. pestis* is primarily wild rodents. Outbreaks occur when bacteria pass from wild rodents to domestic ones and then to humans via flea bites. Person-to-person transmission can also occur via aerosols. Unlike *Y. pestis*, YPT transmission does not involve any vector and occurs through ingesting contaminated food and water. Direct contact with infected animals or humans can also transmit the bacteria to healthy people.

2.3.2. CLINICAL FEATURES

Y. enterocolitica infection is characterized by self-limiting diarrhea, abdominal pain, and low-grade fever. The infection starts with the ingestion of contaminated food or water. After ingestion, bacteria adhere to the small intestinal inner wall, cross the intestinal barrier, multiply in Peyer's patches, and eventually cause lymphadenitis. However, in individuals with compromised immune systems, chronic conditions such as arthritis can also develop (Galindo et al. 2011). Infection sometimes spreads to mesenteric lymph nodes and then disseminates into the spleen and liver. Subsequently, extracellular replication leads to monoclonal abscesses (Trülzsch et al. 2007). Acute gastroenteritis is observed mainly in children owing to their immature immune system. However, in older children and adults, yersiniosis can lead to a range of other complications such as pseudo appendicular syndrome, mycotic aneurysm (Maykel and Steele 2011; Robins-Browne and Hartland 2003), and sepsis from blood transfusions or as a secondary complication. Moreover, yersiniosis can also result in chronic conditions such as erythema nodosum, glomerulonephritis, uveitis, reactive arthritis, and myocarditis (Galindo et al. 2011). Nonetheless, enteropathogenic yersiniosis is commonly self-limiting unless the individual is not immunocompromized. As a result of bacteremia, the mortality rate can reach up to 60% in people with compromised immune systems (Robins-Browne 2012). Y. pseudotuberculosis infection is usually self-limiting. Common Symptoms include low-grade fever, mild diarrhea, and abdominal pain on the lower right side, mimicking appendicitis. But on opening the abdomen, the appendix is found normal

with inflamed mesenteric lymph nodes surrounding it. Infection can rarely lead to sepsis with a high rate of mortality (>70%) (Deacon et al. 2003). There are two forms of Y. pestis infection (plague): bubonic and pneumonic. In bubonic form, bacteria colonize the proximal lymph nodes, causing 'bubon.' Sometimes, bacteria enter the bloodstream and reach the lungs. Lungs are the primary site for Y. pestis multiplication. Bacteria multiply rapidly and spread to the bloodstream, causing septicemia and death within a few hours. Thus, this form of disease is characterized by the absence of clinical symptoms.

2.3.3. TREATMENT

In the case of mild disease, antimicrobial therapy is not needed. However, systemic infection should be treated. Antimicrobial-susceptibility tests should be recommended because YE, YPT, and Y. pestis are resistant to many antibiotics. Before the susceptibility test results, a combination of aminoglycosides and doxycycline can be started. Resistance to penicillin and 1st generation cephalosporins is widespread (Fàbrega and Vila 2012).

3. MISCELLANEOUS BACTERIA

3.1. PLESIOMONAS AND AEROMONAS

These two genera of bacteria are vibrios, gram-negative, and facultatively anaerobic. These are not the ordinary inhabitants of the human GI tract. These have been suggested as causative agents for diarrhea because of their prevalence in patients with diarrhea (Von Graevenitz 2007). The bacteria have not been isolated from poultry, cattle, and pigs (Arai et al. 1980). However, samples from dogs, cats, fish, and river water were found to harbor these organisms. Dogs and cats are not known in detail for their role in transmitting these organisms. A major source of human infection may be contaminated water sources. Clinical symptoms are commonly presented as self-limiting diarrhea and dysentery in infants. *Aeromonas spp*. cause septicemia in debilitated individuals (Janda 2002). These are of low prevalence. However, diarrhea due to an unexplained cause may be suggested to be due to *Plesiomonas* or *Aeromonas*. Antimicrobial sensitivity tests should be adopted for the treatment of these bacteria.

3.2. CLOSTRIDIAL DISEASE

Clostridium difficile causes chronic diarrhea and pseudomembranous colitis in infected persons (Kuipers and Surawicz 2008). It is a gram-positive, obligate, anaerobic, rod-shaped bacterium. This disease cannot be associated with the bacteria in the stool. One study found that 21 percent of people have this bacterium in their stool. The presence of toxigenic strains establishes the disease. Sufficient toxins are necessary to associate this organism as the causative agent for diarrhea in a patient (Sambol et al. 2002). Dogs and pigs harbor *C. difficile* and shed in their feces (Viegas et al. 2020). There is not much evidence that pets may transmit the pathogen to humans. The disease may be nosocomial in humans in some instances. *C. difficile* can also cause disease in dogs. Metronidazole has been effective in dogs. Metronidazole and vancomycin have been curative in people.

3.3. SHIGELLOSIS

Shigellosis is caused by *Shigella* spp., which are gram-negative, aerobic bacteria. The bacterium has been isolated from dogs with low prevalence rates of 0.3 to 0.5 percent. There is no such data available for the cats. This suggests that dogs may be an unlikely but possible source for humans. However, they are immune

to the disease. The bacteria enter the GIT through the oral route and invade the intestines' epithelial cells. The disease in people is characterized by several clinical presentations, of which fever, dysentery, and abdominal pain are best known. However, asymptomatic infection may also occur. In suspected individuals, sulphonamides, tetracyclines, or beta-lactams may be tried (Christopher et al. 2010).

3.4. COLIBACILLOSIS

*Through various mechanisms, Escherichia coli causes diarrhea in humans, cattle, pigs, and many other animal*s. These mechanisms include the action of both heat-stable and heat-labile enterotoxins, invasion of enterocytes, and mechanical obstructions and disruptions to the brush border. Human infection is clinically manifested as severe abdominal pain, diarrhea, which is often bloody, and vomiting. Farm animals are a significant source of infection for humans. In contrast, there is little evidence that companion animals carry non-invasive, enterotoxigenic *E.coli.*

4. PROTOZOAL DISEASES

4.1. COCCIDIOSIS

Coccidiosis, primarily cryptosporidiosis, is a widespread intestinal disorder in animals and humans. It can affect the GIT, respiratory tract, kidneys, and biliary tract (Hunter and Nichols 2002). It can be either a contributing factor or a critical cause of acute enteritis in humans and animals. *Cryptosporidia* spp. are coccidial protozoa with a small size of 4-5µm. It is not confirmed whether it has multiple species; however, it can infect numerous host ranges (Pumipuntu and Piratae 2018).

4.1.1. TRANSMISSION TO HUMANS

Farm animals are considered the most important source of cryptosporidiosis for humans. Researchers in the United States, United Kingdom, Ireland, and Australia have implicated contact with cattle as a significant risk factor for humans to get the infection (Robertson et al. 2002; Roy et al. 2004; Hunter et al. 2004; Goh et al. 2004). However, sheep have also been considered a source of human cryptosporidiosis, with only a few studies pointing to their involvement. Companion animals are less frequently implicated a source of human cryptosporidiosis. However, in the United States, a weak association was observed between cryptosporidiosis in HIV+ persons and dog contact (Glaser et al. 1998).

Transmission to humans can happen in two ways: direct or indirect transmission. Direct transmission occurs through oral exposure to oocytes excreted in feces. Transmission can happen from animal to human and from human to human, usually in hospitals, daycare centers, water parks, swimming pools, and direct contact with human feces during anal sexual contact. This becomes particularly important during sexual intercourse between men (Hellard et al. 2003). Direct transmission can also happen through direct exposure to infected animals. Veterinarians and animal researchers are at high risk of getting the disease through direct contact with the infected animal.

Indirect transmission can occur through cross-contamination, including contaminated food materials, drinking water, and fomites such as contaminated footwear and clothes used in farms and wildlife facilities. Oocytes resist environmental odds and many disinfectants; direct contact is often unnecessary. Each oocyte contains four sporozoites inside it. Once in the intestine, the enterocyte brush border is the primary attachment site for sporozoites. Sporozoites are then converted to merozoites. Merozoites infect more enterocytes and increase in number. The gametogony stage follows, and two types of oocytes are formed: thin-walled and thick-walled oocytes. The former ruptures inside the intestine

and causes hyper infection (Abou-Bakr et al. 2019), while the thick-walled oocytes are shed in the feces. The protozoa can be found throughout the small and large intestines; however, the ileum is most affected (Del Coco et al. 2012). Oocytes are excreted in the feces and contaminate the environment, such as soil and water bodies, sewage, or slurry, mainly insufficiently treated domestic water supplies. High rainfall and flooding can distribute the contamination to longer distances (Jiang et al. 2005).

4.1.2. CLINICAL FEATURES

Cryptosporidiosis symptoms in humans depend on the immunity status of the individual. In immunocompetent people, infection is usually asymptomatic or self-limiting diarrhea for 5 to 10 days, sometimes accompanied by fever, nausea, abdominal pain, constipation, or weight loss (Siciliano et al. 2020). These symptoms usually subside with the development of immunity in the affected people. In immuno-deficient people, symptoms typically comprise severe watery diarrhea, fever, abdominal pain, and weight loss and can persist for more than a year (Tzipori 1983; Current 1985).

4.1.3. TREATMENT

In immune-competent persons, infection usually subsides without treatment; however, supportive therapy with fluids and electrolytes should be considered. In immuno-deficient individuals, anticryptosporidial drugs may be attempted, but the protozoa are resistant to many of the medicines (Al-Matha and Alsalem, 2012). Spiromycin, clarithromycin, paromomycin, and nitazoxanide are recommended treatment regimens (Acikgoz et al. 2012). Immune-suppressive and cytotoxic drugs should be avoided (Angus 1983; Pitlik et al. 1983a, b).

4.2. GIARDIASIS

Giardiasis is a common enteric illness in the human population and domestic animals, including livestock, dogs, cats (Thompson and Monis 2004; Thompson 2004), and wildlife (Appelbee et al. 2005). The species *Giardia duodenalis* is responsible for most of the cases of giardiasis in humans and most mammals. Thus, it is considered a zoonotic disease. In Asia, Africa, and Latin America, about 200 million people are affected annually (Yason and Rivera 2007). Asymptomatic giardiasis is prevalent in the developing world (Hellard et al. 2000; Thompson 2000). Symptomatic giardiasis typically causes self-limiting diarrhea, abdominal pain, bloating, and weight loss.

4.2.1. TRANSMISSION TO HUMANS

The infective stage of *giardia* is a cyst. Once inside the host, the cyst is excysted in the first part of the small intestine and releases trophozoites, a self-replicating stage of the parasite. The trophozoites divide and increase in number. In response to bile salts and other intra-intestinal conditions, the trophozoites are again converted to cysts and excreted in the feces of the affected host. Cysts are then spread in the environment and reach the intestinal tract of the following host by contaminated food, water, fomites, and through direct contact with the infected host. A minimum of 10 cysts can cause disease in a person. Since 1954, 132 waterborne outbreaks have been reported; 104 were associated with drinking water, 18 were linked to recreational water, and 10 were connected to foreign travel (Karanis et al. 2006). Beavers are a significant cause of water contamination with *giardia* (Tsui et al. 2018).

Furthermore, foodborne outbreaks were found to be associated with food handlers infected with *giardia* and the food handlers who had been changing the diapers of infected children before handling food (Hoffmann et al. 2007). Vegetables, ice, and chicken salad have also been responsible for foodborne outbreaks. Outbreaks due to person-to-person transmission of giardiasis in childcare centers are common.

The disease is common in cattle, pigs, sheep, goats, deer, and elk. Dogs are also a common source of *giardia* for humans. 1 to 12 percent of dogs have been reported to have asymptomatic giardiasis, while 25 to 36 percent prevalence of giardiasis has been reported in dogs with diarrhea. Cats usually have a 1.4 to 5 percent prevalence.

4.2.2. CLINICAL FEATURES

Human giardiasis has severe acute symptoms characterized by explosive (severe), watery diarrhea, abdominal cramps, nausea, flatulence, and anorexia. There may be fecal blood and mucous, but it is uncommon (Newman et al. 2001). Acute symptoms are usually eliminated after 1 to 3 weeks without treatment. Chronic disease characterized by weight loss, irritable bowel syndrome (IBS), food allergies, arthritis or chronic fatigue syndrome (Einarsson et al. 2016). Immune-deficient people may present severe symptoms of giardiasis. Less common, long-term consequences of giardiasis include cholecystitis, fever, urticaria, and ocular inflammation (Khalifa et al. 2007; Lamps and Lamps 2010).

Giardiasis in dogs is usually asymptomatic. Symptomatic infection develops after 1 to 3 weeks post-exposure and is characterized by soft stool with mucous, hematochezia, and symptoms related to chronic ulcerative colitis have been reported. Giardiasis symptoms in cats are usually the same as in dogs, except that these are generally eliminated within 4 to 5 weeks.

4.2.3. TREATMENT

Nitroimidazoles (for example, metronidazole, ornidazole, tinidazole, ipronidazole), quinacrine or furazolidone are the recommended medications used against giardiasis. Most drugs have efficacy more than 80 percent (Ordóñez-Mena et al. 2018). Metronidazole and tinidazole usually have efficacy of more than 90 percent (Upcroft and Upcroft 2001). In one Malaysian trial, ornidazole showed 100 percent efficacy (Wright et al. 2003). Metronidazole has been reported to be 100 percent efficacious at high doses in people (Brandborg et al. 1980), while it has been documented to be 67 percent effective in dogs. There is one report of quinacrine and metronidazole having a synergistic effect when a combination of both treated giardiasis in an immuno-deficient person who did not respond to several treatments with quinacrine and metronidazole separately (Smith et al. 1982).

5. MISCELLANEOUS PROTOZOAL DISEASES

5.1. AMOEBIASIS

Unlike other parasitic protozoa, *Entamoeba histolytica* has a simple life cycle. It lives as the motile trophozoite in the host's intestine or the infective cyst. Human beings or primates are the only natural hosts of this protozoa. Cysts are ingested through food and water that has been contaminated. Once inside, cysts are excysted in the intestine and trophozoites invade the intestinal epithelium causing ulcerations. Sometimes, they can spread further and cause abscesses, particularly in the liver. Metronidazole is the recommended drug of choice (Kumanan et al. 2021). However, furazolidone and tetracycline may also be used.

5.2. BALANTIDIASIS

Balantidia coli is a protozoal parasite of humans, non-human primates, rodents, and swine. It primarily causes infection in the large intestine. Transmission occurs through the fecal-oral route, and cysts are ingested through contaminated food and water. Cysts can remain infective in the environment for up to 10 days. One study in captive African great apes found that trophozoites of B. coli can also be infective (Pomajbíková et al. 2010). No vector transmits this parasite (Schuster and Ramirez-Avila 2008). Once ingested, cysts, surviving the stomach's acidic environment, move to the small intestine where they excyst, and trophozoites are released. These motile trophozoites are then passed to the large intestine. Infection can be asymptomatic to severe bloody diarrhea. Watery diarrhea, dehydration, anorexia, and reduced growth are typical clinical findings of Balantidiasis. Metronidazole, furazolidone, and secnidazole can be used against *Balantidia coli.*

5.3. TRICHOMONAS

Two species of *trichomonas* are of zoonotic importance*, Dientamoeba fragilis* and *Pentatrichomonas hominis*. These two species have a broad host range and have been isolated from domestic and farm animals. Little is known about its pathogenesis and transmission. However, it is suggested that the species mentioned above of trichomonas are transmitted by the fecal-oral route and cause mild diarrhea even with a large parasite load. Metronidazole can cure the disease, and there is little chance of an outbreak.

6. HELMINTHIC DISEASES

6.1. STRONGYLOIDIASIS

Strongyloidiasis is a helminthic disease of zoonotic importance, easily transmissible from one host to another. It is caused by a nematode, *Strongyloides stercoralis* that can live as a parasite and a saprophytic organism in the environment. Females produce eggs in the intestine of the host. These eggs hatch, producing filariform larvae in the host's intestine. These filariform larvae cause hyperinfection. Thus, a large load of the parasite builds up in the intestine despite a small number of initial infective exposures. Hyperinfection caused by *Strongyloides stercoralis* has a high mortality rate (15 to 87 percent) (Marcos et al. 2008). Infection begins when the infective form, filariform larvae, penetrates the skin or mucous membrane of the host. The larvae penetrate until they reach a blood vessel and eventually into the lungs. Adults burrow into the small intestine, producing eggs and damaging the intestinal mucosa. Reservoirs for *Strongyloides stercoralis* are people, dogs, cats, foxes, and the environment, especially in hot and humid climates.

Infection is usually asymptomatic, but some people show symptoms like watery and mucoid diarrhea and abdominal cramps (Vadlamudi et al. 2006). Coughing, fever, and creeping eruptions around the anus and buttocks have been documented in some cases. Hyperinfected people present severe, fatal symptoms. These patients can have a hemorrhage, hemoptysis, secondary sepsis, toxic intestinal dilation, and death. Antihelminthic drugs are used to prevent and treat the infection.

6.2. ECHINOCOCCOSIS

Echinococcosis, known as hydatid disease, is a rare but potentially lethal disease in people. The definitive hosts of this disease are members of the dog family (dogs, wolves, jackals, and dingos). The disease is caused by tapeworm *Echinococcus granulosus.*

Once eggs are ingested by an intermediate host (man, cattle, sheep), they hatch in the intestine and penetrate the intestinal mucosa and into the bloodstream. Subsequently, parasites travel throughout the host's body and live in body organs, mainly the liver and lungs, forming hydatid cysts. The cysts enlarge in size and may cause organ dysfunction. If the cysts are not removed, the daughter cysts are included, and the condition worsens. The parasite reaches the definitive host by ingesting meat contaminated with hydatid cysts. The parasite does not cause clinical disease in the definitive host but may be life-threatening in the intermediate host. Praziquantel, bunamidine, and arecoline are used to kill adult parasites in the definitive host. Hydatid cysts in the intermediate host need surgical removal. Care should be taken to avoid rupturing the cyst, as it can be fatal. Non-operable hydatid cysts can be destroyed in humans by mebendazole or praziquantel (El-On 2003).

7. MISCELLANEOUS HELMINTHS

7.1. ECHINOCOCCUS MULTILOCULARIS

Echinococcus multilocularis is similar to *E. granulosus* and is found in northern Asia, Canada, and Alaska. Dogs and foxes act as definitive hosts in the life cycle of this tapeworm. Unlike *E. granulosus*, scoleces of *E. multilocularis* grow uncontrollably, resulting in widespread metastasis, primarily affecting the liver (Hildreth et al. 2000). Hence, surgical removal is generally not feasible. Other considerations apply to *E. multilocularis* as to *E. granulosus.*

7.2. TAENIASIS

Taenia saginata and *Taenia solium* are significant public health considerations. However, human infection principally occurs through ingesting cysticerci-contaminated beef and pork. After ingestion, the cysts undergo evagination and attach themselves to the walls of the small intestine by their scolex. Over about two months, they mature into adult worms (Nyangi et al. 2022).

7.3. COENUROSIS

Another tapeworm, *Multiceps multiceps*, is found in dogs. After ingesting eggs of this tapeworm by humans, it forms cysts (coenuri). These cysts primarily occur in the brain and can cause internal hydrocephalus and posterior fossa syndrome (Haddad et al. 2008).

7.4. DIPYLIDIUM CANINUM

Humans are occasional hosts of this tapeworm (Narasimham et al. 2013). However, it is commonly present in dogs and cats. The flea or dog louse is its intermediate host. It causes few symptoms in dogs and cats, except for anal pruritus, unless an extensive infestation causes obstruction. Occasionally, children may ingest the intermediate host, which results in mild gastroenteritis, eosinophilia, and restlessness caused by the tapeworm. Diagnosis is based on detecting the tapeworm's proglottids in the stool. Praziquantel effectively eradicates adult tapeworms, but the dog can be reinfected unless the fleas and lice are virtually eliminated from the dog's environment.

7.5. CUTANEOUS LARVA MIGRANS

Ancylostoma spp., canine hookworm larvae, can cause a pruritic creeping eruption when they localize in the skin. However, since human beings are not their natural hosts, skin penetration by these larvae causes

this syndrome rather than the intestinal infection as in dogs (Brenner and Patel 2003). After hatching, these larvae are susceptible to drying. Hence, transmission to people must occur relatively shortly after the hatching. In human cases, pruritus is a prominent symptom, and the tract created by the migrating larvae may be seen on the skin.

8. VIRAL DISEASES

8.1. PARVOVIRUS INFECTION

There is an increasing concern about various human illnesses that appear to be associated with parvovirus infection (Anderson et al. 1985; Plummer et al. 1985). These illnesses include pancytopenias and exanthematous conditions in children. However, these diseases and their link to parvovirus are in the early stages of being understood in detail. There is currently no provided evidence that demonstrates their transmission from pets to humans. However, further research is required to confirm or deny the possibility of such transmission.

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Epidemiology of Zoonotic Tuberculosis and its Implications in Asia

04

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ABSTRACT

Mycobacterium tuberculosis, a gram-positive bacterium that causes tuberculosis (TB) in human is still a serious global health concern, especially in Asia where 22 high burden countries are responsible for 80% of the world's TB cases. There are more than 150 known species of M. tuberculosis. Due to its virulence, it is the world's second most infectious cause of mortality. Furthermore, it has a high contribution to the overall disease burden. Causative agent of TB in animals is Mycobacterium bovis which is responsible for zoonotic tuberculosis in humans. The death rates resulting from these mycobacterial infections are impacted by various factors, including insufficient healthcare infrastructure, socioeconomic inequality, a dense population, and co-infection. The tendency of these mycobacteria to escape host immune responses and establish persistent infections is one of the virulence factors that contributes to the severity of tuberculosis. India has a highest 21% of TB infections in overall prevalence in Asia following China has 14%, Indonesia 6%, Nigeria 5%, Bangladesh 4%, Pakistan 3%, other 13 high burden countries for TB contribute 16%, while rest of the world contributes 20%. The early detection, directly observed treatment short-course (DOTS), and vaccination programs such as Bacillus Calmette-Guérin (BCG) have been the primary control methods in Asian countries. However, obstacles like multi-drug resistant (MDR) and dense populations have made these approaches less successful. To control and completely eradicate the disease in future top priority should be given to providing access to high-quality care, upgrading the healthcare system, and tackling socioeconomic factors that contribute to tuberculosis, such as hunger and poverty. To effectively eradicate tuberculosis in Asia and globally, cross border collaboration including cooperation between governments, international organizations, and research institutes is essential.

Key words: Mycobacterium tuberculosis, Mycobacterium bovis, Zoonotic tuberculosis, Persistent infections, Prevalence of TB, Directly observed treatment short-course, Bacillus Calmette-Guérin

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1. INTRODUCTION

Nearly 2 million individuals every year fall victim to (TB), making it the world's second leading infectious cause of mortality. About 8 million new cases of tuberculosis are reported annually, with 80 percent of those infections affecting the working population which falls victim to mycobacterium. Twenty-two high-burden nations are responsible for 80% of the world's TB cases. China, India, Pakistan, Bangladesh and Indonesia account for half of the world's TB burden, while Sub-Saharan Africa has the highest incidence rate.

Overpopulation, poverty, and poor nutrition are predisposing factors in the spread of tuberculosis. Human immunodeficiency virus (HIV) has been blamed for a dramatic increase in tuberculosis cases in Sub-Saharan Africa in recent years. Multiple drug-resistant tuberculosis (MDR-TB) epidemics have caused widespread public concern in developed nations. According to the World Health Organization (WHO), a significant proportion of the worldwide TB burden in humans is related to the zoonotic spread of bovine TB with 142,000 cases causing 12,500 deaths annually (Zimpel et al. 2020).

2. HISTORY OF *MYCOBACTERIUM*

Mycobacteria are gram-positive, aerobic, or micro-republic bacteria that are very picky about their environment. The pigments produced by certain mycobacteria vary in color from yellow to orange, and in very rare cases, salmon pink. They are between 0.2-0.6 x 1 to 10 µm in size. There are more than 150 known species of mycobacteria which are further divided into two categories. Mycobacterial species which are pathogenic to people and animals are slow-growing species that take more than 7 days to develop noticeable colonies on media, whereas fast-growing species take less than 7 days and are often not dangerous (Metzger et al. 2010).

Death records from the seventeenth century show that TB was a major killer in Massachusetts between 1768 and 1773. By the turn of the nineteenth century, the TB pandemic in Europe had reached its height. TB first developed in Asia in the late 19^{th} or early 20^{th} century. Although the contagious nature of tuberculosis was known to Aristotle and Galen, it was not proven until Koch described the tubercle bacillus in 1882 and later Dormandy in 1999 (Barberis, Bragazzi, Galluzzo, & Martini, 2017).

Recent deletion analysis investigations have shown that either *Mycobacterium africanum* or *Mycobacterium canetti* is the natural ancestral member of the genus, from which other species arose (Metzger et al. 2010). Previously, scientists assumed that *Mycobacterium bovis* was the progenitor of *M. tuberculosis*. The bacterium *M. bovis*, which is responsible for bovine tuberculosis, may infect and cause illness in a wider variety of hosts, including humans. When horizontal gene transfer and significant recombination events are disregarded, the alienable sections of the genomes of mycobacterium tuberculosis complex (MTBC) members are approximately 99.95% identical, demonstrating that they have clonally developed from a common ancestor with the tuberculous bacterium *M. canetti.* Single nucleotide polymorphisms (SNPs), deletions of up to 26 kb, duplication of a few paralogous gene families, and insertion sequences are the only mechanisms by which these infections have developed, giving rise to a wide range of host tropism and pathogenicity.

3. INTRODUCTION OF *M. TUBERCULOSIS* AND *M. BOVIS*

TB complex mycobacteria share 99.9% genetic similarity and identical 16S ribosomal RNA (rRNA) gene sequences. The 4411,532 base-pair genome has 3959 protein-coding and six pseudogenes. All 2441 genes have functions, excluding 912 conserved hypothetical genes.

M. bovis is a slow-growing bacterium. This bacterium affects cattle, goats, bison, deer, and badgers. It causes bovine TB. *M. bovis* infects humans, pigs, cats, and dogs. *M. bovis* and *M. tuberculosis* can cause TB in humans (Zimpel et al. 2020).

4. PATHOGENESIS OF *M. TUBERCULOSIS* AND *M. BOVIS*

The immunological response in classic animal testing shows four infection stages. Pathogenic mycobacteria survive and multiply in macrophages during the first stage by stabilizing the phagolysosome at pH 6.2-6.3 and staying in the endosomal recycling channel, where they can get iron. Avoiding acidity requires the cord factor. Phagosomes acquire lysosomal proteins but cannot merge with them. Monocytes are transported to the infection site in the second phase. Monocyte-to-macrophage differentiation maintains mycobacteria. When antigen-specific T cells grow, mycobacterial proliferation decreases. Release activates macrophages and removes the phagosome maturation block. Nitric oxide synthase produces mycobacteria-toxic radicals. After removing the impediment, the mycobacteria-containing vacuole can be acidified, speeding up the process. If immune cells fail to stop bacterium growth, the sickness progresses to the final stage.

Infected macrophages produce large amounts of pro-inflammatory mediators such TNF, IFN-γ, IL-6, IL-11, IL-18, and IL-12. Anti-inflammatory cytokines include TGF- and IL-10. TB symptoms, including fever and wasting syndrome, stem from uncontrolled cytokine production. Wild animal tuberculosis is a zoonotic threat to people and domestic cattle.

In animals, Inhaled *M. bovis* replicates in alveolar macrophages. After a lung infection spreads, lymph nodes in the mediastinum or elsewhere, such as the head and neck, may become infected. The host's immune system attacks the intruder cell by cell. Inflammation forms granulomas. Tubercles, or sores, characterize tuberculosis. After that, a laminar structure resembling an onion slicer emerges around the granuloma. The granuloma's core cells die, leaving dry cottage cheese-like debris. Biting wounds can spread TB in naturally infected groups. Pulmonary infection from infectious aerosols may take time to develop. Thus, a chronic illness develops, and the infected animal may exhibit a range of symptoms, from asymptomatic to severe overt disease with systemic pathology. Respiratory infections stay latent, unlike wound infections, which progress quickly and create sores. Badgers at any stage of sickness can harm vulnerable hosts.

Badgers with many illnesses have best summarized the pathogenic chain. Grossly evident lesions are more common in the thoracic cavity, which contains the lungs, and in the head and body lymph nodes. The abdominal cavity is uninfected, although many other tissues and organs may be. Badgers disperse similarly. Saliva contains TB, which can infect bite wounds. Due to rapid progression, enlarged lesions, and extensive infection, pathogenesis may differ here. Infected bite sputum can cause tiny subcutaneous granulomas or large, skinless sores.

5. VIRULENCE FACTORS ASSOCIATED WITH *M. TUBERCULOSIS* AND *M. BOVIS*

Inhaled *M. bovis* replicates in alveolar macrophages. After a lung infection spreads, lymph nodes in the mediastinum or elsewhere, such as the head and neck, may become infected. The host's immune system attacks the intruder cell by cell. Inflammation forms granulomas. Tubercles, or sores, characterize tuberculosis. After that, a laminar structure resembling an onion slicer emerges around the granuloma. Granulomas lose their core cells over time. Most bacterial infections, such as those caused by *Corynebacterium diphtheriae*, *Escherichia coli* O157:H7, *Shigella dysenteries*, and *Vibrio cholerae* toxins, lack the virulence components of *M. tuberculosis*. Even without pathogenicity data, the virulence of *M. tuberculosis* can be assessed. Bacterial load or burden is the total number of bacteria in a host following

infection, which is another virulence factor. This stage changes the growth curves of *M. tuberculosis* virulence mutants that reduce animal bacterial loads. Histopathological morbidity studies may reveal a distinct category of *M. tuberculosis* mutations that alter virulence but not bacterial burden.

6. EARLY EVENTS OF TB DISEASE ONSET

Alveolar airways are a common entry point for *M. tuberculosis* in susceptible patients. This is where it presumably has its initial touch with macrophages in the area. Pneumocyte, which are more common in alveoli than macrophages, may be infected and survive by *M. tuberculosis* even when they are outside of the body. Dendritic cells, which are far better antigen presenters than macrophages and likely play a major job in activating T cells with *M. tuberculosis* antigens, also play a critical role in the early stages of infection. Alveolar surfaces include a glycoprotein called surfactant protein A, which may increase mannose receptor activation and hence improve *M. tuberculosis* binding and absorption. Surfactant protein D, which is also found in alveolae, binds to mannosyl oligosaccharide residues on the bacterial cell surface, preventing phagocytosis of *M. tuberculosis*. This may stop *M. tuberculosis* from engaging with macrophages through their mannose receptors. *M. tuberculosis* and other intracellular infections first dwell in the phagosome, an endocytic vacuole, upon entering a host macrophage. Multiple infections, including *M. tuberculosis, Listeria monocytogenes*, and *Leishmania* major, are more lethal in mice with mutations in the gene encoding macrophage-localized cytokine-inducible nitric oxide synthase. Activated mouse macrophages create reactive nitrogen intermediates (RNIs), which are the fundamental components of antibacterial activity. A noteworthy conclusion from the latter study is that *M. tuberculosis* penetrates human macrophages and suppresses Ca^{2+} signaling but does not do so when *M. tuberculosis* is destroyed or when *M. tuberculosis* cells are opsonized by antibodies. Increased Ca^{2+} levels were associated with trafficking in late endosomes, which in turn facilitated the formation of phagolysosomes. Cytokines, nitric oxide, the respiratory burst, and other host defensive responses may all be triggered by Ca^{2+}. By preventing Ca^{2+} elevations, *M. tuberculosis* can evade these defensive mechanisms in the host. Some research has also shown that *M. tuberculosis* would fare better if it could remain in an early endosome for as long as possible, since this would reduce the activity of $CD4^{+}$ T cells in the host's immunological response. During infection with *M. tuberculosis*, macrophages are said to produce less major histocompatibility complex class I (MHC-I) protein and display fewer MHC-II bacterial antigens (Chandra et al. 2022).

7. LATER EVENTS OF TB DISEASE ONSET:

Infected lung macrophages generate chemokines that attract pathogen-resistant neutrophils, lymphocytes, and monocytes. Later, lymphocytes and big macrophages develop granulomatous localized lesions. This method usually stops spreading of bacteria. As cellular immunity grows and removes bacillus-filled macrophages, fibroblasts, lymphocytes, and blood-derived monocytes surround the caseous core of the granuloma. Latent TB, often known as chronic TB, can remain dormant and non-transmissible for life. Cell-mediated immunity can eliminate the infection. Granulomas heal into microscopic fibrous and calcified lesions. If the infected person cannot control the lung infection or if their immune system deteriorates due to immunosuppressive medications, HIV infection, malnutrition, aging, or other factors, the granuloma center can liquefy, providing a rich medium for the revived bacteria to replicate uncontrollably. Live *M. tuberculosis* can still enter the lungs, create active pulmonary TB, and spread to other tissues via the lymphatic system and blood (military or extrapulmonary TB).
Researchers studied *M. tuberculosis* "persistence" in mice using two chronic infection models and found that the bacteria may either be stable in the absence of sickness or not cultivable. The bacteria used to represent chronic diseases may be alive but quiescent, reflecting a true latent state, or they may be

constantly dividing and dying. *M. tuberculosis* supports the second notion that growth and death are controlled. In a mouse model of chronic TB, isoniazid is a drug of choice to kill *M. tuberculosis*. Biochemical investigations demonstrate that *M. tuberculosis* intermediate metabolism switches from aerobic, carbohydrate-metabolizing to anaerobic, lipid-utilizing during chronic mice infections (Chandra et al. 2022).

8. EVASION STRATEGY OF *M. TUBERCULOSIS* AND *M. BOVIS*

M. tuberculosis replicates fast inside host. Apoptosis restricts the transmission of attenuated *M. tuberculosis* in macrophages. Dendritic cells gather bacterial antigens from apoptotic vesicles formed by infected macrophages to link innate and adaptive immunity. Antigens presented by dendritic cells may awaken latent T-lymphocytes. The virulent strain of *M. tuberculosis* suppresses apoptosis and promotes necrosis. Infected macrophages die adaptively. Pathogenic *M. tuberculosis* alters several cellular pathways, and investigations have demonstrated that the host's eicosanoid production pathways regulate macrophage death.

Effector proteins and lipids used by *M. tuberculosis* to obstruct host lysosomal transport are briefly discussed. Endosomal markers RAB5 and RAB7 are not recruited by NdkA, and phosphatidylinositol 3-phosphate is dephosphorylated by SapM69. The phagosome-preventing serine/threonine protein kinase PknG binds to the RAB GTPase RAB7L1. Five type VII secretion systems (ESX-1–ESX-5) are encoded by *M. tuberculosis* and are responsible for exporting substrates from the cell. Important for pathogenicity upon contact with macrophages are the effectors ESX-1 and ESX-3. Both EsxA (exported by ESX-1) and PDIM (a lipid found in the cell envelope) are known to disrupt the phagosomal membrane. However, the mechanism by which this occurs is unclear. *M. tuberculosis* can take up nutrients and deliver effectors to the cytosol via permeabilizing the phagosome. *M. tuberculosis* can take up nutrients and deliver effectors to the cytosol via permeabilizing the phagosome. In addition to affecting the necrosis and inflammasomes AIM2 (cytoplasmic sensor) and NLRP3 (Nucleotide-Binding Domain, Leucine-Rich-Containing Family, Pyrin Domain-Containing-3), *M. tuberculosis* effectors modify lysosomal trafficking. The GAS-STING pathway is activated when bacterial or mitochondrial DNA enters the cytoplasm, prompting the cell to produce more type I interferons and initiating the autophagy process. Members of the *M. tuberculosis* PE-PGRS protein family block autophagy, at least in human lymphatic endothelial cells, and cytosolic mycobacteria join to create cords that are immune to selective autophagy. Since *M. tuberculosis* does not generate a large amount of mitochondrial reactive oxygen species, this may contribute to the decrease in NADPH (nicotinamide adenine dinucleotide phosphate) oxidase activity and autophagy that characterizes *M. tuberculosis* infections relative to those caused by other bacilli. When macrophages are activated by interferon before infection, *M. tuberculosis* is less able to obstruct lysosomal trafficking routes. In contrast to the host defense mechanisms of apoptosis followed by efferocytosis, *M. tuberculosis* induces necrosis using chemicals such as CpnT (NAD^+ glycohydrolase), PDIM (Phthiocerol dimycocerosates), and iron overload. Macrophage mortality is also affected by type I interferon and the increased tissue inflammation caused by *M. tuberculosis*. To survive in different intracellular settings and avoid the antimicrobial defenses of macrophages, *M. tuberculosis* employs a wide range of strategies (Chandra et al. 2022). The following are virulence factors related to *M. bovis.*

8.1. MYCOBACTERIAL LIPIDS

The cell wall of mycobacterium is rich in lipids with an exceptional range of physiochemical properties. Some components involved in virulence are lipoarabinomannan, lipomannan, phosphatidylinositol mannosidase, trehalose-6,6'-dimycolate, phthiocerol dimycocerosate and phenolic glycolipids.

8.2. SECRETION SYSTEMS IN MYCOBACTERIA

Mycobacteria have a waxy cell envelope, and it controls the movements of molecules. Some specialized protein structures for this purpose are twin arginine transporter, ESX-transporter, and PE proteins: PE-PPE and PE-PGRS (Polymorphic GC-rich Repetitive Sequences).

8.3. LIPOPROTEINS

Its genome analysis has shown approximately 90 putative lipoproteins out of which mostly are part of the mycobacterial cell envelope and plasma membrane too. Their presence also contributes to the interaction of the host and pathogen.

8.4. IMMUNE EVASION MECHANISMS

Mycobacterium has a prominent ability to infect and reside within immune cells. It can also survive in the dynamic environment of the macrophage phagosome. Some immune evasion mechanisms of this organism are phagosome arresting, resistance to reactive oxygen and nitrogen species, and inhibition of apoptosis.
It is almost certain that most of the virulence factors of *M. bovis* are the same as those of the classical human TB organism, *M. tuberculosis*, as both organisms can cause identical clinical disease in humans and are genetically very similar (Collins 2001).

9. TRANSMISSION OF *M. BOVIS* IN ANIMALS

Bovine TB is typically seen in the throat and lung lymph glands. Thus, this disease-causing bacterium is spread by mouth and nasal emissions. Inhalation and ingestion cause most infections. Infected food and drink are another risk. Badgers and cattle may have bovine TB. In cattle, respiratory, infected milk, placenta, or ambient contamination may spread it. In farm buildings, badgers may transmit this illness directly via close contact with cattle grazing in infected badger-infested areas (Cousins 2001).

10. TRANSMISSION OF *M. BOVIS* IN HUMANS

Bovine TB is contagious to humans. Raw milk and inhalation can spread it. Due to eradication campaigns, it is rare in developed areas, but reservoirs of animals make eradication challenging. *M. bovis* belongs to risk group 3, which causes extrapulmonary TB in infants and toddlers, tainted milk. Thus, milk boiling and pasteurization limit intestinal transmission. Meat and abattoir workers still get airborne illnesses. Humans infect slowly. Tuberculin skin testing and interferon-gamma release assays to screen for this infection. *M. bovis* and TB share symptoms. Fever, nocturnal sweats, and weight loss are common. Symptoms vary by body component. In 2017, WHO reported 142,000 zoonotic TB cases and 12,500 deaths. The lack of routine bovine TB testing understates these statistics (Grange 2001).

11. EPIDEMIOLOGY OF *M. TUBERCULOSIS* AND ITS ZOONOSIS IN ASIA:

11.1. PAKISTAN

Pakistan has an underreported disease burden, with 181 TB cases per 100,000 persons estimated in 2008 and 81 new sputa smear (SS+) cases per 100,000. TB case detection rates have increased from 19% in 2002

to 84% in 2008, while the rate for newly diagnosed SS+ patients has increased from 13% to 74%. However, recent TB incidence estimates have increased case identification rates to 60% for all TB patients and 58% for new SS+ cases. Despite this, the number of TB patients discovered has increased dramatically in Pakistan because to the efforts of the National Tuberculosis Program (NTP), from 11,050 in 2000 to 248,115 in 2008, and treatment success rates have reached 91% as of 2007. From 1995 onwards, Pakistan adopted and piloted the World Health Organization's (WHO) Directly Observed Treatment Short-course (DOTS) strategy for TB, but the "Islamabad Declaration" declaring TB a national public health emergency did not lead to significant progress in TB control until the NTP was revived in 2001. The National TB Program (NTP) operates under the Ministry of Health to provide general coordination, policy direction, and technical advice for TB management, while the Provincial TB Programs (PTPs) and district health authorities are responsible for actual implementation. The NTP headquarters maintain solid communication channels with PTP directors and local TB program administrators. In 2003, the NTP and PTPs surveyed general practitioners (GPs) in the Lahore and Rawalpindi districts and discovered that just 3% of GPs were using the national recommendations for TB diagnosis and care, while 90% were using chest radiography. There were 115,463 physicians registered with the Pakistan Medical and Dental Council as of the end of July 2009, and 42,700 establishments (69% clinics and pharmacies and 550 private hospitals) were offering official and informal medical services.

Pakistan has 410,000 new TB recorded cases with 69,000 TB deaths reported annually. Pakistan ranks sixth internationally and has the highest TB burden of the 22 WHO Eastern Mediterranean Region members. The NTP and its collaborators plan to conduct a 2010 Pakistani TB sickness prevalence research to better understand the problem (Chakaya et al. 2021).

Of 248,115 SS+ cases in 2008, 99,670 were new. The 2007 treatment success rate of 91% of new SS+ patients exceeded the WHO aim of 85% because of a decrease in the default rate to under 4% and low mortality, failure, and transfer out rates (2%, 1%, and 2%, respectively). Since 2007, notification growth has halted. Since then, only Punjab province has experienced a large increase in registered cases, while other districts have seen stable or declining numbers (Metzger et al. 2010).

The World Health Organization reports approximately 500,000 new TB cases each year, with the trend of drug-resistant cases rising. Pakistan accounts for 61% of Eastern Mediterranean WHO TB cases, contributing to the global TB burden. Data collected from Pakistan by WHO's Global Health Observatory has shown an average of 312,222 new and relapse TB cases each year over the past decade (Awan et al. 2022). In the last decade, TB cases were constantly increasing as in 2010, 264235 cases were recorded which further reached up to 356390 in 2016. Later, the disease incident rate remained constant until 2020 (Awan et al., 2022).

Approximately 5.75 percent of cattle and buffalo in Peshawar, Khyber Pakhtunkhwa, have bovine TB. Human sputum PCR indicated 96% *M. tuberculosis* and 4% *M. bovis*. A comparative cervical intradermal tuberculin (CCIT) test on large ruminants in five Central Khyber Pakhtunkhwa districts, Peshawar, Charsadda, Nowshera, Swabi, and Mardan found 5. 88% (141/2400) bovine TB.

Lahore, the second largest city in Pakistan, had 54% bovine TB by PCR. This study suggests government intervention to reduce TB's health effects. Animal farmers require awareness too. Cattle are more susceptible to this disease than buffalo, 6.45% vs. 5.28%. Infected cattle milk can spread *M. bovis*. PCR helps cattle detect *M. bovis.* 556 cattle and buffalo in Peshawar had bovine TB tested. 5.75 percent of 556 animals tested were positive. Whether 0the animals were farm-raised or bought, whether they slept indoors or outdoors, and how many were in the herd affected prevalence. *M. bovis* was tested in 92 retail milk samples. Eight of the ninety-two milk samples had *M. bovis*. 39.6% of participants knew that a three-week cough could indicate TB. Participants thought prayer and healthy nutrition (41.8%), natural therapies (35.7%), and contacting Hakeem (35.7%) could cure TB (Khattak et al. 2016).

11.2. INDIA

India has the world's largest TB burden with 3 million patients and 2 million new cases per year. Human tuberculosis reporting and bovine population and ownership in India highlight the need to address zoonotic risk from bovine TB. Buffalo-rich areas have higher household TB risk than buffalo-poor ones (Willgert et al. 2023). TB kills 280,000 Indians annually. India is second to China in MDRTB cases, with 99,000 cases per year (Claiborne et al. 2012). India has a highest 21% of TB infections in overall prevalence in Asia following China has 14%, Indonesia 6%, Nigeria 5%, South Africa 5%, Bangladesh 4%, Ethiopia 3%, Pakistan 3%, Philippines 3%, other 13 high burden countries for TB (HBCs) 16%, other countries 20% (Kashyap et al. 2013).

New Delhi had 1.1% fewer MDR TB cases in 2008-2009. 20.4% of 196 New Delhi patients with pulmonary TB who had failed earlier TB therapy, relapsed after treatment, or defaulted during treatment developed MDR TB in a 2005-2008 study. According to the Revised National Tuberculosis Control Programs (RNTCP), 20% of Indians have latent TB (Ahmed and Hasnain 2011).

M. bovis is thought to cause 10% of all TB cases in developing nations, posing a global health threat. *M. bovis* caused 25% of pediatric TB. Indian researchers searched scholarly journals for cow and buffalo bovine TB prevalence on September 11, 2017. Quantitative analysis used 82,419 bovine TB prevalence data from 1942-2016 research. 29,037 were buffaloes and 53,382 cows. This meta-analysis comprised previous studies. Incidence rates were estimated. Significant regions were sampled. Following is the data about some states of India showing prevalence of *M. bovis* from 2014-2016:

- Punjab (with a sample size of 121) had reported prevalence of 14%.
- Uttar Pradesh (sample size of 245) showed prevalence of 14.3%.
- Gujrat (sample size of 2310) reported 2.3% prevalence.
- Karnataka (sample size of 45) reported 26.7% prevalence.

In a recent study conducted in Guwahati metropolitan city India, it is shown that occurrence of bovine tuberculosis was highest in animals five years old and above (17.18%), followed by animals belonging to age group of 3 to 5 years (7.14%) and it was lowest in the age group between one to three years of age (6.52%). During the present study, 220 Jersey crossbreeds, 38 Holstein-Friesian (HF) crossbreeds and 102 indigenous crossbreeds of cattle were screened. The prevalence was found to be more in HF crossbreeds (13.15%), followed by Jersey crossbreeds (10.90%) and indigenous crossbreeds (8.82%). Prevalence of bovine tuberculosis in cattle showing symptoms which could be bovine TB (chronic coughing, reduced milk yield, emaciated body condition, respiratory distress, and fever) was found to be higher (19.71%) compared to apparently healthy cattle (4.58%) and is statistically significant (Srivastava et al. 2008).

11.3. RUSSIA

The Beijing BO/W148 clone of mycobacterium is widely distributed in the Commonwealth of Independent States (CIS) and Eastern Europe. It is most common in Siberia and the European region of Russia (to a lesser degree) (Mokrousov 2013). The spread of multidrug-resistant TB has strong ties to the corrections system. Using restriction fragment-length polymorphism analysis and spoligotyping, researchers analyzed 144 TB isolates found in inmates at the Archangel prison (Archangel, Russia) in 2001. Research of the genetic makeup of the isolates pointed to the W-Beijing group accounting for 87 (76.3%). Only 26.9% of the isolates were not found to be grouped in any way around the W-Beijing area. Toungoussova et al. (2003) found that there were 43 patients in the greatest cluster. Overcrowding, lack of air, and prisoners' generally poor health all contribute to the rapid development

of TB in prisons. There were 3,174 new cases and 171 fatalities from TB in Russian prisons in 2001, and the situation is worse when the illness is caused by drug-resistant *M. tuberculosis* (Toungoussova et al. 2003).
The rate of TB among the general population (not including inmates) in the Archangel region in northwest Russia decreased from 48 cases per 100,000 people in 2000 to 20 cases per 100,000 people in 1991. From 1996 to 2000, the incarceration rate-weighted incidence of TB climbed from 55 to 104 per 100,000 people, according to epidemiological statistics. This dramatic rise in TB cases may be traced back to the large jail population (Toungoussova et al. 2003).

11.4. CHINA

Large-scale population-based study on tuberculosis molecular epidemiology in China, with the second highest global prevalence. Nine drug-sensitive Chinese *M. tuberculosis* isolates shared spotlighting and Mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) characteristics. School TB patients have unique disease experiences due to educational systems and socioeconomic circumstances, according to a qualitative study (Li et al. 2021). The northern pig-tailed macaque is a Class I protected mammal in China and a vulnerable species on the international union for conservation of nature (IUCN) Red List. In 24 provinces, milu deer breeding programs have saved them from extinction. China's latest animal quarantine law lacks disease surveillance and efficient quarantine. Quarantine before mixing with other animals and annual disease testing are recommended to protect animal health. The approved deer and monkey test, tuberculin skin test (TST), sometimes misses TB in monkeys. Faeces, urine, and hair should be sampled for disease detection, diagnosis, and surveillance (Chen et al. 2023). Rural extrapulmonary TB is more common in younger, female patients. Extrapulmonary TB patients have greater MDR-TB. Lymph glands, bronchi, bones, braces, urogenital tract, and meninges are affected. Extrapulmonary TB is less contagious and infrequent. Because it can affect any organ, its vast range of clinical symptoms makes diagnosis and treatment difficult. Extrapulmonary TB affects diabetics, HIV patients, and others. Women and individuals from high-TB-prevalence areas are more prone to get TB. Regional, population, and host characteristics affect anatomical positions (Pang et al. 2019). Diabetes reduces pleural TB risk but not extrapulmonary TB risk. In Poland and Romania, bronchial TB is most common, but in the Netherlands, US, and UK, lymph glands are. Due to Bacillus Calmette-Guérin (BCG) ineffectiveness against diverse TB strains, China's main extrapulmonary TB location differs from others. China's BCG immunization may be linked to extrapulmonary TB-affected areas.
Chinese ethnicity and residency affect BCG immunization rates. BCG immunization reduced severe tuberculosis in active TB patients. Severe active TB was rare in immunized people. BCG scars protected children from serious TB. Chinese law requires all newborns to receive the BCG immunization in the same hospital. However, private hospitals struggle to immunize newborns. Vulnerable children need newborn immunization programs, TB risk awareness, and BCG injections (Liao et al. 2022).
Recent transmission makes one in three TB patients' secondary cases. Secondary cases are more likely in MDR TB patients. Beijing strain-infected TB patients are more likely to be in a genomic cluster and develop active TB faster. *M. tuberculosis* dominates China. Intensified case discovery, fast tests with bacteriological confirmation, and suitable treatment can reduce *M. tuberculosis* spread. Isolating infectious TB patients, testing for latent TB, and providing prophylactic treatment may reduce TB rates. Improved diagnosis and screening reduce epidemic risks and prevent TB spread. Better boarding schools, teachers, and school doctors are needed. Controlling tuberculosis requires microbial genetic sequencing and epidemiologic methods, especially at universities (Li et al. 2021).

11.5. MALAYSIA

Tuberculosis (TB) is mainly found in underdeveloped countries and counts to be a major cause of morbidity and mortality due to a lack of diagnostic, prophylactic, and therapeutic tools (Nissapatorn et al. 2007). After starting the National Tuberculosis Control program in 1961 a reduction in TB cases was observed. A gradual increase was observed from early 1995 till 2002 with the highest incidence rate in Sabah state followed by Wilayah Persekutuan, Sarawak, and Pulau Pinang, respectively (Iyawoo 2004) (Aziah 2004) (Goroh et al. 2020). A large proportion of disease has been observed in foreigners living there. A study was conducted on patients from different walks of life admitted to the Institute of Respiratory Medicine (IRM) from May to December 2003. The rate of tuberculosis in this study was equally high in both native and nonnative patients (Nissapatorn et al. 2007). According to a survey, pulmonary tuberculosis is more prevalent than extrapulmonary tuberculosis (Swarna Nantha 2014). The population having diabetes mellitus is a major predisposing factor in the reactivation of tuberculosis, followed by smoking, chronic kidney disease/end-stage renal failure, and age-related issues (Swarna Nantha 2014). Age group, gender, and marital status also count be significant predisposing factors (Nissapatorn et al. 2007). To estimate the incidence rate in Sabah the State Health Department developed a protected electronic database (TB) (Goroh et al. 2020). DOTS (directly observed, treatment, short course) is the most effective treatment strategy available for controlling TB (Aziah 2004). Almost 33% of the world's human tuberculosis is found in the southeast Asia SEA. The most extensively recognized causing agent of human TB is *M. tuberculosis*, yet an ambiguous number of cases are due to *M. bovis* (Che-Amat & Ong, 2018).

Zoonotic tuberculosis is reported to be responsible for almost 3-15% of tuberculosis among humans worldwide and its infections are seen in a few SEA countries such as Malaysia and Thailand (Hassan 2014). A study conducted in Malaysian context show that if ruminant farmers are adequately up to date and educated, positive attitudes may increase higher levels of positive practices towards zoonosis (Sadiq et al. 2021). A recent study indicated that controlling the hunting of wild pigs and deer would help to control the transmission of infection from wildlife (Cantlay et al. 2017). Wild boars are known to contribute to the epidemiology of animal TB in some areas. Serology has been implemented for screening and diagnosis in wild boars and feral pigs due to relaxed procedures and quicker diagnostic outcomes (Lekko et al. 2021).

11.6. INDONESIA

In 2019, 10 million Indonesians had TB and 1.4 million died. The End TB Strategy has reduced TB infection and mortality slightly. Lack of care prevented 3.6 million TB diagnoses. Advanced disease (MDR) TB was difficult to cure and more likely to kill TB-infected patients. Lack of care may have prevented 3.6 million TB diagnoses. Sumatra has the highest TB prevalence at 95%. Due to their underdevelopment, Sumatra and Java have better health care. Males had more TB. The district had more TB cases than the province in Yogyakarta City. Urban areas risk TB. Environmental considerations make Central Java's Kendal District densely populated (Sulistyawati and Ramadhan, 2021). Indonesia's TB surveillance needs improvement. Due to a lack of healthcare workers, transportation, supplies, and knowledge, this could cause major issues (Noviyani et al. 2021).

Smoking increases the risk of chronic obstructive pulmonary disease COPD, including TB (Noviyani et al. 2021). Individual behaviour and family environment are predisposing factors for TB in Indonesia. Males are more probably to be exposed to TB risk factors, including smoking than females, with 33.8% of Indonesians aged 15 and older reporting smoking (Noviyani et al. 2021). Dogs and old-world monkeys are more susceptible to *M. tuberculosis* (Une and Mori 2007). Zoo animals may supply MDR-TB strains through invert zoonoses with *M. tuberculosis* (Kock et al. 2021). MTC causes wildlife TB in water, likely with *M.*

tuberculosis or *M. bovis*. It is widespread in Indonesia, Nepal, and Thailand, allowing human-to-nonhuman primate transmission. Diagnosis is needed to reduce wildlife tuberculosis (Che-Amat and Ong 2018). *M. tuberculosis* poses a risk of cattle to human transmission in resource limited countries (Adesokan et al. 2019).

11.7. MIDDLE EAST COUNTRIES

WHO's approach to eradicate tuberculosis includes early latent tuberculosis infection (LTBI) detection and control. Using a casual effects model, this study examined LTBI incidence and showed substantial heterogeneity. LTBI was 41.78% in Middle East and North Africa (MENA). To meet the WHO target of eliminating tuberculosis by 2035, MENA countries must increase tuberculosis control and LTBI detection (Barry 2021).

The MIRU-VNTR Act was assessed for its significance in TB control in Muslim Middle Eastern nations. Most Iranian and Saudi TB cases involved immigrants. Reactivated cases can spread harmful isolates. To prevent MDR-TB instances in receiving countries, immigrants must be screened for this disease and treated. According to studies, Saudi Arabia and Turkey were risky for adolescents. MIRU-VNTR molecular epidemiology studies will assess Middle Eastern TB control programs by measuring infection rates and effective factors. New tuberculosis cases, both reactivation and newly infected are considerable in each country, but reactivation is more relevant (Pourostadi et al. 2018).

World immigration centers in the Middle East. Migrants risk their health. Migrants cause health issues in both origin and destination countries. Due to discernment, linguistic and social barriers, legal status, and low socioeconomic level, immigrants can have serious fitness issues. Kuwaiti migrant laborers have a higher rate of pulmonary tuberculosis (Adhikary et al. 2011).

BCG vaccination and no tuberculosis mycobacterial (NTM) infection can skew TST results. The tuberculin skin test should be useful in most Middle Eastern states. TST will lose value in these states until BCG immunization is discontinued (Al-Jahdali et al. 2005). Bovine tuberculosis is a zoonotic illness that affects livestock and infrequently humans and is spread by local contact with sick hosts and unpasteurized dairy products. Bovine tuberculosis is a serious health threat across MENA, including developing nations. In the MENA region, bovine tuberculosis in humans and livestock varied widely by population size and state. Our findings show that the MENA region needs appropriate investigative gear and sustained regulatory approaches, especially on human and animal contact surfaces. MENA countries have reported human-only zoonotic tuberculosis (Kasir et al. 2023).

Iran's bovine tuberculosis records go back 50 years. Iranian hereditary cattle have bovine tuberculosis frequently. European livestock ranches now have a record 28% bovine tuberculosis prevalence. Bovine tuberculosis in Iranian sheep is unconfirmed. Typical TB lesions were isolated from sheep slaughter samples in 2003. Tuberculosis complex mycobacteria and microbes grew at 37°C. However, insufficient bacterial growth hampered molecular investigations to identify this isolate. In 2009, cicarin lymph nodes in Hoveizeh, Couzestan revealed an *M. tuberculosis* strain with a SIT587 spoligotyping. Reservoir animals' role in Iran's BTB epidemiology is uncertain and needs further study (Tadayon et al. 2013).

Despite the low prevalence of DM, 21% of tuberculosis patients in Yemen had DM. Most shared dominance studies were from Middle Eastern countries with rates ranging from 10% to 30%. A Saudi Arabian study indicated that diabetes/tuberculosis has more bone illness than non-diabetic TB. Two Turkish examples showed DM/tuberculosis patients with spondylitis and bone disease. In conclusion, DM/TB and non-DM/TB studies in the Middle East are scarce (Yosra and Abdely Scott 2010).

In 17 Middle Eastern countries, single, arbitrary, and multi-drug resistance in fresh and previously treated TB patients differs greatly. This comprehensive research suggests that drug-resistant tuberculosis, particularly MDR-TB, may be spreading in Middle Eastern TB patients. New fast diagnostics are utilized to

identify persons with tuberculosis symptoms, assess medicine vulnerability, and detect primary confrontation to first-line anti-TB drugs to achieve extra-operative tuberculosis control. To prevent further *M. tuberculosis* strains, efficient therapies must be found (Khademi et al. 2017).

12. CONCLUSION

Tubercle bacilli has gained more chances of infection spread as the immune system of people has been drastically reduced in low-income countries. The emergence and success of the antibiotic resistant mycobacteria has further worsened the situation. Mycobacteria has a vast range of hosts from humans to animals. So, to curtail the infection as well as to reduce the chance of zoonosis, medical and veterinary medical surveillances are needed to positively diagnose the disease outbreaks and later for its eradication.

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Molecular Diversity of Bovine Tuberculosis

05

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ABSTRACT

Bovine tuberculosis (BTB), the causative agent of which is Mycobacterium bovis, a member of Mycobacterium tuberculosis complex (MTC), poses a major public health hazard globally, especially in developing countries. Mycobacterium bovis having 4200 genes, pathogenicity is associated with proline-glutamate or proline-proline-glutamate genes. The zoonotic nature of infection underscores its significance on both animal and human health. Though several species are affected, cattle serve as the main reservoirs. Multiple strains of the Mycobacterium tuberculosis complex have been revealed by molecular investigations, assisting in comprehending the virulence and evolution of the organism. BTB continues to affect human well-being, trade, and animal health despite efforts to control it in developed countries. To mitigate the effects of this zoonotic disease, effective control measures—such as testing and slaughter—are essential. RFLP is mainly used to study the prevalence of BTB. For this an insertion sequence IS110 is widely used as genetic marker. Multi drug resistant tuberculosis is detected by GeneXpert technique, which identify the rifampicin resistance sequence in MTC. Timely surveillance of a disease is of utmost importance, mostly gamma-interferon and intradermal comparative tuberculin skin test are the valuable tools and used for prevention and control programs. Various national and international measures should be taken to develop good surveillance strategies and strengthen the current ones. Bovine Tuberculosis (bTB), being a zoonotic disease, still raises severe health and economic concerns in developing countries. Therefore, timely surveillance of disease is vital for appropriate containment measures. Keeping in view the objectives, various approaches may be adopted for designing a suitable surveillance plan.

Keywords: Zoonotic Bovine tuberculosis, Mycobacterium bovis, Pathogenicity, Mycobacterium tuberculosis complex, Clonal complexes, Surveillance

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1. INTRODUCTION

Bovine tuberculosis (BTB) is an inflammatory disease characterized by chronic granulomatous, caseous necrotizing lesions in the lungs and associated draining lymph nodes. Other organs may also be affected depending upon the route of infection. Inhalation is the most commonly known route of infection, causing lesions of the nasopharynx and lower respiratory tract (Domingo et al. 2014). The etiological agents of the disease are mainly *Mycobacterium (M.) bovis* and to a lesser extent *Mycobacterium caprae,* the members of the *Mycobacterium tuberculosis* complex (MTC). The disease doesn't affect all the species equally; rather some species serve as maintenance hosts and others as spillover hosts. All warm-blooded animals are prone to the infection. The bovine species such as bison and buffalo being more susceptible and cattle are the most common reservoir of *M. bovis* (Ayele et al. 2004; Pesciaroli et al. 2014; Yahyaoui-Azami et al. 2017). Since it is a zoonotic disease, it is viewed as a public health concern. Due to insufficiency and unavailability of surveillance and control measures in most of developing countries, zoonotic tuberculosis is widespread in animals. Therefore, the epidemiological and public health concerns still remain unknown (Cosivi et al. 1998). In the context of public health concerns, *M. bovis* is categorized as a Risk 3 pathogen (OIE 2005). *M. bovis* has historically been responsible for extrapulmonary tuberculosis in newborns and children, which typically is brought on by the consumption of unpasteurized or un-boiled milk from infected cows (Thoen et al. 2006).

2. IMPORTANCE OF MOLECULAR DIVERSITY

Regardless of possessing similar 16S rRNA sequences and 99.9% similarity in nucleotide sequences (Boddinghaus et al. 1990), the mycobacteria a group of *Mycobacterium tuberculosis* complex, vary significantly in host specificity, morphology, and pathogenicity. With the assumption that all mycobacteria have been derived from the same ancestor, some of the strains are human-specific (*Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium canettii*), some are rodent-specific (*Mycobacterium microti),* while some have a broad spectrum of host infectivity (*Mycobacterium bovis*) (Brosch et al. 2002). The housekeeping genes in *M. tuberculosis* are highly conserved, the reason suggested for the evolution during speciation, which is thought to have occurred some 15 to 20 thousand years ago (Sreevatsan et al. 1997). *Mycobacterium tuberculosis* strains may be categorized as "ancestral" or "modern" strains depending upon the presence or absence of a particular deletion (TbD1) in the sequence of *M. tuberculosis*, the latter being representatives for major epidemics (Brosch et al. 2002). The most prevalent causative agent of human tuberculosis, *M. tuberculosis* has been hypothesized to be evolved from *M. bovis* through precise adaptation of animal's pathogen to human host (Stead et al. 1995). Both of these theories were put out prior to the availability of the whole genome sequence of *M. tuberculosis*, and the revelation of multiple variable regions in the genome of M. tuberculosis complex (Cole et al. 1998). The study of molecular diversity is important to study the genetic organization of the common ancestor of tubercle bacilli, the factors that contributed to host specification and spectrum, and from where *Mycobacterium tuberculosis* evolved. All these help in better understanding of pathogenicity and worldwide epidemiology of disease that can help in predicting future patterns of disease spread (Brosch et al. 2002). Moreover, for effective TB control programs, the genotyping of *Mycobacterium tuberculosis* strains is crucial as it enables the diagnosis of outbreaks, transmission tracking, species diversity monitoring, and the identification of secondary infections (Mozafari et al. 2013).

3. EPIDEMIOLOGY OF BOVINE TUBERCULOSIS

3.1. GLOBAL DISTRIBUTION AND PREVALENCE

Bovine tuberculosis is a highly adaptable and successful infection, having worldwide distribution. It is geographically distributed all over the globe. Between 2015 to 2017, as per the information of OIE's

Worldwide Animal Health Information Database, out of 182 countries, 91 have reported tuberculosis infection in cattle (Interface 2023). It is a major, costly infectious condition in various countries affecting cattle along with other domesticated, feral and wild animals such as badgers, deer, possums, goats, sheep, and camelids (Pollock and Neill 2002; Carslake et al. 2011). The link between the infection and the disease (TB) and the relation among transmission and disease is key to understanding the epidemiology of bovine tuberculosis. Therefore, the risk factors that hypothetically are thought to facilitate the disease spread, such as contact between animals and their movement should crucially be taken into account. The determination of risk environments and risk factors for disease and transmission is also closely associated with the factors affecting susceptibility (Skuce et al. 2012). The deployment of schemes such as test and slaughter, inspection of meat at slaughterhouses, and pasteurization of milk has successfully controlled the disease in most of the developed countries. Regardless of such intensive control regimes, it is still an important concern in some developed countries (Ireland, New Zealand, UK) (Humblet et al. 2009; (Allen et al. 2018), and in a majority of developing countries, where the control strategies are not implemented or at early stages (Teppawar et al. 2018).

4. ECONOMIC IMPACT

Bovine TB not only has detrimental effects on the health of cattle, but it also affects trade and profitability significantly. It can undo years of genetic advancement for desired production traits. The welfare of the affected farming families is also significantly affected (Boland et al. 2010). The overall productivity of animals is reduced such as milk yield, meat production and fertility. It costs human health and is compensated from control programs (Cosivi et al. 1998; Olea-Popelka et al. 2017).

5. *MYCOBACTERIUM BOVIS*

The Mycobacterium tuberculosis complex (MTBC), includes *Mycobacterium bovis* consisting of 11 species of bacteria with varying host tropism and pathogenicity (Gagneux 2017). The principal cause of tuberculosis in humans is *Mycobacterium tuberculosis*, although *M. bovis* has a wider host range and can infect a variety of hosts, mostly cattle but also includes people, with varying populational persistence (Gagneux 2017).

6. MYCOBACTERIUM TUBERCULOSIS COMPLEX

The MTBC is a clonal group (Supply et al. 2003) that evolved from a common ancestor with the *Mycobacterium Canetti*, thousands of years ago (Comas et al. 2013). The horizontal gene transfer and significant amounts of recombination are not thought to have occurred in the genomes of MTBCs, which have >99.95% identity over homologous nucleotide sequences, including the ribosomal RNA genes (Hirsh et al. 2004), however, Single nucleotide polymorphisms (SNPs), indels (small insertions and deletions), deletions of up to 26 Kb, insertion sequences (IS), and duplication of a few paralogous genes are the only way of diversion.

7. DIFFERENTIATING REGIONS

Some of the large deletions, in the members of MTBC, called “Regions of Difference” (RD), were initially described through physical mapping and differential hybridization arrays to differentiate between *M. tuberculosis* H37Rv, *M. bovis* BCG Pasteur, and *M. bovis* ATCC 19210 (Gordon et al. 1999). Fourteen

evolutionarily stable regions of difference (RD1–14) are present among these strains that are used to differentiate. These range from 2 to 12.7 kb in size. The identification of these RDs emptied the path for the molecular diagnosis and classification of MTBC species, and they are now regarded as the benchmark for identifying the various individuals in this complex. As a result, the deleted regions RD9 and RD4 and the absence of RD1BCG (which is deleted in BCG strains) allow *M. bovis* to be distinguished from other MTBC members with accuracy (Bespiatykh et al. 2021). Fig. 1 shows the differentiating regions between the different species of MTBC.

8. TAXONOMIC CLASSIFICATION OF *MYCOBACTERIUM BOVIS*

The *M.bovis* was officially named in 1970, since the beginning of the 20th century, albeit called this way (Karlson and Lessel 1970). The type strain was defined as *M. bovis* ATCC 19210, still referenced in the most recent Bergey's Manual of Systematic Bacteriology (Kämpfer 2012), along with CIP 105234 and NCTC 10772. Early taxonomic classification was based on specific phenotypic traits of the isolates, for tuberculous mycobacteria, such as host of origin, virulence in animal models, and biochemical tests (e.g., pyrazinamide resistance, niacin accumulation, nitrate reduction, type of respiration, colony morphology).

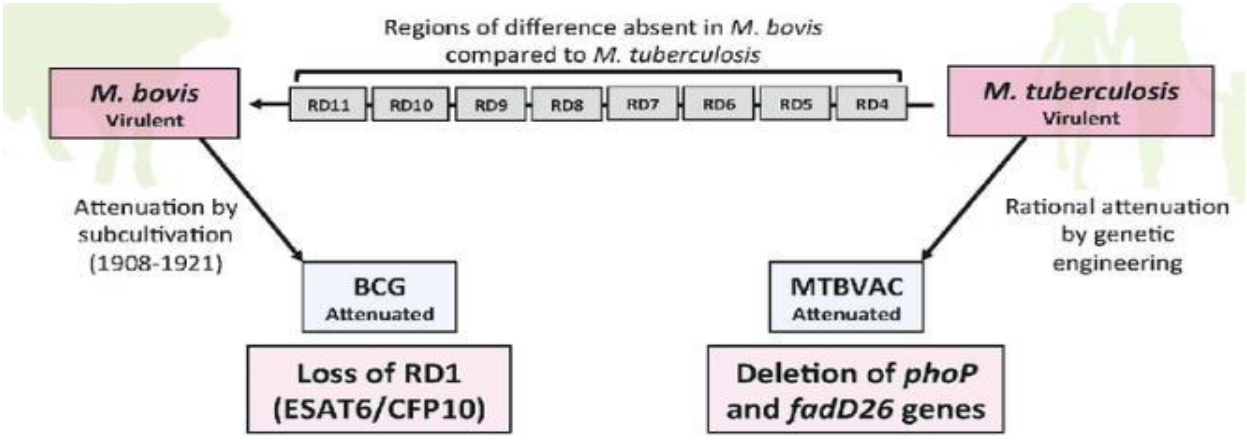

Fig. 1: Differentiating Regions between the different species of MTBC (Gonzalo et al. 2017)

Discussions over the taxonomic categorization of the MTBC species have always been ignited by the close genetic kinship between *M. tuberculosis* and *M. bovis*, as well as among other species. It is sometimes suggested that the MTBC be treated as a single species. However, the epidemiologic and biochemical disparities between diseases, notably between bovine and human infections, bacilli, accentuated the need for differentiating these organisms at some taxonomic level (e.g., species, subspecies, variant).

9. GENOMIC STRUCTURE OF *MYCOBACTERIUM BOVIS*

The M. bovis genome consists of 4.3 Mb, containing approximately 4200 genes, including a single copy of each of the ribosomal RNA genes (5S, 16S, and 23S) and 45 tRNAs. its genome has a high GC content (≈65%), similar to other actinobacteria which suggests the use of suitable sequencing reagents for library preparation in WGS (Tyler et al. 2016). One of the biggest difficulties in analyzing WGS data is the high proportion of repeated sequences in MTBC genomes, especially *M. bovis*. The mobile elements (e.g., insertion sequences—IS), proline-glutamate (PE) or proline-proline-glutamate (PPE) family genes,

integrases, two phage sequences, a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), and the 13E12 repeat family genes are few examples. In particular, PE-PPE gene families account for approximately 10% of MTBC genomes and have been associated with TB pathogenesis (Delogu et al. 2017). The most commonly used sequencing platforms generate short reads, usually ranging from 50 to 300 bp, due to this reason, the repetitive elements are tough to handle in genomic studies because the majority of reads are often shorter than the repeats themselves (Tørresen et al. 2019). Some of these repetitive regions are used for traditional genotyping techniques.

10. CLONAL COMPLEXES

Clonal complexes can be defined as groups of short tandems in which every ST shares at least five to seven identical alleles with at least one other ST in the group.
Till now four *M. bovis* CCs have been described including

- African 1 and 2,
- European 1 and 2)

These are determined based on specific deletions ranging few SNPs and spoligotypes. Similarly, to M. tuberculosis lineages, CCs appear to be geographically segregated, with African 1 and 2 restricted to Africa, European 2 usually found in the Iberian Peninsula, and European 1 distributed globally. However, CCs do not represent the whole genetic diversity as all the M. bovis cannot be classified through CCS. One study projected the four distinct global lineages of M. bovis ranging from (Lb1 to Lb4), geographically segregated and not fully represented by CCs (Zimpel et al. 2020).

11. MOLECULAR TOOLS FOR STUDYING BOVINE TUBERCULOSIS

There are different valuable molecular techniques available to study BTB. Restriction fragment length polymorphism (RFLP) is especially used for epidemiological studies and the differentiation of different strains of BTB. An insertion sequence *IS6110* is widely used as a genetic marker. The *IS6110* fingerprinting has been standardized by the use of restriction enzyme *PvuII*, to digest the genomic DNA of BTB. To perform RFLP 20 to 40 days are required to have sufficient DNA and applied to different strains of all mycobacterium species. International consensus regarding the methodology of *IS6110* RFLP of *Mycobacterium tuberculosis* complex and *IS1245* RFLP of *Mycobacterium avium* has been achieved. RFLP is not so feasible because these bacteria are slow growers which can hinder the RFLP results. Secondly, it is laborious, time-consuming, costly, and requires software for advanced analysis.
Polymorphic GC-Rich repeat sequence (PGRS) is another technique probably considered superior over *IS6110* fingerprinting. PGRS-based probes are the most indiscriminatory type for *Mycobacterium bovis* strains.
PCR-based detection is also widely used which is a more sensitive method than culture. Multiplex PCR is faster than culture and even reduces the time to 2 days from 120 days. PCR targets the 16S rRNA gene sequence of the hypervariable region which is specific for *M. avium*, *M. intracellular*, MTB70 gene which is specific for MTBC. s used to differentiate between different MTBC species. Phylogenetic analysis of MTBC strains from animal origin belongs to RD9 while *M. bovis* have RD4. Spoligotyping is a useful method for the detection and typing of MTBC simultaneously.
The GeneXpert technique is also used for the detection of multidrug resistance (MDR) tuberculosis. It is based upon the principle of DNA sequence-specific detection of Rifampicin resistance mycobacterium tuberculosis complex by PCR. It is a rapid, simple-to-use nucleic acid amplification test (Nabeta et al. 2010). It uses the principle of real-time fluorescent probes, and enables the processing of unprocessed sputum samples, resulting in the generation of results within 90 minutes with minimal biohazard and very little technical training.

12. IMPLICATIONS FOR DIAGNOSTICS AND CONTROL MEASURES

12.1. DETECTION AND SURVEILLANCE STRATEGIES

The term 'surveillance' is a French word that means 'the act of watching over'. Initially, it was acknowledged as the close monitoring of individuals infected with or exposed to contagious diseases for timely detection and later was improved with the inclusion and implementation of appropriate control measures like quarantine (Rojanaworarit 2015). Progress toward the control and elimination of disease significantly depends on the collection, interpretation, and analysis of health-related data (CDC). Surveillance programs refer to the systematized collection, evaluation, elucidation and well-timed dissemination of generated health data for the appropriate planning and implementation of disease control programs through policies and procedures with the ultimate goal of prevention and/or minimization of disease transmission (Berrada 2006).

13. IMPORTANCE OF SURVEILLANCE

Surveillance of a disease is important not only to improve the effectiveness of prevention and control programs but also to monitor the impact of the most effective interventions. Strong disease surveillance helps to identify gaps in health systems that are met with appropriate changes in policies and practices. Effective implementation of a surveillance system faces several challenges while ensuring complete, timely, and accurate data collection which can be used efficiently at various levels of the health system. The lack of trained personnel for the assessment, analysis, and interpretation of data may be responsible for the failure of program priorities and interventions despite the availability of good surveillance systems (CDC). Various measures should be taken at national and international levels to develop good surveillance programs, including developing guidelines and tools for the respective Task Force, vital registration systems, data collection tools and guidance, training workshops, and national-level meetings. Surveillance programs must be strengthened with the assistance of international organizations to ensure that authentic data is used for program improvement, expansion of access to treatment and development of new disease indicators (CDC).

Bovine Tuberculosis (bTB), a chronic zoonotic disease, imposes a serious economic impact on animal production and raises public health concerns. Timely surveillance of disease is important for the appropriate containment measures. In developed countries, single intradermal comparative tuberculin skin test (CITT) and gamma-interferon test are valuable tools for health surveillance and monitoring of animals at the farm level which has resulted in a dramatic decrease in the prevalence of the disease (Berrada 2006; Gonçalves et al. 2022). Many factors like poverty, close association between humans and animals, sociocultural habits and HIV/AIDS are responsible for the widespread dissemination of *M. bovis* infection among humans in developing nations like Africa. Inadequate documentation is available on the zoonotic implications and epidemiology of bovine tuberculosis in developing countries, even though the prevalence of human tuberculosis is quite high in these areas. Several factors account for this situation including the insidious nature of tuberculosis, the simultaneous existence of other more devastating diseases, the lack of cooperation between veterinary and medical experts to deal with the public health significance of bovine tuberculosis, and the limited capacity of planning and implementing surveillance and control programs in developing countries (Berrada 2006).

Various countries tackled the bovine tuberculosis challenge by designing different control strategies for raising awareness, capacity building and skills development (Berrada 2006). Since 1991, Portugal has had an eradication program, PETE, which aims to limit the infection in susceptible animal hosts and hence

reduces the risk of human infection (Gonçalves et al. 2022). The Kingdom of Morocco, a developing country, built organizational and technical capacities at regional and international levels for the surveillance of bovine tuberculosis through a well-designed TCP/MOR/2904 project. At the regional level, this project recommended the establishment of collaborating and reference centers for research, training, diagnosis, and surveillance of bovine tuberculosis. At the international level, the provision of assistance and financing approaches for developing countries to strengthen institutional capabilities and human resources is highly recommended (Berrada 2006).

14. OBJECTIVES OF SURVEILLANCE

The following are the three main objectives of surveillance:

- Description of dynamic disease occurrence patterns that are linked to public health actions
- Elucidation of the historical and epidemiological profile of a disease
- Provision of relevant information and baseline data (Rojanaworarit 2015)
- Identification of current burdens and trends
- Detection of circulating strains and their antimicrobial resistance (Murray and Cohen 2017)

15. METHODS OF SURVEILLANCE

Various approaches for surveillance are based on the objectives of surveillance, clinical presentation of disease, and epidemiology (Murray and Cohen 2017). Bovine tuberculosis (bTB) surveillance may be conducted by two methods, namely, active and passive surveillance. Voluntary reporting to district veterinary staff by cattle herdsmen and peri-urban dairy farms refers to passive surveillance. Whereas, active surveillance includes meat inspection, laboratory testing by Ziehl-Neelsen staining, and targeted screening of cattle in a reported area.

16. SURVEILLANCE SYSTEM ESTABLISHMENT

Surveillance systems propose plans including national activities that should be carried out and updated by State involvement (Connie et al. 2021). Due to resource constraints and additional burdens on the operating system, rational selection of diseases for which a surveillance system is established is of utmost importance. Diseases having severe health outcomes (e.g., rabies), high transmission rates (e.g., Ebola), and epidemic or pandemic nature (e.g., influenza) are generally considered for surveillance (Rojanaworarit 2015).
A surveillance system includes several consecutive methodical steps that are illustrated in Fig. 2 and are subsequently elaborated.

16.1. DATA COLLECTION

In terms of investing in the budget and obtaining quality data during surveillance, data collection is the most crucial step. Several factors account for the adaptation of data sources in terms of public health surveillance. These include accessibility and availability of data, features of health service system, budget constraints, personnel, availability, and quality of facilities. In his article, (Rojanaworarit 2015) provided guidelines that surveillance data should include important information like data on mortality and morbidity, data for epidemic detection and laboratory surveillance, disease occurrence and associated risk factors, data for healthcare and health system surveillance as well as environmental data.

Text

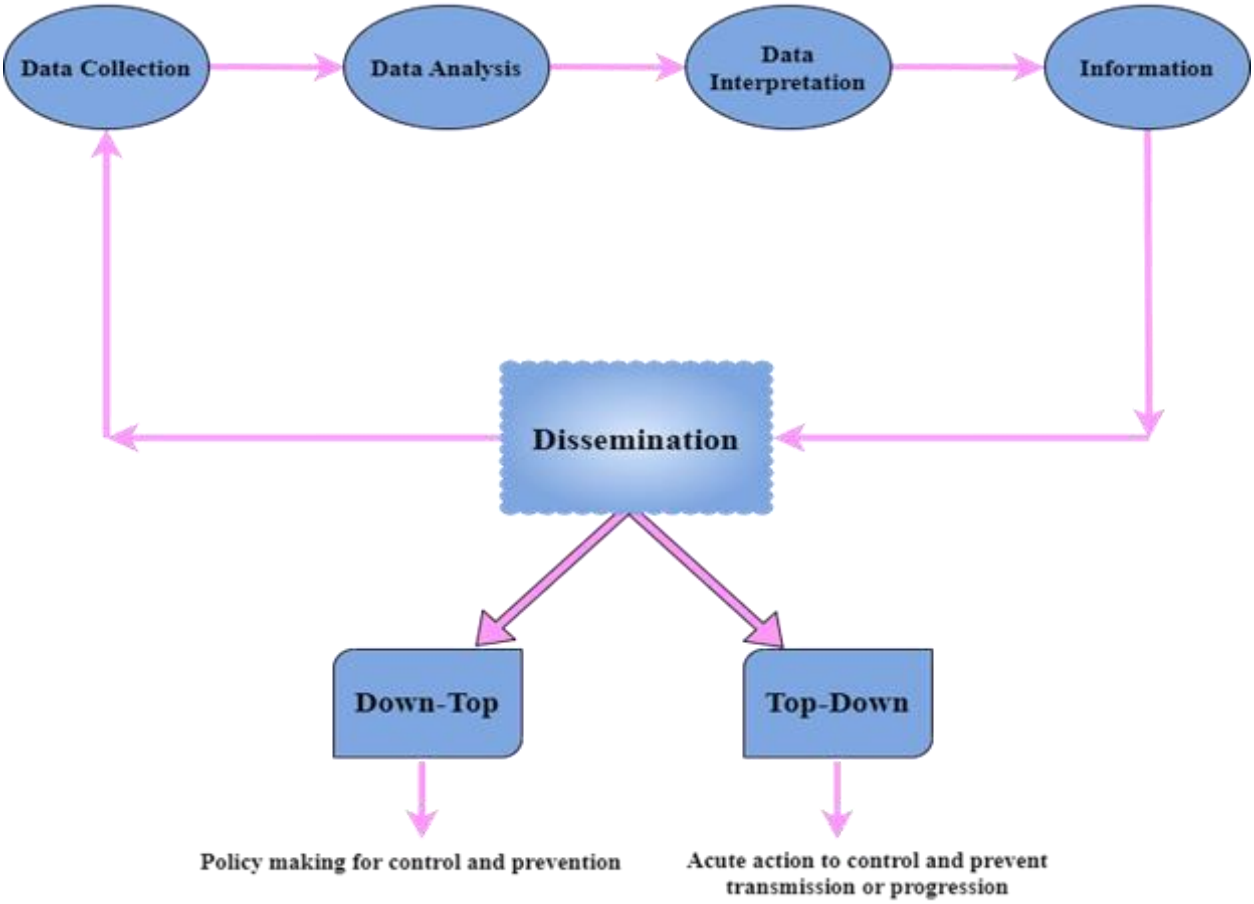

Fig. 2: Surveillance System Workflow

16.2. DATA ANALYSIS

The data analysis approach is adopted to reveal the pattern, magnitude, and trend of a certain health problem. For this, appropriate indicators like prevalence and incidence are used to identify the magnitude of a problem. Elements required in the analysis of surveillance data are:

- Measuring the 'magnitude' of the problem
- Description of pattern and trend
  - Analysis by 'person' characteristics (age, gender, ethnic groups, marital status, occupation, etc)
  - Analysis by 'place' characteristics (international vs intra-country comparison, local disease distribution, etc)
  - Analysis by 'time' characteristics (time onset, secular trend, seasonal pattern, point epidemic, etc (Rojanaworarit 2015).

16.3. DATA INTERPRETATION

Surveillance data interpretation is crucial as it leads to further considerations of whether appropriate public health action is required. The key point at this step is the accurate identification of higher-than-usual disease occurrences that need additional prompt public health action to timely control the disease. Many factors may influence the increase in observed disease occurrence. These include:

- The larger size of the population being investigated
- Altered disease screening campaign
- Improved diagnostic methods
- Better reporting system

Before concluding whether the disease occurrence has increased or not, the above-mentioned factors should be rationally ruled out. Commonly used epidemiological measures like incidence proportion, incidence rate, point prevalence, period prevalence, and case fatality rate must be carefully selected. The interpretation of these measures should be scientifically sound as they are used in the generation of different implications (Rojanaworarit 2015).

16.4. DATA DISSEMINATION

At this stage, the 'who needs to know?' question is addressed. Authoritative health personnel are the ones who must be informed since they require this information for deciding whether to act or not. There are two patterns for the dissemination of data; 'down-top' and 'top-down'. Both of these approaches are important in planning the control and prevention of disease as well as for providing feedback and improving the reporting of surveillance data at the local level (Rojanaworarit 2015).

17. RESEARCH CHALLENGES AND FUTURE DIRECTIONS

Various complications limit the complete eradication of bovine TB. These include: (1) breakdowns due to an anergic carrier or breach in the herd security, (2) complications in administrative and public relations due to non-visible reactor (NVL), (3) large herds, (4) the presence of wildlife reservoirs, (5) lack of diagnostic tests to differentiate between vaccinated and infected animals. International organizations highly endorsed the One-Health approach for TB to address these challenges at the human-animal interface (Borham et al. 2022).

The livestock sector majorly impacts the economy of developing countries by alleviating malnutrition and poverty in rural areas. The rise in the human population has increased the demand for animal-origin foods that are met with the intensification of animal production leading to a higher risk of zoonotic diseases in humans (Berrada 2006). Cattle and other food animals are reservoirs of several diseases of public health importance. Bovine tuberculosis has great potential to infect humans and has thus been considered a neglected zoonotic disease by WHO. Developed countries have greatly reduced the incidence of bTB through efficient eradication and milk pasteurization programs. However, this incidence is still high in developing countries where human TB cases are also on the rise. Several international forums like WHO, World Organization for Animal Health (OIE), and the Food and Agricultural Organization (FAO) have stressed to adoption of strong disease surveillance programs for the prevention and control of both human and bovine tuberculosis in developing and developed countries. There is a need for strong intersectoral collaboration between veterinary and medical professionals to combat zoonotic bTB (Rahman et al. 2015).

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Paratuberculosis; A Potential Zoonosis

06

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ABSTRACT

Paratuberculosis, commonly known as Johne's disease (Yo'-nees), is primarily a disease of ruminants such as cattle, sheep and goats. It is a chronic infectious disease. The name of the disease is derived from the scientist's name who discovered it in 1985 named Johne's along with his colleague Frothingham. The disease is associated with Mycobacterium avium subspecies paratuberculosis (abbreviated as MAP) is an obligate intracellular organism. This bacterium mainly damages the intestines. MAP is a member of Mycobacteriaceae family which also includes M. tuberculosis and M. leprae, causative agents of tuberculosis and leprosy, respectively. Paratuberculosis is also known as "Silent slayer" in USA. The prevalence of this disease is continuously increasing every year due to lack of proper disease control programmes. The reasons behind could be lack of awareness in public as well as the lack of concern shown by respective governmental disease control authorities. Paratuberculosis is chronic in nature due to which there is no accurate treatment for it. The transmission source for this disease is the infected animal. There is an ongoing uncertainty regarding its transmission to human as various researches have produced contradicting results. Its prevalence rate varies in different regions of the world however; it is found most commonly in the countries having intense livestock farming. Crohn's disease (CD) is the term used for the disease in human where the clinical symptoms are similar to those seen in John's disease in animals. MAP is considered the primary cause of CD along with other associating factors however it is yet not confirmed. Primarily, the consumption of dairy products, obtained from infected animals, is considered its mode of its transmission to human. Actual burden of CD is yet unknown as it goes unreported in most of the countries due to lack of awareness among the people & lack of sufficient funding for research purpose. Interdisciplinary research collaborations are necessary to cover the knowledge gaps regarding paratuberculosis, highlighting the significance of surveillance and preventive measures to reduce possible health hazards to people.

Key words: Johne's Disease, Emergent Zoonosis, Threat, Public Health, Crohn's disease.

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1. INTRODUCTION

Paratuberculosis, also known as Johne's disease (Yo'-nees), is a chronic infectious disease that primarily affects ruminant animals, such as cattle, sheep, and goats. History of Johne's disease goes back to the end of 19th century when couple of scientists named; Johne's and Frothingham (Whittington et al. 2019), described it for the first time in 1985. It is caused by Mycobacterium avium subspecies paratuberculosis (abbreviated as MAP) that mainly damages the intestines. MAP is a member of Mycobacteriaceae family which also includes M. tuberculosis and M. leprae, causative agents of tuberculosis and leprosy respectively. The organism is capable of surviving in the environment for a long time posing a continuous threat to all the susceptible species (Hovde and Moum 2012). Since it is an obligate intracellular organism, it always requires a cell named as; Macrophage, within the susceptible host, for its multiplication.

'Silent slayer' is the term used for this disease in USA (Whittington et al. 2019). Its prevalence is increasing globally with every passing year, but still many of the countries, including both developed as well as underdeveloped, lack proper disease control programmes. It has been reported from many countries of Africa, but these countries don't have sufficient information regarding its control and prevention. The reasons behind this might be lack of awareness in public as well as the lack of concern shown by respective governmental bodies. Ultimately, many of the positive cases go unreported (Whittington et al. 2019). Ruminants are the most commonly affected animal species, but it can also affect non-ruminant wildlife species (Collins 2003).

John's disease is much problematic due to its chronic nature and lack of accurate treatment till date. An infected animal is considered primary source of its transmission. Newly borne animal are at higher risk of getting infection due to their weak immune system while fecal-oral route being the most common route of its transmission (Sweeney 2011). By the time, clinical symptoms of disease in animal become noticeable to the farmer, if clinical stage of disease has already reached. It is also thought that when a single positive case is identified in herd, almost 4-8 asymptomatic carriers might have developed, in subclinical form of disease, continuously shedding the bacteria in milk and faeces. This characteristic of disease is veiled danger for the farmer. Despite the exposure to infection at early stage of life, it takes years to exhibit clinical symptoms, and in the meanwhile the infected animal will continuously contaminate the environment of farm putting all the healthy animals at higher risk (Tanaka et al. 2005).

While paratuberculosis has been extensively studied in animals, there is growing evidence suggesting its potential as a zoonotic disease, meaning it can be transmitted from animals to humans (Hovde and Moum 2012). Crohn's disease (CD) is a cataclysmic, chronic and granuloma forming disease of humans affecting their large intestine particularly ileum and colon. This is why it is also known as Crohn's ileitis as well as Crohn's Colitis and regional ileitis (Windsor 2014). According to a report, 0.8 million people in North America are affected by it annually. Its clinical signs and pathological lesions are almost similar to Johne's disease in animals but still their relationship is dubious. Various factors are responsible for the onset of this disease including genetic factors, environmental factors, and impulsive immune response (Uzoigwe et al. 2007). This chapter aims to explore the epidemiology, pathogen biology, host range & transmission, clinical

manifestations, diagnostic challenges, and prevention and control measures of paratuberculosis as a potential zoonosis.

2. EPIDEMIOLOGY

Paratuberculosis has been reported worldwide, with varying prevalence rates in different regions. It is most commonly found in countries with intensive livestock production systems, such as the United States, Europe, Australia, and New Zealand (Windsor 2014). The prevalence of this disease has been reported to increase from 21.6% to 91% in dairy herds of USA just within the period of 10 years (1996-2007). Another report estimates that almost 40% of dairy herds in USA are affected with MAP causing annual losses of more than 1.5 billion dollars to the dairy industry. Most common route of transmission is feco-oral route however the transmission may also occur via other ways like consumption of contaminated milk, vertically from infected sire to dam or from infected dam to fetus. The organism can survive in the body of host for years without causing clinical signs while host keeps shedding the organism in body secretions acting as a silent killer. This organism can survive in the environment either in its normal form (cell wall containing form) or become dormant and survive in vegetative form or gets modified into spore like form. It may even survive pasteurization thus making the milk potential source of transmission to human beings (Uzoigwe et al. 2007).

CD is prevalent worldwide. Its reported prevalence in Canada is found to be 161-319 cases per 100,000 populations. In North America, it is reported to affect 0.4-0.6 million people, while the incidence rate is found to be 6 per 100,000 people every year that is almost equal to Europe. According to reports, it is becoming more common problem in developed parts of the world. Its onset can occur at any age but the most commonly affected age group is 15-30 years (Hovde and Moum 2012). Another study revealed that the people in closed contact with the affected ones are at 3-20 times higher risk of acquiring the disease (Hovde and Moum 2012).

This disease causes significant economic losses in the agricultural industry due to decreased milk production, weight loss, and increased mortality rates in infected animals. Despite all these facts and figures, only few of the countries have proper disease control program while most of the countries don't have even research plans for control of this disease (Uzoigwe et al. 2007). However, the potential public health implications of paratuberculosis have only recently gained attention. Exact burden in humans is still unknown due to lack of awareness in general public, insufficient research funding and under reporting.

2.1. PATHOGEN BIOLOGY

MAP belongs to group known as Mycobacterium avium intracellular complex (MAIC). The majority of its strains differ from other mycobacteria as it requires an iron chelating molecule, mycobactin, from outside sources for its multiplication (Tortoli 2003). It multiplies within macrophages, interrupting the phagocytosis. In its defense, body further attempts to kill the pathogen that ultimately results in granuloma formation. This organism prefers to multiply in cells having abundant iron, pyruvate and calcium in them (De Juan et al. 2006). These bacteria have mycolic acid in their cell wall and are acid fast bacteria due to which they can't be stained via gram staining method and require specific 'acid fast

staining' method (Tortoli 2003). The presence of this substance in cell wall also makes it very difficult to break the wall interrupting the release of DNA required for conducting laboratory tests. Microscopic identification of MAP from human tissue is almost impossible. However, culture method and molecular techniques like PCR can be used for its identification. It survives within human tissue cells and in the form of spheroplast that is cell wall deficient form, due to which it can't attain Ziehl-Neelsen (ZN) stain (Chiodini et al. 1986). Therefore, its confirmation by standard light microscopy using ZN staining method becomes almost impossible. Culture growth of MAP is sluggish taking more than 3 months just for its colonies to appear while culture of samples taken from human may take more time, even a year or more to get its colonies appear.

As it has the ability to go into dormant phase, it survives for years in the environment (Whittington et al. 2004). In addition to this, humidity and acidic soil supports its survival in the environment. Therefore, it is suggested to add lime to soil of farm and its premises before introducing healthy animals. Being a meticulous creature, it requires special media for its growth on which it grows very slowly even for one year to get primary isolation.

2.2. HOST RANGE AND MODES OF TRANSMISSION

Wide range of animal species is reported to be infected with this organism including both the domestic as well as wild animals, and this host range may also extend further. MAP has been isolated from humans suffering from Crohn's disease, AIDs, and other immuno-compromising diseases, indicating its zoonotic significance (Tortoli 2003). Highest number of cases has been reported from Europe, America and Asia that may be probably due to the inadequate reporting system in other regions. The exact route of transmission of MAP to humans remains uncertain, but several potential pathways have been proposed. Direct contact with infected animals, ingestion of contaminated food or water, and inhalation of contaminated dust or aerosols are considered the main routes of transmission (Chiodini et al. 1986). Young animals particularly calves are considered to be at higher risk of acquiring infection probably due to their open gut, presence of Peyer's patches; a specialized lymphoid tissue, believed to accept maternal antibodies. The infection due to MAP is dose dependent; thereby aged animals are susceptible to infection with higher dose. Along with this, onset of disease is also affected by the dose; higher the dose, speedier will be the onset of disease (Weber et al. 2010). Additionally, consumption of unpasteurized milk and dairy products derived from infected animals has been suggested as a potential source of human infection. Regardless of the route of entry, host's intestinal tissue acts as a suitable habitat for this organism. Once entered into enterocytes, the dendritic cells or macrophages carry it to the nearby lymph nodes where it suppresses genes to bypass intestinal barriers. Along with this, it also decreases or quits the immune response against it.

The organism is shed in the faeces of infected animal thereby acting as a continuous source of contamination in the environment. The infected cattle are classified on the basis of the quantity of pathogen being shed in faeces. The quantity is less than 300 CFU per gram in light shedders, 300 to 3000 CFU per gram faeces in moderate shedders while heavy shedders, shed more than 3000 CFU per gram. Another type has been added named as super shedders who can shed more than 10000 CFU per gram. The contaminated pastures can also contaminate the rivers when water efflux into river due

to rainfall acting as a potential threat for both humans and animals (Pickup et al. 2006). The ability of MAP to survive in the environment for extended periods further complicates the transmission dynamics of this bacterium.

2.3. STAGES OF PARATUBERCULOSIS

Tiwari and his fellows in 2006, suggested that MAP infection has 4 phases; silent, subclinical, clinical and advanced clinical. During the first 2 stages, no clinical signs appear in infected animal due to which these 2 has been considered one in the recent classification of disease. Detail of all 4 states is as following: In silent stage, neither the infected animal shed pathogen nor is the development of detectable immune response so at this stage, pathogen detection can only be done via PCR, tissue culture or histopathology (Whittington et al. 2019). During the subclinical stage, diagnosis can be done via different tests used for pathogen detection but discontinuous pathogen shedding, that also in low quantity, leads to higher probability of getting false negative results and there is significant decrease in milk production during this stage. In the clinical stage of disease, feed intake of animal is normal or even increased but its body condition goes down, and also suffers from diarrhoea. At this stage, immune response detecting diagnostic tests are considered to be trustworthy. Emaciated animal with shooting diarrhoea is the clinical manifestation of entering the final stage of disease; the advanced clinical stage. There is development of condition called 'bottle jaw' due to decreased oncotic pressure as a result of severe protein loss (Tiwari et al. 2006).
Paratuberculosis can be explained very well by 'iceberg phenomenon'; which state that for a single advanced clinical case, there must be 1-2 clinical cases, 4-8 subclinical cases while 10-14 silent stage cases. Shedding of pathogen has been described by in 2 ways; Progressive and non-progressive. In the non-progressive shedding pattern, there is irregular shedding and in low number while in progressive shedding, infected animal shed pathogen continuously, in too large number (Behr et al. 2020).

3. ZOONOTIC POTENTIAL OF PARATUBERCULOSIS

A worldwide movement called one health approach is addressing the impact of zoonotic diseases occurring at animal, human, & environment interface (Vandersmissen and Welburn 2014). One health integrated approach got recognition in 2010 as an agreement among World Health Organization (WHO), World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) to ''cope up with health risk issues occurring at the human-animal-environment-interface'' (FAO–OIE–WHO2021). Some Zoonotic diseases got significant attention from the international community while other zoonotic diseases which are less common were not given due attention. The WHO designated these diseases as neglected zoonotic diseases (NZD), one of them is MAP or Johne's disease. The contribution of Johne's disease zoonosis to Crohn's disease has not been established authentically, and needs to be confirmed because it is still controversial. Moreover, MAP is now associated with many inflammatory and autoimmune diseases of humans i.e., granuloma formation in Crohn's disease, Sarcoidosis Blau syndrome, autoimmune type 1 diabetes (T1D), multiple sclerosis autoimmune thyroiditis, lupus, rheumatoid arthritis and possibly, Sjogren's syndrome

(Cossu et al. 2017; Ekundayo et al. 2022). The causal linkage of following diseases with MAP is further elaborated: Crohn's Disease, rheumatoid arthritis, T1D, multiple sclerosis.

3.1. MAP & CROHN'S DISEASE

The chronic granulomatous inflammation of intestines of human beings is called Crohn's disease; MAP was hypothesized to be the causative agent of Crohn's disease back in 1913 when clinical symptoms and post-mortem lesions of this disease in human were found similar to Johne's disease in cattle. CD is believed to have multiple etiological factors like genetic susceptibility, environmental factors (life style, pathogen etc.), and each playing particular role in causing disease. Initially many viruses and bacteria were supposed to be the possible cause, but later on an association between the Paratuberculosis caused by Mycobacterium avium subspecies Paratuberculosis and Crohn disease was established when Thomas K. Daziel, a surgeon in 1901 while operating a patient with chronic inflammation of the intestine observed lesions similar to that of Johne's disease, he was already aware of (Daziel et al. 1913). He collected data from other cases and published his findings in the British Journal in 1913 summarizing that the histological findings of the Crohn's disease in human were similar to that of Johne's disease (Para tuberculosis) in animals caused by MAP, thus suggesting that the disease may be the same. Few other studies have also suggested that four out of six criteria for disease's etiology have been met by MAP in relation to CD. Various studies also suggested the link of MAP with Idiopathic Bowel Disease (IIBD) that includes CD and Ulcerative colitis (UC). The association was confirmed later on when MAP was isolated from Crohn's disease patients in Australia, United States, The Netherlands, and France. The three groups of scientists working with the culture of M. Paratuberculosis isolated MAP from 20, 33, & 38% of humans suffering from Crohn's disease. Only 0.8% (1 in 121) MAP was isolated from healthy humans kept as controls. These isolates were of bovine origin as were found genetically similar to strains isolated from cattle. Confirmation of MAP as the causative agent of IIBD is still controversial but once it is confirmed, it would be a major public health issue in the years to come. Approximately 1-2 million people, across the globe, are believed to be affected by CD. Paratuberculosis in humans, also known as human paratuberculosis or Crohn's disease, shares certain clinical similarities with Crohn's disease, a type of inflammatory bowel disease (IBD). Chronic diarrhoea, visceral sensitization, fatigue, weight loss, and fever are the major symptoms. However, the diagnostic criteria for human paratuberculosis are not well-established, leading to challenges in accurately diagnosing and differentiating it from other gastrointestinal disorders. Further research is needed to establish a clear link between MAP infection and human Paratuberculosis (Grant 2005).

3.2. MAP AND TYPE 1 DIABETES MELLITUS

A chronic autoimmune disease known as type 1 Diabetes (T1D) is related with cow milk exposer in early life. To check the association of cow milk with T1D a study was conducted in 15 countries at various 78 centers and it was established that Mycobacterium avium subsp. paratuberculosis present in the milk triggers the disease, thus establishing a latent MAP infection in infants due to shared genomic risk for both mycobacterial infections and T1D. Further it was studied that MAP's immunodominant heat shock protein 65 (HSP65)

cross reacts with glutamic acid decarboxylase (GAD) of pancreatic origin due to its molecular mimicry resulting in the production of anti-GAD antibodies which cause the immune mediated destruction of pancreatic cells which produce insulin. It was also postulated that MAP may also serve as environmental trigger for T1D in the population genetically at risk, the same was supported by three proposals: shared genetic susceptibilities between mycobacterial infection and T1D, epitopic homologies of pancreatic glutamic acid decarboxylase and HSP65 protein of MAP, and epidemiological correlation with the early age exposure to cow milk. Later on, Sechi and associates on the island of Sardinia which has second highest incidence of T1D in the world also established relationship between T1D and MAP. Map was reported only in T1D patients and not in T2D patients (Sechi et al. 2008). Additionally, MAP peptides homologues to pancreatic proteins were also identified and proved that immune response to these MAP peptides cross react with the classical islet cell antibodies (Niegowska et al. 2016). More than a dozen studies published in various articles implicated MAP in T1D, whereas only one article of Indian origin stated that MAP could not be found in the blood of T1D patient. Interestingly by BCG vaccination of T1D individuals, followed by a booster dose after one month interval, blood sugar level was controlled as BCG provides cross protection against Paratuberculosis. The normal blood sugars level was observed up to eight years after the vaccination (Ku¨htreiber and Faustman 2019). It has been further established that BCG vaccination is quite effective against MAP as it is effective against tuberculosis and non-tuberculosis bacteria (Dow 2018).

3.3. MAP AND MULTIPLE SCLEROSIS

The link between MAP and multiple sclerosis (MS) has been found in various studies in Italy. Similarly in Japan it has been observed that MAP is the risk factor or the microbial trigger of the MS in patients having genetic susceptibility to mycobacterium. Various other studies have also linked MAP along with other microbial triggers of multiple sclerosis in the populations of Italy and Japan (Ekundayo et al. 2022). Anti-myelin basic protein is used to detect Antigenic peptides of MAP and Epstein-Barr virus (EBV) in MS individuals (Mameli et al. 2014). On the other side, spinal fluid of MS individuals was found positive for anti-MAP antibodies (Yokoyama et al. 2018). In 2022 a significant media response was seen when the issue of a journal science published a report revealing the prevalence of multiple sclerosis in association with EPV on the basis of large database (Bjornevik et al. 2022). However, even based on huge data, the revealing was not novel as already it was established that EBV, MAP and human endogenous retroviruses (HERVs) are the microbial triggering agents of the multiple sclerosis (Frau et al. 2021).

3.4. MAP AND RHEUMATOID ARTHRITIS

The uptake and onward survival of MAP in the human cells is favored by the cholesterol enrichment and MAP has the ability to manipulate lipid metabolism process of the host and accumulate cholesterol in the macrophages to enhance infection just like other pathogenic mycobacteria (Johansen et al. 2019). This sort of relationship between host cholesterol level and reaction of MAP is observed in rheumatoid arthritis (RA) along with other diseases like autoimmune diabetes and multiple sclerosis. MAP has been associated with RA as tyrosine phosphatase A (PtpA) and kinase G (PknG) the virulence

factors of MAP necessary for its survival in macrophages are significantly found in RA patients supporting the hypothesis that MAP is involved in the RA pathogenesis. Clinically RA is referring to erosive damage of joints along with cellular and humoral responses to various self-peptides like Interferon regulatory factor 5 (IRF5). The MAP_4027 antigen of MAP also targets IRF5 thus supporting the hypothesis that MAP infections trigger a self-peptide of RA immune response and which involve in disease pathogenesis (Bo et al. 2018). A recent study also established the possible role of many microbial antigens in the triggering and pathogenesis of various diseases. All the studied antigens showed humoral response in RA patients as compared to controls (Jasemi et al. 2021). HERVs are also linked with many autoimmune diseases (Balada et al. 2010).

4. TRANSMISSION OF MAP FROM CATTLE TO HUMANS

4.1. MAP IN THE ENVIRONMENT

MAP is a very resilient microorganism and survives in soil and water up to 120 weeks after its shedding by the infected animals in the environment (Garvey 2020). Map has been detected in grazing areas as well as in runoff water continuing to rivers and municipal waters. These water sources serve as reservoir of MAP as it survives in the biofilm (Botsaris et al. 2016). The efficiency of chlorination or sand filtration methods to inactivate or remove MAP from contaminated water has not been fully investigated. There is only a little information available regarding the effect of chlorination on MAP present in water destined for human's consumption according to which it can only reduce two $\log^{10}$ the numbers of viable MAP in contaminated water (Whan et al. 2001). Cattle manure in different forms is used as fertilizer to agricultural land thus heavily contaminating the grass if the manure is from MAP infected animals. MAP persists on depopulated farms and in the roots & aerial parts of the plants of the pasture plots contaminated with MAP (Kaevska et al. 2014). MAP is also found in aerosol form and inhalation is another possible transmission route of MAP to animals and humans (Rhodes et al. 2014). Due to the inadequacy of MAP diagnostic testing along with latent nature of infection in apparently healthy animals, the farmers are reluctant for the routine testing which result in continuous MAP shedding in the environment, trade of asymptomatically MAP infected animals and delayed culling of infected thus contaminating the environment with MAP (Garvey 2020).

4.2. MAP IN FOOD

Mycobacterium can be found in various food products, like milk and beef, which ultimately act as a potential source of its transmission. Their detail is given below;

4.2.1. MILK AND DAIRY PRODUCTS

Milk and other milk products are the major source of MAP infection in humans. MAP has been detected from yogurt, cheese and muscle meat (Dow and Alvarez 2022). Map has been recovered from the milk of cows both sub-clinically and clinically infected with Johne's disease. It may also be recovered from the milk of other ruminants such as goats and sheep affected by Johne's disease. MAP enters milk directly from within the udder

or by contamination from different sources while milking. The intake of Map infected colostrum or milk is considered as major source of transmission of Johne's disease from cow to calf in an infected herd, thus it is recommended; to control the spread of the disease the calves should not be fed with map infected milk (Collins 2003). Likewise, mostly human's intake cow milk at a very young age, thus cow milk has been considered as a potential source of transmission of the MAP from cattle to humans. As mostly cow milk is pasteurized before its use, many studies have been conducted to check the efficacy of pasteurization and various pasteurization approaches regarding MAP, many of these studies depicted that MAP is more heat resistant as compared to other mycobacteria & low numbers of viable MAP survives milk pasteurization process (McDonald et al. 2003). The factors regarding the survival have not yet been fully understood and it is supposed that the presence of higher numbers of MAP in the forms of large clumps of cells in the milk may lead to the survival of some MAP during pasteurization process.

4.2.2. BEEF

Studies have revealed that Johne's disease affects both beef and dairy cattle so; meat could also be a possible source for the transmission of MAP to human beings. It has been observed that beef of old cattle used to prepare minced meat for human consumption may be a source of MAP infection (Manning 2001). In animals culled due to clinical signs of Johne's disease, MAP infection is thought to be disseminated in the animal tissues including muscles, blood and ileocecal lymph nodes (Grant 2005).

5. DIAGNOSTIC TESTS AND THEIR CHALLENGES

To identify the animal infected with MAP is necessary in order to understand and analyse the problems due to paratuberculosis. It can be done in 2 ways; 1st one is the identification of the pathogen itself that can be done by PCR or culture method, while for other way, ELISA, agar gel immunodiffusion or complement fixation tests can be used to detect immune response. Using pooled samples for diagnostic tests is recommended due to high cost of tests (Collins 2003). True prevalence of this disease is very challenging to be analyzed as there is no availability of standard diagnostic test with 100% sensitivity (Nielsen and Toft 2008). The reported sensitivity of ELISA using milk and serum samples is found to be 25-35% only while the sensitivity of culture test using faecal samples being slightly greater; 55-65%. Using milk samples within 1st 2 weeks of lactation or after 45 weeks can be a way to get ELISA results with better sensitivity (Lombard et al. 2013). Prevalence of paratuberculosis in herd can also be assessed by using environmental samples and performing their culture test or PCR. For this, it is recommended to collect samples from manure storage, particularly pooled samples, in order to get higher sensitivity as well as specificity (Lombard et al. 2013).

Hence, one of the major challenges in diagnosing paratuberculosis as a zoonotic disease is the lack of standardized and validated diagnostic tests. The current diagnostic tests used in animals, such as faecal culture and serological assays, are not suitable for human use due to their limited sensitivity and specificity. Developing accurate and reliable diagnostic methods for detecting MAP in humans is crucial for identifying infected individuals, understanding the true prevalence of human paratuberculosis, and implementing appropriate control measures.

6. PREVENTION AND CONTROL MEASURES

MAP infection is not only causing huge economic losses in dairy industry and acting as a serious public health concern, it also hinders the trade of animals as well as animal products across the borders. These three are major reasons why its control and prevention need immediate and serious attention. By the time, organism is identified in herd; significant damage has already been occurred in the form of its transmission, probably to all the nearby animals (Whittington et al. 2001). Preventing and controlling paratuberculosis in both animals and humans requires a multi-faceted approach. For animals, measures such as culling infected animals, improving herd management practices, and implementing strict biosecurity measures can help reduce the spread of MAP. In humans, promoting awareness about the potential risks associated with paratuberculosis and adopting hygienic practices, such as pasteurization of milk and dairy products, can minimize the chances of infection. Additionally, further research is needed to develop effective vaccines and therapies for both animals and humans.

7. CONCLUSION

Paratuberculosis, is primarily a disease of ruminants, also has the zoonotic potential to be transmitted to humans. Various aspects related to the epidemiology, pathogenesis, clinical manifestations, transmission & diagnostic challenges along with prevention and control measures to minimize the risk of Paratuberculosis zoonosis have been elaborated in this chapter. As the evidence linking MAP infection to human paratuberculosis is still evolving, it is essential to focus more on rendering awareness about the potential risks related with Paratuberculosis zoonosis and encourage further research and development to better understand and manage this complex disease for the benefit of both animal and human health. Continued collaboration between veterinary and human medical professionals is crucial in addressing the challenges associated with Paratuberculosis zoonosis.

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Burkholderia (*Mallei and Pseudomallei*) Related Zoonosis Drastic Zoonotic and Biological Warfare Potential

07

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ABSTRACT

The Burkholderia genus consists of more than 20 species. The important pathogens in this group are B. Mallei and B. Pseudomallei. Burkholderia mallei and pseudomallei are rod-shaped, aerobic, non-spore-forming cocco-bacilli bacteria, which are involved in highly contagious diseases of equids, Glander and Melioidosis respectively. These pathogens are one of the main cause of financial and performance losses in developing nations. Their incubation period varies from days to weeks, or even months in some cases. They are extremely important pathogens if seen as bio-terror due to their highly zoonotic nature. B. Mallei has already been used as a potential biological terror agent in WW-II on both sides, leading to mass killing of horses, mules and donkeys employed in war as well as humans due to zoonoses. They both show typical respiratory and cutaneous signs making equids difficult to ride, or to be used for draught purposes. The diagnosis is based upon the signs and symptoms as well as ELISA, PCR and culture analysis. The Farcy Act was issued in 1899 to deal with Glanders effected equids, states that affected animals should be killed and disposed of properly. For prevention, identification of positive animals and culling is extremely important. Live vaccines for Glanders are available but have no satisfactory results and no vaccine is available for Melioidosis, so all prevention and control rely only on preventive measures. Treatment is quite tough as both bacteria are resistant to a number of antibiotics, making them more important as zoonotic agents, and is possible by a number of antibiotics, including Ceftazidime, Carbapenems, Amoxicillin-Clavulanic acid, Trimethoprim-Sulphadiazine, Danofloxacin, Norfloxacin, and Chloramphenicol and doxycycline.

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1. INTRODUCTION

Glanders is a highly contagious zoonotic disease, mainly present in horses, donkeys, and mules (Rahimabadi et al. 2023). It can cause infection in other animals like cats, dogs, and goats, and also cause disease in humans as well. Glanders is caused by Burkholderia mallei, a gram-negative bacterium (Elshafie and Camele 2021) and is a highly adapted pathogen to harsh environmental conditions. This bacterium can survive within the host cells and also replicate in it. There are different factors that help to survive the bacterium within cell, one being capsule formation, which prevents immune cells to kill the pathogenic bacterium. The route of transmission is primarily through direct contact with the animal and its secretions (Norris et al. 2018). The bacterium can enter the body also by some other routes like ingestion, inhalation, and abrasion of the skin. Mainly it is confined to the respiratory area, but later it can spread to the other parts of the body (Pinho et al. 2023). It is an ancient disease known for centuries. Hippocrates considers it as serious disease of equines.

In 350 BC it was given name 'melis'. In 1822, its etiology was described by isolating it from horse liver and spleen (Schadewaldt 1975).

After the discovery it is classified as Pfeifferella mallei, Loefflerella mallei, Actinobacillus mallei Malleomyces mallei, Mycobacterium mallei, Corynebacterium mallei, Bacillus mallei and Pseudomonas mallei. Now genus is classified as Burkholderia due to 16S rRNA gene typing, cellular lipid and fatty acid composition, DNA-DNA homology values and phenotypic characteristics (Whitlock et al. 2007). Glanders in Asia, America, Europe and Africa causes major morbidity and mortality in 19th and 20th century.

2. CHARACTERISTICS OF *B. MALLEI*

Genus Burkholderia comprises of more than 20 species mostly isolated from water or soil. The important pathogens in this group are *B. mallei* and *B. pseudomallei*. *Burkholderia mallei* is an anaerobic, non-spore-forming, gram-negative coccobacillus. *B. mallei* are non-motile (due to the absence of polar flagella present in other Burkholderia species). *B. mallei* are a non-fermenting bacterium and grow readily on MacConkey agar media (Gilligan et al. 2003). The specimen collection and transport methods, being used normally, are sufficient for recovering burkholderia species due to their capability to survive in hostile environments. Isolation is easier in the field due to survivability in hostile environments and can be isolated by culture or antigen-antibody basis (basis of ELISA in Glanders). *B. mallei*, isolated in 1944 from human postmortem, sequencing revealed two circular chromosomes (Nierman et al. 2004). More than 5,000 protein-encoding open reading frames (ORFs) have been recognized in its DNA.*B. mallei* sis capable to survives in 30% Normal Human Saline (NHS), and serum-sensitive strains lack Lipopolysaccharides. The capacity of *B. mallei* to grow in 30% NHS was assessed, at 2, 4, 8, and 18 hours, with a serum bactericidal assay. The bacterium survived in the presence of 30% NHS for 18 hours (DeShazer 2004). *B. mallei* shares lots of genes with *B. pseudomallei*, and both bacteria have almost same allelic profile (Godoy et al. 2003).

3. PATHOPHYSIOLOGY IN HOST

Pathophysiology of glanders in the host is shown in Fig. 1.

4. ROUTES OF INFECTION

The bacterial invasion of the oral, conjunctival, and nasal mucosa occurs by direct contact or through abrasions, deep lung deposits, and inhalation. The neck, arms, face, and head are the areas of exposed skin most frequently affected by the occupational exposures previously stated. Although penetrations or

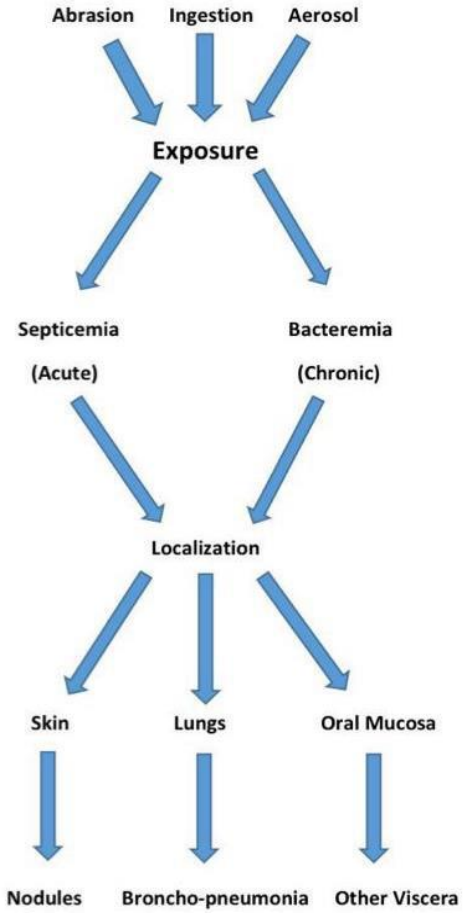

Fig. 1: Pathophysiology of Glanders In Host.

wounds during the anticipated exposure were not discovered, it is considered that *B. mallei* cannot enter normal intact skin (Pal et al. 2022). In fact, the major illnesses picked up in laboratories are not linked to injuries or memories of damage.

5. INCUBATION PERIOD

The incubation period for the acute form of the disease is typically 1–14 days, whereas the incubation period for the chronic form of the disease can last up to 12 weeks or more. Within one to five days of contact, a localized infection usually develops and may be distinguished by swelling of the afflicted area and weeping discharge. Before symptoms manifest, acute lung infection may occur between 10-14 days of incubation. Septicemia may occur right after exposure or up to fourteen days after the exposure. When left untreated, pneumonia develops quickly and is virtually always fatal between 10 and 30 days (Fhogartaigh et al. 2015).

6. SIGNS AND SYMPTOMS

Animals with acute Glanders typically exhibit the following symptoms (Fig. 2) after an incubation period of three to twenty days and death occurs within a few days (Howe et al. 1947).
Numerous Glanders types, such as chronic, disseminated, pulmonary, and septicemia, have been reported (Van Zandt et al. 2013).

7. MUCOSAL INVOLVEMENT

Photophobia and profuse lacrimation are symptoms of B. mallei infection of the eye and conjunctiva. Nasal involvement is characterized by swelling and inflammation of the nose and sinusitis followed by nasal discharge. Local lymph nodes may become irritated, and the face may enlarge. Bronchitis is accompanied by coughing and mucopurulent discharge if the infection spreads to the lower respiratory tract. The first few days after infection are usually characterized by moderate-to-low fever, excruciating headaches, and chills with or without rigors, in noon or evening. However, these symptoms could be very severe and continue through treatment. (Pal et al. 2023).

8. CUTANEOUS INVOLVEMENT

Papular lesions can appear anywhere on the body if the infection progresses chronically. An inflammatory reaction involving pain and swelling usually occurs after B. mallei penetration through an abrasion. In such circumstances, a Glanders node may initially appear as a blister, evolving into an ulcer, which would bleed profusely (Rathish et al. 2022). A localized infection, with a discharge, usually appears at the entering point. Inflammation can travel along local lymphatic, resulting in lymphangitis and several foci of suppuration. Escalating irritation and inflammation of the lymphatic smooth muscles are effects of endotoxins of B. mallei (Pal et al. 2022).

9. PULMONARY INVOLVEMENT

Pleural effusion, pleuritis, pulmonary abscess, and pneumonia are the typical symptoms of a lung infection. Dyspnea, cough, chest pain, and mucus in the sputum are all indications of lung infection. Respiratory infections are frequently accompanied by nonspecific signs and symptoms such as chills, dyspnea, discharge, myalgia, fever (typically above 102°F), headache, lymphangitis, fatigue, pleuritic chest discomfort, cough, tachypnea, sore throat, and gastrointestinal indications (Estes 2010). The onset of various symptoms can take up to two or three weeks. Rigors, weight loss, myalgia, mucosal eruptions, night sweats, dizziness, severe headaches, tachycardia, and nausea are examples of nonspecific symptoms that are frequently present and may point to widespread infection. (Khan et al. 2013).

10. DISSEMINATION OF INFECTION

Localized mucosal or cutaneous infection causes septicemia and the colonization of internal organs such as the liver, spleen, lungs, and the emergence of abscess. Septic shock and high mortality are frequently linked to these diseases.

11. ZOONOTIC ASPECTS OF BURKHOLDERIA MALLEI

B. mallei is an agent which requires a host animal to survive. Members of the family Equidae (donkeys, horses and mules) are the primary natural reservoir for B. mallei. Acute form of the infection usually occurs in mules and donkeys with respiratory problems and fever, whereas horses typically exhibit a more chronic course, especially in endemic areas, and may survive for years. Interestingly, the term Glanders refers to lymphangitis and lymphadenopathy in horses. The condition is known as Farcy when it has cutaneous symptoms. Humans, sometimes felids, wolves, dogs, bears, and camels are susceptible to illnesses, usually to less extent. Other carnivores may get the disease by ingesting the infected meat, but pigs and cattle are immune (Khan et al. 2013).

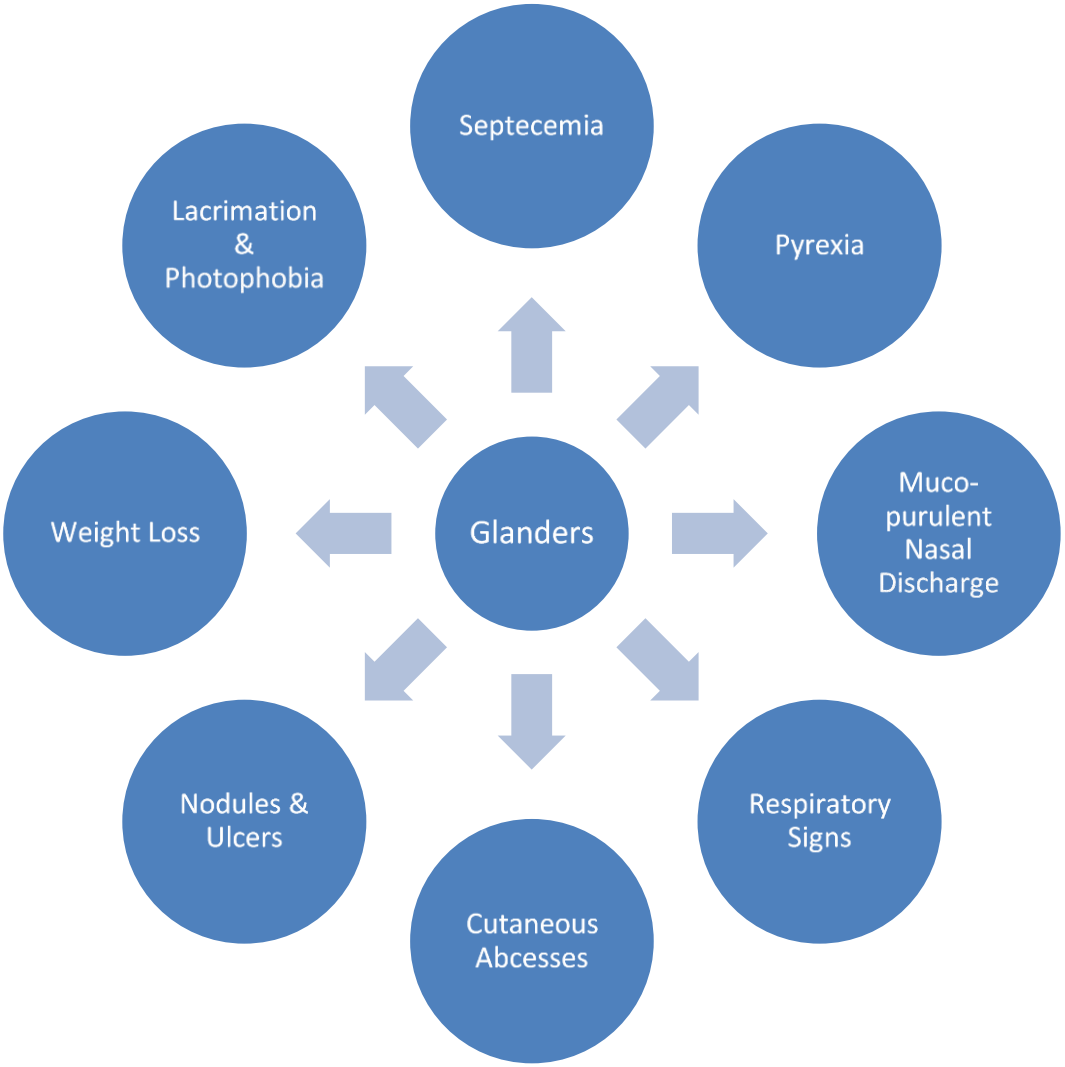

Fig. 2: Mostly observed signs and symptoms in humans in response of zoonotic infection.

The integument, gastrointestinal system, and mucous membranes are all routes for Burkholderia mallei to infect its host (Wernery et al. 2011). The frequency of infections in solipeds and other animals, such as zoo predators (tigers and lions) and camels, has gradually risen over the past two decades, and glanders can be considered a re-emerging illness. B. mallei is thought to be a potential bioterrorism agent due to the lethal and invasive property of the infection in humans (Wittig et al. 2006). The animals most likely to have acute Glanders are mules and donkeys. It can be fatal within days to weeks (Mota et al. 2010). In contrast to horses and donkeys, mules may be somewhat less vulnerable to Glanders, and the illness can have both an acute and chronic course (Khan et al. 2013). Anorexia, sadness, and weakness are the first symptoms of acute Glanders. Clinical symptoms include coughing and fluid flow from one or both nostrils and rapid growth of nodules and ulcerations in nasal sputum with thick, sticky, yellowish-white muco-purulent to blood discharge as these lesions progress, resulting in dyspnea. The sub-maxillary lymph nodes expand bilaterally and often become indurated. They commonly protrude from the jawbones and may even rupture. Septicemia and respiratory failure (bronchopneumonia) cause death within a few days to weeks (Radostits et al 2007).

12. POSSIBLE OUTCOMES OF GLANDERS IN MAN

Regardless of the low frequency of animal to human transmission, the vulnerability at work is still major risk for farriers (those who care for horses' hooves), students, flayers (those who work with leather), stable hands, transport workers, farmers, soldiers, veterinarians, slaughterhouse staff, and horse riders (Pal et al. 2022).

12.1. GENERAL SYMPTOMS

Numerous Glanders types, like chronic, pulmonary, and septicemia, have been stated. The many infection paths account for a considerable portion of the variability of infections. Localized infections are typically restricted to a specific geographic area. The pustules might bleed and ulcerate for a very long time. Localized infections can spread and cause septicemia, multi-tissue, or lung diseases. Furthermore; the following signs most often: moderate-to-low fever in the evening or noon, malaise, fatigue, headache, and myalgia such as backache, lymphadenopathy, and pain in the chest (Khan et al. 2013). After the first wave of disease signs, roughly fifty percent of the patients not only looked well but also became medically better. Within a few days to two months the patients began showing signs and symptoms. (Khan et al. 2013).

12.2. MUCOSAL INVOLVEMENT

Severe lacrimation and photophobia are signs of a B. mallei infection of conjunctiva and the eye. Following B. mallei inhalation, nasal involvement can be marked by inflammation of the nose. There may be lots of nasal discharge with this. Additionally, infection may enter the bone structures of the nasal septum, leading to fistulas and tissue damage. Local lymphatic nodes may enlarge and swell up in the face. Lower respiratory tract infections may also spread, leading to bronchitis, which may be accompanied by coughing up muco-purulent sputum. In the early days after infection, constitutional symptoms like a fever and chills are widespread. These symptoms may also be severe and continue after treatment. Fever during the middle of the day or night; shivers with or without rigors; and a severe headache are a few examples of common signs and symptoms (Pal et al. 2022).

12.3. CUTANEOUS INVOLVEMENT

Papular lesions, which can develop on the body anywhere and have a more extensive, indolent infection, are examples of cutaneous symptoms. After B. mallei's entrance through an abrasion, an inflammatory reaction with indicators swelling and pain is typical. In these situations, a Glanders Node may initially present as the only blister, evolving over time into an ulcer that can bleed rapidly (Waag and DeShazer 2005), at the point of entrance, a discharge-producing localized infection usually appears. Regional lymphatics may become inflamed and develop multiple foci of suppuration along their path, leading to lymphangitis. By producing more irritation and inflammation in the lymphatics, the endotoxins found in some strains of B. mallei have a damaging effect on the smooth muscle cells of the lymphatics (Liu et al. 2014).

12.4. PULMONARY INVOLVEMENT

Influenza, pulmonary abscess, pleuritis, and extensive fluid are common complications of a lung infection. Cough, breathing difficulties, chest discomfort and mucopurulent sputum are all signs of lung infection. Nonspecific signs and symptoms include fatigue, fever (typically above 102°F), a shiver, pain, myalgia, lymphangitis, and pain in the throat, pleuritic, chest discomfort, cough, breathlessness, nasal discharge, and

gastrointestinal indicators. Many symptoms can take up to two to three weeks to manifest. Non-specific symptoms include rigors, fatigue, vomiting, sweating during the night, severe migraines, dizziness, a rapid heartbeat, losing weight and mucosal eruptions, which may indicate a widespread infection. (Khan et al 2013).

12.5. WARFARE POTENTIAL

Burkholderia mallei is a potential bioterrorism pathogen because of its ability to cause severe illness in humans and animals. The bacteria can be spread by aerosolisation, making it a potential weapon for bioterrorism. In addition, B. mallei is resistant to many antibiotics, making it a challenge to treat once the disease has occurred. The use of B. mallei as a biological weapon has been reported previously. During World War I, the bacteria was used to infect military horses and mules. While no recent activities have been reported, it remains a concern for public health officials and military planners due to its potential to cause widespread illness and death (Pal and Gutama 2022).

12.6. DIAGNOSIS

- Clinical Picture of case/Signs and Symptoms
- Culture and Sensitivity (nasal and throat swabs)
- ELISA
- PCR
- Antigen Detection (Ab is produced against pathogen by immune system)
- NAATs (Nucleic Acid Amplification Tests)

12.7. DIAGNOSIS IN EQUIDS AND OTHER ANIMALS

For a clinical diagnosis of Glanders, nodules, ulceration, scarring, and weakened state may be sufficient. Specific diagnostic tests should be utilized as soon as feasible because these symptoms typically do not appear until the disease is well advanced. The diagnosis is confirmed by B. mallei cultured from lesions.

- Based on cutaneous nodules oozing a honey-like discharge or nasal discharge and ulcers on the nasal mucosa, it is suspected.
- Proven using ELISA, PCR, culture, and the complement fixation test (Saqib et al. 2012)

12.8. MALLEIN TEST

Mallein, a secreted glycoprotein of B. mallei when injected is detected in culture supernatant, into the palm, one can test for delayed hypersensitivity. Within 24-8 hours, purulent conjunctivitis and eyelid edema appear in infected hypersensitive horses (Dvorak et al. 2008). Due to reservations about using animals, neither trade testing nor general recommendations are made for the Mallein test. In addition, malleinized sero-conversion may occur and may subsequently exhibit false positive results in additional diagnostic techniques like CFTs. In endemic rural areas, however, the Mallein test may be helpful (Saqib et al. 2012).

13. COMPLEMENT FIXATION TEST (CFT)

Additionally, complement fixation is employed to check for infection. CFT has been used for monitoring, confirmation of outbreaks, and trade testing for decades; also, a required based recommendation from the World Organization for Animal Health (OIE). Studies have demonstrated that the CFT is quite sensitive, but regrettably, a sizable number of false-positive results are generated by this test, which subsequently cause

threats to cross-border trade. Because there are no accessible international standard standards, CFT also has technological drawbacks, being labor-intensive and challenging to standardize (Saqib et al. 2012).

13.1. ELISA

The term ELISA means "Enzyme-linked Immunosorbent Assay" It is an effective method for determining the concentrations of mg/ml to g/ml ordered materials in solutions, including sperm, serum, culture supernatant, and urine. Competitive ELISA has greater sensitivity than CFT and can detect an infection as early as three days after exposure. It is an antigen-antibody reaction. Alkaline phosphatase, Horse Radish Peroxidase, and beta-Galactosidase are just a few of the enzymes deployed in ELISA tests (Richard 2002). Each enzyme utilizes specific substrates for results. For example, Ortho-phenyl-diamine-dihydro-chloride is used for peroxidase, while Para-nitro-phenyl phosphate is utilized for alkaline phosphatase. These substrates react with the enzymes to produce colored end products. Antibodies or antigens present in serum are detected by antigen or antibodies coated on a solid surface by matching. Based on the arrangement of the binding sites for the antibodies and the antigens, three categories of ELISA may be made: indirect, direct, and sandwich (Saqib et al. 2012).

13.2. PCR

It is possible to identify a specific organism using PCR based on 23S and 16S rRNA genes sequences. Burkholderia mallei was specifically identified in clinical samples and pure culture samples from outbreaks using a polymerase chain reaction (PCR) test that targets the flagellin P (fliP)-I S407A genomic region. While other closely related species failed to amplify the 989-bp fragment from each of the 20 B. mallei strains under investigation, primers derived from the known fliP-IS407A sequence of B. mallei American Type Culture Collection (ATCC) 23344T were successful. Horses with a widespread infection of B. mallei had their tissues amplified for B. mallei DNA as well. The created PCR assay can be utilized as an easy, quick approach for detecting B. mallei in clinical samples that is sensitive and specific (Saqib et al. 2012).

13.3. TREATMENT

Treatment is contraindicated due to the lack of availability of effective treatment and antibiotic resistance but some antibiotics are still effective against B. mallei. Isolates of B. mallei are susceptible to Doxycycline, Amoxicillin-clavulanic acid, Chloramphenicol, Gentamycin and Trimethoprim-sulphadiazine. Enrofloxacin is now not very much effective due to its vast use and developing resistance. Horses can be treated by giving the antibiotic course for 12 weeks in which Enrofloxacin (8mg/kg of body weight) and Trimethoprim-sulphadiazine (32mg/kg B/W) are given I.V. once a day for 1 week and their dose is reduced to half during 2nd and 3rd week. Doxycycline (6mg/kg B/W) orally twice a day from week 4 to 12 (Saqib et al. 2012).

In humans and laboratory animals Sulfonamides provide a satisfactory result. Autogenous vaccine (once) and Trimethoprim-sulphadiazine (20 mg/kg B/W P/O) for 1 month alter the disease course if given to infected horses (Al-Ani and Roberson 2007).

A study in mice showed that Finafloxacin controls B. mallei at the organ level and also controls the signs which are going to develop (Barnes et al. 2022).

Bacteria are sensitive to co-Trimoxazole, Danofloxacin, Norfloxacin, and Chloramphenicol. Treatment for 4 days can be given to animals with Ringer's-lactate-dextrose 500ml, 60-80 ml Dimethyl-sulfoxide I.V. and inj. Norfloxacin 5% 35-50 ml I.M. (Muhammad et al. 1998).

14. PREVENTION AND CONTROL

Glanders and Farcy Act of 1899 states that: affected animals should be killed and disposed of. For proper prevention, identify positive animals and eliminate them. According to WOAH, control measures include surveillance, identification, euthanasia, quarantine, cleaning, disinfection, and proper disposal by incineration.

Vaccination for proper prevention is not available. Live vaccines are available but have no satisfactory results. Affected animals should be separated from healthy animals and checked at intervals of three weeks until all animals are negative for Glanders. If the animal dies, don't perform a necropsy. Proper disinfection should be done. Various disinfectants can be used, such as 70% Ethanol (C2H5OH), Potassium Permanganate (KMnO4), household bleach, Iodine, and Mercuric Chloride in alcohol. UV rays and heat also kill the bacteria (Verma et al. 2013).

15. MELIOIDOSIS (BURKHOLDERIA PSEUDOMALLEI)

Melioidosis is infectious and zoonotic disease caused by gram negative bacteria named as Burkholderia pseudomallei (Norman and Chen 2023), also known as Pseudomonas pseudomallei (Somprasong et al. 2023). Genetic makeup is complex, contributing to its pathogenicity (Fang et al. 2016). Commonly found in water and soil, especially in South East Asia and Northern Australia. Main transmission route is contact with contaminated water and soil. Open wounds contribute to bacterial entry into the body. Transmission from person to person is rare (Mohapatra 2023). The symptoms of the disease vary widely, but it affects the liver, lungs, spleen, skin, and joints (Ignee et al. 2023). Diagnosis is confirmed through laboratory tests like sputum analysis and blood cultures (Noparatvarakorn et al. 2023).

Fig. 3: Melioidosis warfare potential (Samy et al. 2017).

15.1. HISTORY

The bacterium *B. pseudomallei* was discovered in Rangoon Burma, now Myanmar, by A. Whitmore and C. S. Krishan Swami in 1912 from the spleen of a man who died from an unknown illness. The pathogen was named Whitmori, and the disease was called Whitmore's disease at first. Again in 1913, the same pathogen was discovered in Malaysia and was named *Pseudomonas pseudomallei.* As the signs of the disease were almost similar to the Glanders, a condo caused by the bacterium mallei, the name was given as *pseudomallei*. A British medical officer cap. J. Simpson was the first to describe the symptoms officially in 1915 in Malaysia and named the disease Melioidosis after the pathogen's name. During World War II, the disease was highly prevalent among soldiers. This disease has gained quite an attention in recent years after the possible zoonotic and bioterrorism risk (Fig. 3) due to its possibility to cause high mortality, antibiotics resistance remains a sound issue yet (Foong et al. 2014). WHO and other authorities are working on it providing efficient medication and vaccines for the prevention and control.

16. AGENT CHARACTERISTICS OF *B. PSEUDOMALLEI*

It is a gram-negative, aerobic, non-sporulating saprophytic coccobacilli. Polar flagella make *B. pseudomallei* motile and on MacConkey agar it appears as a non-fermenter. *B. pseudomallei* shows a typical bipolar staining. Main reservoirs of this bacterium are contaminated soil and water (Wuthiekanun et al. 1995). Epizootic infections are possible and are caused by various animals that come in contact with the agent and act as reservoir hosts (Cheng and Currie 2005).

For isolation Ashdown agar medium is being used, specifically for non-sterile samples such as samples from throat, rectum or sputum (Ashdown 1979). Pathophysiology of melioidosis is shown in Fig. 4.

Smooth colonies of *B. pseudomallei* are formed within 24-48 hours with a putrid odor, mostly yellow to orange-colored, which after a few days, form wrinkled and dry colonies like *Pseudomonas stutzeri*. Earthy and musty odor is produced during growing phase.

B. cepacia medium and *B. pseudomallei* Selective Agar (BPSA) are newly introduced growth media. Growth of mucoid *B. pseudomallei* colonies on BPSA medium is more than compared to the Ashdown medium (Howard and Inglis 2003).

17. CLINICAL SIGNS AND SYMPTOMS

Due to range of signs and symptoms it is frequently misdiagnosed with other diseases (Fong et al. 2015). From mild to severe disease, patients may exhibit a range of clinical symptoms such as headaches, fever, muscle discomfort, abscesses, labored pneumonia and cough. (Karunarathna et al. 2018). Site of the infection/inoculation may have an impact on the clinical appearance. The time that clinical symptoms first appear, or the incubation period, varies greatly for Melioidosis. It could last anywhere from 1 to 21 days or for many years (Chakravorty and Heath 2019).

18. FORMS OF MELIOIDOSIS

According to (Alwarthan et al. 2018), Melioidosis can manifest clinically in a number of ways (Fig. 5):

18. SUBCLINICAL FORM

Subclinical form is caused by the seroconversion the agent in population living in an endemic area. It is considered that all new cases have a recent history of infection and that there is no sufficient evidence to justify the emergence of clinical cases of seroconversion (Currie 2014).

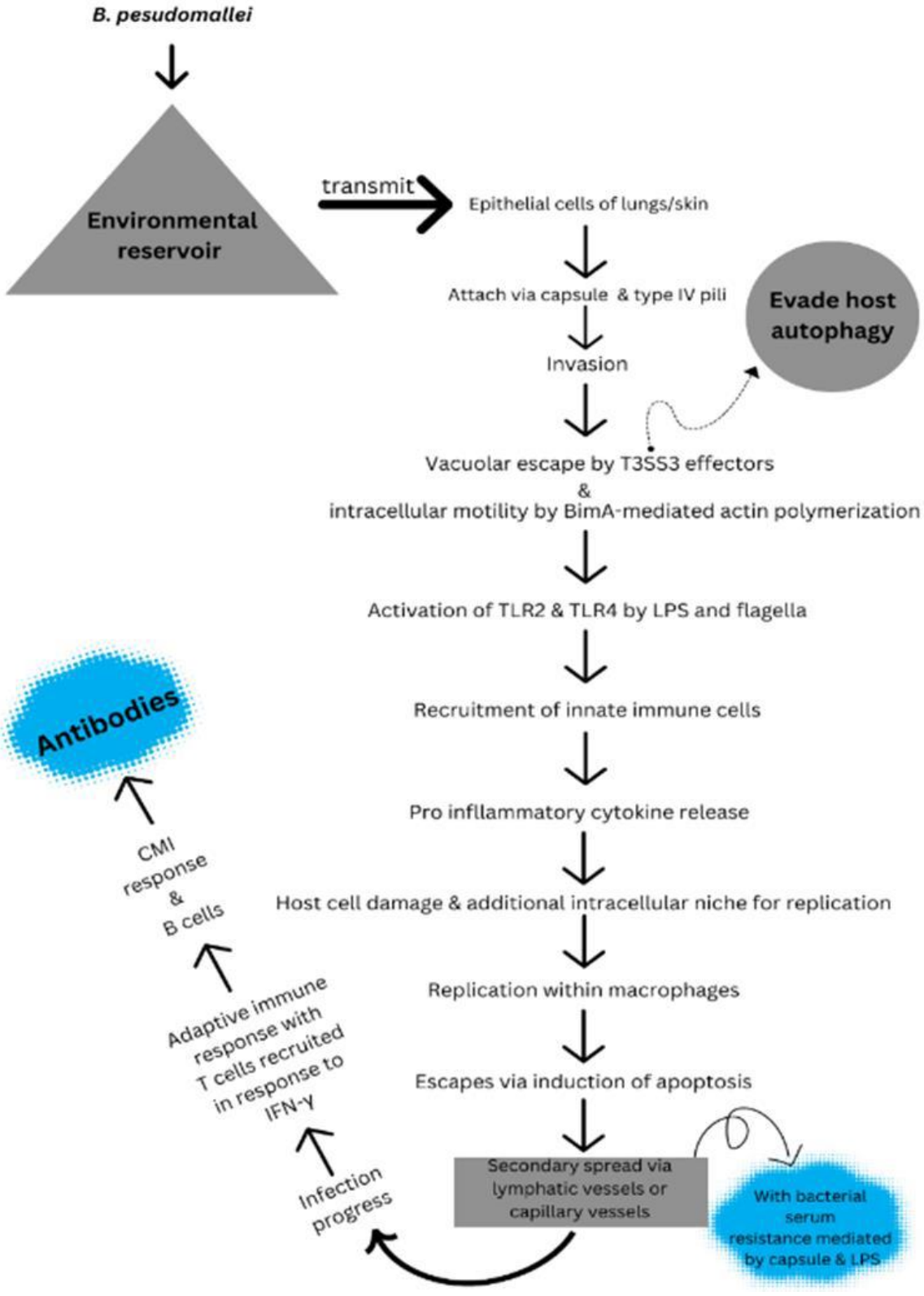

Fig. 4: Pathophysiology of Melioidosis (Adler et al. 2009).

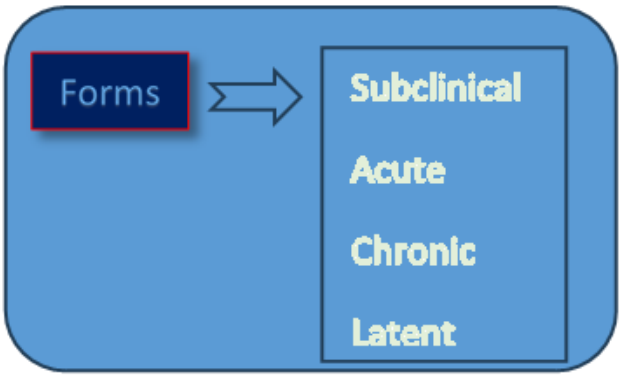

Fig. 5: forms of melioidosis

18.1. ACUTE FORM

A severe case which results in septicemia, shock and death. Exposure through inhalation/aspiration leads to acute form. Lungs are mainly affected (Mahendra et al. 2022). More than half of patients exhibit bacteremia in which 20% suffers septic shock (Chakravorty and Heath 2019). Lungs are more affected in adult cases. Children experience skin infections more frequently. Bacteria enters bloodstream, colonize in organs (liver, spleen, kidney, genitalia and brain) and then cause inflammation and development of visceral abscesses. Central nervous system is less impacted (Currie 2014).

18.2. CHRONIC FORM

Symptoms persist for longer than two months. Chronically infected people make up around 11% of all instances and can resemble the clinical symptoms of cancer, tuberculosis, or fungal infections, such as weight loss, fever and cough that may be bloody (Alwarthan et al. 2018).

18.3. LATENT FORM

Bacteria might occasionally become active or relapse after months or years. It is regarded as being in latent form in that case (Wiersinga et al. 2012).

19. ZOONOTIC ASPECT OF *B. PSEUDOMALLEI*

It is endemic in the north of Australia and Southeast Asia. During the last twenty-five years, it caused significant illness and death in this region (Limmathurotsakul and Peacock 2011). Pneumonia is most common than skin and soft tissue infection, parotitis and prostatitis (Cheng and Currie 2005).
Diabetes mellitus is the risk factor, as evidenced by research in Australia and Thailand, where as much as sixty percent of Melioidosis sufferers are diabetic, primarily type 2 (Cheng and Currie 2005). *B. pseudomallei* have a diverse host range in addition to humans such as cattle, goats, and swine are the most commonly recorded domestic animals (Ouadah et al 2007). The occasional instances or small outbreaks of the disease have been observed in apes, gibbons, orangutan species like cows, zebras, deer, kangaroos, cattle, camels, sheep, wallabies, koalas, pets such as horses, dogs, cats, shoes, parrots, rabbits, rat, guinea pigs, squirrels, dolphins, seals (Sprague and Neubauer 2004). Recently in California *B. pseudomallei* disease in two domestic iguanas was reported (Zehnder et al. 2014). Melioidosis is present in both acute and chronic forms in animals. Anorexia, pyrexia, wheezing, skin dehydration, and lesions are common symptoms in animals (Galyov et al. 2010).

20. POSSIBLE OUTCOMES OF MELIOIDOSIS IN HUMAN

B. pseudomallei can infect humans. This organism is mostly acquired from the environment; however, a few zoonotic examples have been described.

20.1. CLINICAL SIGNS

B. pseudomallei can induce a wide range of clinical symptoms in humans. While many infections appear insignificant, others cause acute lung illness, septicemia, or localized long-term suppurative disorders. The prevalence of various disorders varies by geography (Mariappan et al. 2017). Parotid abscesses, for example, are prevalent in children in Thailand but uncommon in Australia. If the organisms move to other locations, one sickness can evolve into another (Benoit et al. 2015).

20.2. ACUTE LOCALIZED INFECTIONS

Acute localized infections can happen at the point of injection. Localized skin disease was observed to be a prevalent type of Melioidosis among children in Australia. Scars in the skin often manifest as grey or white, hard nodules and ulcers that are frequently but not usually single. Caseating nodules are frequently surrounded by inflammation. Regional lymph nodes and lymphangitis may accompany them. Suppurative parotitis/parotid abscesses, damaging corneal ulcers seen following ocular trauma, and illnesses that simulate necrotizing fasciitis are all examples of acute localized infections. Prostatic abscesses are a common symptom of the genitourinary tract. Although localized infection can spread, systemic illness is not necessarily preceded by localized infections. The skin and subcutaneous tissues can be affected through the hemorrhagic transfer of microorganisms from other sites (Cheng et al. 2015).

20.3. PULMONARY DISEASE

People commonly suffer from lung illness. It can occur as a separate condition or as a part of septicemia, and it can emerge quickly or gradually following a nonspecific prodromal sickness. The severity of pulmonary Melioidosis ranges from moderate acute or chronic pneumonia to respiratory difficulty with severe septic shock. Fever, wheezing, pleuritic chest pain, and, in certain situations, hemoptysis are common symptoms. Individuals with pneumonia as part of septicemia may have a cough or pleuritic pain while being febrile and very unwell. Chronic lung Melioidosis may increase and decrease, and symptoms include a decrease in weight, fevers, sweating during the night, and a productive cough with blood-tinged sputum. Nasal ulcers and nodules are occasionally observed, and the septum can perforate. Pneumothorax, empyema, and pericarditis are all possible consequences. Severe instances can develop into septicemia (Dance 2014).

20.4. SEPTICEMIA

The most severe form of Melioidosis is septicemia. It is common in those who already have conditions like diabetes, cancer, or kidney failure. The onset is frequently abrupt, with fever, rigors, and other characteristic sepsis symptoms. However, it may appear gradually, with a variable fever and substantial weight loss. Fever, severe headache, anxiety, pharyngitis, upper abdomen discomfort, stools, jaundice, and considerable muscle tenderness are common signs of septicemic Melioidosis. Pulmonary symptoms, such as dyspnea, are prevalent, and arthritis or hepatitis may be present. A diffused pustular redness with

regional lymphadenopathy, cellulitis, or lymphangitis is seen in many cases. Septic shock is a common and potentially fatal condition (Limmathurotsakul and Peacock 2011).

20.5. CHRONIC CASES

Chronic cases can result in infections and suppurative lesions in multiple organs. While the spleen, liver, skeletal muscle, and prostate gland are frequently affected, lesions can also be seen in the skin, lung, kidney, heart, bone, joints, lymph nodes, and testes. There are also mycotic aneurysms. Melioidosis can cause brain infections, encephalomyelitis (with multiple symptoms, including flaccid paralysis), or meningitis in rare cases. In situations of encephalitis, there may be significant residual abnormalities (Loveleena and Dhawan 2004).

20.6. DIAGNOSIS

1. Clinical signs of Melioidosis make a clinical diagnosis particularly challenging.
2. Travel history to an endemic region.
3. Cultural analysis, biochemical tests and gram staining.

21. ASHDOWN SELECTIVE AGAR (ASA)

ASA is selective medium in endemic areas, which exhibits huge, round, wrinkled purplish colonies. However, it has recently been found that some bacterial strains are unable to grow on this medium. *Burkholderia pseudomallei* Selective Agar (BPSA) was created for better isolation (enrich gentamicin susceptible strains). Smooth colonies are formed on chocolate agar, horse blood agar and MacConkey agar. The microbe should be cultured for at least 4 days with daily examination because it grows slowly. While there are various commercial kits available for bio typing (Walk-Away, VITEK-2, and 1156576 and 1156577). Their sensitivity and specificity remain debatable. It may be mistaken for contaminant or *Pseudomonas* (Pal et al. 2022).

22. ANTIBIOTIC DISC DIFFUSION TEST

It may be employed as a presumptive test. It is based on the observation that the majority of isolates are susceptible to Colistin but not to Co-amoxiclav (Pal et al. 2022).

22.1. SERUM TESTS

The sensitivity and specificity of serological diagnostic methods such immunofluorescence, complement fixation tests and indirect haem agglutination have been reported to be low. Due to cross-reactions and high background antibody in endemic places and antibody-based tests are prone to producing false positive results. As a result, only the bacterial culture approach may be used for confirmatory assays (Pal et al. 2022).

22.2. LATERAL FLOW IMMUNOASSAY (LFI)

Another diagnostic technique that makes use of monoclonal antibodies (mAb 3C5) is the LFI, created by In BiOS (Active Melioidosis Detect), to identify the capsular polysaccharide. A promising technique for detecting *B. pseudomallei* may be AMD LFI. It is equipment-free diagnostic test, user-friendly, inexpensive, quick and reliable but it has low sensitivity for samples that are not blood.

Many molecular methods for identifying species have been developed, with various advantages and disadvantages. These methods include particular PCRs, rtPCR and 16S rDNA sequencing. Quick and affordable detection method is matrix-assisted laser desorption/ionization of time-of-flight mass spectrometry (MALDI-TOF MS). Additionally, imaging tests like CT, chest radiography or ultrasound are frequently very helpful for figuring out how severe the illness is and for looking for subclinical abscesses (Pal et al. 2022).

22.3. TREATMENT

Prolonged antibiotic treatment is required (Laws et al. 2019). Antimicrobials should be selected by culture and sensitivity tests. *B. pseudomallei* is resistant to Macrolides, Gentamicin, Ampicillin, Polymyxin, 1st and 2nd generation Cephalosporin, Tobramycin, and Penicillin (Currie 2015).
Two phase treatment is given, the acute phase and the eradication phase. In the acute phase, drugs including Ceftazidime (50mg/kg TID), Carbapenems, Amoxicillin-Clavulanic acid, and Trimethoprim-Sulphadiazine are given by parenteral route for 10-14 days, then orally Trimethoprim-Sulphadiazine or amoxicillin-clavulanic acid to prevent relapse for 12 weeks (Dance 2014).
The minimum inhibitory concentration of Imipenem and Meropenem is low. They are more bactericidal than Ceftazidime. Drug of choice for acute phase is Ceftazidime while Trimethoprim-Sulphadiazine for the eradication phase (Wiersinga et al. 2018). Abscesses may be surgically drained.

23. PREVENTION AND CONTROL

To control Melioidosis effectively, its risk factors should be controlled, such as diabetes, alcohol, smoking, immunosuppression, environmental exposure, and chronic diseases of the kidney, heart, liver, and lungs (Mohapatra and Mishra 2022).
After handling aquariums, snails and fish wash hands with soap. Don’t allow less than 5y children to clean the aquarium (Dawson et al. 2021).
According to CDC:

1. Avoid contact with standing water and soil
2. Wear boots specially for agriculture workers
3. Healthcare workers use standard precautions

No vaccine is available, so all prevention and control rely only on preventive measures (Norman and Chen 2023). Drink only treated or boiled water. If an animal outbreak occurs, euthanize infected animals and disinfect the environment. Avoid rain and dust (Pal et al. 2022).

24. CONCLUSION

Glanders and Melioidosis are two main diseases caused by the genus Burkhulderia. The diseases are of primary importance in terms of performance, economics, trade, work force and, above all, zoonosis. Signs and symptoms of both diseases are quite similar and often it becomes important to diagnose the one differently for better treatment and management of infected equines. Due to the zoonotic aspect, both have major health concerns for workers like farriers, grooms, game players, volunteers and veterinarians working closely with them. Moreover, in developing countries, a large number of the population is directly or indirectly involved with horses, mules, and donkeys to meet their financial needs. It is important to devise a regulatory framework to screen and separate the affected ones from healthy ones to minimise the risks. There is also need to develop screening tests which are more specific and easier to perform,

while cost effectiveness remains primary concern. Treatment due to AMR resistance and quarantine of effected equines remains major concern too.

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Salmonella Resistance in Broiler Chicken: Risk to Human Health

08

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ABSTRACT

Salmonella is the most common zoonotic foodborne pathogen of economic status in animals and humans. The natural habitat of Salmonella is the gastrointestinal tract (GIT) of domestic and wild animals, part of diverse foodstuffs of both animal and plant origin, that become infected directly or indirectly with Salmonella. Most domestic and wild animals are infected with Salmonella spp. with no signs of illness. Due to increasing antibiotic resistance of Salmonella, poultry food and feedstuff became the main source of infection for humans. Appropriate risk analysis and effective control measures could reduce the prevalence of Salmonellosis at production and processing sites, that eventually, improves human health and be beneficial for mankind.

Keywords: Salmonella; Animals; Humans; Foodstuff; Poultry; Antibiotic resistance

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1. INTRODUCTION

Animal proteins are the most demanded protein source around the World, accepted by different faith groups. Whereas, poultry meat is considered as the most consumed and economic meat among animal sources. In recent years, poultry meat consumption increased by 5.5% per capita worldwide, indicating the demand for this product for protein availability and food security (Machado Junior et al. 2020). Nonetheless, consumption of contaminated poultry products (meat, egg) was stated to induce 20.6% foodborne diseases in the United States from 1998 to 2008, in which *Salmonella* spp. was one of the main etiological agents (Painter et al. 2013). *Salmonella* natural habitat is GIT of birds and, can be entered into the production system by contaminated water or feed, litter, live vectors and even through human contaminated tools and boots etc. (Wales et al. 2010). Contaminated poultry meat and eggs are considered as the main risk for human infections. Early, incorporation of antibiotics was considered as the greatest solution to get rid of such zoonotic pathogenic bacteria, but later, with emergence of antibiotic resistant *Salmonella* outbreaks changed the vision.

Risk analysis and modelling frameworks were carried out to evaluate and define control measures for the risk of food borne diseases cause by *Salmonella* from layers (Namata et al. 2008), broilers (Namata et al. 2009; Rajan et al. 2017; Kloska et al. 2017), dairy cattle (Nielsen and Nielsen 2012), and pigs (Binter et al. 2011; Hill et al. 2016). Surveillance, vaccination and biosecurity have been associated to ultimate decline in salmonellosis, so, need to focus and put more efforts on serotypes, cause incidence of disease in humans such as *Salmonella typhimurium* and *Salmonella enteritidis* by adopting efficient control measures in poultry and egg production (Hugas and Beloeil 2014). At both processing plants and production site, simple hygiene practices could eliminate and prevent contamination of such pathogenic microorganisms (Hugas and Beloeil 2014).

Poultry food safety requires appropriate management at each level of processing and production in such a way that active antimicrobials can be applied. Steps should be taken to evaluate microbial testing and data analysis for *Salmonella* positive forms and precautionary measures should be adopted to avoid cross contamination. Once the problem, risks associated with execution of the problem, possible solution and their implementation are strategically defined, then application of such solutions are validated and applied for the next step. It is very important that these antimicrobial implementations be validated at that step they are applied and as part of overall food safety. In addition to these antimicrobials, feed additives which generate a positive effect on overall health of animals should be adopted in order to generate safer and up to the mark of standard and quality food and feedstuff. Such food safety practices should be implemented, understood and communicated throughout the poultry production and processing continuum.

2. HISTORY OF *SALMONELLA*

The bacterium *Salmonella* was first found by Soholerin in 1839 (Myruvik et al. 1976), and isolated from a person who died from typhoid fever by Eberth in 1880 from the tissues of spleen and mesenteric lymph nodes. Later, in 1888 was cultured by Smith and Salmon from a pig, perished from hog cholera (Merchant and Packer 1977). Later in 1888, Garter carried out its isolation in human (Bryan et al. 1979). However, the major contribution in isolation of Salmonella was by White Kaufmann-Le Minor, out of 2600 serotypes 1600 of which belongs to *S. enterica*. Over 200 serotypes are reported to cause disease in humans (Xu et al. 2021).

For over a century, foodborne infections have been major health concerns that *Salmonella* instigated (Worku et al. 2022) and are grouped as typhiodal salmonellosis (TS, commonly called enteric fever) and non-typhoidal salmonellosis (NTS, commonly called gastroenteritis) infections (Ngogo et al. 2020; Akinyemi et al. 2021). Typhoid fever is caused by *Salmonella enterica* serovar *typhi* while the para-typhoid fever is caused

by *Salmonella paratyphi* A, B and C (Akinyemi et al. 2021). While the source of NTS infection is group of *S. enterica* serovars *S. typhimurium, S. enteriditis* and *S. cholerasuis*, these are responsible serovars that cause infection in individuals through consumption of contaminated food products and diets (Thung et al. 2018).

3. CLASSIFICATION OF SALMONELLA SPECIES

Salmonella is a genus categorized in the family Enterobacteriacae, which retain thought-provoking genotypic and phenotypic characteristics with peculiar nomenclature compared to bacteria of same and outside the family (Oludairo et al. 2022). The classification of *Salmonella* remained controversial and multifaceted (Euzeby 1999). According to most accepted nomenclature, based on variation in the sequence of the 16Sr RNA gene, this genus comprises of two major species *Salmonella enterica* and *Salmonella bognori* (Crump et al. 2011; Dione et al. 2011). Depending on the geographic distribution, host adaptations, antigenic nature, biochemical reaction and DNA-relatedness the S. enterica is further categorized in six sub-species (Carter and Wise 2004). These were S. enterica enterica (I), *S. enterica salamae* (II), *S. enterica arizonae* (IIIa) *S. enterica diarizonae* (IIIb), *S. enterica houtenae* (IV) and *S. enterica indica* (VI).

While the *S. bognori*, most commonly occurs in cold-blooded animals and surroundings (Brenner et al. 2000). Seventeen serovars are identified and represented with symbol V (Jordain and Pattison 1996; Lake et al. 2002). Among all, serovars of subspecies I (S. enterica) are highly pathogenic although other subspecies and *S. bongnori* are relatively less infectious to animals and humans (Lake et al. 2002; Rahman and Othman 2017).

Salmonella is classified into three species according to the biochemical characteristics of the genus, that includes, *S. Choleraesuis* with only one serovar and specific host is swine, *S.typhi* also have one serovar and it mainly infect humans. While, the third *S. enteritidis* contains about 2000 serovars and it includes all serovars that infect animal and human (Carter and Wise 2004). According to host predilection *Salmonella* can also be divided in three groups: adapted to man S. typhi and *S. paratyphi*, adapted to most of the animals *S. choleraesuis* and serovars of *S. enteritidis* while the third have all the serotypes that are not adapted to particular host. New serotypes are frequently identified and added to many previously classified (Rahman and Othman 2017).

4. *SALMONELLA* OUTBREAKS

Zoonotic outbreaks are when individuals abide from the same diseases from animals, animal products and associated environment (EFSA 2021; Abebe et al. 2020). *Salmonella* grounds for diverse diseases i.e., enteritis, septicemia and abortion, while rarely in less than 1% of clinical cases meningitis is also reported (Gille-Johnson et al. 2000; Koonse et al. 2005). These diseases show less specificity for their host species (Gopee et al. 2000; El-Sharkawy 2017).

In numerous regions of the world, salmonellosis is among the most substantial public health challenges (Padungtod and Kaneene 2006; Vindigni et al. 2007). Occurrence also have been reported in southern Thailand from poultry and eggs (Lertworapreecha et al. 2013), while in USA and Europe from meat and beef (Yavari 2012; Heredia and Garcia 2018). Lecis et al (2011) studied the incidence of Salmonella in fresh milk, pork and chocolate in Europe and parts of Africa. Epidemics have also been reported in Africa, Europe and USA from insects and wildlife in (Hidalgo-Vila et al. 2007; Percipalle et al. 2011; Heredia and Garcia 2018).

In Africa Non-typhi *Salmonella* appeared to be endemic and a major cause of bacteremia with 4100 deaths per year mostly in children (Majowicz et al. 2010). As in South and Eastern Africa *Salmonella typhi* is the foremost source of bloodstream infections with multiple outbreaks since 2012 (N'cho 2019). Very high

incidence was reported in Malawi 444 cases per 100,000 person per year (Meiring et al. 2021). Among the most common serotypes were *Salmonella enteritidis* and typhimurium was reported as a common cause of iNTS (Feasey et al. 2015). In a case study on children from Kenya indicated 1.3% bloodstream infections were caused by *S. typhimurium* and *S. enteriditis* while *Salmonella typhi* caused its 1.4% (Mbae et al. 2020). Worrisome rising trend in NTS cases was reported from the Middle East and Northern Africa as Sudan 9.2% Tunisia 10.2%, and highest in Morroco was 17.9%. Whereas the lowest were reported in Oman 1.2%, Palestine 1.2 % and Jordon 1.1%. (Andrews-Polymenis 2014; Fardsanei et al. 2018; Al-Rifai et al. 2019). In Saudi Arabia, the *Salmonella* based infections become predominant during Umrah and Hajj season (Abd El Ghany et al. 2017).

In the United States of America, a report by CDC (Centers for Disease Control and Prevention) estimated 1.35 million illnesses in 2022, in which 26,500 hospitalizations and 420 deaths occur due to NTS infections each year (Kuehn 2019). In late 2022, because of *Salmonella typhimurium* a multi-country outbreak was reported in the US and UK. In 2014 Salmonellosis remained the second most communal zoonotic disease in the European Union (EU) with the incidence of 9830 hospitalizations and 65 fatalities in humans (ECDC 2015). However, a stability in infection was observed from 2015-2019 but a considerable decrease was reported in the year 2020 (EFSA 2021). In China it was reported in 2015 that outbreaks of food-borne diseases Salmonella were the second most common pathogen (Fu et al. 2019). He et al. 2023 reported the Salmonella infections from 2012-2021 in Zhejiang province with 1614 hospitalizations among 11,269 cases, when the average positive rate was 3.65% for the entire province.

Infections instigated by *S. typhimurium* impose serious health concerns in low and middle-income countries including Pakistan (WHO 2017). Where the residents of Punjab and Sindh provinces were most vulnerable to infections among the all the disease prevailing Asian nations (Rasheed et al. 2019). A study reported in 2018 that *S. Typhi* infections were also on a higher rate in Pakistan among all the south East Asian countries (Watkins et al. 2020). As in Sindh province first large-scale outbreak, 493.5 cases were reported among 100,000 population (Klemm et al. 2018; Fatima et al. 2021), while in Punjab reports were similar (Saeed et al. 2020; Kim et al. 2021; Nizamuddin et al. 2021) as well as among international travellers (Godbole et al. 2018; Chirico et al. 2020; Watkins et al. 2020). Outbreaks of a new subvariant of extensively drug resistance (XDR) *S. typhi* emerged in Pakistan in the province Sindh (WHO 2019). From September 2020, 2883 were reported, the pervasiveness increased from 7 to 15 per 100,000 people per year. Now this lineage is spreading beyond the province (Ahmad et al. 2021; Rashid et al. 2023).

5. ROUTES OF TRANSMISSION-SALMONELLOSIS

Primary reservoir of Salmonella in human and animals are gastro intestinal tract (GIT), nonetheless there is wide range of sources of Salmonella infections i.e., egg, meat, dairy products, vegetables and water (Brenner et al. 2000). Food is the most common source of infection in developed countries and food-borne infections are difficult to identify, while it is the most imperative measure to prevent the infections also (Shi et al. 2015). In Australia 70% increased cases were reported between 2000-2013, in which food was a source relating both Salmonella *typhi* and Salmonella non-typhi (Pires et al. 2014). Water might signify a source of contamination while the egg and meat remain the most important source (Ford et al. 2016). The acquisition of the infection has been associated with exposure with the chicken and other birds can carry microorganisms (Seif et al. 2019). After contact with pets' infection has also been reported, while person to person transmission is also possible (Gut et al. 2018). In China, a multidrug resistant species (MDR) was identified from livestock in several provinces (Kuang 2015; Wang et al. 2020)

Some serotypes are host specific as *S. typhi* only infects humans while others to warm-blooded animals. Around 50 serovars are tangled in the incidence of disease in humans and animals (Ford et al. 2016).

6. EFFECT OF *SALMONELLA* ON HEALTH OF BROILER CHICKEN

Salmonella pass in the host through oral route, and colonizes inside the alimentary canal. Though, it is normally spread by poultry meat and egg shell contamination *via* chicken intestinal innards (Pires et al. 2014). The numeral laying hens placed in cage free systems upsurges the risk of *Salmonella* contamination in egg feces, because eggs are in close contact to chicken's dropping (Whiley and Ross 2015).
Although, *Salmonella enteritidis* follow two main paths of egg contamination;

- Horizontal Transmission
- Vertical Transmission

Horizontal transmission or indirect infection; eggs can be infected by contaminated droppings during or after laying eggs or dissemination over eggshell from the colonized chicken gut (De Reu et al. 2006).
Vertical transmission or direct infection; infestation to eggshells before egg laying, eggshell membranes, albumin, egg yolk or instigating *Salmonella enteritidis* infection to reproductive organs (Wibisono et al. 2020).
Salmonella infection spreads in poultry either horizontal or vertical mode of transmission and its prevalence is greater in 1-day old chicks. Grownup birds are less susceptible to Salmonellosis (Shivaprasad et al. 2013). Severity of the disease depends upon the age of the bird. Younger the bird, the more severe the impact. Besides this, severity of salmonellosis in broiler chicken differs according to various factors in different avian species that includes environmental stress, presence of coinfections, hostage, host immunity, infective dose and management factors etc. (Wibisono et al. 2020). Mainly, *Salmonella* infecting avian species by two ways; *Salmonella gallinarum* and *Salmonella pullorum*. Therefore, Center for Disease Control and Prevention (CDC) also reported the infection from low avian specific serovars for example, *S. Kentucky*, *S. lille*, *S.berta*, *S.anatum*, *S. infantis*, S.*berta*, *S. javiana, S.newport* and *S. eneteritidis* (CDC 2013a; CDC 2013b).
Gram negative bacteria, Salmonella enters the host, through oral route and colonize in the Sgastrointestinal tract. There are numerous factors associated Salmonella colonization in poultry includes;

- Age of the chicken
- Survival of *Salmonella* across gastric barrier
- Diet
- Physiological and environmental stressors
- Animal health and disease status
- Genetic background of the bird
- Usage of antimicrobials and coccidiostats (Dunkley et al. 2008)

So, *S. enteritidis* is habituated to the intestinal villi and colonized in the GIT with the help of the protein, known as adhesions (Beachey 1981). To avoid bacterial infection, the most common method is to avoid it from binding to intestinal epithelial cell receptors (Wizemann et al. 1999). In poultry, macrophages and heterophils, innate immunity cells play a significant role in intestinal infection (Fasina 2010). As the pathogen enters into the host intestinal epithelial barrier, innate immunity cells move to the site *via* oxidative stress and phagocytosis destroy such pathogens (Brisbin et al. 2008). Salmonellosis in chicken increases significantly in inflammatory cytokines such as IL-1β, INF-g and LITAF (Matulova et al. 2013). Though, *Salmonella* disables host defense by collecting IL-10, a suppressive cytokine.
However, adaptive immune response includes humoral and cell-mediated response for *Salmonella* infection. Though, first adaptive immune response against *S. enteritidis* infection is through the mucosal immune system, involving mucosal associated leukocytes and lymphocytes production and mucosal immunoglobulin A (IgA) (Wigley 2014). These mucosal immunoglobulins A inhibits *S. enteritidis* from attachment to intestinal epithelial cells, and prevents mucosal colonization (Wigley 2014). Shape of the intestine is a decisive factor of intestinal health. Due to increased surface area and decreased tissue

turnover rate, enhanced villus height and reduced crypt depth parallel with excessive nutrient absorption within the small intestine (Munyaka et al. 2012). In contrast, Chicks infested by *S*. *typhimurium* showed reduced jejunal villus height, crypt depth and height: crypt depth ratio subsequently results in severe enteritis (Borsoi et al. 2011).

7. *SALMONELLA* RESISTANT IN BROILER CHICKEN

Flemming discovered the first antibiotic called Penicillin in 1928 (Fairley 2007) that greatly reduced the mortality and morbidity during World War II in 1940's. Antibiotics are effective chemical substances to treat patients with bacteria causing infectious diseases (Saylers and Whitt 2005). Antibiotics reproduce their effects either bacteriostatic or bactericidal ways (Croft et al. 2007). Antibiotics are collectively referred to as antimicrobial agents to compounds produced by numerous microorganisms, drugs, synthetic chemicals, disinfectants etc. (Saylers and Whitt 2005). Although, antibiotics lose their efficacy as resistance provokes. Antimicrobial resistance may be intrinsically acquired by exchange of DNA fragment (Croft et al. 2007). Such mutations occur over spontaneous mutation, can be frameshift mutation, point mutation, insertion of large element and deletion of genetic material, naturally taking place with average rate of 1×10^{-6} base pairs exchange of genetic material from other bacteria. This way bacteria acquire adaptation to confront against the deadly effects of antimicrobial agents.

Mechanisms of bacterial resistance vary among different species of bacteria. Bacteria have the remarkable ability to survive. As far as, *Salmonella* is concerned, 3 different methods are elucidated to show antimicrobial resistance;

- By producing specific proteins, in the form of enzymes, which digest and alters the antimicrobial into no longer effective e.g., *Salmonella* β-lactamases inactivates β-lactam class (Croft et al. 2007; Cosby et al. 2015).
- By inducing an efflux pump that effectively pumps antimicrobial out of bacterial cell, such as, antimicrobial conc. inside the cell not approach to threshold to interfere with cell metabolic processes e.g., Chloramphenicol and tetracyclines resistance in *Salmonella* spp. (Foley and Lynne 2008)
- By mutating the target or produce chemical change to target site on which antimicrobial insert effects, known as receptor modification e.g., vancomycin resistant enterococci cause mutation on receptor site induce low affinity to vancomycin (Croft et al. 2007; Cosby et al. 2015)

However, other factors for the development of resistance include inability to detect new phenotypes, multiplication of different clones and selective pressure. Selective pressure commensurate by overuse of antibiotics for treatment of human diseases and in-home disinfectants (Rybak 2004)

Two basic methods for transmission of antimicrobial resistance implemented in *Salmonella*.

- Antimicrobial drug resistant *Salmonella* isolates clonal spread
- Horizontal transfer of antibiotic resistance genes (Molbak et al. 1999; Butaye et al. 2006)

Horizontal transfer of resistance genes could be easily transferred from one strain of *Salmonella* to another or among another bacterium spp. (Guerra et al. 2002). In case of *Salmonella*, Class I integrons and plasmids principally involved in horizontal transfer (Guerra et al. 2002; Dieye et al. 2009). Integrons, genetic determinants of machineries of site-specific recombination systems that establish mobile gene cassettes. Though, Class I and Class II integrons have been found in *Salmonella*; Class I represents resistance integrons, are mainly in the *Salmonella* genomic islands, Class II integrons presents super-integrons, related to TN7 transposon family, not yet fully explained (Carattoli 2003; Fluit 2005).

8. *SALMONELLA* ANTIMICROBIAL RESISTANCE AGAINST ANTIMICROBIAL CLASSES

Antimicrobial resistance develops when microorganisms develop such mechanisms to protect themselves from the antimicrobial drugs, used to cure the infection, induced by such pathogens (Cosby et al. 2015).

8.1. TETRACYCLINE

About 31 antimicrobials along with tetracycline approved by the United States of America in 1951, for use in broiler feed, deprived of any veterinary prescription for the animal growth and production and treatment of coccidiosis (Jones and Ricke 2003). Though, in the late 1960s, each European state passed its own National regulations for the antibiotics use in animal feed (Castanon 2007). Broilers that survived at 35 days of age, developed antibiotics resistance (Diarra et al. 2007). As an antimicrobial, tetracycline inhibits protein synthesis by preventing attachment of tRNA to A site of 30S ribosomal subunit. Salmonella isolates ascribed to the energy dependent efflux pump that potentially eliminates tetracycline from bacterial cells. Another mechanism of antimicrobial resistance reported in other bacterial spp., not in *Salmonella* (Chopra and Robberts 2001). Almost 32 genes are reported that are involved in tetracycline and oxytetracycline resistance such as *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)* etc. in *Salmonella* isolates. Among those genes, *tet(A)* placed within *Salmonella* genomic island (Carattoli et al. 2002), on integrons (Briggs and Fratamico 1999) and on transferable plasmids (Gebreyesm and Thakur 2005), *tet(B)* is also located on transferable plasmids (Guerra et al. 2002). These genes are simply relocated and spread between *Salmonella* isolates and generally considered important markers for identification of *Salmonella* infection (Carattoli et al. 2002). In the poultry sector, consumer opinions and demands are greatly concerned with animal welfare, food and environmental safety (Dibner and Richards 2005). So, consumer and policymakers acquire decreased use of antimicrobial growth promoters in animal feed for human health safety (Dibner and Richards 2005; Rahmani and Speer 2005).

8.2. SULFONAMIDES AND TRIMETHOPRIM

Both of these drugs are competitively inhibiting the enzymes, responsible for synthesis of tetrahydro folic acid (Alcaine et al. 2007). Sulphonamides are basically structural analogue of ρ-amino benzoic acid, involved in the synthesis of dihydrofolic acid that effectively prevent dihydrofolate synthetase in bacteria that confers the synthesis of folates (Duijkeren et al. 1994). Though sulfonamides are not effective in mammalian cells, mammalian cells directly uptake folate from food, not able to synthesize folates (Bushby 1980). While, trimethoprim inhibits dihydrofolate reductase (Mascaretti 2003). Since late 1960s, both of these drugs are bacteriostatic (Alcaine et al 2007), used in combination for bacterial infections. Sulphonamides and trimethoprim are broad spectrum antibiotics, used in the treatment of respiratory tract, alimentary tract, and urogenital tract, joint, skin and wound infections caused by Gram-positive and Gram-negative bacteria (Duijkeren et al 1994). Sulphonamides resistance among *Salmonella* isolates is due to sul gene, which is responsible for inactive dihydrofolate synthetase, instead, trimethoprim resistance ascribed to expression of dihydrofolate reductase that does not fix trimethoprim (Mascaretti 2003; Antunes et al. 2005).

8.3. BETA-LACTAMS

Carbapenems, penicillin and cephalosporins are three major sets of betalactam. Mechanisms of action of these antibiotics are facilitated by their ability to interact with penicillin binding proteins, mainly take part in the synthesis of peptidoglycan, present in bacterial cell walls. Generally, this group of medicine exhibits bactericidal activity but differs among organisms, penicillin binding proteins and beta lactams (Alcaine et al. 2007). Beta lactams move across the bacterial cell wall to approach penicillin binding proteins that are facilitated by OmpF and Omp C porins (Alcaine et al. 2007). Loss and change in porin concentrations are not considered as a way of resistance, but, decline in any one of these porin concentrations resulted in

increased beta lactam resistance against cephalosporin, cefoxitin and ampicillin etc. (Alcaine et al. 2007). Beta lactams are the broad-spectrum antibiotics against Gram positive and Gram-negative bacteria. As far as *Salmonella* is concerned, bactericidal activity is observed due to inhibition of penicillin binding proteins (Angulo et al. 2000). Resistance exhibited by excretion of beta lactamases into periplasmic fluid for Gram-negative bacteria, and into the environment for the Gram-positive bacteria. Beta lactamases hydrolysed the beta lactam ring into beta amino acid, reported with no antimicrobial activity. Plasmid carries the genes for beta-lactamase production (Mascaretti 2003). Resistant *Staphylococcus aureus* has serious health issues due to Staphylococcus resistance to methicillin (Pray 2008). Later on, in the emergence of these beta lactam resistance, six-member ringed cephalosporin and five-member ringed, without sulfur carbapenems, developed. Though, these antibiotics are prescribed in treatment of acute otitis media in United States (Arrieta 2003; Cosby et al. 2015).

8.4. PHENICOLS

Chloramphenicol are the broad-spectrum antibiotics against Gram-positive and Gram-negative bacteria. Additionally, it can move across the blood brain barrier, so effective in systematic infections (Alcaine et al. 2007). But, due to widespread resistance and toxicity limited in use prescribed other than developing countries. In *Salmonella* isolates it showed resistance by two mechanisms; removal of drug through efflux pump and enzyme inactivation of O-acetyltransferase (Cannon et al. 1990). Both of these processes are reported to be effective in chloramphenicol resistance in *Salmonella* serotypes, especially in *Salmonella typhimurium* and *Salmonella agona* (Schwarz and Chaslus-Dancla 2001). Later on, florfenicol was developed and approved by FDA in 1996 for treatment of bovine respiratory pathogens but not for use in humans (White et al. 2000). Usage of florfenicol in animal farming was envisioned to reduce resistance to chloramphenicol in humans. However, chloramphenicol was excluded in Europe (1994) for animal treatment and florfenicol was permitted for veterinary use in 1995 in France (Arcangioli et al. 1999).

8.5. QUINOLONES AND FLUOROQUINOLONES

Nalidixic acid, is a synesthetic bactericidal drug, was first approved quinolone (Mascaretti 2003). These antibiotics target DNA gyrase and DNA topoisomerase IV (Wolfson and Hooper 1989), the actual mechanism is still complicated and not completely stated (Mascaretti 2003). Though, limited salmonella isolates are resistant to nalidixic and low-grade resistance to other quinolones (Molbak et al. 1999; Breuil et al. 2000). High grade resistance to quinolones is rare (Olsen et al. 2001; Casin et al. 2003). However, *Salmonella* show resistance in two ways; Target mutation to quinolone resistance determining region of gyr A, gyr B and in the par C subunit of topoisomerase IV (Cloeckaert and Chaslus Dancla 2001; Baucheron et al. 2004). Another mechanism related to alteration in the expression of AcrAB-TolC efflux system through mutations in the genes encoding system regulators that results in over-expression of the efflux system and reduce quinolone sensitivity (Baucheron et al. 2004; Oliver at al 2005). In short, not a single mutation exhibits quinolone resistance, it is the result of multiple mutations (Heisig 1993). Early fluoroquinolone was first licensed for human therapy, no resistance reported. Later, as far as licensing of fluroquinolone to animal use was authorized, rate of fluroquinolones resistant *Salmonella* in humans and animals and then in food infection vigorously increased in different countries (WHO 2011). Danofloxacin, orbifloxacin, enrofloxacin, sarafloxacin, difloxacin, and marbofloxacin were six approved fluroquinolones for animal use in United States (Martinez et al. 2006). Afterwards, enofloxacin and sarafloxacin, authorized for treatment of respiratory diseases in poultry, have been excluded from approved list,

because of amplified antibiotic resistance to Campylobacter and *Salmonella* spp. improved in human illness (Nelson et al. 2007).

9. CHICKEN- MEAT OR EGG ARE THE CARRIERS OF SALMONELLOSIS TO HUMANS

Salmonella contaminated animal-based products induce 3% worldwide food borne diseases with approximately 80 million infections (El-Saadony et al. 2022). Salmonellosis is the disease condition associated with pathogenic bacteria *Salmonella*, causing severe damage to the poultry sector all over the world. Though, it is a zoonotic bacterium which can be transferred from animals to humans. The transmission can occur by following different ways.

- An infected animal direct contact
- By consuming or handling contaminated animal products like raw meat or eggs from turkey and chicken
- Interaction with contaminated equipment or with infected vectors such as pets or insects.

However, raw chicken products or frozen chicken meat and also eggs of backyard hens are the amplest cause of animal facilitated *Salmonella* infections in humans.

Infective dose of salmonellosis for humans ranges from 10^4 to 10^6 cells or more, or it could be low as 10^1 to 10^2 cells in a low immunity person, or, a human consumes contaminated high fat matrix food like cheese, chocolates, peanut butter, pizza etc. However, *Salmonella enterica* is a documented serovar of *Salmonella*, causing infection in humans based on raw poultry and poultry meat products, well known for economic and public health implications. It's a zoonotic pathogen that can readily pass from animals to humans, while consuming contaminated meat, animal-based products or other food products contaminated with animal fecal material. The infection spread either through direct or in direct contact with colonized birds or by consuming contaminated water (Lammerding 2006; Chai and Mohan 2011). The main niche of *Salmonella* species is the gastrointestinal tract of humans and farm animals, reptiles, birds, fish, amphibians and shellfish (Heinitz et al. 2000; Bailey et al. 2010; Machado et al. 2020). No doubt, fecal contamination is the major source of water and food contamination plays a key role in the spread of salmonellae in the environment and ultimately, the food supply chain. In such a way, meat animals became infected and became a carrier or reservoir of salmonellae.

10. EFFECT OF SALMONELLOSIS ON HUMAN HEALTH

Salmonellosis is one of the most common foodborne infections. The genus Salmonella comprises 2600 serovars, among them, more than 100 because of a cause of infection in humans (CDC 2020). In humans, salmonellosis was exhibited by typhoid fever. Common signs and symptoms of typhoid fever includes high fever, cough, malaise and headache (Fig. 1).

Among humans, the most common serovar is *Salmonella enteritis*, causing typhoid fever or enteric fever. *Salmonella* is motile Enterobacteriaceae that produce variety of GIT infections ranging from simple fever diffuse abdominal pain, and constipation however, untreated typhoid leads to intestinal hemorrhage, delirium, bowel perforation, obtundation and death within 1 month of progression of disease (Christie 1987; Butt et al. 2022). Patients may also suffer with short-term or long-term neuropsychiatric issues. Typhoid is derived from ancient Greek word means, cloud, indicating the prolonged severity of the disease related to long-lasting neuropsychiatric effects among the untreated. About 21 million people in the year suffer from typhoid fever in the World. With time, it has developed resistance to antibiotics. In the year 2016, Multi Drug Resistance (MDR) was reported in Pakistan. Though, only three classes of antibiotics, carbapenems, tigecycline and azithromycin are effective against *Salmonella* serovars (Butt et al. 2022).

Salmonella enters the GIT through phagocytic cells, which then present to macrophages of lamina propria. Macrophages recognize pathogen associated molecular patterns (PAMPS) such as lipopolysaccharides and flagella through their toll like receptor TLR-4, TLR-5/CD-14/ MD2 complexes. In this way, intestinal cells and macrophages mobilize neutrophils and T-cells with interlekin-8. At this stage, inflammation is quiet enough to suppress the infection (Parry et al. 2002; Raffatellu et al. 2006). By distal ileum, *S*. *typhi* and *paratyphi* enter the host system with the help of specialized fimbriae that support to adhere to the epithelium over clusters of lymphoid tissue in the ileum, commonly known as Peyer Patches, main entrance for macrophages to enter into lymphoid system from Gut. Once bacterium enters, induce host macrophages to attract more macrophages (Raffatellu et al. 2006). Additionally, these serotypes are able to produce Quorum sensing, intracellular communication through organism coordinate swarming and produce biofilm (Rana et al. 2021).

Later, typhoid introducing *Salmonella* adopt macrophages cellular machinery for their reproduction (Ramsden et al. 2007), by mesenteric lymph nodes to thoracic duct, lymphatics that leads to reticuloendothelial tissues of the spleen, liver, lymph nodes and bone marrow. As they approach, and establish a favorable environment, they start to flourish by continuing multiplications. This way, they allow *Salmonella* to enter into the bloodstream (Parry et al. 2002).

Though, bacterium infect gallbladder either *via* bacteremia or direct extension of infected bile, reverse back to GIT in the bile and again infect Peyer Patches. However, bacteria were not able to infect the host shed in the stool and became a carrier to infect others (Christie 1987; Parry et al. 2002). So, bacterium shed by a single carrier may have numerous genotypes, quite hard to follow an outbreak of its origin (Chiou et al. 2013).

Typhoid causing *Salmonella*, generally have non-human vectors, even though an inoculum as small as 100,000 germs of typhi able to infect 50% of healthy person (Levine et al. 2001). Besides this, Paratyphi requires much higher volume to infect, less pervasive in rural areas, easily transmitted through street foods and provides a micro-friendly environment to bacterium. In short, their route of transmission is slightly different from one another, for typhoidal *Salmonella* generally modes of transmission are;

- Oral transmission through contaminated water
- Oral transmission *via* food/ beverages handles by infected person
- Hand-to-mouth transmission after using unhygienic commode and wash basin (Earampamoorthy et al. 1975; Ali et al. 2006; Ram et al. 2007)

Typhoidal *Salmonella* have the ability to survive in the low pH (Fig. 2), as much as 1.5. Medicines and procedures like antacids, H2 blockers gastrectomy, proton-pump inhibitors and achlorhydria reduce stomach acidity, which helps to induce *S. typhi* infection (Parry et al. 2002).

11. METHODS TO PREVENT *SALMONELLA* OUTBREAK

Poultry signifies a natural habitat for *Salmonella* and Campylobacter. Though, twice of them are commonly found in GI tract of and considered as commensal bacterium. Best exercise to control *Salmonella* is in live poultry production. So, the best strategic plan for Salmonella control is anticipation in birds based on three main facts:

- By following effective hygiene measures for the prevention of incidence of *Salmonella* into the farm/flock
- By controlling the spread of pathogens with in the farm/flock
- By accompanying prophylactic procedures to recover immune resistance of animals against pathogenic bacterium

To follow these key steps, need to manage or organize short footnotes;

11.1. PROVIDE HEALTHY ENVIRONMENT

Dead animals and Broken eggs (potential source of infection) should be immediately cleared. Troughs must be clean from dropping. Poultry house should be fumigated before restocking. Clean water and feed are very much essential; feed should be stored, kept dry and protected from rodents and pets, while drinking water flow rate should be sufficient to offer the birds with adequate amounts of water, but not too much that the floor becomes wet.

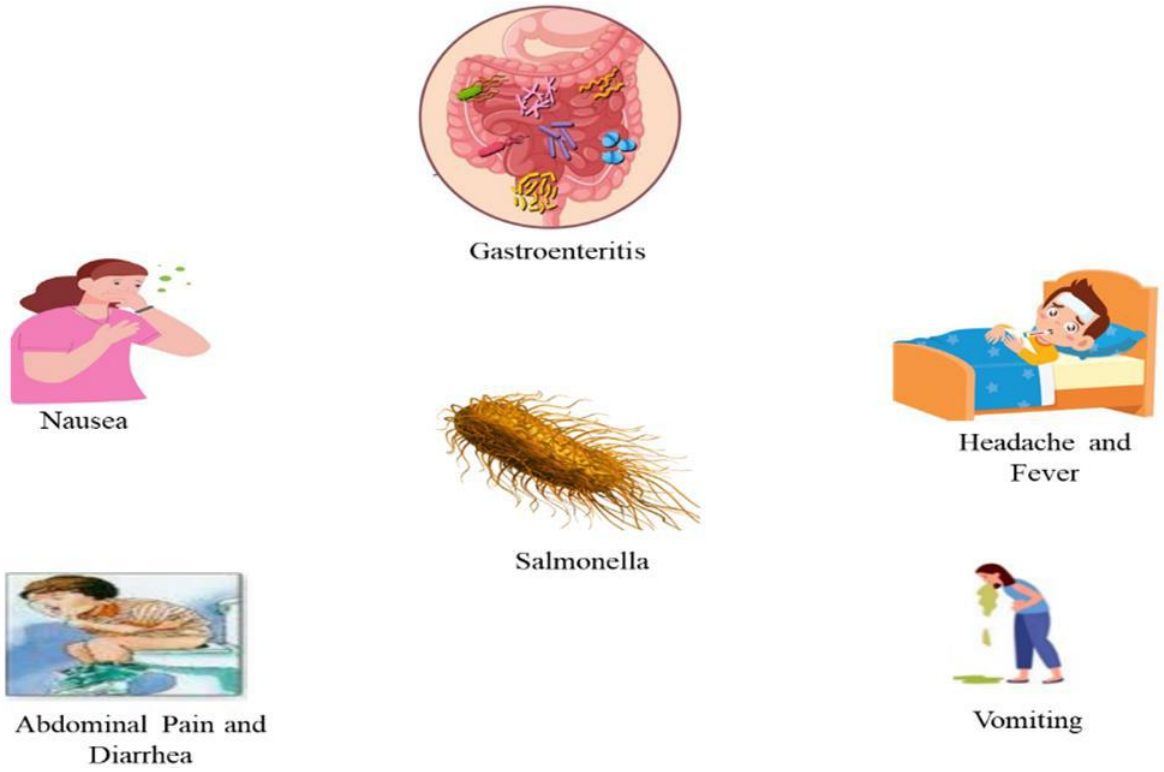

Fig. 1: Clinical manifestation of Human Salmonellosis.

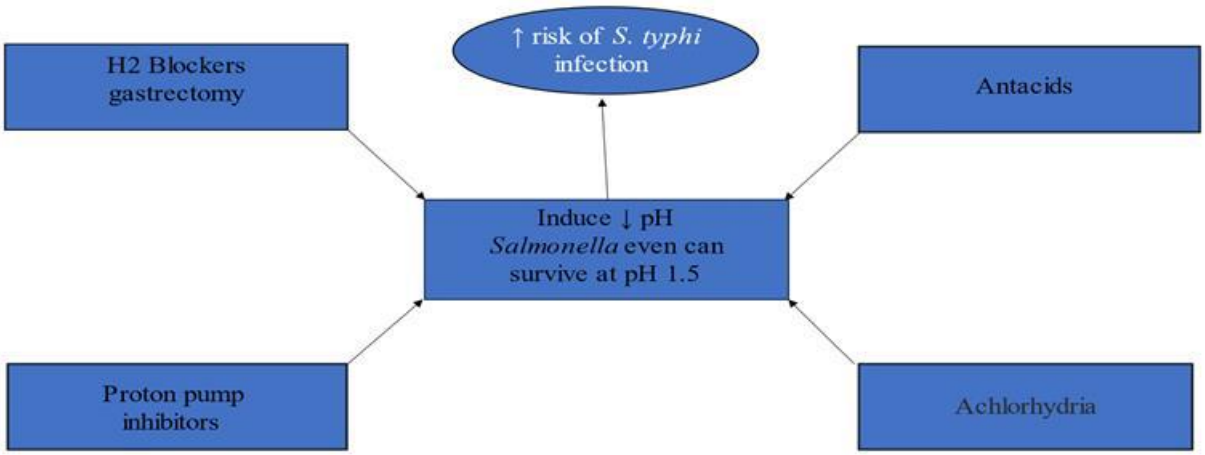

Fig. 2: Typhoidal Salmonella survival in reduce stomach activity.

11.2. LITTER SHOULD BE DRY

Used litter should be removed regularly after specific time material. Well absorptive material such as straw granulates and wood shaving for litter recommended. Adequate water supply granted; excess water supply wet the litter. Birds must be monitored for diarrhea to avoid wet droppings.

11.3. MINIMIZE THE CONTACT

Only a few people were allowed to visit the flock in order to reduce the spread of *Salmonella*. Instruments and wear clothes should be entirely used for the poultry house (Heinzl, 2022) Other than this strategic plan, there are various approaches used to avoid the incidence of Salmonellosis in poultry such as;

11.3.1. CHITOSAN

Chitosan is a polysaccharide, with diverse medical application present in the hard outer skeleton of lobsters, shrimp and shellfish (Attia et al. 2022). Chitosan molecules cause metal ion chelation and vital nutrients of bacteria decrease bacterial growth (Rabea et al. 2003). *In vitro* studies reported that chitosan exhibit antibacterial activity against *S. aureus* and *S. paratyphi* (Islam et al. 2011). Chitosan is the hard sugar-induced host immune modulating response against pathogens (Lee et al. 2009).

11.3.2. NANOPARTICLES

Nanomaterials seek more attention in this era due to their exceptional physical and chemical characteristics (Yousry et al. 2020; Reda et al. 2021; Salem et al. 2021). Zinc oxide nanoparticles (3mg/g) along with poultry ration found effective against *S. typhimurium* and *S. aureus* (De Silva et al. 2021). Similarly, incorporation of gold nanoparticles exhibited antibacterial activity against *S. typhimurium* (Reda et al. 2021). Silver nanoparticles along with rosemary extract revealed antibacterial effects against *E. coli, S. enteritidis* and *S. typhimurium* (Mohamed et al. 2017).

11.3.3. PHYTOCHEMICALS

Plants produce phytochemicals as secondary metabolites to protect themselves from bacteria, yeast, and mold infection. Extraction and purification of such phytogenic substances, found effective against *Salmonella* in poultry. Each phytochemical has its own mode of action, due to diverse phytochemical nature and mechanism of such phytogenic substance, considered as no resistance develop, but lately *S. aureus*, *E. coli*, *E. faecalism,* and *S. typhimurium* showed resistance to sum components of herbal drugs (Khan et al. 2009). Phytochemicals are hydrophobic in nature, *Salmonella*- Gram negative bacteria are less susceptible to such phytochemicals, because only hydrophilic solutes can pass through the bacterial cell wall. Though, invasion inside the cell could be enhanced by mixing phytochemicals with an emulsifier, but efficacy depends upon the chemical composition of these phytochemicals (Heinzl 2022).

11.3.4. ESSENTIAL OILS

Essential oils are the volatile, aromatic, and oily compounds extracted from different parts of the plant (Abd El-Hack et al. 2022). Such beneficial oils are potent digestive stimulants, hypolipemic

agents, immunostimulants, antifungal, antibacterial growth promoter subtances that generate positive effects on egg production and broiler performance (Nahed et al. 2022). *Cymbopogon citratus* (lemon grass) oil extract showed antibacterial activity against *S. enterica* and *S. typhimurium* (Alagawany et al. 2021). Similarly, eugenol, carvacrol, trans-cinnamaldehyde and thymol have effective antibacterial effects against *Salmonella* and Campylobacter in layer and broiler chicken (Johny et al. 2010).

11.3.5. PREBIOTICS AND PROBIOTICS

Prebiotics are not easily digestible substances consumed by valuable microbiota in the intestine (Yaqoob et al. 2021). Fructooligosaccharide incorporation in poultry ration as prebiotic improves growth performance, enlarges small intestinal villi length and enhances bacterial colonization in broilers (Xu et al. 2003). Addition of fructooligosaccharide increases the production of short chain fatty acids that alters gut microbiota towards beneficial bacteria that is causative to growth of pathogenic *Salmonella* and improves innate and immunological responses in broiler chicken (Shang et al. 2015).

Probiotics are the living microorganisms that help in development and growth of the host when administered in suitable concentrations (Mack 2005). Such beneficial bacteria generate bactericidal and bacteriostatic chemicals in the intestine which are toxic to pathogens, such as, in *vitro* studies revealed that Lactobacillus spp. ferment saccharides and produce lactic acid that lowers intestinal pH and inhibits pathogenic bacterial growth like *S. typhimurium* and *E. coli* (Abd El-Hack et al. 2021). This phenomenon is approved in *in vivo* studies, as subset of fatty acids (SCFs) like acetate propionate and butyrate amplified, occurrence of *E. coli* and *Salmonella enterica* reduced in broiler cecum (Van Der Wielen et al. 2000).

In the start of 20th century, the life style is quiet change, life is too busy and hectic schedule tend people to eat outdoor cooked food from restaurants and hotels. Most potently, in developing countries, the food hygiene is compromised, in such a way, these restaurants and hotels are common places for the onset of an outbreak (Spackova et al. 2019). By adopting hygiene conditions, proper cleaning of dishes and washing hands of the employees, storage of food in an appropriate manner and cooking food at adequate temperature can reduce the risk of Salmonellosis (Ibram et al. 2007; Appling et al. 2018). So, poultry and meat farmers need to maintain food security. Though, prevalence of *Salmonella* and virulence of strain is equally important. Eggs are also another source of *Salmonella* infection. Although, different methods are implemented these days for decontamination of eggs like freeze drying, pasteurization, hot air and microwave heating (Oscar 2020; Keerthirathne et al. 2017). But with these techniques the properties of eggs may be affected. For immune compromised individuals or for vulnerable patients microbiologically decontaminated eggs are recommended. Drinking water is also an important source for the spread of infection. Proper storage and supply of clean filtered water to the public is the necessary step, should be taken by the Government and authorities to avoid the risk of Salmonellosis. Besides this, demanding hygiene practices required to be implanted in the food preparation and processing industry, around the environmental controls in ready to cook and convenient food production.

12. CONCLUSION

In conclusion, zoonosis *Salmonella* engenders high cost in the poultry sector. With increasing population and inflation rate, protein demands are mainly focused on the poultry industry. As Salmonellosis is transferred from animal to human *via the food* chain, it needs to be controlled by all means. Antibiotics

are the drugs to combat such pathogens but emerging MDR strains of *Salmonella* are still a potential risk to public health locally and beyond. In this way, implementation of more rigorous preventive and control measures is necessary, because surveillance has major concern and impact. Along these antibiotics, poultry producers need to seek, active but not, resistance generating natural solutions or feed additives against *Salmonella* for further development.

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Role of Escherichia coli, Staphylococcus, Salmonella and Brucella Species in Spread of Antimicrobial Resistance Across Species

09

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ABSTRACT

Antimicrobial Resistance (AMR) has emerged as a critical global concern in the 21st century, presenting a "Quiet Pandemic" with escalating infection occurrences and a dearth of novel antimicrobial drugs. This paper explores the causes, prevalence, and regional implications of AMR, focusing on the One Health Approach initiated by international organizations like OIE and FAO. In Pakistan, a national action plan has been implemented to address AMR. The causes of AMR include the unnecessary use and misuse of antimicrobials, inappropriate prescribing patterns, and the lack of new novel antibiotics. The global prevalence of AMR is particularly high in sub-Saharan Africa and low- to middle-income countries, with specific concerns about colistin resistance mediated by MCR-1 in China. Insufficient public understanding of antimicrobials is also identified as a significant factor contributing to AMR. A comparative study between the UK and India highlights variations in public awareness, potentially impacting the incidence of infectious diseases. Vigilant monitoring of AMR-related infections and mortality rates is crucial, with projections indicating a potential rise in global mortality by 2050. The roles of key bacteria, including Escherichia coli, Staphylococcus aureus, Salmonella, and Brucella, in the spread of AMR are discussed. E. coli, for example, harbors resistance genes from both human and animal sources, posing a threat to both populations. Similarly, the emergence of methicillin-resistant Staphylococcus aureus (MRSA) complicates treatment, while Salmonella contributes to global foodborne illnesses.

Keywords: Antimicrobial Resistance, One Health Approach, Global Prevalence, Public Awareness, Regional Perspectives

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1. INTRODUCTION

Antimicrobial Resistance (AMR) arises when microorganisms, including bacteria, fungi, parasites, and viruses, undergo evolutionary changes that lead to their reduced susceptibility to the antimicrobial drugs, such as antibiotics, that are commonly employed for their treatment (Nabil et al. 2022). In the 21st century, AMR has elevated to become a paramount global apprehension, primarily due to the swift escalation of AMR infection occurrences and the scarcity of novel antimicrobial drugs being introduced to address this global challenge (Prestinaci et al. 2015). AMR is commonly labeled the "Quiet Pandemic," demanding immediate and more efficient measures for management rather than being regarded as a concern that can be addressed in the future (Founou et al. 2021). In the absence of preventive actions, projections suggest that by 2050, Antimicrobial Resistance (AMR) might emerge as the leading global contributor to mortality (O'Neill 2016). In light of AMR, numerous international health organizations and governments have initiated measures to address this concern. The "One Health Approach" was established, necessitating a worldwide collaborative endeavor involving a diverse array of disciplines. This includes organizations like the World Organization of Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), which make sure that each organization operates within its area of competence while working with others to lessen the potential effect of AMR. The Ministry of National Health Services announced the release of Pakistan's national action plan (NAP) for AMR in May 2017. Based on the NAP, strategies have been initiated on a national and provincial scale in Pakistan (Saleem et al. 2022).

2. CAUSES OF AMR

2.1. UNNECESSARY USE OR MISUSE OF ANTIMICROBIALS

When we make misuse of antimicrobials there will be chances of development of resistant bacteria and as a result there will be limited treatment options (Abushaheen et al. 2020).

2.2. INAPPROPRIATE PRESCRIBING PATTERNS

When we prescribe inappropriate prescriptions there will be chances of complications and mortalities as well as increased healthcare costs (Darkwah et al. 2021).

2.3. LACK OF NEW NOVEL ANTIBIOTICS

When we use excess antimicrobials, it reduces effectiveness of other treatments and produces global public health impact and as result lack of new novel antibiotics (Udaondo and Matilla 2020).

3. GLOBAL PREVALENCE OF AMR

As a recent study on the global burden of bacterial antimicrobial resistance in 2019 showed, the AMR burden in that year was highest in sub-Saharan Africa and higher in low- and middle-income countries (LMICs) than in East Asia, Australasia, and Western Europe, with species of One Health importance in their transmission accounting for the highest attributable mortality (Murray et al. 2022).

According to Chinese research, the establishment of colistin resistance mediated by MCR-1 poses a danger to antimicrobial resistance (AMR), especially because zoonosis might result in the spread of this resistance gene from animals to people (Ben et al. 2019). Based on this research, the presence of MCR-1 was identified in E. coli isolates obtained from both animals and commercially available meat sources. MCR-1 is suspected to have experienced extensive transmission among food-producing animals in southern China. Conversely, the detection rate of MCR-1 was notably lower in samples of human origin. This variance in the prevalence of MCR-1 between animal and human populations suggests a potential avenue for zoonotic transmission originating from animals and affecting humans (Hu et al. 2021).

Insufficient understanding of antimicrobials also plays a significant role in the emergence of AMR. In 2017, Public Health England conducted a survey in the UK to assess the public's awareness and comprehension of antibiotics (Parveen et al. 2022). A total of 83% of respondents acknowledged the appropriateness of antibiotics for bacterial infections, while 35% of participants held the belief that antibiotics could effectively combat viral infections. These results indicate improvement over the last poll from 2014, indicating that the general population in the UK has grown awareness and understanding about the appropriate use of antibiotics (Parveen et al. 2022). In comparison to an Indian poll, it was found that 49% of participants thought antibiotics might treat viral illnesses, whereas 45% of respondents used medicines to treat cold symptoms (Niyomyart et al. 2023). As a result, India's incidence of infectious diseases, including those attributed to multi-resistant pathogens, was noted to be among the highest (Torumkuney et al. 2022). These studies suggest a correlation between antimicrobial resistance (AMR) and the level of public awareness.

As the emergence of AMR accelerates, vigilant monitoring of infection and mortality rates associated with AMR remains a priority. The projected incidence of AMR-related illnesses in the United Kingdom increased from the 61,946 patients reported in 2018 to 65,162 diagnosed cases in 2019 (Moser et al. 2018). On the other hand, the European Centre for Disease Prevention and Control (ECDC) has reported that the yearly incidence of AMR-related illnesses has topped 670,000 cases in the European Union alone (Mestrovic et al. 2022). According to the results of a previous study's data analysis, 1.27 million of the estimated 4.95 million global fatalities in 2019 that were linked to bacterial antimicrobial resistance (AMR) were directly attributable to bacterial AMR (Murray et al. 2022). It was previously highlighted in a well-known estimate that the projected yearly mortality rate directly attributable to AMR is expected to reach 10 million by 2050. The regions most affected by this prediction are expected to be Asia and Africa, primarily due to their substantial populations and limited regulatory measures pertaining to the prevention of AMR (Tang et al. 2023). Based on earlier studies, the Sub-Saharan Africa region experiences the highest overall mortality rate across all age groups attributed to conditions directly associated with or connected to Antimicrobial Resistance (AMR). In contrast, Australasia exhibited the lowest rate of mortality linked to AMR-related factors in the year 2019 (Tang et al. 2023). In Pakistan a survey of 411 students, only 6.3% had undergone antimicrobial resistance (AMR) training. 16.1% of students believed that antibiotics are effective for viral ailments. More than half of the students agreed that AMR is a major healthcare problem in Pakistan (65.9%). Most students viewed poor infection control practices (66.9%), the use of too many broad-spectrum antibiotics (68.4%) for a longer duration (62.8%) with inadequate doses (67.9%) as the causes of AMR (Hayat et al. 2022).

Recent research examining antibiotic accessibility in low and middle-income nations revealed that Vietnam and Bangladesh exhibited the highest ratio of unlicensed establishments dispensing antibiotics. These essential medications were commonly present in conventional drug stores for minor ailments, making them easily accessible to the general population (Pulingam et al. 2022). Given the convenient availability of antibiotics within these communities, persisting with this approach could result in various challenges. These challenges encompass inappropriate antibiotic utilization due to a general lack of comprehension regarding antibiotics and limited awareness about antimicrobial resistance (AMR),

compounded by inadequate consideration of the antibiotics' quality. Such factors collectively have the potential to give rise to the development of AMR.

Table 1: A brief overview of losses because of antimicrobial resistance (AMR)

Sr.	Source	Region	DATA of AMR	Reference
1.	ECDC Report	European Union	Over 670,000 cases of AMR infection annually	(Mestrovic et al. 2022)
2.	Data Analysis of 204 countries (1990-2019)	Global	4.95 million deaths worldwide linked to bacterial AMR in 2019 1.27 million deaths directly caused by bacterial AMR in 2019	(Murray et al. 2022)

ECDC; European Centre for Disease Prevention and Control.

Research on this matter presents contradictory results, with a recent study revealing that China, classified as a developing nation, emerged as the leading consumer of veterinary antimicrobials in 2017. This consumption constituted approximately 45% of the global total and is projected to maintain its position as the largest user by the year 2030. Furthermore, there is substantiating evidence indicating that developed nations have witnessed a decline in their overall antimicrobial sales. For instance, the United Kingdom experienced a notable reduction of 39.2% from 2015 to 2017 (Tiseo et al. 2020). These findings indicate that the risk factors observed in emerging countries are starting to converge with those seen in developed nations.

Children from Haiti, Kenya, Malawi, Namibia, Nepal, Senegal, Tanzania, and Uganda participated in the research study, which lasted from May 2006 to December 2016. It was found that antibiotics were prescribed to 80.5% of the children with respiratory illnesses, 50.1% of the children with diarrhea, and 28.3% of the children with malaria. For children between the ages of one and five, the average number of antibiotic prescriptions written was 24.5 (95% CI 22.6-26.7), with differences ranging from 7.1 (6.3-7.9) in Senegal to 59.1 (54.1-64.6) in Uganda (Fink et al. 2020).

In North Africa, the Middle East, and South Asia, there were notable surges in the utilization of fluoroquinolones and third-generation cephalosporin's (Mestrovic et al. 2022). In the high-income region, the utilization of carbapenems exhibited the highest levels, rising from 0.05 to 0.09 Defined Daily Doses (DDD) per 1,000 individuals per day between the years 2000 and 2018 (Tang et al. 2023).

As indicated by recent studies, there has been a notable 67% rise in the utilization of final-resort antibiotics like polymyxin among individuals within the European Union/European Economic Area (EU/EEA). Typically administered within hospital environments, these ultimate-line antibiotics serve as a final option for treating individuals grappling with severe infections, particularly in cases involving multidrug-resistant bacteria.

4. MECHANISM OF ACTION OF ANTIMICROBIAL RESISTANCE

Figure 1 shows the mechanism of antimicrobial resistance.

5. ROLE OF ESCHERICHIA COLI IN AMR SPREAD

E. coli holds a unique position in microbiology, causing serious infections in humans and animals while also being a substantial component of diverse host microbiomes (Riley 2020). "Colibacillosis" refers to an ailment resulting from the bacterium E. coli, typically present in the lower intestines of warm-blooded mammals. Intestinal pathogenic E. coli variants induce gut disorders, spanning mild diarrhea to colitis, while extra-intestinal pathogenic E. coli variants, initially residing in the gut, trigger diseases in distant body sites post-migration, like the urinary tract or bloodstream. Among animals, E. coli ranks among the

primary contributors to diarrhea, often accompanied by pathogens like Rotavirus, Coronavirus (Tchatchouang 2017). Cryptosporidium parvum, or a synergistic blend of these agents. ETEC (Enterotoxigenic E. coli) impacts a range of animal species, primarily affecting young animals including food-producing ones (such as piglets, newborn calves, and chickens) as well as companion animals like dogs. Diarrhea is a significant livestock ailment capable of spreading among animals, potentially resulting in substantial consequences for the entire herd or flock. E. coli harbors numerous genes from both human and animal sources that provide resistance to β-lactams, including blaTEM-1, which is prevalent in animal origin E. coli but encodes for narrow-spectrum β-lactamases targeting penicillin and aminopenicillins. Clinically significant in veterinary medicine, ESBL (Extended-Spectrum β Lactamase) producing E. coli strains confer resistance to a range of antibiotics, including penicillin, aminopenicillins, and various cephalosporin, approved for veterinary use (Christopher 2023).

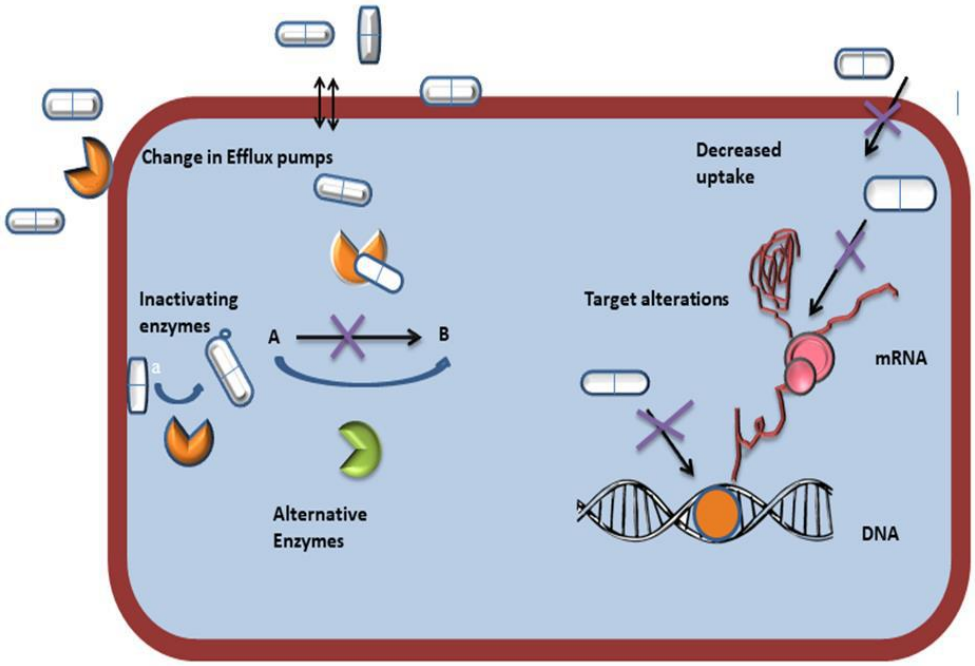

Fig. 1: Antimicrobial Resistance mechanism.

While class A ESBL enzymes are frequently responsible for acquired resistance to broad-spectrum cephalosporins in E. coli, AmpC-type enzymes, classified as class C β-lactamases, also contribute to substantial resistance against these antimicrobial agents. Quinolones and fluoroquinolones, vital for treating infections in humans and animals, exhibit broad bactericidal activity; resistance typically results from mutations in DNA gyrase and topoisomerase IV, the drug targets. Fosfomycin targets the MurA enzyme, crucial for peptidoglycan synthesis, and is employed in veterinary medicine to combat infections from various Gram-positive and Gram-negative pathogens, including E. coli. Sulfonamide resistance in E. coli from animals results from sul genes, namely sul1, sul2, or sul3 (Singh et al. 2023).

6. ROLE OF S. AUREUS IN AMR SPREAD

Methicillin-resistant Staphylococcus aureus (MRSA) is a common nosocomial and community-associated pathogen that can colonize or cause infections in both human and animals. S. aureus is a Gram-positive

bacterium and causative agent of wide range of infectious diseases such as skin infections, bacteremia, endocarditis, pneumonia, and food poisoning (Yildiz 2022). The organism was originally a leading nosocomial pathogen and afterwards epidemiologically distinct clones emerged in community settings. S. aureus expresses number of virulence factors which help to establish infection by facilitating tissue attachment, tissue invasion and evading from host immune response. The ability to acquire resistance to multiple antibiotics classes makes S. aureus, a challenging pathogen to treat. Emergence and spread of S. aureus strains which are resistant to methicillin, referred to as methicillin-resistant S. aureus (MRSA) resulted in high morbidity, high mortality, and increased treatment costs (Willis et al. 2022).

Table 2: Antimicrobial resistance in *E. coli* represented against various classes of antibiotics

Antibiotic Class	Specific Antibiotic	Resistance in *E. coli*	Reference
Beta-lactams	Ampicillin	Commonly resistant due to beta-lactamase production	(Peng et al. 2022)
	Amoxicillin	Often resistant due to beta-lactamase	(Góchez et al.
	Ceftriaxone	Increasing resistance, especially in some strains	2019)
	Carbapenems	Increasing resistance, especially in healthcare settings	(Davies and
Fluoroquinolones	Ciprofloxacin	Increasing resistance, especially in urinary tract infections	Wright 1997)
	Levofloxacin	Similar resistance pattern to ciprofloxacin	(Kenyon 2021)
Aminoglycosides	Gentamicin	Varies, some strains may exhibit resistance	
	Amikacin	Generally less resistance compared to other classes	
Tetracyclines	Tetracycline	Varies, some strains may be resistant	
Sulfonamides	Trimethoprim/ sulfamethoxazole	Varies, resistance can be common	
Nitrofurans	Nitrofurantoin	Generally still effective for uncomplicated UTIs	

Table 3: Host Spectrum of *E. coli*:

E. coli Species	Host Spectrum	Reference
E. coli K-12	Primarily found in humans and mammals	
	Used as a model organism for research	
E. coli O157:H7	Mostly associated with cattle and other ruminants	
	Causes foodborne illnesses in humans	
E. coli B	Isolated from humans and mammals	(Cao et al. 2015)
	Used in various laboratory applications	
E. coli C	Found in humans and some mammals	(Rangel et al. 2005)
	May cause urinary tract infections	
E. coli Nissle 1917	Isolated from human intestines	
	Used as a probiotic for gastrointestinal health	
E. coli DH5α	Commonly used in molecular biology and genetics	
	Easily manipulated for cloning experiments	
E. coli BL21	Widely used in protein expression and biotechnology	
	Not typically associated with a specific host	

Vancomycin remained gold standard drug to tackle these strains for years but the emergence of resistance restricted its clinical utility. Newer anti-MRSA antibiotics which were approved by U.S FDA came as respite for clinicians (Phillips-Jones and Harding 2018). The bacterium typically colonizes the anterior nares, mucous membrane, and skin, and can be carried asymptomatically. However, it can penetrate into deep tissues and other normally sterile sites in the body when cutaneous and mucosal barriers are disrupted (e.g., due to wounds, invasive medical devices, or chronic skin conditions). It is well recognized that S. aureus is a multispecies pathogen, able to cause disease in humans and livestock. The diseases in livestock include mastitis in cows, goats, sheep and rabbits, skin infections in pigs and rabbits and invasive infections in chickens.

Table 4: Medicine Resistance to *Staphylococcus aureus*:

Antibiotic Class	Specific Antibiotic	Resistance in *Staph aureus*	Reference
Beta-lactams	Methicillin	Methicillin-resistant *S. aureus* (MRSA)	
	Oxacillin	MRSA	
	Penicillin	High resistance due to beta-lactamase	
Glycopeptides	Vancomycin	Rare resistance, but emerging cases of vancomycin-resistant MRSA	
	Teicoplanin	Generally effective against MRSA	
Macrolides	Erythromycin	Variable resistance, especially in community-acquired MRSA	(Mwangi et al. 2013)
	Clarithromycin	Similar resistance pattern to erythromycin	
Fluoroquinolones	Ciprofloxacin	Increasing resistance, especially in healthcare settings	
	Levofloxacin	Similar resistance pattern to ciprofloxacin	
Aminoglycosides	Gentamicin	Variable resistance, depending on strain and location	(Yao et al. 2019)
	Amikacin	Generally less resistance compared to other classes	
Tetracyclines	Tetracycline	Varies, some strains may exhibit resistance	
Sulfonamides	Trimethoprim/ sulfamethoxazole	Varies, resistance can be common	

7. ROLE OF SALMONELLA IN AMR SPREAD

Salmonella, a genus-wide species with global health significance, is a primary contributor to foodborne illnesses, resulting in numerous fatalities worldwide (Shang et al. 2023). Salmonella, a Gram-negative, rod-shaped bacterium found within the Enterobacteriaceae family, encompasses two species: Salmonella enterica and Salmonella bongori. This diverse group includes over 2600 S. enterica serovars, many of which have the potential to cause diseases in both humans and animals (Zahra et al. 2023) while certain Salmonella variants like Salmonella gallinarum (SG) and Salmonella pullorum (SP) lack flagella and motility, most Salmonella strains exhibit motility through peritrichous flagella. The presence of SG and SP in poultry is associated with clinical illness and substantial economic impacts on poultry farming, particularly in less developed nations (Syed Abu Thahir et al. 2023). Based on recent data from the United States, Europe, and Low- and Middle-Income Countries (LMICs), Salmonella is a prevalent global source of foodborne illnesses, contributing to widespread food contamination across various natural settings (Shakeel et al. 2023). Salmonella enterica, frequently located in the gastrointestinal tract of food animals, displays persistence through chronic carriers who eliminate the bacteria via their fecal waste. Consequently, these carriers serve as a source for subsequent bacterial contamination, enabling the transmission of Salmonella through contaminated dairy, meat, eggs, and other agricultural products cultivated using manure contaminated with the bacterium (Manyi-Loh and Lues 2023).

Table 5: Host Spectrum of *S. aureus*:

Staphylococcus aureus Species	Host Spectrum	Reference
S.aureus in Humans	Commonly colonizes human skin and mucous membranes	
	Can cause a wide range of infections, including skin, respiratory, and bloodstream infections	
Methicillin-resistant *S.aureus* (MRSA)	Often found in healthcare settings, including hospitals and long-term care facilities	(Olaru et al. 2023)
	Community-acquired MRSA strains can affect otherwise healthy individuals	
S.aureus in Animals	Found in various animal species, including livestock and pets	
	May lead to skin and soft tissue infections in animals	
MRSA in Animals	Increasing concern in animal populations, including companion animals and livestock	
	Can lead to infections in animals and potential transmission to humans	
Methicillin-sensitive *S.aureus* (MSSA)	Common in humans and animals alike	
	May lead to various infections in both hosts	

Table 6: Host Spectrum of Salmonella:

Type of Hosts	Host Examples	Reference
Humans	Causes foodborne illnesses in humans Can lead to gastroenteritis and typhoid fever Infections often linked to contaminated food	
Domestic Animals	Found in a variety of domestic animals Poultry, cattle, swine, and more Can cause enteric infections	
Wild Animals	Present in wild animal populations Birds, rodents, reptiles, etc. May serve as reservoirs for transmission	(Aung et al. 2019)
Livestock	Found in livestock species, such as cattle, poultry, and pigs Contamination of meat products possible	
Pets and Companion Animals	*Salmonella* can infect dogs, cats, and more Contaminated pet food can lead to infections	
Reptiles and Amphibians	Salmonella carriage common in these animals May pose a risk of transmission to humans	
Aquatic Animals	Can be present in aquatic environments May affect fish and shellfish	
Invertebrates	Can be isolated from certain invertebrates Potential role in transmission	

Table 7: Medicine resistance to *Salmonella*:

Antibiotic Class	Specific Antibiotic	Resistance in Salmonella	Reference
Beta-lactams	Ampicillin Amoxicillin	Some Salmonella strains are resistant Varies among different serotypes	(Burke et al. 2014)
Fluoroquinolones	Ciprofloxacin Levofloxacin	Emerging resistance, especially in certain serotypes Similar resistance pattern to ciprofloxacin	(Carey et al. 2021)
Aminoglycosides	Gentamicin Amikacin	Some strains may exhibit resistance Generally less resistance compared to other classes	(McArthur et al. 2013)
Tetracyclines	Tetracycline	Varies, some strains may be resistant	
Sulfonamides	Trimethoprim/ sulfamethoxazole (Co-trimoxazole)	Some Salmonella strains are resistant	
Nitrofurans	Nitrofurantoin	Generally still effective for uncomplicated infections	

8. ROLE OF BRUCELLA IN AMR

Brucellosis is a worldwide, chronic infectious disease caused by small aerobic, non-motile, Gram-negative coccobacilli of the genus Brucella. There are 12 established species within the genus that are recognized based on preferential host specificity (Celik et al. 2023). B. melitensis infection causes abortion, stillbirths and the birth of weak offspring, and occasionally epididymo-orchitis in goats and sheep and is the most virulent Brucella species for humans (zoonotic), responsible for a severely debilitating and disabling illness that results in high morbidity with low mortality. B. melitensis has been controlled in most industrialized countries; however, it remains endemic and associated with an extensive negative impact on the productivity of flocks in low and middle-income nations, where goats and sheep are the major livestock species and the main economical livelihood, such as the Mediterranean region, the Middle East, Central Asia, Sub-Saharan Africa, and parts of Latin America (Rossetti et al. 2022). On the contrary, B. ovis seems to be non-pathogenic for humans and the main clinical sign of infection is epididymitis in rams, with occasional abortions in ewes and increased perinatal death. In small ruminants, there are reports of B. ovis specific antibodies in goats from Brazil (Pereira et al. 2023) and Bulgaria (Arnaudov, 2012). Today, the

disease likely exists in numerous global sheep-farming areas, including Australia, New Zealand, Russia, France, Spain, Portugal, South Africa, the United States, Mexico, Argentina, and Brazil.

Table 8: Host Spectrum of *Brucella*:

Brucella Host Spectrum	Host Examples	Reference
Humans	Causes human brucellosis	
	Acquired through contact with infected animals	
	Can lead to flu-like symptoms	
Domestic Livestock	Affects various livestock species	
	Cattle, goats, sheep, and more	
	Leads to reproductive issues in animals	
Wild Animals	Found in a variety of wild animals	
	Bison, elk, deer, and more	
	Can be transmitted to humans through exposure	(Abdel-Glil et al. 2022)
Pets and Companion Animals	Can infect dogs and other pets	
	Exposure through contact with infected animals	
Wildlife	Carried by various wildlife species	
	Elk, bison, and other mammals	
	Transmission to livestock and humans possible	
Marine Mammals	Isolated from marine mammals	
	Dolphins, seals, and more	
	Potential for transmission to humans	

Table 9: Medicine Resistance to *Brucella*:

Antibiotic Class	Specific Antibiotic	Resistance in *Brucella*	Reference
Aminoglycosides	Streptomycin	Variable resistance	
	Gentamicin	Varies among strains	
Tetracyclines	Tetracycline	Varies among species and strains	
Sulfonamides	Trimethoprim/sulfamethoxazole	Varies among strains	
Rifamycins	Rifampin	Resistance uncommon	(Shahrabi et al. 2023)
Quinolones	Ciprofloxacin	Varies among species and strains	
Macrolides	Azithromycin	Variable resistance	
Aminopenicillins	Ampicillin	Resistance observed	
	Amoxicillin	Resistance reported in some strains	

9. CONCLUSION

Antimicrobial resistance remains a critical global public health challenge in the 21st century, garnering significant political attention from G7 countries and persisting on the agendas of various political conferences. While efforts to implement mitigation strategies are yielding positive outcomes, failure to address AMR could lead to a return to the pre-antibiotic era, where common infections posed life-threatening risks. AMR embodies a complex interplay of factors, encompassing social dynamics, conflicts, healthcare, economics, behavior, climate events, and pharmaceutical innovation. Crafting a comprehensive solution for AMR requires collaborative actions by individuals, communities, and nations to ensure the continued availability of effective antimicrobials for sustaining human and animal health.

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Harnessing Probiotics for Controlling Salmonellosis

10

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ABSTRACT

Numerous serotypes of the genus Salmonella may cause salmonellosis, which is still a major worldwide public health problem with enormous social and economic costs. Alternative tactics are typically required to successfully manage Salmonella infections, since conventional management measures are not always sufficient. This paper investigates the intriguing possibility of using probiotics as a workable and long-term method of managing salmonellosis. Probiotics, which are living microorganisms that provide health advantages when given in sufficient quantities, have shown significant promise in reducing the risk of Salmonella infections. This research comprehensively investigates the strategies that probiotics use to produce antimicrobial compounds against Salmonella. These processes include host immune response regulation, generation of antimicrobial chemicals, and competitive exclusion. The paper also explores the complex relationship between probiotics and the gut microbiota, providing insight into how probiotics may help restore the microbial equilibrium that Salmonella has upset. Additionally, this thorough investigation assesses how well different probiotic strains work to stop Salmonella from colonizing a variety of host species, such as people, animals, and poultry. The review summarizes the most important studies that have advanced our knowledge of the many ways that probiotics may be used to prevent Salmonella infections in a variety of settings. Additionally, covered are the difficulties and factors to be taken into account in the creation and use of probiotics as a salmonellosis management approach, such as strain selection, dose optimization, and the need for uniform regulatory frameworks. Probiotics and conventional therapies are investigated for possible synergies in order to provide an integrated strategy to managing salmonellosis from a holistic standpoint. This review concludes by offering a thorough summary of the status of research on using probiotics to manage salmonellosis. It provides important insights into the mechanics, uses, and difficulties of using probiotics as a long-term and successful tactic in the continuing fight against Salmonella infections by combining data from many research.

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1. INTRODUCTION

The intestinal bacteria *Salmonella* still poses a serious threat to the world's public health. The bacteria that cause salmonellosis is known as *Salmonella* in honor of the study done by American bacteriologist D.E. Salmon. *Salmonella* is responsible for several diseases, including septicemia, enteric (typhoid) fever, and mild gastroenteritis. The Salmonella serovar involved, strain virulence, infective dose, host animal species, age and immune status of the host, and geographic region are just a few of the many variables that affect the nature and severity of infections in various animal species (Mastroeni & Maskell 2006). Only a small number of serovars, which can be divided into three groups based on how frequently they infect different hosts, are responsible for the majority of salmonellosis cases in humans and domestic animals. These serovars tend to cause subclinical intestinal infections or acute enteritis, while those with a narrow host range tend to cause severe systemic diseases. The serovars which are host-specific, are less able to trigger inflammatory reactions in the intestines. This could make immune de and tissue-wide spread easier. This pathogen feature may be attained either passively via the loss of effector proteins responsible for inducing pro-inflammatory responses or actively through the evolutionary acquisition of effector proteins responsible for immune suppression. *Salmonella* may spread among the animals, mostly via feces, which can lead to high levels of transmission and disease. Monitoring and attempting to manage these infections costs the agricultural sectors and public health authorities a lot of money each year. Finding ways to limit this transmission across animals would be made easier with knowledge of the pathophysiology of Salmonella infections in many animal species (Mastroeni and Maskell 2006).

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It is a strong foe due to its frequency in food and water sources, as well as its capacity to cause infections in the gastrointestinal system. Scientists have been looking for alternate approaches to reduce enteric infections since Salmonella is still a widespread disease in industrialized and developing nations, and because antibiotic treatments might have unintended side effects. Probiotics seem like a compelling option to address this issue. Live bacteria known as probiotics may help the host's health when given in sufficient doses (FAO/WHO 2001).

Antibiotics have been the mainstay of conventional methods for treating Salmonella infections, but the emergence of antibiotic-resistant forms has made it necessary to investigate other tactics. In the fight against salmonellosis. The WHO/FAO concentrated especially on probiotics as foods or nutritional supplements, although they may also be employed in medication applications (as live biotherapeutics), microbial feed additives (for animal usage), genetically modified organisms, and live vaccinations if given orally. The International Scientific Association for Probiotics and Prebiotics gave a more thorough insight into the appropriate usage of this word to improve clarity for probiotic terminology. The requirements for using probiotics in meals or medicines are among this definition's important features.

Probiotics—defined as living microorganisms that provide a health benefit when given in sufficient amounts—have emerged as a viable weapon. An in-depth discussion of probiotics' involvement in preventing and managing Salmonella infections is provided in this chapter.

2. UNDERSTANDING SALMONELLOSIS

Before delving into the potential of probiotics, it is crucial to comprehend the pathogenesis of Salmonella. The bacterium gains entry through contaminated food or water, and once inside the host, it employs an array of virulence factors to breach the intestinal epithelial barrier. This leads to symptoms ranging from mild gastroenteritis to severe systemic infections. Non-immune mechanisms, such as the stabilization of the gut mucosal barrier, increasing mucus secretion, and improving gut motility may prevent enteric pathogens from colonizing and infecting the mucosa. Other potential mechanisms include competing and struggling for nutrients and secreting specific low molecular weight antimicrobial secretions (bacteriocins). The effects of probiotic strains on non-immune defenses are mentioned in this chapter. Probiotic suspensions are effective in preventing several Salmonella serovars. According to Mountzouris et al.'s research, an oral probiotic (multi-strain) treatment decreased the amount of *S. enteritidis* in broilers (Mountzouris et al. 2009). They found that probiotic feeding substantially boosted intestinal and systemic levels of IgA and IgG antibodies against *S. enterica* and decreased the susceptibility of infected animals in treated groups (by 50%) as compared to untreated controls (by 100%). By enhancing specific anti-Salmonella antibodies in serum and the intestinal tract, increasing splenocyte proliferative responses to mitogens, and increasing phagocytic activity of peritoneal and blood cells, other probiotic strains, including *L. rhamnosus* HN001 and *B. lactis* HN019, demonstrated protective properties against S*. typhimurium* in mice (Gill et al. 2001; Shu et al. 2000). Mice infected with *S. typhimurium* and given the probiotic strain *L. helveticus* M92 were used in a study to demonstrate the significance of the lactobacillus S-layer protein in protecting the immune system. The authors hypothesized that the mice's intestinal tract's competitive exclusion and the increased immune protection provided by *L. helveticus* M92 and its S-layer protein were responsible for the decreased infection by *S. typhimurium* FP1 (Castillo et al. 2011).

The anti-inflammatory traits of some probiotic bacteria may also be linked to the pathogen defense offered by these microbes. The immune system's inflammatory response is triggered by the host defense against infection and may result in tissue damage (Fig. 1). In this regard, different research showed that probiotic bacteria of human origin, *Bifidobacterium infantis* 35624 when given to mice before they were exposed to *Salmonella typhimurium* or given an injection of LPS, had anti-

inflammatory and pathogen-protective effects. According to O'Mahony et al. (2008), the probiotic's positive impact was due to the production of Treg cells, which inhibited excessive NF-B activation in vivo. (Fig. 1), Using a mouse model, Gobbato et al. 2008) investigated the same LAB's efficacy against *S. typhimurium* in vivo. Particularly in mice given *L. bulgaricus*, they saw a considerable lowering of the number of apoptotic cells in the small intestinal tissue cells. The significant increase in Bcl-2+ anti-apoptotic protein cells seen in the small intestine of the mice who received this LAB may help to explain this outcome. *S. thermophilus* had no impact on apoptosis inhibition and failed to raise the number of Bcl-2+ cells in comparison to the untreated control. Instead, injection of *L. casei* increased the number of Bcl-2+ cells, albeit it had a comparable impact on apoptosis inhibition to *S. thermophilus*. It was also shown that mice administered L. *delbrueckii* subsp. *bulgaricus* had more IFN+ cells in their small intestines. The enhanced microbicidal activity seen in the macrophages isolated from the peritoneum and Peyer's patches after this LAB oral treatment was consistent with this finding. The number of IFN+ cells was similarly raised by *L. casei*, but this increase was insufficient to cause the peritoneal macrophages to become microbicidal. Fig. 2 shows some immune mechanisms generated by different probiotic strains against *Salmonella* infection.

Additionally, probiotics function as immunological adjuvants that affects systemic and mucosal immune responses. They can regulate NK cell activity, modulate the inflammatory response, stimulate the production of specific cytokines and the phagocytic activity of macrophages and neutrophils, and enhance specific antibody responses, particularly mucosal secretory IgA (Alvarez-Olmos & Oberhelman 2001; Galdeano de Moreno de LeBlanc et al. 2007). Given the diverse clinical manifestations, it is evident that a multi-faceted approach is required to combat this pathogen effectively.

3. PROBIOTIC'S MODE OF ACTION

Probiotics are expected to have a variety of different mechanisms of action. Some of these mechanisms influence the suppression of intestinal pathogenic microbes, while others are in charge of enhancing animal performance. Although the precise mechanisms of action by which probiotics perform biological activities are not entirely known, their mode of action is often described as competitive exclusion or colonization resistance (Fig. 3). Oelschlaeger describes three mechanisms in which probiotics function in the host system (Oelschlaeger 2010).

i. Probiotics may be useful in altering the host's innate and acquired immune systems. The direct action of probiotics on other microorganisms will prevent and serve to control infections and re-establish the microbial balance in the gut.

ii. Microbial products such as antimicrobials, toxins, and host metabolites may be the major components for probiotic actions. This will be efficient in preventing infectious diseases and ameliorating the inflammation of the host's digestive tract. The probiotics improve gastrointestinal digestion of meal components and nutrient absorption while also helping in the inactivation of toxins and detoxification of bile salt.

iii. probiotics compete with pathogenic bacteria to bind to mucus, since they may adhere to the mucosal wall and are beneficial in host immune responses (Galdeano et al. 2019).

By providing an additional source of nutrients and digestive enzymes, probiotics may also encourage the production of vitamins in the host (Markowiak 2017). In addition to producing inhibitory compounds such as volatile fatty acids and hydrogen peroxide to strengthen the host's tolerance to infections, these may stop the spread of dangerous bacteria (Plaza-Diaz et al. 2019).

According to research, yeast-based probiotics enhanced the amount of cellulolytic bacteria in ruminants, which demonstrates their impact on microbial fermentation and results in high cellulose breakdown and better microbial protein synthesis (Amin et al. 2021). By generating proteins or polypeptide bacteriocins, both Lactobacilli and Bifidobacteria promote the development of closely related bacterial species,

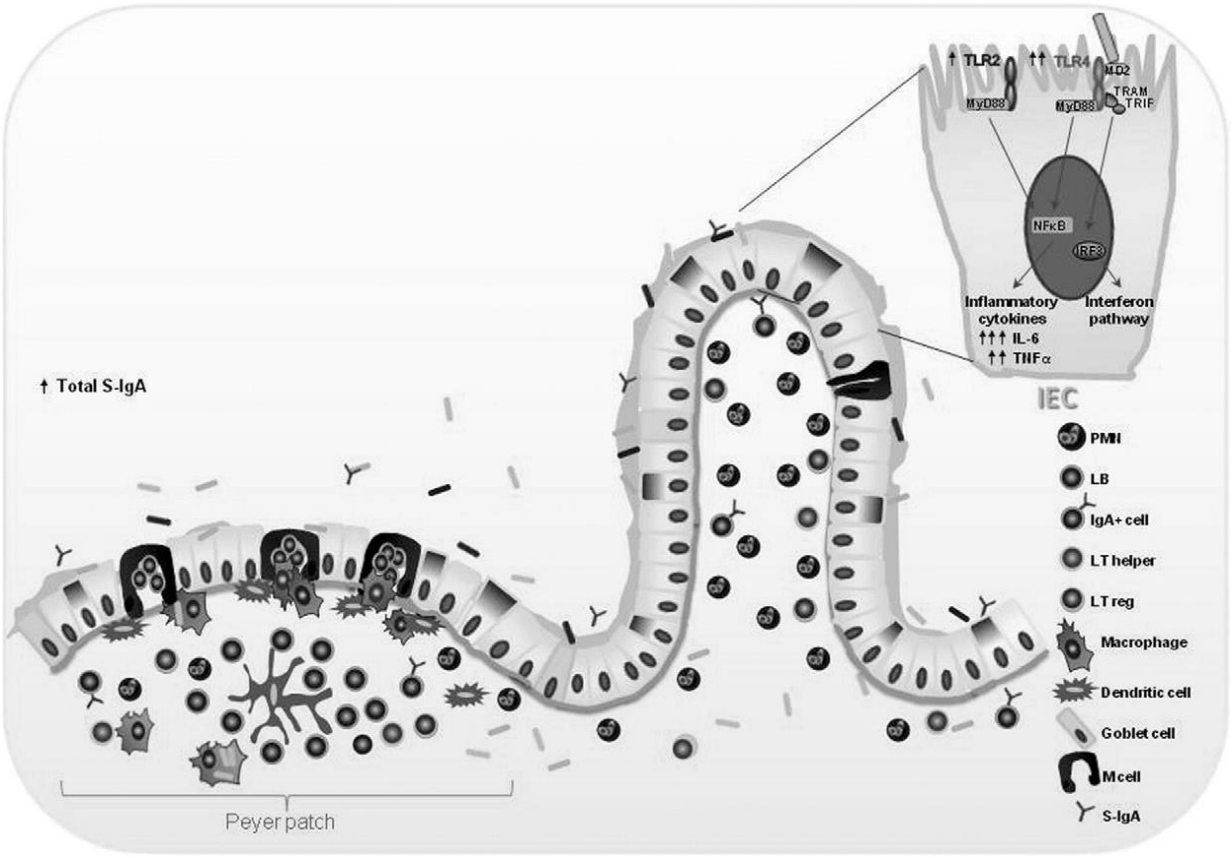

Fig. 1: Schematic diagram of the immune mechanisms generated against *Salmonella* infection in the inductor (Peyer's patches) and effector (lamina propria and epithelium) sites of the gut immune response. *Salmonella* enters through M cells or intestinal epithelial cells (IECs), then internalizes and replicates within phagocytic cells, and induces their cellular death, a mechanism used to disseminate to deeper tissues. The infection usually produces an inflammatory response with infiltration of polymorphonuclear cells, and the activation of inflammatory cascades into the immune cells and epithelial to produce pro-inflammatory cytokines and tissue damage (Castillo et al. 2011).

therefore, reducing the number of harmful germs in the gut. *Bacillus, Staphylococcus, Enterococcus, Listeria,* and *Salmonella* species are just a few of the pathogens that probiotic species like LAB, Bifidobacteria, and Bacillus can combat with the help of a few different types of thermostable bacteriocins (Piqué et al. 2019).

Havenaar et al. (2018) presented some specific characteristics of probiotics based on the various characteristics of a good microorganism, such as having a positive effect on the host, being nontoxic and nonpathogenic in nature, having the ability to survive for a long time with high cell counts, and their capacity to survive through the digestive system.

Some probiotic strains have been shown to exhibit anti-inflammatory qualities that support the equilibrium between pro- and anti-inflammatory cytokines (Cristofori et al. 2021; Pagnini et al. 2010), as well as the generation of antimicrobial compounds such as volatile fatty acids, hydrogen peroxide, and bacteriocins (Vieco-Saiz et al. 2019). According to studies, probiotic bacteria create organic chemicals that have been shown to have inhibitory action on pathogenic bacteria like *H. pylori* (Rezaee et al. 2019). Dai et al.'s investigations show that probiotics may improve tight junction protein expression by stimulating the p38 and ERK signaling pathways, which in turn can strengthen gut barrier integrity (Wang et al. 2018). Probiotics have antiviral activities in animals in addition to the anti-inflammatory effects (Lehtoranta et al. 2020).

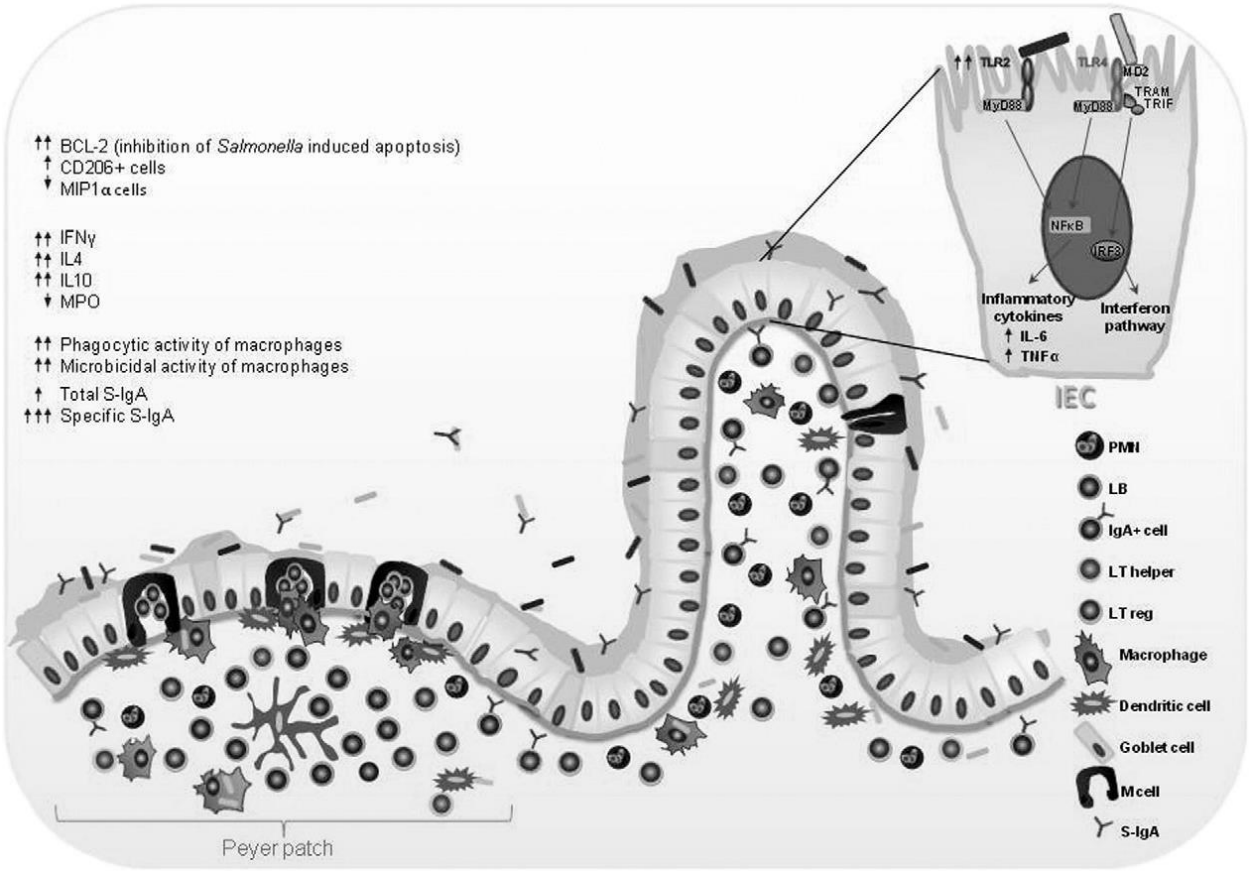

Fig. 2: Schematic diagram of some immune mechanisms generated by different probiotic strains against *Salmonella* infection. Probiotics produce an anti-inflammatory response, with increase of dendritic cells, macrophages, and Treg cells that produce regulatory cytokines such as IL-10. Probiotics also enhance IgA-secreting cells and mucus-producing cells that reinforce the intestinal defenses (Castillo et al. 2011).

Probiotic cultures were thought to have the potential to lessen exposure to chemical carcinogens in cancer studies. This can be performed by:

(i) detoxifying consumed carcinogens

(ii) modifying the intestinal environment which helps to minimize carcinogenic producing bacteria populations or metabolic activities

(iii) Producing metabolic products (e.g. butyrate) that start apoptosis

(iv) producing inhibitory substances to prevent tumor cell growth

(v) propagating the immune system in curtailing of cancer cell propagation for a better defense mechanism.

Probiotics may also boost the activity or synthesis of digestive enzymes in birds and shield them from the harmful effects of enzyme activity. Probiotics may also generate enzymes that hydrolyze or release nutrients in the host's digestive system. According to (Nahashon et al. 1994), layers given diets containing *L. acidophilus* showed a rise in phytase activity in the crop but not in their digestive systems. Additionally, enhanced P retention in layers was linked to higher phytase activity in the lactobacillus-fed birds.

All techniques must be carefully examined in each situation to have a comprehensive grasp of the probiotics' mechanism of action. Probiotic effects are the result of the probiotic bacteria' interaction with the host. Therefore, further research on the relationship between hosts and microorganisms is required

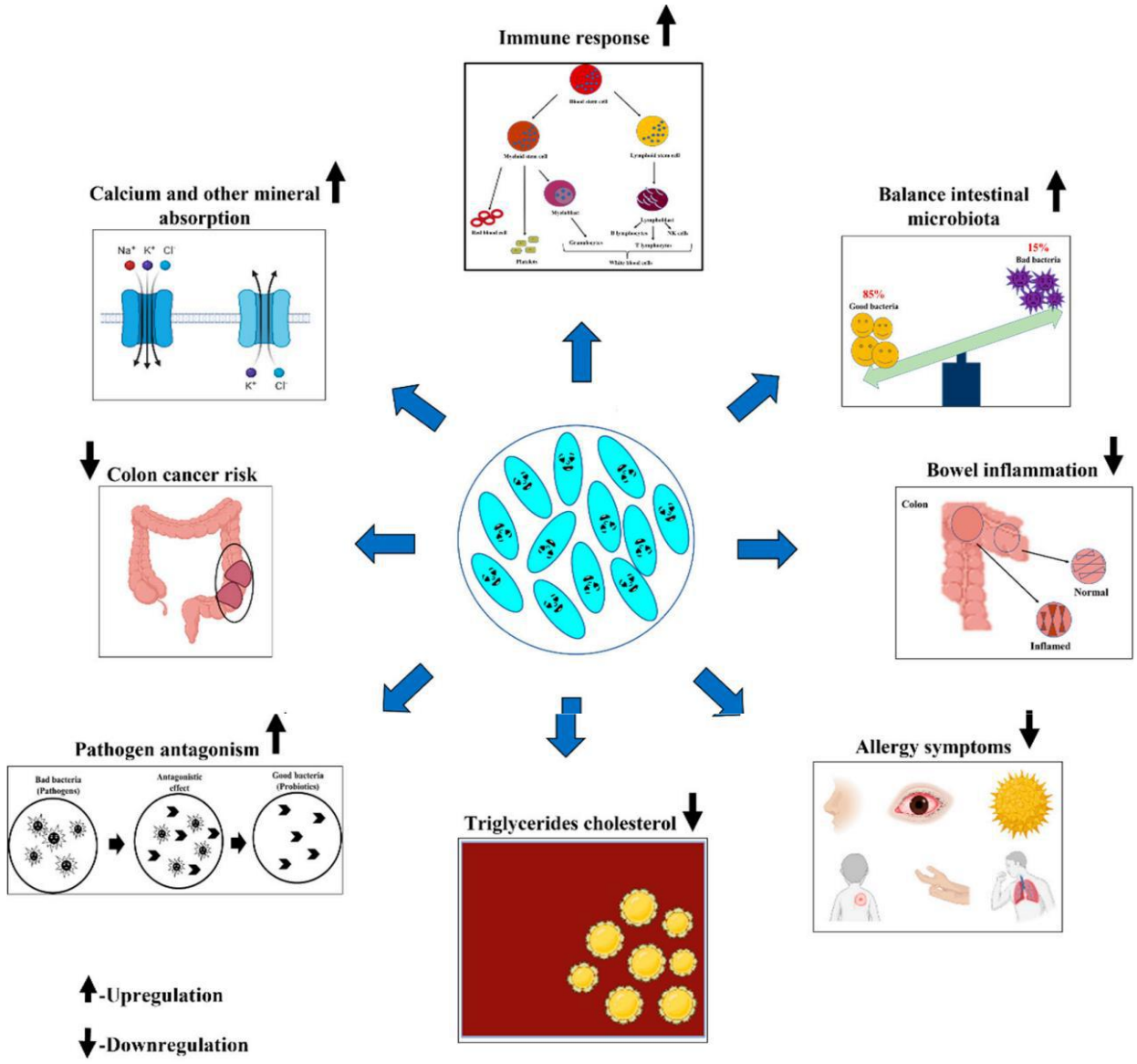

Fig. 3: Schematic view of Modes of action of probiotics (Galdeano et al. 2019)

to better understand how probiotics work. In the past, high research costs and underdeveloped molecular tools might have prevented in-depth examination of probiotic effects. The effects or mechanisms of action of probiotics may now be understood, however, thanks to several wonderful molecular approaches. Understanding microbial ecology and how probiotics function may be substantially facilitated by the rapid advancements in molecular techniques and DNA sequencing.

4. PROBIOTICS: A PRIMER

As per the definition given by the World Health Organization and the Food and Agriculture Organization, probiotics are live bacteria that, when properly provided, confer health benefits to the host and maintain health.

Decomposing bacteria and its components may also have probiotic-like qualities. The microorganism, which is commonly said to display the lactic acid bacteria and Bifidobacterium strains, has probiotic qualities and is employed in many healthful nutritional supplements and foods. Ideally, a genuine probiotic should be of safe human origin, in good health, and devoid of any pathogenic or toxic agents or vectors that might spread antibiotic resistance (Plaza-Diaz et al. 2019). Identification of the compounds one microbe produces that encourage another's development factors has updated the positive effects of symbiotic bacteria on mammals reaching the level of their intestinal fora (Bortoluzzi et al. 2020). Various investigations have shown a variety of potential theories surrounding probiotic classification according to evolutionary history. The development of genomic techniques has improved our ability to classify various probiotic species and mechanisms (Reid 2016). Several lactic acid bacteria (LAB) are regarded as probiotics as a result of the presence of these microorganisms when fermented with sugar-rich foods, the capacity to produce lactose (Plaza-Diaz et al. 2019). In line with their morphological and phenotypic features that are first lactic Termobacterium, Betacoccus, Streptobacterium, Tetracoccus, and Microbacterium were used to classify acid bacteria. Betabacterium. Currently, just Streptococcus is kept, whereas the remaining bacteria were given new names, including Bifidobacterium, Enterococcus species, and Lactobacillus species (Mohania et al. 2008). In terms of morphology, the *Lactobacillus* genus is a member of the *Firmicutes phylum,* class *Bacilli,* order *Lactobacillales,* and family *Lactobacillaceae*. It contains more than 170 species of Gram-positive, facultative, anaerobic, catalase-negative, rod-shaped bacteria. It is used in the creation of fermented foods that come from both plants and animals, including milk and meat (Zhang et al. 2018). *Bifidobacterium* is typically Gram-positive, non-motile, anaerobic, pleomorphic, non-sporting bacteria that result from the fermentation of carbohydrates into acetic, formic, and lactic acids (Vlkova et al. 2002). Because they are obligate anaerobes, *Bifidobacterium* cultivation is more difficult than *Lactobacillus* cultivation and frequently calls for more attention when it is used to make probiotic products and dairy products like yogurt (Abou-Kassem et al. 2020).

Today, encouraging an association of many probiotic species is a problem in probiotic analysis and production. This is due to research showing that it has a greater impact on a person's health than one probiotic usage. Eight different VSL, #3 probiotic substances were found to be effective in the treatment of a variety of conditions, including ulcerative colitis, boosting the immune system, improving diabetes patients' resistance to hepatic insulin, diarrhea, bowel disorders, and ulcerative colitis (Dong et al. 2016; Schlee et al. 2008). Additionally, combining *Bifidobacterium* strains with *Lactobacillus acidophilus* and (LA) has been proven to be effective in reducing the incidence of NEC (necrotizing enter colitis) and NEC-related deaths in infants with severe disorders (Nair and Soraisham 2013). When there is a mutual disruption between the probiotic consortia, the ability of the probiotic items will diminish. Therefore, it is important to guarantee that probiotic consortia won't interact with one another.

A single strain is insufficient; a combination of strains might be more beneficial, as shown by the basic research of probiotic products including bacteria, which indicated an effective improvement in the recovery of disorders (Nair and Soraisham 2013). On the other hand, although many microorganisms are regarded as probiotics, not all of them have the desired properties.

Before considering bacteria as a probiotic, numerous factors need to be considered, say researchers (Mitropoulou et al. 2013). To ensure the safety of probiotic products, the probiotic bacteria should be nonpathogenic and generally recognized as safe (GRAS) by the FDA Drug Administration and US Food.

There are living microorganisms in the gastrointestinal tract in addition to the beneficial bacteria that live in the human stomach. *Clostridium difficile* and *H. pylori* are the two most prevalent of these, but there are other types as well that might pose health risks.

By competing with supplements or adhering to the gastrointestinal region, probiotic use may prevent or minimize the growth of certain pathogens in the gastrointestinal system (Ohashi and Ushida 2009). In

every circumstance, pathogens anticipate nutrients to multiply and either initiate or intensify diseases. The gastrointestinal system is noteworthy for its abundance of nutrients. It makes the environment suitable for the colonization of bacteria to begin. When compared to pathogens, the capacity of probiotics to win over the bacteria favors their growth (Khaneghah et al. 2020).

In the competition for nutrients, probiotics may create specific metabolites, such as unstable unsaturated lipids that disrupt the pH of the gastrointestinal system.

Because most pathogens cannot thrive at low pH, the pH lowering of the gastrointestinal system creates an unfavorable environment for bacteria and will inhibit pathogen development (Biswasroy et al. 2020). The digestive tract contains live bacteria, with *C. difficile* and *H. pylori* being the most prevalent. Other microbes are also present, and some of them pose health risks. Cocktail, a commercial probiotic, has been proven to significantly reduce salmonella infection in chicken tonsils and ceca (Moreno et al. 2010). Salmonella numbers included in the digestive system and further enteric dispersal are reduced by regular injection of Lactobacillus casei CRL, according to an in vivo study using a mouse model (Asahara et al. 2011). Probiotics, which predominantly encompass strains of *Lactobacillus, Bifidobacterium,* and other beneficial bacteria, play a pivotal role in maintaining gut homeostasis. They exert their influence through various mechanisms, including competitive exclusion, production of antimicrobial compounds, and modulation of the host immune response. These properties make probiotics an attractive candidate for preventing and mitigating Salmonella infections.

5. PROBIOTICS VS. SALMONELLA: A BATTLE FOR COLONIZATION

A key strategy employed by probiotics in combating Salmonella is competitive exclusion. By colonizing the gastrointestinal tract, probiotics create an environment that is less conducive for Salmonella to establish itself. This competition for resources and adhesion sites can significantly reduce the pathogen's ability to thrive. One of the most common causes of acute gastroenteritis, known as S. Typhimurium, is Salmonella enterica serovar typhimurium, which is characterized by inflammatory diarrhea. Inflammation facilitates in the making of colonization of S. Typhimurium and other Enterobacteriaceae, while the normal gut is mostly occupied by commensal microorganisms, namely Bacteroides and Firmicutes (Barman et al. 2008: Lawley et al. 2008: Lupp et al. 2007: Stecher et al. 2007). Recent research has demonstrated that *S. typhimurium* nourishes in the inflammatory gut because it can use particular carbon and energy reserves and is resistant to antimicrobial proteins produced by the host as part of the nutritional immune response (Thiennimitr et al. 2011; Liu et al. 2012; Raffatellu et al. 2009). The most significant micronutrient metal that S. Typhimurium uses is iron (Crouch et al. 2008; Raffatellu et al. 2009) which it acquires through specialized transporters (Liu et al. 2012; Raffatellu et al. 2009). Due to binding by host proteins such as heme, transferrin, ferritin, and lactoferrin, levels of free iron are very low in the host environment (Andrews and Schmidt, 2007). Hepcidin secretion, which stops the stomach from absorbing iron from the circulation by blocking the iron transporter ferroportin-1, is one of the additional strategies used by the host to further restrict iron availability during inflammation (Genz and Nemeth 2015).

Bacteria that lack iron produce and export siderophores, which are tiny, high-affinity iron chelators. All Enterobacteriaceae, including commensal E. coli and Salmonella, release enterochelin, a catecholate-type siderophore that is adequate to overcome the host's iron restriction in a normal (non-inflamed) environment (Raymond et al. 2003). However, when inflammatory responses happen, the host secretes antimicrobial peptide and lipocalin-2, which sequesters ferric enterochelin. As a result, strains of *E. coli,* such as *commensal E. coli,* that solely rely on enterochelin for siderophore-based iron acquisition are constrained in their growth (Berger et al. 2006). By producing extra siderophores that are not captured by lipocalin-2, certain infections can circumvent this response (Fischbach et al. 2006a). For instance, Salmochelin (Muller et al. 2009) a C-glucosylated enterochelin derivative that is too big to fit into the

enterochelin-binding pocket of lipocalin-2 (Fischbach et al. 2006b; Hantke et al. 2003), may be produced and secreted by Salmonella.

Commensal bacteria known as probiotics are thought to have positive effects on the host. Probiotic strain *Escherichia coli Nissle* 1917 (*E. coli Nissle,* serotype O6:K5:H1) was first discovered in a soldier who seemed immune to a case of diarrhea *(Nissle*, 1959). Several intestinal disorders, including acute enteritis (Henker et al., 2007), have been treated or prevented with E. coli Nissle (Cukrowska et al. 2002; Kruis et al. 2004;; Mollenbrink and Bruckschen 1994; Nissle 1959), but it is unknown what mechanisms underlie these protective effects. According to Raffatellu et al. (2009), salmochelin-mediated iron acquisition during inflammation promotes *S. Typhimurium* colonization, therefore we reasoned that *E. coli Nissle* may defend the host by using comparable processes to compete with *S. typhimurium* for vital micronutrients.

The *E. coli* Nissle genome shared several fitness traits with strains of the same serotype of uropathogenic E. coli (UPEC), according to a snapshot study of the genome (Grozdanov et al., 2004). Contrary to popular belief, the *E. coli Nissle* genome seems to encode for as many iron uptake systems as UPEC (Martínez-García and Lorenzo 2011). Notable members of this arsenal include salmochelin, the hydroxamate-type siderophore aerobactin, the mixed-type siderophore yersiniabactin, and the hemin uptake transporter ChuA. It is hypothesized that redundancy in iron absorption, which encourages the development of UPEC in the bladder and kidney (Garcia et al. 2016) may also contribute to the colonization of the inflammation of the gut with *E. coli* Nissle.

6. IMMUNOMODULATION: STRENGTHENING THE HOST DEFENSE

Probiotics also play a vital role in modulating the host immune response. They enhance the activity of immune cells, such as macrophages and T cells, which are crucial in mounting an effective defense against Salmonella. Additionally, probiotics stimulate the production of antimicrobial peptides, reinforcing the innate immune system's capacity to combat the pathogen. First presented by Metchnikoff in 1907, the theory claims that intestinal bacteria cause "autointoxication," which is harmful to human and animal health. Additionally, he suggested that the prolongation of Bulgarian peasants was caused by their frequent consumption of fermented milk that contained living beneficial bacteria. As mentioned in previous sections, the gut microbiota has a significant influence on the host's immunology, biochemistry, physiology, and resistance to non-specific diseases (Gordon & Pesti, 1971). These findings have given rise to the hypothesis that altering the gut microbiota's makeup by dietary supplements may improve health (Goldin & Gorbach, 1992). According to Prasad et al. (1999), the two genera of probiotic bacteria that are most often utilized are Lactobacillus and Bifidobacterium. Gram-positive, non-spore-forming, catalase-negative, typically nonmotile rods, lactobacilli do not reduce nitrate and are not catalase-positive. According to Mikelsaar et al. (1998), the most often utilized lactobacilli species are *L. acidophilus*, *L. salivarius*, *L. casei*, *L. plantarum*, *L. fermentum*, and *L. brevis*. Bifidobacteria, on the other hand, are Gram-positive, non-spore-forming rods with unique cellular bifurcating or club-shaped morphologies. The species *B. animalis*, *B. longum*, *B. bifidum*, and *B. infantis* are the most often utilized ones.

It is widely accepted that at least 109 CFU/day of probiotics must be consumed (Ouwehand et al., 2002). The levels of lecithinase-negative clostridia in the feces were found to be much lower in research (Benno & Mitsuoka, 1992). Another research demonstrated that consumption of yogurt supplemented with *B. longum* substantially increased the number of Bifidobacteria in the treated participants' feces as compared to those who received control yogurt (Bartram et al. 1994).

In full-term newborns, Langhendries and colleagues (1995) studied the effects of drinking fermented baby formula containing live Bifidobacteria (106 CFU/g of *B. bifidum*), finding that there were significant increases in resident bifidobacteria. Human adult volunteers drank fermented milk containing Lb acidophilus LA2 for seven days; the number of resident *Lactobacilli* and *Bifidobacteria* rose noticeably in

the excretions (Hosoda et al. 1996). The authors observed that the resident *Bifidobacteria* considerably increased and the clostridia counts dropped after examining the effects of ingestion of follow-up formula (NAN BF) containing *B. bifidum* strain Bb12 on fecal flora (Fukushima et al. 1997).

7. PROBIOTICS AND STIMULATION OF THE IMMUNE SYSTEM

Studies on both animals and people have shown that certain strains of lactic acid bacteria may trigger and control several features of acquired immune responses. Additionally, it has been shown that there are considerable variations in how well the immune system is modulated by various Bifidobacteria and lactobacilli strains and that these variations are dose-dependent. Readers are urged to study the outstanding reviews on the immunomodulatory effects of probiotics that have been published in recent years (Gill 1998).

Probiotics and probiotic-derived products are immunologically detected in the gut by specialized membranous cells (M cells), which are found on top of Payer's patches and epithelial cells. It has also been shown that dendritic cells, which are dispersed throughout the subepithelium, can directly sample lumenal antigens. Antigen-presenting cells (APCs) receive antigens that M cells have picked up, process, and offer them to naive T cells. Through the production of pattern-recognition receptors (such as TLRs and CD14) that identify pathogen-associated molecular patterns (PRRs), APCs can distinguish between closely similar bacteria and their byproducts. Whether T cells develop into T helper 1 (Th1), T helper 2 (Th2), or T regulatory (Treg) cells depends on the kind of cytokine release, phenotype, and level of activation of APCs. Th1 cells are subsequently activated, producing IFN-g, TNF-a, and IL-2, which is linked to the emergence of cell-mediated and cytotoxic immunity. Activated Th2 cells primarily secrete IL-4, IL-5, and IL-13, which promote the production of antibodies and are linked to atopy. Treg cells secrete IL-10 and TGF-b, which suppress both Th1 and Th2 cell activity.

8. ANTI-SALMONELLA COMPOUNDS: NATURE'S ARSENAL

Certain probiotic strains secrete antimicrobial compounds, such as bacteriocins and organic acids, which can directly inhibit the growth of *Salmonella*. This chemical warfare, waged by probiotics in the gut ecosystem, further diminishes the pathogen's ability to proliferate. Many probiotic strains have the capacity to synthesize a range of antibacterial compounds. The most frequent ones are carbon dioxide, hydrogen peroxide and antibacterial compounds like bacteriocins and non-bacteriocin, non-lactic acid molecules (Fayol-Messaoudi et al. 2005; Marianelli et al. 2010). These organic acids (lactic acid and acetic acid) cause a reduction in fecal pH. The ability of six Lactobacillus strains, including probiotic ones, to prevent the invasion of *S. Typhimurium* SL1344 into Caco-2/TC7 cells in culture was examined. Some of them produced just lactic acid, whereas other strains produced both lactic acid and one or more additional inhibitory substances, which were responsible for their antibacterial action (Makras et al. 2006). Another in vitro investigation revealed that the buildup of lactic acid was the cause of *L. rhamnosus* GG's antibacterial action against *S. typhimurium* (De Keersmaecker et al. 2006). In their investigation of the antibacterial properties of *L. plantarum* ACA-DC287, which they isolated from a Greek cheese. Fayol-Messaoudi et al. (2009) found that non-lactic acid molecules present in the probiotic strain's cell-free culture supernatant were responsible for killing the pathogen when it was co-cultured with S. Typhimurium. Additionally, *S. Typhimurium* was prevented from penetrating cultured human enterocytes like Caco-2TC7 cells by L. plantarum. According to Lin et al. (2008), a probiotic strain may prevent Salmonella choleraesuis from invading the human Caco-2 cell line via a variety of processes, including the generation of organic acids and bacteriocins.

There are several cases where bacteriocins produced by probiotic LAB influencing the health of the gastrointestinal (GI) tract (Gillor et al. 2008). Bacteriocins produced by lactic acid bacteria (LAB) have been thoroughly described (Castro et al. 2011: Cintas et al. 2000). However, due to their seldom inhibition of frequently encountered enteropathogenic bacteria as *Klebsiella, Enterobacter, Salmonella* or bacteriocins produced by Gram-positive bacteria have limited probiotic uses in the gastrointestinal tract.

9. STRAIN-SPECIFIC EFFICACY

It is important to note that not all probiotic strains possess equal efficacy against Salmonella. The choice of probiotic strain(s) is critical, as each may have distinct mechanisms of action and affinities for specific Salmonella serotypes. Thus, a tailored approach is necessary to select the most appropriate probiotic(s) for a given scenario.

10. CHALLENGES AND FUTURE PROSPECTS

Despite the promise of probiotics, challenges remain. These include strain stability, host-specific responses, and the need for standardized protocols for administration. Additionally, further research is warranted to better understand the complex interactions between probiotics and Salmonella.

11. CHALLENGES OF PROBIOTICS IN ANIMAL FEEDING

Animal feed products often include probiotic microbes, which are typically thought to be safe. The likelihood of propagating antibiotic resistance is increased by the presence of communicable antibiotic resistance genes in a few probiotic bacteria, and contagions from probiotic microorganisms as well as the occurrence of entero and emetic toxins are the main risks attached to probiotic microbes used in animal feed. most studies on probiotics. In papers, efficiency is more often discussed than safety. The most comprehensive information on probiotics' health is solely based on *Lactobacillus* and *Bifidobacterium.* Therefore, more studies about the safety and usage of probiotics. Probiotics used in animal feed are generally safe for animal protection, although when dealing with dangerous or unfavorable microorganisms, measures for people and the environment should be followed. Threats related to the use of probiotics in animal feed, hypothetically are listed below. the probiotic-eating animal contracting a GIT infection;
ii. GIT infection in customers who consumed animal products made by animals given probiotics;
iii. Transmission of antibiotic resistance to other pathogenic microorganisms through probiotics;
iv. Animal and human food handler infections;
v. Skin or eye sensitivity or discomfort in probiotic administrators;
vi. Probiotics' production of toxins that have negative metabolic or toxic effects on humans
vii. Hyperstimulated immune systems in susceptible hosts.
Animal feed containing probiotics must be considered before recognizing microorganisms as probiotics. should be compared to the dangers listed above. It's necessary to identify microbes down to the strain level to assess the specificity of a certain bacterium and to comprehend its advantageous attributes. When determining whether or not to utilize microorganisms as probiotics in animal feed, as seen in Fig. 4, there are a few issues that need to be resolved. Probiotics are primarily utilized The most secure bacteria are often *Lactobacillus* and *Bifidobacterium.* Numerous fermented foods have exploited microbes widely and historically for quite lengthy (Shortt et al. 1994). The GIT of people often contains a significant amount of these microorganisms. and animals, and diseases caused by these microbes are quite uncommon. According to the US Food and Drug Administration, *Lactobacillus bulgaricus* and *L. acidophilus* are "Generally Regarded as Safe" (GRS). The European Food Safety Authority (EFSA) has determined that a

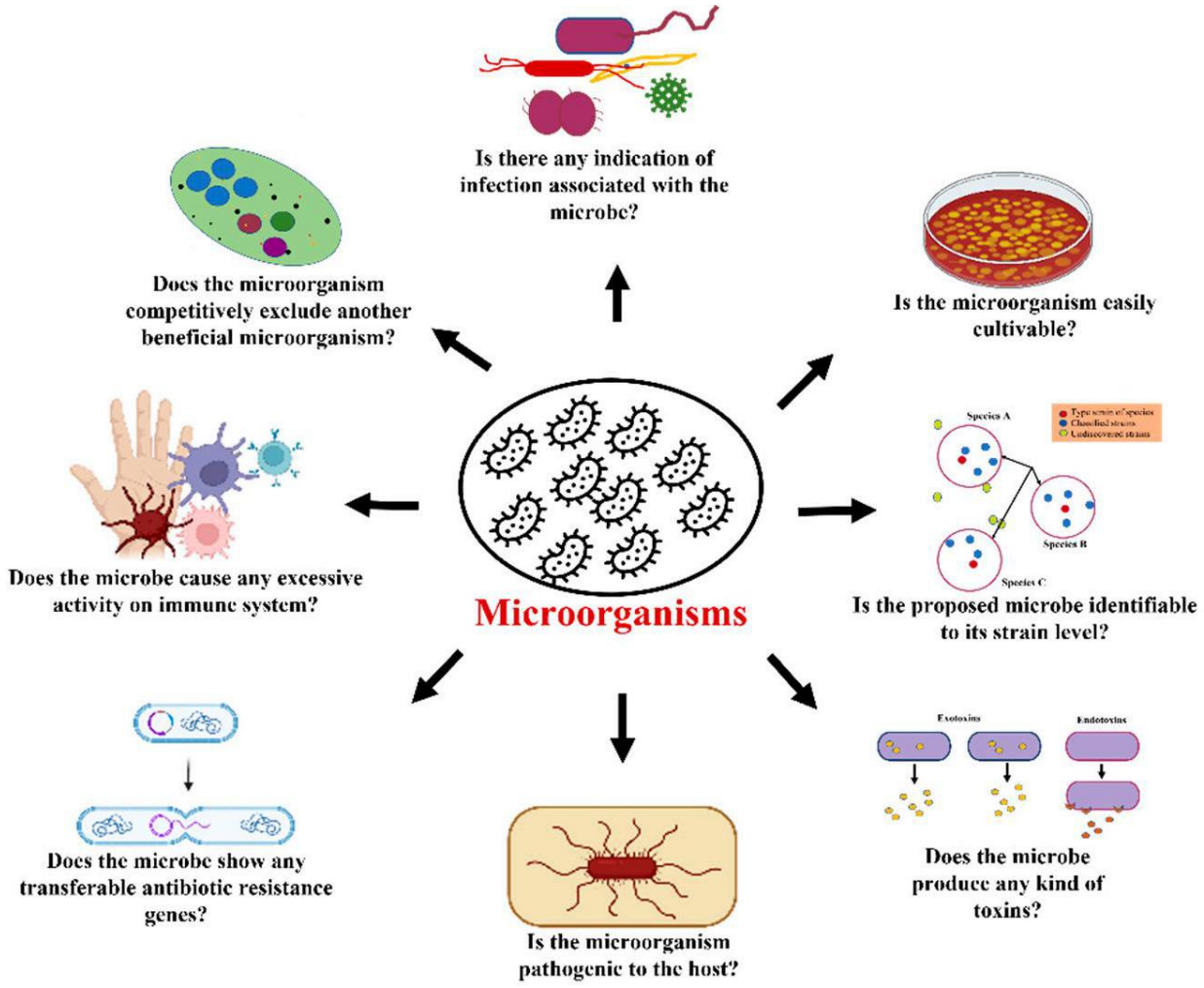

Fig. 4: Evaluation of potential probiotics in animal feeding.

select Bacillus species, such as *B. subtilis, B. megaterium, B. licheniformis*, and *B. coagulans*, are safe since they don't contain any toxins. Even while Enterococcus bacteria have many positive benefits, they have been linked to only a small number of human diseases, such as those that are acquired in public places and hospitals. Therefore, before using *Enterococcus* bacteria as probiotics, rigorous safety assessments are required (Arias et al. 2012).

12. FUTURE DIRECTIONS

According to research, probiotics are a major source of antimicrobials that promote good health and are used as a source of nutrients in the production of animals.

Probiotics may replace antibiotics that promote growth, strengthening the animals' immune systems in the process. Even with the existing understanding of how probiotics affect organisms, research is still being done to better understand some of their mechanisms of action. In the future, it will be crucial to understand how probiotics work in order to combat a certain element of growth or animal performance. Numerous applications of probiotics and their distinct diagnostic and therapeutic functions may be revealed by further study on certain gene expression pathways or metabolic pathways connected to the

impact of probiotics. Targeted probiotic applications may also address a number of disease-related issues in both people and animals. Transcriptome and metabolomics, two cutting-edge molecular approaches, provide detailed information on the mechanisms of action of probiotics, illuminating their positive impacts and how they enhance bird performance. Additional investigation into certain gene expression pathways, such as those identified using metabolomics assays linked to the impact of probiotics, reveals multiple probiotic applications as well as their distinct diagnostic and therapeutic purposes. Probiotics may be used in very particular ways to address problems with a variety of diseases that affect both people and animals. Although probiotics have been praised for improving animal performance, including health, there are drawbacks to giving them to animals. Some probiotic species, like enterococci, may carry genes for drug resistance that are transmissible, while others, like Bacillus cereus, may create enterotoxins that might be hazardous to the host. Lack of knowledge about the probiotics' potential interactions with host cells and their appropriate safe dosages is another major obstacle to their utilization. Therefore, research must be improved to show that probiotics may be used appropriately and efficiently based on the circumstances of the target individuals or host organisms.

13. CONCLUSION

Probiotics represent a compelling avenue in the fight against Salmonella infections. Their multifaceted approach, encompassing competitive exclusion, immunomodulation, and the production of antimicrobial compounds, makes them invaluable allies in this battle. With continued research and a refined understanding of strain-specific efficacy, probiotics hold great promise for controlling salmonellosis and potentially revolutionizing our approach to infectious diseases.

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Avian Salmonellosis and Public Health Concerns

11

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ABSTRACT

Avian Salmonellosis is caused by bacteria from the genus Salmonella species. Intensive poultry production has, regrettably, served as the catalyst for the increased spread of salmonellosis. A noteworthy characteristic of this bacteria is its horizontal and vertical transmission in birds. It is transmitted to humans through the food chain, making this a topic of concern for public health and surveillance. The human consequences of salmonellosis are manifold, ranging from the discomfort of gastroenteritis, watery diarrhoea, and abdominal pain to more ominous manifestations such as headache, nausea, vomiting, fever, and occasionally meningitis, which can sometimes culminate in death. Comprehensive surveillance at local, national, and international levels is necessary to design effective control strategies for salmonellosis. Mitigation strategies for salmonellosis are multi-faceted tasks that include strict biosecurity and vaccination along with the use of probiotics, prebiotics, indispensable amino acids, organic acids, and essential oils in the feed of poultry, which can lessen the contamination of poultry products.

Key words: Avian Salmonellosis, Transmission, Surveillance, Public Health, Prevention

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1. INTRODUCTION

Avian Salmonellosis is caused by a bacterium belonging to the genus Salmonella (S) species. The proliferation and spread of such microorganisms of great importance have been favored by rigorous poultry farming owing to the extension and development of the market. This has led to keeping animals in commercial poultry in such large aggregations (Stella et al. 2021). Avian Salmonellosis proves to be the most damaging disease globally as poultry rearing and farming is progressing. In poultry, contaminated eggs are mainly associated with the cause of fowl typhoid and pullorum disease via its spread from one generation to the next (Wigley et al. 2001). Avian Salmonellosis is a significant disease due to its ability to cause not only a clinical illness in poultry, but also can be transmitted to humans through food, thereby acting as a major source of food-borne transmission (Kabir 2010). Avian salmonellosis, due to its infective strains that are lethal to both humans and animals and its ability for zoonotic transmission via food, has made salmonella not only a concern for public health but also a hot topic in several programs of local, national, and international surveillance. Hence, exposure to avian salmonellosis can result in a health risk (Steve et al. 2004). In the USA, more than 40,000 instances of Salmonella infection, along with 400 fatalities resulting from Salmonellosis of acute nature are documented annually (Fabrega and Vila 2013). Non-typhoidal Salmonella (NTS) infections impose a substantial annual economic burden of approximately $11.39 billion on the U.S. economy, surpassing the costs associated with other bacterial food-borne illnesses. This makes NTS the most expensive food-borne pathogen regarding its impact on health outcomes, resulting in losses of around $3.7 billion (Batz et al. 2014). In a nutshell, Salmonella causes a huge economic loss in the poultry industry via mortality and decreased production (Bierschenk et al. 2017).

The "Avian Salmonellosis" chapter depicts the relationship between Salmonella bacteria and avian populations, focusing mainly on birds as reservoirs and vectors responsible for zoonotic transmission. The chapter digs into various aspects of this zoonotic disease, covering its impact on avian health and its potential risks to human populations. This chapter's sole aim is to provide readers with an understanding of avian salmonellosis as a zoonotic threat. By examining the interplay between avian hosts and Salmonella pathogens, this chapter aims to educate readers about the salmonella prevalence, distribution, transmission routes, particularly to humans from avian hosts, and most importantly, strategies for preventing and controlling avian salmonellosis, emphasizing One Health concept. By fulfilling these objectives, the chapter aims to contribute to the broader understanding of zoonotic diseases within the context of the book "Zoonosis."

2. AVIAN SALMONELLOSIS: AN OVERVIEW

Salmonella is a significant zoonotic pathogen that triggers transmissible ailments in animals and human beings (Li et al. 2018). Annually, Salmonella infection not only results in diminished productivity and, in some cases, fatality in avian, but it also leads to the spoilage of the human food supply, resulting in financial setbacks within the poultry sector. Additionally, it poses a vulnerability to public health (Sylejmani et al. 2016). The Salmonella genus is comprised of two genetically distinct species. One of these species, *Salmonella enterica*, can be additionally classified into six subspecies according to biochemical response patterns. Among these subspecies, only one, namely *Salmonella enterica* subspecies *enterica*, is known to be linked with diseases in animals with regulated body temperature, encompassing a vast array of more than 2,500 motile and non-host-adapted serovars, including examples like *Salmonella enterica* subspecies *enterica* serovar *Enteritidis* and *Salmonella enterica* subspecies *enterica* serovar *Typhimurium* (Gast and Porter Jr. 2020). *Salmonella enterica* has the ability to infect various hosts, and it stands as a prevalent culprit behind foodborne illnesses in both humans and a diverse range of animals. Among these animals,

food-producing ones, specifically, have been identified as sources for non-typhoidal Salmonella illnesses. Within the realm of poultry, Salmonella infections that are specific to the host lead to systemic diseases, primarily attributed to *Salmonella enterica* serovar *Gallinarum*, which causes fowl typhoid and serovar *Pullorum*, which is responsible for pullorum disease (Chappell et al. 2009), and usually doesn't cause a disease in mammals. On the other hand, Salmonella bacteria that are non-selective for the host coexist within birds and can endure in the alimentary tract. While they typically do not produce noticeable symptoms, they are linked to widespread human illnesses (Dunkley et al. 2009; Gantois et al. 2009).

3. PREVALENCE OF SALMONELLOSIS

The frequency of different serotypes of Salmonella within live birds varies from 6% to 30% (Gutierrez et al. 2009; Liljebjelke et al. 2005; Srinivasan et al. 2014; van de Giessen et al. 2006), and in domesticated birds and their derivatives, it extends from 1% to 65.5% (Antunes et al. 2003; Fearnley et al. 2011; Hue et al. 2011; Hyeon et al. 2011; Jordan et al. 2006; Yang et al. 2011). Adult layers had the highest infection rate i.e.,53.25% in contrast to brooding i.e.,14.55%, developing i.e.,16.10%, and young hen i.e.,16.10% (Rahman et al. 2004). In Mymensingh, 45.9% of layer birds had Salmonella infection (Hossain et al. 1970; Talha et al. 2001). Salmonella contamination in poultry samples has been reported worldwide, with rates of 17% in the USA, 35% in Spain, 36% in Korea, 39% in Brazil, and 53% in Vietnam (Lu et al. 2011; Plym and Wierup 2006). The general occurrence of Salmonella in white layers from commercial settings was 25.55%, with the highest seroprevalence in finisher birds (32.22%), followed by grower birds (26.66%) and starter birds (17.77%). The peak seroprevalence occurred in the wintertime (49.07%), trailed by the fall season (25.71%), summer (18.57%), and then spring (15.38) (Shakir et al. 2021). The first outbreak occurred at the Regional Poultry Farm in Mundayad, Kerala, India, in September 2005. Two grower birds displayed symptoms like drooping, diminished feed consumption, and mortality. The subsequent occurrence likewise transpired in September 2005 at the Central Hatchery in Chengannur, Kerala. There was a significant mortality rate among newly hatched chicks, and some died inside their eggshells. Yellow colonies grown on McConkey agar suggested the presence of *Salmonella sp* (Rajagopal and Mini 2013). A case of classical fowl typhoid was identified in Denmark, affecting 18,000 brown layers housed in battery cages. Epidemiological findings suggested that the introduction of the infection might be linked to the collection of spent hens by a German slaughterhouse (Christensen et al. 1994).

An estimate suggests that Salmonella is accountable for about 93.8 million instances of human gastroenteritis along with 155,000 fatalities on a global scale annually (Majowicz et al. 2010). The top two most common serovars in the US includes: *S. typhimurium* and *S. enteritidis,* and they account for 41.5% of the overall epidemics and makes up nearly 60% of all the Salmonella outbursts globally (Hendriksen et al. 2011) and 91% of incidents in Africa (Uche et al. 2017). NTS was 20.39% in hospitalized patients (Gong et al. 2022). In March 1961, there was a *Salmonella typhimurium* outbreak linked to fresh eggs at a fashionable restaurant in northeast Atlanta. Physicians in North Atlanta noticed numerous patients with severe gastrointestinal symptoms, all of whom had consumed blue cheese dressing at the restaurant. Investigation revealed the dressing was made with a rich, unpasteurized mayonnaise base containing about 22 fresh eggs per gallon, seven times more than usual commercial products. Following the outbreak, the restaurant switched to commercially prepared, pasteurized mayonnaise with lower egg content and pH, preventing further incidents (McCroan et al. 1963).

4. TRANSMISSION AND SPREAD

Salmonella infections are a foremost global public health concern (Sanchez et al. 2002; Tariq et al. 2022). The complex host/reservoir of *Salmonella enterica* (Jajere 2019) presence in the intestines of diverse

animals (Joann Colville 2007), acting as reservoirs for potential zoonotic transmission and foodborne illness (Antunes et al. 2016), is facilitated by its broad host range, efficient fecal shedding from carrier hosts, prolonged environmental survival, and effective transmission via various vectors like feed, fomites, and vehicles (Bedi et al. 2022). The transmission sources often involve contaminated poultry, pork, beef, eggs, and dairy products (Acha and Szyfres 2001). Occasionally, foods of plant origin can become contaminated through contact with animal products, human waste, or unclean utensils in commercial processing facilities and household kitchens, leading to incidents of human salmonellosis (Acha and Szyfres 2001).

The heightened risk of zoonotic salmonella transmission to humans through the food chain is attributed to its ability to spread horizontally and vertically within avian communities, occasionally resulting in subclinical infections or remaining completely asymptomatic (Antunes et al. 2016). The primary reservoir for human infections is poultry products, encompassing meat and eggs, frequently sourced from apparently healthy animals (Jibril et al. 2020). Food animals are particularly recognized as a reservoir for non-typhoid Salmonella infections (Revolledo and Ferreira 2012). Recent incidents have underscored the risk associated with raw or undercooked eggs (Humphrey 1994), but broilers are also a significant source of human infection (McGarr et al. 1980).

The primary mode of salmonella transmission involves ingesting contaminated food or direct and indirect exposure to animal feces (Shakespeare 2009). Indirect transmission can occur when individuals come into contact with surfaces or objects where animals reside and move or when they consume food and drinks prepared in environments contaminated with salmonella (Hedican et al. 2010). Even though live poultry infected with Salmonella may seem outwardly healthy, they can sporadically shed the bacteria (Braden 2006). Eggs are particularly susceptible to contamination due to their proximity to chicken feces, and there is a higher likelihood of Salmonella contamination in eggs from hens raised in cage-free systems (Whiley and Ross 2015). Salmonella spp. can be vertically transmitted, passing from parent to offspring or contaminating eggs (Liljebjelke et al. 2005) through the potential contamination of the external eggshell with infected faeces and the infection of the egg's interior before shell formation, encompassing two main routes: horizontal transmission, characterized by potential eggshell contamination through contact with the colonized chicken intestine or exposure to contaminated droppings during or after laying (De Reu et al. 2006) and vertical transmission, which originates from the infection of chicken reproductive organs by *Salmonella enteritidis*, impacting multiple egg components such as the yolk, albumen, eggshell membranes, or the eggshells themselves (Wibisono et al. 2020) as shown in Fig. 1.

Salmonella spp. can quietly inhabit the intestines of poultry and various other animals without causing visible symptoms (Obe et al. 2023). However, when food production practices lack proper hygiene measures, there is a risk of Salmonella spp. contaminating the meat of these animals as it comes into contact with gastrointestinal contents during the slaughter process. A variety of food products, ranging from homemade mayonnaise to omelet mixes, tartar sauce, egg nog, milkshakes, mousse, ice cream, egg sandwiches, scotch eggs, and various other dishes that incorporate either raw or "cooked" eggs, have been associated with instances of infection outbreaks (Hennessy et al. 1996). Infections can result from the consumption of food or water that has been contaminated with bacteria, and the existence of these microorganisms is closely correlated with insufficient hygiene practices (Chlebicz and Slizewska 2018).

Person-to-person transmission is possible when infected individuals fail to follow proper hand hygiene practices after using the restroom and subsequently handling food. Cross-contamination is another concern, especially when clean food items are placed on surfaces that have previously been in contact with contaminated food (Wibisono et al. 2020).

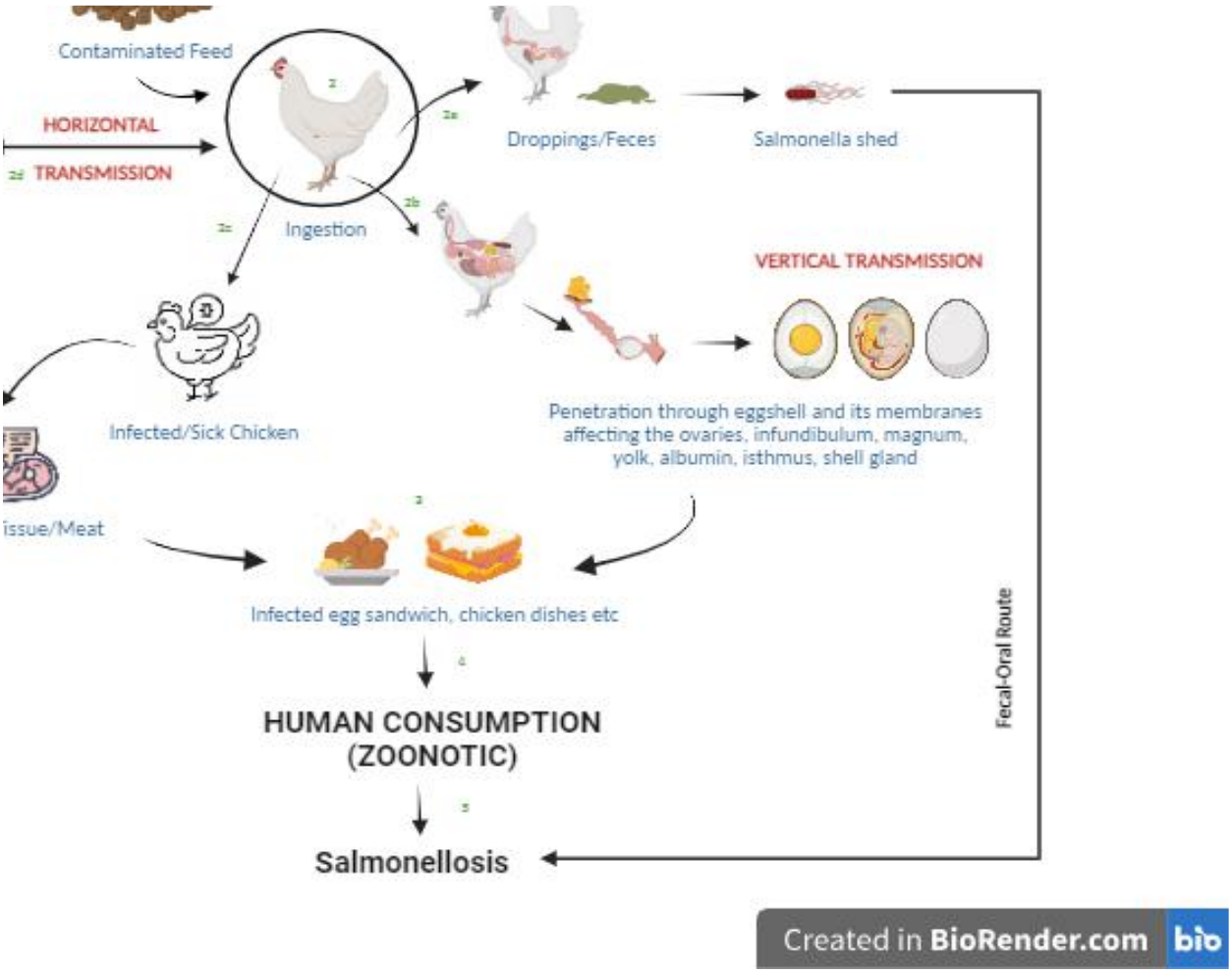

Fig. 1: A schematic diagram showing different modes of transmission of avian salmonellosis as a zoonotic potential to humans: ([1]Healthy bird ingests contaminated feed, [2]represents the initial infection route in avian populations, [2a]Virus shed in droppings which highlights the fecal-oral route as a potential source of human salmonellosis, [2b]Vertical transmission depicting salmonella transmission to eggs and its penetration through eggshell and membranes, affecting various egg components, [2c]Salmnella in poultry meat, [2d]Horizontal transmission depicting transmission through direct contact, [3,4,5]Infected egg and meat products consumed by humans leading to zoonotic transmission of salmonellosis).

Certain animals serve as carriers, intermittently shedding Salmonella. Flies can also play a role in transmitting Salmonella, carrying the bacteria on their feet as they move from contaminated food sources to uncontaminated ones (Thomson et al. 2021). Rodents are potential reservoirs for Salmonella, capable of spreading infections between households and contaminating stored animal feeds. Additionally, insects represent another potential source of Salmonella infection in chickens (Leffer et al. 2010).

5. PUBLIC HEALTH RISKS

Pathogenic bacteria belonging to the Salmonella genus can lead to three distinct forms of salmonellosis in humans: non-invasive and non-typhoid, invasive and non-typhoid, as well as typhoid fever caused by the *S. typhi* serotype, in addition to paratyphoid fever induced by the serotypes *S. paratyphi* A, B, and C (Kurtz et

al. 2017). Salmonella infection can occur due to direct contact with infected animals or indirect exposure to their contaminated environments (Chlebicz and Slizewska 2018). When illness does manifest, it typically presents as diarrhea, which may include blood, accompanied by abdominal cramps and fever within a range of 12 to 72 hours after infection (Colville and Berryhill 2007). In the absence of treatment, most individuals will recover within about a week (Colville and Berryhill 2007). A small fraction of those who appear to have recovered from salmonellosis may subsequently develop Reiter syndrome, which affects the joints, eyes, and urogenital tract (Colville and Berryhill 2007). Generally, human beings serve as the primary source of infection, particularly individuals who become carriers following recovery from the disease, as they may continue to excrete Salmonella in their feces and urine for extended periods (Gunn et al. 2014). The infective dose for salmonellosis typically ranges between 10^6 and 10^8 cells (Chlebicz and Slizewska 2018).

Salmonellosis, a frequently encountered zoonotic disease, presents with gastroenteritis marked by a relatively short incubation period (6–48 hours), leading to watery diarrhea persisting for approximately ten days, accompanied by symptoms such as headache, abdominal pain, nausea, vomiting, and fever (Hubálek and Rudolf 2010). In certain instances, the bacteria can infiltrate the bloodstream and lymphatic system, potentially resulting in conditions like meningitis. However, the fatality rate is generally low at around 0.1% (Hubálek and Rudolf 2010), with fatalities primarily occurring in toddlers and elderly patients due to severe dehydration; disease severity tends to escalate in immune compromised individuals, the young, and the elderly, resulting in various clinical symptoms including nausea, abdominal pain, diarrhea, dehydration, and, in some cases, even death (Elliott 2007). Non-typhoidal serotypes of Salmonella, implicated in foodborne outbreaks, also represent a public health concern in addition to their zoonotic significance (El-Saadony et al. 2022). Salmonella infection not only contributes to poultry morbidity and mortality, with *S. gallinarum* causing fowl typhoid and *S. pullorum* responsible for pullorum disease, but it can also spread from diseased poultry to humans (Lillehoj et al. 2000).

Birds, despite consuming Salmonella-contaminated feed without displaying clinical symptoms, can subsequently introduce the bacteria to processing facilities during evisceration, thereby contaminating poultry carcasses and posing health risks to humans (Wibisono et al. 2020). Salmonella spp. rank among the leading bacterial causes of foodborne gastroenteritis (Sanchez et al. 2002).

6. PREVENTIVE MEASURES

6.1. BIOSECURITY MEASURES

Animals serve as the primary source of non-typhoidal salmonellae responsible for human infections. Human exposure to these bacteria primarily occurs through direct contact with animals, handling of animal waste or manure, or indirectly through food contamination with fecal matter, which are the key pathways for human infection (Sanchez et al. 2002). Implementing effective control initiatives involves integrating sound hygiene and management strategies, complemented by routine serological testing and a clearly defined approach to slaughter (Barrow 1993). Crucial management procedures should encompass the introduction of disease-free and healthy chicks into a meticulously cleaned and sanitized environment, guaranteeing the complete removal of *Salmonella gallinarum* and *Salmonella pullorum* while rigorously enforcing biosecurity measures (Gast and Porter Jr. 2020). It is imperative that both the water and feedstuff stay devoid of any contamination caused by Salmonella. Adequate disposal of deceased birds is of utmost importance. Taking thorough precautions to ward off diseases originating from potential mechanical conveyors such as footwear, attire, hatchery equipment, utensils, bedding materials, crates, vehicles, and processing facilities is essential (Christensen et al. 1994). Hazard Analysis Critical Control Point (HACCP) protocols implementation within both the raw material supply chain and the compound feed mill environment can substantially diminish Salmonella contamination risks in both incoming materials and the

final feed product. Ensuring consistent and effective control of Salmonella contamination within finished poultry feed hinges on the capability to thoroughly decontaminate the feed and prevent subsequent contamination. For efficient heat-based decontamination, it is imperative to apply a precise combination of specific temperature and duration, along with accurate relative humidity levels, consistently to the finished feed. Equally vital is the safeguarding of the decontaminated feed post-heating to prevent any potential re-contamination, extending these protective measures to the poultry flock. This encompasses the careful management of personnel and equipment access, while employing procedures to maintain the integrity of the hygiene barrier and avoid the introduction of contamination (Totton et al. 2012).

The initiative to manage Salmonella in the poultry sector is a cooperative endeavor that requires the active participation of producers, processors, and consumers, all collaborating to ensure the consumption of safe products (Shariat 2023). Within the pre-harvest environment, Salmonella has the potential to disseminate through diverse transmission routes. These pathways includes the transmission via eggs, inter-bird transfer, exposure to salmonella contaminated water, feed, and litter materials, as well as environmental contact resulting from insufficient biosecurity precautions and pest management techniques (Service 2021). Pre-harvest strategies encompass a range of measures, such as the implementation of robust biosecurity plans, vaccinating breeding stock against Salmonella, utilizing feed ingredients that are free of pathogens, efficient bedding maintenance, and the use of water with added acidity (Ruvalcaba-Gomez et al. 2022). One of the first steps to consider in reducing the presence of pathogens in manufacturing units is the enforcement of good manufacturing practice and sanitation standard operating procedures. It is of utmost importance for facilities to give precedence to the well-being of employees and the cleanliness of the processing area to safeguard both the workforce and the products delivered to consumers (Service 2021). Once a food product enters the food service chain or is purchased by a consumer, it becomes crucial to follow proper handling procedures, ensuring that poultry is cooked to the prescribed internal degrees of heat such as at 165°F (74°C), following the guidelines established by USDA-FSIS, thereby, providing an additional safeguard for consumers (Service 2022) .

6.2. FEEDING STRATEGIES

Antibiotics affect feed efficiency by influencing the gut microbiota, where a competition for nutrients occurs between the host and pathogenic bacteria like *Escherichia coli*, Salmonella species, and *Clostridium perfringens* (Abd El-Hack et al. 2022; Swelum et al. 2021). Due to concerns about antibiotic resistance, the use of antibiotic growth promoters is declining (Danzeisen et al. 2011). *Salmonella enteritidis* being the primary serotype in poultry farming, has developed resistance to several antibiotics (Ray and Bhunia 2007). There's a global effort to reduce antibiotic use and promote organic substitutes for antibiotics, such as prebiotic agents, probiotics, indispensable amino acids, organic acids, essential oils, and more.

Competitive exclusion" occurs when microorganisms in the alimentary canal vie for resources, including nutrients and adhesion points (Nurmi et al. 1992). Disease-causing bacteria like *E. coli* along with Salmonella requires attachment with the enteric mucosal membrane in order to initiate infection in avian (Lan et al. 2005). Lactic acid producing microorganisms, like *Lactobacillus* strains, produce lactic acid through carbohydrate fermentation, diminishing intestinal acidity and inhibiting bacteria like *S. typhimurium* along with *E. coli* and *C. perfringens* (Murry et al. 2004). In vivo studies have confirmed the findings, demonstrating a decrease in Enterobacteriaceae in broiler ceca when levels of acetate, propionate, and butyrate (SCFA's) increased [46]. Incorporating fructo oligosaccharide in daily broiler feed restricts *Salmonella enteritidis* colonization (Shang et al. 2015). Combining probiotics and prebiotics (symbiotics) such as 0.1% of fructo oligosaccharide, reduced Salmonella enteritidis inhabitation in chick intestines more effectively than when used separately (Fukata et al. 1999).

Cymbopogon citratus (extract of lemongrass) demonstrates powerful bactericidal properties towards a range of pathogenic bacteria, including *S. typhimurium* and *S. enterica* (Alagawany et al. 2021). Oregano and Thyme extracted essential oils efficiently reduce Salmonella species establishment in chickens' alimentary `tracts (Koščová et al. 2006).

6.3. VACCINATION

Various vaccines, including bacterins, weakened, subunit, and nanoparticle-founded vaccines, protect birds from Salmonella infections (Fig. 2) (El-Saadony et al. 2022). Inactivated whole bacteria (bacterins) offer variable safety towards salmonella (Davison et al. 1999). Chickens receiving formaldehyde-inactivated *Salmonella Enteritidis* in decomposable microspheres at an age of 2 weeks old showed reduced shedding of salmonella in feces and its colonization in various organs (Liu et al. 2001). Layer herds inoculated with *Salmonella Enteritidis* bacterin vaccines around 14 and 20 weeks showed that Salmonella establishment was absent, unlike the unvaccinated cohort (Davison et al. 1999). Oral administration of an attenuated *S. Enteritidis* vaccine to 9-week-old chickens reduced Salmonella establishment within spleen, liver and in ceca (Cerquetti and Gherardi 2000). Chickens receiving a subunit vaccine at 9 weeks old, followed by two booster doses, exhibited reduced Salmonella colonization in the ceca (Khan et al. 2003). A study by (Salman et al. 2005) involved engineering of vaccine (nanoparticles-based) that is Salmonella-like by attaching *S. enteritidis* flagellin to mimic natural colonization in the gut. These flagellin-coupled nanoparticles effectively prompted specific absorption in the intestinal mucosa, encompassing Peyer's patches. In another study, a Salmonella subunit vaccine was orally administered to layer chickens, having antigenic outer membrane proteins and flagellar proteins of Salmonella within and on exterior of polyanhydride nanoparticles (Fig. 2). This elicited a distinct immune reaction and restricted Salmonella establishment in the intestinal mucosa (Renu et al. 2018).

7. SURVEILLANCE AND MONITORING

In 1885, an American scientist, Daniel E. Salmon, successfully isolated an enteric pathogen discovered in the intestines of pigs (El-Saadony et al. 2022). Over time, various factors such as industrialization, large-scale food production, reduced trade barriers, and increased human migration have contributed to the global dissemination and heightened prevalence of foodborne illnesses (Todd 1997). This development has given rise to significant public health concerns and an increased risk of zoonotic transmission associated with Salmonella, prompting the introduction of numerous surveillance initiatives on international, national, and local scales (Steve et al. 2004). In developing nations, it is estimated that approximately 16 million cases of typhoid fever occur annually, resulting in around 600,000 deaths worldwide (Ivanoff 1995). In contrast, developed countries have largely controlled the transmission of this disease through the practice of good hygiene and sanitation measures, resulting in a decrease in its prevalence (Ahmed et al. 2005). In Pakistan, Salmonellosis is considered endemic, and various studies have been conducted across the country, including Karachi (Hafiz et al. 1993; Saqib and Ahmed 2000), Lahore (Khalil et al. 1993), Peshawar (Gandapur et al. 1993) and Rawalpindi/Islamabad. From 2000 to 2002, in Asia, specifically in Japan, Korea, and Thailand, *S. Enteritidis* was collectively the most commonly reported human serotype, with *S. Weltevreden* ranking second in 2000 and 2001 but dropping to fourth place in 2002, being overtaken by *S. Rissen* and *S. Typhimurium*. In 2002, *S. Enteritidis* constituted 38% of human isolates but only 7% of non-human isolates. Meanwhile, *S. Anatum, S. Rissen, and S. Stanley* were the most prevalent non-human serotypes in Asia (Galanis et al. 2006).

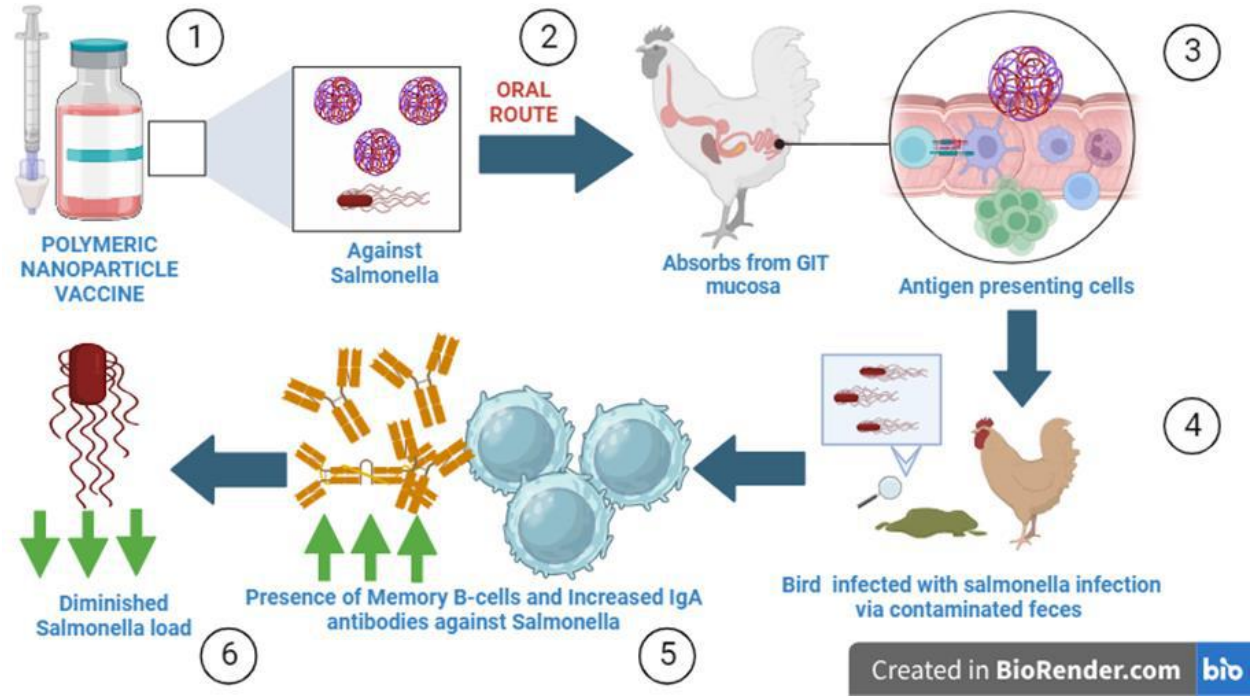

Fig. 2*:* Schematic representation of the defense system of the chicken's body against Salmonella when administered with a polymeric nanoparticle vaccine.[1]Polymeric nanoparticle vaccine is administered to the bird via oral route, [2]The vaccine is absorbed from gastrointestinal tract mucosa, [3]Antigen presentation of vaccine antigen by antigen-presenting cells, [4]Bird infected with salmonella contaminated droppings, [5]Memory B-cells and IgA antibodies are already present in infected chicken's body (virus-neutralization), [6]Decreased salmonella load (Acevedo-Villanueva et al. 2021)

In the United States, there are over 40,000 documented cases of Salmonellosis annually, resulting in approximately 400 deaths each year (Fabrega and Vila 2013). Worldwide, animal products contaminated with Salmonella account for 3% of bacterial foodborne illnesses, causing an estimated 80 million infections and 155,000 fatalities. The literature provides a wide range of estimates for the annual global incidence of salmonellosis, spanning from 200 million to over 1 billion cases (Bierschenk et al. 2017). Effective surveillance programs that can promptly detect and prevent human Salmonella infections require comprehensive monitoring of Salmonella contamination along the entire food supply chain, encompassing animal feed, live animals, slaughterhouses, the retail sector, and restaurants (Newell et al. 2010). In 1997, the United States reported 37,200 confirmed cases of salmonellosis, with 92% of cases based on positive stool samples and 7% from blood samples collected in seven states (Sanchez et al. 2002). The overall incidence of Salmonella infections in the U.S. population in 1998 was 17.4 cases per 100,000 individuals (Sanchez et al. 2002). In the European Union, the number of salmonellosis cases in humans decreased by 5.4% in 2011 compared to 2010 and by 37.9% compared to 2007, indicating a statistically significant declining trend between 2008 and 2011. Salmonellosis is closely linked to poultry production, and substantial efforts are in progress to manage and restrict the spread of this pathogen during various stages of poultry production.

During the summer months, specifically from June to August, approximately 40% of Salmonella infections are reported, with food consumption as the primary associated factor (Sanchez et al. 2002). Children under the age of 1 year are notably susceptible to infection, with an incidence rate exceeding 175 cases per 100,000 individuals. In the age group of 1 to 9 years, the incidence is approximately 50 cases per 100,000

persons, while beyond the age of 9, the incidence decreases to around 25 cases per 100,000 individuals and remains relatively steady across other age groups (Sanchez et al. 2002). Globally, the anticipated fatality rate linked to salmonellosis exceeds 150,000 cases (Chlebicz and Slizewska 2018). Concerning the prevalence of avian salmonellosis based on age, the highest infection rate is observed in adult layers, accounting for 53.25% of cases, in contrast to brooding (14.55%), growing (16.10%), and pullet (16.10%) chickens (Kabir et al. 1970). Worldwide data on Salmonella serotype distribution in humans and various food products have helped establish an epidemiological connection between salmonellosis and poultry items, with diverse serotypes being common to both humans and poultry meat, such as chicken and turkey (Antunes et al. 2016).

8. FUTURE DIRECTIONS AND CHALLENGES

Efforts to combat the rapid spread of antibiotic resistance in poultry should explore alternative control methods like bacteriophages, antimicrobial peptides, and combination antibiotics. Future research focused on development of cost-effective vaccines and innovative delivery methods, particularly automated *in ovo* injection devices, are crucial for Salmonella vaccine development in the broiler industry. Ensuring traceability in poultry-related salmonellosis outbreaks is vital, requiring enhanced record-keeping practices, especially in atcheries and feed stores, to prevent future occurrences and investigate potential alternate transmission routes. Furthermore, addressing Salmonella's animal and public health concerns involves maintaining rigorous management standards, exploring innovative approaches like lytic bacteriophages, and combining established and novel strategies. Poultry breeding companies should focus on researching genetically resistant chicken lines to enhance Salmonella control and identify associated genomic regions for their inclusion in the breeding programs. Contemporary society's shift towards ready-to-eat and takeout foods has reduced home cooking, emphasizing the need for comprehensive collaborative efforts among primary industries, health departments, and food branches to control Salmonella in the egg supply chain, beyond farm-specific strategies. Additionally, public engagement in food safety practices such as post-egg handling handwashing is essential. While numerous national and international regulations should be established to mitigate Salmonella contamination in chicken, significant challenges persist in determining practical and cost-effective control methods within the poultry sector.

9. CONCLUSION

In conclusion, avian salmonellosis represents a noteworthy public health challenge of global significance, characterized by its potential for zoonotic transmission, prevalence in diverse animal populations, particularly poultry species, and association with various food products. These infections, posing risks of gastroenteritis and related health complications, are especially concerning for vulnerable populations, including children. Effectively addressing this issue necessitates the exploration of alternative control strategies, enhancing traceability, and implementing rigorous management standards in collaboration with various stakeholders to ensure food safety and public health protection.

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Methicillin-Resistant Staphylococcus aureus (MRSA) and its Intersection with Animals

12

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is a multidrug-resistant bacteria that poses a serious risk to public health. In addition to human populations, MRSA has become a problem in the context of animals, posing issues with reservoirs, interspecies transmission, and possible effects on veterinary and human treatment. This succinct study delves into the relationship between MRSA and animals, looking at important factors such genetic diversity, transmission patterns, and prevalence. The report addresses the several animal species—companion, livestock, and wildlife—that have been linked to MRSA colonization and infection. The bidirectional transmission of MRSA between people and animals is highlighted in particular, highlighting the need of a One Health approach in understanding and managing the intricate dynamics of this zoonotic infection. To understand the genetic processes underlying MRSA adaption and transmission across species borders, genomic research and molecular epidemiology are examined closely. Additionally, the paper evaluates how animals may serve as reservoirs for rare strains of MRSA and how they contribute to the pathogen's overall genetic diversity. The study also discusses the effects of MRSA on livestock production systems, veterinary treatment, and the dangers it presents to human and animal populations that live near to one another. The need of cooperative efforts between the human and veterinary healthcare sectors is emphasized in the discussion of strategies for monitoring, prevention, and control at the human-animal interface. To sum up, this multidisciplinary summary offers a concise examination of the intricate interactions that exist between MRSA and animals. It seeks to improve our comprehension of the complex dynamics of MRSA transmission in various animal populations by combining existing research and promoting an all-encompassing strategy to reduce the dangers related to this disease that is clinically relevant.

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1. INTRODUCTION

The bacteria Methicillin-Resistant *Staphylococcus aureus* (MRSA) has attracted a lot of interest in the medical community due to its resistance to antibiotics, including methicillin and other beta-lactam medications. The *Staphylococcus aureus* is a Gram-positive, human-host-adapted bacterium that is frequently discovered on people's skin and in their nasal passages. Though sometimes thought of as an opportunistic pathogen, it is also a commensal organism that may cause invasive infections of the skin. The first reports of methicillin-resistant *S. aureus* (MRSA) appeared in the early 1960s, not long after the drug's release. MRSA infection rates sharply rose in the late 1970s, mostly among hospitalized patients. Another important development in the 1990s was the identification of MRSA infections among previously healthy persons contracted in the community (Weese 2010). The terms HA-MRSA (healthcare-associated) and CA-MRSA (community-associated) now apply to these two MRSA origins. MRSA has always been linked to diseases that affect people who get treatment, but it has now developed a presence in animals as well, posing new problems and causing concern in the veterinary and public health fields. This chapter explores the complex interactions between MRSA and animals, including the patterns of transmission, the distribution of reservoirs, and any possible effects on both animal and human health.

2. MRSA: A BRIEF OVERVIEW

MRSA is a strain of the bacterium *Staphylococcus aureus* (SA) that has shown and developed resistance to beta-lactam antibiotics, primarily methicillin. *Staphylococcus aureus* is a well-known nosocomial bacterium that causes epidemics in humans and cattle mastitis, according to studies (Biedenbach et al. 2004) (Barton et al. 2006). *S. aureus* includes different virulence factors like Panton-valentine leukocidin (PVL), certain enzymes such as proteases, lipases, and elastase, and slime factor i.e., biofilms, which promotes the mutilation of host tissues and proliferation to other sites (Gordon and Lowry 2008). The staphylococcal cassette chromosome mec (SCCmec), which carries the mecA gene, is the main mechanism via which MRSA spreads (Deurenberg et al. 2007). Five different kinds of SCCmec elements were identified, including types I, II, III, IV, V, and VI. Inappropriate antibiotic use, underdosing, and improper delivery play a significant influence in the development of resistance to antibiotics. MRSA has emerged as a significant contributor to infections that are hospital- and community-related, as well as isolated from milk (Livestock-associated) (Klein et al. 2007) (Devriese and Hommez 1975). The emergence of MRSA is largely attributed to the overuse and misuse of antibiotics, which has led to the selection of resistant strains. Initially recognized in human healthcare settings, MRSA infections are challenging to treat due to their resistance profile.

3. MRSA IN ANIMALS: AN EVOLVING CONCERN

In recent years, MRSA has been identified in a variety of animal species, both domestic and wild. The transmission of MRSA between humans and animals, as well as within animal populations, has been

documented, highlighting the complex interplay between these reservoirs. The main known method of MRSA transmission between hosts is through direct physical contact with the source. The defining property of MRSA lineages is the capacity to spread to many hosts, including humans as well as animals. When a person comes into personal touch with an animal or their surroundings, LA-MRSA can spread to humans (Pantosti 2012). Before the Hungarian cow was identified as the root cause of livestock-associated MRSA transmission to its keeper by analyzing swabs taken from throat in 1961, LA-MRSA was solely confined to animals (Cefai et al. 1994). This report was the preliminary account of MRSA spreading from an animal to a man, demonstrating the potential for horizontally transmitting MRSA between humans & animals. Following that, several reports on numerous animal species, including poultry, pigs, cattle, sheep, goats, equines, and companion animals, were published by a variety of authors from different parts of the world. These findings showed that both MRSA strains either from animals or humans shared several clonal complexes (CCs) with multi-locus sequence types (STs), including CC5, CC1, CC8, CC9, CC59, CC22, CC30, CC45, CC97, CC130, and CC398. However, other HA-MRSA and CA-MRSA strains have also been identified, and they are comparable to other LA-MRSA strains that are shown in Fig. 1. Bovine mastitis is brought on by a human clone called ST1 that was discovered in animals (Grundmann et al. 2010). comparable to human clones CC398 and ST398 that cause diseases like HA-MRSA and CA-MRSA, animal clones of these strains induce comparable infections in humans (Witte et al. 2007). A global ST5 poultry clone was also

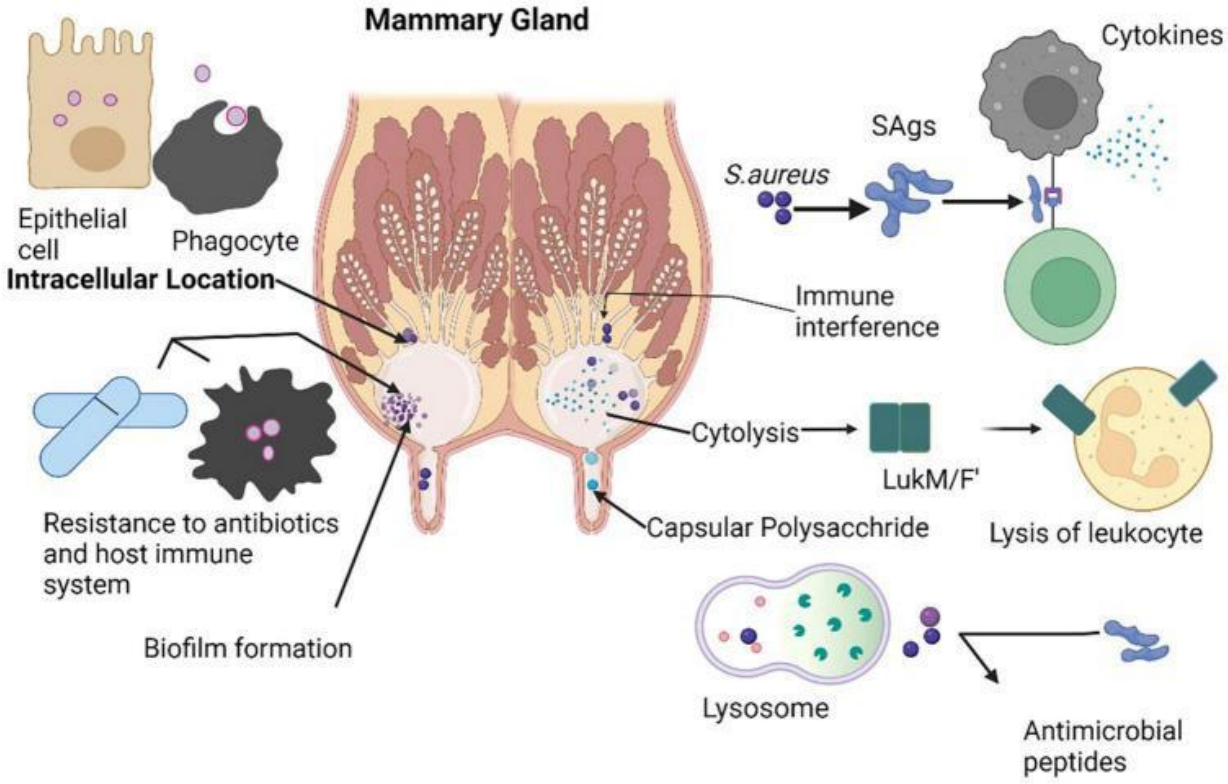

Fig. 1: Different strategies used by *Staph. aureus* to cause infection in mammary glands

discovered in individuals working on poultry farms (Lowder et al. 2009). Numerous studies have also established that MRSA may spread from companion animals to people. For instance, research by across the United States and Canada found that 18% of pet owners carried MRSA. Similar stress was observed in patients, hospital personnel, and nursing cats in different research conducted in a UK nursing home (Scott

et al. 1988). The same MRSA lineage ST22 was spread from sick canines to veterinary staff members in research done at a veterinary facility (Baptiste et al. 2005). However, a household member in the Netherlands discovered that dogs were also occupied with a strain which is human PVL-positive CA-MRSA (Van Duijkeren et al. 2005). Another research by Shoaib et al. (2020) outlined the dangers connected to LA-MRSA transmission from companion animals. The owner's sex, the sample spot, and the dog's size were found to be insignificant risk factors for MRSA transmission. Amongst these risk factors, it was discovered that having access to the bedroom by a pet, veterinarians, body infection, use of antibiotics for longer periods and animal health records were also important risk factors for the transmission of MRSA to humans. According to a different study by Mulders et al. (2010) the type of slaughtering process, the surroundings of the abattoir, and farm employees who come into touch with live birds, are all important risk factors for MRSA transfer from poultry to humans.

4. TRANSMISSION DYNAMICS

Transmission of MRSA between animals and humans occurs through direct contact, environmental contamination, and shared living spaces. Companion animals such as dogs, cats, and horses have been found to carry MRSA, often without showing clinical signs of infection. S. aureus, in contrast, does not adapt to either dogs or cats as hosts. This is why *S. aureus* colonization in pets, including both methicillin-resistant and methicillin-susceptible strains, often lasts little more than a few weeks (Loeffler et al. 2010) (Morris et al. 2012). *S. pseudintermedius*, which can also be methicillin-resistant (MRSP), is the most prevalent commensal *Staphylococcus* in dogs and cats. MRSA is often obtained from people in dogs and cats. The strains discovered in animals closely resemble those discovered in residents of that area (Loeffler et al. 2010) MRSA colonization rates in cats and dogs typically vary from 0-4%, but they can reach as high as 7-9% in certain populations (Weese 2010). Contact with an MRSA-infected human, multiple courses of antibiotics, going to a veterinary facility, having surgery, or being hospitalized for a prolonged period of time are the main risk factors for MRSA colonization in dogs.7,8 The risk of MRSA colonization is also higher for veterinary professionals than it is for the general public (Weese 2010; Loeffler et al. 2010). Recent studies have revealed a frequency of 4–18% among veterinary staff compared to 1-3% in the general population (Weese 2010), highlighting the need of proper hand hygiene and glove use in the veterinary field. Livestock, including pigs, cows, and poultry, have also been identified as reservoirs of MRSA, raising concerns about potential foodborne transmission.

5. ZOONOTIC IMPLICATIONS

The zoonotic potential of MRSA cannot be understated. While MRSA strains in animals may differ from those in humans, the exchange of genetic material between species can lead to the emergence of novel strains with enhanced virulence and resistance traits. Individuals who work closely with animals, such as veterinarians, animal handlers, and pet owners, are at an increased risk of contracting MRSA infections. Although MRSA infections present in companion animals and food were once believed to spread more slowly, it is also becoming a severe issue for food sector companies and food animals. According to LA-MRSA is a significant contributor to mastitis in buffaloes and cows, which results in reduced or nonexistent milk output. poultry's infections such as chondronecrosis, septic conditions and comb necrosis are also caused by LA-MRSA . Nearly all pets such as dogs, cats, and horses, have the potential to spread LA-MRSA to people who come into contact with them directly or indirectly. Mastitis is a serious condition that affects dairy cows and is associated with excessive antibiotic usage, which results in significant economic losses. Among all other potential causes of mastitis across the world, *S. aureus* is the most common

pathogen. According to Grinberg et al. (2004), LA-MRSA is a significant factor in the pustular dermatitis that affects their milkers. However, none of the bovine MRSA clones, which cause subclinical mastitis in cattle, are often found in dairy cows (Aqib et al. 2018; Abdeen et al. 2021). MRSA was initially found in cattle in Belgium in milk samples in 1972, where it was presumed that it had been contaminated by the hands of the milkers (Lee 2003). According to (Holden et al. 2013), mammary gland's infection due to MRSA in calves reduces milk production and, in extreme cases, might result in the termination of milk production from the mammary glands.

Healthy poultry birds' cloaca and nares have also been shown to have LA-MRSA. It can result in pyoderma, omphalitis, UTI, arthritis, and otitis in poultry birds (Pickering et al. 2022). following the use of several antimicrobial drugs. MRSA of spa types t011 and t157 were discovered. While ST398 is a brand-new MRSA strain linked to livestock that is also present in poultry (Nemati et al. 2008). According to a different analysis, all MRSA isolates of chicken origin belonged to spa type t1456.

5.1. DIFFERENT DISEASES CAUSED BY MRSA IN DIFFERENT SPECIES

Numerous human illnesses, including acne, wound suppuration, food poisoning, urinary tract infection (UTI), endocarditis, otitis, pyogenic pneumonia, osteomyelitis, nosocomial infections, health-care associated infections, mastitis, and septicemia, may be brought on by MRSA strains (Boucher et al. 2008). Horses are susceptible to botryomycosis, a "peculiar disease" caused by bacteria that causes pyogenic inflammation of the spermatic cord and a localized purulent infection. Localized pyogenic infection, severe acute mastitis, and apparent toxemia in cattle and ewe. Similar to caseous lymphadenitis in sheep, anaerobic strains produce abscesses in sheep. Food poisoning and pustular dermatitis in cats and dogs. In pigs, exudative epidermatitis “greasy pig disease” and in avians, suppurative arthritis “Bumble-foot”.

5.2. MRSA AND COMPANION ANIMALS

Most families now include pets like dogs, cats, and horses, especially in advanced countries like the USA and the UK (Chomel and Sun 2011). As a result, there is a substantial likelihood that these animals may colonize humans or infect them with MRSA (Mustapha et al. 2014). Dogs are more likely than cats to be infected or colonized with MRSA, according to Morgan (2008), and 1.5% of MRSA was found in samples from diseased companion animals in the UK. Infections of the skin and soft tissues are the most common way to present the diseases. EMRSA-15 (ST22) and EMRSA-16 (ST36) are the MRSA strains that have been isolated in the majority of UK hospitals (Ellington et al. 2010), whereas USA100 (ST5), which has been linked to HA-MRSA infections in humans, has been recovered from US pets (Ellington et al. 2010). Additionally, an MRSA clone (ST398) that was typical of farm animals was found in dogs and horses in a UK investigation (Loeffler et al. 2009). The majority of events and outbreaks of MRSA infections have been linked to problems following surgery and large stables (Weese et al. 2005; Morgan 2008). MRSA strains identified from horses were different from those recovered from people (Loeffler and Lloyd 2010). The prevalence of MRSA infection in different species of animals has been shown in Table 1.

Table 1: Prevalence of MRSA infection in different species of animals

Countries	Animals	Sample type	Prevalence(percentage)	References
Germany	Dog, cat, horses	Wounds	62.7, 46.4, 41.3	Vincze et al. 2014
Germany	cats	Clinical	10	Walther et al. 2008
Netherland	calves	Nasal samples	88, 28	Graveland et al. 2010
Belgium	Cows/broilers	Nasal/Cloaca	5.0, 5.0	

6. VETERINARY HEALTHCARE SETTINGS

Veterinary clinics and animal hospitals can serve as potential hotspots for MRSA transmission. In these settings, animals receiving medical treatment can act as reservoirs, while human-to-human transmission can exacerbate the problem. Implementation of rigorous hygiene and infection control measures is crucial to prevent MRSA spread within veterinary facilities.

7. ONE HEALTH APPROACH

The One Health approach, which recognizes the interconnectedness of human, animal, and environmental health, is paramount in addressing the MRSA challenge. Collaborative efforts between medical and veterinary professionals, researchers, and public health authorities are essential for understanding and mitigating the impact of MRSA on animals and its potential to affect human health.

8. FUTURE DIRECTIONS

As MRSA continues to adapt and evolve, ongoing research is needed to elucidate the dynamics of MRSA transmission in animal populations and its implications for human health. Improved surveillance, diagnostic tools, and antimicrobial stewardship programs are vital components in managing MRSA across species. according to a study, a post-antibiotic era, in which common diseases and mild infections might kill, is a very real prospect for the twenty-first century. According to the World Health Organization (WHO), the rise in antibiotic-resistant microorganisms is one of the biggest threats to public health.
A-lactam antibiotic and potassium clavulanate were discovered to be the most efficient combination against MRSA (De Araújo et al. 2013). According to several studies, phytochemicals have a significant organic potential to demonstrate antibacterial action and function as modulators of antibiotic resistance, either alone or in combination with antibiotics. According to (Lakshmi et al. 2013), plants' active secondary metabolites are responsible for the bulk of these healing benefits. According to (Coutinho et al. 2009), phytochemicals modify bacterial enzymes and inhibit efflux pumps in addition to altering active sites, increasing plasma membrane permeability, and inhibiting efflux pumps as shown in Fig. 2.

8.1. SYNERGISTIC EFFECT OF ANTIBIOTICS WITH NSAIDS

Numerous investigations have demonstrated that NSAIDs have antibacterial capabilities, however, the precise route of action is unknown. Diclofenac, aspirin, and ibuprofen have been found to exhibit antibacterial properties at 5 mg/ml against some gram-positive bacteria, except mefenamic acid. The only NSAID that is effective against gram-negative bacteria is aspirin because gram-negative bacteria have lipopolysaccharide, which is hydrophilic and hinders most medicines' metabolism. Antimicrobial medicines can penetrate gram-positive bacteria cells with ease since their cell walls lack lipopolysaccharides (Khalaf et al. 2015).

8.2. NANOPARTICLES AS THERAPEUTIC AGENTS

Metal nanoparticles (NPs) are increasingly common and affordable production materials that are finding many uses in the modern world due to their unique properties. The market for nanometals based on metal oxides was said to have reached USD 4.2 billion in 2016. The rising usage of metal-based nanoparticles in biomedical research is observed to be expected to enhance the need for NP manufacture by 2025 (Gudkov et al. 2022).

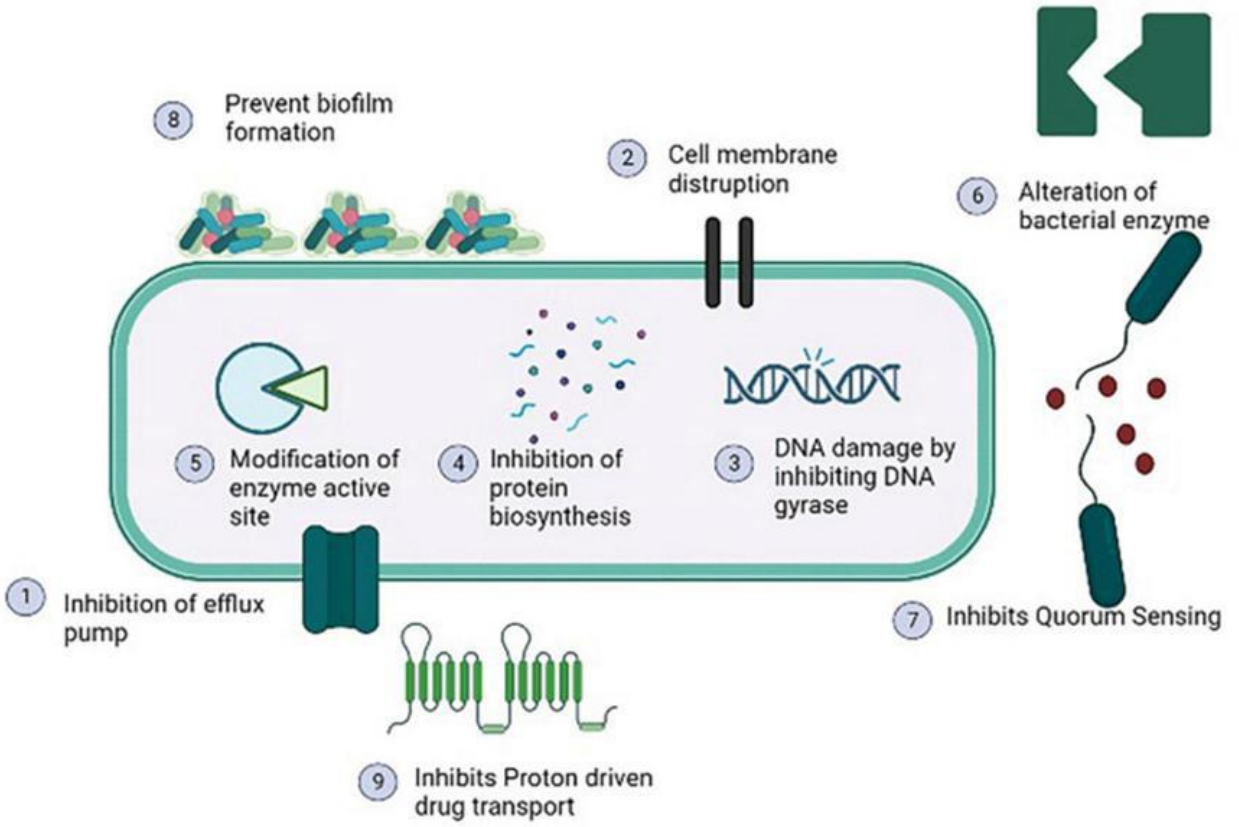

Fig. 2: Antibacterial mechanisms of various phytochemicals against methicillin resistant strain of *Staphylococcus aureus* (MRSA).

9. CONCLUSION

The emergence of MRSA in animals underscores the intricate relationship between human and animal health. Understanding the transmission dynamics, reservoirs, and zoonotic potential of MRSA is imperative for effective control and prevention strategies. By adopting a One Health approach, society can work collaboratively to address the challenges posed by MRSA and promote the well-being of both humans and animals. Methicillin-resistant *S. aureus* strain (MRSA) is a flexible and unpredictable pathogen with a variety of lineages shared by humans and animals, suggesting that it spreads from people to animals. The lineages CC398, CC9, CC130, CC97, and CC398 were those shared by humans and animals. Except for this, few HA-MRSA and CA-MRSA lineages were found in animals, although LA-MRSA lineages, which resemble HA-MRSA and CA-MRSA, were found. Because of its growing genetic adaptability and ubiquity in both animal and human populations, this disease poses a danger to public health. This disease has a wide variety of hosts, including people, food animals, pets, and many more. Work on effective MRSA vaccination and vaccine manufacturing is a further strategy.

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Zoonotic Risks of Antimicrobial Resistance: Alternative Strategies to Combat this Silent Pandemic

13

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ABSTRACT

Over the past 20 years, antimicrobial resistance (AMR) has grown to be a global concern to public health systems everywhere. From the invention of the initially developed medicines that consistently improved human health throughout the antibiotic time period, antimicrobial overuse and abuse in both veterinary and human medicine has contributed to the global AMR epidemic. This chapter provides a thorough summary of the epidemiology of antimicrobial resistance (AMR), emphasizing the relationship between people and animals that produce food as well as the laws and policies that are now in place throughout the world. The challenges posed by antimicrobial resistance (AMR) will be addressed through a variety of strategies, such as developing novel antimicrobials, bolstering the monitoring system for AMR in both human and animal groups, better understanding the ecology of resistant bacteria and resistant genes, raising stakeholder awareness of the responsible use of antibiotics in animal production and clinical settings, and addressing the effects of AMR on public health and the environment. Given the worldwide scope of antimicrobial resistance (AMR) and the fact that bacterial resistance is impervious to barriers, the essay concludes with particular recommendations aimed at various stakeholders and organized around a comprehensive approach. The well-known zoonotic illnesses include hemorrhagic colitis caused by Escherichia coli, brucellosis caused by Brucella abortus, bovine tuberculosis caused by Mycobacterium tuberculosis, or anthrax caused by Bacillus anthracis. Similar to this, the majority of antibiotics are not entirely broken down before being released into the food chain where they bioaccumulate and impact different ecological niches. The comprehension of AMR mediated by zoonoses is a global issue that affects not only scientific researchers but also farm animal producers, medical personnel, patients, and consumers. In order to maintain the efficient use of antibiotics in human as well as animal medicine many alternative methods used.

Key words: Zoonosis, Antibiotics, Resistance, Risk factors, Control.

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1. INTRODUCTION

Over the past 20 years, antimicrobial resistance (AMR) has grown to be a global concern to public health systems everywhere. From the invention of the initially developed medicines that consistently improved human health throughout the antibiotic time period, antimicrobial overuse and abuse in both veterinary and human medicine has contributed to the global AMR epidemic. This chapter provides a thorough summary of the epidemiology of antimicrobial resistance (AMR), emphasizing the relationship between people and animals that produce food as well as the laws and policies that are now in place throughout the world. The challenges posed by antimicrobial resistance (AMR) will be addressed through a variety of strategies, such as developing novel antimicrobials, bolstering the monitoring system for AMR in both human and animal groups, better understanding the ecology of resistant bacteria and resistant genes, raising stakeholder awareness of the responsible use of antibiotics in animal production and clinical settings, and addressing the effects of AMR on public health and the environment. Given the worldwide scope of antimicrobial resistance (AMR) and the fact that bacterial resistance is impervious to barriers, the essay concludes with particular recommendations aimed at various stakeholders and organized around a comprehensive approach. The well-known zoonotic illnesses include hemorrhagic colitis caused by Escherichia coli, brucellosis caused by Brucella abortus, bovine tuberculosis caused by Mycobacterium tuberculosis, or anthrax caused by Bacillus anthracis. Similar to this, the majority of antibiotics are not entirely broken down before being released into the food chain where they bioaccumulate and impact different ecological niches. The comprehension of AMR mediated by zoonoses is a global issue that affects not only scientific researchers but also farm animal producers, medical personnel, patients, and consumers. In order to maintain the efficient use of antibiotics in human as well as animal medicine many alternative methods used.

It is hard to develop a world that can control human and animal health. Globally, there is serious worry about the rise of new infectious agents and resistance to drugs in existing pathogens. In the early 19th century, the discovery of antibiotics revolutionized the healthcare system by preventing millions of deaths. Humans use different types of antibiotics such as Aminoglycosides, Beta-lactams, Glycopeptides, Macrolides, Oxazolidinones, Quinolones, Tetracyclines, and Sulphonamides in the treatment of most diseases (Etebu and Arikekpar 2016). These antibiotics are used as bactericidal (to kill bacteria) as well as bacteriostatic (to inhibit the growth and replication of bacteria). Bacteriostatic antibiotic classes are macrolides, sulphonamides chloramphenicol, lincosamides (clindamycin), and tetracyclines while beta-lactams, aminoglycosides, glycopeptides (vancomycin), quinolones polymyxins (colistin) are bactericidal drugs (Patil and Patel 2021). Each group of antibiotics has its specific mode of action. For example, aminoglycosides, chloramphenicol, lincosamides, tetracycline, and macrolide have a direct action on RNA to inhibit the synthesis of protein and thus have a bactericidal effect. Cephalosporin, penicillin, and glycopeptide inhibit cell wall synthesis, sulfonamides inhibit folate synthesis, and fluoroquinolone inhibits DNA synthesis. Frequent and continuous usage of antibiotics has led to drug resistance called antimicrobial resistance (AMR) globally but is more prevalent in developing countries (Chokshi et al. 2019).

Pathogens can become resistant to antibiotics over time, making infections more difficult to cure and raising the risk of disease spread, life-threatening sickness, and death. AMR is among the greatest threats to human and livestock populations in terms of productivity and public health noticeably in countries like Pakistan, India, Bangladesh, Nepal and Sri Lanka. AMR is becoming dangerous in developing countries, because of the easy availability and frequent use of antibiotics (Sharma et al. 2018). The majority of antibiotics that enter the bodies of humans and livestock are not completely utilized, causing the release of unmetabolized forms into the surroundings. Antibiotic resistance among the native microbiome and altered sensitivity of the bacterial population are results of the increased level of antibiotics in varied environments. The presence of antibiotics in surroundings causes changes in the genomes of bacteria leading to the emergence of genes that are resistant to antibiotics. During a process called horizontal gene transfer, microorganisms acquire antibiotic-resistance genes (Tang et al. 2023). Integrons (genetic components with a site-dependent recombination mechanism), transposons(DNA sequences represent a kind of mobile genetic component), and plasmids (A little circular DNA structure that exists in bacterium) serve as carriers of genetic material between infectious agents through transformation (Foreign genetic material (DNA) is taken in by bacteria from the environment), transduction (The genetic material of one bacterium is carried to another by a virus.), and conjugation (Direct contact between bacteria results in the transfer of genetic material) (Bello-López et al. 2019).

Livestock is the most common carrier for the propagation of resistant pathogens. A large population of bacteria resides in the gastrointestinal (GI) tract of animals and humans (Argudín et al. 2017). The spread of infectious germs in the GI tract that are resistant to antibiotics passes the resistant gene to additional pathogens, changing the microbial community structure already present there. Injecting overdose antibiotics in livestock herds produces AMR not only in the normal flora of the GI tract but also in the zoonotic pathogens (Guardabassi et al. 2018). The term “Zoonoses” is derived from the Greek word “Zoon”, which means animal, and “noses”, which means illness (Narayan, et al. 2023). According to the World Health Organization (WHO), any disease or infection that is naturally transmissible from vertebrate animals to humans or from humans to animals is classified as zoonosis 61% of human pathogens have a zoonotic nature. AMR in animals and the human population is threatening in this century. Researchers are also working on zoonotic infectious agents but there is still a lack of appropriate administration, legislation, and controlled usage of antibiotics. Humans and animals with resistance to several drugs have few or no antibiotic alternatives. AMR may result in rising expenses and the instability of medical systems. When a new antibiotic gets approved, resistance to it will eventually develop (Inoue, 2019).

An enhancing distance between rising AMR and the creation of new antibiotics is noticed. The increase in AMR and failure to develop new effective drugs reached an alarming level. AMR spreads briskly and effective antibiotic development is too slow as a result new pathogens mainly bacteria that have zoonotic importance are out of control (Chandra et al. 2019). The sensitivity of pathogens decreases in response to previously used antibiotics and newly developed drugs as a result resistant genes are transferred. AMR especially in zoonotic pathogens is like a time bomb that will be threatened in the future more aggressively. One cause of this threat is that, despite the medical demand for new antimicrobials, the healthcare sector hesitates to invest and develop novel medicines because doing so would require spending more than eight hundred million dollars for up to ten years for each approved agent. The risk of freshly discovered molecules may prove unsuccessful in the short term but could serve as an additional prevention factor. The drugs industry has given preference to long-term utilization of medications for combating persistent diseases because it considers that study into inventing fresh antibiotics is less profitable (Gostin, 2021).

2. AMR MECHANISMS

As a result of AMR bacteria evolve and stop responding to antibiotics, making infections more difficult to cure and raising the risk of infection development, life-threatening diseases, and mortality. Bacteria utilize different mechanisms to counter antibiotic effects. Methods of enzymatic degradation (beta-lactamases synthesis by bacteria), efflux pump (through eliminating antibiotics that enter the cell), new metabolic pathways (the development of modified proteins), changes in membrane permeability to antibiotics(resistant to hydrophobic substances e.g bacteria like Escherichia coli have an outer membrane that offers a form of impermeability to substances like macrolide and beta-lactam drugs), and alteration of bacterial proteins acting as antimicrobial target(Antibiotics intrinsic receptor modification, such as ribosomal changes) are utilized by bacteria (Reygaert, 2018). Utilization of antimicrobial agents does not affect some bacterial strains: innate resistance is a feature that is present from birth. Resistance is acquired through changes in genetics such as mutation and horizontal transmission of the genome from different forms of bacteria. Mostly resistance is acquired through mutation and horizontal transfer of genetic material from one strain to another strain. A higher growth curve of bacterial replication also plays a role in the increasing number of resistant bacteria. Viruses (bacteriophages) and plasmids transfer resistant genetic material by transduction and conjugation. Plasmids transmitted both vertically and horizontally in acquiring resistance. Resistance in many categories of antibiotics is developing as a result of new patterns. Redundancy and infidelity at the molecular level are included in these patterns (Ferri, et al. 2017).

3. ZOONOTIC PATHOGENS AS A CARRIER OF AMR

Environmental contamination, drug resistance, and persistent illnesses that cause severe mortality along with elevated morbidity have all put livestock and human communities at risk**.** The emergence and rapid growth of epizootics, zoonotic, and epidemics have brought attention to the health risks at the global level and the significance of comprehending how infectious agents are transferred between humans and livestock. Most infectious diseases spread from the animals through different biological agents like gram-positive and gram-negative bacteria, viruses, prions, parasites and fungi. More than two hundred zoonotic diseases are present in different geographical regions and some spreading worldwide. Inhalation, ingestion, and other routes that contaminate mucosal membranes are the methods of zoonotic disease transmission. Animal tissue found in undercooked meat, infected vegetables, dairy products, shellfish, and unpasteurized milk are also sources of infection (Mohammed et al. 2016).

Several researchers have documented numerous instances of antimicrobial-resistant bacteria present in livestock. ESBL (extended-spectrum beta-lactamase) producing bacteria, MRSA (Methicillin-resistant *Staphylococcus aureus*), vancomycin-resistant enterococcus, MDR (multi-drug resistant) *Salmonella,* and *E. coli* are some of them. Many AMR zoonotic pathogens are also present in aquatic life transferring infection to the water bodies, human population, and soil microbiota. *Streptococcus iniae*, *Aeromonas hydrophila*, *Vibrio vulnificus*, *Photobacterium damselae*, and *M. typhi* are the pathogens of fishery that are zoonotic and AMR spreading (Nisa et al. 2023). The misuse of antibiotics in animals and seafood is exclusively related to ineffective regulations and monitoring systems. Lack of awareness regarding modifications in AMR pattern in the system which indicates the challenge cannot be successfully tackled from an ecosystem viewpoint by the research that has already been done in the field of AMR. A crucial step in lowering the hazards to people's health is to identify potential human exposure points to zoonotic infections in the animal business using an ecosystems approach (Michael and Schwsarz 2016).

3.1. *SALMONELLA ENTERITIDIS*

Salmonellosis is among the most common diseases caused by food in the world. *S. enterica* is the primary cause of salmonellosis illnesses in both humans and livestock (Pal et al. 2015). *S. enteritidis* mostly spreads infections in human communities through tainted poultry meat and eggs. To be able to comprehend ways of spreading and managing salmonellosis, it is crucial to know the evolutionary history and distribution of S. Enteritidis strains originating from chickens. To identify the origins and modes of *S. enteritidis* disease spread, genomic approaches offer unbiased and trustworthy techniques. Pulsed-field gel electrophoresis and gold standard methods are used for genotyping of this bacterial strain. Antimicrobial resistance is a global issue in the animal sector and as well as in the human population Salmonella strains have increased the resistance against various types of antibiotics. Salmonella resistant to multiple drugs is a serious threat in modern days in the perspective of public health (Vågene et al. 2018).

3.2. *CLOSTRIDIUM PERFRINGENS*

A pathogen called *Clostridium perfringens* can be detected in the digestive tract of mammals, avian species, and their surroundings. In favorable conditions, this gram-positive, rod-shaped, anaerobic, spore-forming bacterium shows clinical signs in animals when its growth curve rises and causes economic damage in the poultry industry of broilers and other birds (Borriello, 2018).

4. ANIMALS AS POTENTIAL CARRIERS OF ANTIBIOTIC RESISTANCE AND ZOONOTIC INFECTIONS

As zoonotic bacteria develop resistance to antibiotics, they pose more risks. Different MRSA strains from animals, including ST 130 throughout Europe, CC93 in Denmark, and ST398 in the Netherlands, have been discovered to spread among people. Growing worries are being expressed concerning animals as a potential reservoir for zoonotic diseases that can circulate among individuals due to the likelihood of zoonosis. There is evidence linking the overuse of antibiotics in animals to rising antimicrobial-resistant bacteria in people. Fluoroquinolones are frequently used to treat infectious diseases in livestock. Overuse of fluoroquinolones resulted in AMR Campylobacter diseases. According to a Food and Drug Administration (FDA) report on human health, eating chicken contributed to the development of fluoroquinolone-resistant Campylobacter in the human population. The microbe colonies of both healthy humans and livestock have been shown to have Enterococcus faecalis expressing the vanA resistance genes (VRGs), however, before 21 century, neither healthy humans nor livestock in the US had this infection. This difference was anticipated due to the widespread usage of avoparcin in farming practices (Dafale et al. 2020).

Due to the abundance of VRGs in animals, there are numerous potential for human infection and antimicrobial-resistant bacteria colonization. Numerous microorganisms are responsible for zoonotic illnesses (Ahmed and Baptiste 2018). Zoonoses can be categorized according to their etiological causes into viral, bacterial, parasitic, fungal, chlamydial, and microbial zoonosis (MacGregor and Waldman 2017). Bacterial zoonosis includes anthrax, salmonellosis, tuberculosis, Lyme disease, brucellosis, and plague (Asante, 2019). Viral zoonosis includes Ebola, AIDS, and rabies. For those who work with animals, understanding the spread of diseases, management methods, and disease prevention is crucial. As a work-related risk, it will aid in zoonotic disease prevention and management. Rabies, avian influenza, Rift Valley fever, Zika fever, dengue fever, hantavirus infection, and AIDS are famous zoonotic diseases that spread through viruses. Irrational use of antibiotics in the fields of agriculture, aquaculture and human health systems results in AMR (Ghasemzadeh and Namazi 2015).

Table 1: Gram-positive and gram-negative bacteria transferring infections and AMR.

Diseases	AMR	Antibiotics	Animals	Reference
Cellulites, septicemia and meningitis	*E. coli, Salmonella sp. Campylobacter sp. Streptococcus suis*	Methicillin, Cephalosporin, Cefotaxime	Birds	(Maciel et al. 2017).
Allergies and blood infection Pyoderma	*Staphylococcus pseudintermedius, Staphylococcus sp.*	Methicillin, Ofloxacin, Methicillin, Clindamycin, Fluoroquinoles	Cats, Dogs and Horses	(Cavana et al. 2023).
Avian Tuberculosis	*Mycobacteria, Neisseria gonorrohoeae*	Azithromycin, Gentamycin, Chloramphenicol	Avian (Poultry, Duck and Chicken)	Dai et al. 2020).
Diarrhea	*E. coli, Salmonella, Campylobacter*	Sulfamerazine, Dihydrostreptomycin		
Enterocolitis	*Campylobacter, Salmonella*	Ciprofloxacin, Tetracycline, Enrofloxin, Nalidixic acid		
Enteritis	*Campylobacter sp.*	Nalidixic acid, Ciprofloxacin, Tetracycline		
Urinary tract infection	*E. coli*	Ampicillin,	Livestock	
Mastitis	*Staphylococcus aureus, Klebsiellapneumoniae, Salmonella*	Fluoroquinolones, Cefotaxime	Dairy Cattle	
Bovine Tuberculosis	*Brucellaabortus, Mycobacterium bovis*	Methicillin, Amoxicillin,		
Typhoid	*Salmonella typhimurium, Salmonella enterica*	Clavulanic acid Norfloxacin, Amoxicillin Penicillin, Oxytetracycline, Streptomycin, Sulfonamide		
Diarrhea, gastritis	*Salmonella* and *Vibrio spp.*	Cephalothin Ampicillin Chloramphenicol	Sea fish	(Yena et al 2020).
Elementary tract infection	*Klebsiella pneumoniae, Salmonella spp.*	Various Beta lactamases	Pigs	(Bader et al. 2017).
Septicemia	*Staphylococcus aureus, Campylobacter, Salmonella*	Levofloxacin, Penicillin, Tetracycline		
Urinary tract infection	*Enterococcus faecium, Campylobacter sp*	Nalidixic acid. Quinolones, Fluorquinolones		

5. VECTOR-BORN ZOONOTIC INFECTIONS

Antibiotic-resistant zoonotic disease is often thought of as originating from a site of direct host–receiver interaction. The relationship between resistant vector-borne disease commonly impacts the pathogen, host, and human, resulting in unanticipated complications. Vector-born zoonotic diseases (VBZDs) are spreading more quickly in terms of infectious diseases that are directly transmitted and resistant to antibiotics. Infectious diseases that affect people make up a disproportionate amount of newly emerging infectious disorders. Predominantly spread by hematophagous, blood-feeding, and arthropods. Over 90 percent of all vector-borne diseases are spread by flies, ticks, mites, and mosquitoes. Several widespread bacterial diseases, such as Lyme disease, Rocky Mountain spotted fever, and ehrlichiosis spread by Ixode and Ehrlichia (Khan, 2015).

6. DYNAMICS AND DETERMINANTS OF VECTOR-BORNE DISORDERS

The spread of infection from the native host to the receiver of a vector-borne disease depends upon a variety of circumstances. One of the main factors causing an upsurge in vector-borne diseases is a decline in host availability. Rats are the main targets which are decreasing and as a result, vector-born diseases increasing. Yersinia pestis attacks the human population when there is a decline in the rat population. Human behaviors and alterations to the environment are also main factors in the spreading of vector-

borne diseases. Deforestation, urbanization, pollution, and other factors are also important in the spread of VBZDs (Cator et al. 2020).

7. ANTIBIOTICS RELEASE IN THE ENVIRONMENT

Antibiotics that fail in complete digestion in the body are consistently released in the ecosystem through veterinarians' workplaces, medical debris, drug manufacturers, dairies, poultry and other sources such as both household and urban filth. Remnants of drug residues are regularly found in a variety of aquatic entities, including streams, water in the ground, and ponds that are the result of aquatic operations and the result of water runoff directly from wastewater treatment plants (WWTPs). Since WWTPs absorb un-metabolized antibiotics from many sources through sewage and urban waste, they are widely recognized sites for the growth of antibiotic-resistant bacteria (ARBs) and play a role in the spread of antibiotic-resistant bacteria (ARB) and antibiotic-resistant gene (ARG). A small amount of antibiotic residues are also found in soil. The main source of antibiotics in soil is farm animals, waste from sanitation facilities, and dung. Bacteria in aquatic entities and soil frequently face exposure to antibiotics. These bacteria develop resistance to antibiotics using any mechanism for the development of resistance. Even a minimum concentration level of antibiotics in both aquatic and soil environments will be a risk to human and animal health (Arshad and Zafar 2020).

Table 2: Zoonotic infections by bacteria.

Etiology	Disease	Organs involved/ signs, symptoms	Animal host	Reference
Bacillus anthracis	Anthrax	respiratory organs, Skin, or GI tract	Bison, elks, white-tailed deer, goats, sheep, pigs, dogs, mink, cattle, equine	(Ngetich, 2019).
Actinomyces bovis	Actinomycosis	soft tissues, skin, and abscess, Swelling of lymph nodes	cattle, equine	(Murakam et al. 2018)
Brucella abortus, Brucella melitensis, Brucella suis, Brucella canis	Brucellosis	Weight loss, back discomfort, joint pain, poor appetite, and a fever	Cattle, dogs, goats, sheep, and pigs	(Alamian and Dadar,2020).
Burkholderia mallei	Glanders	Fever, sweating, muscle aches, chest pain, muscle tightness, headache	Horses, donkeys, and mules	(Khakhum et al. 2019).
Borrelia burgdorferi	Lyme disease	Fever, headache, skin rash, erythema migrans	Cats, dogs, and horses	(Steere et al. 2016).
Bordetella bronchiseptica	Bordetellosis	breathing issue	Canine and feline	(Brockmeier et al. 2019).
Yersinia pestis	Bubonic plague	bleeding from a natural opening, chills, diarrhea, fever, stomach ache, and vomiting	prairie dogs, mice, voles, chipmunks, dogs, rabbits, ground squirrels, Rock squirrels, wood rats	(Barbieri et al. 2020).
Salmonella enterica, S. bongor	Salmonellosis	Enteritis	Domestic animals, birds, and dogs	(Wibisono et al. 2020).
Mycobacterium bovis, M. caprae, M. microti	Tuberculosis	Respiratory organs bone marrow	Cattle, sheep, swine, deer, wild boars, camels, and bison	(Michelet et al. 2020).
Leptospira interrogans	Leptospirosis	Fever, abdominal pain, jaundice, and red eye	Wild and domestic animals including pet dogs	(Philip et al. 2020).

An increase in antibiotic-resistant bacteria and the transfer of antibiotic-resistant genes leads to infectious diseases. Antibiotics spread from their source to the environment involving bioaccumulation, biotransformation, and slowly accumulated antibiotics in water and soil. Antimicrobial drugs build up in larger quantities and then propagate further as an outcome of their stability in the environment. Some antibiotics are degraded easily like penicillin and most antibiotics take a long time for their degradation like macrolides, tetracyclines, and fluoroquinolones. The higher half-life of these long-lasting antibiotics increases their persistence in the environment (Grenni et al.2018).

8. ECONOMICALLY THREAT OF AMR

AMR may result in rising expenses and the instability of healthcare systems. People with AMR nosocomial infections (primarily circulation illness) or those who become ill after consuming food tainted with resistant germs recover more slowly, and septicemic infections and mortality are more common. Due to the prolonged hospitalizations and the consumption of costly drugs, the expense of healthcare has risen in this scenario. Also, new medications carry increased toxicological concerns as well as more frequent adverse drug reactions (ADRs). Healthcare expenses are thought to be about €1.5 billion each year in Europe, including €600 million in production. In the USA $55 billion lost results as AMR-related infections

Table 3: Antibiotics, Half-life, and their concentration in different environments.

Group of Antibiotic	Antimicrobial drugs	Conc (mg/kg/L)	Half-life (days)	Reference
Aminoglycoside	Vancomycin	L0 (sandy loam)	L6	Chung et al.
	Gentamicin		4	2017).
Beta-Lactam	Ampicillin	).2 (soil)	).43	Lonsdale et
	Amoxicillin	L0 (aquatic environment)	3.89h	al. 2019).
Cephalosporin	Cefradine Cefuroxime Ceftriaxone	L0 (surface water)	6.3	Shahbaz,
			3.1	2017).
			L8.7	
Fluoroquinolones	Ofloxacin Levofloxacin Norfloxacin	).045 (soil)	990–1386	Mohammed
	Ciprofloxacin	).225 (wastewater)	4.2	et al. 2019).
		30 (soil)	52	
		).542 (soil)	L153–3466	
Macrolides	Azithromycin Clarithromycin	L(soil)	L2.82	Dinos, 2017).
		L(soil)	36.48	
Sulfonamides	Sulfamethoxazole Trimethoprim	L0(water waste)	49	Tačić et al.
		).17(water waste)	11.5	2017).
Tetracycline	Doxycycline	L0(water)	49 578	Kasumba et
	Tetracycline	).1 (soil)		al. 2020).

*As per EUCAST database

higher cost than HIV infections (Ahmad and Khan 2019). Experts caution that because reporting system constraints affect the death and morbidity data connected to drug-resistant bacteria, the cost impact on the healthcare industry is expected to be greater. In developing countries, the situation is alarming and the economic cost of the health care system is higher as compared to European countries. Under this circumstance, a successful AMR plan must concentrate on global collaboration, enhanced national efforts, discovery of innovative medications, successful governmental and public awareness campaigns, and government intervention, as well as improved communication between higher education institutions and drug companies, clients, veterinary professionals, and healthcare providers (Watkins and Bonomo 2020).

9. USE OF ANTIBIOTICS IN THE AGRICULTURE SECTOR AND AMR

The antimicrobial-resistant bacteria and their antibiotic-resistant genes have been extensively shown to be present in the ground, animals, and plants that produce food, and throughout the entire food chain. Following the use of enrofloxacin and moxifloxacin on farms that raised poultry in the Netherlands, the prevalence of fluoroquinolone-resistant strains of Campylobacter rose from 0 to 14% in broiler chicks and from 0 to 11% in farm staff members (Mdegela et al. 2021). Many antibiotic-resistant bacteria like Salmonella, Campylobacter, and *E. coli* are zoonotic and present in domesticated animals. Staphylococcus aureus methicillin-resistant (MRSA), Campylobacter spp multiresistant, and *E. coli* ß-lactam-resistant are pathogens present in farm animals. These microbes from livestock that produce food can infect people through a variety of channels, including aquatic or polluted environments, straight animal interaction, and food-borne pathways such as eating polluted or cross-infected foods of animals. There is also strong evidence that beneficial bacteria, including those found in the digestive tracts of humans and livestock, like *E. coli* and *Enterococcus spp*., may serve as a possible storehouse for resistance genes that can be passed between bacterial species, including those that can infect both human population and livestock with infections (Mshana et al. 2021).

10. ANTIMICROBIAL-RESISTANT AND PUBLIC HEALTH CONCERNS

Recently, a large number of international medical organizations recognized AMR as a major issue in global healthcare and a risk to the current healthcare system that could make it challenging to control many infectious diseases and drastically degrade current treatments. AMR, a global epidemic that is on the rise, is typically linked to the "selective pressure" brought on by the incorrect, excessive, or improper use of antibiotics in animals as well as humans (Bortolaia et al. 2016). A poorer standard of living, invasive infections by bacteria, an upsurge in recurrence rates of infections, and long-term and subsequent opportunistic infections of gram-positive and gram-negative bacteria are all linked to infections by antibiotic-resistant strains. These issues are unmistakably becoming worse, just like infections caused by salmonella-resistant human pathogens, *Campylobacter spp*. and enterococci with vancomycin-resistant connected to an increased risk of issues, a rise in disease incidence, a rise in treatment errors, and a rise in fatalities (Sanderson et al. 2019). According to the European Centre for Disease Control (ECDC), antibiotic resistance by bacteria accounts for twenty-five thousand fatalities annually ((EFSA and ECDC 2019). AMR caused 0.1 million deaths in the United States of America and eighty thousand fatalities in the Chinese mainland. According to figures, five thousand individuals in the United Kingdom per year pass away from infections such as E. coli and K. pneumonia, with bacterial resistance to medicines accounting for 50% of these deaths. Microbes that produce carbapenemase which include Enterobacteriaceae producing New-Delhi metallo-protease-1, antibiotic-resistant Acinetobacter, have emerged and are spreading quickly. The resistance to multiple drug diseases caused by oxacillinase 48, carbapenemase, and Klebsiella pneumonia poses a major risk to the general population since they expose people to inadequate antibiotic alternatives (Bakthavatchalam et al. 2016).

11. CONTROL STRATEGIES FOR AMR (ANTIMICROBIAL RESISTANCE)

Stewardship of antibiotics plays a crucial role in AMR management measures. It is utilized in one program that is relevant to human, veterinary, and WHO health. Antimicrobial stewardship is a rational set of actions that promotes the appropriate use of antibiotics. AMR curve decrease is also

aided by effective infection prevention and control. Antibiotics used effectively and research into new antibiotic alternatives are both important in controlling this dangerous threat to both the human and animal communities. AMR-related bacterial infections and zoonotic diseases can also controlled by vaccination, health promotion, and research into the AMR process. AMR monitoring and surveillance programs play a vital role in checking the spread of infections and AMR (Ayukekbong et al. 2017).

12. PLANTS EXTRACT

Plant extracts and their products used in the treatment of bacterial infections since ancient times. Menthol as a primary component of peppermint oil effective in bacterial infections hence products containing menthol able to eradicate gram-positive and gram-negative resistant bacteria. Some plant extracts used in combination with antibiotics have a great effect on microbial activity and growth. Guaco, guava, clove, garlic, lemongrass, ginger, carqueja, and mint are used in different studies. The synergistic effect of these extracts on *S. aureus* was evaluated. Antibiotics like tetracycline have good synergistic effects (Jouda, et al. 2016).

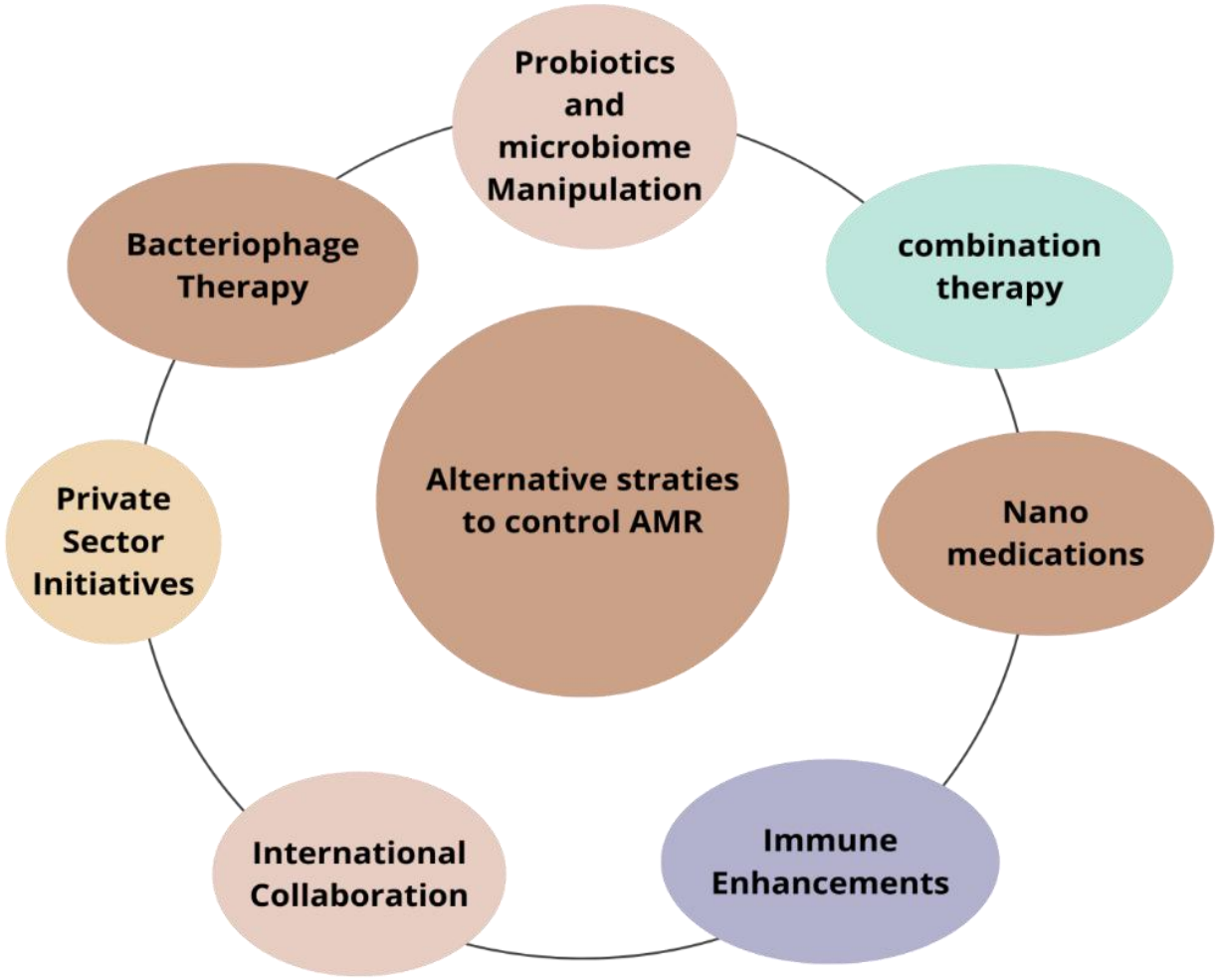

Fig. 1: Alternative control strategies for controlling antimicrobial resistance.

13. ESSENTIAL OILS

Due to the significant amount of biologically active compounds, including volatile and aromatic components, essential oils have been extensively used over the years in medical treatments. Essential oils from *Lippia alba*, *Salvia officinalis*, *Salvia triloba*, *Salva triloba,* and other medicinal plants have a great effect on bacterial growth. Oils from eucalyptus, peppermint, palmarosa, and orange are also very effective (Chouhan et al. 2017).

14. PREBIOTICS AND PROBIOTICS

A class of nutrients known as prebiotics is broken down by the gut flora. Throughout recent years, there has been a growing interest in how they relate to human health in general. They can nourish the gut bacteria, and as a result of their decomposition, fatty acids with short chains circulate into the bloodstream and influence not only the gut but also other organs. Carbohydrates are the most known prebiotics. Probiotics are live microorganisms when given to a host in sufficient quantities, improve their health (Joseph et al. 2023).

15. NANOMEDICINE

The use of nanotechnology in medicine is Nanomedicine. It is possible to target a specific organ, tissue, cell, and bacteria with manufactured particles. Targeting provides the advantage of concentrating high levels of antibiotics at the location where bacterial eradication is required, which should reduce the total therapeutic antibiotic dose. These advantages of nanocarrier formulations might tackle current resistance issues and expand the range of antibiotics used in clinical settings (Wang et al. 2020).

16. BACTERIOPHAGE THERAPY

The phages include viruses that attack their bacterial hosts. There are several common characteristics to take into account while selecting phages for therapeutic application. The phages must first effectively eliminate the target bacterial pathogen with negligible bacterial survival. The phages should also be simple to create in high-titer preparations, multiply, and purify. Third, the phages must be stable throughout a range of concentrations so that prolonged storage at frigid temperatures does not result in a significant loss of infectivity. The third need is that the phage preparations be sterile and free of endotoxins or other hazardous impurities. No harmful genes are known or suspected to be present in the phage genomes. Fifth, the phages shouldn't have the capacity to function like generalized transducing phages (Luong et al. 2020).

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Zoonoses and AMR: Silent Spreader of Superbug Pandemic

14

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ABSTRACT

The accelerated and indiscriminate use of antimicrobial agents in livestock, driven by their short reproduction period and abundant intestinal microbes intensifies the emergence of resistance. Livestock gut serves as a breeding ground for antimicrobial-resistant bacteria, perpetually disseminating them across diverse ecosystems. Through horizontal gene transfer and quorum sensing, resistant genes proliferate within native flora. Zoonotic pathogens, acting as carriers, may transmit antibiotic-resistant genes to humans, underscoring their pivotal role in human resistance development. To mitigate this threat, a comprehensive understanding of zoonotic illnesses, early detection, and effective management strategies are imperative. The one health approach integrates diverse disciplines to achieve optimal medical outcomes by acknowledging the interconnectedness of humans, animals, and their shared environments. According to new speculation from the Center for Disease Control and Prevention, the globe is about to break into a "post-antibiotic era" in which illness caused by bacteria will be the leading cause of death instead of tumor. Over 2 million incidents of serious diseases, which comprise 23,000 fatalities annually in the United States, are brought on by bacteria that are resistant to antibiotics. Over 95% of all emerging infectious diseases described in the second half of the 20th century are zoonotic and antimicrobial-resistant (AMR) infections. Each year, drug-resistant illnesses brought on only by tuberculosis (TB), HIV, and malaria claim the lives of almost 700,000 people. Drug-resistant diseases are predicted to imperil 10 million individuals annually by 2050 if nothing is accomplished. This chapter underscores the urgency of curbing antimicrobial resistance in ecosystems and its potential impact on human health and the collaborative endeavors of global authorities such WHO, CDC, and OIE and other pertinent health and agriculture agencies are crucial in addressing and mitigating the challenges posed by the zoonosis in spread of superbug pandemic.

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1. INTRODUCTION

The groundbreaking development of antimicrobial agents, which provided the cornerstone of antibiotic treatment to cure and manage infections caused by bacteria, was seen as a turning point in the field of medical discipline and is one of the most important medical discoveries of the 20th century. Due to the advent and rapid growth of antibiotic resistance among many microbial species, which has turned into a worldwide problem, its usefulness has since been constrained (Kumar 2019). Before antibiotics were discovered, created, or made available for purchase, antibiotic resistance already existed. In actuality, bacteria collected from glacier waters over 2000 years ago had ampicillin resistance, and microbes from permafrost more than thirty thousand years ago have vancomycin resistance. (Morrison and Zembower 2020). In accordance to a World Health Organization (WHO) research from 2019, antimicrobial resistance caused 7 million fatalities, and it's predicted that in the year 2050, that number is expected to increase to 20 million, incurring more than $2.9 trillion. The majority of antimicrobial agents were produced in the "golden era" of antimicrobial studies, which spanned the years 1950 to 1960. The first "superbug" in the past was eventually referred to as methicillin-resistant *Staphylococcus aureus* (MRSA) and it is transmitted to humans via zoonotic animals (Uddin et al. 2021). Antimicrobial resistance bacteria are becoming more prevalent within zoonotic pathogens and typical bacterial species as a result of the overuse of antibiotics in veterinary treatment and food-producing animals.

2. IMPORTANCE OF RELATIONSHIP BETWEEN AMR AND ZOONOSES

Owing to the short reproduction period and the greater abundance of the intestinal microbes, the inappropriate and increased use of antimicrobial agents in livestock production places substantial strain on the emergence of resistance (Fig. 1). The gut serves as a biological reactor for the generation of antimicrobial resistance bacteria, which are then constantly discharged in various ecosystems. Through horizontal gene transfer processes, carriers, and quorum sensing, these antimicrobial resistant bacteria spread resistant genes across native flora. Antimicrobial resistant genes that could be spread to human beings may be carried by pathogenic zoonotic organisms (EFSA Panel 2009). These antibiotic resistant genes reach the human gut via zoonotic pathogens and therefore, plays an important role in development of resistance in humans. To limit the development of antimicrobial resistance in the ecosystem and ultimately to humans via livestock and food chain, understanding of zoonotic illness, encompassing ARBs transfer, early detection, and management strategies must be established (Dafale et al. 2020).

3. UNDERSTANDING ANTIMICROBIAL RESISTANCE

According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), pathogenic antibiotic resistance in bacteria can be described in 2 distinct manners: first, in terms of the usual microbe population that exists prior to being exposed to the antibacterial agent, and secondly, in the context of the negative clinical consequences associated with an unregulated illness if an individual gets that antibiotic (MacGowan 2008). Since penicillin was first used in medicine, *Staphylococcus aureus* has been documented to be resistant to β-lactam medications (Yoshii et al. 2017; Ahmed et al. 2019).

3.1. MICROBIAL

a) The majority of microorganisms have a quick growth rate, ensuring favorable alterations,
b) Gene mutation/change in genetic sequence,

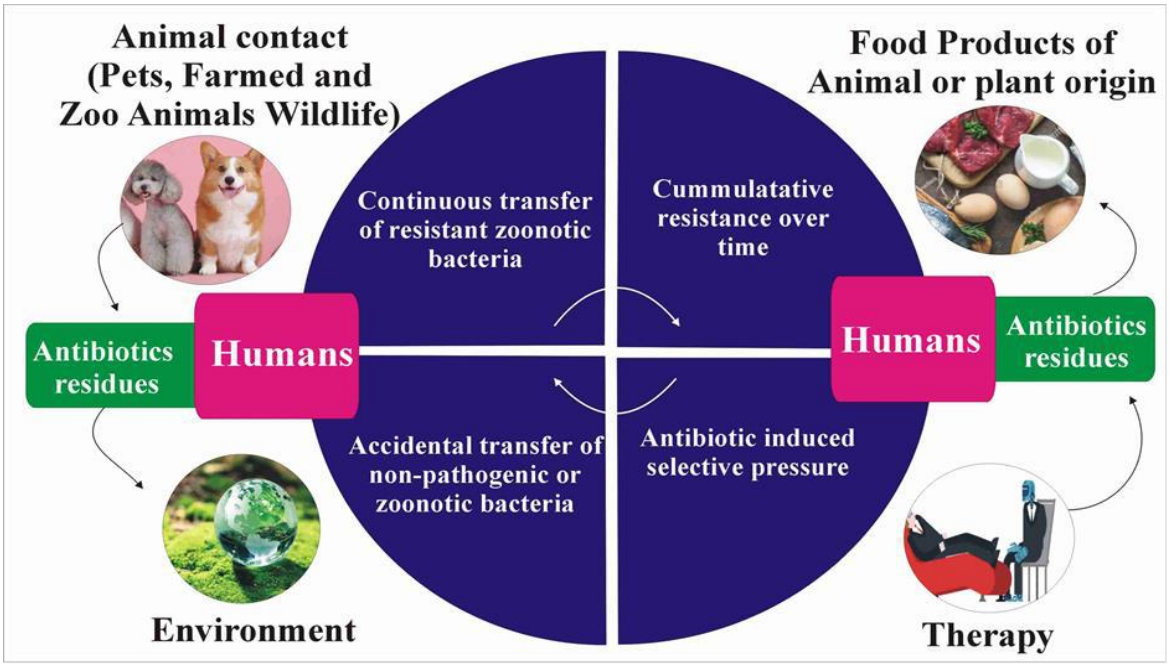

Fig. 1: The transfer of antibiotic residues form animal to human and then to environment. On the other side the treatment of human being and transfer of these residues to food products of animal and plant origin. Zoonotic resistance bacteria are transfer and results in cumulative resistance, similarly the antibiotic selective pressure and transfer into nonpathogenic zoonotic bacteria.

c) The influence of selective pressure on the population of microbes, and
d) Applied selective stress: Humans use antibiotics extensively and intensively, creating an aggressive and polarized selective stress that is going to persist to elicit a powerful adaptive response in the microorganisms (Michael et al. 2014; Nadeem et al. 2020).

3.2. HUMAN

a) Increase in population also provides bacterial culture with ample nutrients and suitable conditions and exhibits exponential growth,
b) The quick propagation of infectious illnesses is greatly facilitated by a large percentage of people residing nearby, and
c) Rapid travel can carry diseased people, causing infections to spread over the world even prior to the manifestation of visible symptoms (Michael et al. 2014).

3.3. OVERUTILIZATION OF ANTIBIOTICS

Antibiotic overuse has contributed to the current increase in AMR rates (Manohar et al. 2020; Tang et al. 2023).

3.4. THERAPEUTIC PRACTICES

a) Inappropriate prescription: Antibacterial recurrent use can culminate in excessive use of antibiotics and AMR,

b) Consecutive antimicrobial treatment: Combination of antibiotics results in the development of AMR, and
c) Established practices: Overall bacterial adaptive response is caused by the prescription of long-term antibiotics that have been prolonged up to 14 days, and also by the prescription of large doses (Michael et al. 2014; Cantón et al. 2022).

3.5. MASS PERCEPTION AND BEHAVIOR

Antibiotics are seen by the general population as a fast and effective cure for a wide range of illnesses, which has led to behaviors that successfully subvert the authority of a prescriber and therefore promote AMR (Michael et al. 2014; Lyall et al. 2023).

3.6. CULTIVATION APPLICATIONS

The productivity of farms can be considerably boosted by using antibiotics on both cattle and crops. The impact of farming practices on microbial ecology has led to the presence of numerous and diversified AMR genes in urbanized agricultural nature, and seemingly natural environments (Michael et al. 2014; Hassell et al. 2019; Nadimpalli et al. 2020; WHO 2022c).

4. ZOONOTIC ANIMALS

Implication of antimicrobials in livestock for growth promotion leads to the emergence of antibiotic resistance in humans towards bacterial species that can infect both animals as well as humans (Wassenaar and Silley 2008).

5. MECHANISMS OF ANTIMICROBIAL RESISTANCE DEVELOPMENT

Antibiotic pathways to resistance can be generally divided into Intrinsic or Acquired resistance.

5.1. INTRINSIC RESISTANCE

Certain particular genera of bacteria (or species) have distinctive structural/functional traits that lead towards the development of resistance to antibiotics (Impey et al. 2020). These bacterial populations typically lack a target site for the particular antibiotic, rendering it useless i.e., Resistance to β-lactam antibiotic towards Mycoplasma species due to the absence of cell wall (Maes et al. 2020). Moreover, the existence of an outer layer preventing an antimicrobial agent from entering cells of bacteria. Presence of efflux pumps i.e., the ATP-binding cassette (ABC) superfamily and inactivation of antibiotics by bacterial enzymes i.e., β-lactamase.

5.2. ACQUIRED RESISTANCE

In which ordinarily sensitive bacteria can acquire genes from different strains of bacteria in order to acquire resistance to specific drugs. Three mechanisms by which bacteria acquire resistance (Christaki et al. 2020; Biondo 2023).
a) Alteration of bacterial enzymes and inactivation of antibiotic
b) Decreased intrinsic antibiotic concentration

c) Modifications at the antibiotic agents' target sites
d) Resistance by propagation of resistance gene (Fig. 2).

6. RESISTANCE DUE TO QUORUM SENSING AND BIOFILM PRODUCTION

Bacterial quorum sensing, a technique that controls bacterial transcription of genes, is dependent on the adaptable chemical messengers referred to as auto-inducers. By employing auto-inducers for quorum sensing, microbes are able to interact both between and within species. Bacteria use quorum sensing both for the development of resistance as well as to increase each other's virulence in disease conditions. For example, *Vibrio cholerae*'s insidious releases auto-inducer which at lower concentrations functions as kinases and promotes the formation of LUXO-Phosphate from phosphate which promotes the development of biofilm as bacteria reproduces concentration of autoinducer increases and binds to its receptor and it results in alteration in activity of auto-inducer from kinase to phosphate and dephosphorylation of LUXO and halting of biofilm gene expression and start of new sequence of quorum sensing events. This ultimately results in the production of highly toxic cholera toxins and increases deaths (Holoidovsky and Meijler 2020; Ramamurthy et al. 2020).
There is a 1000-fold increase in the development of resistance in bacteria in biofilm and quorum sensing because it results in a better exchange of genetically altered information within and between bacterial strains (Athulya and Chaturvedi 2020).

7. CONSEQUENCES AND IMPLICATION OF AMR IN HUMAN AND ANIMAL HEALTH

According to research (de Kraker et al. 2016) by 2050 AMR might result in a 1% yearly Economic decline, with this number reaching as high as 5-7% in underdeveloped nations. This would result in loss of between 100 and 200 trillion euros globally. The frequency of antimicrobial intake in a given bacterial population is strongly connected with the degree of antibiotic resistance generated by the microorganisms in the population of animals. Overuse and long-term administration of antibiotics in livestock at sub-clinical concentration stimulates growth, changes in gut microflora and promotes cross resistance among different classes of antibiotics.
From the zoonotic spread of illnesses, antibiotic-resistant bacteria enter the human gastrointestinal tract where they disrupt the natural biodiversity of the gastrointestinal tract (Esposito et al. 2022). When zoonotic pathogens containing antibiotic-resistance genes enter in human body they spread the resistance genetic material to the gut microflora of the host, upsetting the balance of the ecology in the stomach (Adem 2022).
Additionally, human beings excrete ARGs (antibiotic resistance genes) or ARBs (antibiotic resistance bacteria), which then enter the ecosystem from soil or water from municipalities. ARBs penetrate the food chain through the soil or sewage and harm the well-being of animals, continuing the process of ARB dissemination (Dafale et al. 2020). The incorporation of antibiotics in pesticides in edible crops causes the contamination of antibiotics in soil and water ultimately dissemination of multidrug resistance bacteria via metabolic processes i.e. manure application in commercial swine farms results in resistant Salmonella serotypes (Pokharel et al. 2020).

8. CURRENT GLOBAL TRENDS AND CHALLENGES IN COMBATING AMR

AMR is among the 10 most prevalent worldwide public health concerns (Sanderson et al. 2019; Tang et al. 2023). The idea of "One Health" affirms that there exists a direct link between the health of human,

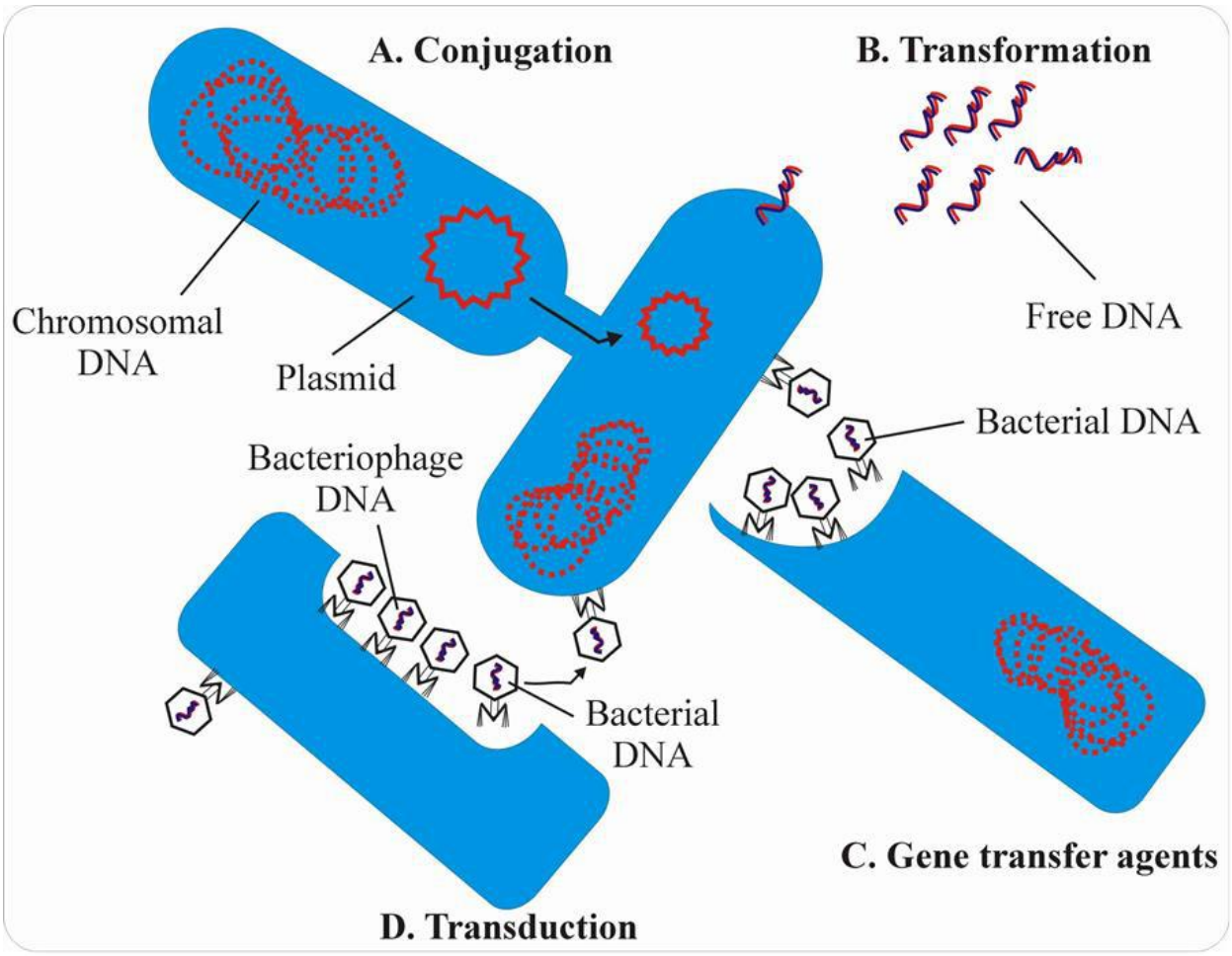

Fig. 2: Diagram representing the resistance by propagation of resistance gene A) Conjugation B) Transformation, C) Gene transfer agents, and D) Transduction (Von Wintersdorff et al. 2016).

animals, and their shared environment. The World Organization of Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO), and the WHO have closely collaborated to make sure that viable measures are implemented in every field to reduce the potential hazards of antimicrobial resistance through this strategy (Tang et al. 2023).

Along with the "One Health Approach" the World Health Organization (WHO) introduced the Global Action Plan focused on AMR (GAP-AMR), an intervention program for AMR in 2015. By responsibly preserving antibiotics and providing proper accessibility and quality controls, this approach seeks to guarantee that infectious illnesses can always be effectively treated and managed. In order to accomplish the objectives of the general Global Action Plan, it is anticipated that nations would present their national plans in AMR. The Global Antibiotic Resistance and Use Surveillance System (GLASS) was the other strategy that was introduced. This initiative's main objectives are to promote worldwide antimicrobial monitoring and identify its underlying causes. This would entail giving guidance and suggestions to assist states in putting appropriate corrective measures into place. One must include social sciences like economics and politics to completely comprehend the scope of the antimicrobial resistance dilemma. Therefore, understanding how each person integrates with reality via mental models is crucial (Calvo-Villamañán et al. 2023).

9. ZOONOTIC DISEASES

In 1951 WHO defined zoonoses as "diseases and infections that are naturally transmitted between vertebrate animals and man". Whereas, Rudolph Virchow in the 19th century introduced the term “zoonosis” (plural zoonoses) which is derived from two Greek words “zoon” (animals) and “noson” (sickness) (Leal Filho et al. 2022; Singh et al. 2023). Zoonotic infections account for more than 75% of developing infections. The rapid appearance of influenza A/H1N1 in Mexico in late April 2009, the Nipah Virus in Southeast Asia in 1998, SARS in early 2003 and Highly Pathogenic Avian Influenzas (HPAI) first appeared in 2004 and reemerged in 2009 are a few examples of zoonotic infections (Cáceres and Otte 2009).

9.1. IMPACT OF ZOONOTIC DISEASES ON HUMAN AND ANIMAL POPULATIONS

Each year, zoonosis causes over 2.7 million fatalities and 2.5 billion cases of human disease while also having an effect on cattle production and the security of food. Animal goods were significantly reduced by more than 70% due to zoonotic diseases. The lack of high-protein foods of animal origin, such as milk, meat, and eggs, has an impact on both the health of humans and their nutrition. Zoonotic illnesses like toxoplasmosis and brucellosis can cause infertility, abortions, and poor progeny. Farmers and the entire nation may suffer significant financial losses as a result (de Silva et al. 2023).

Notably, the SARS pandemic and highly virulent avian influenza, which affected several industries notably tourism, had a major detrimental effect on the world GDP. According to the World Bank, outbreaks that affected sheep and beef caused Australia's livestock sector to lose 16% of its economic value. The latest COVID-19 pandemic has had a big effect on the world economy. All societal sectors, notably the tourism and hospitality, banking, educational and health services, and sports sectors, have been profoundly affected by COVID-19 (Rahman et al. 2020). The consequences of zoonotic diseases extend beyond the negative effects on human and animal health to include significant economic loss as a result of decreased livestock productivity, environmental disruption, and the expense of human disease.

9.2. EMERGING ZOONOTIC DISEASES AND THEIR POTENTIAL FOR GLOBAL SPREAD

An average of more than 3 novel viral species that harm people are identified annually. Some of the common emerging diseases include Ebola, MERS, HIV, SARS, etc. Variations in human demographics and the community, along with shifts in the utilization of land and agricultural practices, were recognized by Woolhouse and Gowtage-Sequeria as the 2 kinds of variables most frequently linked to the reemergence of infectious diseases among humans.

When efficient control and biosecurity protocols are not effective, the intensification of farming for livestock, which is linked to rising animal population and assists in the spread of diseases by encouraging invasion into wildlife habitats and creating new possibilities for encounters among humans, livestock, wildlife, and vectors (Otte and Pica-Ciamarra 2021).

9.3. INTERPLAY BETWEEN ANTIMICROBIAL RESISTANCE AND ZOONOSES

The majority of developing illnesses have penetrated virtually every ecosystem and breached transboundaries. Animals are the most typical biological carriers for the propagation of pathogens with resistance (Vidovic and Vidovic 2020; Sivagami et al. 2020). Microbial populations found in animal guts

work in concert to benefit their hosts. By passing the resistant gene to other pathogens in the gut, the introduction of a drug-resistant pathogen changes the microbial community structure. Opportunistic diseases travel from animal to human either directly (via zoonoses) or indirectly (through vectors), which has an impact on human health (Gnat et al. 2021; Mohamud et al. 2023). The majority of antimicrobial agents that enter the bodies of humans and animals are not fully metabolized, causing the release of metabolized forms into the environment. Antibiotic resistance genes (ARGs) are becoming more prevalent as a result of the presence of antimicrobial agents in the atmosphere (Zhuang et al. 2021). The widespread presence of ARBs in the surroundings is increased by the spread of ARGs via related mobile genetic elements (MGEs) such transposons, plasmids, and genomic islands to other microbial populations. These ARBs develop into powerful pathogenic zoonotic agents that may sicken the global population of people severely (Dafale et al. 2020).

Moreover, both direct contact with animal feces having resistant microbes and indirect contact with polluted water or food can result in the spread of resistant germs from animals to humans. For instance, a significant fraction of the bacteria in the fecal ecology of hens are resistant to antibiotics. This has resulted in the development of resistance against most critical and remarkably important antibiotics (Table 1) i.e., streptomycin, gentamicin, ampicillin, sulfamethoxazole, sulfisoxazole, trimethoprim, chloramphenicol, spectinomycin, tetracyclines) in animals as well as in humans (Roug et al. 2013).

Table 1: Resistant antibiotic classes and resistant bacterial species

Sr.no.	Resistant Drugs	Bacterial species	Zoonosis Occur via species
1	a. Nalidixic acid, Ciprofloxacin b. Amino penicillin (amoxicillin and ampicillin) c. Cephalosporin i.e. cefotaxime	*E. coli*	Poultry, Swine, Cattle
2	a. Glycopepetide i.e. vancomycin b. Macrolide c. Quinupristin and delfopristin	*E. faecium* *E. faecalis*	Poultry, Swine, Cattle
3	a. Fluoroquinolones	*Campylobacter jejuni* *Campylobacter coli*	Broiler, Pig
4	a. Carbapenem	*Acinetobacter baumannii*	Cattle, Pig
5	a. Methicillin b. β-lactams, lincosamide and fluoroquinolones	Staphylococcus aureus, *Staphylococcus pseudintermedius*	Dogs, Cats

10. CASE STUDIES ILLUSTRATING THE INTERCONNECTION BETWEEN AMR AND ZOONOSES

E. coli resistance to cefotaxime, a third-generation cephalosporin, became more prevalent in poultry in the Netherlands after 2003, reaching an extent of over 20% in 2007; this resistance incidence reduced significantly following the utilization of ceftiofur. A one more 3rd generation cephalosporin, was banned in hatching facilities in 2010, reaching a degree of 2.9% in 2014. The first cases of livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) in pig farms were identified in the beginning of the 2000s. Contact directly between cattle and humans is the main threat for MRSA transmission, which happens often. Exposure can result in colonization, and LA-MRSA can then be detected asymptomatically on the epidermis or in the nasal cavity. LA-MRSA can result in a local skin infection (such as an abscess) if it enters the body through the skin (for example, through a wound or cut). There are times when it can result in severe illnesses like pneumonia or bloodstream infections (BSIs).

The WHO which classifies vancomycin as a crucial AM for the well-being of humans, says that avoparcin is a glycopeptide that was formerly utilized in veterinary treatment as an antibiotic growth promoter. After learning that the application of avoparcin as an antibiotic growth promoter predisposed vancomycin-resistant enterococci (VRE) to occur, Denmark became one of the earliest nations to outlaw it in 1995 (Bennani et al. 2020).

11. POLICY AND REGULATORY INTERVENTIONS AT THE NATIONAL AND INTERNATIONAL LEVELS FOR COMBATING AMR

This set of public health problems includes global epidemics, antibiotic-resistant bacteria (AMR), food-borne illnesses, and recurrent zoonotic infections. A collaborative and integrated multi-sectoral competence is needed for the mitigation, early detection, and efficient response, and to minimize the consequences. This strategy is commonly known as One Health. Initial calls for a One-Heath policy were made following the H5N1-caused avian influenza epidemic.

One Health, according to the World Bank, is an approach for improved cooperation in fields of mutual concern (intersections), with a focus initially on zoonotic diseases and AMR, that will lower threat, strengthen public health worldwide, and combat poverty and economic expansion in developing nations (Gebreyes et al. 2014). Two primary objectives for implementing One Health include improving national capacity for zoonotic illnesses and AMR for avoidance, identification, and response, as well as improving collaborative cooperation and communication among key stakeholders for combating zoonoses and antimicrobial resistance (WHO 2022a).

India's National Action Plan on AMR is a great illustration of the One Health strategy and may be utilized as a reference to create a practical outline for a nation's efforts to deal with other comparable public health issues, particularly in the event of future outbreaks of disease India’s National Action Plan includes

a) The use of antimicrobials for the purpose of promoting poultry production has been outlawed.

b) Need for cross-sectoral collaboration for the establishment of a national policy for antimicrobial resistance containment that includes approaches that are largely focused on using antibiotics in the human health sector.

c) A regulation for livestock encouraging the responsible application of antimicrobial agents in livestock.

d) Defining the duration of the antimicrobial discontinuation period for poultry, livestock, and seafood.

e) Guideline about the discontinuation period and residual cap for antibiotics in meat and related products.

Moreover, agricultural, food and environmental sectors should be listed as crucial bodies in mitigating AMR. The national action plan’s objectives include raising information through effective interaction, instruction, and training, enhancing monitoring methods, reinforcing the control and prevention of infections, research and development, supporting investments, and cooperative actions to fight antimicrobial resistance (Biswas et al. 2023).

12. PROGRAMS ADVANCING THE ONE HEALTH PARADIGM AT THE NATIONAL AND INTERNATIONAL LEVEL INCLUDES

a) Global congress on influenza and birds (El Mellouli et al. 2022)

b) Collaborative partnership between UNESCO, World Health Organization, Food and Agriculture Organization, World Organization for Animal Health (Ossebi et al. 2022)

c) Quadripartite agreement for combating AMR, rabies, TB, and SARS (WHO 2022b)
d) OH alliance comprising the globe healthcare and animal associations (Otu et al. 2021)
e) Functional ecological, individual, and veterinary healthcare outline for boosting One Health

13. RESEARCH AND DEVELOPMENT OF NEW ANTIMICROBIAL AGENTS AND VACCINES

The discovery of new antibiotics is a crucial step in combating the AMR epidemic, yet from 2017 to 2022, the US FDA and/or the EMA only granted approval for 12 novel antimicrobials. The bulk of the most recently licensed antibacterial shows only little therapeutic improvement over current therapies, and about 80% of these medications come from families of antibiotics that are currently in use and where resistance mechanisms have already been identified. In the clinical and preclinical research, there were as of late 2021, 77 antimicrobial or combination medicines and 217 antibacterial candidates, respectively, intended for the 13 WHO essential infectious agents, notably *Mycobacterium tuberculosis* and *Clostridium difficile* (Eisinger et al. 2023).

It is important to discover novel medications within an established antibiotic class since this can result in enhanced safety characteristics, more practical dosing regimens, and the gathering of information for pathologies or populations that have not yet been researched for the drug class in question. When new medications are developed within an established class, the antibacterial spectrum may also evolve gradually (for example, compare cefazolin to ceftriaxone to cefepime). However, the escalating resistance to drugs in ordinary diseases and the potential worry of mutated, multidrug-resistant agents in bioweapons can only be adequately addressed by the creation of novel classes of antibiotics with unique modes of action (Spellberg et al. 2004).

Vaccination is a key component of antimicrobial resistance prevention because it decreases infections brought on by bacterium that are equally vulnerable to and resistant to antimicrobial agents, which lowers the total need for antimicrobial agents. It is evident that vaccines have been successful in minimizing the incidence of diseases over the past century; outbreaks of *Corynebacterium diphtheriae*, *Neisseria meningitidis*, *Hemophilus influenzae* type b (Hib), and Bordetella pertussis have all decreased significantly. Pneumococcal vaccinations have reduced both invasive illness and nasopharyngeal carriage of S. pneumoniae (including isolates resistant to antibiotics), suggesting vaccination's ability to tackle antimicrobials in action. The use of vaccines has been proven to minimize the use of anti-microbial over a wide range of species, including fish, pigs, and poultry, in addition to improving human well-being.

The capsular polysaccharides (CPS) of the bacterial species S. pneumonia and H. influenza are the targets of the pneumococcal and Hib vaccines, respectively. The effectiveness of the pneumococcal conjugate vaccine for invading pneumococcal illness ranges from 86% to 97%. The capsular polysaccharides are among the most effective and commonly targeted antigens of bacteria in vaccine development as a result. Clinical studies are underway for a number of vaccinations for strains of bacteria with substantial antimicrobial burdens that attack capsular polysaccharides as of 2021, including the 12-valent vaccine for extra-intestinal pathogenic *E. coli* (Mullins et al. 2023).

14. INNOVATIONS IN DIAGNOSTICS FOR TIMELY IDENTIFICATION OF ZOONOTIC INFECTIONS

Since traditional approaches for identifying zoonotic infectious agents, such as those centered on bacterial culture, polymerase chain reaction, and immune-mediated methods, offer certain benefits they also have some disadvantages, such as a lengthy timeframe for outcomes and the need for challenging tasks, costly supplies, and specialized tools in some situations. Thus, biosensors appear to be considered among those cutting-edge, effective instruments of diagnosis for this purpose. These components are highly efficient and trustworthy with excellent specificity and sensitivity, and when

supported by nanoparticles, their practicality can further be enhanced in medical systems for the diagnosis (Ahangari et al. 2023).

Nanotechnology which is based on liposomes has mostly improved animal medicinal molecules and diagnosis. Liposomes have been used for targeted medication and delivery of genomes as well as for imaging purposes. Enhancing sensitivity, exclusivity, and efficiency using nanotechnology is crucial for disease management and surveillance.

The ongoing study of HIV-1 and influenza viruses has identified applications for nano-diamonds, nano-traps, and nano-fibers. Frequently affecting both humans and species of birds, avian influenza is now a threat to both individuals and the worldwide financial system. The majority of avian influenza detection methods employ immunochromatography, which makes use of colloidal particle-conjugated antibodies and antiretroviral nuclear protein antibodies. Early research on a fluorescent monochromatic test strip with a threshold of detection (LOD) of ng/ml for hemagglutinin-specific europium nanoparticles (NPs) has been reported. Currently, a colloidal immunochromatographic strip test with two MAbs for H7 N9 avian influenza viral antigen identification has been invented as an experimental gold-related diagnostic. Compared to other tests, this strip test had a 98.6% precision and a 71.40% sensitivity (Arshad et al. 2022).

The phage display technology has a lot of applications for broad-spectrum antibody identification of different diseases. However, it is a pricy and labor-intensive technology being employed for research. High throughput sequencing, commonly referred to as next-generation sequencing, is utilized for unambiguous genomic identification, particularly for unidentified infections. This approach also enables risk evaluation. The surveillance of virus dissemination, i.e., continuous propagation vs reappearance in a population, is being done using NGS approaches. Heartland virus, Bas-Congo virus, Sosuga virus, and SFTS virus are among the diseases that have been identified.

NAAT (Nucleic Acid Amplification Test) which includes Quantitative Real-time PCR/RT-PCR is a quick detection method for already identified microorganisms like methicillin-resistant Staphylococcus aureus (MRSA) colonization in the sinus cavities as well as Marburg virus in a bat reservoir (Mehmood et al. 2023).

15. FUTURE PERSPECTIVES AND CHALLENGES

15.1. ANTICIPATED TRENDS IN AMR AND ZOONOTIC DISEASE DYNAMIC

The propagation of resistance genes is significantly influenced by intricately related environmental and socioeconomic variables. The effectiveness of healthcare frameworks, the facilities for water, sanitation, and hygiene (WASH), per-capita GDP, and weather have all been highlighted as key contributors to the development and spread of antimicrobial resistance**.**

Antibiotics are used improperly and excessively for purposes other than for consumption by humans (Bungau et al. 2021; Abdellatif and Mohammed 2023). The anticipated global sales of antimicrobials for use in livestock raised for human consumption in 2017 totaled 93309 tons. By 2030, this amount is anticipated to increase to 104079 tons. The growing need for meat-based goods and over-the-counter sales, especially in countries with low or middle incomes (LMICs), where demographics are expanding and becoming economically advanced, is the cause of this rise in the consumption of antibiotics. A variety of variables, such as the migration of people and animals, surface water runoff, and the trading of agricultural goods, contribute to the fast dissemination of AMR across ecosystems. Numerous anthropogenic variables, including population growth (urban density) and increasing wealth, have been

determined to be linked to AMR at the human-animal interface by higher preferences for animal-based foods and goods (Allel et al. 2023).

15.2. POTENTIAL IMPACTS OF CLIMATE CHANGE AND URBANIZATION ON THE SPREAD OF AMR AND ZOONOSIS

Ecological changes impacting infectious disease prevalence and spread are linked to climate change. Sand flies, mosquitoes, and ticks are examples of species of arthropods that carry vector-borne diseases. Being ectothermic, vectors of arthropods are directly affected by temperature, which also has an impact on the growth, existence, and replication of infectious agents within vectors as well as the dissemination, prosperity, environmental suitability, frequency, and chronological cycle of vector behavior (such as biting rates). Heavy rains create more potential hatching grounds for mosquitoes and other vectors. Additionally, the lush vegetation that develops after rain offers vectors with cover and rest places Fields (Rupasinghe et al. 2022).

New ecosystems with an elevated human population concentration are upsetting widespread ecotones. As a result, there is greater potential for zoonotic diseases and resistance to spread between organisms, particularly as there is more interaction between people and wild animals. The function of ecosystems in the human-animal health interaction is further affected by human-induced stresses such as land use change, biodiversity loss, climate change, and environmental damage (WHO 2022c).

15.3. RESEARCH GAPS AND AREAS FOR FUTURE EXPLORATION

The development of One Health studies shows an increase in international involvement by comparing the study ecosystems among the first and sixth world one health congresses (ten years later). Despite increased awareness of the challenges facing global sustainable development, there is presently little representation of "Environmental and Ecological issues" and "Sustainable Food Systems" inside the World One Health Congress. In order to monitor disease outbreaks and respond quickly to them, it can be helpful to determine the main animal groups that could be the cause of outbreaks of disease. Additional investigation is required to pinpoint the environmental reservoirs and carriers for zoonosis, which include a diverse array of African bats, apes, and bird species. The methods and modes of transmission, notably the function of vectors, should also be covered in such investigations. It is necessary to identify the kind, degree, and gene mutations of antibiotic resistance in pathogenic zoonotic bacteria in distinct environmental niches. By implementing national laws, developed nations are able to keep the spread of antibiotic resistance to a minimum (Ahmed et al. 2023).

Tragically, due to insufficient laboratory resources and insufficient funding for health services, monitoring of antimicrobial-resistant organisms is often minimal or nonexistent in nations with low incomes. This is especially concerning when taking into account the increased likelihood of the spread of infectious diseases in LICs due to their restricted availability of sanitary facilities and clean water. Nevertheless, with international collaboration, exchange of information, and modifications to policy, these problems can be resolved (Gwenzi et al. 2022).

In epidemiology, investigations involving infections caused by bacteria, whole genome sequencing has come to be regarded as the gold standard because it allows for strain differentiation at the point of single nucleotide polymorphism. Utilizing a structure-based drug discovery approach has sped up the development of new antimicrobial drugs, pharmaceuticals, and vaccine candidates. In order to lessen the impending threat of resistance to antibiotics in the future, there is a need for multidisciplinary investigation and strategic thinking to enhance awareness of genetic variation (Sharma et al. 2023).

16. CONCLUSION

Antimicrobial resistance (AMR) has now become a significant global public health issue, with ten million annually fatalities predicted by 2050. AMR happens when viruses, bacteria, fungi, and parasites in people and animals do not respond to antibiotics, thereby allowing microbes to survive inside the host's body. The One Health Approach is a system including a variety of disciplines in order to attain the optimal medical outcome by recognizing the obvious interactions among humans, animals, and their common surroundings. The Sustainable Development Goals (SDGs), to address the issue of antimicrobial resistance. As a part of both regional and global action strategies, it is still necessary to underline the significance of awareness among the public and lay audiences' understanding of health issues. The 21st century's biggest public health challenge is still resistance to antibiotics. The Group of Seven nations (G7 nations) have already made significant political contributions to this issue, and it is still on the radar of many political summits. It is estimated that if AMR is not fully addressed, we will soon revert to the pre-antibiotic age, when routine diseases related to childbirth, surgery, and open shattered limbs may be fatal. The COVID-19 pandemic has clearly established the need to understand the relationship between human-animal disease patterns as well as the spread of zoonotic diseases and antimicrobial resistance globally among human-animals, animal-animal and human-human disease, and antimicrobial resistance transmission. In order to guarantee that the globe has access to a substantial stockpile of potent antibacterial substances that will support human and animal health both currently and in the future, people, communities, and governments must collaborate *(*Tang et al. 2023).

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Antibiotic Resistance from Zoonotic Point of View and Possible Alternative Treatments

15

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ABSTRACT

Globally, zoonotic infections are major contributor to the rise and spread of antibiotic resistance, and becomes a major threat to the public health. Infections may spread easily from animals to people due to close contact between the two species, which in turn foster the development of antibiotic resistance. Humane face an important risk to the potential transmission of animal originated, including milk, egg, meat and protein source. The need for alternative antibiotics is the deem need of current era. Bacteriophages, nanoparticle and antimicrobial peptides are proven to be effective. Results from different studies underscore the significance of these alternative treatments as a highly effective antibacterial for combating potentially pathogenic agents I animals, thereby enhancing their growth performance.

Key words: Zoonotic, Antibiotic resistance, Alternative, Bacteriophage, Nanoparticles.

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1. INTRODUCTION

The emergence and spread of various infectious diseases are influenced by the interactions between humans, animals, and the environment (Thompson and Kutz 2019). The majority of human infectious diseases are animal-borne. According to the "Asia Pacific strategy for emerging diseases: 2010" study, 60% of newly identified diseases affecting humans are zoonotic and 70% of these pathogens come from wildlife species (Rahman et al. 2020). The most recent human diseases were animal-related and directly linked to animal-derived food items (Slingenbergh 2013). Zoonosis comes from the Greek words "zoo" (animal) and "nosos" which means illness. Zoonoses are diseases or infections that may be transmitted from vertebrate animals to human beings or from humans to animals, according to the WHO (World Health Organization 2020). There are about 61% of human pathogens that are of zoonotic type (Taylor et al. 2001). Zoonosis poses a significant public health concern and represents a direct threat to human health, with potentially fatal outcomes. The majority of these diseases have a detrimental impact on animal well-being and result in a decline in livestock productivity (Grace et al. 2012). In this chapter, we summarized how Antibiotic resistance in livestock is spread? an alternative to these antibiotics: bacteriophages, nanoparticles, and antimicrobial peptides.

1.1. CLASSIFICATION OF ZOONOSES

Zoonotic diseases emerge as a result of a wide range of pathogens. Zoonoses are categorized based on their etiology into various groups, including bacterial zoonoses (e.g., Anthrax, Salmonellosis, Tuberculosis, Lyme disease, Brucellosis and Plague), viral zoonoses (e.g., rabies, acquired immune deficiency syndrome (AIDS), Ebola, and avian influenza), parasitic zoonoses (e.g., trichinosis, toxoplasmosis, trematodosis, giardiasis, malaria, and echinococcosis), fungal zoonoses (e.g., ringworm), rickettsial zoonoses (Q-fever), chlamydial zoonoses (psittacosis), mycoplasma zoonoses (*Mycoplasma pneumoniae* infection), protozoal zoonoses, and diseases caused by acellular non-viral pathogenic agents (e.g., transmissible spongiform encephalopathies and mad cow disease) (Schaechter 2009).

Zoonoses were formerly classified as anthropozoonoses, zooanthroponoses, amphixenoses, and euzoonoses (Hubálek 2003). Anthropozoonoses refers to a class of diseases originating in animals that have the potential to be transmitted to humans, exemplified by the case of rabies. Zoonotic diseases can pass from humans to animals, such as TB in cats and monkeys. The term "zooanthroponoses" describes this phenomenon. Amphizoonoses refers to diseases that have the potential to be transmitted bidirectionally, encompassing transmission from humans to animals as well as from animals to humans. An example of disease is staphylococcal infection. In some cases, humans assume the role of obligatory host for various parasitic diseases. Both Gram-negative and Gram-positive bacteria have the ability to induce zoonotic diseases. Zoonotic diseases are predominantly caused by bacteria, as per their etiology. According to estimates, 42% of zoonotic diseases with bovine origins are bacterial, 22% are viral, 29% are parasitic, 5% are fungal, and 2% are prion-base (McDaniel et al. 2014).

In the same way, it is noteworthy that zoonotic diseases can be attributed to both DNA and RNA viruses; however, RNA viruses cause Zoonosis more often than DNA viruses (Bae and Son 2011). Pathogens can be transmitted to humans directly or indirectly from animals. Diseases that are transmitted directly to humans from animals through media such as air are known as direct Zoonosis (Mortimer 2018). The majority of zoonotic diseases are transmitted to humans via animal hosts. Illustrative instances of such pathogens involve methicillin-resistant *Staphylococcus aureus* (MRSA), *Campylobacter spp.*, and *Salmonella enterica*. Furthermore, the term "reverse Zoonosis" is used to describe zoonotic diseases that arise from pathogens transmitted intermittently between humans and animals, with transmission occurring from humans to animals and subsequently from animals back to humans.

1.2. ANTIBIOTIC RESISTANCE IN ZOONOTIC PATHOGENS

One health approach identifies that most animals who serve as a reservoir for zoonotic diseases are domesticated with which man is commonly associated. Animal husbandry is an intrinsic part of the agricultural economy and plays a vital role to support the livelihood of the rural population. Animal husbandry provides milk, meat, eggs, and wool which directly interact with the human population. Despite the much risk of zoonotic diseases, livestock plays a crucial role in the income of many farmers, nutrition for households, and animal product consumption. Recently many zoonotic diseases have been reported to carry antibiotic resistant genes (ARGs) that reach the human population upon infection. The emergence of ARGs in zoonotic pathogens can be attributed to the inappropriate and irrational utilization of antibiotics (Dafale et al., 2020).

Over the course of previous years, the well-being of both animals and humans has been put at risk by the presence of environmental pollution, the emergence of antimicrobial resistance, and the prevalence of chronic diseases, leading to increased rates of mortality and morbidity (Kalia et al. 2014). Most infectious diseases are considered to be serious health issues having a zoonotic origin. Zoonotic diseases may also be spread through consuming undercooked meat, unpasteurized milk, shellfish, and infected vegetables. In the field of animal husbandry, it is common practice to administer antibiotics to the entire herd, even if only some animals exhibit clinical symptoms, in order to mitigate the spread of infectious diseases within the herd. The practice of administering a large number of antibiotics for a brief duration is referred to as metaphylaxis, whereas prophylaxis involves the addition of antibiotics to animal feed in small doses over an extended period, typically spanning several weeks (Kalia and Purohit 2011).

Despite the absence of clinical symptoms in animals during this period, the risk of infection remains present. The unwise utilization of antibiotics gives rise to selective pressure in pathogens, leading to the development of antibiotic resistance at sub-therapeutic levels of antimicrobial concentration. Bacterial species acquire resistance through a range of mechanisms, which include mutation, alterations in cell permeability, horizontal gene transfer, drug efflux, and quorum sensing (Dafale et al. 2015). Zoonotic infections spread between people and animals through several methods. However, the most likely pathway for transmission occurs via the food chain (Fig. 1 Antibiotic resistant in the food chain). Humans face an important risk to the potential transmission of animal-originated antibiotic-resistant bacteria (ARBs) due to the consumption of animal-derived food products, including milk, eggs, meat, and protein sources. ARBs are more likely to be present in fermented, minimally processed, or raw food products that contain a higher concentration of microbial cells. Pathogenic bacteria in animal-derived food cause stomach problems and resistant strains in humans.

2. ALTERNATIVE TO ANTIBIOTICS

2.1. BACTERIOPHAGE THERAPY FOR ZOONOTIC PATHOGENS

Since antibiotic-resistant bacteria may continue to spread disease, there is an urgent need to identify and develop effective alternative treatments to antibiotics to prevent bacterial infections (Gambino and Brøndsted 2021). Bacteriophages (phages); are viruses that infect bacteria and have been used as medicines since before antibiotics were discovered (De-Melo et al. 2018). Bacteriophages, or phages, are highly prevalent and widely distributed entities across the planet, existing in both natural and artificial settings, particularly in environments conducive to the proliferation of their bacterial hosts (Golkar et al. 2014).

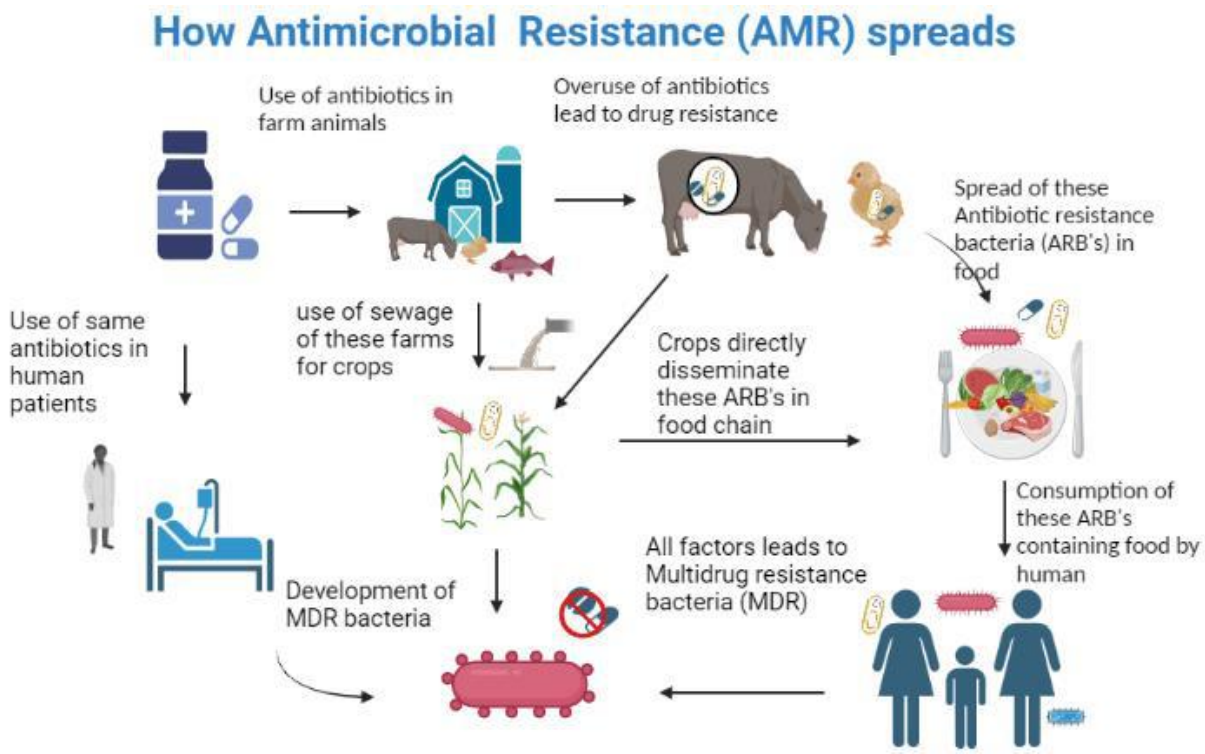

Fig. 1: Antibiotic resistance in the food chain

The discovery of bacteriophages can be attributed to Twort in 1915, who initially identified them as unknown entities capable of impeding bacterial growth. However, it was D'Herelle in 1917 who first succeeded in isolating and characterizing phages. Additionally, he pioneered the development of phage therapy, utilizing it for the treatment of fowl typhoid caused by *S. gallinarum* in poultry (Wernicki et al. 2015). Bacteriophages are widely believed to be the most prevalent life forms on Earth, boasting a population estimated to range from 10^{30} to 10^{32} virions within the biome (Rohwer and Edwards 2002). Extensive documentation exists regarding the prevalence of bacteriophages within the entirety of the human food chain. Certain phages exhibit the capacity to serve as advantageous biocontrol agents (LeLièvre et al. 2019). The primary focus of phage therapy experiments on animals used for food production has been on combating significant zoonotic pathogens, specifically *E. coli, Listeria* spp., *Campylobacter* spp., and *Salmonella* spp. The issue of AMR among certain bacterial strains is a significant and escalating concern (Threlfall et al. 2000).

The European Union (EU) has implemented regulations that prohibit the routine administration of antibiotics in farm animals and impose limitations on the use of chemical treatments for carcasses during processing. Consequently, there is an urgent need for alternative interventions (Dibner and Richards 2005). The utilization of bacteriophages in combatting bacterial infections has yielded favorable outcomes, thereby fostering the advancement of investigations into the prospective application of bacteriophages as therapeutic agents for diseases affecting both humans and animals.

2.1.1. USE OF PHAGE-THERAPY IN POULTRY

Poultry has been widely employed as a primary model for Phage therapy in the context of food-producing animals. Poultry production is large-scale, high-throughput, and mechanized, unlike big animal meat

production, making phage treatment especially useful. Conventional chicken-rearing methods, that house hundreds of thousands of birds, increase disease transmission and economic losses. In a series of investigations, Phage therapy has been employed as a therapeutic approach for the treatment of chickens afflicted with experimental air sac infections caused by *E. coli*. In their initial investigation, the researchers employed an aerosolized solution comprising two distinct bacteriophages, resulting in a 50% reduction in chicken mortality when administered concurrently with the *E. coli* challenge (Huff et al. 2002).

The aerosol spray containing a higher concentration of phages demonstrated a notable decrease in mortality rates when chickens were exposed to *E. coli* three days after primary treatment. In a subsequent investigation, a comparable infection and treatment paradigm was employed, resulting in a notable decrease in mortality among the chickens subjected to phage therapy, with a rate of 7%, in contrast to the approximately 48% observed in the untreated specimens (Huff et al. 2006). The role of contaminated egg and poultry products as a significant reservoir of *Salmonella* spp. is widely acknowledged in academic literature. Furthermore, certain serovars of *Salmonella* have the capacity to induce illness and death in chickens, while also exhibiting the ability to persist in the agricultural setting for extended durations. Bacteriophages derived from human sewage were employed to mitigate the intestinal colonization of the poultry by *S. typhimurium* in experimental settings, resulting in a reduction of approximately 1 log10 cfu. Furthermore, the utilization of these phages led to a significant decrease in mortality rates when compared to untreated animals. Nevertheless, the phages exhibited persistence solely during the period in which *Salmonella* spp. may have been retrieved (Berchieri et al. 1991). Bacteriophage resistant *Salmonella* colonies were retrieved after undergoing phage treatment displayed a coarse physical structure and demonstrated reduced virulence compared to the original strain used for the challenge. Bacteriophages derived from free-range chickens were utilized in order to mitigate the colonization of broiler chickens by *S. enteritidis* PT4.

The experiment involved exposing one-day-old broiler chickens to *S. enteritidis* bacteria, followed by treatment with a mixture of three bacteriophages at a concentration of 10^{11} PFU seven days later. A reduction of 3.5 log10 in caecal carriage was observed five days following phage treatment, in comparison to the control group. A significant decrease in *Salmonella* colonization in the PT group was observed and persisted for the duration of 25 days following phage treatment (Fiorentin et al. 2005). Oral treatment of phage titer of 10^{11} PFU may inhibit caecal colonization of *S. enteritidis* and *S. typhimurium* in broiler chickens by up to 4.2 log10 cycles. A third serovar was targeted for reduction, but without success. Within 72 hours of being treated with bacteriophages, the birds were recolonized by phage-resistant subpopulations of salmonellas. Salmonellas collected from these hens seemed to return to a phage-sensitive phenotype after being challenged with phage-resistant mutants, indicating that phage resistance in this circumstance conferred a fitness cost (Atterbury et al. 2007).

Disease-causing strains of *E. coli* (pathogenic *E. coli*) are a major source of death and illness in chicken farms. When the death rate from *E. coli* septicemia in untreated hens reached about 100 percent, researchers turned to phages for an effective preventative measure. Phage therapy was remained very effective at reducing infection severity even when delayed until the beginning of clinical signs (Barrow et al. 1998). A research study was conducted to examine the impact of bacteriophages on the occurrence of neonatal diarrhea in poultry. A comparison was made between the impact of phage therapy and antibiotic on the chicken survival subjected to *E. coli* (strain 3-1) challenge. The application of phage therapy resulted in a notable decrease in the occurrence of diarrhea among the chicken population, with a reduction to 26% to that of 51.6% observed in the control group. The survival rate in the Phage therapy group exhibited a tenfold increase compared to the control group, and a six-fold increase compared to the group treated with antibiotics (Xie et al. 2005).

Campylobacter spp., an essential zoonotic origin pathogenic bacterium in poultry, has also a focus of phage therapy interventions. The prevalence of *Campylobacter* in poultry has long been recognized as a

major source of contamination in the human food chain (Skarp et al. 2016). One risk study revealed that reducing carcass *Campylobacter* contamination by 2 log10 cfu may reduce human Campylobacteriosis by 30 times (Rosenquist et al. 2003). After repeated phage dosages, broiler chicken caeca's contain 1–2 log10 cfu less *Campylobacter* (Wagenaar et al. 2005). The utilization of phages obtained from the same ecological niche as the host bacterium in numerous phage therapy trials raises inquiries regarding the cohabitation dynamics between viruses and their host organisms within their natural habitat. A recent study has revealed that the presence of phages in broiler chickens naturally colonized with *Campylobacter* which is associated with reduced populations of the bacteria in the caeca. This observation implies that phages may naturally prey upon *Campylobacter* within commercial flocks. The variability in bacteriophage titers observed in the intestinal tract could potentially be attributed to the dynamic nature of *Campylobacter* spp., as well as the presence of a susceptible host that is subject to change over time (Atterbury et al. 2005).

2.1.2. USE OF PHAGE-THERAPY IN LARGE FARM ANIMALS

Phage cocktails were administered to calves, piglets, and lambs as a potential treatment for enteritis, building upon their previous experiments with mice. The calves were subjected to either colostrum feeding or colostrum deprivation, followed by exposure to *E. coli* (strain O9:K30.99). All nine calves that were administered a phage titer (10^{11} PFU) cocktail of two distinct phages via colostrum did not exhibit any signs of illness, in contrast to the control group where 93% of the calves became ill. Among the cohort of 13 calves that were deprived of colostrum and subsequently administered phages upon the onset of diarrhea, a mortality rate of 15.4% was observed, in contrast to the control group which experienced a 100% mortality rate. The findings of this study provide evidence that the administration of phages can significantly decrease both the incidence of illness and the number of deaths, even when administered at the early stages of clinical manifestations. Comparable achievements were documented in the context of utilizing enterotoxigenic *E. coli* strains to induce challenges in piglets and lambs (Smith and Huggins 1983). Bacteriophages in combination can effectively diminish the population of *E. coli* O157:H7 within the gastrointestinal tract of sheep. In comparison to certain other studies on phage therapy (PT), it was observed that the utilization of higher phage MOI (multiplicity of infection) values, specifically 100 and 10, resulted in lower efficacy when compared to a MOI of 1(Callaway et al. 2008). Lytic bacteriophage exhibits specific affinity towards the *E. coli* K1 capsular antigen. This bacteriophage was utilized for the treatment of colostrum-deprived calves that had been subjected to *E. coli* challenge. The onset of bacteremia was observed to be postponed in animals subjected to phage treatment, resulting in a significant extension of their lifespan (Barrow et al. 1998). According to a study conducted by the application of phage therapy did not result in a reduction in the colonization of sheep by *E. coli* O157 (Sheng et al. 2006). It has been reported that an estimated 5% of bovine livestock are believed to harbor significant quantities of *E. coli* O157 in their gastrointestinal tract, thereby presenting the most substantial threat to human well-being (Matthews et al. 2006). Implementation of phage therapy to decrease the presence of this pathogen in a specific subset of highly colonized animals has the potential to generate significant advantages for public health.

2.2. NANOPARTICLE AS AN ALTERNATIVE OF AMR

To control the spread and emergence of antibiotic resistance, specifically resistance in humans against clinical antibiotics, an alternative approach is needed. In the past few years, nanoparticles have grabbed the attention worldwide due to their toxicity against microbes (Vidovic et al. 2015). Nanoparticles (NPs) are particulate substances having sizes ranging from 1 to 100nm on a nonmetric scale that permits

modification in the chemical and physical properties of materials. The size of NPs has a role in antimicrobial activity (Jeevanandam et al. 2018). NPs have been used in biological imaging, photo ablation therapy, biosensors, and drug administration (McNamara and Tofail 2015) and as an alternative in the reduction of antimicrobial resistance (Kanwar et al. 2021). NPs are categorized depending on their size, morphology, and chemical properties. Based on the chemical and physical characteristics of NPs, various classes of NPs are carbon-based NPs, metal NPs, polymeric NPs, Semi-conductor NPs, lipid-based NPs, and ceramics NPs (Khan et al. 2019).

Among them metal NPs showed higher antimicrobial activity (Jones et al. 2008) and no harmful effects to humans (Reddy et al. 2007). The reactivity of NPs depends on the size of NPs; the smaller the NPs, the greater the area-to-volume ratio resulting in high production of free metal and reactive oxygen species (Thakkar et al. 2010) resulting in the alteration of bacterial cellular components (Abdal et al. 2017). Absorption of the nanoparticles on the surface of bacteria causes damage that depends on the NPs size; the smaller the size greater will be the damage. The reason behind this damage is due to the interaction of the negative charge on the bacterial surface with the positive charge on the surface of NPs (Slavin et al. 2017; Gupta et al. 2019). There are 5 different mechanisms (Fig. 2 Antimicrobial action of metal Nano-particles) through which nanoparticles act on microbes, including the generation of reactive oxygen species, cell wall, and cell membrane interaction, adsorption at the cell membrane, blockage of protein synthesis, DNA destruction and disruption of the metabolic pathway (Pérez-Díaz et al. 2015; Nisar et al. 2019).

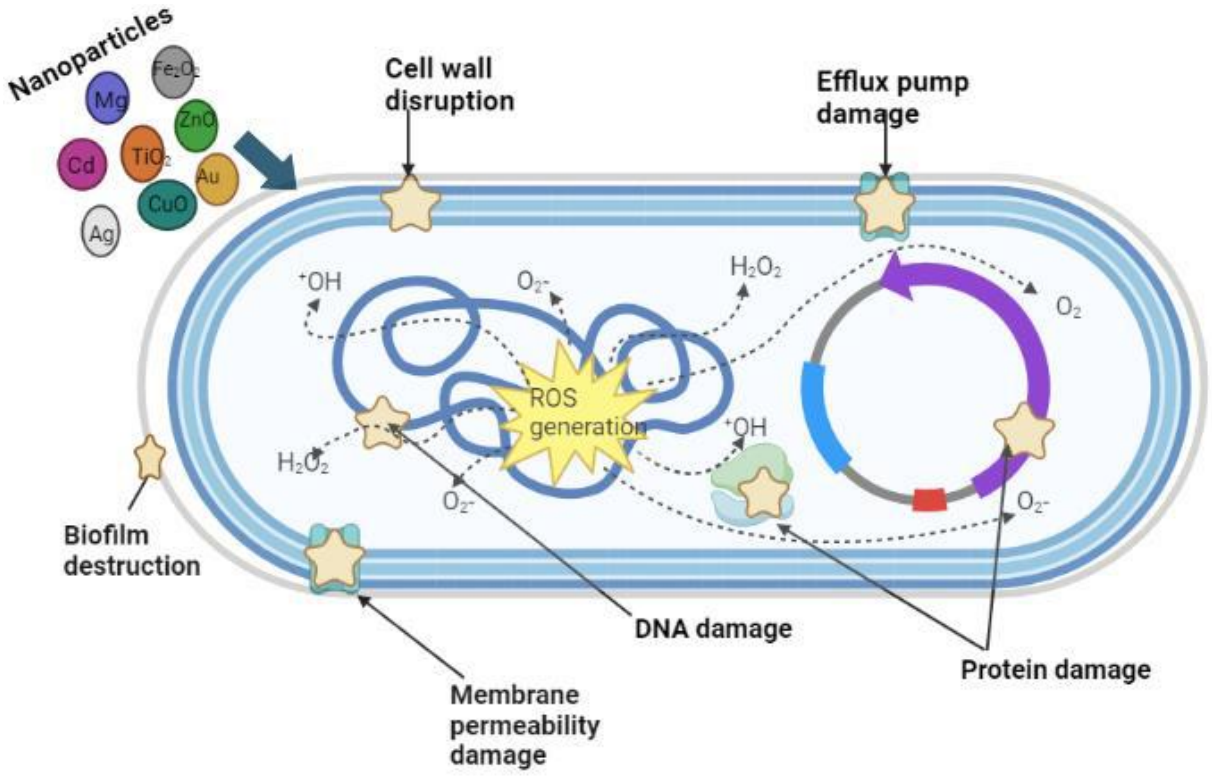

Fig. 1: Antimicrobial action of metal Nano-particles

Antimicrobial resistance against zoonotic diseases is an alarming situation for both animals and humans. The antimicrobial activity of various NPs against zoonotic pathogens has been evaluated. The efficacy of NPs can be enhanced when combined with antibiotic drugs. Silver nanoparticles were combined with

various drugs like ioniazid, ofloxacin, rifampicin, and various others. All these drugs except kanamycin showed greater growth inhibitory activity along with silver nanoparticles (AgNPs). Through these combinations, a minimal dose can be found to overcome antimicrobial resistance (Kreytsberg et al. 2011). The effects of bacterial-resistant antibiotics were evaluated in combination with AgNPs against *P. multocida, S. enterica, S. aureus,* and *E. coli*. Due to the combination of antibiotics such as gentamicin, colistin, amoxicillin, etc. with the AgNPs bacteria like *P. multocida* resistant to these antibiotics became sensitive to these antibiotics and the antimicrobial effect of AgNPs was enhanced (Smekalova et al. 2016). Administration of drugs in combination with nanoparticles showed higher antimicrobial activity as compared to free drug administration. The research was conducted in which chitosan nanoparticles were combined with tetracycline and silica nanoparticles with chlorpromazine to evaluate their efficacy against multi-drug resistant (MDR) *S. enterica* and *S. aureus*. These combinations reduce the microbial load by 83.02±14.35% by inhibiting the AMR mechanism (Brar et al. 2022).

The antimicrobial activity of AgNPs was evaluated against MDR *S. enterica* and *S. typhimurium*. These isolates were sensitive against only meropenem but resistant against tetracycline, ampicillin, and amoxicillin. Proteins present in the bacterial membrane contain sulphur that interacts with AgNPs resulting in bacterial death. During different storage times, the growth was seen to be decreased while after 48hrs no bacterial growth was present (Abou-Elez et al. 2021). The growth of both MDR *Salmonella* spp. and *E. coli* was inhibited due to the antimicrobial property of gold nanoparticles (AuNPs) (Abdalhamed et al. 2021). Some biofilm-producing bacteria induce chronic mastitis resulting in inflammation of the udder. Due to the antimicrobial resistance an alternative treatment is needed for bovine mastitis. Plant-derived drugs having antimicrobial activity are naturally effective agents. Quercetin and AgNPs (QA NPs) together inhibit the growth of MDR *E. coli.* QA NPs efficiently decreased the transcription of genes associated with biofilm formation resulting in the inhibition of *E. coli* biofilm formation (Yu et al. 2018).

Another bacterial pathogen that causes bovine mastitis is *S. aureus*. Various kinds of Nanoparticles like polymeric nanoparticles, nanogels, liposomes, inorganic nanoparticles, and solid lipid nanoparticles are developed to enhance intracellular drug delivery and inhibit biofilm formation. NPs have played a key role in the treatment of bovine mastitis (Algharib et al. 2020). AgNPs when combined with vancomycin could overcome the resistance in *S. aureus* and increase cell death (Esmaeillou et al. 2017; Mohamed et al. 2020). Zinc oxide (ZnO) can also be used to treat mastitis. Also, Saeb et al. (2014) suggested that the AgNPs can make methicillin-resistant *S. aureus* sensitive and inhibit their microbial activity (Abd EL-Tawab et al. 2018). Both AgNPs and AuNPs have enhanced activity when combined with ampicillin against *S. aureus*. However, the activity of AuNPs increases when ampicillin attaches to their surface (Brown et al. 2012). Brucellosis caused by *Brucella* spp. is difficult to treat. Virulence of *Brucella* spp. is associated with intra-macrophage survival. The antimicrobial activity of AgNPs of a concentration of 4-6 ppm is effective even inside macrophage cells (Alizadeh et al. 2013). Solid-liquid nanoparticles encapsulated with doxycycline significantly decrease the load of *B. melitensis* in macrophages by 3.5 log as compared to free doxycycline (Hosseini et al. 2019). AgNPs and AuNPs have toxic effects against *B. melitensis* and *B. abortus* and have no side effects on organs and bacteria showed no resistance against these NPs even after extensive exposure (Elbehiry et al. 2022). Glutathione combined with AgNPs can be used effectively against MDR *Campylobacter* spp. in chickens (Silvan et al. 2018).

2.3. ANTIMICROBIAL PEPTIDES

The identification of antimicrobial peptides (AMPs) has provided insights into the mechanisms by which plants or insects, which do not possess adaptive immune systems, are capable of defending themselves

against microbial infections (Nayab et al. 2022). The increase in antibiotic resistance has prompted an extensive investigation into the exploration of alternative options to existing antibiotics. Antimicrobial peptides (AMPs) are being increasingly recognized as potential candidates for standalone or adjunctive use with existing antibiotics, thereby getting renewed attention (Fox 2013).

Nisin is a cationic peptide with antimicrobial properties that is synthesized by *Lactococcus* and certain species of *Streptococcus*. In 1969, nisin achieved the distinction of being the inaugural antimicrobial agent to attain the status of a food-safe additive. Nisin has been employed as a therapeutic intervention for bovine mastitis resulting from a diverse array of bacterial pathogens, including *Enterococcus*, *Streptococcus*, and *Staphylococcus*. One primary benefit is that the presence of nisin in milk is limited to a duration of 12 hours following its application, at concentrations that pose no discernible risk to consumers. Additionally, the application of nisin helps to mitigate the development of bacterial resistance (Cao et al. 2007).

The concurrent application of the nisin or another bacteriocin, enterocin DD14 in conjunction with colistin exhibited a synergistic impact on colistin-resistant strains of *E. coli* that were obtained from porcine sources. The observed phenomenon can be attributed to the destabilization of the cell membrane caused by the interaction between colistin and LPS, which subsequently facilitates the penetration of bacteriocins into the cell wall, resulting in its damage (Al-Atya et al. 2016). The effective application of bacteriocins' antimicrobial characteristics has been employed for the purpose of managing the harmful microflora in poultry. Plantaricin, derived from *L. plantarum* F1, has been suggested as a potential substitute for antibiotics in the treatment of colibacillosis in grill chickens (Ogunbanwo et al. 2004). New bacteriocins with potential application in poultry have been discovered within the microbiota of the gastrointestinal tract of domestic broiler chickens. The bacteria *P. polymyxa* NRRL B-30509 was obtained from domestic Russian grill chickens and found to produce the bacteriocin ribosomally synthesized AMPs “paenicidin A”. This bacteriocin has demonstrated antimicrobial activity specifically against *C. jejuni* (Stern et al. 2005).

Pediocin A was given in the feed of broilers infected with *C. perfringens* type A, which is known to produce the Net B toxin and is associated with the development of necrotic enteritis. The administration of the treatment resulted in a notable enhancement in the development and performance of the avian subjects. Nevertheless, the bacterial load did not exhibit a decrease (Keyburn et al. 2008). The application of microencapsulation techniques to bacteriocin can potentially mitigate the degradation of bacteriocin caused by digestive processes in broilers (Grilli et al. 2009). The application of the divercin AS7 along with nisin as dietary additive for broilers has demonstrated a positive impact on the enhancement of body weight gain (Jozefiak et al. 2011). Nisin exhibits antimicrobial properties against microbiota that are associated with reduced productivity in grill chickens, akin to the ionophore monensin. The addition of nisin had a beneficial impact on the gut microbiota by decreasing the presence of potentially harmful bacterial populations in the jejunum and ceca (Kierończyk et al. 2020). These results underscore the significance of bacteriocins as a highly effective antibacterial alternative for combating potentially pathogenic agents in animals, thereby enhancing their growth performance.

3. CONCLUSION

Antibiotic-resistant zoonotic diseases threaten human and animal health. It reduces antibiotic efficacy, prolongs sickness, increases healthcare expenditures, and potentially kills patients. It also hinders our capacity to fight infectious illnesses, undermining modern medicine and public health. Promoting appropriate antibiotic use in people and animals is vital. Antibiotics should be used sparingly in veterinary medicine. Novel therapeutics like phage therapy, bacteriocins, or nano-particles are intriguing alternatives. These focused methods may solve certain problems without causing general resistance.

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Antimicrobial Resistance in Syphilis: An Emerging Public Health Crisis

16

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ABSTRACT

Syphilis, caused by the spirochete bacterium Treponema pallidum, has posed a global public health challenge since the fifteenth century. This chapter provides a comprehensive exploration of the multifaceted nature of syphilis, its historical background, and the emerging threat of antimicrobial resistance (AMR) in its management. A mother infected with syphilis during pregnancy can transmit the disease to her unborn child, and this sexually transmitted infection sy can progress through various stages, each presenting different symptoms and characteristics. The initial focus is on the escalating concern of resistance in syphilis, as it compromises the effectiveness of conventional treatment strategies, particularly the shortage of Benzathine penicillin G reported in some regions. Certain populations, including pregnant women, individuals with HIV, and men who engage in intercourse with males, are particularly at risk due to the increasing prevalence of bacteria that are resistant to penicillin and its replacements, tetracycline and macrolides. To overcome syphilis resistance, a combination of therapies is one of the most effective strategies. While a single antibiotic is frequently used for the duration of syphilis therapy, mixing several antimicrobial medications can improve treatment efficacy and reduce the likelihood that resistance will develop. The synergistic effects of antibiotic combinations such azithromycin, doxycycline, and benzathine penicillin have been researched. This chapter aims to raise awareness about the urgent public health crisis posed by AMR in syphilis. Examining the causes, background, along with mechanisms of syphilis highlights the need for a worldwide strategy to tackle the growing issue of antibiotic resistance. In order to create effective strategies to stop the dissemination of resistant strains, give appropriate care, and lessen the public health burden of syphilis, it is essential to understand the many aspects of antimicrobial resistance (AMR) in syphilis.

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1. INTRODUCTION

Syphilis is a sexually transmitted infection caused by the spirochete bacterium Treponema pallidum. (Carbone et al. 2016) It has a lengthy history that dates to the fifteenth century and continues to be a global public health issue. A mother who has the virus during pregnancy can pass it on to her unborn child through sexual intercourse. There are different phases of syphilis, and if ignored, it can have a serious negative impact on health (Wasserman et al. 2007). Treponema are members of the Spirochaetaceae family. Many Treponema species are part of the microbiota of animals and humans. Spirochaetes are opportunistic bacteria that are associated with human gingivitis and chronic periodontitis. The most important species of Treponema is the Treponema pallidum, which is a causative agent of syphilis. Syphilis is a chronic, multi-stage infectious disease (Stamm 2015). Syphilis progresses through different stages; each has its own Symptoms and characteristics.

In Primary Syphilis, single painless lesions occur usually but can be multiple and painful later on (Ward et al. 2007). The chancre appears 3 Weeks after infection and lasts for 3 to 8 weeks and heals. regardless of whether a person receives treatment (Pagani et al. 2021). After primary syphilis, Secondary syphilis occurs between 4 to 8 weeks. Symptoms include rashes, mucosal lesions, and sores in the mouth, vagina and anus. Rashes are mainly papular and rarely papules ulcerate and can be associated with mucosal ulceration. Condylomata lata (wart like lesions) may develop in the moist areas like the mouth and groin region. Symptoms may also include fever malaise, headache, swollen lymph and neurological problems (Ward et al. 2007). Without treatment secondary syphilis becomes latent and non-infectious, there are no sign and Symptoms show and deteriorate changes may occur in early latent disease (the two years of latency) but less in late latent disease (Nyatsanza and Tipple 2016).

T. ertiary syphilis can appear typically 15 to 30 years after onset of infection. It is rare, and can affect any tissue (granulomatous), cardiovascular system. nervous system and mainly skin and boner affected. (Nyatsanza and Tipple 2016). Antimicrobial resistance (AMR) is the term used to describe a microorganism's capacity to resist the effects of antimicrobial medications, which can result in treatment failures and the spread of resistant strains. Antimicrobial resistance in syphilis has become a growing source of worry in recent years. This resistance can limit the efficacy of conventional treatment plans and make illness management and control more difficult. Because vaccine is not available for syphilis. So antibiotics is the main component for control. Benzathine penicillin G is used for the early syphilis. Shortage reported in this antibiotic in France so there is a need alternative treatment (Sanchez et al. 2020). Syphilis has become a public health problem in vulnerable population due to mutation in antibiotic resistance. In Vulnerable population (such as men who have sex with men, HIV patients and pregnant women) antimicrobial resistance is emerging in macrolides and tetracycline. These medicines use as alternate treatment for syphilis in those patients allergic to penicillin. So, there is a need to focus on macrolide and tetracycline resistance (Orbe-Orihuela et al. 2022b). This chapter's goal is to give readers a thorough grasp of the rising public health concern known as antibiotic resistance in syphilis. It aims to investigate the causes, spread, and historical context of syphilis, as well as the mechanisms. The overall goal of this chapter is to raise awareness of the danger that syphilis poses due to antibiotic resistance and to stress the necessity of tackling this problem globally. Understanding the different facets of antimicrobial resistance in syphilis can help us create efficient plans to stop the spread of resistant strains, provide proper care, and ultimately lessen the impact of syphilis on the general public's health.

2. UNDERSTANDING SYPHILIS

2.1. HISTORICAL BACKGROUND OF SYPHILIS

Treponema pallidum is the bacteria that causes the sexually transmitted disease syphilis. Its history dates back centuries, with the earliest recorded outbreaks occuring in Europe in the late 15th century. There were

many possibilities in the time before 15th century where syphilis would have been discovered but they were not fully supportive in account of proof (Rothschild 2005). Girolamo Frasastoro came up with the term "syphilis sive morbus gallicus" in 1530. The phrase "great pox" emerged as a way to distinguish the illness from smallpox due to the associated rash (pox). Despite the two centuries of usage for the phrase, syphilis grew to be a cultural disgrace, leading to a number of attributions. The Germans and the English referred to it as "the French pox", the Russians and the Poles called it "the Polish sickness", the French called it "the Neapolitan sickness", some Flemish, Dutch, Portuguese, along with North Africans called it "the Spanish sickness" or as "the Castilian sickness", and the Japanese called it "the Canton rash" or as "the Chinese ulcer". In India, Muslims and Hindus both held the other accountable for their actions. In the end, the Europeans were held entirely to blame (Hayden 2003). The phrase "lues venera" (or else "venereal pest") was originally used in a book on the ailment authored in the sixteenth century by the Parisian educator called Jean Fernelius, who work and interests focused primarily in treating the condition using mercury.

2.2. SYPHILIS AND WAR

The French army's conquest of Naples was commonly blamed for the spread of syphilis throughout Europe. Less well-liked hypotheses have, however, been evolved since then (Winspeare 2016). The Edict of Expulsion of the Jews, issued in 1492 by Ferdinand de Aragon and Isabel of Castilla, ordered the expulsion from Spain and the remainder of its lands of all people of Hebrew descent who refused to convert to Catholicism. About 200.000 Jews have currently departed the nation for Northern Africa and Southern Europe. A portion of them briefly settled at Rome's walls while traveling there; they were barred entry, and an outbreak killed 30.000 people in the new Diaspora. After all efforts, the sickness that ultimately came to be known as syphilis makes its way into the ancient city of Rome. As a result, some historians of the time claimed that Jews were to blame for the spread of syphilis in Europe because the illness was already present in Italy before to the French invasion of Naples in 1495(Tampa et al. 2014).

2.3. PATHOGENESIS AND STAGES OF SYPHILIS

2.3.1. SPECIES OF SYPHILIS

The chronic infectious condition known as syphilis is caused via the spirochaete *Treponema pallidum* strains pallidum. The order Spirochaetales, which consists of spiral-shaped pathogenic bacteria, includes the genus *Treponema*. The genera Leptospira and Borrelia are further members of this group. In addition to *T. Pallidum* subspecies pallidum, which causes venereal syphilis, other pathogenic treponemes that affect humans involve *T. Pallidum* subspecies pertenue, which causes yaws, *T. Pallidum* subspecies endemicum, which causes endemic syphilis, and *T. carateum*, which causes pinta (Jaiswal et al. 2020). The so-called 'endemic' treponemes are morphologically identical to each other as well as to *T. Pallidum* subspecies pallidum, have strong antigenic relationships, and exhibit a high degree of DNA similarity. However, they differ in terms of geographic distribution, host tissue specialization, and animal infectivity.(Antal et al. 2002).

2.4. AGENT OF SYPHILIS

Syphilis' causal agent, *Treponema pallidum*, was discovered in 1905. Since then, other pathogenic isolates have been discovered, including the *Truffi*, *Gand, Gent,* and *Ami* strains as well as the Nichols pathogenic isolate. The Nichols pathogenic strain is the one that has received the greatest research attention. It was

first isolated from a person with secondary syphilis' cerebrospinal fluid in 1912, and it has since been passed through rabbit testes. It has remained harmful for humans even after 65 years. No pathogenic strain of *T. Pallidum* has been in vitro grown to date, not even this one.(Miao and Fieldsteel 1978). The pathogenesis and stages of syphilis is enlisted in Table 1.

Table 1: Pathogenesis and stages of syphilis**.**

Stages of Syphilis	Symptoms	Time period	Diagnosis	References
Primary	A chancre, painless sore, appears at the infection site, most frequently on or close to the mouth, genitalia, and the abdomen	Usually, 3 to 6 weeks	Delayed diagnosis or remains undetected	(Andersen 1978)
Secondary	One of the most typical symptoms is a non-itchy rash which appears on the soles of the feet, palms of hands, and the backs of the human body. Fever, exhaustion, a sore throat, pains in the muscles, enlarged lymph nodes, and hair loss are further symptoms.	4 to 10 weeks	Delayed Diagnosis	(CHAPEL 1980; Anderson et al. 1989)
Latent	Usually, no symptoms	Months to years	Serological tests	(Stamm 2016c).
Tertiary	Tertiary syphilis symptoms might include dementia, inability to coordinate an individual's movements, paralysis, numbness, and blindness	Several years	Serological Tests	(Singh and Romanowski 1999).

2.5. PATHOGENESIS

T. Pallidum is a helically formed, micro-aerophilic bacterium with a length of 6–20 m long and 0.1-0.18 m in diameter. It is made up of a central protoplasmic cylinder that is encircled by a peptidoglycan layer, the outer membrane, and the cell membrane (Wang 2015). Motility is provided by 2 to 3 flagella that protrude from both ends of the organism. The outer membrane of *T. Pallidum* lacks lipopolysaccharide but does contain a small amount of surface-exposed transmembrane proteins. *T. Pallidum* has been classified as a stealth pathogen due to the absence of immunological targets on the outer membrane. The uncommon membrane proteins of *T. Pallidum* have the potential to be virulence determinants, and at least one of them has been identified as a porin despite the fact that nothing is known about them.(Blanco et al. 1997).

A repeat gene Tpr gene has been identified in recent studies. It has been demonstrated that opsonic antibody binds to Tpr K and that the Tpr proteins are highly immunogenic in rabbits. Activated macrophages can phagocytize toxic treponemes, eliminating them from circulation. In a rabbit model, Tpr K varies in seven different places. Only homologous protection is offered by antibodies for these variable areas; they do not offer protection against heterologous strains(Leader Hevner et al. 2003). These TprK V region genes thirty vary as they are passed down over successive generations. Antigenic variation via gene conversion during illness has been believed as a further method using which the microbe evades the human immune response, allowing extended infection and survival in the midst of a robust host response. Similar processes have been identified in the syphilis-causing spirochetes belonging to the genus Borrelia (Morgan et al. 2003).

Because *T. Pallidum* cannot be grown for extended periods of time on artificial media, research into the pathophysiology of syphilis has been hampered. *T. Pallidum* can be passaged for a finite number of generations with a generation time of 30-33 h using monolayers of rabbit epithelial cells at 33-35 °C, but at this time, neither the quantity of organisms nor the flexibility in their manipulation make these techniques useful for studying *T. Pallidum*-host interactions. The most often used technique for producing

organisms for study is in vivo propagation by inoculation of rabbit testis, which produces significant numbers of organisms (Edmondson et al. 2018).

2.6. STAGES OF SYPHILIS

2.6.1. PRIMARY STAGE

The early stages of syphilis often appear three weeks after bacterial encounter. A chancre, painless sore, appears at the infection site, most frequently on or close to the mouth, genitalia, and the abdomen. The chancre can persist for three to six weeks and frequently remains undetected, delaying diagnosis(Singh and Romanowski 1999; Brown and Frank 2003; Stamm 2016c).

2.6.2. SECONDARY STAGE

Syphilis normally progresses to the secondary stage four to ten weeks after the chancre first appears. The illness is now spreading throughout the body. One of the most typical symptoms is a non-itchy rash which appears on the soles of the feet, palms of hands, and the backs of the human body. Fever, exhaustion, a sore throat, pains in the muscles, enlarged lymph nodes, and hair loss are further symptoms. These symptoms may go away on their own in a few weeks or they may come and go even in the absence of medical care (Mattei et al. 2012).

2.6.3. LATENT STAGE

When syphilis is in its latent stage, there are no outward symptoms, but the infection can still be detected using a variety of serological tests, showing that the associated microbes are still present within the body. Years may pass during the latent period while the patient is not aware of their illness. Without treatment, the illness can advance to a later stage, or some people might never have any further symptoms (Essig and Longbottom 2015).

2.6.4. TERTIARY STAGE

Syphilis can advance to the tertiary stage if it is not treated, which can result in serious consequences that could be life-threatening. Typically, the appearance a tertiary stage follows a latent period that may last for few to many years. It damages several organs, causing degenerative lesions in the blood vessels, heart, brain, bones, joints, and other tissues. Tertiary syphilis symptoms might include dementia, inability to coordinate an individual's movements, paralysis, numbness, and blindness. The sexually transmitted spread of syphilis is extremely uncommon since there are so few treponemes present during tertiary syphilis. This stage can lead to neurological abnormalities, cardiovascular complications, blindness, deafness, and mental disorders (Stamm 2016a).

2.7. GUMMAS

The skin, bones, liver, or any other organ, such as the stomach and eyes, may develop little lumps or tumors known as gummas. The soft palate and the bones of the nose frequently develop gummas. The legs, trunk, face, and scalp are other frequent locations (Heston and Arnold 2018).

2.8. NEUROLOGICAL ABNORMALITIES

Syphilis can result in a variety of nervous system issues, including sudden, intense pains. People may vomit as a result of these painful spasms, which commonly damage the stomach among other organs. Additionally, you can get sudden, lightning-like sensations in your bladder, rectum, and throat. Syphilis may also make it more difficult for you to detect and respond to temperature changes. Stroke, meningitis (inflammation of the brain), visual problems, and blindness are all possible. Nervous system issues can also result in incontinence and impotence in men(Tramont 1987; Rufli 1989).

2.9. PITUITARY GLAND COMPLICATIONS

Rarely, syphilis can lead to hypopituitarism, a condition that occurs when the pituitary gland produces low amount of hormones than usual. In addition to other problems, this can result in accelerated aging in adults as well as dwarfism in children (Pekic and Popovic 2017).

2.10. CHILDBIRTH AND PREGNANCY COMPLICATIONS

If you develop syphilis while pregnant, your unborn child might get the disease. Infants who are infected are more likely to be born or to develop a variety of abnormalities. Infants who get syphilis from their mothers have a high risk of dying during or soon after delivery, and syphilis during pregnancy dramatically increases the chance of miscarriage or stillbirth (Benedetti et al. 2019; Manolescu et al. 2019).

2.11. GASTRIC COMPLICATIONS

This fairly typical condition, which most frequently affects people in their early 20s or 40s, has an impact on the stomach. It may result in discomfort, hunger loss, motion sickness, nausea, and weight loss (Choi et al. 2006).

2.12. CONGENITAL SYPHILIS

Congenital syphilis is a disorder that develops when a syphilis-infected pregnant mother transmits the virus to her fetus. Both the mother and the child's health and wellbeing are seriously at stake. If neglected, this treatable illness may result in fetal death, early delivery, or death soon after birth. Rash is one sign of congenital syphilis that neonates may experience other include fever, enlarged liver and spleen, anemia, and bone malformations (Cooper and Sánchez 2018; Rowe et al. 2018).

2.13. CLINICAL MANIFESTATIONS

There are a wide range of syphilis clinical manifestations and symptoms. Ocular, otic, or neurosyphilis syphilis can develop at any phase of the illness(Dourmishev and Dourmishev 2005; Forrestel et al. 2020). Syphilis' symptoms have been known for hundreds of years. In his description of the "French sickness" in 1514, Juan de Vigo mentioned genital pustule. The original chancre was accompanied by a reddish rash that was later recorded by several other people. This rash's apparent similarity to smallpox is what gave rise to the word "pox." Protean late manifestations affecting all organ systems are being observed, introducing syphilis as the "great imitator." A strong index of suspicion is still necessary for diagnosis (Singh and Romanowski 1999).

3. ANTIMICROBIAL RESISTANCE

The capacity of bacteria to resist and thrive while being targeted by antimicrobial drugs is known as antimicrobial resistance (AMR) (Abushaheen et al. 2020).
A major concern globally is the phenomenon of antimicrobial resistance (AMR). AMR, also known as acquired resistance, is the capacity of bacteria to withstand the effects of antimicrobial drugs that were earlier successful in treating illnesses brought on by such pathogens. Microorganisms like bacteria become insensitive to certain antibiotic treatments after developing resistance to them(Holmes et al. 2016; Frost et al. 2019).

3.1. MECHANISM OF AMR

As there are different ways by which antimicrobials can act on microbes similarly there are several ways in which microbes can evade antimicrobial drugs and develop resistance against them by making them no longer effective (Moo et al. 2020a).

3.1.1. LOW UPTAKE OF DRUG

Many factors play functional role in lowering and delaying the uptake of drug by bacterial cell Biofilms are one of them as they limit the penetration of drug within the cell by interfering with it and also by acting as a barrier between cell membrane and drug. Like P. aeruginosa, other microbes can produce a permeability barrier to prevent drugs from penetrating their cell membrane(Zhou et al. 2015).

3.1.2. DRUG INACTIVATION

Resistance can be acquired by neutralizing the effect of drug by using various mechanism like production of enzymes, β-lactamases are enzymes that hydrolyses the Beta-Lactam ring in case of Beta-lactams (Zhou et al. 2015; Pulingam et al. 2022).

3.1.3. DRUG EFFLUX

These efflux pumps play roles in transportation of molecules or antimicrobial drugs in and out of the cell and are of different types depending upon their functions. Multiple drug efflux pumps are the one aiding in resistance as they can transport a large number types structurally and functionally different drugs in and out of the cell (Uddin et al. 2021).

3.1.4. CHEMICAL MODIFICATIONS

It may be one of the prescription drugs, which is achieved by bacterial development of various enzymes that may connect to the drug and stop it from adhering to the target site, as is done using chloramphenicol when acetylation is carried out(Uddin et al. 2021), Or it can be of the target site like modifications in the penicillin binding proteins to decrease its affinity for beta-Lactams as observed in MRSA (Pulingam et al. 2022).

3.2. FACTORS CONTRIBUTING TO ANTIMICROBIAL RESISTANCE IN SYPHILIS

It appears that *T. Pallidum* is resistant to macrolides like azithromycin, which is brought on by point mutations or methylation of the peptidyl transferase region between nucleotide positions 2058 and 2059

in domain V of the 23S ribosomal RNA gene. *T. Pallidum* Street Strain-14 was the first strain to incorporate the A2058G mutation(Stamm 2015; Beale et al. 2019).
This resistance is caused by a change in the 23S rRNA target site carried by the mutation A2058/9G. There is currently widespread macrolide resistance to treat syphilis, including in Australia, Canada, China, Europe, and the USA (Tien et al. 2020b).
Tetracycline resistance in *T. Pallidum* isolates has been linked to mutations in the 16S rRNA gene(Wu et al. 2014). Additionally, recent examination of the *T. Pallidum* genomes revealed amino acid alterations in penicillin-binding proteins, however the therapeutic significance of these alterations is not yet clear. This finding serves as a reminder of the need to look for mutations in pertinent genes and assess the prevalence of resistances in a given area (Liu et al. 2021).
Additionally, it is believed that three penicillin-associated protein genes (tp0500, tp0760, and tp0705) and the 23S rRNA and 16S rRNA genes for possible mutations linked to antibiotic resistance in the *T. Pallidum* isolates (Liu et al. 2021).

3.3. GLOBAL TRENDS AND EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE IN SYPHILIS

Antimicrobial resistance (AMR) is a global issue that compromises the ability to effectively treat a variety of infectious illnesses, including syphilis (Dadgostar 2019). *Treponema pallidum* is the bacteria that causes the sexually transmitted disease syphilis. Syphilis is a chronic illness, and the only recognized natural host of *T. Pallidum* is the human. Direct sexual contact with acute primary or secondary sores is necessary for the transmission of syphilis. The emergence of antimicrobial resistance in syphilis is a growing problem that demands attention worldwide(LaFond and Lukehart 2006) .
Monitoring antibiotic resistance in syphilis is essential for informing public health policies and directing efficient treatment strategies. This involves monitoring the incidence of AMR strains, detecting risk factors linked to the emergence of resistance, and evaluating the effectiveness of novel therapeutic approaches. For countries to fully understand global patterns and adopt preventive actions, cooperation and data exchange are critical(Klaucke et al. 1988; Crofts et al. 1994).
A combined approach is required to address the worldwide threat of AMR. This involves enhancing surveillance programmes, encouraging appropriate antibiotic use(Dadgostar, 2019) funding the discovery of novel antimicrobial medicines, and increasing public and professional knowledge. Syphilis must be treated as a top priority in terms of global health issues, and everyone needs access to appropriate treatment alternatives (Majumder et al. 2020b).

4. MECHANISMS OF ANTIMICROBIAL RESISTANCE IN SYPHILLIS

4.1. GENETIC MECHANISM

4.1.1. GENETIC MECHANISM OF AMR IN SYPHILIS

Antimicrobial resistance (AMR) is the term used to describe a microorganism's capacity to endure and proliferate in spite of being exposed to antibiotics. A sexually transmitted infection called syphilis is brought on by the bacterium *Treponema pallidum*. The genetic mechanisms underlying AMR in syphilis are poorly known, despite indications of rising resistance to several antibiotics used to treat syphilis, such as azithromycin (Liu et al. 2021).
Nevertheless, there are a few potential mechanisms that could contribute to amr in syphilis:

4.1.2. TARGET GENE MUTATIONS

Bacteria can acquire resistance by mutations in the genes targeted by antimicrobial medications. For instance, if a particular antibiotic works by attaching to a specific protein involved in bacterial replication or metabolism, mutations in the gene producing that protein may block or diminish the drug's ability to bind to that protein, rendering the medicine useless (Giedraitienė et al. 2011).

4.1.3. EFFLUX PUMPS

Efflux pumps are proteins that actively pump medicines out of the bacterial cell. They can be found in bacteria. By doing this, they can lessen the drug's effectiveness by lowering its concentration inside the cell. AMR may be facilitated if syphilis bacteria produce efflux pumps that can expel antibiotics (Giedraitienė et al. 2011).

4.1.4. ACQUISITION OF RESISTANCE GENES

Through horizontal gene transfer, bacteria can obtain resistance genes from other bacteria. Processes like conjugation, transformation, or transduction may be used to achieve this. It is possible to develop resistance to syphilis if the bacteria that cause it pick up resistance genes from other bacteria that are already resistant to particular antibiotics (Roe and Pillai 2003).
It's important to keep in mind that the precise genetic processes causing AMR in syphilis haven't been well investigated or defined yet. In order to create efficient preventative and treatment plans for syphilis, further study is required to better understand the genetic basis of antibiotic resistance in this disease.

4.2. MOLECULAR MECHANISM OF RESISTANCE OF AMR IN SYPHILIS

A sexually transmitted infection called syphilis is spread on by the bacterium *Treponema pallidum*. Similar to other infectious diseases, antibiotic resistance in syphilis develops when the bacteria mutate and build defenses against antibiotics. Penicillin or its derivatives, the recommended treatment for syphilis, were quite effective as shown in Fig. 2 (Stamm , 2010).
However, it is crucial to remember that antibiotic resistance may develop in any bacterial illness, including syphilis. If resistance developed, it would probably be the result of genetic mutations or the acquisition of resistance genes through horizontal gene transfer. Other microorganisms that have gained antibiotic resistance frequently exhibit these methods (Baker et al. 2018).
Depending on the particular antibiotic and bacterium involved, different molecular pathways might cause bacteria to become resistant to antibiotics (Roe and Pillai, 2003). A broad review of some typical ways by which bacteria acquire antibiotic resistance are as follows:

4.2.1. EFFLUX PUMPS

The concentration and efficiency of antibiotics can be decreased by bacteria producing efflux pumps that aggressively pump them out of the bacterial cell (Abebe et al. 2016).

4.2.2. TARGET SITE MODIFICATIONS

Bacteria can change the enzymes or proteins that antibiotics target to make them less vulnerable to the medications (Blair et al. 2015).

4.2.3. ENZYMATIC INACTIVATION

Antibiotics can lose their efficacy due to the production of enzymes by bacteria.
Reduced permeability: The effectiveness of antibiotics can be decreased by bacteria altering their cell membranes to prevent drug entrance (Abebe et al. 2016).

4.2.4. ANTIBIOTIC MODIFICATION

Antibiotics can lose their effectiveness when bacteria develop enzymes that change them chemically (Fig. 1) (Gupta and Birdand 2017).
It is crucial to underline that penicillin or its derivatives continue to be the suggested treatment for syphilis and that antibiotic resistance is not currently a serious concern. To stop the emergence and spread of antibiotic-resistant strains of any bacterial infection, however, it is essential to maintain surveillance and use antibiotics responsibly. For the most recent information and suitable treatment choices, it is essential to speak with a healthcare provider if you suspect you have syphilis or are worried about antibiotic resistance (Liu et al. 2021).

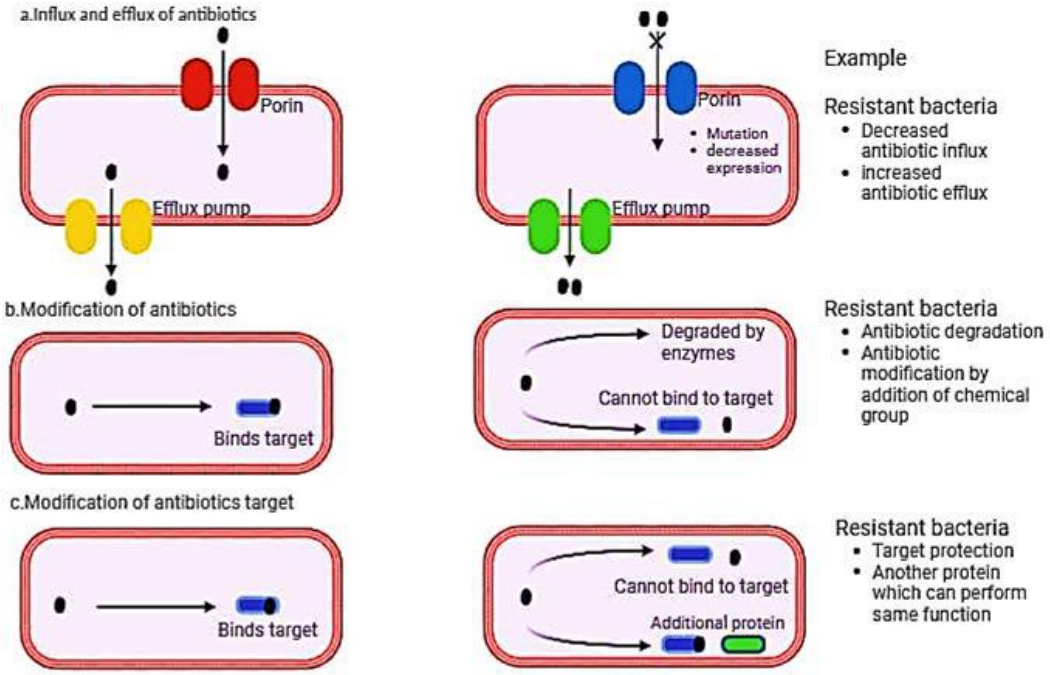

Fig. 1: Mechanism of AMR in bacteria.

4.3. ACQUISITION OF ANTIMICROBIAL RESISTANCE (AMR)

4.3.1. SPONTANEOUS MUTATION

Resistance can be acquired by bacteria, including *Treponema pallidum*, by unintentional alterations in their DNA. These mutations may naturally take place during DNA replication and may result in alterations to specific bacterial genes that are susceptible to antibiotic action. As a result, the bacteria develop an immunity to the antibiotics' actions (Che et al. 2021).

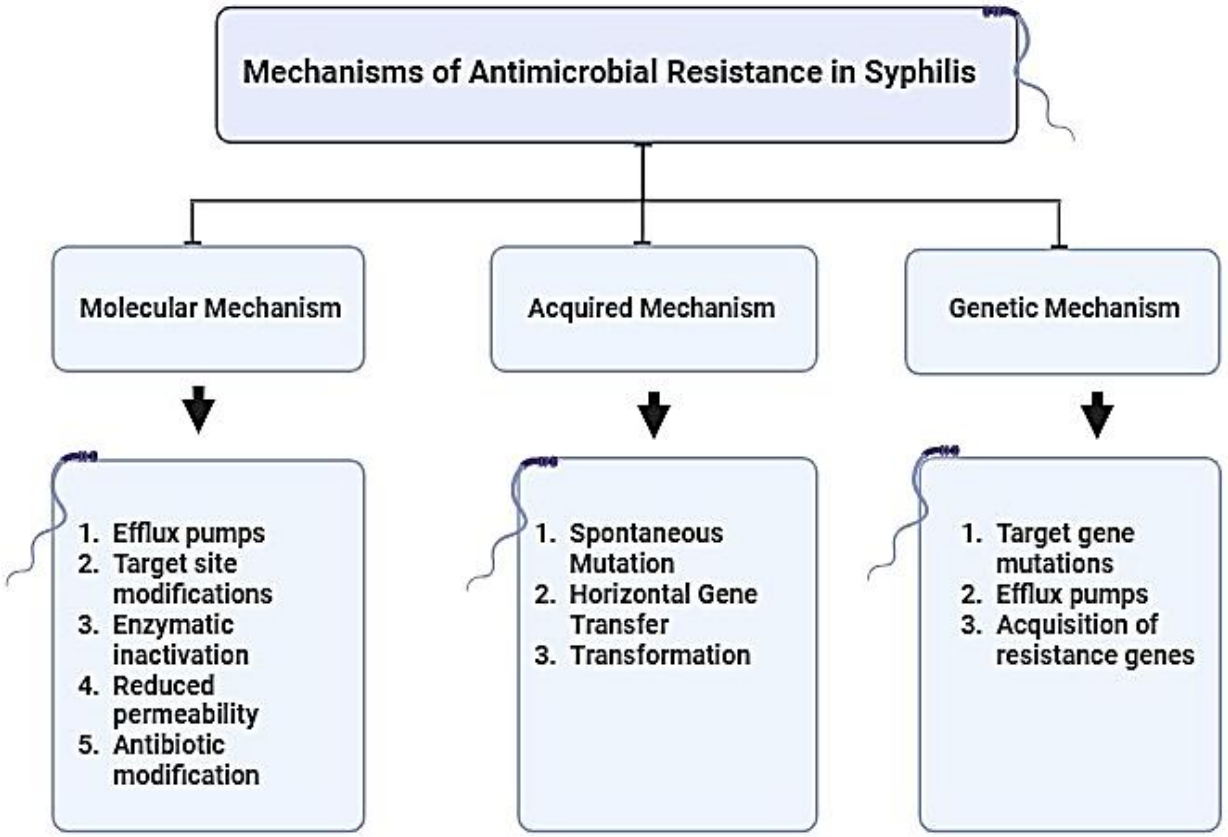

Fig. 2: Mechanism of AMR Syphilis.

4.3.2. HORIZONTAL GENE TRANSFER

This is a crucial way by which bacteria acquire AMR genes. Genetic material is exchanged between various bacteria, even those of different species. The following are the top three horizontal gene transfer mechanisms: a. Conjugation: During conjugation, two bacteria physically unite by means of a pilus, which resembles a bridge. Plasmids (tiny, circular DNA molecules) encoding AMR genes are transferred from the donor bacteria to the receiving bacterium as shown in Fig. 3. (Baker et al. 2018).

4.3.3. TRANSFORMATION

Bacteria transform by consuming free DNA pieces from their surroundings, which may contain AMR genes. The resistance is conferred via the integrated DNA, which is incorporated into the recipient bacterium's genome (Che et al. 2021).

4.3.4. TRANSDUCTION

Through the use of a bacteriophage, a bacterial virus, genetic material is transferred between bacteria during transduction. It is possible for the bacteriophage to unintentionally package and transfer bacterial DNA, including AMR genes, to another bacterium throughout the course of an infection (Jamil et al. 2023).

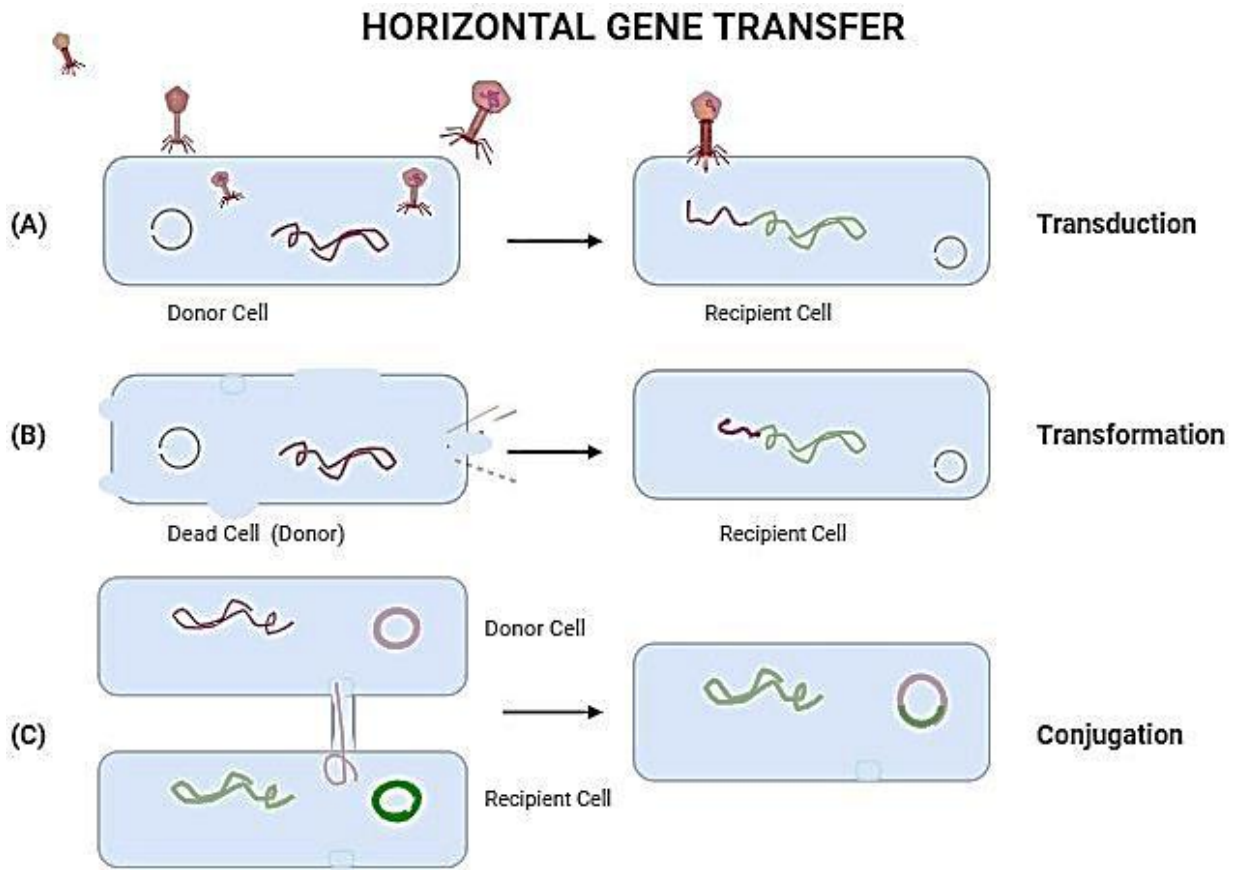

Fig. 3: Horizontal gene transfer.

4.4. EVOLUTIONARY ASPECTS OF ANTIMICROBIAL RESISTANCE IN SYPHILIS

Antimicrobial resistance (AMR) in syphilis has become a troubling problem over time. The bacteria *Treponema pallidum* is the source of the sexually transmitted disease syphilis. Penicillin has been the mainstay of syphilis treatment for many years and is still quite successful today. Antibiotic-resistant *T. Pallidum* strains have, nevertheless, started to appear in recent years (Roe and Pillai 2003). Here are some key evolutionary aspects of antimicrobial resistance in syphilis:

4.4.1. NATURAL SELECTION

Natural selection is what causes the syphilis antimicrobial resistance to emerge. Antibiotics used to treat syphilis put the bacteria under selective pressure, favoring the survival and reproduction of those with genetic mutations or other resistance-granting processes. These resistant strains increase in population prevalence over time (Santos-Lopez et al. 2021).

4.4.2. GENETIC MUTATIONS

An important factor in the emergence of antibiotic resistance is genetic mutation. In the case of syphilis, mutations can develop in genes involved in antibiotic uptake, efflux, or modification, as well as in genes responsible for the binding of penicillin to the target location within the bacteria. These alterations may

change the bacterial target's structure or function, making it less vulnerable to the antibiotic's effects (Barbosa and Levy 2000).

4.4.3. HORIZONTAL GENE TRANSFER

Antimicrobial resistance in syphilis can be acquired through horizontal gene transfer in addition to genetic alterations. The transfer of resistance genes between diverse bacteria, including unrelated species, is a part of this process. Plasmid exchange, transposon-mediated transfer, and bacteriophage-mediated transfer are a few examples of methods that might result in horizontal gene transfer. The receiving bacteria can quickly develop resistance features thanks to this transfer of resistance genes (Baker et al. 2018).

4.4.4. ANTIBIOTIC MISUSE AND OVERUSE

Antibiotic misuse and overuse are major factors in the emergence and dissemination of antimicrobial resistance in syphilis. Self-medication, incorrect antibiotic use, and inadequate or incomplete treatment plans can all contribute to the selection and spread of resistant bacteria. Antibiotic usage is common in many industries, including medicine, agriculture, and animal husbandry, which accelerates the creation and spread of resistance (Moo et al. 2020b).

4.4.5. GLOBAL DISSEMINATION

Antimicrobial resistance to syphilis is not isolated to any particular area. The international movement of people, including migration and travel, promotes the cross-border transmission of resistant strains. These resistant strains can further evolve and adapt to local conditions once they are established in a new population, which adds to the continuous difficulty in properly treating syphilis (Stamm 2010).

4.5. RATIONAL USE OF ANTIBIOTICS

Rational use of drugs requires that patients must be given medication appropriately to their clinical needs, at the lowest cost to them and their community, for an adequate period and in doses that meet their particular needs (WHO 1988). In short,

4.5.1. PROPER INDICATION

(antibiotics only prescribed for serious infections)

4.5.2. PROPER DRUG

(for disease, bacterium, patient conditions)

4.5.3. PROPER DOSAGE

(patient characteristics- weight, renal function, interactions)

4.5.4. COST EFFECTIVE

(should not be expensive)

4.5.5. PROPER TIME

(not too late, not too early)

4.5.6. PROPER DURATION

(for the disease to be cured)
Presently, there is no vaccine available to prevent syphilis. Antibiotics are among the most consistently used drugs of choice for treatment of syphilis globally. Doxycycline and macrolide were used as alternative therapies. At the present time massive use of Macrolides causes its resistance in syphilis patients in many geographical regions including Australia, Canda, China and Europe. Antimicrobial resistance poses severe threats to human healthcare costs and dramatically increasing resistance causes mortality as well as morbidity (Tien et al. 2020a).
Irrational use of antibiotics are main drivers in the development of drug resistance among syphilis patients. Antimicrobial resistance causes the antibiotics to become less effective, as well as cause adverse health effects and it becomes hard to treat patient with serious infectious diseases.
In order to address the growing issue of antimicrobial resistance in syphilis, it is essential to encourage appropriate antibiotic use, create novel treatment options, improve monitoring systems to track patterns of resistance, and fund studies to comprehend the genetic mechanisms underlying resistance. In addition, public health initiatives including thorough sexual education, improved access to screening and care, and the application of combination therapy can lessen the effects of antibiotic resistance in syphilis (Barbosa and Levy 2000).

5. DIAGNOSTIC CHALLENGES IN ANTIMICROBIAL RESISTANCE

Syphilis can be treated and cured with antibiotics, but if left untreated, it can cause serious health problems, such as damage to the heart, brain, and other organs. It is important to practice safe sex and get tested for syphilis regularly to prevent the spread of the disease. The text is talking about a part of the brain called the pallidum. In the late 1990s, many people believed syphilis was no longer a concern. Many years later, this hidden disease became a problem for public health again. It affected certain groups of people more, like men who have sex with men, people living with HIV, female sex workers, male sex workers, people in prison, and pregnant women (Workowski and Bolan 2015a),
In 2017, the CDC said that 15-20% of individuals in the United States have a certain problem or condition. In the past, they had a syphilis infection, and from 2013 to 2017, there was a 76% rise in infection cases. According to the Health Data Organization, about 49. 7% of syphilis cases worldwide have been estimated to be prevalent. For women, about 18. 7% of them have this condition. And for men, about 31% of them have it. It seems that there are a lot of men getting sick again worldwide. The World Health Organization (WHO) says that around 7 million people got syphilis for the first time in 2020.
Even though syphilis is required to be reported in every country, it becomes a bigger issue when it doesn't show any symptoms. Many people with acquired syphilis, around 43%, do not show any signs of the disease. Because of these things, the illness could be wrongly identified by doctors. Many countries, like Mexico, do not have a lot of information or reports. We really need proof that there are more cases happening, and we have to be extra careful because this old enemy is affecting people who are already in a tough situation (Dombrowski et al. 2015; Mabey et al. 2006).

5.1. CURRENT DIAGNOSTIC METHODS FOR SYPHILIS

5.1.1. SEROLOGICAL TESTS

Serological tests are the main way to diagnose syphilis. They work by finding specific antibodies that the immune system makes to fight T, which causes syphilis. Pallidum is a difficult word to simplify, but it refers to a part of the brain. These tests can be put into two main groups: tests that are not for syphilis (Non-treponemal Tests) and tests that are for syphilis (Treponemal tests) (Dombrowski et al. 2015).

5.1.2. NON-TREPONEMAL TESTS

They find antibodies that attack cardiolipin, which is a part of cells that are harmed. The two most common tests used for certain diseases are the VDRL test and the RPR test. These tests can sometimes be sensitive but might give incorrect positive results, especially in patients who have other infections or autoimmune disorders (Workowski et al. 2021).

5.1.3. TREPONEMAL TESTS

Treponemal tests can find antibodies that specifically target the bacteria called Treponema. Pallidum antigens are substances found in the body that can be used to detect a specific disease. They are very precise, but not often used for first testing because they sometimes give incorrect positive results. Enzyme immunoassays (EIA) and fluorescent treponemal antibody absorption (FTA-ABS) are two common tests used in medical practice (Workowski et al. 2021; Ong et al. 2018).

5.2. MOLECULAR DIAGNOSTIC TESTS

Molecular diagnostic tests like PCR and NAATs can find T directly. Pallidum DNA or RNA means the genetic material of the pallidum organism. These tests are very good at finding if someone has syphilis, especially during the early stages when the body may not have produced enough antibodies to detect the disease. However, they cost a lot of money and may not be easily found in all healthcare places, (Orbe-Orihuela et al. 2022a).

5.3. POINT-OF-CARE TESTS (POCTS)

Point-of-care tests (POCTs) are fast diagnostic tests that can be done at the bedside or in the field, giving quick results. Some tests for syphilis can detect specific antibodies. Although convenient, these tests may not be as good at detecting things as tests done in a laboratory (Organization 2016).

5.4. DARK-FIELD MICROSCOPY

Dark-field microscopy is a way to look at samples under a special microscope that makes them easier to see. It's often used to examine things like genital sores or wounds. This technique helps us see T more easily. Pallidum motility means the movement of a certain type of bacteria called pallidum. It can help doctors make a quick guess about a person's illness. However, not everyone may have the necessary skills, and it may not be easily found (Ong et al. 2018).

5.5. SEROLOGICAL ALGORITHM

To make sure syphilis is diagnosed correctly, doctors often use a serological algorithm. This algorithm takes into account the strengths and limitations of different tests to improve the accuracy of the diagnosis. This method uses various tests in a certain order. It starts with one kind of test and if it shows positive results, another kind of test is used to confirm it (Janier et al. 2014).

5.6. POTENTIAL ISSUES IN DETECTING RESISTANCE OF SYPHILIS

Recognizing resistance to anti-microbials in syphilis is basic for viable treatment and administration of the malady. In any case, a few potential issues and challenges may emerge within the handle of distinguishing resistance in syphilis cases. This chapter points to supply a point by point diagram of these potential issues, which can complicate resistance location endeavors and affect persistent care (Wu et al. 2012).

5.6.1. LOW AWARENESS AND TESTING

One of the main problems in finding resistance in syphilis is that healthcare providers are not aware that resistance is possible. Because penicillin has been effective in treating syphilis in the past, doctors may not always think about resistance as the reason why treatment is not working. This means that sometimes, resistance testing that can help detect things early is not done, causing missed chances to find problems sooner (Organization, 2016). (Siedner et al. 2004).

5.6.2. OVERRELIANCE ON NON-TREPONEMAL TESTS

In regular medical practice, tests like the RPR and VDRL are commonly used to screen for syphilis. While these tests are sensitive, they require accuracy, which can lead to false-positive results. If serological tests don't show the desired results or if treatment doesn't work, healthcare providers might not think about resistance right away because the information from non-treponemal tests is limited (Organization 2016).

5.7. CHALLENGES IN REFINED TREPONEMA PALLIDUM

The bacteria called *Treponema pallidum* is the main cause of syphilis. This type of bacteria can actually be difficult and take a long time to treat. The bacteria have strict needs and are particular, making it hard to find usable samples for testing their vulnerability. So, it may not always be possible to do resistance testing based on culture, especially in places with limited resources (Workowski et al. 2021).

5.8. LACK OF STANDARDIZED METHODS FOR RESISTANCE TESTING

When there are no standard rules for testing resistance in syphilis, different labs and areas may use different methods for testing. This lack of sameness can make it hard to compare resistance data and make it difficult to fully understand resistance patterns (Workowski et al. 2021).

5.9. GENETIC HETEROGENEITY OF TREPONEMA PALLIDUM

Treponema pallidum is a type of germ that can have different genes, and some types might be more or less sensitive to antibiotics. The bacterium having different genetic characteristics can make it difficult to

accurately predict and detect resistance because different strains may have different ways of resisting (Janier et al. 2014).

5.10. SMALL SAMPLE SIZES

Syphilis resistance is not very common compared to other types of infections. So, when researchers study resistance, they sometimes only have a small number of people to study. This means they might not have enough data to accurately understand how common resistance is (Janier et al. 2014).

6. DIFFICULTY IN DISTINGUISHING REINFECTIONS FROM TREATMENT FAILURES

Differentiating between when a treatment isn't working and when someone gets infected again with a different type of T. "Pallidum can be difficult." People getting infected again can have the same symptoms as before. This might be mistaken as the treatment not working, leading to a wrong diagnosis (Wu et al. 2012).

7. LIMITED AVAILABILITY OF MOLECULAR TECHNIQUES

The use of advanced methods, like whole-genome sequencing, can give us important information about mutations that cause resistance. However, not all healthcare places have these methods available, especially in areas with limited resources (Dombrowski et al. 2015; Cantor et al. 2016).

8. ETHICAL CONSIDERATIONS

Resistance testing in syphilis involves taking samples from patients to see if the infection is resistant to treatment. However, it may not always be possible or acceptable to collect these samples from patients. The ethical concerns of collecting samples from private areas like genital sores may affect how resistance testing is done (Dombrowski et al. 2015; Cantor et al. 2016).

8.1. EMERGING DIAGNOSTIC TECHNOLOGIES FOR ANTIMICROBIAL RESISTANCE IN SYPHILIS

People are worried about syphilis becoming resistant to antibiotics. This has led to research to find better ways to diagnose it. It is very important to find resistance quickly and correctly, so we can give the right treatment and avoid treatment not working. This chapter talks about new tools that can help us diagnose if a person is resistant to antibiotics for syphilis. These tools can help us understand how resistance works and help us choose the right treatment for each person.

8.2. MOLECULAR-BASED RESISTANCE DETECTION

8.2.1. WHOLE-GENOME SEQUENCING (WGS)

Is a technique that helps scientists find out the full genetic code of the *Treponema pallidum* strain causing an infection. WGS can find genetic changes linked to resistance to medicines. This method gives detailed information about the pathogen's genetic make-up, which helps understand how it becomes resistant to treatments. WGS can help keep track of resistance trends and better understand the genetic differences of *T. Pallidum* strains are types of bacteria that are weak or pale in color (Cantor et al. 2016).

8.2.2. POLYMERASE CHAIN REACTION (PCR)-BASED ASSAYS

PCR tests are commonly used in labs for diagnosing things because they are really good at detecting specific things and are very accurate. We can make special tests called PCR assays that target specific genes in syphilis which are linked to resistance against antimicrobial drugs. For instance, changes in the 23S rRNA gene have been connected to macrolide resistance in *T. Pallidum* is a word that means a pale or light color. PCR tests can quickly find mutations that cause resistance to medicine. Doctors can use different samples like swabs from private parts or blood to do these tests (Grimes et al. 2012).

8.3. NEXT-GENERATION SEQUENCING (NGS)

New sequencing technologies have greatly changed genomic research and are now being used more often to study diseases like syphilis. NGS is a technology that can quickly read the DNA code of many samples at once. Next-generation sequencing (NGS) can give us in-depth information about the variety of genes in T. We study groups of pallidum bacteria and find mutations that make them resistant to treatment in patient samples (Tien et al. 2020a). (Cantor et al. 2016)

8.4. DIGITAL POLYMERASE CHAIN REACTION (DPCR)

This technology can be used to find uncommon strong types of T. The pallidum is not affected by most strains. Digital PCR (dPCR) is a better and more accurate type of PCR than the traditional one. It can be really useful in keeping track of how resistance is developing and spreading (Tien et al. 2020a).

8.5. MASS SPECTROMETRY-BASED TECHNIQUES

Scientists have used a method called mass spectrometry, specifically one called matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, to identify bacteria that cause diseases. This method has been effective. We can use these methods to find proteins or biomarkers that show resistance in *T. Pallidum* can be described in simpler terms as a part of the brain. Mass spectrometry techniques can quickly and accurately study bacterial proteins, and may be useful for identifying resistance (Orbe-Orihuela et al. 2022a).

8.6. MICROFLUIDIC-BASED ASSAYS

Microfluidic technologies are small platforms used for different diagnostic tests. These tests have benefits like needing less sample and chemicals, analyzing quickly, and possibly being used at the patient's location. Microfluidic-based tests can be created to find specific resistance markers or check how effective antibiotics are against infections using patient samples (Orbe-Orihuela et al. 2022a).

8.7. MACHINE LEARNING AND ARTIFICIAL INTELLIGENCE (AI)

Machine learning and AI can be used to study big sets of data collected from genetic sequencing or other diagnostic methods. These methods can help find connections between genes and resistance to syphilis, which can help predict and detect resistance earlier (Tucker et al. 2010).

8.8. METAGENOMIC SEQUENCING

Metagenomic sequencing is a technique used to study different types of germs found in a sample from a sick person. This method can find T. Study the genetic makeup of pallidum and identify resistance markers without growing bacteria. Metagenomic sequencing is a new method that shows potential for quickly and thoroughly detecting resistance (Grimes et al. 2012).

8.9. IMPACT OF ANTIMICROBIAL RESISTANCE

8.9.1. CLINICAL IMPLICATIONS OF RESISTANT SYPHILIS INFECTION

Syphilis is transmitted by sexual contact and is caused by the bacterium *Treponema pallidum*. It can have substantial clinical implications if left untreated.

8.10. PUBLIC HEALTH CONSEQUENCES

Resistant syphilis infection, also called as antibiotic-resistant syphilis, is a serious public health concern due to the following consequences

8.10.1. INCREASED TRANSMISSION

Syphilis strains that are resistant to treatment may spread more readily among people in a community. Syphilis outbreaks could result from this, especially in high-risk groups like those who engage in sex work and males who have intercourse with other men (Fernandes et al. 2015).

8.10.2. DELAYED TREATMENT AND DIAGNOSIS

Usually it is more difficult to detect the strains of resistant syphilis by using standard methods of testing as it lead to late diagnosis. Delayed identification and treatment can lengthen the course of the infection, raise the risk of consequences, and keep the transmission of disease to other people (Bowen et al. 2015).

8.10.3. LIMITED TREATMENT CHOICES

Resistant syphilis strains decrease the efficacy of conventional antibiotics for syphilis, like penicillin. This restricts treatment choices to make it more challenging to efficiently control and manage syphilis infection (Workowski and Bolan 2015)

8.11. HEALTH CARE IMPLICATIONS

8.11.1. RISK ASSESSMENT

Syphilis education must be a important part of STI interventions, along with the ability to obtain accurate sexual histories from patients. Concentrating on "sexual health" as opposed to "sexual illness" is vital due to the latter's negative meaning, which might make patients feel more relaxed discussing sex with their doctor (Nelson 2014).
Additionally, it is crucial to note that if the lesion is not shielded by the condom, condoms are less effective in preventing infections like syphilis (as well as chancroid and maybe herpes simplex virus and human papillomavirus) that are spread through sores.

In the clinical context, handouts with Internet and social media links to support in-office talks might be useful (Workowski and Bolan 2015a)

8.12. SCREENING

Numerous studies propose insufficient provider adherence to STI screening procedures; they include poor syphilis testing rates among HIV-positive people, missed chances to prevent congenital syphilis cases and also low syphilis testing for women who gave birth to stillborn children. Third party spenders should think about introducing reimbursement inducements that reward health systems for improved screening practices, and health care systems should work to implement quality control activities that track provider adherence to syphilis screening (Patel et al. 2017).

8.13. PUBLIC HEALTH'S ROLE

Successful illness therapies require the involvement of public health (containing municipal, state, and federal health departments) at all levels, from the individual to the public policy levels. Public health can persuade lawmakers to make rules that decrease obstacles for those who require medical preventative treatments and guarantee the financial stability of STI clinics.
Public health can also make sure that healthcare professionals are properly educated and trained about syphilis in terms of prevention, interpreting diagnostic tests, suggestions for therapy, and current disease incidence surveillance (Schmidt et al. 2019).

8.14. ADDITIONAL TREATMENT OPTIONS

Additional therapy choices are another area of biomedical research that is in requirement. *T. Pallidum* has not yet shown any signs of penicillin resistance, although syphilis therapy has not advanced in 75 years.
According to the CDC, penicillin is the solitary available therapy for pregnant women to prevent congenital syphilis and it gets worse by the presence of penicillin allergies
Ceftriaxone is a alternative for penicillin that the World Health Organization (WHO) classifies as having "very low quality evidence" in situations when it is either not available or desensitization is not a choice for pregnant women (Workowski and Bolan 2015a).

9. FUTURE PERSPECTIVES AND RESEARCH DIRECTIONS

9.1. PROMISING AREAS OF RESEARCH ON ANTIMICROBIAL RESISTANCE IN SYPHILIS

One of the most important areas of study involves creating novel syphilis therapies. Penicillin, which has been used to treat syphilis for more than 70 years, is currently the first line of defence. However, the increase of *T. Pallidum* resistance strains has occasionally rendered penicillin useless. Alternative antibiotics like macrolides, cephalosporins, and tetracyclines are being researched. Azithromycin, which is frequently used to treat other STDs including chlamydia and gonorrhea, may be useful in treating early syphilis, according to recent research. Further research is required to evaluate azithromycin's long-term effectiveness because it has also been observed that some bacteria are resistant to it. (Clement et al. 2014; Stamm 2015).
In order to identify *T. Pallidum* DNA in blood or urine samples, researchers are investigating novel diagnostic methods including loop-mediated isothermal amplification (LAMP) and polymerase chain

reaction (PCR). Studies are being conducted to determine these tests' accuracy and viability in clinical settings since they have demonstrated encouraging results in detecting early-stage syphilis. (Larsen et al. 1995; Luo et al. 2020).

Another crucial area of study is figuring out how *T. Pallidum*'s molecular mechanisms of resistance work. Penicillin-binding proteins (PBPs), which are the target of penicillin, are assumed to be the source of antibiotic resistance in *T. Pallidum* due to gene mutations. According to research, *T. Pallidum* contains three PBP homologs, and changes in any of these genes can result in penicillin resistance. Targeting these genes is a topic of research (Liu et al. 2021).

9.1.1. INNOVATIVE APPROACHES TO COMBAT RESISTANCE IN SYPHILIS

A serious concern to world health is antimicrobial resistance. However, the existing pipeline of antibiotics lacks enough innovation to address this challenge due to a commercial framework that does not give a return on investment, which discourages investment in antibiotic R&D. The antimicrobial stewardship program, which is in charge of defining, promoting, and monitoring the appropriate use of antibiotics, is crucial in tackling current issues (Vickers et al. 2019). In Addition to Antimicrobial Stewardship Program other innovative approaches to combat resistance in syphilis include Antibiotic combination therapy, developing Nanotechnology-based Therapies (Worthington and Melander 2013; Murugaiyan et al. 2022; Koh et al. 2023). Here we discussed these approaches one by one:

9.1.2. Antimicrobial Stewardship Program

Antimicrobial stewardship strategies are coordinated initiatives within a healthcare environment that support the prudent application of antibiotics, enhancing patient outcomes, lowering antibiotic resistance, and limiting the spread of diseases brought on by antibiotic-resistant microorganisms (Srinivasan 2017; Vickers et al. 2019). In this section, we'll look at a few of these innovative strategies and see how effectively they could work against resistance:

9.1.3. Optimizing Treatment Regimens

Antimicrobial stewardship programs are essential for optimizing treatment regimens in combating against syphilis resistance. They make sure that patients get the best antibiotics at the right dosages and intervals (Doron and Davidson 2011). Antimicrobial stewardship programs lessen the possibility of treatment failure and the consequent establishment of resistance strains by using a patient-centered approach (MacDougall and Polk 2005).

9.1.4. PROMOTING SAFE USE OF ANTIBIOTICS

Antimicrobial stewardship programs provide a strong emphasis on the safe use of antibiotics, specifically for treating syphilis. This involves educating medical professionals about the proper uses, doses, and time periods for antibiotic treatment (Doron and Davidson, 2011). For example, Penicillin is the only CDC-recommended regimen for the treatment of syphilis during pregnancy and the prevention of congenital syphilis in the newborn (Workowski and Bolan 2015b)

9.1.5. COLLABORATION AND EDUCATION

The training and education of medical professionals and students on prudent antimicrobial prescription or antimicrobial stewardship is necessary for reducing antimicrobial resistance since it is linked to

antibiotic abuse. Healthcare professionals collaborate to put stewardship initiatives into action and enforce them, including physicians, chemists, and microbiologists. Initiatives to educate healthcare professionals on the value of safe antibiotic usage and the effects of resistance on syphilis. These programs also inform patients of the need of adhering to their recommended treatment plans, emphasizing the overall effectiveness (Majumder et al. 2020a)

9.1.6. ANTIBIOTIC COMBINATION THERAPY

A combination of treatments is one of the most effective methods for overcoming syphilis resistance. While a single antibiotic is often used throughout the course of therapy for syphilis, combining different antimicrobial drugs can increase the efficacy of the treatment and lower the risk of resistance emerging. Antibiotic combinations such benzathine penicillin, doxycycline, and azithromycin have been studied for their synergistic effects. Combination treatment enhances patient outcomes, has greater rates of effectiveness, and stops the emergence of resistance (Worthington and Melander 2013; Stamm 2015; Murugaiyan et al. 2022; Koh et al. 2023).

9.2. NANOTECHNOLOGY BASED THERAPIES

Antibiotic resistance may now be treated in novel ways thanks to the application of nanotechnology in medicine. Nanoparticles can deliver antibiotics more efficiently and increase medication penetration by getting to places that are challenging for traditional medicines to reach. In addition, researchers are looking at the possibility of using nanoparticles as carriers for gene-editing instruments like CRISPR/Cas9 to target particular antibiotic-resistant genes in *Treponema pallidum* making the bacteria susceptible to therapy. These developments in nanotechnology have the potential to completely transform the way syphilis is treated and successfully deal with resistance (Wan et al. 2021).

9.3. POTENTIAL STRATEGIES FOR PREVENTING RESISTANCE EMERGENCE

Even if there are no symptoms, syphilis can be transmitted through sexual contact with an infected individual. Consequently, a combination of personal and public health actions is needed for syphilis prevention (Klaucke et al. 1988).
Potential strategies for preventing the emergence of syphilis has been mentioned in Fig. 4 which includes:

9.3.1. SURVEILLANCE SYSTEM

Monitoring the changing patterns and trends of syphilis occurrences and outbreaks, sorting out at-risk groups, and measuring the effectiveness of treatments all lie under this category (Catchpole, 1996). The establishment and execution of successful preventative programmes can be promoted by surveillance data. In order to ensure that only significant problems are being monitored while ensuring that surveillance systems are operating effectively, analysis of surveillance systems must encourage the best possible use of public health resources (Klaucke et al. 1988).

9.3.2. SEX EDUCATION

Since there is no vaccine to prevent syphilis, it is crucial to promptly diagnose and treat infected people and their sexual partners as part of syphilis prevention programmes that involve the use of condoms

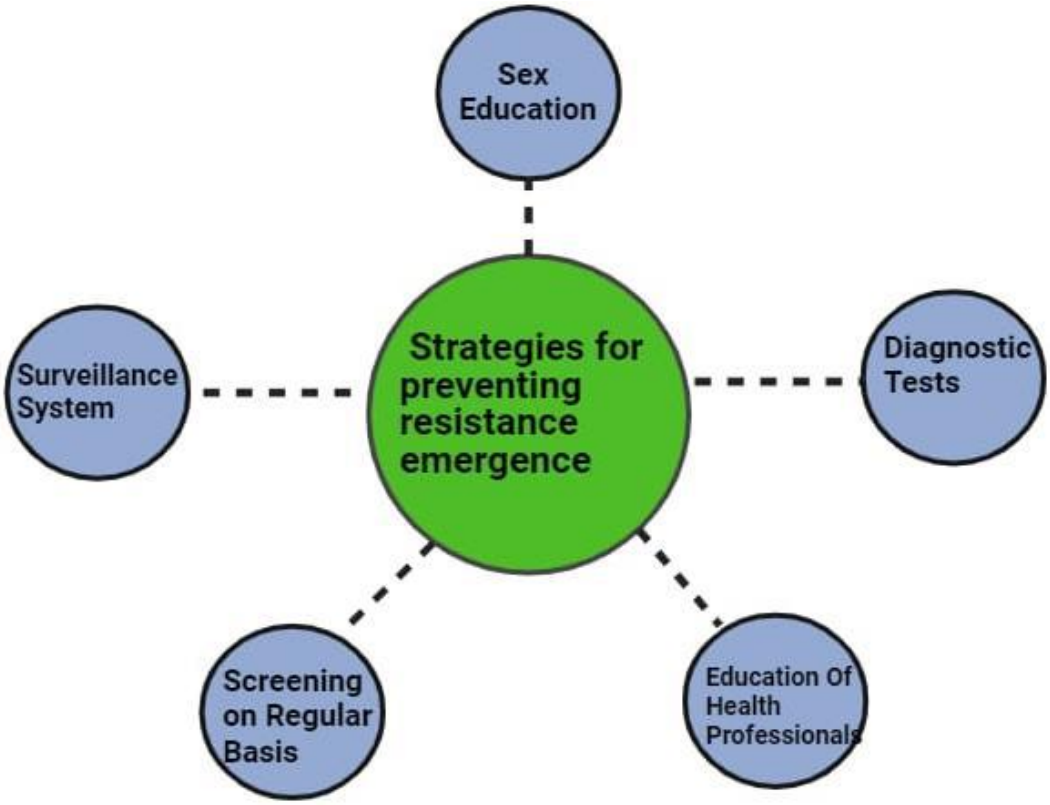

Fig. 4: Potential Strategies for Preventing Resistance Emergence.

promotion & sex education (Stamm 2016b). In order to minimize sexual activity's exposure to contaminated body fluids, physical barriers must be used. If used appropriately and regularly, condoms can reduce the risk of developing or transmitting syphilis infection (Peterman and Furness 2015).

9.3.3. SCREENING ON A REGULAR BASIS

This includes testing individuals who are at a high risk of developing syphilis regularly such as every three to six months depending on their level of exposure and behavior. Asymptomatic or infections that are latent that may go undetected and mistreated could potentially be found by screening (Peterman and Furness 2015).

9.3.4. EDUCATION OF HEALTH PROFESSIONALS

This includes providing current knowledge and training on the recognition, treatment, and avoidance of syphilis to medical professionals, laboratory workers, and public health personnel. Education can create awareness and encourage adherence to policies while further improving the standard and range of syphilis care (Peterman and Furness 2015; Lazarini and Barbosa 2017).

9.3.5. PERFORMING DIAGNOSTIC TESTS

This means improving the availability and affordability of syphilis testing services, particularly for high-risk populations including pregnant women, MSM, sex workers, and HIV-positive individuals. Testing can help in early syphilis finding, treatment, and preventing the disease's spread (Peterman and Furness 2015).

10. CONCLUSION

Antibiotic resistance in syphilis is a major public health issue that makes treatment and management difficult. *Treponema pallidum* is the bacterium that causes the several phases of syphilis, which can have major health effects if untreated. Lesions and rashes are present throughout the primary and secondary stages, however other tissues and organs may be affected at the tertiary stage. Syphilis is primarily treated with antibiotics, mainly benzathine penicillin G. Treatment choices are currently being hampered by the development of antibiotic resistance, particularly in vulnerable groups like HIV patients, pregnant women, and men who have sex with men. It is especially important to be aware of the resistance to tetracycline and macrolides, which are frequently used as alternatives for people allergic to penicillin. Finding out if syphilis is resistant to treatments is really important for controlling and managing the disease. But there are several things that make it difficult to accurately assess resistance, such as not enough data, difficulties in growing the bacterium in a laboratory, no standardized ways to test for resistance, and limited access to advanced techniques for studying the bacteria at a molecular level. To address these problems, healthcare providers, researchers, and policymakers need to work together to improve surveillance systems, make testing methods uniform, and enhance access to new technologies. By finding solutions to these difficulties, we can have a better understanding of and fight against antibiotic resistance in syphilis. This will ultimately help improve the outcomes of treatment and overall public health. Antimicrobial resistance in syphilis is being identified using new techniques. By using these techniques, we can improve therapy options while learning more about how resistance functions. We can discover crucial details about the genes of T using molecule-sequencing methods like whole-genome sequencing, PCR tests, and next-generation sequencing. Pallidum is the name of a particular region of the brain. Resistance mechanisms refer to the body's defenses against injury or means of combating noxious substances. Other approaches to diagnosing and treating syphilis are under investigation. These consist of metagenomic sequencing, digital PCR, mass spectrometry, microfluidic tests, and machine learning.

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Antimicrobial Resistance and Zoonotic Pathogens

17

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ABSTRACT

The development of antimicrobial resistance (AMR) poses serious threat to the environment, animal and human health in terms of treatment of the diseases especially zoonotic infections. The ability of microorganisms to withstand the action of antimicrobial therapy has serious consequences for treating bacterial infections and has emerged as a global health concern. Antibiotic resistance has been increased by the irrational use of antibiotics in veterinary care, agriculture and human medicine. This irrational use has made antimicrobial ineffective to treat diseases especially diseases of zoonotic importance. The phenomenon of AMR presents a serious risk to public health in addition to impairing the efficacy of medical interventions. The likelihood of spillover events is raised by the interdependence of ecosystems and the growing connection between humans and animals. The management of infectious diseases is made more challenging by the coexistence of zoonotic pathogens and antimicrobial resistance. The transboundary pathogens have infected the environment globally due to their spread and by developing antimicrobial resistance. The growing issue of antibiotic resistance, which has become a serious health hazard in practically every country in the globe, including Pakistan, has been vigorously advocated for by WHO. 'One Health' offers a variety of strategies to stop the transboundary and zoonotic spread of AMR and maintain the efficient use of antibiotics in both human and animal treatment. Resistant zoonotic pathogen strains have the potential to undermine the efficacy of current therapies, resulting in illnesses that worsen and last longer. Implementing ethical and rational use of antimicrobials, surveillance, and control strategies between human medical care, veterinary care and environmental professions can develop effective measures to reduce the threats of AMR and protect the world from this threat. WHO has started to adopt mitigation measures to have fruitful consequences and pleasant results to cope these conditions effectively.

Keywords: Antimicrobial Resistance, Zoonotic Pathogens, transboundary diseases, Public Health

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1. INTRODUCTION

The continuous and misuse of antimicrobials against bacteria, fungi, viruses and parasites had led to the development of resistance in them. Antimicrobial resistance is one the most discussed topic around the globe due to increased risk against disease spread and its cure. It is estimated that around 10 million deaths will occur yearly by 2050 worldwide. The pathogen becomes resistance against the therapeutic effects of antimicrobials and remain inside the body of host intact. It takes time for a pathogen to develop resistance against any specific drug, when microbe is evolved and become resistant it becomes very difficult to eliminate it from the body (Vidovic et al. 2020).

Providing a healthy and disease-free environment for human as well as for animals is now a greater challenge for researchers due to increasing threat of zoonotic pathogens. The transboundary pathogens have infected the environment globally due to their spread and by developing antimicrobial resistance (Jansen et al. 2018). These resistant pathogens are evolved to great extent in recent times, their presence in the hosts vary greatly. Biological host is required for growth and multiplication of these pathogens. Animals are considered as the perfect host for resistant pathogens (Greig et al. 2015).

2. WHAT IS RESISTANCE TO ANTIMICROBIALS?

Antimicrobial resistance is the resistance against antibiotics or any antimicrobials by virus, bacteria and some parasites to inhibit their function. It must be due to their overuse or misuse for a long time against the disease. With the advancement of time, microbes become resistant to different antimicrobials and spread the disease without any hindrance. The widespread use and abuse of antimicrobials in the fields of both animal and human health has caused the expansion of resistant bacteria that are resistant to the wide range of medicines that are currently accessible (Tang et al. 2023).

The growing issue of antibiotic resistance, which has become a serious health hazard in practically every country in the globe, including Pakistan, has been vigorously advocated for by WHO. The Pakistani government has committed to taking on the problem as a top priority on a global scale. The Ministry of National Health Services Regulations and Coordination is collaborating with the provinces, the veterinary industry, and health development partners to increase national capacity in strengthening laboratory diagnostics and surveillance, promoting the prudent use of antibiotics, preventing and controlling infections, and educating communities on the prevention and management of antibiotic resistance (Greig et al. 2015).

Pakistan just finished creating a national action plan to combat antimicrobial resistance with WHO's assistance; this plan will now be converted into priority-based province operational plans (Jansen et al. 2018).

3. MECHANISM OF ANTIMICROBIAL RESISTANCE AND ITS TRANSMISSION

Antimicrobial substances may prevent the growth of different bacterial colonies (bactericidal substances, such as beta-lactams) or kill them (bacteriostatic substances, such as macrolides and lincosamides). Penicillin, aminopenicillins, and cephalosporins, for example, are beta-lactam antimicrobials that prevent the production of the cell wall (peptidoglycan) (Nhung et al. 2017).

Beta-lactamases, which destroy the beta lactam ring of the gram negative bacteria, is one mechanism of beta-lactam resistance. Alterations to the cells, such as penicillin-binding proteins in Gram-positive cocci. The Beta-lactamases can hydrolyze cephalosporins, beta-lactam/beta-lactamase inhibitor combos, and narrow-spectrum penicillin in Enterobacterales (e.g., *Escherichia coli*) (Rodríguez et al. 2019).

AMR can be caused through chromosomal or extrachromosomal gene alterations, as well as by acquiring resistant genes from different organisms. The treatment protocol by the use of antibiotics, encourages the interchange of resistant elements both within and across bacterial growth and results in the formation, survival, and proliferation of resistant clones. The pathogens have certain genome which has the ability to become resistant to certain bacteria and increase the risk of AMR. The antimicrobials like carbapenems and several generations of cephalosporins, plasmids are of particular significance. Bacteriophages can also be used for transformation or transduction of genetic material (Migura et al. 2022).

4. DRIVING FACTORS OF ANTIMICROBIAL RESISTANCE

There are numerous causes for the occurrence, selection, and spread of AMR. Antimicrobial resistance is well known to be stimulated by the continuous and overuse of antimicrobials by the humans. Between 2000 and 2015, usage of antibiotics in people grew by 65%. Different antimicrobial drugs are utilized for prophylactic and growth stimulation in addition to treating animal diseases as a way to sometimes combat poor hygiene. Since resistant bacteria can spread through human and animal interactions, the medicine in humans as well as in animals should be considered to have a therapeutic index and might be used according to requirement of infection (Mader et al. 2021).

5. DISEASES BY ZOONOTIC PATHOGENS

A zoonotic illness is one that spreads spontaneously between humans and vertebrate animals, such as wild animals, domesticated animals, or cattle. According to estimates, these diseases account for roughly 60% of all emerging diseases and 58% of all human infections (Dafale et al. 2020). A foodborne disease is one that is contracted after consuming tainted food, whether it is due to a parasite, viral, bacterial, or chemical agent (Mader et al. 2021).

6. PREVALENCE OF ANTIMICROBIAL RESISTANCE IN BACTERIAL ZOONOTIC PATHOGENS

One of the biggest risks to global food security and human health is the propensity of bacterial diseases to develop resistance to antibiotics. The MIC (minimum inhibitory concentration) value, or the lowest amount of the medicine necessary to stop the growth of a bacterial culture, can be used to measure antimicrobial resistance (AMR) to a certain medication. While bacterial isolates are frequently categorized as sensitive or resistant for practical purposes, MIC is a continually fluctuating feature (measured discontinuously) (Bhat 2021).

6.1. ANTI-MICROBIAL RESISTANCE IN *STREPTOCOCCUS SUIS*

Streptococcus suis is primarily found in pigs as a commensal, colonizing in the gut area, nasopharynx and vaginal region. However, it can also cause severe respiratory and systemic illness, especially in young ones. *S. suis* causes a dangerous zoonotic illness that was also the main reason for bacterial meningitis in Vietnam for a long period of time. Moreover, certain autogenous vaccines are employed in the pig industry, they are serotype-specific and offer patchy cross protection against heterogeneous *S. suis*. Antimicrobials are still the go-to treatment for *S. suis* as a result, and as a result, *S. suis* is a major factor in the use of antibiotics in pig farms (Dafale et al. 2020).

In addition to being a major issue in and of, this also offers special advantages as a model for researching antimicrobial resistance. *S. suis* is present in the majority of pigs, if not all of them, pork is the most widely consumed meat in the world by weight. Furthermore, compared to other animals, such as cattle (45mg) and fowl (148mg), pigs use more antibiotics (172mg per population corrected unit). They are comprised of antibiotics which are taken directly against *S*. *suis*, whether as a treatment, prophylactic, or meta-phylaxis, but also, and maybe more frequently in relation to different bacterial infections and in certain countries as growth enhancers. It is anticipated that the high selection pressure brought on by the extensive use of antibiotics in pig farming will result in AMR in *S. suis* (Hadjirin et al. 2021).

6.2. ANTI-MICROBIAL RESISTANCE IN *M. TUBERCULOSIS*

In contrast to *M. tuberculosis* (mTB), which primarily affects humans, *M. bovis* (bTB) is the main cause of tuberculosis (TB) in different domestic and wild animals. *M. bovis* can transmit zoonotic TB (bovine tuberculosis) to humans through eating, inhalation, and less frequently contact with mucous membranes and torn skin. Despite having a close genetic link, members of the MTBC, particularly *Mycobacterium tuberculosis* (mTB) and *Mycobacterium bovis* (bTB), have different host preferences and a different geographic distribution for the formation of tuberculosis. The prevalence of *M. bovis*-caused bovine tuberculosis is rising worldwide, especially in poor countries. *M. bovis*-caused bovine tuberculosis is becoming more common worldwide, especially in developing nations. However, there are few researches in Pakistan that concentrate on *M. bovis* illness in humans and its risk factors (Borham et al. 2022).

6.3. ANTI-MICROBIAL RESISTANCE I N ESBL-*E. COLI*

Escherichia coli that produces extended-spectrum beta-lactamases (ESBL) is most commonly found in chicken, therefore there is a chance that ESBL-producing *E. coli* could be imported into Africa through poultry products. Since chicken is the food source of choice for *Escherichia coli* that generates extended-spectrum beta-lactamases (ESBL), there is a possibility that poultry products could be used to introduce ESBL-producing *E. coli* into Africa. Although the proportion of *E. coli* that must be ESBL in aquacultures can be high (27%) the majority of our knowledge of how marine animals affect human health is limited by the generally subpar quality of published studies. *E. coli* that produces ESBLs has a colonization rate of 1-9% or 3-63% in bats and birds, respectively. Since the majority of them are migratory animals, they can spread bacteria resistant to antibiotics over long distances. Said "filth flies" are a significant carrier of resistant bacteria and gastrointestinal disorders in places with poor sanitation systems (Sajeev et al. 2023).

6.3.1. PREVALENCE OF ESBL IN ASIA

In comparison to Africa, Asia has a significantly greater frequency of ESBL-producing E. coli in meat samples and cattle. It is estimated that 54-93% of chicken meat samples and 35-75% of hog meat samples had ESBL-producing *E. coli*. In contrast, research done in Thailand and Cambodia found that contamination rates for both pork and poultry were less than 4%. In addition to ESBL, meat from Asia is frequently infected with microbes that carry the mcr-1 gene (Sajeev et al. 2023).

7. COMMON ZOONOTIC BACTERIA WITH AMR IN THE FOOD CHAIN

Resistance among zoonotic bacteria, which spread through food, has increased as a result of the overuse or rather misuse of antimicrobials combined with poor hygiene in the food production chain. They are the

major risk to people's health. Since more than a decade, campylobacteriosis has been the most often reported zoonosis in humans in the European Union and the most common bacterial food-borne infection overall (Álvarez-Molina et al. 2021).
Food-borne outbreaks found in places where salmonellosis is most frequently diagnosed. Fluoroquinolones are regarded as being of vital importance for treating both zoonosis in humans with severe instances. Additionally, the efficacy of treating human Campylobacter infections with fluoroquinolones has been impaired as a result of an extraordinarily high prevalence of resistant isolates, particularly from broilers and meat (Korsgaard et al. 2022).

8. ANTI-MICROBIAL RESISTANCE IN AQUACULTURE

According to Food and Agriculture Organization (FAO), aquaculture is the farming of aquatic species such as fish, mollusks, crustaceans, and aquatic plants in inland and coastal environments. The lack of environmental separation between aquaculture production systems and the environment in many nations causes an increased risk of AMR residues in animal farming and nearby waters that damage wild fish, plants, and sediments (Olaru et al. 2023). This alters the makeup of environmental bacteria and promotes the selection of bacteria resistant to antibiotics. There are significantly varied numbers of antimicrobial compounds authorized for use in aquaculture in different nations. The use of different medication in aquaculture must be based on some clinical and research based knowledge, proper assessment and diagnosis of diseases must be discussed and set some sort of SOPs to cope different ailments at farm level. Any attempts for the administration of antimicrobials on the surrounding or worldwide basis, however, are hindered by the lack of defined AMU indicators for aquaculture (Olaru et al. 2023).

9. ANTI-MICROBIAL RESISTANCE IN VETERINARY MEDICINE AND FOOD ANIMALS

The overuse of antibiotics in veterinary care and in animals raised for food is encouraging the evolution of antibiotic-resistant bacteria in both zoonotic pathogens and the normal bacterial flora. The connection between AMR and animal and human morbidity is a significant issue for contemporary medicine. Even though zoonotic infections are the subject of substantial research, there is still a need for adequate control, regulation, and human usage of antimicrobial agents (Thapa et al. 2020) . Additionally, the use of antibiotics in manure for agricultural purposes spreads the antibiotics throughout several biological niches, including the soil and water. These antibiotics when enters into water bodies cause the proliferation and the chances of AMR spread accordingly. The spread of antibiotic resistance in the environment is seen as a worldwide danger, and AMR is recognized as preeminence in the World Bank's most recent One Health approach framework (Torres et al., 2021).

10. ZOONOTIC PATHOGEN AS AN AMR CARRIER

- Environmental pollution, antibiotic resistance, and chronic diseases have in the past jeopardized the health of both humans and animals, causing significant rates of death and morbidity (Jones et al. 2008).
- The majority of infectious illnesses are regarded as serious health problems with zoonotic origins. The World Health Organization (WHO) defines zoonotic illness or zoonosis as "any disease or infection that are naturally transmitted between vertebrate animals and humans." An infectious agent that causes disease could be a virus, fungus, bacteria, parasite or prions (Alexander et al. 2018).

- Approximately 200 zoonotic pathogens are known to exist in the globe today; some are restricted to a certain region, while others are said to have a global distribution. Transmission methods for zoonotic pathogens include ingestion, inhalation, and other methods that contaminate mucosal membranes (Schmeller et al. 2020).
- Additionally, zoonotic infections can be consumed through undercooked meat, unpasteurized milk, dairy products, shellfish, and infected vegetables, all of which include animal tissue. Anthrax, animal influenza, bovine tuberculosis (BTB), brucellosis, hemorrhagic colitis, zoonotic diphtheria, rabies, and Q fever are additional well-known zoonotic diseases (Shanks et al. 2022).

11. ZOONOTIC PATHOGENS FEATURING ANTIBIOTIC RESISTANCE

According to one health approach, domesticated animals which are frequently linked with humans are the ones that act as a reservoir for zoonotic infections. Animal husbandry is a crucial component of the agricultural economy and supports the rural population's way of life. Milk, meat, eggs, and wool are products of animal husbandry that have a direct impact on the human population. Animal husbandry has a very high risk of transmitting zoonotic diseases to humans from their hosts. Despite the high danger of zoonotic infections, livestock is essential for many farmers' livelihoods, household nutrition, and the consumption of animal products. There have been recently numerous reports of zoonotic illnesses carrying anti-microbial resistant genes that, when contracted, can infect humans (Binot et al. 2015).
The inappropriate use of antibiotics has led to anti-microbial resistant genes in zoonotic diseases. Animal husbandry treats the entire herd with antibiotics for any infectious diseases to stop the spread of illness, even when some animals have clinical symptoms. Meta-phylaxis is the term for the procedure of administering a high dose of antibiotics for a brief course, whereas prophylaxis refers to the blending of antibiotics with the feed in modest doses for an extended period of time, typically for many weeks. Even though the animals are not showing any clinical symptoms at this time, the risk of infection still exists (Chang et al. 2015).
The improper use of antibiotics places microorganisms under a selection pressure that causes antibiotic resistance at concentrations of antimicrobials below the therapeutic range. Various mechanisms, including as mutation, alterations in cell permeability, horizontal gene transfer, drug efflux, and quorum sensing, are used by different bacterial species to acquire resistance. Increased prevalence of resistant bacteria in the intestinal flora of pigs, chickens, and other agricultural animals has been linked to the transmission of anti-microbial resistant genes and heavy antibiotic use (Dafale et al. 2020).

12. VARIOUS METHODS FOR ANTIBIOTIC DEGRADATION

Antibiotic use and disposal have increased, which disrupts other biological processes and water quality. Antibiotic degradation is therefore required to prevent the formation of new resistant bacteria and anti-microbial resistant genes (Wester et al. 2017). Both abiotic and biotic processes break down antibiotics. It is aided by hydrolysis, metal-assisted photolysis, adsorption mechanisms, bio-electrochemical processes, oxidation, and reduction. (Chang et al. 2015).

12.1. ANTIBIOTIC PHYSICAL-CHEMICAL DEGRADATION

- Antibiotics physicochemical characteristics and molecular structure play a significant role in abiotic degradation. Compared to macrolides and sulfonamides, ß-lactam antibiotics are reportedly more vulnerable to hydrolytic breakdown (Liu et al. 2016).

- Fluoroquinolone antibiotics are primarily broken down via photo-degradation. Waste water is decontaminated and its antibiotic content is reduced through chlorination and UV radiation treatment (Robbiati et al. 2023).
- To limit the extent of anti-microbial resistant microorganisms and anti-microbial resistant genes, natural polysaccharide chemical conjugates can be used in addition to degrading antibiotics to stop the growth of resistant bacteria (Collignon et al. 2019).
- An antibiotic's antibacterial capabilities are lost when it attaches to soil particles and forms a complex, according to research. Adsorption and desorption of antibiotics are two terms that describe this process. In this approach, the soil's pH and water holding capacity are crucial factors (Wester et al. 2017).
- The cationic form of antibiotics like sulfonamides transforms to the neutral and anionic form as they are absorbed in soil. Antibiotic breakdown is dependent on abiotic factors. Waste water is decontaminated and its antibiotic content is reduced through chlorination and UV radiation treatment. In order to limit the extent of anti-microbial resistant microorganisms and anti-microbial resistant genes, natural polysaccharide chemical conjugates can be used in addition to degrading antibiotics to stop the growth of resistant bacteria. An antibiotic's antibacterial capabilities are lost when it attaches to soil particles and forms a complex, according to research. Adsorption and desorption of antibiotics are two terms that describe this process (Falenski et al. 2011).
- In the sorption approach, the soil's pH and water holding capacity are crucial factors. The cationic form of antibiotics like sulfonamides transforms to the neutral and anionic form as they are absorbed in soil (McEwen et al. 2018).
- Abiotic antibiotic degradation is influenced by a number of physical and chemical factors. The abiotic treatment may be hampered by pH changes, salt concentrations, and the presence of other compounds in the system. In such circumstances, biotic mechanisms that include the use of microorganisms may be essential for removing lingering antibiotics from the environment (Sajeev et al. 2023).

12.2. BIO AUGMENTATION-INDUCED ANTIBIOTIC DEGRADATION

- Antibiotics are subject to biotic breakdown when they pass via a possible microorganism metabolic pathway. anti-microbial resistant genes are primarily involved in microorganisms that efficiently break down the parent antibiotic or functional group and release the byproducts, which contribute in the bioremediation of antibiotics (Hong et al. 2020).
- It has been demonstrated that bio augmentation can successfully remove antibiotics from industrial wastewater. Numerous bacterial strains have been used to remove antibiotics from soil and wastewater (Xu et al. 2017).
- Similar to this, it has been reported that bio augmentation of membrane bioreactors with Achromobacter dentrificans improves sulfamethoxazole elimination (Falenski et al. 2011).
- An effective way to remove antibiotics from the environment is bio augmentation (Chang et al. 2015).

13. THE WIDESPREAD RESISTANCE: ONE HEALTH APPROACH

AMR can be fatal, yet there are effective, adoptable remedies that are still hidden. Due to its multidimensional, linked, and diverse ecological aspects, understanding the AMR pattern is difficult. The right usage of antimicrobial products in diverse areas must be decided upon by individuals and society as a whole in order to control widespread resistance (Tang et al. 2023).

A multi-sectoral approach to oversight, involving teams from the veterinary, environmental, and healthcare sectors as well as stakeholders, is necessary to understand the complex AMR situation

(McEwen et al. 2018). A method known as "One Health" entails interdisciplinary cooperation between academics, policymakers, and leaders operating at the regional, municipal, national, and worldwide levels. This strategy aims to improve human, animal, and environmental health outcomes. A priority of the "one health" approach is the onset of epidemic illnesses involving AMR (Sajeev et al. 2023). In order to comprehend the AMR issue and identify competent solutions to create appropriate usage guidelines and deliver efficient risk messaging, this strategy brings together many sectors operating in the field. Another issue that requires a broader explanation is the spread of AMR. This problem includes the transfer of germs between human hosts, animals (both domestic and wild), and the environments in which each can thrive (Wester et al. 2017).

Understanding zoonosis-mediated AMR is a problem that affects everyone, including the scientific community, producers of food animals, healthcare workers, patients, and customers. 'One Health' offers a variety of strategies to stop the trans-boundary and zoonotic spread of AMR and maintain the efficient use of antibiotics in both human and animal treatment (Robbiati et al. 2023).

14. ONE HEALTH STRATEGIES TO COMBAT ANTIBIOTIC RESISTANCE

In order to address the antimicrobial resistance challenge, the WHO, other international organizations (such as the Food and Agriculture Organization FAO) and the World Organization for Animal Health (OIE), as well as numerous individual nations, have established detailed action plans. The five main goals of the WHO Global Action Plan are described in the subtitles of the sections that follow. In order to address antibiotic resistance, the WHO Plan adopts a One Health strategy, and it encourages member nations to follow suit when creating their own action plans. The WHO Global Plan is supported by five fundamental pillars (Wester et al. 2017).

1. Enhance Antimicrobial Resistance Awareness and Understanding Through Effective Communication, Education, and Training
2. Using surveillance and research, improve the body of knowledge and evidence
3. Utilize Effective Sanitation, Hygiene, and Infection Prevention Measures to Decrease the Incidence of Infection
4. Improve Antimicrobial Drug Use for Human and Animal Health
5. Increase investment in new drugs, diagnostic tools, vaccines, and other interventions while developing the economic case for sustainable investment that considers the needs of all countries (Álvarez-Molina et al. 2021).

World Health Organization has devised some plans under the umbrella of one help to limit the usage of antimicrobial and to hinder the antimicrobial resistance, some recommendations and strategies are also proposed at domestic and international levels to set some criteria and SOPs to work on minimizing the antimicrobial resistance worldwide. The main difficulty which scientists and researchers face today is the availability of data regarding disease spread and antimicrobial usage hence it’s a long journey to continue and work for the betterment of human as well as for animal health. The concept of one health helps a lot to cope up this condition but still there are many things to do regarding our strategy and still more work needs to be done to minimize the risk of antimicrobial resistance from our ecosystem (Collignon et al. 2019).

15. CONCLUSION

Antimicrobial resistance is still a significant problem for worldwide public health in the twenty-first century. The G7 nations have already made significant political contributions to this issue and is a priority for a number of political conferences. Zoonotic pathogens are of great concern in this regard as they

directly affect our lives and their spread leads to pandemic situation. WHO has started to adopt mitigation measures to have fruitful consequences and pleasant results to cope these conditions effectively.

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Zoonosis: An Emerging Link to Antimicrobial Resistance Under ‘‘One Health Approach’’

18

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ABSTRACT

The current state of infectious diseases has given rise to a new era in which the management and sharing of etiological agents with their effects on ecosystems is understood through the lens of "One Health." In this regard, the importance of zoonotic illnesses gives rise to serious concerns. Because of their high density and shorter generation times, the indiscriminate and increased use of antibiotics in animal agriculture puts significant pressure on the gut microbiota to acquire resistance. In this case, the gut serves as a bioreactor for the spawning of ARBs, which are then continuously discharged into other habitats. Through quorum sensing, vectors, and horizontal gene transfer events, these ARBs spread resistance genes among the natural flora. The possibility for zoonotic infections to carry ARGs that could be transmitted to humans, accounts for about 60% of infectious illnesses that affect humans. The well-known zoonotic illnesses include hemorrhagic colitis caused by Escherichia coli, brucellosis caused by Brucella abortus, ovine tuberculosis caused by Mycobacterium tuberculosis, and anthrax caused by Bacillus anthracis. Similar to this, the majority of antibiotics are not entirely broken down before being released into the food chain where they bioaccumulate and impact different ecological niches. Antibiotics have an environmental persistence duration ranging from less than one day to 3466 days. This review has covered the effects of antibiotic misuse in cattle as well as their future in different ecological niches. Additional information is provided about the persistence of antibiotics and their biodegradation in the environment using various abiotic and biotic methods. To manage the development of antimicrobial resistance (AMR) in the environment and, eventually, to humans through food webs, comprehensive surveillance systems for zoonotic diseases, including ARB transmission, prevention, and control measures, as well as knowledge on personnel hygiene, should be built.

Keywords: Antibiotic resistance, antimicrobial resistance, Livestock, Zoonosis, Half-lives of antibiotic, Gut microbiome

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1. INTRODUCTION

In recent years, the management of human health has encountered new challenges. The global concern primarily revolves around emergence of novel opportunistic pathogens and drug resistance among existing pathogens. These emerging diseases have transcended geographical boundaries, permeating various environments. The evolving hypothesis postulates that the altered disease patterns are a result of pathogenic determinants proliferating within ecosystems, exploiting any available biological host. Among these hosts, animals stand out as the most prevalent. Animals' gastrointestinal tracts harbor diverse microbial communities that collaborate to enhance their host's well-being (Purohit 2018). By spreading their resistant genes to other gut pathogens, drug-resistant pathogens disrupt the community structure when they are introduced to the gut microbiome. Opportunistic infections spread from animals to humans with the help of direct contact (zoonoses) or vectors, having an adverse effect on human health. The intricate connection between humans and animals through ecosystems has prompted the adoption of the "one health" approach.

The global increase in concern lies in the transmission of diseases from animals to humans through the zoonotic route. Zoonotic diseases have the potential to trigger sporadic disease outbreaks, novel epidemics, or remain geographically limited occurrences. The "one health" paradigm includes the thorough management of all elements causing the introduction of novel zoonotic illnesses. This approach interconnects animal health, human health, and environment. The one health strategy must be used due to the rise in antibiotic resistance among humans, which is spread via zoonotic infections. Managing zoonotic diseases through the one health approach requires collaborative efforts from multidisciplinary teams consisting of mammalogists, entomologists, ecologists, ornithologists, physicians, and epidemiologists ensuring successful investigations.

A substantial change in human health resulted from the discovery of antibiotics in the early 1900s, which helped to save countless lives. Antibiotics are complex substances that obstruct the growth of microorganisms through a number of mechanisms, including alterations to cell membranes, antimetabolite effects, hindrance of cell wall synthesis, disruption of protein synthesis, competitive antagonism, and inhibition of nucleic acid synthesis. Antibiotics are used in livestock and animal husbandry to prevent infectious diseases and increase the meat and dairy production. Furthermore, they are employed to facilitate substantial increases in animal growth and weight. Despite the advantages of antibiotics, the unregulated utilization of these agents and their release into the environment raise significant concerns (Parmar A et al. 2018). (Fig. 1)

The majority of antibiotics introduced into human and animal bodies undergo incomplete metabolism, leading to the release of unmetabolized forms into the environment. These unmetabolized compounds are expelled from the body and find their way into the environment through sewage sludge, municipal wastewater, and animal manure. The heightened presence of antibiotics across diverse environments exerts selective pressure, fostering the growth of antimicrobial-resistant bacteria (ARBs). This growth triggers antimicrobial resistance (AMR) within the indigenous microbiome and alters the sensitivity of bacterial populations (Parmar KM et al. 2017). Environmental Antibiotics bring about modifications in the

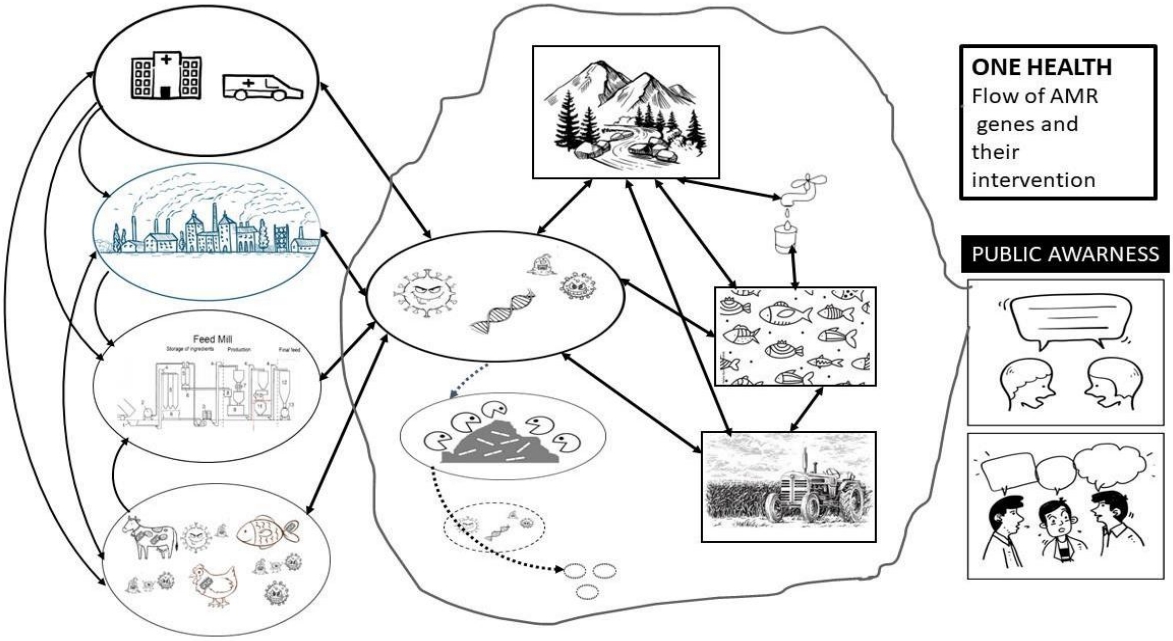

Fig. 1: Diagram showing the movement of resistance genes in the environment and how the "One Health" approach can be used to control it. ARB breeding hotspots are connected by a black solid line, and the bioremediation strategy in the environment is shown by a dotted line.

genetic composition of bacteria that results in the proliferation of antibiotic resistance genes (ARGs). These ARGs are transferred via mobile genetic elements (MGEs) like transposons, genomic islands, and plasmids to other microbial populations, consequently amplifying the prevalence of ARBs within the environment and these ARBs can develop into potent zoonotic pathogens, contributing to severe infections within the global human population.

The utilization of antibiotics in the treatment of animals used for food production is fostering the emergence of ARBs among both zoonotic pathogens and normal bacterial communities. The correlation between AMR and the occurrence of illnesses in animals and humans is a significant concern within modern medical practices. Despite the ongoing comprehensive research concerning zoonotic pathogens, the effective regulation and management of antimicrobial agents, combined with their careful use by humans, still lack appropriate measures. Additionally, the practice of using antibiotics in manure for agricultural purposes leads to the dissemination of antibiotics into various ecological niches, including water and soil. Antibiotics from the soil can leach into water bodies, and thus becoming distributed throughout different ecological environments. The dispersal of antibiotic resistance within the environment is recognized as a global threat, and AMR has attained paramount importance within the recent approach of One Health framework introduced by the World Bank (Thakur and Gray 2019). This One Health approach to AMR encompasses considerations for wildlife, environment, and the aquaculture. The approach recognizes the pivotal roles that various sectors play in the advancement and proliferation of AMR, concentrating efforts on actionable and impactful research.

2. THE ONE HEALTH STRATEGY TO TACKLE WIDESPREAD RESISTANCE

AMR can have fatal consequences, yet the implementation of effective strategies to counter AMR remains intricate. Deciphering the pattern of AMR is challenging because of its intricate, multifaceted ecological,

and interconnected characteristics. Addressing the extensive resistance requires decisions made by individuals and societies, which must prioritize the judicious use of antimicrobial products across various sectors. Gaining a comprehensive understanding of the intricate AMR landscape necessitates holistic oversight involving multi-sectoral teams comprising healthcare professionals, environmental experts, stakeholders, and veterinarians. One Health approach emphasizes interdisciplinary collaboration among researchers, policymakers, and leaders operating at regional, local, national, and the international levels (Binot et al. 2015). It aims to enhance health outcomes for humans, animals, and the environment. The mitigation of epidemic infections associated with AMR is a central focus of the "one health" strategy. This approach interconnects diverse sectors engaged in comprehending the AMR challenge and devising effective solutions, including the establishment of appropriate usage guidelines and the provision of effective risk communication. Furthermore, dissemination of AMR poses another challenge, necessitating a broader exploration of bacterial transmission among human hosts, both domestic and wild animals, and the corresponding environments in which genes of resistant bacteria can propagate.

The management of AMR under the one health approach involves considering various factors, including

1. The establishment of regulations and guidelines for the prudent use of diverse antimicrobial classes in both human and animal health.
2. Comprehending the environmental dissemination of AMR through mediums such as water, air, and soil, which harbor resistant bacteria and ARGs and serve as pathways for AMR transmission between human and animal populations.
3. The implementation of innovative and established solutions using novel technologies to quantify AMR across domains like herd management and animal husbandry.
4. Effective communication strategies to raise awareness of consumer about the risks related to AMR dissemination from sources such as food production, and other non-human origins.

The grasp of zoonotic-mediated AMR is a universal concern that spans the food animal producers, scientific community, patients, healthcare professionals, and consumers. In order to sustain the judicious use of antibiotics in both human and animal medicine, the "One Health" approach employs a variety of strategies to curb the cross-border and zoonotic propagation of AMR.

3. ZOONOTIC PATHOGENS AS VECTORS OF ANTIMICROBIAL RESISTANCE (AMR)

Over the previous years, both human and animal health have faced challenges stemming from environmental pollution, AMR, and chronic diseases, consequencing in elevated morbidity and mortality rates (Kalia et al. 2014). The emergence and dissemination of zoonoses, epidemics, and epizootics have underscored the heightened health risks on a global scale, emphasizing the significance of comprehending the interface between humans and animals in the transfer of infectious agents. Many of these infectious diseases are regarded as severe health concerns originating from a zoonotic source. As defined by the WHO, the zoonotic diseases, or zoonoses, refer to "any disease or infection that is naturally transmitted between vertebrate animals and humans." These diseases can be caused by viruses, fungi, bacteria, parasites, or prions. Currently, there are approximately 200 recognized zoonoses, with some confined to specific geographic regions and others having a global distribution. Zoonotic pathogen transmission occurs through various routes, including ingestion, inhalation, and other means that lead to mucous membrane contamination. Furthermore, zoonotic pathogens can be transmitted through dietary habits involving undercooked animal tissue, unpasteurized milk, dairy products, seafood, and contaminated vegetables. Several other well-documented zoonotic diseases include anthrax, animal influenza, bovine tuberculosis (BTB), brucellosis, hemorrhagic colitis, zoonotic diphtheria, rabies, and Q fever.

The prevalence of zoonotic diseases is the outcome of intricate interactions among various factors, encompassing biological, genetic, political, social, ecological, environmental, and physical aspects. Zoonotic pathogens are accountable for over 60% of infectious diseases in humans. Within this group, ARBs represent one of the most prevalent zoonotic pathogens that can be found widely within the environment. These bacteria exhibit a short generation time and are subject to intense selection pressure from the host immunity and antimicrobial agents. Consequently, these bacterial communities undergo notable evolutionary changes, impacting human health by their interactions with pathogen-host spp., serving as infection reservoirs. Specific bacteria, such as *Proteus mirabilis, Pseudomonas fluorescens, Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Staphylococcus* species, have demonstrated resistance against certain antibiotics (Hathroubi et al. 2018). The one health approach underscores the direct correlation between the emergence of ARBs and the escalated use of antibiotics within the farm animal and aquaculture sectors.

ARBs enter the human gut via zoonotic disease transmission, leading to disruptions in the natural gut diversity. Zoonotic pathogens harboring ARGs are introduced into the body of humans, where they can transfer these resistance genes to the gut microbiome, thereby causing disturbances in the ecosystem of gut. Moreover, the ARGs or ARBs are expelled from the body of human and find their way into the environment either through the soil or municipal wastewater. These ARG genes from the soil or wastewater are then transmitted to ARBs, which enter the food web, affecting animal health, and perpetuating the ARB transmission cycle (Hathroubi et al. 2018).

4. ZOONOTIC PATHOGENS AND THEIR ANTIBIOTIC RESISTANCE

The One Health strategy highlights that the majority of animals acting as reservoirs for the zoonotic diseases, are domesticated and frequently interact with humans. Animal husbandry is an integral component of agricultural economy, and holds significant importance in sustaining the rural population's livelihood. This practice supplies milk, meat, eggs, and wool that have direct interactions with human population. Potential for zoonotic disease transmission from animals to humans is particularly elevated in the context of animal husbandry. Despite the substantial risks associated with zoonotic diseases, livestock continues to play a pivotal role in the income of numerous farmers, as well as in providing nutrition for households and contributing to the consumption of animal products.

It has been shown that the ARGs, which are passed to the human population upon infection, have recently been found to be present in a number of zoonotic diseases. The misuse of antibiotics is thought to be a contributing factor in the rise of ARGs in zoonotic diseases (Dafale et al. 2016; Yadav et al. 2019). In the context of animal husbandry, even when only certain animals show clinical symptoms, entire herds are often treated with antibiotics to prevent disease transmission within the herd. This practice, known as metaphylaxis, involves administering high doses of antibiotics for a short duration. In contrast, prophylaxis involves incorporating antibiotics into feed at lower doses over an extended period, typically spanning several weeks. While animals may not exhibit clinical symptoms during this period, the risk of infection remains. The imprudent use of antibiotics subject's pathogens to selective pressure, leading to antibiotic resistance even at sub-therapeutic antimicrobial concentrations. Bacterial species acquire resistance through various mechanisms, including mutation, changes in cell permeability, drug efflux, quorum sensing and horizontal gene transfer. The increased prevalence of resistant bacteria in the intestinal flora of swine, chickens, and other agricultural animals has been related to the transmission of ARGs and the excessive use of antibiotics. The close contact between farmers, workers, and animals greatly increases the likelihood of ARB transmission. Numerous routes facilitate the transmission of zoonotic pathogens between humans and animals, with the food chain being the most probable pathway. Humans face a

significant risk of exposure to ARBs originating from animals through the consumption of products like milk, meat, eggs, and protein. The ARBs are particularly likely in raw, less processed, or fermented foods with higher microbial loads. The presence of pathogenic bacteria in animal-derived foods creates a concerning scenario within the human gut, leading to the emergence of the resistant strains in the humans (Kalia and Purohit 2011; Dafale et al. 2015).

5. LIVESTOCK AS POTENTIAL RESERVOIRS FOR ANTIBIOTIC RESISTANCE

Threats posed by zoonotic diseases have escalated as a result of their adaptation to antibiotic resistance. Methicillin-resistant Staphylococcus (MRSA) strains that have emerged from farm animals, including CC93 in Denmark, ST 130 across Europe, and ST398 in the Netherlands, have been seen to spread among human populations (Chang et al. 2015). Concerns about animals acting as reservoirs for illnesses that could evolve to spread among humans are brought up by the possibility of zoonotic transmission. Research has indicated a correlation between antibiotic usage in food animals and a rise in ARBs in humans. The importance of fluoroquinolones, a class of antibiotics frequently used to treat infectious illnesses, has been highlighted (Dafale et al. 2015). Fluoroquinolone-resistant Campylobacter infections were reported by Barza and Travers (Barza and Travers 2002), who linked them to an overuse of the drugs in animals. Following this, the Food and Drug Administration (FDA) of the United States determined that chicken eating was directly related to fluoroquinolone-resistant Campylobacter in people. This result finally helped to justify the US decision to stop using fluoroquinolones on poultry (Pereira et al. 2018).
There are differences in the epidemiology of vancomycin resistance components between Europe and the USA. The gastrointestinal microbiome of healthy individuals and livestock animals in Europe has been found to have *Enterococcus faecium* expressing the vanA resistance gene (VRGs), however in the United States, neither healthy humans nor livestock animals had been found to contain this organism until 2008. This discrepancy was anticipated due to the extensive utilization of avoparcin in European agricultural practices since the 1970s, while this glycopeptide had never been authorized for agricultural use in the United States. It has been proven that avoparcin exerts selective pressure favoring the establishment of VRGs in mice by conferring cross-resistance to vancomycin (Birkegård et al. 2019). ARBs have several opportunities to colonize the human environment and to infect humans when VRGs are present in animals. Molecular and epidemiological studies have demonstrated the similarity between VRGs-containing organisms isolated from both animals and humans, confirming the potential for the transmission of VRGs. A thorough understanding of zoonotic pathogens, transmission of disease, control measures, and prevention is indispensable for individuals working with livestock. This knowledge is essential to mitigate zoonotic diseases as occupational hazards, contributing to the prevention and control of such diseases in both human and animal populations.

6. TRANSMISSION OF ANTIBIOTIC RESISTANCE GENES FROM LIVESTOCK

Zoonotic AMR pathogens can move from farm animals and affect humans through the consumption of food or water that has been contaminated, as well as through direct contact with animals. In the various stages and processes of the food production chain, there are opportunities for the transfer of ARBs. Physical environment, including soil, water, and air, significantly contributes to the transfer of ARBs from animals. Within a farm setting, there is a potential for the direct movement of the resistant bacteria among animals within the same herd and those in close proximity. Additionally, interactions between farmers and their animals can also facilitate the transmission of resistant

bacteria. Moreover, when animals consume water, feed, or waste contaminated with ARBs, the likelihood of transfer increases (Barton 2014). Several instances of ARBs in livestock have been recorded by numerous researchers. These instances encompass bacteria that produce extended-spectrum beta-lactamases (ESBLs), vancomycin-resistant *Enterococcus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli,* and multidrug-resistant *Salmonella* (Barton 2014). Other ARGs, such as plasmid-mediated colistin resistance, *Klebsiella pneumoniae* carbapenemase-2, and New Delhi Metallo-β-lactamase-1 found in the livestock animals, pose threats to both human and animal health (Liu et al. 2016; Tafaj et al. 2019).

In the livestock industry, inappropriate antibiotic usage contributes to the rise of the specific resistant bacteria. The extent of antibiotic use correlates directly with specific resistance level observed in commensal *E. coli* isolates from poultry, cattle, and swine. Metagenomic data has also demonstrated that the imprudent administration of antibiotics to animals leads to resistance development in the commensal microbial populations of human (Chang et al. 2015). Vounba et al. (2019) reported instances where use of 3rd generation cephalosporins in poultry led to human fatalities caused by the resistant *E. coli*. The GIT of animals functions as a notable reservoir for ARBs. It is well established that bacteria can transfer genes within the same species or between different species through mechanisms such as phagocytic transduction, natural transformation, and plasmid transfer. Resistance genes for sulfonamides, quinolones, tetracycline, aminoglycosides, β-lactams, and vancomycin have been identified in animals. ARBs and ARGs perpetually circulate within soil, plants, animals, and contribute to the spread of resistant microorganisms among diverse living organisms.

7. VECTOR BORNE ZOONOTIC DISEASES

Most instances of zoonotic diseases characterized by antibiotic resistance are often perceived as resulting from direct contact between the host and recipient. The intricate interplay among the resistant pathogen, the host, and humans is frequently influenced by vector-borne diseases, introducing unforeseen complexities. Compared to directly transmitted antibiotic-resistant infections, vector-borne zoonotic diseases (VBZDs) represent a sizable category of zoonotic illnesses with an increasing occurrence rate. Since VBZDs make for 22% of all newly developing infectious illnesses in humans, they are disproportionately prevalent (Jones et al. 2008). The main vectors of these diseases are hematophagous or blood-feeding arthropods. Mites, ticks, flies, and mosquitoes make up more than 90% of the vectors that cause emergent VBZDs. Ticks like Ixodes and Amblyomma transmit a number of common bacterial illnesses, including ehrlichiosis, Rocky Mountain spotted fever, and Lyme disease.

8. DYNAMICS AND DRIVERS OF VECTOR BORNE DISEASES

Vector-borne diseases are influenced by multiple factors that facilitate the transmission of infection from the original host to the recipient. A significant driver of vector-borne diseases is the reduction in host abundance. This phenomenon has been closely linked to a decline in rodent populations, which serves as the primary host, causing Yersinia pestis to transition to human populations through vectors as an alternative host (Jones et al. 2008). Environmental changes and human activities stand as crucial factors that shape the dynamics of vector-borne diseases. Over the past three decades, vector-borne zoonotic diseases (VBZDs) have extensively emerged in new geographical areas. The emergence of VBZDs primarily results from the pathogen's shift from its original host to humans due to human-induced activities. While local environmental changes like urbanization and deforestation play a role in local regions, trade and travel are the primary drivers of events in new regions. In order to treat patients

and promote behaviors that lower the risk of infection, doctors and public health officials must work together to control vector-borne zoonotic illnesses. In order to combat the ecological factors that drive the spread of disease, urban planners, disease ecologists, and medical entomologists must all work together to help direct the growth and restoration of ecological communities and put vector control strategies into place.

9. QUORUM SENSING

By accumulating multiple genotypic and phenotypic mechanisms that confer resistance against widely used antibiotics, bacterial populations are evolving into superbugs. Quorum sensing (QS), which enables microorganisms to interact using tiny signal molecules, is one such method (Kalia et al. 2019). In order to create biofilms that enable bacteria to withstand antibiotic dosages up to 1000 times higher, QS relies on the cell density phenomenon. By producing dangerous molecules through QS regulation and circumventing defensive mechanisms, resistant bacteria use information exchange to spread infectious diseases among animals and people (Kalia 2013). A promising approach is Quorum Sensing Inhibition (QSI), which involves inhibiting the QS process.

Quorum sensing inhibitors (QSIs) exert their effects through diverse mechanisms, encompassing:

1. Halting the synthesis of signal molecules
2. Enzymatically breaking down signal molecules
3. Competing with signal molecules for receptor binding sites
4. Disrupting the binding of signal molecules to gene promoters, thus inhibiting gene expression
5. Scavenging autoinducers through antibodies and macromolecules like cyclodextrins (Fetzner 2015).

QSIs come from a variety of sources and have shown promise as anti-pathogenic substances (Dafale et al. 2016). It has been shown that some enzymes, including as lactonases, acylases, oxidoreductases, and lactonases with properties similar to phosphotriesterase, can degrade QS signal molecules (Fetzner 2015). It is known that a variety of plants produce QSIs that either weaken QS signals or compete with them for signal receptors. *Emblica officinalis, Curcuma longa, cinnamon,* grapefruit, *Medicago truncatula,* and other edible fruits and plants have all demonstrated usefulness as extracts against diseases brought on by plant pathogens. By concentrating on the QS signaling pathway, a QSI reduces the pathogenicity of pathogenic bacteria that are resistant to it. It's important to note that QSI doesn't necessarily affect bacterial growth (Koul et al. 2016; Koul and Kalia 2017), but rather impedes the establishment of a bacterial community among resistant pathogens. Therefore, the combined application of QSIs and antibiotics holds promise as an approach for treatment.

10. FATE OF ANTIBIOTICS IN ENVIRONMENT

Non-metabolized antibiotics are consistently released in the environment, including water and soil, from sources such as veterinary practices, hospital waste, pharmaceutical industries, poultry and dairy operations, households, and municipal waste disposal (Cycoń et al. 2019), posing pollution concerns. Antibiotics are frequently identified in various aquatic settings like rivers, groundwater, and lakes, originating from wastewater treatment plants (WWTPs), surface runoff, or aquacultural activities. Due to the fact that they acquire unmetabolized antibiotics through wastewater from various sources, WWTPs are acknowledged as important nodes for the evolution of ARBs and ARGs (Kapley et al. 2016). A few nanograms to milligrams of antibiotics per kilogram of soil are also discovered in soil, in addition to water bodies (Jiang et al. 2010). Sewage sludge, manure, and locations where there are livestock populations are the main causes of antibiotic presence in soil.

Antibiotics are continuously being introduced into the environment, exposing aquatic and soil species to them. Through gene mutations or horizontal gene transfer from other resistant germs, environmental microbes may gain resistance to these particular medicines. Antibiotics entering different water bodies and soil pose serious dangers to human health and also harm numerous biological ecosystems, even at low amounts. Antibiotic use and distribution contribute to the growth and ubiquity of ARGs worldwide. Severe infections are linked to the ongoing growth in ARG and ARB strains.

Following a cycle of bioaccumulation, partial biotransformation, and slow deposition in soil or water, antibiotics diffuse from sources into the environment (Binot et al. 2015). Antibiotics naturally persist in the environment, which causes them to build up at higher quantities and spread farther. Although certain antibiotics, like penicillins, are easily degraded, others, including fluoroquinolones, macrolides, and tetracyclines, demonstrate greater persistence because of their longer half-lives, enabling them to stay in the environment for longer periods of time (Nimonkar et al. 2019). The environmental half-lives of antibiotics range from 1 to 3466 days. According to Walter et al. (Walters et al. 2010), azithromycin had half-lives ranging from 408 to 3466 days and ofloxacin had half-lives ranging from 866 to 1733 days in a sand clay loam in the United States. Notably, even antibiotics within the same groups can have differing half-lives, potentially influenced by varying conditions, compositions, and soil variations. The degradation of antibiotics in the soil is often impacted by diverse functional groups, which exhibit a wide range of half-lives in the soil. The extended persistence of antibiotics in the environment provides ample time for genetic changes to occur in bacteria residing within their respective niches.

11. DIFFERENT APPROACHES FOR ANTIBIOTIC DEGRADATION

The increased use of antibiotics and their release into the environment affect the biological processes that affect water quality. Therefore, controlling the formation of novel resistant strains and ARGs depends on the degradation of antibiotics. Antibiotics undergo degradation through both non-biological (abiotic) and biological processes. This degradation is facilitated by various methods, including bioelectrochemical approaches, hydrolysis, mechanisms involving adsorption, metal-assisted photolysis, oxidation, as well as reduction.

12. PHYSICO-CHEMICAL DEGRADATION OF ANTIBIOTIC

The abiotic degradation is predominantly influenced by the molecular structure of antibiotics and physicochemical properties. For instance, ß-lactams are more susceptible to hydrolytic degradation compared to sulfonamides and macrolides. The degradation of fluoroquinolone antibiotics primarily occurs through photo-degradation. In a study by Wajahat et al. 2019, two methods were investigated for the degradation of commonly used antibiotic ciprofloxacin: anatase-facilitated photocatalysis and ozonation. Their findings indicated that the photocatalysis process resulted in a higher degradation rate in comparison to ozonation. The treatment of wastewater through UV radiation and chlorination aids in decontaminating wastewater, thereby reducing the antibiotic levels. Natural polysaccharide chemical conjugates can be used to suppress the growth of resistant bacteria, halting the proliferation of ARBs and ARGs, in addition to degrading antibiotics. A process known as adsorption and desorption of antibiotics occurs in the soil when drugs bind to particles and produce complexes that reduce their antibacterial effectiveness. In this sorption process, the soil's pH and water-retentiveness both play significant roles. For instance, sulfonamides, an antibiotic, may shift from their cationic form to neutral

and anionic forms as they are absorbed by the soil. Degradation of abiotic antibiotics is influenced by a number of physical and chemical factors. Abiotic treatment may be hindered by changes in salt concentration, pH, and the presence of other substances in the system. In such circumstances, biotic activities that employ microbes may be crucial in eliminating lingering antibiotics from the environment.

13. DEGRADATION OF ANTIBIOTIC THROUGH BIOAUGUMENTATION

Antibiotics are broken down through the metabolic processes of bacteria during biotic degradation. ARGs are the main component of microorganisms that take part in the bioremediation of antibiotics, as emphasized in reference (Purohit et al. 2016). ARGs effectively break down the parent antibiotic or its functional groups, releasing byproducts in the process. Antibiotics can be successfully removed from industrial effluents via the bioaugmentation technique. Antibiotics have been extensively removed from soil and wastewater using bacterial strains. For instance, *Labrys portucalensis F11* and *Rhodococcus sp. FP1* were used by (Maia et al. 2018) to break down routinely used antibiotics like Ofloxacin and Levofloxacin (Walters et al. 2010). Similar to this, it has been reported that bioaugmentation of a membrane bioreactor with *Achromobacter dentrificans* improves the elimination of sulfamethoxazole (Leng et al. 2017).

Antibiotic-degrading bacteria are primarily found in anthropogenically altered habitats such soil, excrement, sludge, and seawater. In a study by Hirth et al., they added *Microbacterium sp. C448* to soil that contained sulfamethazine, and the antibiotic was reportedly degraded by 59.23% as a result (Hirth et al. 2016). Another investigation revealed that *Ochrobactrum sp.* degraded erythromycin at a rate of 97% (Zhang et al. 2017). Bioaugmentation has become a useful remediation method for antibiotics in the environment. This method plays a key role in shortening the half-life of the antibiotic by enhancing the breakdown of unwanted chemicals and completing the functions of native bacteria.

14. TRANSBOUNDARY SPREAD OF AMRS

The global dissemination of ARGs and their potential transmission between animals, humans, and environment presents a significant challenge in mitigating the threat of AMR. Notable contributors to the spread of resistance include animal farming, aquaculture industries, wastewater treatment plants, and waste generated by hospitals. ARGs can be transferred by various mechanisms, including zoonotic transmission, horizontal gene transfer, or by the environment to the opportunistic human bacteria, often facilitated by genetic elements. In addition to these factors, airborne microorganisms are frequently found to be resistant to multiple drugs, posing a significant risk of health to both animal and human populations through airborne transmission. The existence of airborne AMR has been documented in the scientific literature. For instance, Pal et al. (2016) examined the smog in Beijing and discovered the presence of a wide variety of 64.4 different types of ARGs, above levels observed in other environments (Pal et al. 2016). Tetracycline-resistant genes were found, according to Li et al. (2016), in the indoor air of human-inhabited places in Colorado (Li et al. 2016). Beta-lactam resistance genes were also found in a Californian urban park (Echeverria-Palencia et al. 2017). The distribution of airborne AMR is influenced by factors such as antibiotic usage, meteorological conditions, physicochemical parameters, and the microbial community. The efficient global transportation network and frequent human travel significantly contribute to the dissemination of airborne ARGs and ARBs across various cities. These airborne AMRs are now recognized as biological pollutants, capable of disturbing the gut flora of human and impacting the immune system when inhaled. Even in isolated areas where antibiotics are not often used, the aerial spread of AMRs exposes these areas to second-hand ARGs that have grown in other regions and been transported there.

Research in the field of airborne AMR development remains underexplored and requires attention to understand the patterns and spread of AMRs through the air.

15. CONCLUSION

Escalating environmental pollution represents a significant global threat that has garnered increased attention in recent times. In the livestock industry, the imprudent use of antibiotics fosters resistance within the gut microbiome, essentially acting as a bioreactor for cultivating pathogens. This practice elevates the likelihood of new ARGs emerging and spreading throughout the environment. Similarly, unmetabolized antibiotics are excreted into the environment, disrupting the biogeochemical cycle through the food chain.

The presence of resistant bacteria and the suppression of indigenous microorganisms lead to alterations in microbial community, nitrogen cycle, enzyme activity, and carbon assimilation. Future research efforts should prioritize the examination of antibiotic persistence, bioaugmentation, accumulation, biotransformation, and biostimulation, in the environment, as these factors heighten the risk of breeding ARBs through horizontal gene transfer (HGT) events. In pursuing these objectives, it's essential to consider the interplay between biotic and abiotic degradation processes.

Given that the animal gut serves as a significant breeding ground for antibiotic resistance, it is crucial to regulate and investigate the emerging resistance patterns using a "one health approach." This approach involves establishing an ARBs monitoring system to facilitate a timely public health response. Furthermore, the creation of a coordinated response involving numerous agencies is urgently needed. By coordinating efforts, it would be easier to communicate, deliver timely information, and spread awareness of the proper use of antibiotics in the aquaculture, animal husbandry, and agricultural sectors. These actions are anticipated to improve the environmental efficacy of both novel and existing antibiotics in addition to managing human and animal infections. Additionally, there will be a decrease in the propagation of antibiotic resistance among zoonotic diseases, which will lower the incidence of life-threatening infections in people that are resistant to treatment.

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Zoonotic Aspect of Methicilin Resistant Staphylococcus Aureus

19

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ABSTRACT

Antibiotic-resistant bacteria have become an astoundingly painful issue for the security of global public health due to their exponential rise and persistence. A major player in antibiotic-resistant groups of bacteria is *Staphylococcus aureus*. It is a ubiquitous organism with superior adaptive and survival capabilities making it a perfect study model and an extremely hard enemy to deal with. *S. aureus* can gain resistance against various antibiotics and the most important one of those is the methicillin group. The specific group that gains resistance against this antibiotic group is known as Methicillin Resistant *Staphylococcus aureus* or MRSA. The emergence of MRSA is a primary issue for the healthcare sector as most of the antibiotics commonly used for the treatment of regularly occurring infections are the ones belonging to the methicillin group. Such circumstances call for the initiatives to battle antimicrobial resistance through the development of new antibiotics, the use of alternative medicine and the reduction of the misuse of antibiotics. Hence the researchers need to thoroughly comprehend the mechanism of methicillin resistance gene transmission to prevent its spread and save the human population from the abhorrent future of multi-drug resistant bacteria where every disease will become fatal and all antibiotics will be useless against them.

Key word: Resistant, Bacteria, MRSA, Antibiotic, Methicillin, *Staphylococcus aureus*.

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1. INTRODUCTION

AMR or Antimicrobial resistance is one of the major global health challenges of the 21st century. The approach of “One Health” is being thought out holistically as an important tactic to prevent the rise and spread of multi-drug resistant or MDR bacterial agents. This can help in the preservation of the efficacy of antibiotics already being used publically. The main concept of “One Health” is emphasizing the security of global health through an inter-connected approach of linking the health of humans with that of the environment and animals (pets, livestock and wild). Out of several bacterial pathogens, staphylococci are the ones that have been used as a model organism for the study of “One Health'' relevant subjects. Many of the other organisms cannot be used because those species and their clones have been shown to “jump” across different types of ecosystems being considered for the study. *Staphylococcus aureus* or (*S. aureus*) generally behaves as a commensal. However, it can also act as an opportunistic pathogen that serves as an etiologic agent for causing various types of infectious diseases in both humans and animals. This bacterium has a major effect on public health, the general ecosystem and the production capacity of livestock production (Rossi et al. 2020). The presence of characteristics of *S. aureus* like anti-microbial resistance, virulence and, most importantly, adaptation to the host systems, are of immaculate importance for the public health sector. These characteristics raise concerns regarding livestock, pets and wild animals as they can serve as zoonoses reservoirs or intermittent hosts for reservoirs of zoonoses (Rossi et al. 2020). There has been an increase in interest regarding the exploration of methicillin-resistant *S. aureus* (MRSA) and its characteristics saving from the impact of antibiotics. Though old this trend has rejuvenated since the last decade. However, only a small amount of information is available regarding the global occurrence of MRSA isolates in wild animals. These trends need proper research despite wild animals being claimed as potential transmission vehicles or reservoirs for MRSA (Silveira et al. 2021).

2. METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* OR MRSA

Among the normal *S. aureus* organism, MRSA is present which is the reason for increased cases of hospital-based or nosocomial infections from 1980 to 2000. This rise in the number of cases was noticed in almost all parts of Europe and the world too (Cuny et al. 2015). Only a few countries including the Netherlands and the Scandinavian countries, take serious actions to control and prevent MRSA in the populations (Köck et al. 2014). After this many other European countries also took action against MRSA which resulted in the stop of the high spread of MRSA infection in the last five years. The control measure was very effective in that the rise in infection declined which is seen in some European countries (Cuny et al. 2015). The major and basic control measure which became the cause of the rise in infection rate was the introduction of mandatory surveillance in some countries of Europe for the MRSA bacteria (Johnson et al. 2012). The surveillance was then followed by some search and follow-up techniques region-wide programmed for the control of MRSA in Germany (Ciccolini et al. 2013; Jurke et al. 2013). The healthcare-associated MRSA (HA-MRSA) is separated from the community-associated MRSA (CA-MRSA) based on epidemiological criteria and then grown independently according to the associated risk factors (Salgado et al. 2003). In some regions of the world for example South America and the United States, the infections by CA-MRSA are becoming a great problem of public health security. In European countries, the ratio of cases of CA-MRSA is very low (Li et al. 2014). The major difference between HA-MRSA and CA-MRSA is find out through their functional genomic and structural traits (Otto 2013). The precision of epidemiological differentiation between HA-MRSA and CA-MRSA can be decreased by the presence of HA-MRSA in the community (Tavares et al. 2013) and the presence of genotypic CA-MRSA infection through the nosocomial routes (Liu et al. 2008). Livestock-associated MRSA

(LA-MRSA) can be the reason for infection of MRSA in the community. LA-MRSA normally belongs to livestock (Huijsdens et al. 2006; Lewis et al. 2008; Layer et al. 2012). Using genotypic traits both the HA-MRSA and CA-MRSA can be differentiated due to their genome. The structure of the *S. aureus* species population is clonal and it involves the exchange of pieces of genetic information between different strains which is a rare condition. That is why, a test called multi-lit sequence typing(MLTS), identifies seven housekeeping genes and their allelic profiles. This resulted (sequence types also written as STs) in a strong genomic framework which shows the spread of MRSA at a high level. The clonal complexes (CCs) which are being grouped are sharing at least five of the total seven MLST alleles (Feil et al. 2003). For gene typing, Spa typing (Harmsen et al. 2003) is used around the world. The base of this test is the sequence polymorphism of the X-region of the spa gene. It also gives preliminary attribution to CCs. MRSA was first described as the methicillin-susceptible *S. aureus* (MSSA) which after time evolved with antibiotic resistance. By acquiring the SSCmec elements that had a mec gene (mecA, rarely mecC) it becomes MRSA. These mec genes have codes which can make an additional penicillin-binding protein. This protein has less attraction for the beta lactum group of antibiotics. Due to this low attraction, the capacity of resistance increases against all the members of the antibiotic group except ceftobiprole and ceftarolin. Almost 11 structurally different types of SCCmec have been discovered now (Palavecino 2014). Above mentioned methodologies are the base for major knowledge about the rise and spread of LA-MRSA. The next-generation sequencing techniques are economical, best for sequencing and make it possible for researchers to study the genome of whole bacteria. Ultimately this helps in studying the transmission and evolution of LA-MRSA more effectively as compared to the past studies (Price et al. 2012).

3. TRANSMISSION

The movement of animals across different habitats and their frequent contact with other livestock, wild animals and along with their indirect interception with the human population can increase the transmission of bacteria. This contact often increases the risks of MRSA colonization. Simultaneously, both animals and humans may also acquire infections (Porrero et al. 2014; Swift et al. 2018; Penna et al. 2021). Usually, the sources spreading antimicrobial-resistant bacteria are anthropogenic. These sources may include domestic wastewater effluents and industrial sanitary disposal, runoff from agricultural sites and their wastes. Such sources are suspected to play a major role in linking wild animals with AMR bacteria (Porrero et al. 2014; Rousham et al. 2018). Once a specific type of bacteria gets transmitted to the wild animal populations they can quickly become responsible for transmission of a large number of AMR genes, mobile genetic elements and epidemic clones (Porrero et al. 2014; Rousham et al. 2018). These examples increase the importance of control of bacteria and the implementation of control strategies throughout the ecosystem to decrease the rise in AMR traits of bacteria. MRSA is recognized as a multi-drug resistant (MDR) bacteria. It is most effective against beta-lactam antibiotics because it synthesises a modified form of penicillin-binding protein 2 (PBP2a/c) which cancels the effect of beta-lactam antibiotics. This PBP2a/c protein is encoded by mec genes which are the parts of staphylococcus cassette chromosome mec (SCCmec). These SSCmec genes are also known to be mobile elements of the genetic information which can harbour the gene's AMR for too long (Becker 2021). The origin of the mecC gene for causing MRSA has not been identified till now. In the beginning, the mecC was thought to be related to livestock-associated (LA)-MRSA but after that it was found that the gene is more related to the wild-life (Zarazaga et al. 2018). It means that mecC-MRSA can also be called wildlife-associated MRSA (WA-MRSA) (Zarazaga et al. 2018; Dube et al. 2021). The *S. aureus* is a suitable pathogen for study because of the large range of its hosts. Some of the clonal complexes (CCs) of MRSA have only specific hosts for example MRSA-CC398in pigs and MRSA-CC5 in poultry. On the other hand, CC1 and CC130 show a large number of hosts in which they can cause infection (García-Álvarez et al. 2011; Raafat et al. 2020).

Some of the clones of MRSA can transmit disease in different domains of "One Health". Due to this wide range of hosts, surveillance should be done at a high level to control the infection. The nasal oral discharge from wild animals (WHO 2014) is a major source of MRSA transmission in humans and other animals (Rahman et al. 2020). The rate of prevalence of CCs depends on the area (farmland or urban) and the frequency of interaction with the animals (Morand et al. 2014). The anatomy of the nasal cavity and buccal cavity of animals shows that there is a small path to the trachea and pharynx respectively which makes the entry of microbes in the trachea and pharynx way easier (Morand et al. 2014).

4. ZOONOSIS OF LA-MRSA TO HUMANS

Transmission of *S. aureus* from one host to another primarily happens through physical contact made with the infected host (Fig. 1). The stables of pigs have dust with large amounts of MRSA-colonized contamination in them (Schulz et al. 2012). So, it can be safely assumed that the likelihood of a human getting an infection of MRSA increases in areas with high colonization of it which can be inhaled by humans (Bos et al. 2016). Colonization in the nasal region of humans has been found in 77%–86% of the cases while analyzing the MRSA-positive stables (Cuny et al. 2009; Van Den Broek et al. 2009). The growth of these colonies appears to be dependent upon the period of exposure to the aetiology along with the intensity of contact with the affected animals (Graveland et al. 2011).

5. ZOONOSIS OF MRSA THROUGH CONTACT

For a large number of farmers, the colonisation of MRSA continued even when regular contact with animals was terminated due to holidays (van Cleef et al. 2011; Köck et al. 2012). Other people living on the contaminated farms (e.g., household members) were found to be colonised with lesser frequency ranging from 4%–5% (Cuny et al. 2009). A longitudinal study with comparative analysis performed in Belgium, Denmark, and the Netherlands helped researchers discover that contact with pigs was the most important factor for the carriage and transmission of MRSA by farmers and their household members (Belgium 29%, Denmark 0%, Netherlands 6%). An increase in MRSA carriage rate was observed only in Belgium among household members of farmers. This increase was linked to the differences in country-specific species of pigs facing exposure (Garcia-Graells et al. 2013). A study that was performed in the region of Netherlands was used to conclude that living with an MRSA-positive pig farmer and working with sows meant you have to regularly come in contact with MRSA making it a major determinant of the carriage of MRSA to the household members of the farmers (van Cleef et al. 2015). In 2010 Netherlands initiated a crucial program at the national level to promote reduced usage of antibiotics in farm animals. A longitudinal study of MRSA in Germany considered the colonization of pigs and humans working on pig farms as well as their household members to study the decline in antibiotic resistance with a reduction in the usage of antibiotics. The report claimed a 44% fall in the use of antibiotics at the farms, that had entered in this study was linked with the declining prevalence of both MRSA in pigs as well as LA-MRSA in humans irrespective of the frequency and intensity of animal contact (Dorado-García et al. 2015).

6. RELATION OF HERD SIZE WITH MRSA OCCURRENCE

A study held in Taiwan revealed that the nasal carriage of LA-MRSA ST9 among the pigs was greater in larger herds which is 34% as compared to those with less amount of animals which is only 7%. Also, the risk of infection was high in humans dealing with larger herds was about 36.8% as compared to those who deal with smaller herds which is almost 9.1% (Fang et al. 2014). The colonization of LA-MRSA in the nasal region was found in the workers of slaughterhouses (Mulders et al. 2010) and veterinarians in

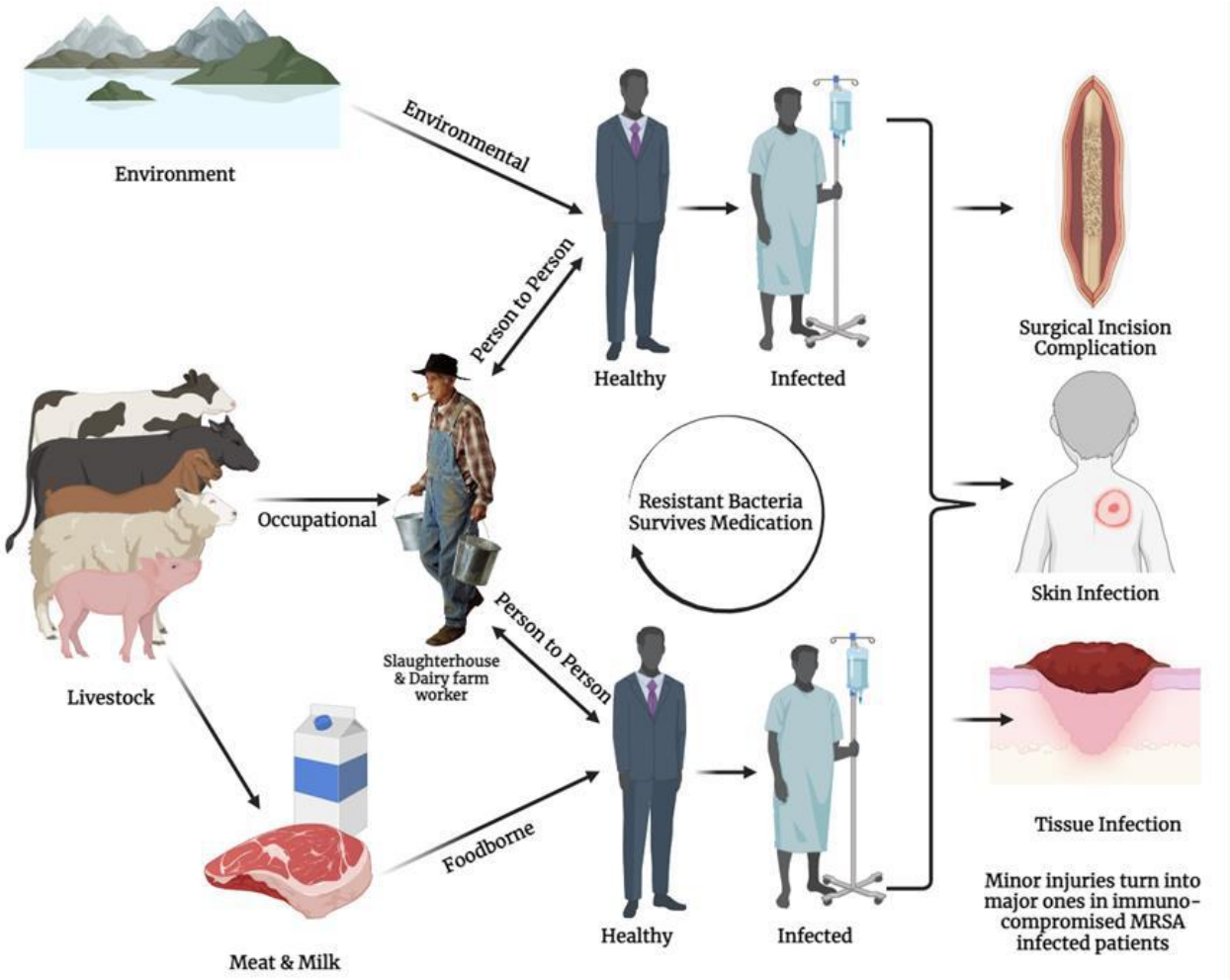

Fig. 1: Zoonotic Transmission of Methicillin-resistant *Staphylococcus aureus*.

Germany (Hermes et al. 2012) and in Belgium (Garcia-Graells et al. 2012). The risk of getting an infection of MRSA is greater in veterinarians due to the close association with livestock. The colonization of MRSA has been seen in the household members of veterinary industry staff (Verkade et al. 2014). To understand the colonization of LA-MRSA in family members of veterinarians whole-genome maps are used to check the possibility of LA-MRSA transmission in humans (Bosch et al. 2015). The major part of this data has been taken from studies which are about conventional farms. The research study in Germany shows that there was no CC398 strain of LA-MRSA in both humans and pigs at organic farms (Cuny et al. 2012). This study revealed that the prevalence of the CC398 strain of LA-MRSA is much less in organically grown pigs compared to those which are reared through conventional methods of farming in the Netherlands (Van de Vijver et al. 2014). The prevalence of LA-MRSA in humans far from farms is less, as proved by research on students of an institution with a large number of pig farming facilities in the northwest of Germany (Cuny et al. 2009). However, the tests of patients admitted to the hospital revealed that the prevalence of LA-MRSA is very high in the northwest region of the country as compared to the whole country (Köck et al. 2013). In the Netherlands, the number of livestock was identified as a risk for livestock-associated methicillin-resistant *S. aureus* (Feingold et al. 2012). Explosions of LA-MRSA were seen many times in the air emitted from pig stables. Research proved the

presence of LR-MRSA in the air expelled from pigs' stables ranges from 350 m downwind to 500 m on the surface of the soil (Friese et al. 2013a).

7. TRANSMISSION ROUTES OF LA-MRSA

The presence of LR-MRSA has also been recognized in the poultry droppings at poultry farms and the soil which is fertilized by poultry litter containing fertilisers (Friese et al. 2013b). Based on this, the discovery of LA-MRSA in the samples of stool collected from rocks in Austria is surprising for the researchers (Loncaric et al. 2013). After all these the main question was about the people living in the area which is surrounded by the livestock farms have a greater chance to get infected by LA-MRSA. So in these areas, the control and eradication of LA-MRSA is more important. In Lower Saxony in Germany, research has been conducted regarding the colonization of LA-MRSA cc389 in the nasal region of humans. This study revealed that the people living around traditional livestock farms have 1% of the nasal colonization of LA-MRSA CC398 (Bisdorff et al. 2012). An epidemiological survey was done in the state of Pennsylvania, USA and the outcomes of the survey were interesting. The state was well known for the high rate of skin problems and soft tissue infection due to the LA-MRSA in comparison to other states. Also, it has been recorded that the people living near the areas around the farms which are fertilized by animal dung are at greater risk of getting infection. This study was limited to some areas due to unfavourable circumstances. The major problem of this was the absence of the typing of several MRSA isolates which are enough to determine both the animal and human origin (Casey et al. 2013). It has also been reported that the isolates of MRSA have been found in dogs and cats. This transmission of LA-MRSA from pets to their human owner enlightens the need for more hygienic conditions in the daily routine (Idelevich et al. 2016). This proves that the case of human-to-human transmission of LA-MRSA CC398 is much less as compared to the zoonotic transmission. The latest studies reveal the data regarding LA-MRSA infection in humans having no contact with animals from Spain (Benito et al. 2014) and Germany (Deiters et al. 2015). The research data from the Netherlands shows that out of all the cases of LA-MRSA CC398 cases, 15% case were humans who never had contact with veal calves or pigs (Lekkerkerk et al. 2015) LA-MRSA infection is also found in person who has to deal with contaminated meat products. The main routes of transmission are human-to-human contact and environmental exposure. Most preferably this applies to food-handling personnel. A statistical study in the Netherlands shows that people with regular consumption of chicken meat have a high risk of getting LA-MRSA infection (Van Rijen et al. 2013).

8. PHENOTYPIC IDENTIFICATION OF MRSA AND VRSA

For the colonies of MRSA and VRSA identification can be done using respective antibiotics like the oxacillin (1µg) and vancomycin (30µg) placed on the activated colonies of *S. aureus* (0.5 McFarland) on agar plates of Muller Hinton respectively. The plates should be then incubated at 37°C for 24 hours. Growth inhibition zones can be observed around antibiotic discs and are measured by vernier callipers. These measurements are then compared with the standard inhibition zone diameters of the respective antibiotics as described in the Clinical and Laboratory Standard Institute (Javed et al. 2021). The isolates of bacteria with resistance towards the antibiotics are then named respectively. So, if one isolate is resistant to oxacillin discs it will be declared as the methicillin-resistant strain of *S. aureus* also known as (MRSA). On the other hand, the strain that was killed by antibiotics and found to be sensitive to oxacillin will be named methicillin-sensitive *S. aureus* or (MSSA). Similarly, the bacterial isolates showing resistance towards vancomycin discs were termed vancomycin-resistant *S. aureus* or (VRSA). Now the rest of the isolates that did not survive against vancomycin discs were claimed to be vancomycin-sensitive *S. aureus* or (VSSA) (Javed et al. 2021).

9. CONCLUSION

Methicillin-resistant *S. aureus* or MRSA has emerged on the horizon of public health as a cloud of disease blurring out the sunshine of health. It is an *S. aureus* with additional genes of resistance against the methicillin group of antibiotics. It can be simply identified by an antibiotic sensitivity test with antibiotic discs of the methicillin group. MRSA has antibiotic resistance due to special mec genes that encode for penicillin-binding protein. This protein helps the bacteria in nullifying the effectiveness of beta-lactam antibiotics.

MRSA genes are highly transmittable due to the ubiquitous nature of *S. aureus* bringing the difficulty of its control and eradication up by many levels. Although the rapid spread of MRSA worldwide has raised an alarm among public health entities. Additionally, its abundant routes of transmission make its control even harder. There are several varieties of MRSA known as clonal complexes (CCs) that infect both animals and humans. Most important and widespread of all these are CC398 and CC130 etc. Other classifications of MRSA include livestock-associated MRSA and wildlife-associated names according to their sources and reservoirs. Knowledge of types and sources can help humans understand MRSA transmission and formulate control policies accordingly as done successfully by various European countries.

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One Health Approach: Combating Antimicrobial Resistance and Zoonotic Diseases in a Connected World

20

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ABSTRACT

The complex interaction among humans, animals, and the environment significantly influences the emergence and transmission of infectious diseases. Zoonotic diseases, originating from animals and transmissible to humans, pose substantial global health risks. This chapter delves into the multifaceted nature of zoonotic infections, emphasizing their prevalence, impact on public health, and ties to antimicrobial resistance (AMR). Approximately 61% of human pathogens have zoonotic origins, highlighting the critical need for a comprehensive 'One Health' approach. Zoonoses not only cause significant illness and mortality, with approximately 2.7 million deaths annually and a staggering 2.4 billion cases of illness, but also impact livestock workers in low- and middle-income countries disproportionately. These diseases create a cascading effect, influencing both human health and animal productivity. The interconnectivity between humans, animals, and ecosystems serves as a breeding ground for opportunistic infections and the proliferation of drug-resistant pathogens. Antibiotic misuse in both human and animal settings exacerbate the development of antimicrobial resistance, which presents a growing global concern. The persistence of antibiotics in the environment further amplifies this issue, contributing to the proliferation of antibiotic-resistant bacteria (ARBs). The spread of ARBs, facilitated by various factors including animal husbandry practices, agricultural activities, and environmental contamination, amplifies the challenge of managing infectious diseases. Addressing the intricate relationship between zoonotic infections, antimicrobial resistance, and environmental impact necessitates a holistic 'One Health' approach. Collaboration across disciplines, encompassing veterinarians, ecologists, epidemiologists, and healthcare professionals, is crucial for effective surveillance, prevention, and control strategies. Integrated efforts involving rigorous surveillance systems, technological advancements, and public awareness campaigns are imperative to combat the spread of zoonotic diseases and antimicrobial resistance.

Keywords: Zoonotic diseases, Antimicrobial resistance, One Health approach, Environmental impact, Interdisciplinary collaboration, Public health

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1. INTRODUCTION

An important factor in the formation and spread of several infectious diseases is the interaction between people, animals, and the environment (Rahman et al., 2020). Animals are the primary vector of transmission for most infectious diseases that affect people. The "Asia Pacific Strategy for Emerging Diseases: 2010" paper states that over 60% of new human illnesses are caused by these viruses, most of which have their origins in wildlife species (Organization, 2011). There have been recent findings linking animal-based diets to human diseases with animal origins (Slingenbergh, 2013).

The word "zoonoses" comes from the Greek words "zoon" (animal) and "nosos" (disease). The World Health Organization (WHO) defines zoonosis as any illness or infection that can spread naturally from vertebrate animals to humans or from humans to animals (Organization, 2022). The origin of about 61% of human pathogens is zoonotic (Taylor et al., 2001).

Zoonoses is a direct risk that poses a major harm to human health and has the potential to be lethal. An estimated 2.7 million people die and 2.4 billion cases of illness are caused by the 13 most frequent zoonoses worldwide each year, with an emphasis on the impoverished livestock workers in low- and middle-income nations (Fig. 1) (Grace et al., 2012). The majority of these ailments have an impact on animal health and reduce cattle output (Grace et al., 2012).

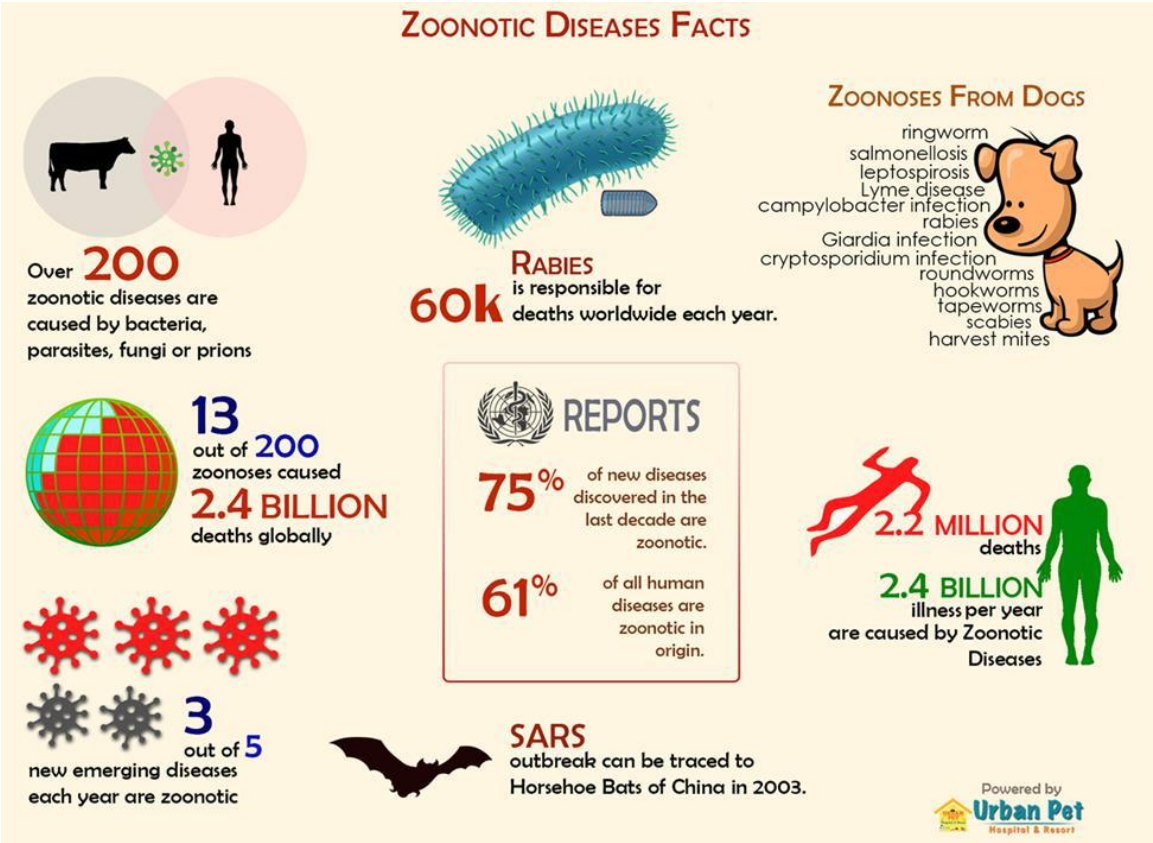

Fig. 1: Zoonotic diseases facts.

In recent years, managing human health has presented new difficulties. A major global problem is the introduction of new opportunistic infections and medication resistance in existing diseases. The majority of developing illnesses have penetrated virtually every ecosystem and crossed transboundaries. According to the emerging hypothesis, the development of disease patterns takes into account how pathogenic determinants breed in ecosystems using any potential biological hosts that may be present. Animals are the most typical biological hosts for the propagation of pathogens with resistance. Animal guts are made up of a variety of microbial communities that work together to benefit their host (Purohit, 2018).

The introduction of a drug-resistant pathogen modifies the community structure by dispersing the resistant gene to other pathogens in the gut. Human health is at risk from opportunistic infections that spread from animals to people through direct contact (zoonoses) or vectors. The ''one health'' approach is now taking into account a situation where humans and animals are interconnected through ecosystems (Purohit, 2018).

Zoonoses, or the transmission of illnesses from animals to humans, are a serious global problem. The zoonotic infections may ultimately result in new epidemic diseases, irregular disease outbreaks, or an odd curiosity specific to the area. All factors influencing the emergence of novel zoonotic illnesses are managed under the "one health" philosophy. One health strategy connects the environment, animals, and people. One health approach is required to address the rise in antibiotic resistance in the human population brought on by zoonotic diseases. For efficient investigations, the one health approach to zoonoses management uses interdisciplinary teams comprised of doctors, ecologists, entomologists, epidemiologists, and ornithologists (Zakaria, 2020).

The discovery of antibiotics in the early 1900s has revolutionized human health and saved millions of lives. Antibiotics are complex chemicals that stop microorganisms from growing in a number of different ways, such as changing cell membranes, blocking the formation of cell walls, inhibiting the synthesis of nucleic acids and proteins, and creating a competitive environment. To avoid infectious infections, antibiotics are used in livestock and animal husbandry to boost the output of dairy products and meat. On a broad scale, it is also employed for enhancing the weight and growth of animals. Even though antibiotics are helpful, their unchecked use and environmental spread constitute a serious worry (Parmar et al., 2018).

The majority of antibiotics used by people and other animals do not completely break down in the body, releasing unmetabolized forms into the environment (Kalia, 2015). They leave the body and enter a different environment where they pass through sewage sludge, animal waste, and municipal wastewater. The development of ARBs is under selection pressure due to the increasing concentration of antibiotics in various settings, which changes the sensitivity of the bacterial population and increases antibiotic resistance in the local microbiome (Parmar et al., 2017).

Antibiotic resistance genes (ARGs) are becoming more prevalent due to environmental antibiotics altering the genetic makeup of bacteria. The frequency of ARBs in the environment is increased by the spread of ARGs to other microbial communities via related mobile genetic elements (MGEs) such as plasmids, transposons, and genomic islands. These ARBs grow into potent zoonotic pathogens that can seriously infect people worldwide (Parmar et al., 2017).

The use of antibiotics in veterinary care and on animals used for food production is encouraging the formation of ARBs in both normal bacterial flora and zoonotic pathogens. A critical issue for modern medicine is the link between AMR and animal and human illness. Even though there is a lot of research being done on zoonotic infections, there is still a lack of adequate management, regulation, and human usage of antimicrobial agents (Thakur & Gray, 2019).

Additionally, the use of antibiotics in manure for agricultural purposes spreads the antibiotics throughout several ecological niches, including the soil and water. Antimicrobial spread from the soil to water sources and subsequent ecological niches. Antimicrobial resistance (AMR) in the environment is the leading health concern, according to the World Bank's most recent One Health approach framework ((Berthe et al.,

2018)). Environment, aquaculture, and wildlife are the focus of one AMR prevention strategy (Thakur & Gray, 2019).

2. THE ONE HEALTH APPROACH

AMR can be fatal, yet there are effective, adaptable remedies that are still hidden. Due to its multidimensional, linked, and diverse ecological properties, understanding the AMR pattern is difficult. The right usage of antimicrobial products in diverse areas must be decided upon by individuals and society as a whole to control widespread resistance. The complex AMR scenario necessitates a multi-sectoral approach to supervision. Teams from the veterinary, environmental, and healthcare fields as well as stakeholders should be involved in this strategy. An approach known as "One Health" encourages interdisciplinary collaboration between academics and decision-makers who work at the local, state, federal, and global levels (Fig. 2) (Binot et al., 2015).

Fig. 2: One Health Policy

This strategy aims to improve environmental, animal, and human health outcomes. The ''one health'' strategy places a high priority on the spread of AMR-associated pandemic diseases.

Through the development of appropriate usage guidelines, the transmission of effective risk messages, and an appreciation of the AMR problem, this approach brings together a wide range of sectors with active players in the field. Another issue that requires a more thorough explanation is the spread of AMR. This problem is related to the migration of bacteria between human hosts, animals (both domestic and wild), and the environments in which each of these groups of bacteria can proliferate (Binot et al., 2015).

Understanding how AMR spreads through the air, water, and soil is essential because the environment serves as a reservoir for resistant microorganisms and ARGs as well as a conduit for the disease's transmission between host animal and human populations. AMR can be managed within a single health approach by implementing several factors, such as (1) standards and restrictions for the critical usage of different kinds of antibiotics used for animal and human health, and (2). (3) Well-established and cutting-edge approaches using cutting-edge technologies for assessing AMR, encompassing herd management and animal husbandry (4) Successful communication techniques to raise customer knowledge of the dangers of AMR transmission from the food industry and other nonhuman sources (Binot et al., 2015).

Everyone is impacted by the issue of understanding zoonoses-mediated AMR, including the scientific community, food animal producers, medical professionals, patients, and consumers. "One Health" suggests several strategies to stop the transboundary and zoonotic spread of AMR to maintain the efficient use of antibiotics in the treatment of both humans and animals.

3. ZOONOTIC PATHOGENS AS AMR CARRIER

In the past, chronic illnesses, antibiotic resistance, and environmental pollution have all put human and animal health in jeopardy, increasing death and morbidity rates (Kalia et al., 2014).

The incidence and dissemination of zoonoses, epizootics, and epidemics have brought attention to the elevated risk to global health and the significance of comprehending the transfer of infectious agents from animals to humans. The majority of infectious diseases are thought to have zoonotic origins and are considered serious health issues A zoonotic disease, also known as a zoonosis, is defined by the World Health Organization (WHO) as "any disease or infection that is naturally transmitted between vertebrate animals and humans". An infectious agent that causes disease could be a virus, fungus, bacteria, parasite, or prions. Approximately 200 zoonoses are known to exist in the globe today; some are restricted to a certain region, while others are said to have a global distribution (Kalia et al., 2014). Transmission methods for zoonotic pathogens include ingestion, inhalation, and other methods that contaminate mucosal membranes.

Furthermore, zoonotic diseases can be transmitted by eating foods containing animal tissue, such as raw meat, unpasteurized milk, dairy products, shellfish, and contaminated vegetables. Anthrax, animal influenza, bovine tuberculosis (BTB), brucellosis, hemorrhagic colitis, zoonotic diphtheria, rabies, and Q fever are other well-known zoonotic illnesses (Kalia et al., 2014).

Numerous factors, including biological, genetic, social, ecological, political, physical, and environmental ones, interact to cause the incidence of zoonotic diseases. More than sixty percent of human infectious diseases are caused by zoonotic pathogens. The most common zoonotic infections among them are ARBs, which are frequently present in the environment. The host immune system and antimicrobial medications exert considerable selection pressure on the bacteria and shorten their growth time. These interactions with pathogen-host species that act as a reservoir of infection have caused these communities to undergo major evolutionary modifications that impact human health. Few antibiotics are effective against Staphylococcus species., Proteus mirabilis, Pseudomonas aeruginosa, and Klebsiella pneumonia (Hathroubi et al., 2018).

According to one health strategy, the development of ARBs and the rising use of antibiotics in agriculture and aquaculture are related. Through the transmission of zoonotic diseases, the ARBs intrude into the human gut and disrupt the gut's natural diversity. When humans contract zoonotic infections with ARGs,

the resistance genes are transferred to the human gut microbiome, which modifies the equilibrium of the gut environment. Human excretion of ARGs or ARBs finds its way into the environment through soil or municipal wastewater. After being dispersed by soil or wastewater, ARBs damage animal health and enter the food chain, perpetuating the cycle of ARB transmission (Hathroubi et al., 2018).

4. GLOBAL CHALLENGES AND FUTURE DIRECTIONS

The soil microbial community is negatively impacted by the ongoing release of antibiotics into the environment through animal feces, manure, urine, and sewage sludge.

Antibiotics are given to animals to treat illnesses, but often the antibiotics do not break down in the bodies of the animals, and a sizeable amount of the unmetabolized antibiotics end up in either the soil or urban effluent. The body excretes 60, 50–90, and 75–80% of the dosages of erythromycin, tetracycline, and lincomycin, respectively, according to numerous reports. Tetracyclines are the next most commonly found compound in manure, after fluoroquinolones, sulphonamides, and macrolides. The overabundance of antibiotics in soil eventually alters the susceptibility of the microbial population to antibiotics by promoting the growth of ARBs (Kapley et al., 2016).

The use of antibiotics in soil environments helps bacteria change their genetic makeup and spread ARGs among themselves. Autochthonous soil bacteria become resistant to ARGs as a result of the spread of ARGs, which also makes them a source of ARGs in the environment (Kapley et al., 2016).

In addition to changing genetic, structural, and functional diversity, antibiotics in the soil also have an impact on microbial activity, enzyme activity, the nitrogen cycle, and carbon mineralization. The human body absorbs ARBs from the soil and water environment, where they multiply in the stomach and change how susceptible people are to antibiotics. The development of novel enzymes and genes that confer bacterial resistance has also been linked to the presence of antibiotics in soil, according to research (Kapley et al., 2016).

5. SURVEILLANCE AND EARLY WARNING SYSTEMS

For the protection of public health and global health, it is essential to monitor zoonotic illnesses (diseases that can be spread from animals to people) and antimicrobial resistance (AMR). To efficiently identify, track, and respond to these risks, a variety of technologies and strategies are used. The following are some crucial tools and techniques for tracking AMR and zoonotic diseases:

5.1. GENOMIC SEQUENCING

The rapid sequencing of pathogen genomes made possible by next-generation sequencing (NGS) can be used to find zoonotic pathogens and monitor their evolution. Metagenomics is useful for surveillance since it can identify a variety of infections and AMR genes in a single sample.

5.2. POLYMERASE CHAIN REACTION (PCR) IN REAL-TIME

In clinical samples, PCR can rapidly and precisely identify particular infections or AMR genes, assisting with early diagnosis and tracking.

5.3. GISP: GEOGRAPHIC INFORMATION SYSTEMS

To aid in surveillance and response planning, the spatial distribution of zoonotic illnesses and AMR hotspots are mapped using GIS technology.

5.4. TECHNOLOGIES FOR MOBILE AND WEARABLE HEALTH

Mobile apps and wearable technology can monitor people's health and gather information on disease symptoms and potential exposure, helping early warning systems.

5.5. TELEHEALTH AND TELEMEDICINE

Telemedicine makes it possible to monitor and confer with patients from a distance, lowering the danger of disease transmission and enhancing access to medical care.

5.6. TECHNOLOGIES FOR MONITORING ANIMALS

Animal populations are tracked using tracking and monitoring tools (such as GPS collars and RFID tags) to assist in finding potential disease reservoirs.

5.7. MODELING OF THE EPIDEMIOLOGY

Public health decision-making is aided by the use of mathematical models and machine learning algorithms to predict the development of zoonotic illnesses and AMR.

5.8. LABORATORY TRUCKS

To quickly test for zoonotic infections and AMR, portable laboratories with diagnostic equipment such as PCR machines can be deployed to remote locations.

5.9. AI ANALYTICS WITH BIG DATA

Large amounts of data from many sources, including social media, news articles, and clinical records, can be analyzed with the aid of big data analysis and artificial intelligence to find disease outbreaks and novel AMR patterns.

5.10. ONE HEALTH ARRANGEMENTS

The interdependence of human, animal, and environmental health is emphasized by the one health movement. This strategy encourages cooperation across many industries and academic fields to monitor zoonotic diseases and AMR thoroughly.

5.11. OBSERVATIONAL NETWORKS

Regional, national, and international surveillance networks and reporting systems enable information sharing and early warning of zoonotic disease outbreaks and AMR trends.

5.12. TECHNIQUES FOR MOLECULAR TYPING

AMR-causing pathogens can be tracked down using two methods: multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE), which both identify the source of the pathogens and their routes of transmission.

5.13. SEROLOGICAL EXAMINATIONS

Retrospective diagnosis and seroprevalence investigations are made easier by the ability of serological testing to identify antibodies to certain diseases.

5.14. BIOSENSORS

Pathogens or AMR indicators can be found in many samples using biosensors, which are frequently based on antibody-antigen interactions or molecular recognition. To assure the early detection and prompt reaction to growing threats to human and animal health, effective monitoring of zoonotic illnesses and AMR necessitates a multi-pronged approach that incorporates these technologies, as well as worldwide collaboration and data sharing.

6. THE IMPORTANCE OF RESEARCH AND COMMUNICATION

One kind of wildlife interaction with discernible potential drawbacks is wildlife-associated zoonotic illness (Vaske et al., 2009; Wobeser, 2013). However, despite worries that public support for animal research may decline due to misperceptions about zoonotic disease risks, nothing has been done to better understand these beliefs and potential consequences for the One Health Initiative (Brook & McLachlan, 2006; Stronen et al., 2007). Trends and predicted trajectories for zoonotic illness in North America, where infectious disease outbreaks occur more frequently than ever in contemporary times, show the necessity for such research (Jones et al., 2008). Even after accounting for enhanced monitoring and reporting efforts, an analysis of 335 distinct illness occurrences in the global human population from the 1940s to the 1990s showed that the frequency of new zoonoses originating in wildlife rose every ten years (Jones et al., 2008).

The primary drivers of rising disease incidence and prevalence include an increasing human population, global migration of people and animals, and the encroachment of agricultural and urban development on wildlife habitat (Vaske et al., 2009; Wobeser, 2013). Since these trends are likely to continue, it becomes sense to incorporate animal health as a keystone of the One Health concept.

Public support for healthy wildlife populations can be increased by health communications if they convey the idea that safeguarding the health of wildlife also protects human health and wellbeing. People may distance themselves from wildlife by simply becoming more aware of the diseases that are linked to it (e.g., spending less time outdoors where wildlife may be encountered or lessening their support for wildlife protection). Health specialists should better anticipate public reaction to one health message by understanding how and why individuals create their beliefs about diseases related to wildlife. Will people choose to eradicate the diseased species exclusively, or will they accept the notion that shielding wildlife from illness may also shield humans from zoonotic illnesses? A better way to address this question would be to examine the drawbacks of One Health messaging.

7. EXISTING AND NEEDED RESEARCH ON PERCEPTIONS OF ZOONOSES AND WILDLIFE HEALTH

Public worry about zoonoses has hardly been explored by research. Vaske et al. (2009) conducted a recent meta-analysis of human dimensions research on wildlife diseases, which highlights the paucity of studies on most diseases associated with wildlife as well as the incompleteness of these studies' assessments of the variety of possible consequences of these diseases (Vaske et al., 2009).

Rather, risk perception analysis for zoonoses has mostly concentrated on the kinds and intensities of anxiety associated with specific diseases within specific populations (e.g., rabies concerns among recreational cave divers and speleological societies, or chronic wasting disease concerns among Midwesterner hunters).

Each study adds something to the body of knowledge, but they only begin to explain how or why different animal diseases have different risk estimations. We concur with the 2009 recommendation made by Vaske and colleagues for a thorough approach to research on the human aspects of animal-related illnesses (Vaske et al., 2009).

Information regarding risk perceptions and disease development is scarce for most diseases. Given the wide variations in wildlife-associated diseases and the environments in which they arise, it is critical to determine the factors influencing risk perceptions of various diseases in various contexts. For communicators to create effective One Health messages, information on how cultural and other elements, such as social responses, affect risk perceptions of various diseases as well as the same disease across settings would be beneficial. Some researchers have looked at how a wildlife-associated disease's traits affect people's perceptions of danger, although this area of study also requires more development (Peterson et al., 2006).

Perception of current risk Retrospective studies on zoonoses describe how specific groups saw a disease outbreak. It doesn't reveal how a population with a particular set of traits could react to a future outbreak. Further study is needed on a component of risk analysis that can forecast reasonably both the degrees and types of worry about a risk related to wildlife.

Research on zoonoses has concentrated on a variety of issues, such as how they affect the local economy in addition to how they affect the health of people, domestic animals, and wildlife. While some studies have looked at a variety of potential issues, the majority have only looked at those that have to do with human health. Other studies have asked respondents merely "how concerned" they are about a particular disease without disclosing the specifics of their worries (Dorn & Mertig, 2005; Figuié & Fournier, 2008; Peltz et al., 2007).

The narrow focus of the study makes it difficult to comprehend how zoonotic disease is perceived from a One Health standpoint. The fundamental tenet of one health is that people may be concerned about a disease for non-public health reasons (e.g., aesthetic concerns or the health of the ecosystem). Health risk communicators will not be able to determine whether a message has negative side effects if they do not have a comprehensive understanding of people's anxieties.

Although some studies suggest factors that may influence public concern, empirical research does not identify the specific cultural, social, and health characteristics that influence perceived hazards and for whom these characteristics are relevant. Similarly, studies have not demonstrated the relative importance of zoonoses versus other potentially hazardous wildlife interactions. Given the epidemiology and modes of transmission of certain diseases, it is not surprising that a large number of people are ignorant of the risks connected to specific illnesses. The danger is also questionable because human zoonoses like Lyme disease are difficult to detect. While it's unclear if increased ambiguity makes people worry more about zoonoses than other wildlife issues, risk perception theory indicates that people may feel more "dread" when faced with these kinds of threats (Slovic & Peters, 2006).

8. CONCLUSION

The globe is seriously concerned about the growing environmental degradation, which is why it is getting more attention now. Inappropriate use of antibiotics in the cattle industry causes the gut microbiome to become resistant, serving as a bioreactor for the growth of pathogens and raising the likelihood that novel

ARGs will emerge and spread throughout the environment. By moving down the food chain, it disturbs the biogeochemical cycle like how the unmetabolized antibiotic excretes itself into the environment. The growth of resistant bacteria and the suppression of native microorganisms have an impact on the microbial population, enzyme activity, nitrogen cycle, and carbon absorption. Future work plans should concentrate on antibiotic persistence, accumulation, bioaugmentation, biostimulation, and biotransformation since these factors increase the chance of ARBs breeding through HGT events.

As you concentrate on these strategies, it's important to consider how biotic and abiotic degradation processes interact. The "one health approach" should be used to regulate and research the developing resistance trend through ARBs monitoring systems in order to permit a quick public health response. This is because antibiotic resistance thrives in the intestines of animals. It's also critical to develop multi-agency coordinated solutions that will improve coordination, spread information, and raise awareness of the appropriate use of antibiotics in agriculture, animal husbandry, and aquaculture. The results of these actions will boost the environmental efficacy of both novel and existing antibiotics while also controlling human and animal diseases. Additionally, there will be a decrease in the transmission of zoonotic diseases that cause life-threatening, incurable infections in people.

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Mitigation Strategies for Vancomycin-resistant Staphylococcus aureus

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ABSTRACT

In just two hundred years the prevalence of infectious disease started soaring as new modes of travelling were invented. Now people could one place to another quickly carrying several kinds of disease with them. With the advent of diseases, life expectancy was reduced to around 35 years and death was rampant. These dire circumstances led to the discovery of antibiotics that became the main weapon of mankind against infectious diseases post-infection. Humanity was saved from the looming threat of constant epidemics and reduced life expectancy. Soon mankind began to thrive and people started increasing the use of antibiotics to battle all kinds of diseases. With overuse came the problem of misuse of antibiotics. People soon started using antibiotics without proper protocols and dosing regimens. This malpractice soon resulted in the emergence of a capability in bacteria to nullify the effects of antibiotics. The antibiotic resistance meant that the easily curable diseases once again became untreatable maladies. One such bacteria that gained antibiotic resistance was Staphylococcus aureus. S. aureus gained resistance against the methicillin group of antibiotics as they were commonly used against it. After that vancomycin became the drug of choice against methicillin-resistant S. aureus. Soon, people started misusing vancomycin too which quickly led to the development of vancomycin-resistant S. aureus (VRSA). VRSA were usually multi-drug resistant bacteria that effectively rendered, many of the antibiotics being used against them, ineffective. These conditions forced the researchers to look for alternative medication modes and techniques for countering antibiotic resistance.

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1. INTRODUCTION

In the groups of Gram-positive cocci Staphylococcus aureus is an organism often linked with gastroenteritis that occurs due to consumption of contaminated food products like milk (Ayele et al. 2017) and shrimp. The phenomenon of multidrug resistance in food-borne bacteria is a concern of worldwide occurrence (Song et al. 2015). S. aureus and several other bacteria resistant to beta-lactam drugs have been isolated from milk products (Ayele et al. 2017) and shrimp (Arfatahery et al. 2016). Out of many factors acting in the selection of drug-resistant bacterial pathogens, some important ones are misuse of antibiotics and improper disposal of antibiotics in the environment (Cheng et al. 2015; Roca et al. 2015). Thus, the improper use of antimicrobial drugs in marine (di Cesare et al. 2013; Tajbakhsh et al. 2015) and livestock (Yang et al. 2017a) animals ultimately leads to the production of resistant bacterial species. This rise in turn results in increased levels of contamination in foods obtained from animal sources. An alternative tactic to control the rise of antibiotic-resistant bacteria has been suggested. This proposal emphasizes the research on antimicrobial compounds present in plants (Atanasov et al. 2015).

After the identification and reporting of Staphylococcus aureus with Methicillin-Resistant also known as (MRSA) in the 1960s physicians started using vancomycin (VA) as the drug of choice against it. However, soon reports started coming out about the occurrence of Staphylococcus aureus with Vancomycin-Resistant also known as (VRSA) (Alakeel et al. 2022; Saber et al. 2022; Pinheiro et al. 2023).

The isolates of S. aureus from Saudi Arabia were also tested positive for resistance against several drugs of antibiotic nature including vancomycin. This discovery forced the scientists to begin their search for new alternatives against the Vancomycin-resistant Staphylococcus aureus (VRSA). One such alternative option was the use of medicinal plants. Since ancient times, plant extracts have been used for the treatment of diseases caused by bacteria. Now issues like the increase in bacterial resistance against conventional antimicrobial drugs, necessitate the shift of attention to medicinal herbs. A commonly known plant Ziziphus nummularia, also known as sidr in Arabic is potential is a potential candidate for battling antibiotic resistance. It is a branched thorny bush. This plant is native to Saudi Arabian land and it grows in arid, dry regions (Mesmar et al. 2022). Several parts of Ziziphus nummularia can be used for the treatment of a broad range of diseases. Recently it has gained scientific approval for possessing beneficial bioactive substances that act as antimicrobial, antioxidant, antitumor, anti-hypotensive, anti-inflammatory, anti-hypoglycaemic, liver protective and immune system stimulants (Mustafa et al. 2019; Khurshid et al. 2022).

2. Healthcare Challenges against the Issues of *Staphylococcus aureus*

One of the more severe problems concerning infection of *S. aureus* is the outbreak of infections of drug-resistant pathogens and their impact on global human health. The emergence of MRSA had a major effect on the settlements of hospitals that later turned into community-based infections in a particular trend. It mostly happens in people who lack proper medical attention (See et al. 2017; Rowe et al. 2021). In a cascade-effect manner, MRSA has started showing resistance to an extended range of antibiotics belonging to the beta-lactam group (ESBL). This group includes the most commonly used antibiotics like penicillins, carbapenems and cephalosporins (Rasheed and Hussein 2021). 80% of the mortality rate recorded in hospitalized people with MRSA infection is due to the formation of biofilm by invading bacteria (Alonso et al. 2022). The recurrence of MRSA infections can be of any type ranging from cystic fibrosis disorders, soft tissue and skin infections, endocarditis, bacteremia, UTIs, colonization of nares and osteomyelitis (Rowe et al. 2021). MRSA is also a major pathogen of the infection associated with implants (Khatoon et al. 2018). The typical infections caused by *S. aureus* include issues of soft tissues and skin infections. These infections may also include impetigo and purulent cellulitis (Cruz et al. 2021). Occurrence of impetigo is common in

infections caused by *Staphylococcus*. It can be specifically seen at the extremities in the crusty lesions (Alegre et al. 2016). The toxic shock syndrome toxin produced by *Staphylococcus* can lead to the occurrence of toxic shock syndrome (Wang et al. 2007). These infections mainly happen due to the use of absorbable tampons. It involves severe clinical symptoms such as multi-organ coupled septic shock. With the consideration in mind that the important part of *S. aureus* is the resident of the nasal region in humans, Nurjadi et al. (Nurjadi et al. 2015) its connection between pvl (Panton-Valentine leukocidin) can underline, which is mostly discovered in intercontinental travellers, and the genes linked with the harshness of soft tissue and skin infections are lukL/lukS genes. MRSA has been identified as the main cause of health-care setting-associated pneumonia in the statistical analysis performed by Walter et al. (Walter et al. 2018) in the countries of Europe. After *Pseudomonas aeruginosa*, the second major colonizing bacteria of the lungs is *S. aureus,* especially in people suffering from cystic fibrosis. *S. aureus* affects the regulator protein for transmembrane conductance found in the epithelium layer of cystic fibrosis. This effect then causes the mucus to accumulate in the respiratory tract. The mucus engorgement leads to difficulty in breathing and ultimately to the disease and fatality of patients with cystic fibrosis (Stauffer 2017). The main sources for getting infected with nosocomial pneumonia are the endotracheal tubes and overuse of intensive care unit ventilators for the patients which may also lead to infection with biofilm-forming bacteria (Bauer et al. 2002). Medical equipment like pacemakers, defibrillators, and heart valve implants are the sources of cardiovascular infections of *S. aureus* that can usually lead to early-onset endocarditis due to prosthetic valves (Viola and Darouiche 2011).

The most significant cases of increased mortality by endocarditis and sepsis were observed in several types of vascular catheters (Alonso et al. 2022). The clumping factors (Clfs) are fibrinogen-binding proteins such as ClfA and ClfB, along with SdrE, which induce the aggregation of platelets. This aggregation then leads to endocarditis (O´Brien et al. 2002). Past studies have discovered *S. aureus* as the second major etiologic agent of shunt infection (Bhatia et al. 2017; Yakut et al. 2018). Intracranial pressure and meningeal irritation were the notable clinical signs seen during cerebrospinal fluid shunt infections (Kulkarni et al. 2001). An elevated risk of shunt infection was seen in people facing spinal fluid leakage post-surgery of shunt reimplantation. Additionally, *S. aureus* tries to produce a viscous infection of joints and bones called osteomyelitis (Chang et al. 2013). Another recent issue concerning *S. aureus* is the prevalence of its infections during orthopaedic procedures, especially hip or knee arthroplasty (Beam and Osmon 2018).

UTIs caused by *S. aureus* are rarely observed. However other issues like older age, hospital exposure, urologic surgical procedures, long-term urinary tract catheterization, urinary tract obstruction, and malignancy favour induction of hematuria caused by *S. aureus*. Similarly, it may also cause dysuria, bacteriuria, or bacteremia (Gad et al. 2009). The research by Gjodsbol et al. (Gjødsbøl et al. 2006) denoted that *S. aureus* can be identified in more than 80% of chronic wound infections typified by diabetic foot ulcers, venous ulcers, and pressure sores.

3. Herbal Mitigation of VRSA

In the context of herbal medication, the plants belonging to the genus *Plectranthus* have 3000 well-identified species found in all countries of Africa, Australia, Asia and South America. The plants are well known by local folks as a popular medicine (Figure 1). They are often used by locals for treatment of digestive, respiratory problems, infectious and inflammatory (Waldia et al. 2011; Daglia 2012). There are several species of *Plectranthus* (Kiraithe et al. 2016; Crevelin et al. 2015) including *P. amboinicus* (Swamy et al. 2017), have been under consideration by researchers due to their unique pharmacological characteristics. These studies will help validate the proper use of these medicinal herbs. *P. amboinicus* has its bioactivity by 76 volatiles and 30 non-volatile compounds present in it. These compounds belong to

various types of phytochemicals (Arumugam et al. 2016). Research regarding the pharmacological activities of *P. amboinicus* was conducted from its extracts which are sophisticated volatile compounds. These compounds are naturally synthesized in several portions of the plant by secondary metabolism. These substances have great potential in the biomedicine sector (Swamy et al. 2016). An increased sensitivity of methicillin-resistant *S. aureus* (MRSA) has been observed against the *P. amboinicus* extracts (de Oliveira et al. 2013; Santos et al. 2015).

These researchers studied the crude methanolic extracts of *Ziziphus nummularia* and used them against the VRSA (Vancomycin-Resistant Staphylococcus aureus). The phytochemicals present in these extracts included tannin, phenols, saponin and flavonoids. No steroids and alkaloids were found in the plant extracts although it was separated using TLC. The GC-MS analysis was applied to discover that it contains 2-Octene, (E)- and Eugenol. These substances are the major antimicrobial factor found in the extracts of this plant. A study with similar objectives was presented by Odongo et al. (Odongo et al. 2023). In this study, they checked the antimicrobial potential of extracts from various plants like *Toddalia asiatica, Aloe secundiflora*, *Camellia sinensis* and *Senna didymobotrya* against several types of clinically important pathogens including *S. aureus*. The study made several revelations about the potential antibacterial effect that was achieved through the combination of the extracts from *Aloe secundiflora* and *Clonorchis sinensis* used against *S. aureus*.

Akinduti et al. (Akinduti et al. 2022) studied the antimicrobial activity of the plant extracts of several plants including *Vernonia amygdalina, Azadirachta indica, Acalypha wilkesiana* and *Moringa oleifera*. These extractions were tested against the isolates of multi-drug-resistant *S. aureus*. The results of these tests revealed the potential impact of these plant extracts as antibacterial agents due to the presence of compounds like saponin, alkaloids and terpenoids in the plant extracts. These discoveries suggested that the extracts of plants might be an alternative to the herbal formation with biologically active substances that can target *S. aureus* even if it is vancomycin-resistant. The antimicrobial activity of plant extracts from *Calpurnia aurea* and their portions were tested against several important pathogenic bacteria including *S. aureus*. The antimicrobial activity was evident in targeting *S. aureus*. It was attributed to all of the fractions found in the plant extract. The other studies regarding the effectiveness of extracts from plants like *Calpurnia aurea* also supported its usability for the treatment of skin infection. These extracts include compounds like saponins and alkaloids that improve their effectiveness (Wasihun et al. 2023). In another research, the leaves of *Artemisia afra* were used to obtain crude extracts. These extracts were then tested for effectiveness against several clinically important pathogens including *S. aureus.* These extracts have shown promising activity with bactericidal potential. Thus suggesting that the plant extract under consideration is a less toxic and economical antibiotic from *Artemisia afra* against *S. aureus* (Haile and Jiru 2022). Edet et al. filtered the raw extracts from *Annona muricata* to verify its effectiveness against multi-drug resistant (MDR) *S. aureus* and the result determined the composition of extracts to be several phytochemicals including glycosides, saponin, flavonoids, alkaloids, hydroxyanthraquinones, polyphenols, anthraquinone, phlobatannins and tannin. The GC-MS study showed the presence of carbonic acid 2-dimethylaminoethylpropyl ester, 1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one and trichloromethane, bicyclo[4.1.0]heptan-2-one 6-methyl. Conclusively, all the studies proved that the phytocompounds present in the raw methanolic extracts obtained from *Ziziphus nummularia* had active compounds for anti-VRSA action against pathogenic bacteria (Edet et al. 2022).

4. Use of PAMAN Nanoparticles

Infections of bacterial origin are a serious threat to the security of global human health (Outterson et al. 2016). Another severe case of infectious disease is its occurrence by antibiotic-resistant bacteria that is

estimated to cause 10 million fatalities by the year 2050 (Humphreys and Fleck 2016). Even with an ongoing series of publications regarding innovative and "improved" antibiotics, the clinical presentation of their effects is still not very promising (Burrowes et al. 2011). Strict policies, high costs of development and shorter lifetime effectiveness life-times of new antibiotics, before the first strains of bacteria emerge to develop new antibiotics at a commercial level, have little attraction (Spellberg 2014). Hence formulating new strategies is a matter of urgency to discover options for improved usage of presently available antibiotics. Simultaneously, a change of paradigm is necessary for the aversion of research and development to focus away from the production of antibiotics towards introducing new antibiotics that do not lead to the emergence of antibiotic resistance in pathogenic bacteria. Such a shift in the paradigm of research might be more attractive for companies if it becomes commercially feasible for marketing and later on for clinical uses. A large number of innovative nanotechnology-based antibiotics are already known to mankind (Liu et al. 2019a) Some examples of nanoparticles include metal nanoparticles (Yang et al. 2017b; Zheng et al. 2017; Wang et al. 2019), particularly Ag nanoparticles (AgNPs) (Xiu et al. 2012) that have the capability for disrupting membranes of the cell by releasing Ag ions (Rizzello and Pompa 2014; Wang et al. 2016). The main challenge hindering the practical use of AgNPs is the formation of aggregates in suspension. The aggregation leads to a reduction in the antimicrobial action of AgNPs (Martínez-Castañon et al. 2008). AgNP aggregation can be prevented by using block copolymers (Ji et al. 2020), micelles (Huang et al. 2017) and vesicles (Lu et al. 2013) as a template. The disadvantage of template synthesis is that they are complex and expensive. Both of these issues hinder clinical application. Dendrimers are also a good, alternative template to be used with AgNPs. Such an example is Poly-(amido-amine) (PAMAM) dendrimers which are dendritic molecules with extensive branching and large molecular mass. Its size distribution is narrow with a distinct globular structure (Avila-Salas et al. 2020; Song et al. 2020). It has been used in conjunction with gold nanoparticles as contrast agents in computerized tomography (Liu et al. 2019b) or immune-sensor coatings (Razzino et al. 2020). Hence PAMAM dendrimers are also useful as a suitable template for AgNPs. The relatively larger molecular size of PAMAM dendrimers facilitates the integration of AgNPs and also assists in the conjugation of an additional antibiotic. Conjugation of two antibiotics can result in the reduction of the chances of the pathogenic bacteria developing resistance (Ejim et al. 2011).

A frequently used antibiotic for controlling clinical infections is Vancomycin (Ozcan et al. 2006; Dalton et al. 2020). Vancomycin has been used in the past as a part of single-conjugated systems for antibiotic applications (Choi et al. 2013). Conjugation of vancomycin with PAMAM dendrimers has shown five orders of better-targeting magnitudes against cell surfaces of bacteria. These are much better than the simple vancomycin solution (Choi et al. 2013). However, Choi et al. (Choi et al. 2013) only discussed the targeted attacking and killing efficacy of vancomycin in PAMAM dendrimers with single-conjugation without conjugating a second antibiotic or evaluating the possibility of antimicrobial resistance being developed in the bacteria under attack. Recently, a new type of PAMAM-based dendrimer for antibiotics, with dual-conjugation has been developed. It is hetero-functionalized as it kills the vancomycin-resistant *S. aureus* strain in vivo and in vitro while preventing tissue damage that may occur upon usage of AgNPs (Gu et al. 2019) or vancomycin (Abdullah et al. 2016) in high concentrations. In vivo, the killing of bacteria was confirmed by an experiment on a murine-infected wound model through the application of a single low dose of topical Van-PAMAMAgNP dendrimers (2 mg/kg vancomycin, which is a lower dose as compared with other animals under consideration as the literature suggests that topical application necessitates up to 40 mg/day/kg dose for seven days continuously) (Ozcan et al. 2006). The most significant role of these innovative hetero-functionalized Van-PAMAM-AgNP dendrimers having dual-conjugated is their ability to prevent the development of antibiotic resistance in vancomycin-sensitive strains of *S. aureus*. These two notable characteristics of Van-PAMAM-AgNP dendrimers were developed using unmodified AgNP. On the

other hand, Van-PAMAM-AgNP dendrimers were discovered to be blood and tissue-compatible up to an Ag equivalent concentration of 8 µg/mL in vivo and in vitro. In contrast to unmodified ones the modified AgNPs often display a reduction in biocompatibility (Li et al. 2021). The combination of vancomycin with AgNPs seems crucial for low-dose bactericidal action against vancomycin-resistant staphylococci. Both single-conjugated PAMAM-AgNP dendrimers and Van-PAMAM cannot efficiently kill strains of *S. aureus* at low doses, irrespective of their vancomycin resistance. AgNP can induce damage to the cell walls of bacteria by releasing Ag ions (Kaur et al. 2019), thereby allowing entry of vancomycin into the intracellular spaces (Kaur et al. 2019).

Fig. 1: Herbal Treatment options for Vancomycin-resistant *Staphylococcus aureus*.

5. Conclusion

Staphylococcus aureus is a globally prevalent bacteria found in nearly all kinds of media making it a prevalent organism suitable for study models. Sometimes it is also found in isolates from infection sites like in case of ectopic skin infections in Saudi Arabia. The infections caused by *S. aureus* were easily treated in the past using regular antibiotics like the methicillin group. Soon, people started misusing this antibiotic leading to the development of resistance in bacteria. These became the methicillin-resistant *S. aureus*

(MRSA). After the discovery of methicillin resistance scientists started using Vancomycin as the drug of choice against MRSA. Soon, people also started vancomycin haphazardly that soon led to antibiotic resistance in bacteria against it. The discovery of vancomycin-resistant *Staphylococcus aureus* (VRSA) was the last nail in the coffin of humanity battling against pathogenic *S. aureus*. This discovery was an alarming situation for mankind as this meant there were no more antibiotic options for humans to use against pathogenic *S. aureus*. Soon, researchers started shifting their attention towards the alternative remedies of generally all infectious diseases particularly *S. aureus* infection, besides the use of traditional; antibiotics. This shift in the research and development paradigm soon resulted in the emergence of new herbal and nanoparticle options for battling infectious diseases in the modern era. The herbal approach implements the use of plant extracts as antibiotics or antibiotic carriers. Similarly, the nanoparticles may be used as lone antibiotics or antibiotic carriers.

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Mitigation Strategies for Methicillin-resistant Staphylococcus aureus

22

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ABSTRACT

Staphylococcus aureus (S. aureus) is a spherical Gram-positive bacterium that is naturally non-motile. It is a pathogen of virulent nature that causes various skin and soft tissue infections in humans. Although it was easily treatable through the use of easily available antibiotics like methicillin this relief was short-lived as the bacteria started gaining resistance against the antibiotics. These bacterial pathogens were called methicillin-resistant Staphylococcus aureus or MRSA. MRSA defends itself against attacks of antibiotics by various mechanisms including modification of the target, efflux of drug and blocking the penetration of antibiotics. Additionally, it also codes a blaZ gene that manufactures the beta-lactamase enzyme. This enzyme renders the beta-lactam antibiotics useless against bacteria. This phenomenon is alarming as it means that antibiotics are now becoming useless for curing diseases and hence there is a need for the development of mitigation measures to control the prevalence of antibiotic-resistant bacteria. The administration of antibiotics is also problematic because it cyclically leads to antibiotic resistance. As the new antibiotics are being developed the bacteria are also developing resistance against them. Such problems necessitate a focus shift of researchers from antibiotics towards new treatment options that have no vulnerability towards resistance and will remain effective throughout usage. Some of the remedies that have been presented by researchers as alternatives to traditional antibiotics include nanoparticles, nanotechnology delivery systems and plant-based extracts. All of these alternatives need extensive research to thoroughly comprehend their action mechanism. Once completely explored these remedies can be exploited to their full potential for gaining the best results with minimum consequences.

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1. INTRODUCTION

Staphylococcus aureus (S. aureus) is a pathogenic Gram-positive bacterium with a sphere shape and natural non-motility. It belongs to the genus of staphylococcaceae. It has an average size of 1 µm in diameter (Ondusko and Nolt 2018). S. aureus is a pathogen of virulent nature that colonizes different parts of its human hosts to produce life-threatening ailments. Additionally, S. aureus also prevents the initiation of immune responses of both endogenous and adaptive nature which can lead to the induction of human infection of either locally bound or systemic type. It is the widespread and leading etiologic agent causing infections of skin and soft tissues including problems like endocarditis, bacteremia and osteomyelitis (David and Daum 2017). The commensal or frequent extant of this bacteria symptomlessly in several portions of the body of humans including mucous membranes, glands of the skin, nasal epithelial cells, and the gut of well folks (Ogston 1881). Direct contact, usually through the touch of the skin surface with the skin of a colonized or infected person, is the core transmission method for the spread of S. aureus. Another alternative mode for transmitting S. aureus occurs through contact with contaminated surfaces and objects which can play a significant role in its spread from infected to healthy populations (Miller and Diep 2008). The data from reports of prevalence have shown that twenty percent of the population were constant nasal carriers of S. aureus while thirty percent had a recurrence of infection (Ogston 1882).

S. aureus is one of the major infections caused by communal bacteria in both humans and animals (Chang et al. 2003; Stryjewski and Corey 2014). S. aureus can colonize for a short or long period. It may even be treated spontaneously with the existing options of remedies. However, S. aureus is famous for its potential for antimicrobial resistance. Around 30% of the global population is infected with S. aureus asymptomatically and enduringly also by the MRSA (methicillin-resistant S. aureus). High-intensity of infection and severe condition of symptoms may cause high morbidity and fatality. This may result in a further increase in the cost of healthcare (Schmidt et al. 2015). MRSA colonization in infections also appears in several types of animals like horses, cats, dogs, cattle and birds (Weese 2004; Šťástková et al. 2012). Latest studies regarding the prevalence of S. aureus have reported a high colonization rate for MRSA bacteria in infected humans that tend to live in close contact with the animals (especially infected ones) (Weese et al. 2006). The re-emergence of infections with S. aureus occurs commonly even after complete treatment of infected hosts with antibiotics due to the hindrance in protective immune response development. Hence, S. aureus has seen a rapid rise in its ranks to become one of the major agents causing several health-relevant diseases (Gill et al. 2019).

Penicillin is an effective remedy to defend patients against S. aureus. However, recently it has become a short-lived relief due to the development of antibiotic resistance in bacteria. It started showing up in disease surveillance reports during the era around the 1940s that antibiotic resistance in bacteria can develop by the gene mutation phenomenon of the blaZ gene also known as the β-lactamase encoding gene. This gene helps the bacteria to resist the action of the antimicrobial drugs by producing the β-lactamases enzymes that cause the β-lactam ring disruption of the penicillin, hydrolytically, rendering it ineffective (Kardos and Demain 2011).

Various researchers have provided reports of the trends regarding antimicrobial resistance (Chambers and DeLeo 2009; Vanamala et al. 2021). There has been a proposal regarding the widespread administration of penicillin being the sole reason for the emergence and development of MRSA rather than the introduction of methicillin (Harkins et al. 2017).

MRSA or methicillin-resistant S. aureus is a new strain of S. aureus that was identified and isolated during the era of 1960s. It happened within a year's time after the first introduction of methicillin by Beecham at the clinical level (Harkins et al. 2017). Since that time the incidence and prevalence of MRSA infections

has increased at a global level dramatically. An alternate study regarding the prevalence of MRSA infections claimed the number of infections going up each day, especially in developed countries. This trend indicates the prevalence of bacteria in communities despite good well-being practices and the communal (Miller and Eells 2008). The infection of MRSA is still a notable risk for the population as 60 to 80% of colonization happens in case of hospital-acquired infections in well-developed countries with sufficient resources (Williams et al. 2009). A recent meta-analytical study of inpatients in the US reported that there are admissions of approximately 4 million S. aureus infection patients each year. Simultaneously, infections of MRSA increase the fatality rate (~19,000) of the US population in hospitals, each year (Klevens et al. 2007).

ABSTRACT

Staphylococcus aureus (S. aureus) is a spherical Gram-positive bacterium that is naturally non-motile. It is a pathogen of virulent nature that causes various skin and soft tissue infections in humans. Although it was easily treatable through the use of easily available antibiotics like methicillin this relief was short-lived as the bacteria started gaining resistance against the antibiotics. These bacterial pathogens were called methicillin-resistant *Staphylococcus aureus* or MRSA. MRSA defends itself against attacks of antibiotics by various mechanisms including modification of the target, efflux of drug and blocking the penetration of antibiotics. Additionally, it also codes a blaZ gene that manufactures the beta-lactamase enzyme. This enzyme renders the beta-lactam antibiotics useless against bacteria. This phenomenon is alarming as it means that antibiotics are now becoming useless for curing diseases and hence there is a need for the development of mitigation measures to control the prevalence of antibiotic-resistant bacteria.

The administration of antibiotics is also problematic because it cyclically leads to antibiotic resistance. As the new antibiotics are being developed the bacteria are also developing resistance against them. Such problems necessitate a focus shift of researchers from antibiotics towards new treatment options that have no vulnerability towards resistance and will remain effective throughout usage.

Some of the remedies that have been presented by researchers as alternatives to traditional antibiotics include nanoparticles, nanotechnology delivery systems and plant-based extracts. All of these alternatives need extensive research to thoroughly comprehend their action mechanism. Once completely explored these remedies can be exploited to their full potential for gaining the best results with minimum consequences.

2. MRSA-ASSOCIATED RISK FACTORS

Risk factors associated with MRSA may lead to fatality, hospitalization for a long period or admission to of a patient in the unit for intensive care, open wounds, an overdose of antimicrobial drugs, hemodialysis, the addition of drugs to treatment enhancing the toxicity profile, and placement of a urinary catheter for an extended amount of time. Identification of MRSA colonization during hospitalization of infected patients can reduce the danger of the development of infection or its spread to others (Ayliffe 1997). An impact of this MRSA infection is an increase in resistance against multiple antibiotics. This trend limits the options of therapeutic management options in the treatment of MRSA and its relevant infections. MRSA has also been discovered as an agent of poor infection control and its rapid resistance development towards almost all antibiotic therapy options in case of severe infections. The antibiotics mostly prescribed for the treatment of MRSA infections are aminoglycosides, fluoroquinolones and erythromycin.

Vancomycin is still considered as a crucial weapon necessary for the defence against the infection of MRSA (Cuny et al. 2010).
Another cause of the development and spread of antibiotic resistance is the misuse of antibiotic drugs. On the other hand, the world is facing this challenge due to the lack of suitable innovative antimicrobial drugs that can act effectively against resistant bacteria (Yeaman and Yount 2003).

3. BLOCKED PENETRATION

The mechanism of diffusion transports drug agents across a cell membrane by porin, diffusion through the bilayer of lipids and automatic uptake by bacteria. The minute hydrophilic pieces of antibiotics (β-lactams and fluoroquinolones) can easily cross the outermost membrane of the bacterial cell only through the porins passage. The reduction in the number of porin passages leads to the reduction in the amount of β-lactam and fluoroquinolone antimicrobial drugs that enter the cell. This phenomenon of under-dosing at the cellular level leads to the development of resistance against these classes of antibiotic drugs (Yeaman and Yount 2003).

4. EFFLUX PUMPS

Antibiotic inflow in the bacteria during the administration of drugs that act by passive diffusion. On the other hand, efflux devices of the bacteria tend to pump out the antibiotics that are bioavailable within the bacterial cell. The reduction in concentration of the antibiotic drug within the bacteria is unable to achieve the objective of stopping the bacterial populace growth (Yeaman and Yount 2003). These efflux pumps are always present in the cytoplasmic membrane of the bacteria. On the other hand, particular porins are present on the outermost surface of the bacterial cell. These efflux pumps can precisely target the antibiotics. The efflux system pumps drain the substances of toxic nature out of the cell to maintain a suitable environment internally. Many of these efflux pump systems are carriers of multiple drugs. On the other hand, they are also responsible for the efflux of toxic metabolites along with the externalization of antibiotics including fluoroquinolones, macrolides and tetracyclines. The drug efflux mechanism is significantly notable the Gram-negative bacteria (Dˇzidi´c et al. 2008; Soto 2013).

5. MODIFICATION OF TARGET MOLECULE

The modification of the target site often results in a spontaneous mutational modification of a gene present on the bacterial chromosome. This mutation can effectively differ the adhesion capability of antimicrobial drugs. Modifications in the ribosomal subunit 30S or 50S indicate the emergence of resistance towards the antimicrobial drugs that act by inhibition of protein synthesis in bacteria (Tenover 2006). Similarly, PBP adaptation favours the Gram-positive bacteria for developing antibiotic resistance. However, the production of β-lactamases enzyme is still an important mechanism of resistance progression in Gram-negative bacteria (Hiramatsu et al. 2001).

6. HERBAL MITIGATION

The mitigating impact of *C. latifolia, A. officinalis, T. vulgaris* and *Z. jujuba* has been considered for studies by several scientists. Animals that were administered with *T. vulgaris* and *Z. jujuba* presented a decrease in scores of gross lesions of both lungs and heart. On the other hand, *C. latifolia*

administration led to decreased gross lesions but only in the lungs. Development of gross lesions in the tissues of the host due to the virulence of bacteria. MRSA release toxin substances that can lead to various blood issues including hemolysis in RBCs, adhesion of RBCs in the tissue walls and hindrance against phagocytosis by macrophages (Otto 2014). The reduced score of gross lesions for lungs denotes the importance of the role played by extracts of these plants in preventing infections with MRSA in the systems of in-vivo nature. In the beginning, *S. aureus* circulates in blood vessels and, later on, it starts adhering to the extracellular matrix constituents, of the blood hence leading to the formation of thrombi, or host cells of endothelium and initiation of the cell colonization process (Niemann et al. 2004). *S. aureus* can also interact with cells of the endothelium in blood vessels (Peacock et al. 1999), the matrix of extracellular spaces (Flock 1999) and platelets (Sullam et al. 1996; Kerrigan et al. 2002). Several *S. aureus* surface proteins have a high degree of selective permeability to plasma proteins and matrix. They have been proven to moderate the processes of attachment to these surfaces (Van Belkum et al. 2002). Another well-known feature of *S. aureus* is binding to host RBCs (red blood cells). This binding occurs when plasma proteins like fibrinogen are present in blood (Croize et al. 1993; Wilkerson et al. 1997). Isolation and calculation of bacterial load from the blood, throat, joint, lungs, and heart were undertaken as an experiment to indicate the persistency and adhesion of *S. aureus* in host tissues. The data from these studies has revealed a notable decrease in a load of bacteria in

(a) the throat region of infected people of the group treated with the *T. vulgaris*
(b) the lungs of the group that were given antibiotic-treatments
(c) blood and heart of all groups that were treated except the ones with *A. officinalis* and *Z. jujuba* administration
(d) joints in the group treated with *Z. jujuba* and *A. officinalis*.

The decreased laid of bacteria has been witnessed due to several reasons including bacteriostatic and bactericidal effects of the phytochemicals like flavonoids, and phenolic and alkaloid compounds found in crude plant extracts (Benariba et al. 2013). These findings are in exact alignment with the research done by Owais et al. (Owais et al. 2005). He reported that a decrease in load of bacteria was observed in the vital organs of mice post-treatment with extracts of *Withania somnifera L. Dunal*. Haematology of samples from infected hosts can be studied to obtain a good understanding of the stage of bacterial infection, its progress and the impact of treatment. Elevations in levels seen during tests of ESR, neutrophils, and lymphocyte TLC count are credible infection indicators for the body of the host. Once the patient is cured after effective treatment these levels quickly drop to normal range (Piper et al. 2010). In the past *S. aureus* was known to contain an enzyme hemolysin, which helped it in killing of host blood cells in a targeted manner. A decrease in Haemoglobin, PCV, Red Blood Cells and platelets was observed in an unattended group of infected hosts being studied. This might be explained by to lysis effect of enzymes on the RBCs of the host during the invasion of bacteria or due to the removal of iron from the body of the host to indirectly inhibit red blood cell formation. There is evidence that *S. aureus* has the capability of using haemoglobin as a source of iron for bacterial growth (Mazmanian et al. 2003; Pishchany et al. 2013). Among the group being treated except for the ones receiving *A. officinalis* all other groups being administered plant extracts displayed an improvement in their haematological parameters. The best improvement of ESR and lymphocyte count towards normal was seen in the group being treated with plant extracts from *C. latifolia, Z. jujube* and *T. vulgaris*. Additionally, the groups being administered *T. vulgaris* and *C. latifolia* extracts had their count of neutrophil and platelet pretty close to the counts of their negative control group counterparts proving their higher potential for limiting infection. During this study, Sur and Ganguly (Sur and Ganguly 1994) observed a comeback to normal in haematological parameters of the infected host post-treatment with root extracts from tea plants in the case of Ehrlich ascites

carcinoma-induced mice. Various other experiments have also proved the antimicrobial activity of *A. officinalis* through in vitro procedures (May and Willuhn 1978; Mazmanian et al. 2003; Zarei et al. 2013; Rezaei et al. 2015), although its effectiveness in the in-vivo system is still vague indicating the necessity for in vivo studies.

7. NANOTECHNOLOGICAL MITIGATION

For many years, efforts have remained prime work to increase the effectiveness of treatment of MRSA by increasing the bioactivity and efficiency of the therapeutic measures. First time in 1994, nanotechnology was used to treat MRSA and *S. aureus* infections. The arrival of nanoparticles gives small-sized particles, defensive inner environments and shapes to increase the activity of drugs in organs, tissues and cells of the infected host. The use of nanoparticles for treating diseases shows better therapeutic results as compared to the traditional way of treatment (Kesharwani et al. 2014; Kesharwani et al. 2015; Kesharwani et al. 2019; Pandey et al. 2019; Bapat et al. 2020; Singh et al. 2020). The approval of the use of nanotechnology in treating infections of MRSA and *S. aureus* was first given by the FDA in 1994. The major advantage of the use of NPs are their size range, usability shape, and formation of a protective environment which helps to enhance the drug activity in organs, tissues and cells of the infected host. The formation of these nanoparticles requires far more effort than other antibiotic preparations (Aldawsari and Singh 2020; Singh et al. 2020a). Some major properties of these NPs include improving the stability of drugs in blood serum, increasing their time of retention in blood circulation, and delivering the drug at a specific site for activities regarding the pharmacology of that drug at a specific time and rate (Kingsley et al. 2006; Singh et al. 2020b). Another astonishing property of NPs is that they can be phagocytosed by the cells of the host and help in the treatment of the intracellular infections of the bacteria (Onyeji et al. 1994). The first delivery vehicle of NPs was made for vancomycin and teicoplanin in 1994 against MRSA infection (Taylor 2013). Esmaeili et al. used PlGA NPs for an antibiotic named Rifampicin. The drug therapy for mycobacterium infection and leprosy with Rifampicin is still in use. Fusidic acid is also practised against MRSA contagions (Esmaeili et al. 2007). Duran et al. used the in vitro Rifampicin fabricated with NPs (20 to 60 um) antibacterial activity, this combination can sustain the bioactivity and cytotoxicity of Rifampicin against MRSA (Durán et al. 2008). As discussed earlier, the infections of MRSA can be controlled with proper use of antibiotics but it is still a challenge to get effective antibiotics to treat the infection of MRSA. The majority of antibiotic drugs are of hydrophobic nature which causes low bioavailability and solubility (Delcour 2009). This is the main reason why the majority of the antibiotic drugs that are administered lead to the appearance of severe side effects, toxicity in normal body cells and the emergence of drug resistance against multiple antibiotics (MDR) (Prestinaci et al. 2015). Another major problem is the traditional delivery system of drug transfer which does not have sufficient competency for treating bacterial infections completely. Such shortcomings made it necessary for the researcher to formulate a novel system of drug delivery or NDDS to improve the index of the therapeutic window for antibiotic drugs. It is suggested that the use of NDDS can lower the adverse effects and decrease bacterial resistance against antimicrobial agents. Many new methodologies have been introduced under the umbrella of nanotechnology to steal the spotlight in the medical field (Choudhury et al. 2019a; Choudhury et al. 2019b). The challenge of the traditional delivery system has been solved by using nanosized carriers called sas nanocarriers. The use of nanotechnology-based therapeutic agents has been implemented in the enhancement of treatment regimens against different diseases for example cancer (Gorain et al., 2018; Choudhury et al. 2019b; Kesharwani et al. 2019; Gorain et al. 2020). These nanocarriers have

remarkable properties which include increased therapeutic efficacy, increased circulation time for systemically administered drugs, and improved stability of serum their overall stability is also far better than the standalone drugs. Along with these benefits, this approach also decreases the adverse effects on normal cells, decreases the resistance of microorganisms against antimicrobial agents, and uses combined regimens of therapy for reaching specific sites according to the drug requirement. The antibacterial properties of nanomaterials depend on the following mechanisms:
(1) formulation of reactive oxygen species (ROS) through photocatalytic activity which in turn leads to the destruction of the components of cellular and viral nature,
(2) the constituents that make the membranes of the cell and cell wall,
(3) discontinuation in energy supply,
(4) blockage of enzyme production and suppression of DNA synthesis (Huh and Kwon 2011).

8. NANOPARTICLES OF INORGANIC NATURE

These are nanometric particles that are chemically inorganic and have various benefits. They are beneficial because of the peculiar physicochemical properties they display when compared with individual molecules, atoms, or bulk materials of the same type (Choudhury et al. 2017; Numan et al. 2021). Some particular inorganic nanoparticles for example oxide derivatives of metals and nanoparticles of metallic nature are thought to have some antibacterial activities. Examples of these nanoparticles are gold nanoparticles (AuNPs), nanoparticles of silver (AgNPs), titanium dioxide nanoparticles (TiO2 NPs), and nanoparticles of zinc oxide (ZnO NPs) (Choudhury et al. 2020). Nanoparticles of an inorganic nature have the property to adhere and accumulate on the cell wall of bacteria cell wall quickly, and then penetrate the cell through pores due to their size being smaller than the pores (Sánchez-López et al. 2020). The higher ratio of surface area to volume means that even small doses of inorganic nanoparticles can show higher levels of antibacterial activity as compared to free antibiotic drugs (Choudhury et al. 2020). Hence proved that the inorganic nanoparticles show bactericidal action by disrupting the structural and functional integrity of microorganisms.

9. METAL NANOPARTICLES

The immense use of MNPs as medicine has drawn the attention of researchers and several studies done to find out the mechanism of MNPs as antibacterial medicine and also their role against resistance (Huh and Kwon 2011). The properties of metal nanoparticles regarding their physicochemical nature are their size, charge, shape, zeta potential, morphology of surface, and structure of the crystal, which makes them significant and regulate their actions on the bacterial cells (Figure 1). Recent research work shows that MNPs act in three different ways which are stress of oxidation (Gurunathan et al. 2012), non-oxidative stress (Leung et al. 2014), and release of ions by the metal (Zakharova et al. 2015). Different metal nanoparticles have been utilized to assess their efficacy against MRSA for instance silver (Ag) and gold (Au) NPs (Lambert 2002; Morones et al. 2005; Nabikhan et al. 2010; Sobrova et al. 2012). According to commercial use of MNP applications, the most commonly used MNP is AgNPs which are used in cosmetics, nanomedical devices, and food products.
Besides the fact that they are less toxic the silver ions, the ability of these NPs to induce oxidative stress for long periods in eukaryotic cell lines and subcellular organelles, for example, mitochondria, shows that they could contribute to the early onset of some metabolic diseases for example neurodegenerative and cardiac diseases (Dos Santos et al. 2014; Strauch et al. 2017; Grzelak et al. 2018; Holmila et al. 2019). The reason for this toxicity is still not found out but many studies show that it is not the uncontrolled release of silver ions but the size of the particles causing toxicity. Besides the uncontrolled ion release, there are

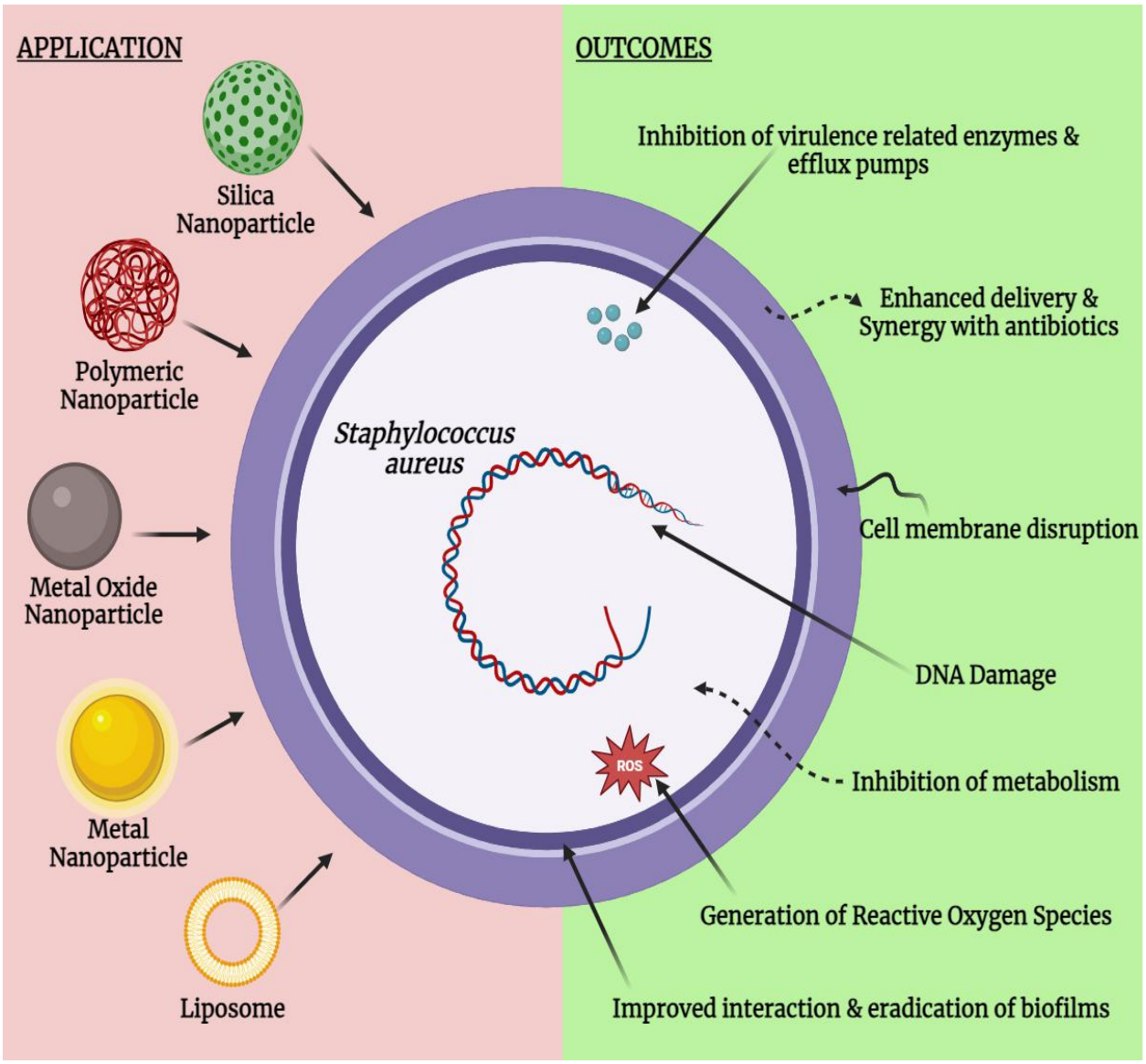

Fig.1: Nanotechnological mitigation of Methicillin-resistant *Staphylococcus aureus*.

different studies regarding AgNP toxicity and the animal modules used. Solvents employed during the particle synthesis are another major source of toxicity. As a result of this many researchers have now used the greener methods which results in a major reduction in the geno and cytotoxicity in the cell line by increasing the particle stability, the big example is the graphene (Strauch et al. 2017). The use of this particle in biomedicine, the lifetime matching i.e. the stability of the particle and the device function, to minimize the toxic events, is routinely applied in implants and topical applications. The less expensive alternatives of Au and AgNPs, are the zinc oxide (ZnO) and titanium oxide (TiO2) NPs which are seen to be used against MRSA, killed it under in vivo and in vitro conditions (Ansari et al. 2012; Rauf et al. 2017). In MRSA-associated skin infection, ZnO NPs have been used to reduce the burden of bacteria when used in murine models (Umamageswari et al. 2018) also one study shows the antibacterial activity of ZnO NPs against MRSA when used at a concentration of 1875mg/ml (Kadiyala et al. 2018). Another study revealed the bactericidal activity of these NPs and also their mechanism of action of these NPs which is through

inhibiting different pathways including amino acid synthesis in *S. aureus* (Roy et al. 2010). TiO2 NPs also show anti-MRSA activities in the disk diffusion method when used with different antibiotics including cephalosporins, glycopeptides, and azalides. TiO2 NPs form free radicals under UV photoactivation, which enhances their ability to kill MRSA (Wahab et al. 2014).

10. CONCLUSION

The development of methicillin resistance among bacteria has caused an alarm for the security of public health. This means that the antibiotics of today will soon be useless for the treatment of disease and hence there is a need for the development of alternate remedies. Other remedies that can be used effectively as a replacement for antibiotics include nanotechnology and herbal medicinal extracts. The main benefit of using these remedies is that they do not induce antibiotic resistance but reduce bacterial growth effectively. Additionally, these remedies do not have adverse effects that are usually seen during the administration of antibiotics, especially for an extended period. One important example of herbal remedies is the plant extract. On the other hand, an important example of nanoparticle remedies is nano-metals. These nanomaterials are usually prepared by finely ground metals and their derivative compounds. Some important examples of nano-metals include Gold and silver nanoparticles. Two important metal-derived nanoparticles are Zinc Oxide and Titanium Oxide.

These alternatives to antibiotics require further research and experimentation to understand their mechanism of action to exploit their full potential. These compounds can be extremely useful once their mechanism of action and methods of commercial preparation are thoroughly comprehended. These can help us in lowering the dosage of drugs, reducing dose frequency and lowering the required amount of antibiotics for treating patients. These effects will consequently lead to a lower toxic effect window, lesser cost of treatment and an overall reduction in prevalence of disease due to reduced transmission of antibiotic resistance.

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Glanders: A Treatable Disease?

23

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ABSTRACT

Equine glanders, often known as farcy, is an infectious zoonotic disease caused by Burkholderia mallei. The only known natural B. mallei reservoir is found in horses, donkeys, and mules. Even though glanders has been completely eradicated in the majority of countries, due to multiple recent outbreaks, the illness is once again considered to be reemerging. Pre-symptomatic or carrier animals are important in the propagation of the infectious agent and can be a source of infection for the healthy horse population. Ulcerating nodular lesions of the skin and mucous membranes are characteristic of glanders. Fever, lethargy, depression, coughing, anorexia and weight loss are examples of generalized symptoms. B. mallei can enter a host by way of the integument, gastrointestinal system, and mucous membranes. We still don't fully understand its pathophysiology and virulence processes. The frequency of false-positive and false-negative results, which cause difficulties in international equine trade and the spread of glanders to disease-free regions, is a significant issue when utilizing serological tests for the diagnosis of glanders. Furthermore, inadequate testing greatly contributes to inadequate disease control. These assays are not only unable to distinguish between antibodies against B. pseudomallei and B. mallei, but they are also unable to distinguish between animals that are naturally infected and those that are malleinized. The detection rate of glanders is increased by the combined use of molecular and serological diagnostic techniques. Early disease detection in susceptible animals, strict quarantine regulations, testing and safe carcass destruction, fair compensation to animal owners, disinfection of infected premises, and veterinary extension services that raise awareness of glanders and their potential zoonotic consequences are all examples of countermeasures against glanders. Also provided is a description of the clinical presentation and effective experimental treatment of spontaneous equine glanders.

Keywords: Glanders, farcy, Horses, Equines, Burkholderia mallei, zoonosis.

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1. INTRODUCTION

Glanders is a zoonotic disease that spreads through contagion and is triggered by the Burkholderia (B.) mallei infection. While glanders were initially believed to have acute or chronic manifestations exclusively, there is emerging evidence that B. mallei has the capacity to induce latent infections resembling those initiated by Burkholderia pseudomallei. (Kettle and Wernery 2016) This ailment has been denoted by a variety of alternate terms, such as cutaneous Droes, Farcy Pipes, Farcy, Malleus, Farcy Buds, and Equinia. (Jubb et al. 2012).

Glanders is an extremely infectious bacterial illness affecting horses, mules, and donkeys, identifiable by the development of nodular lesions in the respiratory, cutaneous, and lymphatic systems. This zoonotic ailment can be transmitted to individuals who are in close proximity to infected animals or those who handle the organism in laboratory settings. (Dvorak and Spickler 2008).

Human infection with this disease is relatively rare, even during outbreaks among horses. The recognition of this illness dates back to ancient times, with Hippocrates documenting clinical symptoms around 425 BC. Nearly a century later, Aristotle mentioned the disease within the broader context of epizootics and referred to it as 'melis'. (Al-Ani and Roberson 2007) (Whitlock et al. 2007) The causative agent, B. mallei, was officially documented in 1882 when researchers isolated it from the infected spleen and liver of a horse. (Schadewaldt 1975) It is believed that horses serve as the natural source of infection, with humans being accidental hosts. Human infection can happen through either the cutaneous or inhalational routes. (Kettle and Wernery 2016).

The formation of the present Burkholderia genus was determined by a combination of factors, including DNA-DNA homology values, phenotypic traits, cellular lipid and fatty acid composition, and 16S rRNA gene typing. This taxonomic revision occurred in 1992. (Yabuuchi et al. 1992) Within the Burkholderia genus, there are several notable species, among which Burkholderia pseudomallei stands out as the causative agent of melioidosis. Additionally, Burkholderia cepacia is recognized as a significant pathogen, particularly in patients suffering from cystic fibrosis. (Burns et al. 1996) Another member of this genus, Burkholderia thailandensis, is recognized as a bacterium with relatively lower virulence in comparison to certain other Burkholderia species. (Glass et al. 2006).

Glanders is believed to have been one of the earliest instances of a biological weapon in the 20th century. Germany initiated a program of biological sabotage against multiple countries during World War I. These countries also include the United States. Covert operatives were provided with cultures of B. mallei and anthrax, which they employed in efforts to infect livestock intended for Allied nations. The objectives were twofold: to disrupt livestock and to facilitate the transmission of the extremely contagious, deadly agent from animals to humans. Presently, there are concerns about attempts to create an aerosolized, antibiotic-resistant form of B. mallei, which has the potential to become a bioweapon of significant potency, similar to anthrax. (Srinivasan et al. 2001).

2. CAUSATIVE AGENT

Burkholderia mallei is a Gram-negative bacterium that lacks motility, does not form spores, and is an obligate intracellular pathogen in mammals (Whitlock et al. 2007). Its dimensions span 0.3-0.8 µm in width and 2-5 µm in length, and its staining affinity with basic dyes is weak because of the presence of lipoid granules, resulting in irregular staining patterns. (Worley Jr and Young 1945) Rod-shaped forms of *B. mallei* both inside and outside macrophages in the spleen, liver, and lungs are observed. (Ferster and VIa 1982) Although the existence of a capsule in *B. mallei* was first reported by Miller et al. (1948), it wasn't confirmed until almost forty years later, emphasizing its critical function as a virulence component. Subsequent studies indicated that 19% and 33% of the tested strains developed a capsule after about 1

and 3 hours of incubation period, respectively. (Khan et al. 2013) When grown on appropriate culture media for a period of 1 to 2 days, *B. mallei* typically forms small, circular, shapeless, and translucent colonies. This distinctive colony morphology plays a significant role in the laboratory identification of the bacterium. (Wetmore and Gochenour Jr 1956).

3. TRANSMISSION

Glanders transmission takes place through diverse means. The number of documented instances of glanders in humans has been quite limited. Typically, these cases are linked to individuals who have had close contact with infected animals, with a particular focus on veterinarians and those involved in caring for animals afflicted with glanders. (Gangulee et al. 1966) (Howe and Miller 1947). Typically, transmission involves contact between infectious substances from contaminated animals and mucous membranes or open skin. Moreover, the organism can be inhaled through contaminated aerosols or dust particles. Infections in laboratory settings have occurred due to both skin contact and inhalation. (CDC, 2000). Human-to-human transmission is uncommon, with only a few documented cases, which include potential instances of sexual transmission and infections in family members who provided care to individuals with glanders. In rare situations, humans can acquire the infection by consuming meat that is contaminated.
In animals, the disease spreads by ingesting food and water contaminated by the nasal discharge of carrier animals. Carnivores are also vulnerable to infection if they consume meat that is contaminated with the pathogen. (Pal et al. 2016)
In addition, glanders can spread by direct skin-to-skin contact, inhalation that results in deep lung deposits, and bacterial invasion of the mucous membranes of the mouth, nose, and conjunctiva. Exposure to the skin during work is common, especially on the neck, face, hands, and arms. Normally, *B. mallei* cannot penetrate intact skin, although many cases lack evidence of wounds or penetrations during exposure. Interestingly, most laboratory-acquired infections occur without any injury or a clear recollection of injury. In the eighth case mentioned, there was no recollection of a skin break or a specific exposure-related incident, such as a needle stick or broken glassware. However, the patient did mention taking a finger-stick sample of their own blood for diabetic monitoring before entering the lab. Since gloves were not worn, the finger-stick site might have served as a bacterial entrance point. The presence of unilateral axillary lymphadenopathy in this patient aligns with a percutaneous infection (Van Zandt et al. 2013).

4. CLINICAL SIGNS AND SYMPTOMS

Glanders manifests clinically in horses, mules, and asses and is likely transmitted to humans from affected animals. Interestingly, cattle seem to be immune to the disease. In animals, glanders can manifest as either an acute or chronic condition, with the chronic form being more prevalent. During acute glanders, there is significant constitutional and functional disturbance, marked by a high fever reaching 105 or 106 degrees and lasting several days. Rapid weight loss, frequent chills, and unexplained lameness may occur, along with swelling in the limbs. Diagnosis during this stage is challenging. Subsequently, the fever subsides, and local lesions develop. Glanders nodules develop on the nasal septum, progressively softening and giving rise to discharge, which results in the formation of deep, irregular-edged ulcers. The disease rapidly spreads to the lungs and lymphatic glands, causing severe inflammation in the joints' synovial membranes. Fever reappears, extensive cheesy deposits form in the lungs, and death occurs within one to two weeks. Four distinct types of equine glanders can be identified as cutaneous, nasal, pulmonary, and asymptomatic carriers. (Falah et al. 1987) Cutaneous glanders can occur as a result of skin trauma or may manifest as a secondary condition stemming from the respiratory form of the disease. This variety of glanders is marked

by the existence of nodules, pustules, and ulcers, which may manifest on various parts of the horse's body, even though they can be most frequently seen on legs. (Mohammad 1989) Infections induced by *B. mallei* are recognized to result in profound anemia, which is probably attributable to the inhibition of erythropoietic activity within the bone marrow. (Al-Kafawi et al. 1977) One of the most prevalent clinical presentations of horse glanders is its pulmonary manifestation. It is recognized by the development of solid, encapsulated, spherical, grayish nodules that are dispersed throughout the lung tissue. (Al-Ani and Zubaidy 1978) The upper respiratory tract is where the glandes that cause nasal manifestations appear as nodules or ulcers. These ulcers are usually located on the cartilaginous nasal septum and lower portions of the turbinate. (Jubb et al. 2012)

Chronic glanders typically begins with a mild acute attack, often unrecognized and mistaken for a minor cold or lymphangitis. The acute variant of the chronic glanders usually exhibits an incubation period that spans from 1 to 14 days (Al-Ani et al. 1998), whereas the incubation time for the chronic form might last up to 12 weeks.

Localized infection usually appears 1-5 days after exposure and is marked by swelling of the affected region and the onset of a discharge. After a period of 10 to 14 days of incubation, signs of acute lung infections may manifest. Septicemia may appear right after exposure or take up to 14 days to manifest. If left untreated, pneumonic illness almost always results in death within 10 to 30 days and frequently has a quick start. Notably, a significant observation in the eight cases since 1943 is that at least half of the patients experienced temporary improvement in both their overall condition and clinical signs after the initial symptoms, before a second wave of symptoms emerged. This transient improvement might be mistakenly perceived as the disease being eliminated by both the patient and the physician. However, it is crucial to understand that this temporary improvement should not discourage physicians from recommending necessary treatments. (Van Zandt et al. 2013).

Pre-symptomatic or carrier animals can provide a risk of infection to healthy horses and are essential to the spread of the infectious agent. Ulcerating nodular lesions on the skin and mucous membranes are the hallmark of glaucoma. Fever, lethargy, depression, coughing, appetite loss, and weight loss are typical symptoms. *Burkholderia mallei* can enter a host by way of the gastrointestinal system, the integument, or mucous membranes. Despite ongoing research, the virulence mechanisms and pathogenesis of the bacteria remain incompletely understood. A notable challenge in diagnosing glanders stems from the reliance on serological tests, which frequently produce false-positive and false-negative results. These inaccuracies not only complicate international trade involving equids but also contribute to the spread of glanders to disease-free regions. (Khan et al. 2013).

5. DIAGNOSIS OF GLANDERS

5.1. CLINICAL EVALUATION

The clinical manifestations of glanders in equids can often resemble those of other respiratory diseases, highlighting the significance of veterinarians in distinguishing them from conditions such as ulcerative lymphangitis (*Corynebacterium pseudotuberculosis*), strangles (*Streptococcus equi* spp. *equi*), pseudotuberculosis (*Yersinia pseudotuberculosis*), epizootic lymphangitis (*Histoplasma farciminosum*) and sporotrichosis (*Sporotrichum* spp.) (Kettle and Wernery 2016).

Radiology can detect the existence of abscesses in various organs, including the lungs, liver, and spleen. It is significant to note that these abscesses may not be specific to glanders and can also be caused by other diseases. Therefore, specific diagnosis through the isolation and positive identification of the causative organism, *Burkholderia mallei*, is essential for confirming glanders (Van Zandt et al. 2013).

5.2. LAB TESTS

Glanders can be detected through several techniques, encompassing clinical indicators, the mallein test, serological examinations, and bacterial isolation. (Al-Ani and Roberson 2007)
Burkholderia mallei can be cultivated on various types of agar media, including nutritional agar, MacConkey agar, and blood agar, among others. When grown on these commonly used culture media, *B. mallei* typically forms colonies that are viscid, smooth, and creamy in appearance. These colonies can be observed after 48 hours of incubation at 37°C. Importantly, *B. mallei* exhibits the ability to thrive both as an aerobic organism and as a facultative anaerobe, and it can do so in the presence of nitrogen. (Pal and Gutama 2022) The low concentration of *B. mallei* in the tissues and biological fluids of infected equids (horses, donkeys, mules, etc.) makes pathogen isolation difficult. PCR (Polymerase Chain Reaction) and real-time PCR are two of the molecular techniques that have been developed to increase the detection of *B. mallei*. However, many of these tests encounter difficulty in differentiating between infections caused by *B. mallei* and those brought about by B. pseudomallei, a closely related bacterium responsible for melioidosis. (Kettle and Wernery 2016)
Currently, the diagnosis of glanders involves various methods, including the use of mallein (an allergic hypersensitivity test) and serological assays like the complement fixation test (CFT), counter immunoelectrophoresis test (CIET), indirect hemagglutination test (IHAT), enzyme-linked immunosorbent assays (ELISAs), and indirect fluorescent antibody test (IFAT). (Cravitz and Miller 1950).

6. ISOLATING AND IDENTIFYING THE RESPONSIBLE PATHOGEN

Cultivating *B. mallei* from an intact cutaneous nodule, pulmonary lesion, or lymph node is a valuable diagnostic approach. (Al-Ani et al. 1998) Culturing swabs from the pus-filled insides on glycerin agar usually produces diminutive, circular, shapeless, and translucent colonies. *B. mallei* can be identified by gram stain morphology, biochemical responses, and male guinea pig inoculation (Al-Ani et al. 1998). Several culture media were formulated to facilitate its cultivation, (Rogul et al. 1970) and 3% supplementation of glycerin in brain heart infusion agar is a frequently employed medium for extensive propagation.
The Straus reaction entails injection via intra-peritoneal route in male guinea pigs with suspected substances to facilitate the diagnosis. The manifestation of swelling and periorchitis within 3-7 days after immunization, offering additional confirmation of the existence of a *B. mallei* infection. (Al-Ani and Roberson 2007).

6.1. MALLEIN TEST

The test for delayed hypersensitivity is conducted through the intrapalpebral inoculation of mallein, which is a glycoprotein secreted by *B. mallei* and can be found in the culture supernatant. When hypersensitive horses infected with *B. mallei* are subjected to this test, they typically exhibit the development of purulent conjunctivitis within 24 hours, along with swelling of the eyelid. The mallein test is a valuable tool used in the field to diagnose glanders in equines. (Pal et al. 2016).
Serodiagnosis via the Complement Fixation Test (CFT) is recommended by (OIE), the World Organisation for Animal Health, for international trade purposes and is likewise endorsed for surveillance investigations. Although this test is acknowledged for its high sensitivity, it's important to note that it has a tendency to produce a significant number of false-positive results. These false-positive outcomes can cause unjustified limitations on the international trade of animals and related products, leading to economic losses for both proprietors and the equine industry. (Elschner et al. 2019).

6.2. TESTS ASSESSING CELL-MEDIATED IMMUNITY

A frequently utilized in-vivo test to assess cell-mediated immunity for glanders diagnosis is the intradermopalpebral mallein test. (Verma et al. 1994) This test entails injection of 0.1 mL of mallein intradermally adjacent to the inferior eyelid. An optimistic reaction typically emerges in 48 to 72 hours and becomes prominent in pronounced eyelid swelling, accompanied by the blepharospasm and very severe purulent conjunctivitis. Mallein test demonstrates a positive prognostic value of 92% in acute as well as chronic cases and a negative prognostic value of 96% in progressive cases. (Wilson and Miles 1975) Nevertheless, it has been reported to exhibit sensitivity limitations, particularly in progressive clinical cases, (Jana 1982) and there have been documented cases of false positives linked to Streptococcus equi infections. (Falah et al. 1987) Additionally, the mallein test may at times trigger the production of antibodies against *B. mallei* in uninfected horses, potentially resulting in a positive complement fixation test (CFT) (Hagebock et al. 1993).

A cell-mediated immunity test conducted in vitro is the lymphocyte stimulation test. Combining this test with CFT and bacterial culture for glander diagnosis has been shown to offer high sensitivity and specificity rates, enhancing the accuracy of diagnosis.

The diagnostic utility of Rose Bengal plate agglutination test (RBT) has been tested for equine glanders (Naureen et al. 2007).

7. TREATMENT OF GLANDERS

Many disease-free nations ban treating animals exhibiting glanders infection symptoms, such as positive serological titers. Considerable research has been conducted regarding in vitro antibiotic susceptibility of *B. mallei*. Although medication sensitivity of the isolates studied thus far varied significantly, the organism is generally susceptible to sulfadiazine, sulphamethazine, sulphathiazole, sulphamdimidine, neomycin, tetracyclines, oleandomycin, erythromycin, polymyxin B, kanamycin, nystatin, and sigmamycin. It exhibits reduced sensitivity to chloramphenicol, furazolidone, and nitrofurazone, and varying degrees of resistance to streptomycin, para-aminosalicylic acid, penicillin, and isoniazid (Ipatenko 1972; Kovalev and Gnetnev 1975a,b; Lozovaia 1989; Al-Izzi and Al-Bassam 1989; Thibault FM et al. 2004a). Researchers from Pakistan found that thirteen *B. mallei* isolates demonstrated sensitivity to co-trimoxazole, chloramphenicol, danofloxacin, and norfloxacin (Muhammad et al. 1998), in contrast, cephalexin and penicillin exhibited the lowest in vitro effectiveness. It is reported that erythromycin, oxytetracycline, gentamicin, sulfamethoxazole/trimethoprim (TMP), ampicillin, and Baytril (enrofloxacin) all shaped significant zones of inhibition against *B. mallei* (Al-Ani et al. 1998).

Pakistani researchers conducted a study on the antibiotic vulnerability of 41 *B. mallei* isolates obtained from naturally occurring horse glanders epidemics in province of Punjab, Pakistan. As reported by, Naureen et al. (2010), every isolate exhibited resistance to ampicillin., whereas 94% of the isolates exhibited susceptibility to ofloxacin as well as enrofloxacin.

In 2009, Dr. Saqib employed antibiotic therapy to treat 23 horses affected by a glanders outbreak at Polo Club in Lahore city, Pakistan. The treatment regimen involved oral administration of doxycycline (doxycycline, Belgium; twice daily), followed by intravenous administration of enrofloxacin (Enrotil; Dae Sung Microbiological Labs, Korea; once daily), and trimethoprim (TMP) + sulphadiazine (Tribrissen®; GlaxoSmithKline, Pakistan; once daily). This treatment regimen was utilized to address the glanders infection in the affected horses. The following table provides the dose and dosage schedule. (Khan et al. 2013).

8. DOSE AND DOSAGE REGIME

Total no. of animals: 23
Duration of treatment: 84 days
Total number of doses: Parenteral=42; Oral=126; Total=168

8.1. ANTIMICROBIAL THERAPY

Sr. #	Antibiotic	Dose	Duration	Time
1	Enrofloxacin	8 mg/kg	SID	1st week
		4 mg/kg	SID	2nd and 3rd week
2	Trimethoprim+Sulphadiazine	32 mg/kg	SID	1st week
3	Doxycyline	16 mg/kg	BID	2nd and 3rd week
		6 mg/kg	BID	4th to 12th week

SID = once in a day; BID = twice in a day
By the end of the 12-week therapeutic session, none of the horses had glanders returning. Aside from one horse that experienced delirium, which vanished entirely within three hours, no adverse effects associated with any antibiotics were seen. The clinical symptoms of the glanderous animals are shown in Fig. 1 and 2 before and after anti-biotic medication.
The treated horse was infection-free, according to the subsequent lines of evidence. (Khan et al. 2013).
1. After receiving high dosages of corticosteroids, the condition did not recur. Additionally, 6 of the 23 horses were put to sleep after receiving corticosteroid therapy. Lung tissue, submandibular lymph nodes, and mediastinal lymph nodes all diagnosed negative for *B. mallei* in culture tests. The suspension of the tissues after intraperitoneal inoculation in guinea pigs likewise tested negative for B. mallei. (Khan et al. 2013).
2. Throughout the one-year post-therapy monitoring period, 2 out of the 110 Sentinel horses that were introduced to the group of horses treated for glanders (totaling 17) continued to yield negative results for mallein, indicating their lack of infection.
3. At one month after birth, 3 foals (n = 2) of mares treated with glanders confirmed negative for mallein, and they remained negative throughout the observation period. (Khan et al. 2013).
The animal ethics committee at the Faculty of Veterinary Science, University of Agriculture Faisalabad, Pakistan, provided its approval to conduct an experimental treatment trial. (Khan et al. 2013).

8.2. PREVENTION AND CONTROL

For both humans and animals, there is currently no vaccination available to protect against glanders. In regions where glanders are prevalent among animals, human disease prevention primarily revolves around the identification and eradication of the infection within the animal population. (Pal and Gutama 2022) Occurrences of glanders in animals are required to be informed to the World Organization for Animal Health (OIE). The presence of this disease in a country can lead to international trade restrictions affecting horses and other animals that have been affected by glanders. Following notification, government veterinarians, who possess expertise in diagnosing exotic diseases, conduct thorough investigations to confirm the presence of glanders. When glanders is identified, a comprehensive set of control measures is put into place. These measures encompass:

8.2.1. STRINGENT QUARANTINE

All animals, both infected and those exposed to the disease, are placed under strict quarantine.

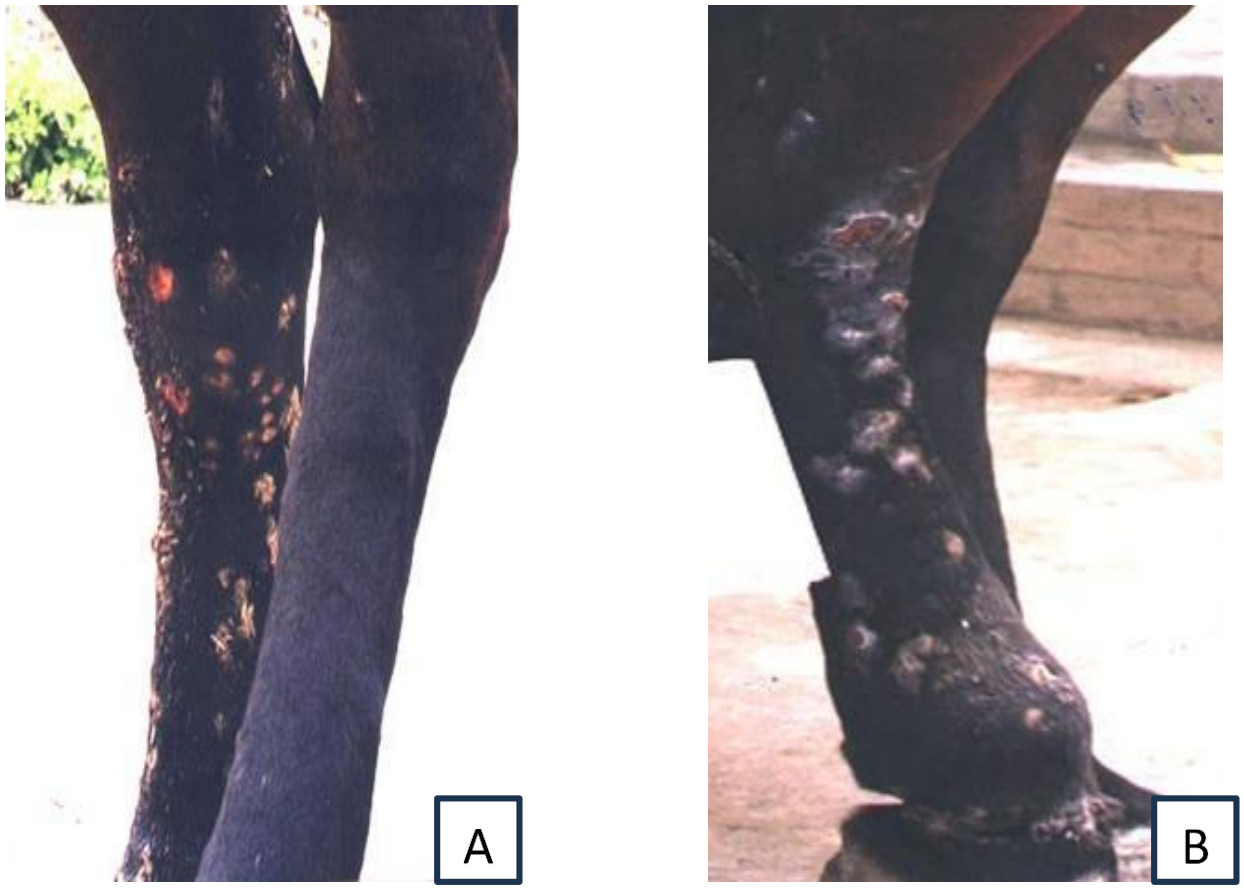

Fig. 1: The images illustrate the impact of an intravenous course of experimental therapy on a horse affected by glanders. In Figure (A), you can see the right hind leg with ulcers prior to treatment, and in Figure (B), the same leg shows signs of improvement with curative ulcers and scarring after completion of the second week of intravenous medication. This demonstrates the positive response to the treatment in addressing the ulcers caused by the glanders infection.

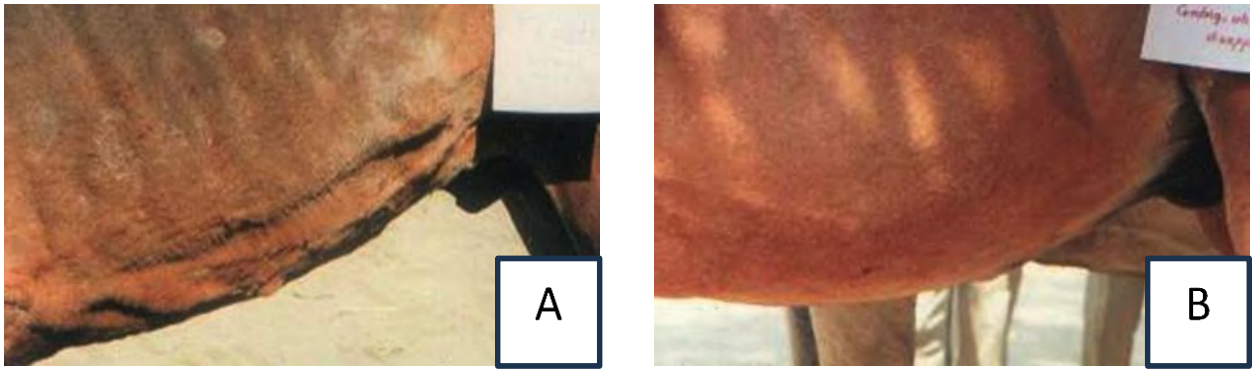

Fig. 2: Shows the results of a glander therapy experiment after six months. Note the horse's (A) deteriorated or shabby body coat and the presence of lymphangitis on the flanks and the medial part of the abdomen. The complete eradication of lesions (lymphangitis) and noticeably better physical condition are seen by the absence of rib demarcation (B).

8.2.2. DIAGNOSTIC TESTING

Animals displaying clinical signs suggestive of glanders undergo diagnostic tests to confirm the presence of the disease.

8.2.3. ASSESSMENT OF APPARENTLY HEALTHY EQUIDS

Equids that appear to be healthy but have been exposed to *Burkholderia mallei* undergo screening tests. Those that test positive in these screenings are removed.

8.2.4. EUTHANASIA

Sick animals and those with positive results in mallein test are compassionately euthanized.

8.2.5. ISOLATION AND RETESTING

Equids that have been exposed to *B. mallei* and initially tested negative in mallein tests are isolated and retested after a waiting period of 2 to 3 weeks.

8.2.6. PROPER DISPOSAL

Carcasses of euthanized animals, as well as any contaminated bedding or feed, should be disposed of in accordance with state regulations, typically through burning or burial.
These measures are critical for containing the spread of glanders and ensuring the protection of both animal and human health (Dvorak and Spickler 2008). *B. mallei* is notably vulnerable to conventional disinfectants, including benzalkonium chloride, iodine, mercuric chloride in alcohol, potassium permanganate, 1% sodium hypochlorite (chlorine bleach), 70% ethanol, and 2% glutaraldehyde. However, phenolic disinfectants are less effective against it. *B. mallei* can also be eradicated by heating it to 55°C for 10 minutes or by exposure to UV light. To ensure containment and prevent further dissemination, strict adherence to isolation, hygiene, and sanitation protocols is crucial. When cleaning contaminated materials, a solution comprising one-part household bleach (0.5 percent sodium hypochlorite) and nine parts water should be used. These measures are essential for controlling the spread of *B. mallei* and maintaining a safe environment (Pal and Gutama 2022).

9. QUARANTINE AND ISOLATION PROCEDURES

The spread of *B. mallei*, especially by asymptomatic carriers during animal importation or exportation, represents a significant transmission route. Therefore, it is imperative to implement infection control measures, ensure accurate screening before importing animals, prevent contact between infected and healthy animals, and advance rapid diagnostic techniques along with appropriate therapeutic interventions. These steps are crucial in managing and mitigating the dissemination of *B. mallei*. (Kianfar et al. 2019).

10. CONCLUSION

In conclusion, this chapter has shed light on the intricate aspects of glanders, an infectious disease caused by *Burkholderia mallei*, affects horses and poses a zoonotic threat to humans. We explored the historical

context of glanders, their transmission, clinical signs and symptoms, diagnosis, treatment, and prevention. Notably, while it was once used as a biological weapon, there is hope in modern medicine, as recent research suggests that antibiotic therapy can successfully treat equine glanders. Nevertheless, the disease remains a significant concern, both for animal and human health, with the need for stringent prevention measures, diagnostic accuracy and effective treatments. The history and current understanding of glanders serve as a reminder of the ongoing importance of vigilant surveillance and international cooperation in managing infectious diseases, whether natural outbreaks or potential bioterrorism threats.

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Global Prevalence of Listeriosis

24

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ABSTRACT

Listeria monocytogenes, a Gram-positive bacterium, is the causative agent of listeriosis, a severe foodborne infection affecting both animals and humans. Despite being initially recognized as a rare disorder, recent outbreaks in various regions have brought attention to its significant impact on public health. This zoonotic pathogen, categorized as a rare condition by ORPHANET, has become the sixth most prevalent zoonotic disease in Europe, with high mortality rates. Listeriosis cases have been on the rise in Europe since 2008, posing a substantial threat to vulnerable populations. The adaptability of L. monocytogenes to diverse environmental conditions, including food processing and agricultural areas, highlights its ability to persist and spread. Virulence factors, bacterial strain characteristics, and host susceptibility contribute to the severity of listeriosis. Insufficient epidemiological evidence hampers the estimation of contamination severity in most outbreaks. Government regulatory agencies enforce strict guidelines and programs in the food industry to control L. monocytogenes spread. Listeriosis primarily spreads through contaminated food, with infective dosages varying based on health conditions. High-risk groups, such as the elderly and immunocompromised individuals, are advised to avoid consuming high-concentration L. monocytogenes foods. The disease's protracted incubation period, propensity for severe clinical signs, and challenges in controlling its spread contribute to its severity. Global prevalence, outbreaks, and risk factors underscore the need for effective control measures. L. monocytogenes can contaminate various food sources, emphasizing the importance of stringent food safety practices. Laboratory data, microbiological diagnosis, and health risks associated with listeriosis further contribute to understanding and managing this public health threat.

Key words: Listeria monocytogenes, Listeriosis, Foodborne infection, Virulence factors, Food safety

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1. INTRODUCTION

Listeria monocytogenes is a common Gram-positive bacterium that can be found in different environmental conditions and is the cause of listeriosis in humans. In the 1980s, this zoonotic illness was initially recognised as a foodborne infection. Due to being considered a relatively rare disorder, listeriosis frequently does not receive the acknowledgement it needs (Chowdhury and Anand 2023). It is categorised as a rare condition and is listed with the reference ORPHA533 in ORPHANET, a European Consortium concentrating on Rare Diseases and Orphan Drugs. The 28 member states of the European Union registered 2,480 identified cases of human detrimental listeriosis in 2017. This illness is the sixth most prevalent zoonotic disease in Europe; because of its high death rate, it stands out as the most severe in terms of clinical indicators (Finn et al. 2023). Because it primarily affects vulnerable populations, this is very alarming. The disease has garnered attention again in the medical community and media due to recent outbreaks in South Africa, the United States, and Andalusia in 2019, which have impacted hundreds of individuals (Lepe 2020).

Listeriosis is caused by the bacteria *L. monocytogenes*, a rare but severe illness that affects both animals and humans. It is one of the most serious foodborne illnesses due to its uncommonly high hospitalization and case fatality rates. There are twenty-one known species of tiny, Gram-positive, rod-shaped, common bacteria in the genus Listeria. Only *L. monocytogenes* and *Listeria ivanovii* are among these that are dangerous to mammals. In the later part of the 20th century, pathogenic strains of Listeria became a major foodborne pathogen in Western countries. Listeria outbreaks in humans and animals have had a substantial economic effect on humankind and the food sector. Interestingly, listeriosis cases have increased in Europe since 2008 (Tuytschaever et al. 2023).

Originally a saprophytic bacterium, certain members of the Listeria genus have effectively adapted to a variety of environmental conditions attributed to human activities. These ecosystems include food processing and agricultural areas, where there is a presence of animal and bird waste (Feng et al. 2023). Their remarkable ability to detect and adapt to environmental stressors facilitates this flexibility. Additionally, because of their ability to withstand adversity, Listeria can more easily enter the digestive systems of mammals by way of contaminated food (Quereda et al. 2021).

The virulence of the bacterial strain determines the effect of listeriosis on human health. Essential factors also include the number of bacteria consumed, the genetic variety within a population, the host's general health and immune system, and any food properties that may affect the microbial ecosystem and host state (Yang et al. 2024).

For most food-related listeriosis outbreaks, there is currently insufficient reliable epidemiological evidence to estimate the severity of contamination. However, the infective dosages of *L. monocytogenes* have been evaluated by researchers to be between 10^7 and 10^9 colony-forming units (CFUs) for individuals in good health and only between 10^5 and 10^7 CFUs for those who are more vulnerable. Recent events have shown that, even in cases when products contaminated with *L. monocytogenes* are widely available, most consumers are not impacted when the levels of contamination are low, and the circumstances necessary for the growth of bacteria are met. It is important to note that reports of *L. monocytogenes* in human faeces have not been linked to any apparent illness. These studies' results, along with the data currently available on epidemics in the United States and Italy. It demonstrates that even the less severe form of illness, which manifests as feverish gastroenteritis in healthy individuals, requires the consumption of substantial amounts of bacteria. On the other hand, even when consuming minimally contaminated products, persons with a high vulnerability may have severe clinical signs of listeriosis (Akram et al. 2021; Félix et al. 2023; Petrišič et al. 2023).

Government regulatory agencies have ordered the food industry to set up programs for analysing dangers at crucial control points in order to regulate the spread of *L. monocytogenes* through food. They have also enforced strict guidelines regarding the allowable thresholds of *L. monocytogenes*

contamination in food items (Sarghaleh et al. 2023). All strains of *L. monocytogenes* are currently treated in the same way from the perspective of regulation. It's crucial to remember that some strains, like those in the CC1, CC2, CC4, and CC6 groups, show increased virulence (Keane et al. 2023). These strains are known to afflict people with minimal or no immunosuppressive comorbidities, and they are often associated with clinical cases. Conversely, strains such as CC9 and CC121 exhibit lower virulence and are less usually linked to clinical infections. It is recommended that people in high-risk categories (such as the older, immunocompromised, and pregnant women), should refrain from consuming food that contains a significant concentration of *L. monocytogenes* due to the seriousness of the illness, the variability surrounding the minimum infectious dosage and the varying pathogenicity levels throughout Listeria strains. For the broader public, it is essential to deal with high-risk foods with care and store them at low temperatures for short periods (Quereda et al. 2021).

Due to the high death rate and seriousness of listeriosis, which can cause brain inflammation, blood poisoning, and abortion, it poses a severe threat to public health. Its protracted incubation time and propensity to afflict people with underlying medical issues, intensify this disease even further (Ding et al. 2023). The elderly, expectant mothers and their unborn children, and people with cancer, cirrhosis, diabetes, chronic kidney disease, rheumatoid arthritis, collagen-vascular disorders, and alcoholism are among those groups which are most susceptible to listeriosis. Remarkably, this microbe can survive at temperatures below those of cold storage. Furthermore, it has been found in the digestive tracts of animals as well as on the outside of living things. There have been separations from agricultural runoff and animal faeces. *L. monocytogenes* is a significant threat to the food business and challenging to control because of its capacity to persist and spread in environments associated with food (Bialvaei et al. 2018).

2. TRANSMISSION OF LISTERIOSIS

Sources of infection for *L. monocytogenes* include the soil and the digestive systems of asymptomatic animals such as fish, crabs, birds, and wild mammals. *L. monocytogenes* can be secreted by infected animals through their faeces, milk, and uterine fluids. The bacteria can also be found in the remains of miscarried babies and frequently in the urine and respiratory secretions of sick animals (Chen et al. 2023). The presence of Listeria monocytogenes in plants and straws is typically attributed to contamination from faecal matter or soil. Listeria infestation is most commonly contracted by consumption of contaminated food, though it can also be transmitted by direct physical contact and inhalation. Furthermore, sexual transmission could be a possible means of dispersal. Listeriosis usually develops in ruminant animals after they eat contaminated silage or other feed. Uncooked vegetables, unprocessed meat, seafood and unpasteurised dairy goods are examples of spoiled food sources for people. Additionally, contaminated post-processing goods, specifically Ice cream, sliced and grated cheese, soft cheddar cheese and deli cold cuts, have been linked to *L. monocytogenes* (Chen et al. 2023; Wang et al. 2023). Although the infectious dose for oral transmission is uncertain, the host sensitivity and the bacterial strain are assumed to play a role. Processed food is prone to contamination at every stage of production, from the unprocessed materials to the primary buyer. In response to the notable rise in contamination levels and the resulting danger of eating food contaminated with *L. monocytogenes*, food security and safety have become critical worldwide concerns (Wu et al. 2023). The pathogen's resistance to low temperatures is a significant challenge in the production chain, as it can cause a spike in infection rate and an increase in fatality rate. It is imperative to emphasise that listeriosis, which is caused by *L. monocytogenes*, is not exclusive to humans and can cause a variety of severe symptoms that can be fatal. In most cases, healthy people can eat items contaminated with Listeria without experiencing any symptoms. However, it is estimated that an infective dose of roughly

10 to 100 organisms is required to initiate the infection in those who are susceptible. The most common form of disease in both ruminants and newborn humans is vertical transmission. The placenta or an infected birth canal are the main routes by which these illnesses are spread. In addition, proximity with diseased livestock during birth, lambing, or post-mortem investigations might result in human infection. There have been isolated cases documented after coming into contact with infectious birds or the corpses of chickens that don't appear to be sick (Kaptchouang Tchatchouang et al. 2020).

3. PATHOPHYSIOLOGY

Although contaminated food is the primary cause of the spread of infection with *L. monocytogenes*, the main route for the bacteria to enter the body is through the gastrointestinal tract. Consequently, the pathophysiology of listeriosis has been thoroughly understood. In the early hours following infection, the bacteria settle inside phagocytes and antigen-presenting cells in the lamina propria, having first invaded the enterocytes that line the absorptive epithelium of microvilli (Shen et al. 2023). The liver and spleen are the main organs of concern that are eventually targeted by a spread through the lymphatic and circulatory systems. The majority of the bacteria is located in the parenchyma of the liver, where it causes pyogranulomas. Within the first twenty-four hours after infection, this series of events takes place. Then, in healthy people, the pyogranulomas go away entirely a week after the infection first appears; in patients on immunosuppressive treatment, however, the infection may worsen and become an invasive illness. This procedure proceeds somewhat quickly and is not the same as the disease's protracted incubation period, which by definition includes a subclinical stage of infection. When it comes to treating disorders in immunocompromised people, the subclinical stage is crucial because lipophilic medicines, like rifampin or quinolones, may be able to reach granulomas more easily than ampicillin and completely eradicate the bacterium (Koopmans et al. 2023).

L. monocytogenes is recognised as a model cytosolic microorganism at the cellular level. Whereas the bacteria actively mediate entry into phagocytic cells through the cell, the formation of internalin (InlA and InlB) on their surface starts the process of penetrating non-phagocytic cells. These internalin interact with cellular receptors, including Met (the hepatocyte growth factor receptor) and E-cadherin, which causes the bacterium to internalise and form a vacuole that stays affixed to the cell membrane. After that, the bacteria use two phospholipases, PlcA and PlcB, and a pore-forming toxin called listeriolysin O (LLO) to help them to exit the internalisation vacuole (Adhikari et al. 2023). These enzymes cause the vacuolar membrane's structure to change, which makes it easier for *L. monocytogenes* to move into the cytoplasm of the cell. In the pathogenesis at the cellular level, this stage is crucial. The bacterium initiates a variety of metabolic pathways in this intracellular milieu to aid in its development and multiplication. The bacteria use several strategies to avoid the innate cytoplasmic immune responses. Among these is the induction of actin polymerisation via the bacterial surface protein ActA, which allows *L. monocytogenes* to spread to nearby cells. Recent studies have suggested that the bacteria can become persistent inside the vacuoles. The prolonged phase of incubation of the illness and the restricted efficacy of antimicrobials in treating it may be explained by this latent infection. Moreover, these enduring vacuolar forms may make regular microbiological identification of the bacteria more challenging (Lepe 2020).

4. INCIDENCE

According to reports, there are between 0.1 and 1 percent cases of listeriosis per 100,000 people per year. *L. monocytogenes* is responsible for 17 and 19 per cent of the recognised reasons for foodborne disease-related mortality in the United States of America and France as well, despite listeriosis being

more uncommon than other foodborne infections. Listeriosis may not be diagnosed as often as it should since it was not considered an infectious disease in the United States until 2000. The number of cases of listeriosis recorded each year has gone up in a number of European countries in recent years. This increase may be the result of a rise in the population of people over age 60 or those under 60 who have a predisposing immunocompromised state. There were 782 instances of listeriosis recorded in a report from 20 countries in 1991. It revealed that 43% of the infections were associated with pregnancy, 29% with septicemia, 24% with infections of the central nervous system (CNS), and 4% with unusual types of illnesses (Hernandez-Milian and Payeras-Cifre 2014).

5. OVERVIEW OF LISTERIOSIS PREVALENCE WORLDWIDE

According to estimates from the World Health Organization (WHO), 600 million individuals acquire foodborne illnesses each year. Foodborne diseases have a negative impact on the nation's economy, trade, and tourism, as well as the healthcare system and socioeconomic development. Globally, foodborne gastroenteritis cases are frequently linked to certain foodborne organisms, including Vibrio spp., Listeria spp., and Salmonella spp. Listeriosis is recognised to be caused by *L. monocytogenes*, and particular dietary sources are rarely found in its infrequent instances. A cluster is created when there are three or more cases of listeriosis with the same pullover strain during a given time frame. When a source strain produces larger-than-expected groups of patients at a particular time and location, it's referred to as an epidemic. Due to the prolonged and variable incubation period of listeriosis (3 to 70 days), which can cause recall bias and make it difficult to determine an appropriate exposure period for food histories, it is difficult to investigate the cause of an outbreak. Additionally, it will be difficult to identify foods that are rapidly spreading and not usually known to be an origin of human contamination (Letchumanan et al. 2018).

Foods that have not been cooked can have the contamination of Listeria. Under normal circumstances, listeria infections result in diarrhoea and other digestive issues; in 20% of cases, they are fatal. Listeria contamination in this study was notably lower than that of other pathogens under investigation. Previous reports have indicated comparable results (de Silva et al. 2013). Nonetheless, a number of studies have revealed a greater frequency in samples of fresh food items such as cheese sprouts (Samad et al. 2018). The first case of listeriosis in humans was documented in Pakistan, where the incidence rate was 1.66%. In Pakistan, the percentage of cow's milk containing *L. monocytogenes* ranged from 2.25 to 6%. Previous research in the South Asian region has revealed that tainted milk serves as a conduit for the spread of *L. monocytogenes*. Globally, the occurrence of *L. monocytogenes* in cow's milk samples was stated 5.3%, 4%, 1.66%, and 7.5% (Yakubu et al. 2012; Nayak et al. 2015; Dalzini et al. 2016); (Obaidat and Stringer 2019). The majority of cow's milk consumers in Pakistan are susceptible to *L. monocytogenes*. Pakistan and especially Punjab, has inadequate information about the risks of cow's milk. According to this study, 3.43% of cow's milk contains *L. monocytogenes* (Munir et al. 2022).

Over the years, there have been several listeriosis outbreaks reported globally. In 2018, South Africa experienced the world's worst epidemic of *L. monocytogenes*, with 937 reported cases and 193 fatalities caused by mortadella intake (Thomas et al. 2020). A total of 59 patients were examined in 2020 after outbreaks of listeriosis were documented in the United States. Soft cheese (11 illnesses, one fatality), meats including Prosciutto, mortadella and salami (12 illnesses, one fatality), and enoki mushrooms (36 infections, four deaths) have been associated with these outbreaks. Every product connected to the epidemic was withdrawn (CDC 2021). There is no official evidence of *L. monocytogenes* contamination in foodborne illness cases in Brazil. However, specific investigations have found the microbe in various Brazilian foods (Barancelli et al. 2011; Camargo et al. 2017; Maistro et al. 2012; Oliveira et al. 2018).

6. RISK FACTORS OF LISTERIOSIS

Listeriosis is a dangerous condition that can be treated and prevented. Those who are elderly, pregnant, or have damaged immune systems, such as those with HIV, cancer, kidney transplants, or steroid therapy, are more likely to develop severe listeriosis and are advised to stay clear of high-risk foods. Soft cheeses, cold-smoked fishery products, deli meat, and ready-to-eat meat products such as fermented meats and sausages are examples of high-risk foods. In nature, *L. monocytogenes* is found in enormous amounts. They can contaminate food and are present in soil, water, plants, and some animal excrement. The bacteria *L. monocytogenes* is the source of the infectious disease listeriosis. One of the most dangerous and severe foodborne illnesses is foodborne listeriosis. *L. monocytogenes* is the bacterium that causes it. With 0.1 to 10 percent cases per 1 million individuals annually, based on the countries and areas of the world, it is a relatively rare illness. Despite the low number of occurrences, listeriosis is a serious public health concern due to the high fatality rate linked to this condition.

In contrast to numerous other prevalent bacteria that cause foodborne illnesses*, L. monocytogenes* can endure and proliferate at low temperatures, often seen in refrigerators. The primary mode of infection is eating contaminated food that contains a high concentration of *L. monocytogenes*. Human-to-human condition is also possible, most notably from pregnant women to unborn children.

In the natural world, *L. monocytogenes* can be originated in soil, water, and the digestive tracts of animals. When manure is used as fertiliser or in the ground, vegetables might become polluted. Additionally, during preparation, germs can infect ready-to-eat food, and during distribution and storage, those bacteria can grow to deadly proportions.

Foods that are most frequently linked to listeriosis include:

- Foods that keep well under refrigeration (If given adequate time and refrigerated temperatures, *L. monocytogenes* can multiply in food) and have a long shelf life.
- Foods that are eaten raw, without being cooked or subjected to other treatments that would destroy *L. monocytogenes.*

Meat products that are ready to consume, such as smoked salmon, meat spread, frankfurters, and fermented raw meat sausages, were among the foods linked to previous outbreaks. There were also prepared salads like bean sprouts and coleslaw, fresh fruits and vegetables, and dairy goods like soft cheeses, unpasteurized milk, and ice cream (WHO 2018).

7. LABORATORY DATA

Public health researchers used the Pulse Net system to determine which illnesses were linked to this outbreak. CDC Pulse Net maintains a nationwide database of the DNA fingerprints of the microbes that cause food-borne diseases. In bacteria, whole genome sequencing (WGS) is used to achieve DNA fingerprinting. WGS demonstrated the tight genetic relatedness of bacteria found in samples from sick individuals. This implies that the same food may have caused the outbreak's sufferers' illnesses (CDC 2023).

8. HEALTH RISKS AND CHALLENGES

L. monocytogenes is the primary pathogen that causes disease in people, while *L. ivanovii* is a rare bacterium that causes disease in animals—both the genus Listeria and its subtypes cause infections in humans. The majority of the time, food contamination by microorganisms is the cause of listeria disease. The majority of the time, the illness that causes frequent, liquid-like bowel movements, fever, persistent headache discomfort, and muscle soreness are minor symptoms. In humans and animals, severe signs of listeriosis include blood poisoning, inflammation of the brain's membranes and surrounding cerebral

tissue and miscarriage. The most vulnerable are immunocompromised individuals, expectant mothers and newborns. Listeriosis has been associated with consuming a wide variety of foods such as meat and fish products and food that has already been cooked and doesn't require cooking (Zahra et al. 2020).
Because of its widespread distribution and capacity for adhesion, *L. monocytogenes* can endure in the food processing industry. Persistent strains of this bacterium, once adhered to, have the ability to form biofilms, contaminate food goods, and exhibit far more resistance to sanitising agents, including benzalkonium chloride, anionic acid and hypochlorite sanitiser than sporadic strains. Additionally, it has been reported that certain strains of *L. monocytogenes* are resistant to common antimicrobial agents like clindamycin, penicillin, and tetracycline. The presence of this resistance in food poses a threat to public health and results in significant financial losses because contaminated products must be removed from the market, and industry operations must be suspended until the contamination is resolved. The sale of implicated products is declining as a result of the erosion of consumer trust (Dos Santos et al. 2021).

9. MICROBIOLOGICAL DIAGNOSIS

The medical diagnosis of *L. monocytogenes* infection is able to be determined by microbiological investigations despite the fact that it can be clinically identified. When *L. monocytogenes* is isolated from a sample that should be sterile, such as blood, cerebrospinal fluid (CSF) or less commonly peritoneal, pericardial, pleural and articular fluid, etc, it indicates an invasive infection. Sample isolating from faeces is not recommended or regarded as an invasive illness criterion unless it is needed for epidemiological reasons. A microbiological diagnosis is mainly made using standard methods of staining and cultivating cultures. Some components of the diagnosis, meanwhile, are still up for debate. In instances with suspected neurolisteriosis, a recent study found that the susceptibility of Gram-staining in cerebrospinal fluid is low; in CSF, 83% of cases had positive results, whereas 64% of patients had positive results in blood culture. These issues can be avoided because *L. monocytogenes* is currently included in the molecular system for the syndromic examination of meningitis, which increases sensitivity and shortens the time required to identify bacteria. Diagnosing illness in expectant mothers and newborns is crucial as well since cultures in these cases may be unusable because of antibiotic use throughout the peripartum period. Additional samples, such as the amniotic fluid, newborn tracheal aspirate and placenta, may also need to be studied during the initial 48 hours following delivery, even though the clinical recommendation for the assessment of early-onset blood infection in newborn don't specifically address or recommend using these samples in comparable clinical scenarios (Lepe 2020).

10. CONTROL MEASURES AND TREATMENT

Because *L. monocytogenes* has been shown to remain along the cheese food chain, from the farm to the fork, its removal from cheese is crucial (Lahou and Uyttendaele 2017). This is why various tactics to regulate and lessen the pathogen's prevalence in food are presently being researched (Tumbarski et al. 2018). In Spain, conventional, outstanding quality soft-ripened cheeses made with unprocessed milk present a more severe and complex battle against the presence of this hazard due to the fact that they are manufactured under Protected Designations of Origin (PDO). One example of such a cheese is "Torta del Casar," a traditional cheese made in Extremadura, an area in the southwest of the country, in compliance with Regulation (CE) 1491/2003 of the European Commission. The inclusion of materials or microbes that are not part of the product itself is typically prohibited under P.D.O. laws.
The hazards related to the abundance of *L. monocytogenes* in traditional foods have been controlled and minimised through the widespread use of conventional preservation procedures such as salting, drying, severe heat treatments, chemical preservation and acidification (Amit et al. 2017; Jan et al. 2017). New

methods of conservation, such as pulsed electric fields, innovative packaging, high hydrostatic pressure and biopreservation, have begun to replace them gradually. The technique of bio-preservation has minimal impact on the organoleptic and sensory qualities of cheeses; it has been deemed an excellent alternative to other methods or procedures for enhancing the safety of food and increasing its shelf life.

Biopreservation is defined as the utilisation of harmless natural entities or controlled conditions, along with their metabolites. Consumers today avoid foods that contain chemical additives and instead seek better options created with natural or little refined ingredients. Therefore, bio-preservation could guarantee that customers' expectations are met.

Conversely, current research indicates that natural food safety additives could include antimicrobial peptides produced by employing gastrointestinal enzymes to hydrolyse milk proteins. The proliferation of both pathogenic bacteria and non-pathogenic bacteria has been demonstrated to be strongly controlled by these peptides or even inhibited (Martín et al. 2022).

However, the quantity of food-originating *L. monocytogenes* strains that are resistant to antibiotics has grown over time. According to some research, the widespread use of growth boosters in animal growth, human and veterinary medicine, and agriculture is to blame for the establishment of resistant strains (Sakaridis et al. 2011; Wang et al. 2013; Sugiri et al. 2014; Oliveira et al. 2018; Teixeira et al. 2020).

Antibiotic therapy is the mainstay of treatment for listeriosis because of its high death rate. For patients allergic to penicillin, tetracycline sulfamethoxazole-trimethoprim and gentamicin are used as second-choice or alternative therapy. The first line of treatment is the use of β-lactam antibiotics, such as penicillin G and ampicillin. The majority of medications used to treat Gram-positive bacteria, including erythromycin, gentamicin, and clindamycin, are effective against isolates of *L. monocytogenes* (Haubert et al. 2016; Rugna et al. 2021).

11. CONCLUSION

Listeria monocytogenes remains a significant public health threat, causing the severe illness of listeriosis. Despite its classification as a rare disorder, recent outbreaks and the escalating number of cases in Europe emphasize the urgency of addressing this pathogen. Listeriosis stands out as the sixth most prevalent zoonotic disease in Europe, marked by a high death rate and severe clinical indicators, particularly affecting vulnerable populations. The adaptability of *L. monocytogenes* to diverse environmental conditions, especially in food processing and agricultural areas, underscores its persistence and spread. Government regulatory efforts have been implemented to analyze dangers at critical control points in the food industry, yet challenges persist in determining minimum infectious dosages and addressing variability in virulence among different strains. The transmission of listeriosis involves contaminated soil, asymptomatic animals, and various food sources. The pathophysiology primarily targets the gastrointestinal tract, with subsequent organ involvement. Globally, the incidence of listeriosis varies, with an increasing trend possibly linked to demographic changes. Antibiotic therapy remains the mainstay of listeriosis treatment, though antibiotic-resistant strains pose a growing concern. The complex nature of *L. monocytogenes* necessitates ongoing research and comprehensive strategies in food safety, regulatory practices, and medical interventions to mitigate its impact on public health. As we mark the one-year anniversary of this exploration, continued vigilance and research are imperative to address the evolving challenges posed by *L. monocytogenes* and protect susceptible populations from the severe consequences of listeriosis.

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Listeriosis: Clinical Perspectives

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ABSTRACT

Listeriosis, caused by the bacterium Listeria monocytogenes, is a rare but severe food-borne illness primarily affecting individuals with compromised immune systems, pregnant women, and the elderly. The clinical manifestations of Listeria infection include chronic and asymptomatic bacteremia, meningitis, encephalitis, and adverse outcomes in pregnancy. This bacterium, a facultatively anaerobic, gram-positive organism, thrives at 37°C and is found in various agricultural and natural habitats, contaminating raw materials that enter food processing facilities. The epidemiology of listeriosis reveals its significant impact on mortality rates, with an increasing number of cases reported in the United States, particularly affecting the elderly. Listeria persists in diverse environments, from soil to meat products, water, and decaying vegetation, raising concerns about its transmission and potential sources.

Clinical predisposing factors for listeriosis include involvement of the central nervous system, initial bacteremia, age over 60, and various comorbidities. Listeria's pathogenicity is multifactorial, involving factors such as hemolysin, phospholipases, internalin, ActA, p60 (iap), and mechanisms for metal ion uptake. The bacterium's ability to grow within cells and its virulence factors contribute to the severity of listeriosis. Recent developments in Listeria detection encompass various methods, including culture-based, immuno-based, and molecular approaches such as PCR and biosensor-based techniques. The detection of L. monocytogenes remains a significant challenge due to the bacterium's persistence in different environments. Molecular methods, particularly DNA microarrays, PCR, and biosensors, are considered reliable for sensitive and specific detection. In conclusion, Listeriosis poses a substantial health risk, especially to vulnerable populations. Preventive measures involve decontamination of livestock and food products. Ongoing research focuses on understanding the complex pathogenicity of L. monocytogenes, and molecular methods play a crucial role in its detection and control.

Keywords: Listeriosis, Listeria monocytogenes, food-borne pathogens, Polymerase chain reaction, immune system, molecular methods

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1. INTRODUCTION

1.1. INTRODUCTION TO LISTERIOSIS

Listeriosis is an uncommon but deadly food-borne illness that can be brought on by Listeria monocytogenes. The illness normally only affects people with low immune systems, such as babies, older adults (Suominen et al. 2023), pregnant women and their fetuses, new mothers, and those with impaired immune systems. On occasion, adults and children who are otherwise healthy are also affected (Donovan 2015).

1.2. CLINICAL MANIFESTATIONS

L. monocytogenes infects people when they eat food that is contaminated. It is believed to move from the mesenteric lymph nodes to the spleen and liver after being able to breach the intestinal barrier (Fig. 1). It is still unclear how much intraluminal multiplication occurs and exactly where it breaches the intestinal barrier. Infection of L. monocytogenes may result in chronic and asymptomatic bacteremia if the immune system cannot regulate it, particularly at the level of liver and spleen. Meningitis or encephalitis may develop because of it getting into the brain or placenta (Yousif et al. 1984) Pregnancy-related abortions, generalized infections in infected neonates (granulomatosis in antiseptic), and immunocompromised patients are the most common cases (Lecuit 2007).
(C). *L. monocytogenes* can lead to acute hepatitis. This condition typically presents as a sudden onset of fever and jaundice, with positive blood cultures for *L. monocytogenes*.
(D) In order to move into the blood stream and cause potentially fatal systemic infections, *L. monocytogenes* can easily get through this lymph node barrier.
(E) Listeria may grow unchecked in the liver, leading to increased low-level bacteremia and spread of preferred secondary target organs (tumour necrosis factor alpha).
(F) and (G) Listeria goes through an internal life cycle which involves early phagocytic compartment escaping, a speedy intra cytoplasmic process of replication via actin-based motility, and a speedy spread to adjacent cells, where they reactivate the cycle. *Listeria* was prevented from infecting the humoral portion of the immune system by this technique of spreading via human tissues. Several virulence factors have been discovered over the past 15 years at significant stages of this intracellular life cycle.
Pathogenic Listeria enters the body through the intestine. After intestinal translocation, the liver is believed to be the first organ to be targeted. Until a cell-mediated immune response removes the infection, Listeria actively grow in the liver (Werbrouck et al. 2006). In healthy individuals, exposure to listerial antigens over time likely contributes to the maintenance of memory T cells that are anti-Listeria. However, in immuno-compromised and weakened patients, Listeria may grow unchecked in the liver, leading to increased low-level bacteremia and spread of preferred secondary target organs (tumour necrosis factor alpha) (Longhi et al. 2004). Both *L. monocytogenes* and L. ivanovii are facultative intracellular parasites that can infect a range of typically non-phagocytic cells, including endothelial,

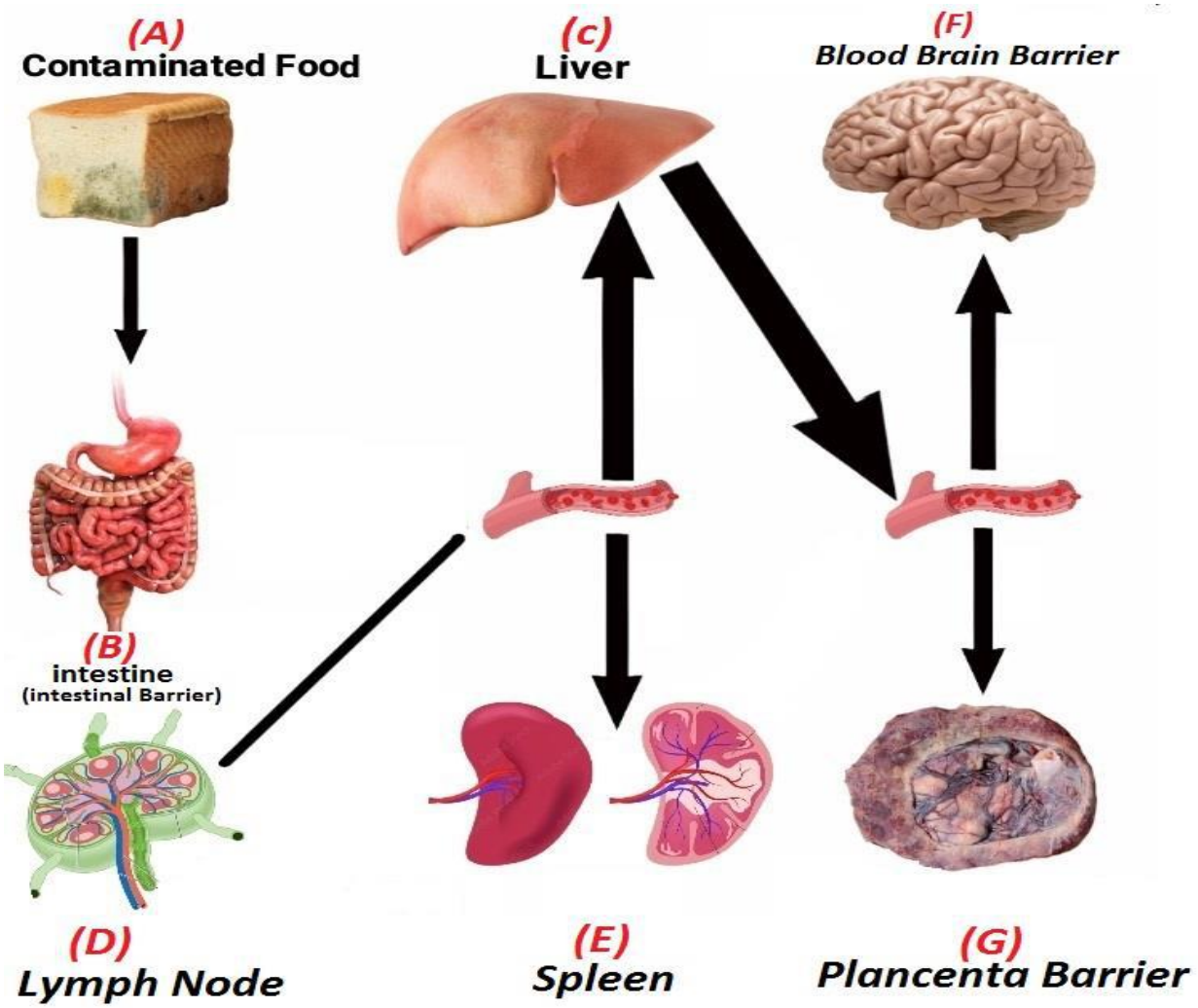

Fig 1: Clinical manifestations of *L. monocytogenes*: (A) main route of transmission is through contaminated food. (B) It causes Listeria gastroenteritis, a typical foodborne illness, a large proportion of people exposed to the contaminated food can become infected, with attack rates reaching up to 72%. The high infection rate is likely due to the presence of a substantial number of Listeria organisms in the contaminated food.

epithelial, and hepatocyte cells. They can also live in macrophages. In all the aforementioned cell types, pathogenic Listeria goes through an internal life cycle which involves early phagocytic compartment escaping, a speedy intra cytoplasmic process of replication via actin-based motility, and a speedy spread to adjacent cells, where they reactivate the cycle. Listeria was prevented from infecting the humoral portion of the immune system by this technique of spreading via human tissues. Several virulence factors have been discovered over the past 15 years at significant stages of this intracellular life cycle (Glaser et al. 2001).

Clinical symptoms might range from mild, invasive conditions like febrile gastroenteritis to more serious ones like sepsis, meningitis, rhombencephalitis, prenatal infections, and abortions. Numerous European nations have seen an increase in listeriosis cases in recent years. These increases are not related to socioeconomic status, gender, geography, ethnicity, or infectious serotypes and are mostly due to the greater risk of bacteremia in listeriosis in those under 65 years old (Allerberger and Wagner 2010).

Consuming contaminated foods including raw meat, unpasteurized dairy products, frozen foods, already wrapped foods, factors influencing the environment, sporadic cases of listeriosis, and illness outbreaks are the primary manifestations of *L. monocytogenes* infection (Weis and Seeliger 1975; Wilson 1995; Beumer and Hazeleger 2003; Thevenot et al. 2006; Ramaswamy et al. 2007).

2. INTRODUCTION TO BACTERIUM

L. monocytogenes is a facultatively anaerobic, gram-positive bacteria that thrives at a temperature of 37°C. Between 22-28°C, it is movable, but around 30°C, it becomes immobile (Allerberger 2003).
L. monocytogenes can be found in agricultural and natural habitats, contaminating raw materials that are then brought into food processing facilities. If allowed, the bacterium can even grow at temperatures below freezing and pose a risk to human health when swallowed (Todd and Notermans 2011).

2.1. EPIDEMIOLOGY OF LISTERIOSIS

Bacteria account for 40% of yearly mortality rates (Kumar and Neelam 2016; Pourakbari et al. 2019). Bacteria and their toxins have been found to pollute water and food supplies (Tauxe, 2002). According to estimates, almost 48 million people in the United States of America are diagnosed with various foodborne illnesses every year, resulting in 128,000 hospital admissions and a 3000 case mortality rate (Mehrannia et al. 2023). The combined information from the nationwide listeriosis surveillance in Finland, patient replies to patient interviews, lab results from patient samples, and comparison with listeria findings from food and food manufacturing facilities gathered as part of studies into the outbreak between 2011 and 2021. In Finland, invasive listeriosis occurs more frequently than the norm for the EU (1.3/100000 in 2021), and the majority of cases are seen in elderly people with a inclining condition. Numerous cases mentioned eating high-risk foods and storing food improperly (Suominen et al. 2023). Intrusive listeriosis cases totaling 253 were documented from 2011 to 2016 in 19 provinces, with a case-fatality rate of 25.7% overall and no deaths among minors or expecting women (Li et al. 2018).
According to CDC, since 2000, listeriosis has been a notifiable illness in United states (Donovan 2015).

3. OCCURRENCE OF LISTERIOSIS

Listeria occurs in our environment and many food products. The organism was isolated from the soil (Welshimer 1960), meat products (Gomez et al. 2015) water (Gartley et al. 2022), and decaying vegetation (Welshimer, 1968). *L. monocytogenes* was recovered using cold enrichment techniques from samples of manure, river water, and sewage mud, providing quantifiable evidence of the organism's capacity to persist in the environment. They discovered that *L. monocytogenes* quantitative counts from sewage sludge sprayed on farmland remained stable for at least 8 weeks. When this technique was suspected of being a factor in a significant epidemic of listeriosis in humans in Nova Scotia, the ramifications of utilizing fecal material as fertilizer for agriculture became clear.

3.1. HUMAN LISTERIOSIS

Bojsen-Moller investigated fecal transport in several population groups using cold enrichment. In hospitalized adult patients (1.2%), patients having diarrhea (1%), healthy abattoir employees (4.8%) and household contacts of listeriosis patients (26%). Up to eight samples were obtained from each patient's household contact. Because up to eight samples were taken from each patient's household contact, the prevalence of listeria isolates in this group cannot be directly compared to data from other populations.

At least one member of five out of 14 households had *L. monocytogenes* positive in their stools. Only two of the families, though, had a family member who was infected with the same serotype as the patient. All the cultures were processed by cold enrichment, but comparisons across groups were made more difficult by the non-hospitalized patients' delayed delivery of specimens to a central laboratory and their increased use of antibiotics before culture (Schuchat et al. 1991).

Animal Listeriosis: It's important to keep in mind that *L. monocytogenes* only unintentionally contributes to the clinically visible human infection, even though this is what gives the organism popularity in the media. Therefore, it is doubtful that it would have created its pathogenic collection with a focus on humans. L. *monocytogenes* is largely an animal illness, and it can produce both solitary instances and outbreaks in both domestic and wild animals (Lecuit 2007).

3.2. CLINICAL LISTERIOSIS PREDISPOSING FACTORS

The meta-analysis supported the following set of listeriosis-related mortality risk factors: 1. involvement of the central nervous system, initial bacteremia, and Age 60 years were clinical predisposing factors; 2. non-hematological malignancies, alcoholism, chronic renal disease, cardiovascular disease, and pulmonary illness were the predisposing comorbidities (Huang et al. 2023).

3.3. PATHOGENICITY OF LISTERIA MONOCYTOGENES

Even though *L. monocytogenes* is frequently present in the atmosphere and human exposure to it is likely prevalent based on carriage studies, invasive listeriosis is a rare complication. Three factors can influence whether an invasive disease will manifest: the host's susceptibility, the virulence of the infecting organism, and the quantity of the inoculum.

The software "Find Target" is used to compare these genome sequences in order to find probable virulence genes and, more generally, to comprehend the pathogenicity of *L. monocytogenes* and its capacity to contaminate food. Additionally, a comparative genomics technique based on DNA arrays is being used to characterize clinical and environmental isolates of Listeria (Ramaswamy et al. 2007). A comparative genomics method using microarrays for the assessment of the biodiversity of Listeria, and that of the species L. monocytogenes, has shown amazing accomplishments in gene expression investigations (Jacquet et al. 2004).

3.4. CLINICAL FEATURES OF LISTERIA INFECTIONS

In all vulnerable hosts, *L. monocytogenes* infection manifests clinically in a fairly similar way. Perinatal listeriosis and listeriosis in mature patients are the two main ways in which these infections manifest. The CNS is affected by either a localized infection or a widespread illness in both cases. Even with early antibiotic therapy, listeriosis has an average fatality rate in humans of 20 percent to 30 percent or more, making it one of the deadliest bacterial illnesses presently known (Allerberger 2003; Mclauchlin 1990; Schuchat et al. 1991).

4. VIRULANCE DETERMINANTS

4.1. HEMOLYSIN (HLY)

This gene (hly) was the foremost virulence determinant factor recognized and sequenced in the Listeria species. Further investigation of the hly locus led to the finding of a chromosomal virulent gene group,

which comprises the majority of the genetic elements compulsory for the intracellular developmental process of this pathogenic Listeria species (Fig. 2). The Hly product was also the pioneer pathogenic factor to play a specific role in the Listeria infection illness process. Hly is critical virulence factor required for the infection and participates in some different processes that occur when listeria interact with their vertebrate host, including intracellular parasitism (Goebel et al. 2013).

4.2. PHOSPHOLIPASES

Pathogenic *Listeria* spp. produces three different phospholipase C (PLC) enzymes with virulence properties. PlcA and PlcB are present in both *L. ivanovii* plus *L. monocytogenes*, but SmcL is exclusive to the *L. ivanovii*. The first description of *L. monocytogenes* producing phospholipase activity was made in 1962 (Fuzi and Pillis 1962), this study showed that the strength of the opacity reactions in egg yolk agar associated with the tested strains' hemolytic ability.

4.3. INTERNALIN

Pathogenic Listeria spp. include a novel family of virulence-related genes, which generates the protein internalins. By examining a collection of mutants that are transposon-induced for reduced intrusiveness in Caco-2 cell monolayers, researchers revealed the first two members of this family to be characterised, InlA and InlB, encoded by the inlAB operon. Internalin was administered to InlA when it was shown that it behaved as an invasin, promoting bacterial internalisation by these typically nonphagocytic epithelial cells (Gaillard et al. 1991). Since then, many internalin homologs in L. ivanovii and *L. monocytogenes* have been discovered (Dramsi et al. 1997; Engelbrecht et al. 1998b, 1998a; Raffelsbauer et al. 1998).

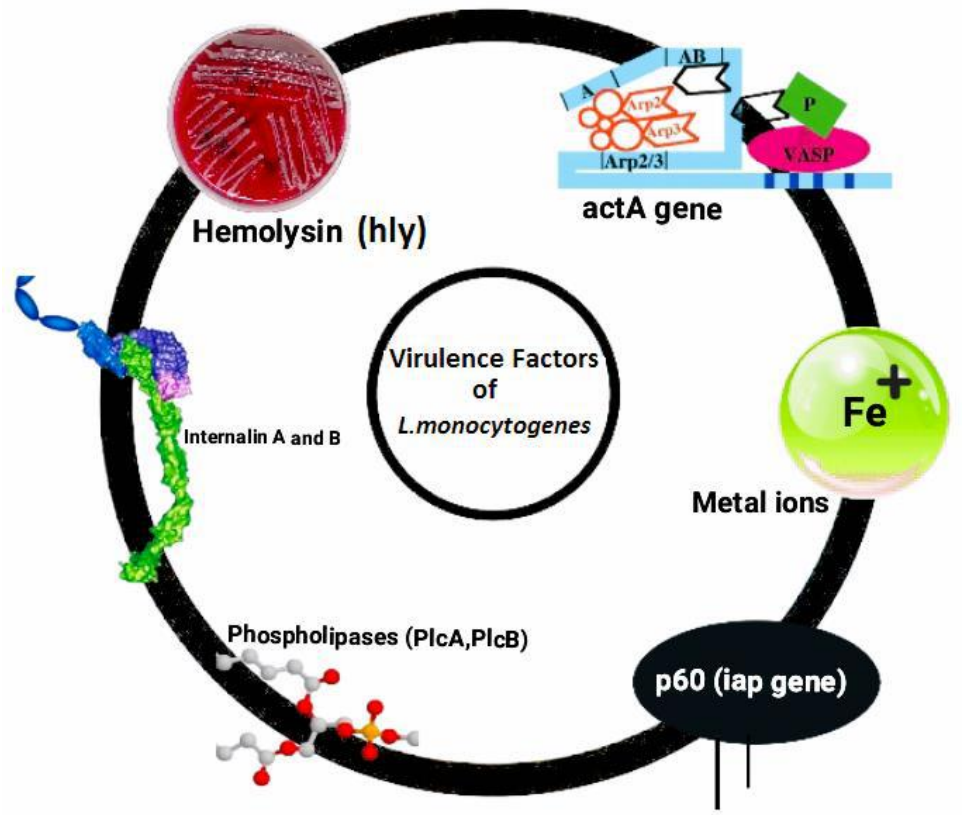

Fig. 2: Different Virulence Determinants present in L. monocytogenes

A component known as a leucine-rich repeat (LRR) domain, that is a tandem repeat pattern of a sequence of amino acids with leucine repeats at specific places, is a feature shared by all internalins (Kajava 1998). Leucine or isoleucine residues are found at locations 3, 6, 9, 11, 16, 19, and 22 in the typical LRR unit of internalins (— —L— —L— —L—L— —N—I— —I/L— —L). This sequence produces a new, right-handed helix known as a parallel b-helix with a turn next to each LRR unit. It was initially found in the pectate lyase of Erwinia chrysanthemi (Heffron et al. 1998; Yoder et al. 1993).

4.4. ACTA

The crucial title role of ActA in listerial intracellular mobility and virulence was first discovered through a mutation of L. monocytogenes-infected cultured tissue cells (Kocks et al. 1992). After entering the host cell, these bacteria were able to exit into the cytoplasm, but they gathered as micro-colonies in perinuclear region of the cell because they were unable to migrate about the cell. Phalloidin, a fungus toxin that is attached with F-actin and paralyses actin cytoskeleton, was used to dye the mutant to demonstrate that it was unable to recruit actin. Additionally, a significant diminution of the actA mutant was seen in the research of mouse contamination example (Domann et al. 1992; Kocks et al. 1992).

4.5. p60 (iap)

L. monocytogenes suddenly produces colonies with a modified, stable, rough phenotype on plates of agar. These bacteria grow in the form of lengthy filaments made of chains of individual cells. Impaired invasiveness is correlated with this lack of virulence, especially in fibroblasts (Kuhn and Goebel 1989). The synthesis of the important 60-kDa extracellular protein p60, which is present in both the culture supernatant and the invasion deficit, is defective (Kuhn and Goebel 1989) and connected with the cell wall (Ruhland et al. 1993) and when exogenously introduced, breaks apart the bacterial networks and recovers invasiveness (Kuhn and Goebel, 1989). The iap gene, which codes for a 484-residue of polypeptide with core repeat regions of Thr-Asn units in L. monocytogenes, is responsible for producing the p60 protein (Kohler et al. 1990).

4.6. METAL ION UPTAKE

Every living thing, even prokaryotic cells, must have iron because it serves as the cofactor for huge collection of enzymes as well as necessary proteins that are associated in electron transport process. Because ferric transferrin and heme molecules in cells' interiors and ferritin in serum bind iron, it is not easily available in the tissues of animal hosts. Because of this, bacterial pathogens have created distinct methods for obtaining iron for development in host tissues. These procedures are essential for pathogenicity (Payne 1993).

4.7. RECENT DEVELOPMENTS IN LISTERIA DETECTION

One hypothesis states that food needs contain 100 CFU/mL/g of Listeria to be infectious. Because of overdue and ambiguous signs, it is problematic to detect at the very initial stage. According to Australian study, listeriosis can be brought on by 10 colony forming units in 25 g of fast food and can be reactivated by 100 CFU/mL. So, Scientists had settled a number of methods to address the requirement for a dependable, delicate, and repeatable method to discover *L. monocytogenes*. Below is a discussion of the most useful and accessible detection methods that have been created to yet.

4.8. CULTURE-BASED TECHNIQUES

The difficult yet exact cold enrichment technique was developed in the 1990s, (Lorber, 2002). The separation of the chromogenic substrate by the enzyme known as -D-glucosidase and the hydrolysis of lecithin appeared to be because of the blue or green colonies. Their cities resembled hazy haloes. After the bacteria's existence was confirmed, it was re-dissolved in non-selective media to get ready for the 4-5 days biochemical test. Additionally, there used to be a significant risk of results that were false positives and a requirement for numerous chemicals, media, and reagents, and also the effort and time investment (Jadhav et al. 2012). A researcher got comparable outcomes using the ISO 11290-1 technique, which was created in 2004 and used a LOD of 1 CFU/g. They later learned that the LOD was 1 CFU/g utilising the 2013 USDAFSIS methodology (Valimaa et al. 2015). The most probable number technique (Dwivedi and Jaykus, 2011) was more sensitive than a chromogenic medium. Listeria was identified too rapidly, indicating that MPN-PCR was a more promising technology than earlier methods (Law et al. 2015).

4.9. IMMUNO-BASED TECHNIQUES

The use of antigen-antibody biochemistry in illness screening and diagnosis seems to have promise. To light this, (Gasanov et al. 2005) stated that an immunological procedure have sensitivity more than a conservative method, that is 105 cells per mL.

4.10. ELISA

ELISA was used for examining food models in 2010 (Ueda and Kuwabara, 2010). Another scientist used an indirect ELISA to test blood samples at a dilution of 1:200 for listeriosis; positive P/N ratios were set to greater than 2. Synthetic LLO-2 peptide (0.40 g/well) and rLLO (0.50 g/well) were used as antigens during this method. A LOD of 105-106 CFU/mL was found to be reliable based on the pH and basicity of the food specimen (Malla et al. 2021).

4.11. IMMUNO-MAGNETIC SEPARATION

In order to improve the sensitivity of the detection approach, a researcher first demonstrated a methodology in 2006 that required connecting a magnetic field with a substantial number of cells of bacteria (Amagliani et al. 2006). In order to identify the hlyA gene in milk samples, a researcher created a prototype in 2006 that combined real-time PCR with an immune-based technique employing rabbit anti-Listeria and beads coated with immuno-magnetic nanoparticle. The Limit of detection observed was >102 CFU/0.5 mL (Yang et al. 2007). Similar to this, a study from 2010 employed paramagnetic beads covered with the Listeria endolysin-derived cell wall domain from contaminated uncooked milk. This LOD ranges from 102 to 103 CFU/mL (Walcher et al. 2010).

5. MOLECULAR METHODS DETECTIONS

5.1. DNA MICROARRAYS

DNA microarray was used to recognize the Listeria virulence genes plcB, inlB, plcA and clpE in 2002. Using this technique, he claimed that the Listeria test was positive (Volokhov et al. 2002). By joining 585 mixed genomic DNA probes, a researcher explored serotype-specific probe differentiation and discovered that 29 probes were successful (Borucki and Call, 2003). As a follow-

up, it was employed as a confirmatory approach to evaluate the sensitivity of PCR amplification and polymorphism. with an 8 log CFU/mL limit for detection, Another study found that 9/16 of the microarrays that were used to analyse the purposely contaminated milk returned positive results. He emphasised the accuracy and dependability of this strategy. Although encouraged, it requires persistence and has a chance to cross-hybridize, which could produce an inaccurate test result (Bang et al. 2013).

5.2. PCR BASED METHODS

In molecular diagnostics, PCR is frequently employed as a promising method for the identification of small samples. A specific set of specialised primers were needed for specific target amplification during a heat cycle in PCR. Gel electrophoresis is then utilised to analyse the outcomes. Below is a discussion of the adjustments that were made to allow for the detection of Listeria utilising PCR:

5.3. CONVENTIONAL PCR

Since PCR employs primers to identify pathogens in a sample, it is a promising technique. Aznar and Alacron (2003) claim that whereas only 17 cases were discovered to be positive during culture, 56 out of 217 instances in naturally infected testers obtained positive PCR results with an edge of detection of 1 CFU/g. They used primers to check for the presence of hypersensitivity protein and the proteins phospholipase C and fibronectin-binding protein, as well as the genes hlyA, iap, inlB, inlA, 16S, and 23S rRNA (Aznar and Alarcon 2003).

5.4. MULTIPLEX PCR

The immediate detection of numerous species in contaminated samples using multiplex PCR has been characterised as a reliable, efficient, and time-saving method (Alarcon et al. 2004). Samples with different LODs, including 260 CFU/ml of S. aureus, 79 CFU/ml of L. monocytogenes, and 57 CFU/ml of Salmonella species (Bang et al. 2013). Using LODs of 1-100 CFU/ml, this method was used to find six prevalent food-borne pathogens in RTE meals (Lei et al. 2008). The hly gene of L. monocytogenes, the nuc gene of S. aureus, the invA gene of S. enterica, the stx gene of E. coli, and the intimin gene of E. coli are targets of the MPCR method, which was developed and has a detection limit of 1 CFU/mL (Zhang et al. 2009). Another study from 2006 claimed that MPCR was not specific for amplicons of similar size and optimization (Liu 2009).

5.5. REAL-TIME PCR (RT-PCR)

With a detection limit of 10CFU per 25 g of food, a 3-day PCR-based test was created that is equal to the EN ISO 11290-1 or ISO 10560 protocols for Listeria discovery. The LOD was 1104 CFU/mL (Kaclikova et al. 2002). According to another study, the total viable count found in the salad was 1.35, 2 and 1.8 CFU/g and in the broccoli it was 0.35, 1.9 and 1.8 CFU/g. In which *L. monocytogenes* had a limit of detection of 1.74, 1.1, and 1.6 CFU/mL in salad and 6.37, 1.2 and 1.3CFU/mL in broccoli, with a total of less than 1000 cells/m (Bhagwat 2003). In 2005, a hly-IAC Q-PCR assay for the detection of Listeria was developed, the detection limit of 8 was established by using varied amounts to spike the sample (Rodriguez-Lazaro et al. 2005). A researcher created quantitative real time-PCR to determine the fluorescence released by the spiked sample in order to broaden the reach of the procedure (Berrada et al. 2006). The obtained LOD

was 10–105 CFU/mL (Berrada et al. 2006). Targeting the ssrA gene in naturally and intentionally contaminated foods (dairy foodstuffs, vegetables and meat) led to a detection limit of 1–5 CFU/25 g/mL, according to another researcher (O' Grady et al. 2008). He therefore concluded that it was a wise scheme for the specific sample. The results of a qRT-PCR analysis on both naturally and intentionally infected ground beef, chicken, turkey, and pork with a detection limit of 18 CFU/10 g were published in a study in 2010 (Suo et al. 2010).

5.6. BIOSENSOR BASED TECHNIQUES

The biological specimen analyzer known as a biosensor uses an analyte as the object and an electrochemical setup as the transducer to provide legible data. He passed the antibody through a biosensor chip immobilised on polyclonal goat anti-rabbit Fab antibodies in 2004 to detect *L. monocytogenes* (Leonard et al. 2004). The procedure of surface plasmon resonance to identify *L. monocytogenes* was found to be promising, with a detection limit of 102 CFU/mL, according to another study that advanced the sensor platform (Poltronieri et al. 2009). Au-labeled secondary antibodies were applied on this platform. A study reported using collagen matrix-merged mammalian B-lymphocyte Ped-2E9 cells as a sensing tool to detect listeriolysin O from the contamination of a food section with an acceptable detection limit of 102-104 CFU/g in a subsequent study (Banerjee and Bhunia 2010).

6. CONCLUSION

Even though listeriosis is not a serious medical issue, the high death rate of apparent listeriosis in younger, older, and immune-compromised patients poses a difficulty for veterinary professionals, food microbiologists as well medical microbiologists, and doctors. Decontaminating domestic livestock and food products has been a crucial preventive measure since food has been identified as the primary source of illness.

Its ability to grow inside cells, iron compounds, catalase and superoxide dismutase, surface components, and hemolysins are just a few of the factors that have been suggested over time to affect L. monocytogenes' pathogenicity, which This suggests that it is their virulence is multifactorial.

Additionally, since meningitis and encephalitis are the most common symptoms of disease, it is important to choose medications that are easy to pass the blood-CSF barrier and the blood-brain barrier. It will be necessary to use unconventional techniques in the future to lessen the health danger that listeria poses. Listeria monocytogenes can be detected using a variety of approaches, but molecular methods such as DNA microarrays, PCR-based methods, and biosensor-based methods are thought to be the most reliable.

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Leptospirosis: A Zoonotic Disease with Reproductive Implications

26

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ABSTRACT

Leptospirosis, a long-standing zoonotic threat that has been recognized for more than a century, has drawn more attention because of its significant effects on public health, especially when it comes to reproductive health. This bacterial disease, that is caused by the spirochete bacterium of genus Leptospira, is quite common around the world and affects both developed and developing countries. The complex nature of leptospirosis transmission, which is deeply intertwined with eco-epidemiological contexts, demands comprehension of its multiple manifestations. The disease's diverse epidemiology is attributed to the vectors that carry it from urban to rural areas, including contaminated water sources and rats. Within the complex landscape of pathogenesis, leptospirosis presents as an acute bacterial septicemic febrile sickness that affects multiple organs and systems. The disease's severity is highlighted by its chronic form, referred as Weil's syndrome, which affects both people and animals. It also has a major impact on reproductive health, since it increases the risk of infertility, abortion, and stillbirth in females. Diagnostic techniques, essential for prompt intervention, involves dark-field microscopy and serological testing. The diagnosis is complicated, necessitating careful specimen collection. The zoonotic nature of the disease, as evidenced by the facts, demands heightened awareness, especially among the people who are at risk. Effective control strategies such as vaccination, chemoprophylaxis, and herd management are crucial since the disease can impact a wide range of populations, including farmers, sewage workers, and medical personnel. This chapter offers a thorough examination of leptospirosis, covering its etiology, epidemiology, pathophysiology, diagnostics, and complex zoonotic network. The emphasis on reproductive implications highlights the need for more knowledge and investigation to improve animal and human health outcomes in the face of this persistent public health issue.

Keywords: Leptospirosis, Reproductive implications, Weil's syndrome, Zoonotic nature, Public health.

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1. INTRODUCTION

Leptospirosis is a zoonotic disease that has been recognized as a public health threat for over a century (Bharti et al., 2003). Both humans and animals are infected by the disease caused by the *spirochete* bacterium of genus Leptospira (Adler & de la Peña Moctezuma, 2010). This disease is commonly found in livestock, wild animals, pets and can infect humans who come into contact with infected animals. Leptospirosis is prevalent in many parts of the world, with more than one million cases reported annually (Picardeau, 2015). The transmission of leptospirosis occurs through direct or indirect contact with the urine of infected animals and contaminated water or soil (Hartskeerl, Collares-Pereira, & Ellis, 2011). The disease can cause a wide range of clinical symptoms, from mild flu-like illness to severe multisystem organ failure (Gouveia et al., 2008). However, recent studies have found that leptospirosis can have significant reproductive implications, especially in women.

Leptospirosis is a global public health concern with an estimated incidence of 1.03 million cases and 58,900 deaths annually (Costa et al., 2015). The disease occurs in both developed and developing countries, with higher incidence rates reported in low- and middle-income countries. Studies have suggested that leptospirosis can cause infertility, abortion, stillbirth, premature birth, and other complications in women (Puliyath & Singh, 2012). Study conducted in Brazil showed that women with a history of leptospirosis infection had a higher risk of miscarriage and premature birth compared to the uninfected ones (Plank & Dean, 2000). In boars, the disease can lead to epididymitis and decreased sperm quality (Cilia, Bertelloni, Cerri, & Fratini, 2021) . Recently, Lilenbaum and Loureiro proposed that the silent reproductive type of leptospirosis, also known as bovine genital leptospirosis, should be treated as a separate disease (Loureiro & Lilenbaum, 2020). It is most frequently caused by strains of the Sejroe serogroup that have modified, and it is connected to early embryonic losses and subsequent oestrus repetition, perhaps as a result of inflammation of the uterus or Leptospira attack on the embryo directly (Libonati, Santos, Souza, Brandão, & Lilenbaum, 2018; Mori et al., 2017). In recent times, it was shown that sheep with leptospirosis had substantial levels of oxidative damage, which contributed to the pathophysiology of reproductive disruption (Silva et al., 2019). Like other ruminants, abortion is the most significant clinical outcome of caprine leptospirosis (Dehkordi & Taghizadeh, 2012). Stillbirth and abortion are two reproductive problems, and the delivery of diseased foals is a frequent outcome in horses (Whitwell, Blunden, Miller, & Errington, 2009). Furthermore, the presence of Leptospira in the uterine environment has related to a localized inflammatory reaction that results in pregnancy losses (Pinna, Martins, Souza, & Lilenbaum, 2013).

Despite growing evidence of the reproductive implications of leptospirosis, the disease remains under-recognized in this context. This chapter aims to provide a comprehensive overview of leptospirosis as a zoonotic disease with reproductive implications. It will review the current epidemiology, transmission, diagnosis, and treatment of leptospirosis, with a specific focus on its impact on reproductive health. It will also discuss the prevention and control strategies for leptospirosis in the context of its reproductive implications.

Overall, this chapter will highlight the need for increased awareness and research into the reproductive implications of leptospirosis to improve health outcomes for animals and humans.

2. EPIDEMIOLOGY: THE PREVALENCE OF LEPTOSPIROSIS

Leptospiral transmission can occur in a variety of eco-epidemiological contexts, including urban, rural, recreation-related, and disaster-related situations. Rats infesting sewage networks, sewage overflowing

during rainstorms, flooded roads, and human being exposed to flooded roadways all contribute to a perfect environment for leptospirosis transmission in urban settings. The source of leptospiral infection in rural areas is frequently agricultural contact with moist fields that may be polluted with rat or farm animal urine (Himani, Suman, & Mane, 2013).

The attack rate, afflicted population, and predominate pathogenic serogroups of leptospirosis in Israel have all altered during the past 15 years. In Israel, the reported assault rate dropped from 2 to 3.6 per 100,000 people between 1950 and 1970, to 0.2 per 100,000 people in the 1980s, and to about 0.05 per 100,000 people throughout the time of their investigation (Kariv, Klempfner, Barnea, Sidi, & Schwartz, 2001). Over the course of the research period, leptospirosis epidemics extended widely and were more frequent, especially in tropical ecoregions. Due to the frequent occurrence of massive outbreaks and high death rates, the effect may be substantial (Munoz-Zanzi et al., 2020).

3. TRANSMISSION

Leptospirosis may be spread by nearly all animals, which contain and expel the organisms from their proximal tubules of the kidneys (Haake & Levett, 2014). The rat is by far the most significant vector of leptospirosis in humans. Because they are found close to human homes and they frequently excrete significant amounts of microorganisms, even months after becoming infected. The most frequent way the disease transferred to people is by skin abrasions and mucous membranes coming in contact with water that has been polluted with infected rat urine. Because they are accidental hosts, people are more at risk when they work or live near the maintenance hosts, particularly rats and farm animals (Rajapakse, 2022). Leptospirosis transmission can be done either by direct contamination with infected rodents and carrier animals or indirect transmission through environment. Routes of transmission of leptospirosis are illustrated in Fig. 1.

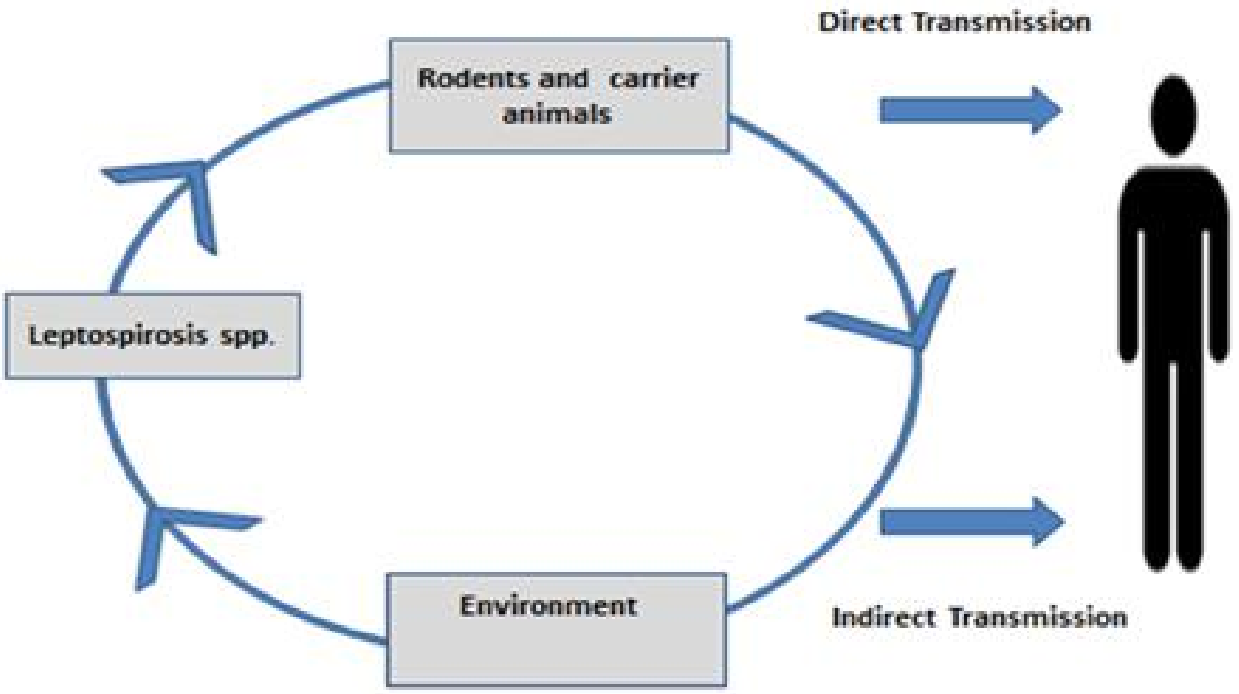

Fig. 1: Routes of Transmission of Leptospirosis.

Some researchers found that a high rate of leptospirosis in cattle presents a significant risk to both human health and agriculture economics (Talpada et al. 2003). *L. interrogans* can be spread to human beings and other animals by being excreted in the urine of sheep and goats (Haji et al. 2022).

4. PATHOGENESIS: UNDERSTANDING THE MECHANISMS OF LEPTOSPIROSIS

Pathogenic leptospires are the source of acute bacterial septicemic febrile illness known as leptospirosis, which can affect both humans and animals worldwide (Costa et al., 2015). Direct contact with infected animals can result in transmission, but this is less common. Instead, transmission is more likely to occur when infected animals' urine contaminates water or soil. The Weil syndrome is the chronic form of leptospirosis, characterized by multiorgan impairment, including renal, vascular, skeletal muscle injury, hepatic, and pulmonary (Goris et al., 2013). Widespread in nature, pathogenic species of Leptospira are maintained in the environment by their life cycles, which involve hematogenous and intercellular diffusion to the proximal kidney tubules of the numerous reservoir hosts.

Leptospira is frequently discharged through urine for short periods of time by humans, who are accidental hosts (Levett, 2015). The urine contaminated soil is cleaned of leptospires after heavy rains and deposited in water bodies, which is the cause of epidemics of leptospira infection. Leptospires can be removed from environmental water sources such as sewage, agricultural fields, wet soil, lakes, water dams, ponds, springs, rivers and decorative water fountains (Escandón-Vargas, Bustamante-Rengifo, & Astudillo-Hernández, 2019). Most severe symptoms of human leptospirosis, including fever, icterus, renal failure, and mortality, are referred to as Weil's disease (O'Toole, Pathak, Toms, Gelding, & Sivaprakasam, 2015). Similar to Weil's disease, domestic animals are susceptible to contracting this acute, potentially lethal illness. Significant kidney damage may develop, especially in dogs under certain circumstances. Horses have persistent uveitis (Verma et al., 2012) and have lower physical ability (Hamond, Martins, Lilenbaum, & Medeiros, 2012). The acute and life-threatening type of leptospirosis in cattle, like that in other ruminants, is uncommon and mainly associated with sporadic outbreaks in calves triggered by accidental strains (Loureiro & Lilenbaum, 2020). In fact, a subclinical chronic form of animal leptospirosis, which is frequently neglected, is the most typical presentation. In this type, reproductive symptoms predominate (Adler & de la Peña Moctezuma, 2010). The chronic form of Leptospira causes significant reproductive abnormalities as a result of colonizing the reproductive canal, which has contributed to economic decline (Mori et al., 2017).

Sant'Anna et al. showed that (living in endemic areas) subclinical leptospiral infection in dogs may be linked to chronic renal disease (Sant'Anna, Vieira, Oliveira, & Lilenbaum, 2019). Cuts and abrasions, mucous membranes like the conjunctiva, weak, wet skin, and mucous membrane of the nose are the primary routes for leptospires into the body. A 7-day bacteraemia is typical. Although thought to be comparable to that in humans and dogs, the pathophysiology of the disease in cats is yet unclear (Murillo, Goris, Ahmed, Cuenca, & Pastor, 2020).

5. CLINICAL MANIFESTATIONS: SYMPTOMS AND DIAGNOSIS OF LEPTOSPIROSIS

Between 20% and 40% of acute febrile diseases are caused by Leptospira (Abela-Ridder, Sikkema, & Hartskeerl, 2010). Weil's Disease, commonly described as a febrile sickness with no discernible symptoms, to multiorgan failure with renal and pulmonary signs (Holla et al., 2018). Additionally, pathophysiological anomalies such as increased blood creatinine levels, hyperbilirubinemia, thrombocytopenia and leukocytosis were seen (Holla et al., 2018; Organization, 2003).

Subfertility and early embryonic mortality are two modest symptoms that are frequently linked to bovine leptospirosis (Loureiro & Lilenbaum, 2020). Although abortion does occur, the chronic phase of an adapted

infection seems to be silent. Cattle breeders and doctors commonly ignore it since it usually manifests in a subclinical form (Ellis, 2015). Adapted leptospiral infection in animals has related to less evident reproductive failures such as early embryonic losses and the resulting estrus repeat. Despite the complex aetiology of these symptoms, two recent investigations on cattle have found a substantial correlation between estrus recurrence and seroreactivity against the Sejroe serogroup (Libonati et al., 2018; Mori et al., 2017). Horses may also be affected with genital leptospirosis, which is a quiet chronic reproductive illness that is frequently misdiagnosed and untreated. The main cause of EGL (Equine Genital Leptospirosis) globally is Serovar Bratislava. The most frequent consequences are estrus recurrence and subfertility (Di Azevedo & Lilenbaum, 2022). Long-term reproductive production of herds can be negatively affected by genital leptospirosis (Loureiro & Lilenbaum, 2020). However, severity of the disease varies based on the species that is afflicted and the infecting strain (Ellis, 2015). Researchers have discovered *L. santarosai* in the testes and semen of a boar's reproductive system. As the animal was not excreting significant numbers of leptospires at the time of urine collection and the emphasis of the infection appeared to have been in the reproductive system, they were also able to find the bacteria in kidney tissue but not in urine (Diaz et al., 2022). Ruminant leptospirosis can manifest as an acute illness or, more frequently, subclinically. Loss of appetite, irritability, diarrhea, opaque furs, epidemic abortions, and milk drop syndrome are all symptoms of acute illness (G Martins, Brandão, Hamond, Medeiros, & Lilenbaum, 2012). Severe sickness is typically linked to accidental serovars, primarily Pomona, Ballum, Icterohaemorrhagiae, or Grippotyphosa, and is frequently associated with lambs and goat kids (Vermunt, West, Cooke, Alley, & Collins-Emerson, 1994). Subclinical infection, on the other hand, is mostly characterized by reproductive issues, such as infertility, an increase in the number of services per conception, longer calving intervals, abortion, and the frequency of stillbirths and poor lambs/goat kids (Gabriel Martins & Lilenbaum, 2014). In women, the results of pregnancies have been as diverse, including foetal loss and miscarriage (often within the first few months of pregnancy) (Carles, Montoya, Joly, & Peneau, 1995; Shaked, Shpilberg, Samra, & Samra, 1993), congenital infection (Shaked et al., 1993), stillbirth (Baytur et al., 2005) and oligohydramnios, as well as successful deliveries of healthy newborns (Gaspari et al., 2007). Foetal CTG (cardiotocography) monitoring also seems relevant, especially in late pregnancy and severe stages of illness, given the potentially bad pregnancy outcomes of stillbirth and miscarriage linked with leptospirosis in pregnancy (Koe, Tan, & Tan, 2014). A diagrammatic representation of disease manifestation is shown in Fig. 2.

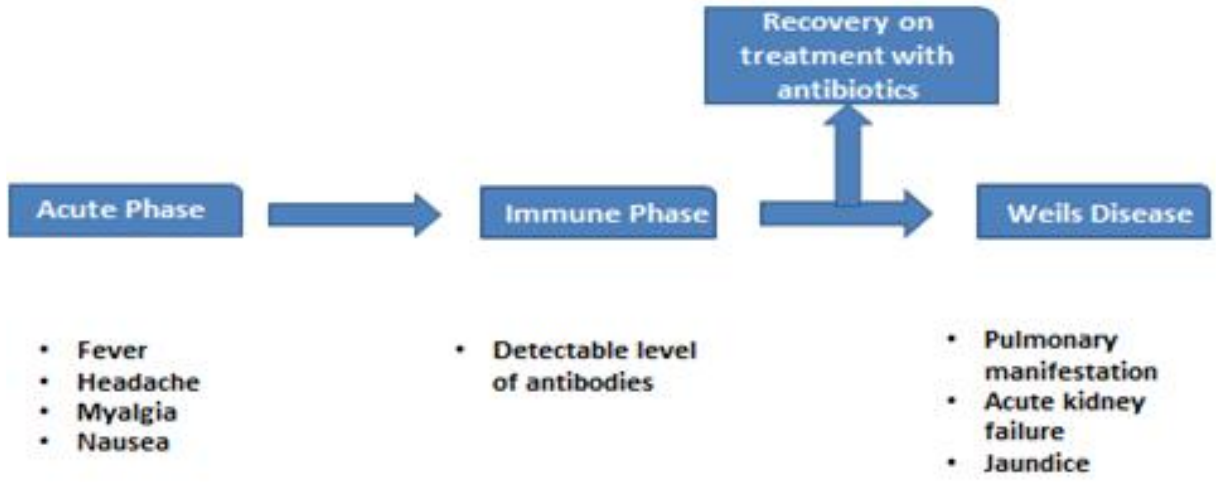

Fig. 2: Diagrammatic Representation of Disease Manifestation.

6. DIAGNOSIS

Leptospirosis diagnostic success is influenced by the kind and timing of specimen collection. During the acute phase, the organism is known to spread quickly into bodily fluids and tissues including CSF (cerebrospinal fluid) and blood (Mullan & Panwala, 2016). Blood, bodily fluids, and urine are the specimens that were collected. The information on specimen collecting is provided in Table 1.

Table 1: Specimen Collection Guide: Comprehensive Information Table for Proper Sampling.

Sample	Objective	Collection Time	Preservatives
Blood	Culture, dark field microscopy, and isolation. Serological test	Prior to seven days of antibiotic treatment.	Fresh within 4h. Chilled and fresh within 4h
Serum	Serological test	After 5 to 7 days	Chilled and fresh within 4h.
Urine	Culture, DFM, and isolation	After 5 to 7 days	Urine is collected immediately after urination and then diluted with phosphate-buffered saline (pH7.2). Within 4 hours, you must arrive at the lab.
CSF	Serological test	After 10 days	Chilled and fresh within 4h.
Aqueous humor	Serological test	After 10 days	Fresh, chilled, within 4h. Aqueous humor tends to gel.

7. DETECTION METHODS

Serological procedures, such as the Microscopic Agglutination Test (MAT), culture isolation of Leptospira, genomic DNA identification using molecular methods, antibody detection, and dark-field microscopy (DFM), are used to diagnose leptospirosis. Fig. 3 shows pictorial representation of diagnostic methods.

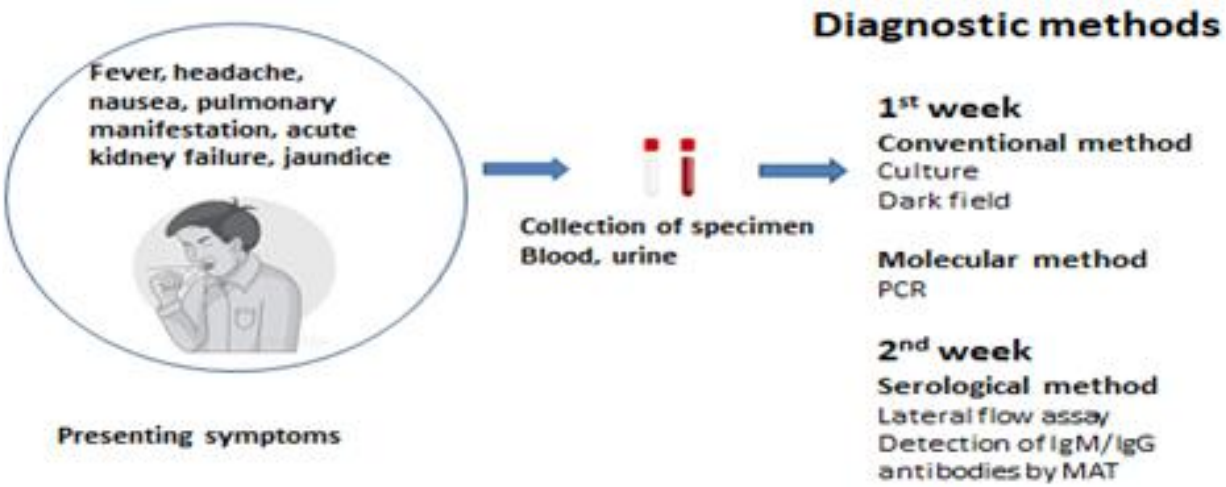

Fig. 3: A Pictorial Representation of Diagnostic Methods

For the diagnosis of leptospirosis, there are two conventional methods i.e, dark field microscopy (DFM) and cultural method (Pinto et al., 2022). Using DFM, it is possible to show that Leptospira is present in bodily fluids such as blood, serum, urine, and CSF. Clinical specimens must be analyzed using experienced people who must recognize the organisms, a sophisticated dark field microscope, and the clinical samples must be handled with biosafety (Niloofa et al., 2015). Biological fluids isolation and culture of Leptospira

including blood, CSF, and urine are regarded as standard methods for the diagnosis of leptospirosis (Gökmen, Soyal, Kalayci, Önlen, & Köksal, 2016). Serological tests such as microscopic agglutination tests, IgM ELISA, and rapid diagnostic tests are used for the diagnosis of leptospirosis. Some molecular methods are used for the diagnosis of leptospirosis such as polymerase chain reaction (PCR), chip-based RC-PTR kit, real-time PCR, and loop-mediated isothermal amplification (LAMP) (Pinto et al., 2022). Leptospirosis can be detected by various techniques as described in Table 2.

Table 2: Comparative Analysis of Different Diagnostic Techniques

Tests for Diagnosis	Specimen required	Advantages	Disadvantages	Reference
Microscopic agglutination test	Serum CSF Aqueous humor	• Easily available • Determines serogroup	• Only in specialized laboratory • May be negative for the first 5-7 days • Cross-reaction might lead to unclear interpretation	(Cole Jr, Sulzer, & Pursell, 1973)
ELISA	serum	• May detect infection earlier than MAT	• Not serogroup specific • Not widely available	(Rosa et al., 2017)
Indirect Haemagglutination	Serum	• May detect an infection before MAT • Effective in a variety of host species	• Not serogroup specific • Not easily available	(Sykes, Reagan, Nally, Galloway, & Haake, 2022)
Bacterial culture	Urine Blood Serum Tissue CSF Aqueous humor	• Clearly demonstrated presence of the organisms	• Technically challenging • Long turnaround time	(Bhatia, Umapathy, & Navaneeth, 2015; Fornazari et al., 2012)
Dark field microscopy	Urine Blood CSF Aqueous humor	• Fast results	• Handling of potentially infective samples	(Bhatia et al., 2015)
Polymerase chain reaction	Urine Tissue Plasma	• Fast results • Available at specific laboratories	• Not serogroup specific	(Brown et al., 1995)
Real time PCR	Urine Plasma Tissue	• Early acute disease • Detects response to treatment	• Not serogroup specific • Equipment cost	(Riediger et al., 2017)
LAMP assay and modification	Plasma	• Acute stage • To recognize relatedness of leptospira	• Not serogroup specific • Lack of specificity	(Sengupta et al., 2017)
Lateral flow assay	Serum	• Pen side test • Screening test • No specialized equipment/training	• High titer is mandatory • Low stability • Only qualitative detection	(Deenin et al., 2022)

8. ZOONOTIC NATURE: THE ROLE OF ANIMALS IN THE TRANSMISSION OF LEPTOSPIROSIS

Since both domestic and wild animals can carry leptospires, everyone is at risk of contracting the disease. Those most at risk include medical professionals, people who care for animals, farmers and agricultural workers, fishermen, rodent catchers, water sports enthusiasts, members of the National Disaster Response Force (NDRF), volunteers for rescue efforts in flood-affected areas, sanitary and sewage workers, etc. (Karpagam & Ganesh, 2020). Direct human-to-animal contact has a lower risk of transmitting Leptospira than indirect contact. Leptospira infection in humans is spread by accidental or intentional contact with contaminated water or soil by carrier animals (De Brito, Silva, & Abreu, 2018). Domestic animals, wild animals, and peri-domestic animals that are asymptomatic carriers keep a variety of Leptospira spp. in their renal tubules and excrete them in their urine for a period of time that can vary from a few weeks to a few months. In rare cases, life-long perseverance without an animal carrier has been observed (Herman, Mehta, Cardenas, Stewart-Ibarra, & Finkelstein, 2016). In urban slum areas, rats are the most common carrier and infection source because they show no symptoms. They spread disease by urinating in public places, contaminating soil and water sources, and acting as a reservoir for the pathogen. There are reports of 104-107 leptospires in the urine of infected or carrier rats (Witchell et al., 2014).
Leptospires are carried from the urine-contaminated ground and deposited in water bodies by heavy rain, which is the reason leptospire infection epidemics are usually linked to floods and storms. Environmental water sources including sewage, farm fields, moist soil, ponds, rivers, lakes, streams, water reservoirs, springs, and even beautiful water fountains may all be treated to eliminate leptospires (Escandón-Vargas et al., 2019). When there are floods, rain washes the fertilizer out of the soil and raises the pH, which encourages leptospire development and survival (Shekatkar, Harish, Menezes, & Parija, 2010). Pathogenic leptospires may live in fresh water and damp soil for weeks to years, particularly in slightly alkaline conditions (Trueba, Zapata, Madrid, Cullen, & Haake, 2004). Humans can become infected by contact with contaminated water and soil, as well as very infrequently through ingestion and inhalation while engaging in work- or leisure-related activities. Only a few cases of leptospirosis spreading between people and indirectly through animal bites have been reported. (Musso & La Scola, 2013). (i) exposure at work, such as farmers, veterinarians, slaughterhouse workers, animal caretakers, gardeners, fishers, sewage workers, and rice mill laborers; (ii) traveler exposure, such as those who visit leptospirosis endemic areas without taking the necessary precautions; (iii) freshwater sports participation, such as canoeing, caving, surfing, etc.; and (iv) Volunteers that labor in flooded areas to provide disaster assistance (Karpagam & Ganesh, 2020). The zoonotic nature of the disease is illustrated in Fig. 4.

9. REPRODUCTIVE IMPLICATIONS: EFFECTS OF LEPTOSPIROSIS ON REPRODUCTIVE HEALTH

Host-adapted leptospires infections, such as those caused by strains from the Sejroe serogroup, are frequently linked to bovine leptospirosis. Adapted strains of bovine leptospirosis can cause abortions, foetal deaths, premature births, and the birth of weak and/or underweight calves, however these symptoms are less common and are more closely associated to subfertility and early embryonic death (Loureiro & Lilenbaum, 2020). Leptospirosis frequently results in no or only mild acute clinical symptoms after bacterial contact to mucosal membranes. Serovar Hardjo infection can cause abortions, stillbirths, or the birth of weak calves, however these effects often only manifest themselves in pregnant cows that get the infection for the first time (Grooms, 2006). In Rio de Janeiro, Brazil, leptospirosis has recently been identified as the most prevalent and potentially the main disease affecting reproductive in small ruminants (G Martins et al., 2012). Some researchers find out, in several of Rivers State's coastal settlements, caprine leptospirosis is endemic. These goats have subclinical Leptospira infections, which are extremely important for public health and affect the reproductive health of goat (Oruene & Bekwele, 2020).

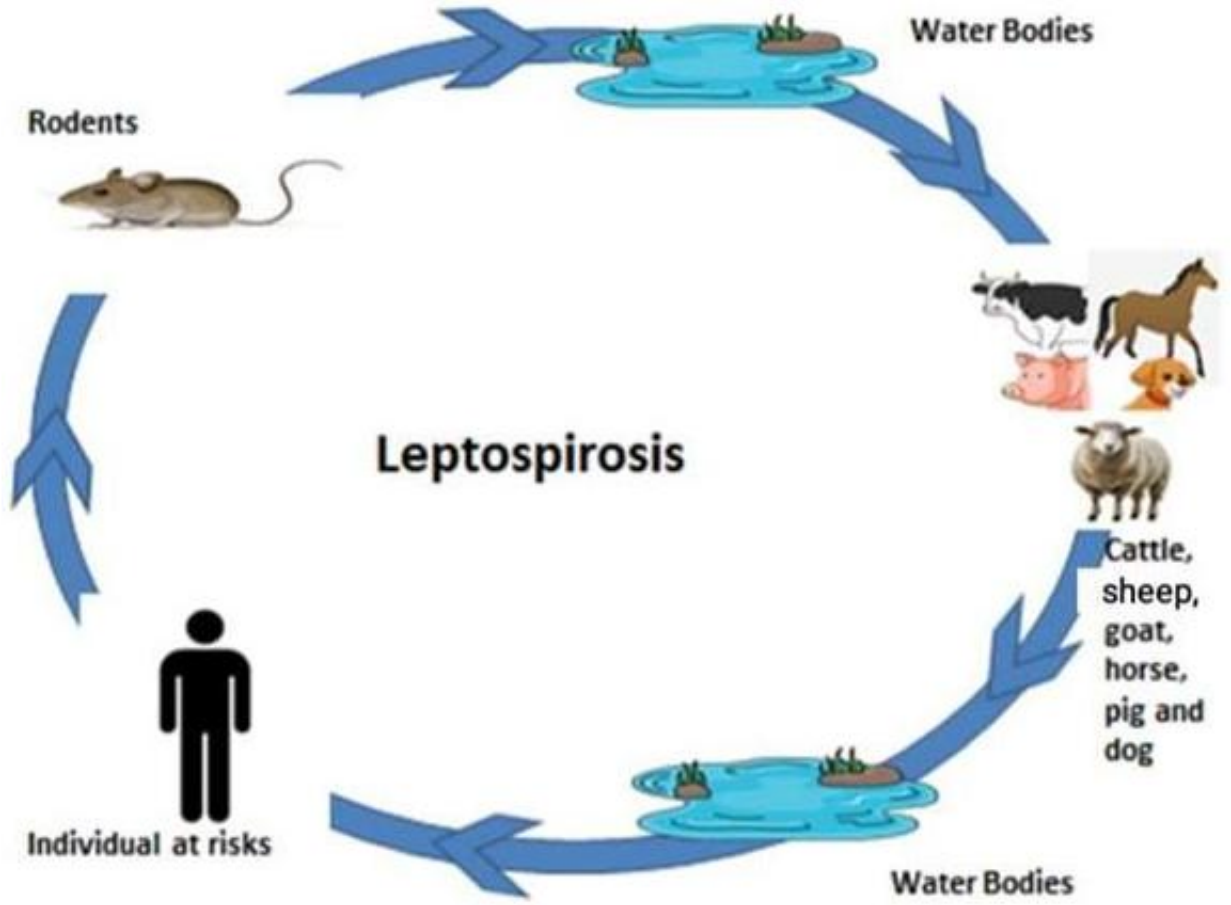

Fig. 4: Zoonotic Nature: Illustrating the Interspecies Nature of the Disease"

Leptospirosis is a serious condition that affects the reproductive system in horses. It causes significant economic damage because of the high cost of treatment, animal fatalities, and, most importantly, decreased reproductive efficiency that is characterised by subfertility, abortion, foetal death, and a poor incidence of embryo recovery (Di Azevedo & Lilenbaum, 2022). The majority of seropositive animals were over 6 years old, and females were more likely to get the disease than males. However, location, breeds, interaction with dogs or other domestic animals, and gender were not risk factors for infection (Da Silva et al., 2020). Leptospirosis in pigs is typically a subclinical disease that contributes significantly to economic losses for pork producers in the form of stillbirths, abortions, and abnormalities in the estrous cycle (Moreno et al., 2017). Pigs have been shown to have infections from Leptospira interrogans, L. borgpetersenii, and serogroups such Canicola, Pomona, Australis, and Tarassovi (Fernandes et al., 2020). The current study's findings confirm that the genital-urinary system is a significant extrarenal source of leptospire infection. The recent identification of L. interrogans serogroups suggests that this serovar is linked to infections of the reproductive system and has to be taken into consideration in swine production enterprises (Gomes et al., 2022).

Dogs are frequently affected by leptospirosis, although studies on chronic infection have just currently been studied. Reproductive failure is also included (Johnston et al. 2019), but less frequently than in ruminants. Leptospira infection has also been related to feline stillbirth (Reilly et al. 1994).

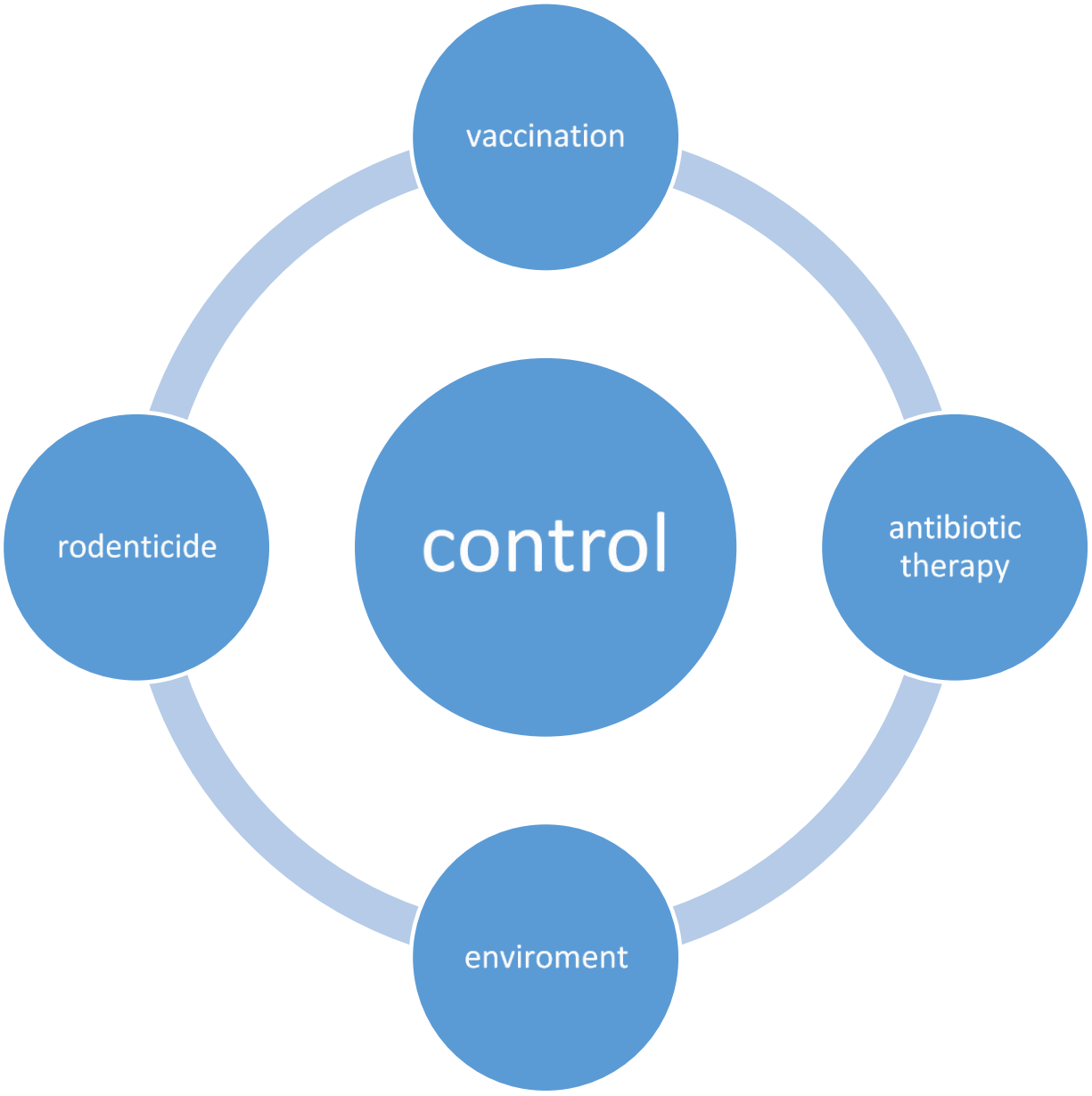

Fig. 5: Effective Disease Control Strategies

10. TREATMENT

The main goal of therapy is to stop the infection before liver and kidney damage becomes severe. When symptoms appear, antibiotic treatment is suggested as soon as possible. The results of treatments are frequently unsatisfactory since animals typically are brought in for treatment after the septicemia has subsided. The secondary goal of therapy is to control carrier animals' leptospiruria and make them safe to remain in the group. Leptospirosis treatment depends only on the type of pathogen involved and how severe the illness is (Grassmann, Souza, & McBride, 2017). Oral azithromycin, doxycycline, ampicillin, and amoxicillin are all choices in a moderate case of leptospirosis (Charan, Saxena, Mulla, & Yadav, 2013). The

recommended medications for severe leptospirosis include doxycycline, tetracycline, ampicillin, amoxicillin, penicillin, and azithromycin, which is also very effective against Leptospira species in the early stages of the disease. Leptospirosis-related fever and acute renal failure in horses have been effectively treated with ticarcillin, penicillin, and enrofloxacin (Frellstedt & Slovis, 2009).

11. CONTROL STRATEGIES

There are not any general recommendations for the prevention and management of leptospirosis in humans because of the complicated and dynamic epidemiology. Domestic animal control methods, however, are frequently much simpler since they may be applied to populations and have the potential to isolate those populations. A few successful strategies are now being used to eradicate the illness. One of the preventative ways to manage the illness in healthy individuals is vaccination. There is no vaccine for leptospirosis in humans, but there are a variety of animal vaccines that can prevent the disease, although they are more effective in preventing disease in domestic animals than in wild animals. Controlling the incidence of the disease in domestic and wild animals will help to eradicate it in people (Bashiru & Bahaman, 2018). Chemoprophylaxis is used with doxycycline 200mg once a week when there is a high chance of exposure to illness. While it might be feasible for travellers, it is more critical in a big city (Gopi, Sri, Krupamai, Magesh, & Dhanaraju, 2021). Further abortions in beef herds are prevented by vaccination and antibiotic treatment of all animals, but in dairy herds, only diseased animals are typically treated because of the probable loss of milk sales.

Wearing protective clothing (such as gloves, safety glasses and boots) helps stop the spread of the disease, although this is not always feasible; for instance, wearing boots in a paddy field is not an option (Hartskeerl et al., 2011).

The only way to control rodent populations is to handle them constantly and actively. The use of rodenticides is dangerous (creation of resistance population) and requires expertise of such control (Painter et al., 2004). Herd management techniques can lower the risk of disease transmission inside and between domestic animals. These consist of vaccination and/or carrier treatment. Effective control strategies are illustrated in Fig. 5.

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Dog-mediated Leptospirosis

27

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ABSTRACT

Leptospirosis is a waterborne zoonotic melody faced globally by both animals and humans. Based on the emergence and losses caused by leptospirosis, it is a recurring and neglected disease of public health importance all over the globe. Lack of public awareness and negligence on a massive scale combined with expeditious and unplanned urbanization in developing countries cause the re-emergence of this disease under unsanitary conditions. As most wild and domestic animals can be the carriers of the pathogen causing this acute febrile illness, everyone is at risk of getting infected including all the healthcare professionals, pet owners, farmers, workers and volunteers at animal daycare centres and shelters for stray animals, fishermen, sanitary workers, rodent catchers, sewage cleaners, etc. The clinical signs and symptoms include a variety of flu-like to acute kidney failure in severe or untreated cases. Typical cases of leptospirosis depict signs like pneumonia, pulmonary haemorrhages and jaundice but many cases are reported worldwide with very rare and uncommon clinical manifestations. This chapter will cover all the possible aspects of dog-mediated leptospirosis from the morphology of the pathogen, its transmission, occurrence, clinical signs, diagnosis and prevention of this disease.

Key word: Leptospirosis, Animal, Haemorrhages, Jaundice, Prevention, Awareness.

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1. INTRODUCTION

Leptospirosis is one of the most underrated tropical diseases. It is a waterborne malady that can spread from animals to humans by infection of spirochetes belonging to the genus Leptospira (Chatterjee et al. 2017; Esteves et al. 2018). The recently published reviews and reports have stated that leptospirosis is nominated as a major emerging and recurring disease by the World Health Organization (WHO). It is widespread among humans and animals all across the world (Wasiński and Dutkiewicz 2013; Chatterjee et al. 2017). More than 350 serovars and 30 serotypes of leptospiral strains both pathogenic and saprophytic have been identified till now (Trott et al. 2018). Leptospira has infected more than a million people worldwide. It is also responsible for about 60,000 deaths worldwide each year (Thibeaux et al. 2018). Researchers suspect that the actual load of the disease of leptospirosis could be greater than the value of the estimates about it (Herman et al. 2016). There are several reasons for that, some important factors in this regard for this situation are probably as follows:

(i) Fewer cases are reported about the actual number

(ii) Established point-of-care-test for diagnosis; therapies and prevention of the disease are not operating effectively

(iii) The inaccurate reporting has led to a seemingly doubtful epidemiologic situation

(iv) Poor hygiene practices, insufficient sanitation, and rapid urbanization of countryside regions without proper planning (Thornley et al. 2002; Kamath and Joshi 2003; Vijayachari et al. 2008; Bandara et al. 2014).

Reports by researchers show that Leptospira was first mentioned by DJ Larrey in 1812. He reported it in Cairo among the troops of Napoleon's army. However, in 1886 Adolph Weil reported the presence of the same organism in kidney tissues. Later on, he named the infection after himself calling it "Weil's disease." Arthur Stimson was the first one to describe and differentiate spirochetes from one another in 1907. He worked on differentiating the *Leptospira interrogans* from other types of spirochetes such as the *Spirochaeta interrogans* from the luminal portion of the kidneys. These kidney tissues were sampled from yellow fever patients. He studied leptospires extract from kidney tissues by using Levaditi silver deposition. The kidney tissues he mostly studied were sampled from people who had died of yellow fever. Hubener, Uhlenhuth, and Fromme in Germany and Noguchi and Inada in Japan simultaneously experimented with transmitting leptospirosis to a guinea pig in 1915. Later on, they were able to successfully isolate the leptospira from infected guinea pigs marking the success of the experiment. Ido et al., reported in 1917 that rats are the carriers of leptospires. Leptospira was identified as the etiological agent of cattle yellow fever in 1940. In the 1980s, leptospirosis was being thoroughly documented only as a disease of veterinary aspect with a major economic impact on cattle, dogs, swine, perhaps sheep, and horses. Many pieces of research regarding Leptospira and leptospirosis have been published starting from this era till the present. Latest advancements in terms of next-generation sequencing (NGS), DNA studies, and genome sequencing have helped researchers comprehend the Leptospira organism and its infection mechanism at the base molecular level (Stimson 1907; Levett 2001; Adler and de la Peña Moctezuma 2010; Trivedi and Kamath 2010; Taylor et al. 2015; Goarant 2016; Kim 2019). The disease was known by many names at the time before its proper identification in several regions. Different regions had different names for it. Chinese used to call it "rice field jaundice". On the other hand, Japanese called leptospirosis "autumn fever" or "seven-day fever." Some other names traditionally used for leptospirosis were "Cane-cutter's disease," "swine-herd's disease, and "Schlammfieber" (mud fever). These names were formed in association with the occupations of the infected patients. Generally, leptospirosis is also known as canicola fever, Fort Bragg fever, cane-field fever, 7 days fever, rat catcher's yellows, Weil's disease, nanukayami fever, harvest fever, field fever, mild fever, pretibial fever, etc. Leptospirosis is mostly prevalent in countries that are tropical and humid or have subtropical climates. It is known to be prevalent in regions of tropic climate. Currently, leptospirosis is also being seen in other areas such as temperate regions. Its appearance in other areas can be

attributed to various factors such as human migration and climate change due to deforestation, urbanization with poor and unplanned sanitation systems, improper waste disposal mechanisms, and lack of hygiene management (Desai et al. 2009; Costa et al. 2015; Dunay et al. 2016). Transmission of leptospira is favoured in regions with tropical climates. The biological load and spread of leptospirosis are still underscored due to underreporting incidence. Hence its re-emergence is often observed to occur notably more than other maladies and in various regions of the world (Holla et al. 2018). During the past two decades, leptospira infections have been on the rise in the southern region of Indian territory. These regions include areas of the Andaman Islands, Kerala, and Tamil Nadu (John 1996). Recently, leptospirosis infections have reached a score of DALY which is around 2.90 million per year. It has been reported worldwide that more males are infected by leptospira with numbers going over 2.33 million per year. The main reason for this increase is the existence of occupational risks. Leptospira infections also impact the economic status of developing countries burdening their financial resources. The biological burden of leptospirosis has been identified to be much greater than filariasis and rabies which carry a DALY score of 42/100000 as tropical and neglected maladies (Costa et al. 2015; Taylor et al. 2015; Torgerson et al. 2015; Goarant et al. 2019).

2. MORPHOLOGY & CLASSIFICATION OF PATHOGEN

Morphologically Leptospires have hair-like forms on their bodies and are slender commas or spiral in shape. Leptospira can be distinguished from other spirochetes based on their characteristic hooks on both ends of the body. The length of Leptospira ranges from 6 to 20 µm and they have an average diameter of around 0.15 µm. They possess a specific periplasmic endoflagella which contributes to its peculiar cork-screw-like movement differentiating it from other spirochetes and are also responsible for their pathogenicity (Slamti et al. 2011). High viscosity of fluids leads to high swimming speed of Leptospira species (Trueba et al. 2004). Their fragile structural makeup makes them best viewed under stain-free dark-field microscopy. Leptospires have characteristics of both gram-positive and gram-negative bacteria as they are gram-variable in appearance. Leptospira has lipopolysaccharides (LPS) on its surface, which adds to its virulence, in contrast to other important spirochete genera like Treponema or Borrelia which don't possess such layer. Leptospira virulence factors that may be involved in the infection include adhesion molecules, lipopolysaccharides (LPS), hemolysins, outer membrane proteins (OMPs), and other surface proteins (Adler and de la Peña Moctezuma 2010). Initially, due to being 8-azaguanine and low-temperature growth Leptospira were broadly categorized under the saprophytic biflexa and pathogenic interrogans groups. Later, as science advanced, serological and genetic traits were used to classify it. There are now three main clinically significant groups of Leptospira that cause leptospirosis in humans. There are 16 pathogenic strains in the interrogans group associated with human and animal infection. Among these strains, nine species of pathogens are linked to human leptospirosis. Mild or chronic infection-causing pathogens also known as host-mediated pathogens are included in the intermediate group (Chiriboga et al. 2015; Trott et al. 2018). There are fourteen nonpathogenic or saprophytic strains in the saprophytic or biflexa group, including *L. biflexa*; and *L. wolbachii* which are not involved in causing leptospirosis in animals or humans (Bharti et al. 2003; Trueba et al. 2004; De Brito et al. 2018; Trott et al. 2018; Escandón-Vargas et al. 2019).

3. TRANSMISSION

Direct contact is the main route of transmission of Leptospira rather than indirect contact. Indirect contact of infection is transmitted by contaminated water or soil by incidental and/or by carrier mammals, from which humans get Leptospira infection (De Brito et al. 2018). Many wild, domestic, and

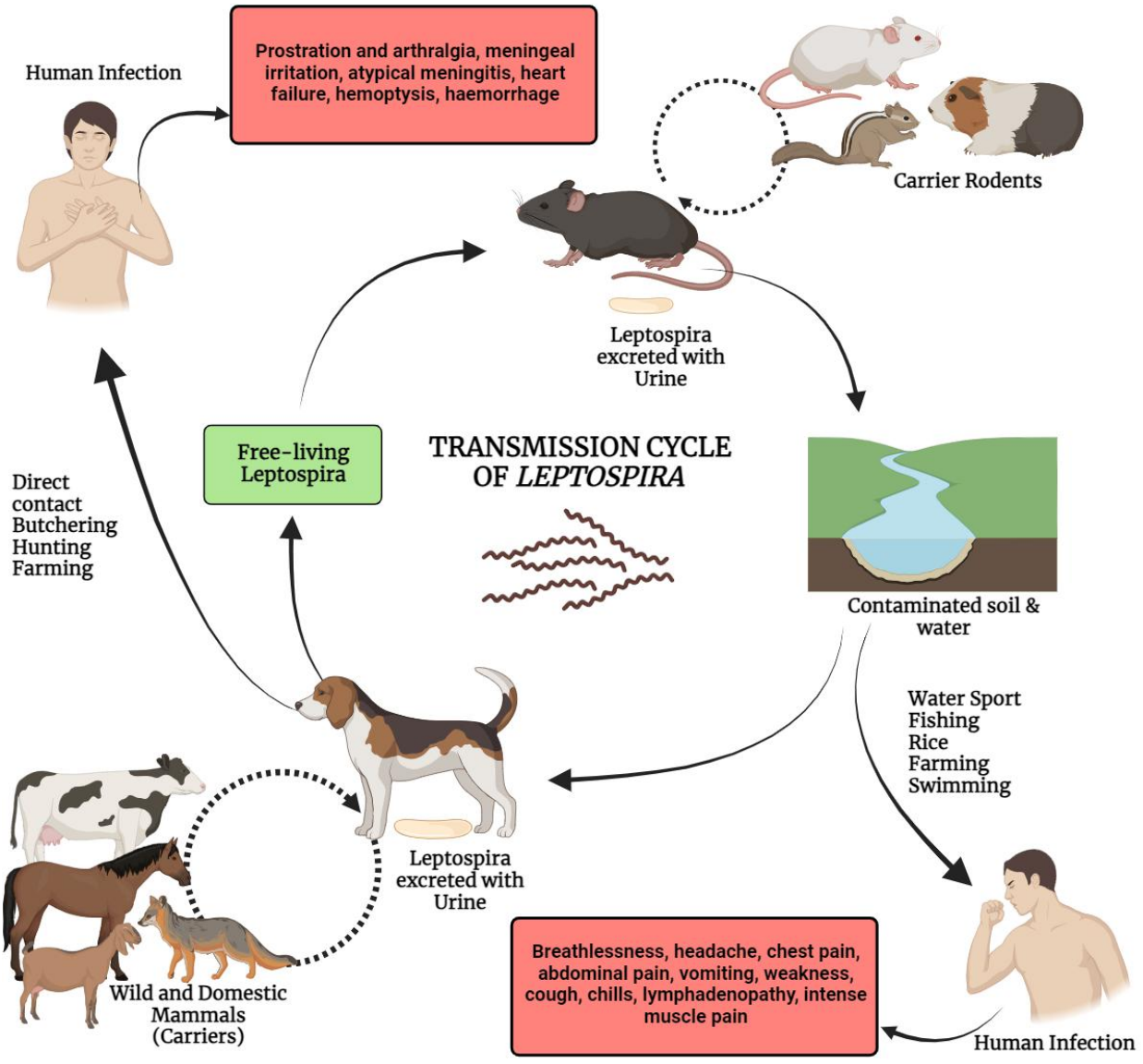

Fig. 1: Transmission cycle of *Leptospira*.

pre-domestic animals are asymptomatic carriers of Leptospira (Fig. 1). They carry a variety of Leptospira species in their renal tubules and within 2 weeks to a few months release them in urine. Leptospira may survive for a lifetime in the environment (Witchell et al. 2014; Herman et al. 2016). In urban slum areas, the infection mainly spreads via rats. They behave as asymptomatic carriers and contaminate soil and water sources with their urine. According to a study carrier rats disseminate infection at the rate of approximately 104-107 Leptospires/1 thus helping in the infection cycle as a reservoir (Witchell et al. 2014). Outbreaks of leptospiral infections are often linked to storms and floods after periods of intense rainfall when leptospires are swept into water bodies from the urine-contaminated soil. Leptospires can be kept far from natural water systems such as lakes, rivers, ponds, puddles, water dams, springs, cosmetic fountains, sewage, wet soil, and agricultural fields (Rawlins et al. 2014; Escandón-Vargas et al. 2019). Rain produces an alkaline pH that promotes leptospira growth and survival in the environment by washing away the fertilizer from the soil during floods etc. (Shekatkar et al. 2010).

For weeks to years, pathogenic leptospires may thrive in freshwater and damp soil, particularly when the pH is mildly alkaline (Trueba et al. 2004). Leptospires' deleterious effects on soil are highly underestimated as it is considered only a waterborne disease (Costa et al. 2012; Casanovas-Massana et al. 2018). The main route of infection in humans is pre-cutaneous from contaminated water bodies and soil and less commonly through inhalation and consumption during work-related or recreational pursuits. Seldom are reports of indirect transmission via animal bites and interhuman transmission (Sharma and Kalawat 2008; Musso and La Scola 2013). The following are the risk factors associated with Leptospira:

(1) Not taking precautionary measures while travelling to an area where Leptospira is endemic

(2) Exposure while working in the veterinary profession, agriculture, animal caretakers, fishermen, abattoirs, gardeners, rice mill workers, and sewage workers.

(3) Freshwater sports participants during canoeing, surfing, and caving.

(4) Volunteers who assist in disaster relief operations in flood-hit areas (Londeree 2014; de Sainte Marie et al. 2015; Desai et al. 2016; Pissawong et al. 2020). Leptospirosis in slum areas is related to exposure to infected rats and improper hygiene and drainage systems (Costa et al. 2014; Hagan et al. 2016; Santos et al. 2017).

4. PATHOGENESIS

The pathogenesis and mode of transmission of Leptospirosis is not well understood although it was reported two centuries ago. However, a lot of genes have been checked to understand their function and their role in the pathogenicity and immunogenicity of the causative agent. This was achieved through research using bioinformatics and molecular approaches. The virulent genes of Leptospira are unique to it and are not found in any other bacterial species and also possess a distinctive virulence system. The genetic analysis and comparison of pathogenic and saprophytic leptospira species expressed approximately 900 distinct genes to pathogenic leptospira species. Important proteins like H-binding proteins that attach to sphingomyelinase, extracellular matrix laminin, hemolysins etc, proteins needed for motility, flagella, and chemotaxis, and virulence-producing proteins like OMP and LPS are all encoded by these genes (Picardeau et al. 2008; Adler et al. 2011). Leptospira manifests its infection in two stages:

(i) mild anicteric phase.

(ii) classical icteric phase.

The former exhibits a slight infection that is usually self-limiting and treatment is also not needed in most cases. Anicteric infection covers approximately 80-90% of leptospira infections (Levett 2001). It consists of an incubation period of 1 to 2 weeks with a range of 2 to 30 days after which characteristic clinical signs begin to appear (Lau et al. 2010). It is present in the environment and enters the body through cuts, abrasions, or contact with mucous membranes. Followed by its entry into the host body, it passes through the membranes and enters the bloodstream with the help of chemotactic factors (Fig. 2). It usually occurs within 2 to 7 days of inoculation.

The main chemoattractants include haemoglobin, long-chain fatty acids, pyruvates, and sugars nervous system, and lungs. However, the main diagnostic factor of Leptospira infection is the infection in the hepatorenal system. According to studies, various Leptospiral proteins are involved in virulence and pathogenicity (Yuri et al. 1993; Lambert et al. 2012; Affroze et al. 2016). They attack the defense mechanism of the host cell by attachment to the fibrinogen network and extracellular matrix (ECM) proteins. According to studies, some toxic substances and proteins are produced by the pathogen which damages the cell membrane and destroys the host cells' vascular network (Martinez-Lopez et al. 2010; Evangelista et al. 2014; De Brito et al. 2018). They are also involved in the production of several virulence factors which play a major role in pathogenesis. These factors include phospholipase, immunoglobulin-

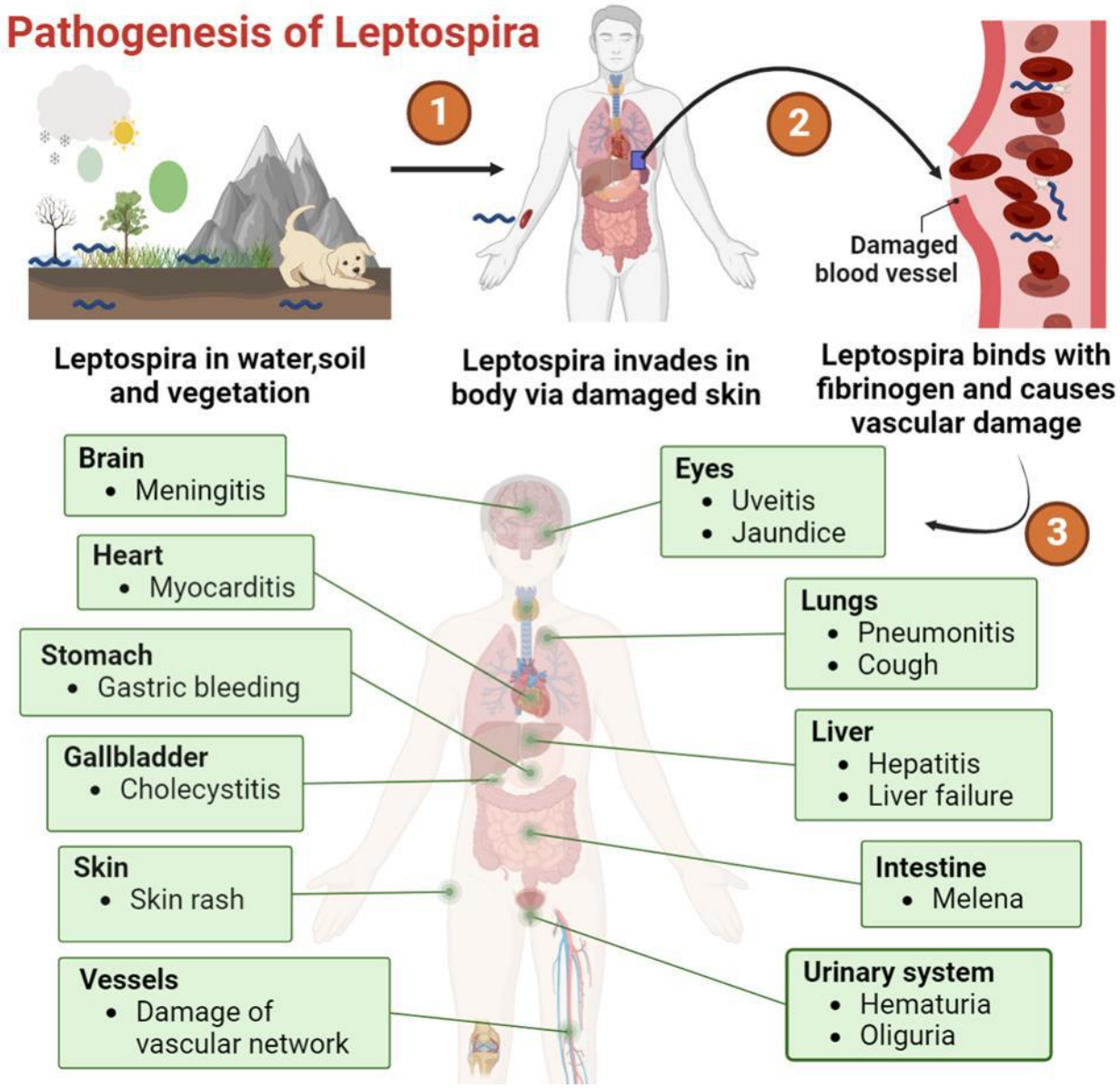

Fig. 2: Pathogenesis of *Leptospira*.

like proteins Lig A and Lig B, lipoproteins, hemolysin, sphingomyelinase, other putative outer proteins, hyaluronidase, collagenase, surface adhesion proteins (del Real et al. 1989; Segers et al. 1990; Atzingen et al. 2008; Gomez et al. 2008; Figueira et al. 2011; Shylaja et al. 2011; Kassegne et al. 2014). The acute septicemic stage in patients includes leptospiremia and septicemia 90% of patients diagnosed with anicteric leptospirosis normally recover. But the infection is remittent and appears again causing haemorrhages of vital organs like kidneys, liver, intestines, lungs, etc, and extreme infection after 2 to 3 days although the patient might have recovered from other signs and fever. Patients in later stages of infection exhibit uveitis, meningitis, and rashes (Rajapakse et al. 2015). The second form of leptospirosis called the classical form is also known as Weil's disease named after the scientist who discovered it. It mainly affects the liver and affects 5-10% of individuals approximately. In this severe form of infection,

erythrocytes can be seen in urine resulting in anuria and oliguria due to the invasion of hepatics and alteration in the function of aminotransferases and leukocytes by the pathogens. There is an increase in the concentration of creatinine and urea which serve as major indicative of Leptospira infection. The infection is systemic and involves major systems like the gastrointestinal tract, and hepato-renal system organisms (Natarajaseenivasan et al. 2012). In Leptospiruria, pathogens appear in urine between 7 to 30 days of infection. Leptospires inhabit and multiply within the renal tubules and continue to be within blood circulation adding to the longevity of infection. Patients mostly recover with proper treatment. Treatment will improve after completely understanding the pathogenesis (Tullu and Karande 2009; De Brito et al. 2018).

5. CLINICAL SYMPTOMS

Because leptospirosis produces varied signs, it might be challenging to distinguish it from other etiologies that cause acute undifferentiated febrile illness (AUFI). Leptospirosis can produce a wide range of clinical signs from flu-like symptoms to multiple organ failure. Acute febrile illness, breathlessness, headache, chest pain, abdominal pain, vomiting, weakness, cough, chills, lymphadenopathy, distinct and intense muscle pain that is limited to thighs, back or calf muscle, prostration and arthralgia, accompanied by any of the following: meningeal irritation, atypical meningitis, heart failure, hemoptysis, haemorrhages (from the lung, intestines, etc.) and less commonly rashes on the skin are the symptoms that usually appear during the initial stages of infection that are related to leptospiremia and lasts for approximately 7 days (Ding et al. 2001; Lin et al. 2008; Bhatia et al. 2015; Holla et al. 2018). The symptoms of the acute phase, observed after weeks or even years of illness include conjunctival suffusion or uveitis (Verma and Stevenson 2012). Leptospiral uveitis is linked with Lru A and B gene product proteins. The protein products of Leptospiral genes A and B are responsible for causing Leptospiral uveitis (Faber et al. 2000; Shylaja et al. 2011). In equines, uveitis is intermittent and occurs more frequently (Pearce et al. 2007). Because of the proximity of endemic geographic locations the symptoms are sometimes misdiagnosed as other causes of acute febrile syndrome, including dengue, hepatitis, and malaria (Esteves et al. 2018). These symptoms fade in 5-7 days, and the patient is capable of recovering even without medications and therapy, or it simply retreats to asymptomatic. A transient recovery phase begins after it, during which IgM is detectable in the blood due to the immunological response. The infection seems to reappear 1-3 days following the initial phase. This is the second phase. The infection is serious at this stage and affects vital organs. Meningitis symptoms such as stiffness of the neck are evident in the second phase. In some rare cases, encephalitis is also observed. The unusual involvement of the circulatory, pulmonary, neurological, ophthalmic, gastrointestinal, and various other systems results in clinical manifestations. Hematologic characteristics such as reticulocytopenia, normocytic normochromic anaemia, thrombocytopenia, hypogonadism, etc. have also been recorded in conjunction with pulmonary haemorrhage that does not outcome kidney failure or jaundice, transverse myelitis, and rhabdomyolysis (Bracho et al. 2010). By binding to the chemotactic factors Leptospires also result in the manifestation of thrombocytopenia and a disease known as TTP or thrombotic thrombocytopenic purpura (Lin et al. 2008; Musso and La Scola 2013; Şükran et al. 2013; Herman et al. 2016). In 20-70% of infected individuals, respiratory system is involved resulting in pneumonia, respiratory distress syndrome (ARDS), severe pulmonary hemorrhagic syndrome (SPHS), acute alveolar haemorrhage, and other complications. Pulmonary Leptospirosis results from delayed antibiotic therapy which is more deadly than other types of leptospirosis (Singh et al. 1999; Dolhnikoff et al. 2007; Gulati and Gulati 2012; Vijayachari et al. 2015; Schönfeld et al. 2019). The most common manifestations of hepatic and renal involvement in icteric infection are anuria and/or oliguria. There is also a rise in the

blood creatinine and urea levels. It may cause a variety of complications in pregnant women from abortion and stillbirth to various other complications and eventually leading to the death of the foetus. It can potentially kill both the mother and the foetus in rare situations. However, the birth of healthy infants can occur with the proper use of antibiotics (Rahimi et al. 2018).

6. IMMUNITY

The basic way through which immunity responds against leptospirosis is the humoral response (Adler and Faine 1977). The main targeting agent for the protective antibodies is LPS. The levels of agglutinating LPS-specific antibodies describe the passive transfer of immunity in the transferred sera (Adler and Faine 1978). Other than these antibodies, LPS-specific monoclonal antibodies also provide passive immunity against leptospirosis in healthy animals (Jost et al. 1986). This is not confirmed if there are any other types of antibody-antigen responses against leptospirosis except LPS. However, the latest studies reveal that the humoral response of immunity is not the only way of protection against leptospirosis. The activation of intact TLR2 (Chassin et al. 2009) and TLR4 (Viriyakosol et al. 2006) are necessary to avoid lethal infection in case of mice. There is a variation of immune response in the case of hosts which are prone to acute leptospirosis while the bovine reservoir host has the cell-mediated response against the *L. borgpetersenii* serovar Hardjo. This cell-mediated response was also confirmed by the immunization trails in cattle, using whole leptospire-based vaccines, which proved that it is a T-helper 1 cell response other than the agglutinating antibody titers (Naiman et al. 2002; Brown et al. 2003; Blumerman et al. 2007).

7. VACCINES

It was first demonstrated in 1916 by Ido et al. that immunization can be done using killed leptospires in case of experimental infection (Ido et al. 2005). After that, all the livestock, domestic animals, and human population have been vaccinated with whole-leptospire-based vaccines routinely6. However, the use of these vaccines causes serious side effects and produces immunity only for short-term defense which is serovar-specific-1. In the case of circulating serovar agents polyvalent vaccines are used which give complete coverage and they should be formulated at affordable prices if there is an emergence of new serovars (Gonzalez et al. 2005). Also, the whole-leptospire-based vaccines do not completely control the transmission of the infection and prevention of the disease which limits the use of these vaccines. Due to all the above-mentioned reasons, the main effort is the production of subunit vaccine candidates to identify surface proteins associated with the bacterial surface that are reserved among the bacterial serovars and also the targets for immune responses concerned with the killing of bacteria. The prime evidence of using this method was seen in the past use of the outer membrane of E. coli. One is vesicles containing recombinant Lip41 and OmpL1 for immunization in hamsters which protects them against some lethal types of leptospires (Haake et al. 1999) Later on, it was seen that the LipL32 produced the best immunoprotection when it is delivered through naked DnA136, Mycobacterium bovis bacille Calmette-Guérin (bCG) (Seixas et al. 2007) and adenoviral systems (Branger et al. 2001). When used in experimental animals the efficiency of these candidate vaccines is low ranging between 40-70%. The high level of protection is shown to be produced by the Lig proteins subunit vaccine candidates which are almost 100% in mice (Viriyakosol et al. 2006) and hamsters (Palaniappan et al. 2006; Silva et al. 2007; Yan et al. 2009). The Lig proteins produced the maximum cross-protective immunity against a range of serovar agents that has been determined also due to the amino acid sequence of these proteins which is 70-100% similar to Leptospira spp. (McBride et al. 2009). The presence of this multiple genome

sequence helps in using different techniques to find out new vaccine candidates (Gamberini et al. 2005). The main purpose is to generate a single vaccine candidate which protects against a wide range of Leptospira species. The genome of *L. interrogans* and *L. borgpetersenii* have 2780 same open reading frames out of which 656 are not shown in *L. biflexa* genome (Bulach et al. 2006; Picardeau et al. 2008). Techniques to purify several target candidates are done by sequencing the genome of a larger number of Leptospira species specifically pathogenic and also the bioinformatics analysis of that genome, selection of open reading frames and specifically those genomes which encoded the outer membrane proteins (Yang et al. 2006). The major hurdle to implementing these strategies is the lack of in vitro correlates for immunity against leptospirosis. The screening at a high throughput level is not feasible in experimental animals given the expected number of antigen candidates. The major purpose of the production of a vaccine is to find out if the infection with leptospira protects against reinfection in the population with a high risk of disease and to find out the mode of action of immunity that is involved. As far as the epidemiologically produced immune correlates are found, different types of vaccine candidates are produced to get new virulence factors and outer membrane proteins.

8. CONCLUSION

Leptospirosis has become one of the biggest public health concerns all across the globe especially in the tropical, temperate, and subtropical regions. An emergence and then re-emergence of this disease has been observed due to rapid unplanned urbanization, improper sanitation, poor surveillance programs, and unhygienic waste management practices and control plans. Additionally, the negligence of this disease added fuel to the fire. As mentioned earlier, there are more than a million people infected with leptospirosis. The mortality rate has been observed to be around 6% per year worldwide. This situation necessitates careful planning for its control and prevention. Leptospira infection is one of the most neglected 17 diseases. These diseases have been categorised by WHO as maladies getting the least consideration from local and international health institutes. Regions of South Asian areas are being reported as the center points of rampantly endemic leptospira infections. The reports indicating an increase in the prevalence of leptospirosis have been published recently regarding the South-Indian regions including the states of Tamil Nadu and Kerala. Despite the systemic availability of control and prevention programs against the infection. A troublesome and hard-to-eliminate portion of sequelae left by leptospirosis is the kidney tissue carrier state. It can last several months or even years. Leptospira also co-aggregate with other environmental bacteria to make the conditions suitable for their survival, hence helping them to persist for a long time. This phenomenon requires the study of the bioburden of Leptospira, especially the pathogenic strains from the environment with extensive research to comprehend transmission patterns. This research can also help us in the development of appropriate prevention and control strategies against leptospirosis.

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Leptospirosis in Cats

28

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ABSTRACT

Leptospirosis, a zoonotic disease, is caused by pathogenic spirochetes belonging to the genus Leptospira. It is widely considered one of the most prevalent zoonoses in terms of both geographic distribution and the variety of animal species susceptible to acute illness or acting as renal carriers. Tropical and subtropical regions, such as Thailand, experience a higher incidence of leptospirosis in humans and animals, making it a significant public health concern in those areas.While clinical signs of leptospirosis in cats have yet to be thoroughly investigated, previous studies have shown that cats with polyuria and polydipsia are more likely to have anti-Leptospira antibodies. However, in cats, the clinical signs are usually mild, despite the presence of leptospires in their blood and urine. Reported clinical signs in infected cats (confirmed through MAT and/or PCR) include polyuria, polydipsia, haematuria, uveitis, lameness, lethargy, anorexia, weight loss, ascites, vomiting, diarrhea, pain on handling, and inflammatory lesions on the skin and digits. Various diagnostic tools can be employed, such as the microscopic agglutination test (MAT), indirect hemagglutination assay (IHA), or immuno-enzymatic assays (ELISA) to detect specific antibodies. Leptospira or their components can also be identified in urine or tissues through culture, dark field microscopy, immunostaining, or PCR.Human infections of leptospirosis can be acquired by individuals in certain occupations, such as veterinarians, farmers, animal caretakers, and researchers, as well as people exposed to pet dogs or domestic livestock during their daily activities. Farmers, veterinarians, and abattoir workers are particularly at risk due to their occupation.

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1. INTRODUCTION

Adolph Weil's description of a distinct case of jaundice accompanied by splenomegaly, renal problems, conjunctivitis, and skin rashes initiated the modern study of leptospirosis in 1886 (Haake and Levett 2014b). It later became as "Weil's disease". Despite the fact that its specific etiology was still unknown, there were signs of contagiously and frequently affecting to those who worked outside and around water. Persons susceptible to epidemics were, sewage workers, rice farmers, and coal miners. Over the following decades, there was a notable advancement in the comprehension of leptospirosis. An important discovery was the fact that leptospirosis could infect practically all mammalian species, particularly rodents. Additionally, the domestic animals were the source of human infection (Thayaparan et al. 2013). Dutch researchers, isolated a strain from canines and continue to use this strain as the type strain for serovar Canicola (Francey et al. 2020). The sickness in cattle was first reported in 1940 in Russia, when it was called "infectious yellow fever of cattle". The variety of leptospirosis serovars and host animals that impacted in 1950s significantly increased. Especially in dogs, cattle, swine, horses, and possibly sheep, this disease had been well-documented by 1980s as a substantial veterinary concern with significant economic ramifications (Ryan et al. 2012).

Over the next decades, it became clear that leptospirosis may present itself in both humans and animals in a wide variety of ways. These symptoms ranged from a moderate febrile sickness, frequently mimicking "influenza-like" symptoms, to severe and rarely deadly illnesses marked by acute liver and kidney failure as well as pulmonary bleeding (Bharti et al. 2003; Gouveia et al. 2008). Evidently, the serovar has an impact on the development of infection for example, human infections caused by serovar Hardjo do not frequently cause death. But it is also obvious that a variety of host and environmental factors can have an impact on the disease progression. Even serovars frequently linked to serious and deadly illnesses can sometimes result in mild infections (Gouveia et al. 2008). Leptospirosis is now known to be the most common zoonotic disease in the world due to the isolation of leptospires from all mammalian species in all continents, with the exception of Antarctica. Additionally, it continues to be a major disease factor in many domestic animal species (Adler 2014). The first documentation of feline leptospirosis dates back to 1972. Prevalence studies have shown that the primary serovars found in cats depending on the region and different species (Schuller et al. 2015). Cats can get leptospiral disease through consuming contaminated prey, particularly when it comes in contact to serovars from the Autumnalis and Ballum serogroups. This shows how cats' propensity for predatory behavior makes them vulnerable to infection (Adler 2014). Due of their near proximity to reservoir hosts, outdoor cats are more likely to develop leptospires. In rural areas, cats can get sick through contact with pig and cow urine, which are potential sources of the bacterium. These elements work together to increase the likelihood that cats living outside or in rural regions may become infected with leptospirosis (Azócar-Aedo et al. 2014; Garoussi et al. 2015). This suggests that an infected cat can potentially increase the likelihood of disease incidence and propagation to leptospirosis for both people and other animals living in the same habitat. It highlights how crucial it is to be aware of risk and implementation of the necessary precautions to safeguard health of all family members living in the home as well as pets (Rodriguez et al. 2014).

2. LEPTOSPIRA THE ORGANISM

Leptospires, are members of the spirochete family, around 0.1mm in diameter and 6–20mm long. Both pathogenic and saprophytic species can be found in the genus Leptospira (L.) (Krøjgaard et al. 2009), having 64 species that are divided into 24 serogroups and over 300 serovars (Vincent et al. 2019). These bacteria are highly motile with an elongated, helically coiled structure, and are distinguished by their particular morphological characteristics among spirochetes. Their ends are distinctively fashioned like a

"question mark" or hook (Pn 2001). *L.interrogans* (which comprised the pathogenic serovars) and L.*biflexa* (which included the non-pathogenic saprophytic serovars) were initially considered to be the two species that made up the genus(Levett 2015). A genetic classification system has essentially supplanted the traditional phenotypic approach of classification. All serovars of *L. interrogans sensu lato* and *L. biflexa sensu lato* are included in this updated method. Taxonomy frequently refers to a species complex as "sensu lato," which is a Latin phrase that meaning "in the broad sense" (Adler 2014). Currently, about 20 species of Leptospira have been identified, and at least ten of those are known to be pathogenic (Levett 2015). The discovered Leptospira species include five species with uncertain pathogenicity and seven saprophytic species (Bulach et al. 2016). The cytoplasmic membrane and peptidoglycan cell wall of leptospires are linked intimately, and both layers are further covered by an outer membrane (Cullen et al. 2004). Leptospires, are thought to require oxygen for growth. 28°C to 30°C is the range in which they grow most effectively. They choose simple fortified media with vitamin B1 and B12, ammonium salts, and long-chain fatty acids. It is to noteworthy that they only use long-chain fatty acids because they can convert these fatty acids through beta-oxidation due to their ability of carbon supply (Krøjgaard et al. 2009).

3. LEPTOSPIROSIS

The spread of pathogenic Leptospira spirochetes results in the zoonotic disease known as leptospirosis (Vincent et al. 2019). According to Adler (2014), leptospirosis is a systemic disease that can affect both humans and domestic animals. Dog, cattle and swine are the main afflicted animals. Fever, renal and hepatic insufficiency, pulmonary signs, and reproductive failure are the symptoms of disease. Clinical manifestations can differ greatly, and many instances might go unrecognized. Leptospirosis is linked with particular serovars that are tailored to the hosts such as Canicola in dogs, Bratislava in horses and pigs, Hardjo in cattle, and Australis and Pomona in pigs (Adler 2014; Andre-Fontaine 2006).
The definitive host of Leptospira serovars are known with their association to hosts for Canicola, Hardjo, Pomona and Bratislava includes dog, cow, and sheep, respectively. These hosts are essential in order for germs to spread across the environment. Humans are unlikely to be important sources for the transmission due to disease severity, making them unlikely to be incidental hosts. Definitive hosts normally contract the infection and rarely display clinical symptoms. Animals infected with serovars are anticipated to display more severe clinical symptoms. Rodents are the primary asymptomatic Leptospira reservoir, which is well acknowledged and well-documented (Levett 2001). Cats are susceptible to develop leptospirosis from rodents. In this aspect, rats are the primary source of infection for cats (Weis et al. 2017).

4. EPIDEMIOLOGY OF LEPTOSPIROSIS

Due to vast geographic distribution and capacity to infect a variety of animal species, either producing acute infections or functioning as kidney carriers, leptospirosis, caused by pathogenic species of Leptospira, is known as one of the most common zoonotic disease. It is widely recognized about the clinical manifestation and progresses of disease in both domesticated animals and humans (Adler 2014; Haake and Levett 2014a). Leptospirosis is endemic on earth, and tends to become more common in summer season. Most diseases in tropical areas tend to happen during and after rainy seasons (Adler 2010; Levett 2001). In tropical and subtropical areas, leptospirosis is extremely common in both humans and animals, and it is of major public health concern in Thailand (Hinjoy 2016). Leptospirosis normally affects 6.6 out of every 100,000 people in Thailand each year on average. The incidence can dramatically

increase during outbreaks, reaching up to 25 cases per 100,000 individuals. They are unable to reproduce and can survive in damp soil for several months with on the host(Adler 2014; Levett 2001). The three main risk factors for leptospirosis transmission in people are: (1) exposure to water; (2) interaction with rodents and (3) transmission through livestock or pets. These elements greatly influence how leptospirosis spread throughout communities (Mwachui et al. 2015).

Table 1 offers a summarized view of the limited researches conducted in multiple locations to ascertain the frequency of DNA sheds in the urine of cats indicating Leptospira. The data available in the table illuminates the scope of investigation regarding leptospirosis in feline populations throughout the world. However, due to the limited nature of the research, further studies may be required to fully grasp the prevalence and significance of Leptospira DNA shedding in cats, both for public health and veterinary purposes (Murillo et al. 2020). The studies cited revealed a considerable variation in the frequency of DNAs indicating the Leptospira sheds in faline urine, with percentages ranging from 0% to 67.8% (Murillo et al. 2020).Notably, these findings did not establish a definite connection to medical illness. The recorded incidence rate appears to be influenced by factors such as the real-time location, specific primers chosen by PCR, and additional variables, contributing to the divergence in results.

Table 1: An overview of recent studies on the frequency of Leptospira DNA shedding in cats' urine.

Location	Number of samples	Gene target/primer set	Prevalence (%)	Reference
Reunion Island	172	rrs2, lipL32 and lipL41	0.6	(Murillo et al. 2020).
Thailand locations	260	lipL32	0.8	(Sprißler et al. 2019).
Algeria – Algiers	107	rrs (16S) and hsp	0	(Zaidi et al. 2018).
Germany Munich	215	lipL32	3.30	(Weis et al. 2017).
Australia Christmas Island	59	23S	42.4	(Dybing et al. 2017).
Canada Quebec	200	G1 and G2 and B64-I/B64-II	3.5	(Bourassi et al. 2021).
Réunion Island	30	lipL32	26.6	(Holzapfel et al. 2021).
Taiwan Southern Taiwan	236	G1/G2 and Leptospira rrs (16S)	67	(Chan et al. 2014).

5. SIGNS

Leptospirosis in cats has not yet been fully investigated in terms of its clinical symptoms. Research from past has indicated that cats who exhibit polyuria and polydipsia symptoms (increased urination and thirst) are more likely to have anti-Leptospira antibodies. This shows a possible connection between these clinical symptoms and feline leptospirosis infection. However, more investigation is required to fully comprehend the disease's clinical presentation and effects on feline (Langston and Heuter 2003; Moinet et al. 2010). Even though their serum and urinary excretion contained leptospira traces, cats often exhibit only modest clinical symptoms. Based on confirmation through MAT (microscopic agglutination test) and/or PCR (polymerase chain reaction), infected cats have displayed a variety of clinical signs, including polyuria (increased urination), polydipsia (increased thirst), haematuria (blood in the urine), uveitis (inflammation of the eye), lameness, lethargy, anorexia (loss of appetite) and weight loss (Lapointe et al. 2013; Mylonakis et al. 2005; Ojeda et al. 2018; Rodriguez et al. 2014; Shropshire et al. 2016; Weis et al. 2017).The thoracic and peritoneal cavities of the animals exhibit pathological signs, including hemorrhagic or straw-colored secretions (Arbour et al. 2012). Like in canines, feline leptospirosis can lead to acute renal injury, which has the potential to develop into CKD (chronic kidney disease) (Schuller et al. 2015; Sykes et al. 2011). Compared to affected dogs, liver lesions are more seldom found in affected cats. Leptospiruria, or a leptospire's existence in the urinary excretions, has been observed in experimentally disease induced faline. In addition, pathogenic Leptospira species' DNA has been found in the urine of both stray and domestic cats (Chan et al. 2014).

6. PATHOGENESIS

From asymptomatic to severe, acute sickness, leptospiral infection can present itself in a variety of symptoms. Factors like features of the host and specific serovar of the infecting organism have an impact on specific outcomes (Adler 2014). There aren't many detailed reports on the clinical illness that Leptospira species induce in house cats. Though the precise disease pathophysiology in cats is still unknown and can be differentiated from dogs to human disease (Hartmann et al. 2013).

Small incisions, mucous membranes like the conjunctiva, or even damp skin are all possible entry points for leptospira in the body. When the bacteria are in the body, they circulate in the bloodstream, and this bacteremic phase can last up to seven days (Hartmann et al. 2013). Usually, the bacteraemic phase of the infection is when the acute clinical disease first manifests (Greene 2006). The endothelium in small blood arteries is harmed by the infection, which leads to localized ischemia in organs. This can result in meningitis, myositis, placentitis, renal tubular necrosis, hepatic damage, and pulmonary damage (Goldstein 2010). The bacteria reproduce and remain within the cells of the renal tubule epithelium, which allows them to colonize the kidneys in the majority of infected animals. Nephritis, an inflammation of the kidney tissue, is brought on by this replication process, which also causes the production of cytokines and the recruitment of inflammatory cells (Adler 2010; Greene 2006; Levett 2001). According to reports, cats infected with leptospirosis may experience chronic interstitial nephritis, a disorder that can harm the kidney tissue over the long term. Cats with this condition may develop persistent and deteriorating renal impairment as a result of interstitial tissue inflammation in the kidney (Millán et al. 2009). Leptospires reach the tubular lumen of the kidneys about 10 days after infection and are subsequently eliminated in urine. Nephritis, an inflammation of the kidney tissue, can occur as a result of this excretion process, which can last for days to months (Adler 2010; Greene 2006; Levett 2001). In fact, various species and individual animals can range greatly in the length and severity of leptospires' urine-based elimination. The amount and time of leptospires' excretion in urine are also influenced by the particular serovar that infects them. These differences add to the complexity and variety of leptospirosis presentations seen in various instances (Adler 2014). Leptospiral DNA was found in cats' urine for more than 8 months after infection, according to an epidemiological investigation, with little to no correlation to clinical symptoms. This leptospirosis carrier state in cats increases the risk of leptospirosis transmission and environmental maintenance even when the feline hosts show no outward symptoms of sickness (Weis et al. 2017).

It has been noted that both humans and dogs can develop Leptospiral Pulmonary Hemorrhage Syndrome (LPHS), which is thought to be prevalent in 70% infected dogs. Affected people may experience severe respiratory discomfort and consequences as a result of this serious illness, which is characterized by lung bleeding and inflammation (Kohn et al. 2010). Acute clinical indications of canine LPHS are frequently present, and they are characterized by significant alveolar and sub-pleural hemorrhages that cause dyspnea (difficulty breathing). Studies have demonstrated that feline instances of leptospirosis may present with multifocal liver necrosis, fibrosis, and chronic hepatitis, even though LPHS has not yet been recorded in cats. According to these observations, leptospirosis can cause hepatic problems in cats as opposed to the pulmonary hemorrhage syndrome seen in dogs (Arbour et al. 2012; Millán et al. 2009; Rodriguez et al. 2014).

Hemorrhages, jaundice, and a drop in platelet count are just a few of the symptoms that might appear in severe leptospirosis cases. Additionally, frequent findings include mild granulocytosis and splenomegaly, or enlargement of the spleen. Once there are circulating antibodies, opsonophagocytosis is used by the body to remove leptospires from the blood and tissues. Even though the condition can seriously harm tissue, it is frequently treatable, and total organ recovery, particularly in the kidneys and liver, is possible. But there could be issues, such recurrent injury like myocarditis that leaves scarring. It is generally known that "white spots," or scarring, can be observed macroscopically in the kidneys of pigs and dogs (Adler

2010). Leptospires can harm the host's tissues and make them ill, although the precise methods by which they do this are yet unclear. Understanding the molecular basis of Leptospira pathogenicity has been hampered until recently by the paucity of genetic tools to modify them. Even though reports suggesting pathogenic processes have been around for a while, the precise leptospiral component that causes these consequences frequently went undiscovered. Cats may be a source of infection for humans, according to recent research that have demonstrated that they can excrete harmful Leptospira in their urine. This emphasizes the necessity to take into account cats as potential carriers and contributors to the transmission of the bacterium to people. In order to prevent and control the spread of the illness from cats to humans, it is important to recognize the contribution of cats to the spread of leptospirosis (Chan et al. 2014; Weis et al. 2017).

7. CLINICO-PATHOLOGICAL DATA

The leukocyte count can fluctuate depending on disease progression and severity. Low levels of leukocytes, may occur during leptospiremia (the blood having leptospires), followed by a transition to leukocytosis, characterized by an increase in leukocytes, particularly neutrophils. Progressing towards the critical stage, level of white blood cells reaches a range of 16.5–45 x 10^9/L (with the reference interval being 2.75–11.75 x 10^9/L) (Moritz et al. 2004; Schuller et al. 2015; Sykes et al. 2011).

7.1. SERUM BIOCHEMISTRY

Around 80-90% of canine leptospirosis cases show elevated levels of urea and creatinine concentrations (Sykes et al. 2011). Upon diagnosis, most infected cats typically exhibit azotemia, with the condition often ranging from moderate to severe intensity (Lapointe et al. 2013; Pn 2001; Rodriguez et al. 2014; Weis et al. 2017). Leptospira toxins hinder the activity of Sodium/Potassium ATPase in the renal tubular epithelium of both cats and dogs. This interference results in considerable renal electrolyte losses, ultimately leading to the development of severe hypokalemia, a condition characterized by low blood potassium levels (Sykes et al. 2011). Observations suggest that cats affected by leptospirosis experience elevated serum phosphorus concentrations, which are probably connected to a reduction in the glomerular filtration rate (Arbour et al. 2012).

7.2. ULTRASONOGRAPHIC FINDINGS

Renal ultrasonographic results are similar to those of canine leptospirosis, according to few published data on feline leptospirosis. The kidneys appear granular, larger and the cortical region is comparatively smaller to medullar region, the cortical region of kidney is slightly hyperechogenic, & the corticomedullary junction has less definition (Arbour et al. 2012; Beaudu-Lange and Lange 2014).

8. SPECIFIC TESTING

Due to the wide range of clinical symptoms, diagnosing leptospirosis is difficult and depends on a number of laboratory tests. Using techniques like the MAT, the indirect hemagglutination assay (IHA), or immuno-enzymatic assays (ELISA), these procedures can identify certain antibodies. Leptospira or their components can also be found in urine or tissues using PCR, dark field microscopy, immunostaining, or culture (Bharti et al. 2003; Levett 2001; Vincent et al. 2019).The advantage of specificity for serovars or serogroups makes MAT the most often used diagnostic test. However, it is unable to distinguish between antibodies produced by infection or immunization, which may present particular issues in animals, particularly when determining

disease status for import or export screening purposes (Vincent et al. 2019). High levels of sensitivity and specificity are displayed by the MAT. However, because live cultures of distinct Leptospira serovars are common in particular geographic regions are required, it might also provide difficulties (Miller et al. 2011). Leptospiral sonicates, recombinant lipoproteins like LipL32, LigA, or the outer membrane porin OmpL1, as well as a wide variety of antigen preparations have all been used to create various ELISA assays. These automated ELISA assays do away with the requirement to maintain live cultures. It's crucial to remember that they could not have the same sensitivity and specificity as MAT, thus depending simply on ELISA is not advised. The following techniques can also be used to identify antibodies: macro-agglutination, latex agglutination, lateral flow assays, and IgM dipstick testing. The only reliable diagnostic method for leptospire detection is culture-based detection, but this method is hampered by some Leptospira strains' sluggish growth rates and the lengthy incubation times needed to generate an isolate in culture. Fresh tissue, blood, or urine samples that were taken prior to the start of antibiotic therapy are necessary for the successful isolation of Leptospira. A minimum of two ten-fold dilutions of tissue fluid or homogenate must typically be inoculated, and depending on the degree of contamination, specific antimicrobial drugs such 5-fluorouracil may be employed to prevent contamination (Levett 2001). It takes up to 13 weeks of incubation at 30°C and weekly dark field microscopy (DFM) inspection for cultures to be deemed negative. Culture is not seen as practicable as a standard diagnostic test for specific individuals due to this extended time frame. However, it is still important for epidemiological studies (Vincent et al. 2019).

The sensitivity and specificity of other methods for finding leptospires in urine, blood, or other tissues, such as DFM, immunofluorescence, antigen ELISA, or immunoprecipitation, are constrained. The infecting serogroup or serovar are not specified by PCR, which is a direct approach for identifying leptospiral DNA. However, it is capable of identifying the Leptospira species. Blood, urine, cerebrospinal fluid, and different body tissues can all be tested with this method. PCR is the best method for evaluating blood and urine in cases of acute feline leptospirosis. Compared to culture, PCR yields quick results, enabling an early diagnosis (Hartskeerl et al. 2006). Because of their increased sensitivity and specificity, the method used in instantaneous PCR is strongly suggested. For the enhancement of precision of the method, genes with numerous copies in the genome, such as lig or rrs, are preferable when choosing genes for the test. Further increasing the test's specificity is the incorporation of genes that are only found in the pathogenic species (Bourhy et al. 2011).

9. TREATMENT

Animals with leptospirosis need supportive care, which includes giving intravenous fluids to correct fluid and electrolyte imbalances. Centrally acting antiemetics and parenteral gastric protector therapy may be beneficial for cats who develop concomitant renal failure. Proper pain treatment should be used to reduce discomfort, particularly in the early stages of the disease when gastrointestinal tissue, muscles, joints, and kidneys may swell and hurt (Schuller et al. 2015). In order to lower the danger of subsequent complications in cats with anorexia, enteral feeding tubes should be used until they regain the ability to feed on their own (Greene 2006).

9.1. ANTIMICROBIAL THERAPY

Ampicillin intravenously may be the recommended antibiotic during the stabilization stage. A 6-week regimen of oral suspension doxycycline has been recommended after the cat is stable to get rid of the carrier condition (Hartmann et al. 2013). For cats, doxycycline monohydrate is available as tablets or suspension and is a less irritant than the hyclate or hydrochloride forms. Doxycycline monohydrate tablets

should be given prior to a feast or a meal so the subsequent oesophagous inflammation can be prevented (Frowde et al. 2011; German et al. 2005).

10. PREVENTION

Animal and human vaccines have both been in use since the 1920s. Nearly every one of these vaccines was made from complete leptospiral cells that had been inactivated using a variety of techniques, including heat, formalin, phenol, and irradiation e.t.c. (Vincent et al. 2019). There isn't a cat-specific commercial vaccine in the market right now. However, experimental inoculation of cats with a commercial dog vaccination containing four separate serovars can result in the production of antibodies, albeit at lower titres than in vaccinated dogs (Shropshire et al. 2016). Cats can prevent infection most successfully by avoiding exposure in the absence of a vaccination. Cats housed indoors are less likely to develop the disease (Hartmann et al. 2013). As part of precautionary measures, it is advised to eliminate predatory possibilities and steer clear of standing water, animal urine, and canines at risk of developing clinical leptospirosis (Arbour et al. 2012; Mwachui et al. 2015; Ojeda et al. 2018). For a period of two weeks, cats who live in the same environment as an animal with a positive diagnosis can get doxycycline at a dosage of 5mg/kg or 10mg/kg PO once a day (Schuller et al. 2015; Sykes et al. 2011).

11. LEPTOSPIROSIS THE ZOONOSIS

Incidences of leptospirosis in humans vary considerably over the world, ranging from 0.1 to 1 case per 100,000 people per year in locations with temperate climates to over 10 cases per 100,000 people in humid sub-tropical areas. Over 100 instances per 100,000 people may get afflicted during outbreaks (Hartskeerl et al. 2011). Veterinarians, farmers, animal caregivers, animal researchers, and those who often come into touch with pet dogs or domestic cattle throughout their daily activities are just a few of the people who can get human leptospirosis (Langston and Heuter 2003). The majority of leptospiral infections in farmers, vets, abbatoir staff, and meat inspectors are contracted through close contact with diseased animals, which is a key risk factor (Barmettler et al. 2011). There isn't much evidence available on this subject, however pet ownership has been proposed as a potential risk factor for Leptospira infection. For instance, 10% of the 61 human leptospirosis cases reported between 1982 and 2001 in a research conducted in California, USA, had dogs as the source of their exposure (Meites et al. 2004). Despite the low seroprevalence of *L. interrogans* in cats, it is nevertheless a public health concern because of the possibility of cat-human interaction. This interaction creates a link between the environmental reservoir and human populations (Mosallanejad et al. 2011). Cats are just as likely as dogs to spread leptospirosis, according to the evidence. However, the practice of hiding their urine and the typical squatting posture they use when urinating could significantly diminish the organism's chances of long-term survival (Lomar et al. 2000). Domestic cats can still carry leptospirosis even in the absence of outward signs of the disease, such as chronic leptospiuria or high antibody titres. As a result, they ought to be considered possible sources of infection for both people and other domestic animals (Bonhomme and Werts 2022).

12. CONCLUSION

In conclusion, leptospirosis is a relatively rare but potentially serious bacterial infection that can affect cats. It is caused by various species of Leptospira bacteria and is typically transmitted through contact with contaminated water or soil, as well as exposure to infected animals or their urine. While cats are generally less susceptible to leptospirosis compared to other animals like dogs, they can still contract the disease and develop symptoms ranging from mild to severe. Symptoms of leptospirosis in cats can include fever, lethargy,

vomiting, diarrhea, muscle pain, jaundice (yellowing of the skin and eyes), and kidney or liver dysfunction. Diagnosing leptospirosis in cats can be challenging, as the symptoms can overlap with those of other illnesses. Laboratory tests, including serology and PCR, are often used to confirm the presence of the bacteria and determine the specific Leptospira causing the infection. Treatment of leptospirosis in cats typically involves antibiotics, such as doxycycline or ampicillin, along with supportive care to manage the symptoms and help the cat's immune system fight the infection. Early detection and treatment are crucial to improve the chances of a positive outcome. Prevention of leptospirosis in cats involves minimizing their exposure to potentially contaminated environments, ensuring proper hygiene, and avoiding contact with rodents and other animals that could carry the bacteria. Vaccines are available for dogs to protect against certain serovars of Leptospira, but vaccines for cats are less common and might not provide complete protection.

In summary, leptospirosis is a bacterial infection that can affect cats, though it is less common compared to other animals. Owners should be aware of the symptoms, take preventive measures, and seek veterinary care promptly if their cat shows signs of illness, as early intervention can greatly improve the prognosis.

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Aspergillosis: An Occupational Zoonotic Disease

29

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ABSTRACT

This chapter provides a comprehensive analysis of Aspergillosis as a major occupational zoonotic disease, examining the complex linkages between human activity, animal relationships, and the spread of Aspergillus species. The zoonotic aspect of the fungal infection, which is mainly brought on by breathing Aspergillus spores, highlights the serious health risk it poses to those who work with animals. The chapter begins with an outline of the epidemiology of Aspergillosis in work contexts and highlights the increased risk faced by professionals in veterinary medicine, agriculture, and other similar industries that involve direct contact with animals. It explores the various work environments where Aspergillus exposure is common, focusing on particular sectors and occupations where people are more vulnerable. In addition, the chapter delves into a variety of risk variables that impact susceptibility, such as immunocompromised states and underlying medical disorders, with a focus on identifying vulnerable groups in industrial settings. The difficulties in diagnosing Aspergillosis in these environments are explored, emphasising the need for improved surveillance and diagnostic instruments designed to address the unique difficulties of zoonotic transmission. The text provides information on control tactics and preventive measures, including the use of personal protective equipment, appropriate immunization regimens, and environmental management. By utilising an interdisciplinary approach, this chapter seeks to bridge the gap between veterinary and human health considerations by offering insightful information on diagnosing, treating, and preventing Aspergillosis in occupational settings. In the end, it remains an invaluable tool for scholars, medical practitioners, and legislators attempting to understand the complexities of Aspergillosis as a zoonotic illness in work environments.

Keywords: Zoonosis, zoonotic aspergillosis, occupational risk, public health, respiratory

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1. INTRODUCTION

Aspergillosis is a fungal infection caused by a specie of fungi known as Aspergillus which can affect the both humans and animals.. Occupational exposure is the main cause for occurrence of this disease especially working with contaminated material such as agriculture, construction, healthcare as Aspergillus spores are found in soil, decaying vegetation, and animal droppings from which these can be inhaled by humans and animals (Malik et al. 2003, Williams et al. 2004, Wild 2010). Aspergillosis is considered a zoonotic disease as it can be transmitted from animals to humans and humans to animals. It also affects the animals particularly birds, but it is more common in domesticated animals like dogs and horses. In humans, it possesses high risk for development in those individuals having weak immune systems such as those undergoing chemotherapy, transplant recipients or those infected with HIV/AIDS. Workers in the industries like agriculture, construction, healthcare, food processing have chances of Aspergillus spores inhalation while working with contaminated material such as hay, grain or compost or may be while cleaning or maintaining contaminated environments. However, there are some occupational groups which are at high risk of developing aspergillosis such as farmers and veterinarians as they both have maximum exposure to Aspergillus contaminated material (Malik et al. 2003, Williams et al. 2004). The symptoms may vary from case to case as in some cases it can cause respiratory infections, allergic reactions, or invasive disease that can lead to serious health complications because it is the most severe form of disease that can affect lungs, brain, kidneys (Morris et al. 2000, Falvey 2007).

Aspergillosis can be prevented by proper hygiene and safety measure in the workplace to prevent occupational exposure, use of personal protective equipment, and prompt diagnosis and treatment of infections. Aspergillosis can be cured by using antifungal medication if the case is complicated then surgery may be necessary (Azie et al. 2012, Blot et al. 2012).

In this chapter, we will discuss different types of aspergillosis and the occupational and zoonotic aspects of Aspergillosis. Besides this, we will also contribute towards prevention and treatment of Aspergillosis as well as proper hygienic conditions to prevent occupational exposure. Impact of Aspergillosis on public health and challenges faced in managing this disease will also be discussed in this chapter.

2. ZOONOTIC TRANSMISSION

There are different ways for humans to be affected with *Aspergillus* species such as handling with infected living birds, poor management and hygienic conditions, inhalation of spores from contaminated feed and litter, examination of dead infected carcasses as well as consumption of raw cooked contaminated poultry carcasses (Fig. 1). Birds of all ages are susceptible to this infection but mostly very young or old birds are affected. During growth of the chick, colonization of conidia occurs in the caudal air sacs because birds get infected due to conidia inhalation. This infection basically occurs in the hatchery where cracked eggs shell gets infected with *Aspergillus* spores and infect the embryos during the hatching process in hatchery or during the brooding time and commonly called as “brooder pneumonia”. Stressors also increase the susceptibility to aspergillosis like poor ventilation, high level of humidity of litter, overcrowding, warm temperature, antibiotic use, vitamin A deficiency. Besides of these all, wild birds are also involved in transmission of *Aspergillus* species. Presence of conidia in bird’s dropping is also a source of infection. So, humans can be infected either directly or indirectly (Small and Nicholls 2003).

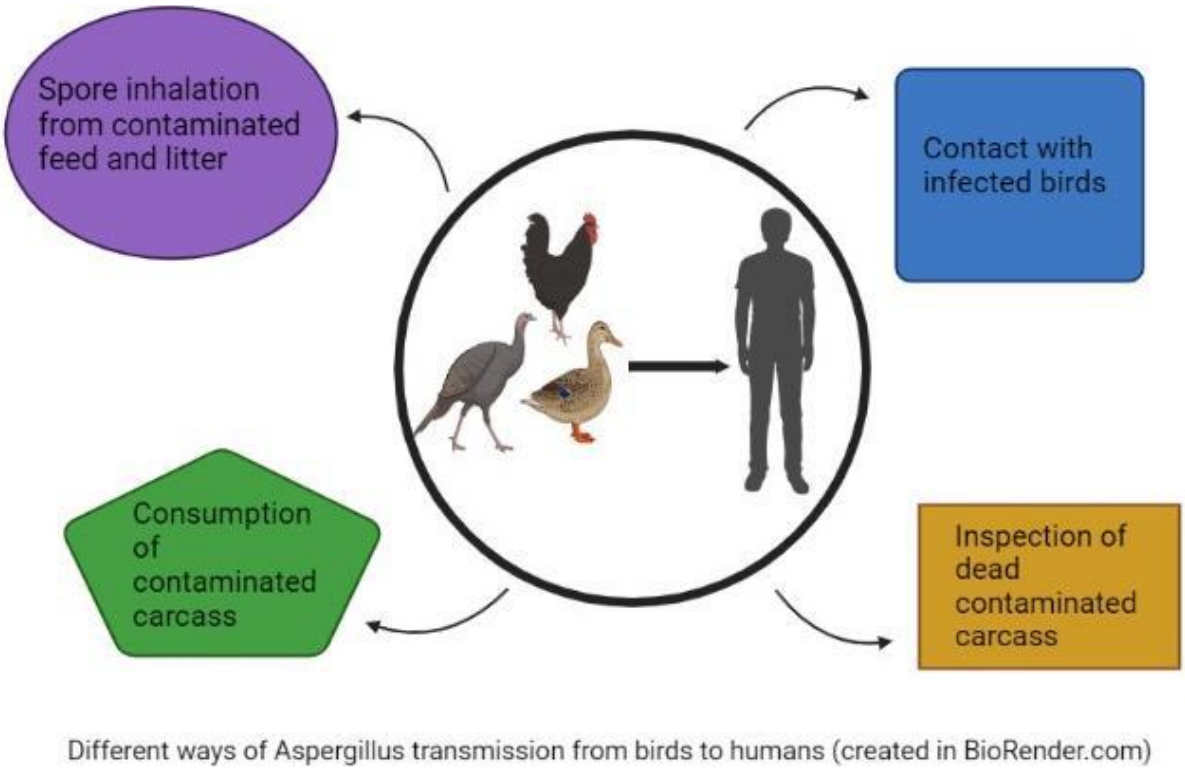

Fig. 1: Methods of *Aspergillus* transmission from animals to humans.

A study has shown the affection of humans in a way that about 15 million people can be infected by aspergillosis with more than 1 million deaths annually (Global Action Funds for Fungal Infections 2020). *Aspergillus Fumigatus* is the most commonly found specie of *Aspergillus* in case of humans (Lamoth 2016). In most of the countries, fungal cause of invasive and allergic infection in humans is *Aspergillus* flavus (Chakrabarti et al. 2008; Hadrich et al. 2010, 2013). However, in other affected areas different species have been reported such as *Aspergillus lentulus*, *Aspergillus thermomutatus*, *Aspergillus pseudofischeri* and *Aspergillus felis* (Barrs et al. 2013; Howard 2014; Negri et al. 2014). *Aspergillus* infection has been characterized in 4 ways depending upon clinic spectrum like invasive aspergillosis, chronic pulmonary aspergillosis, *Aspergillus* bronchitis and allergic bronchopulmonary aspergillosis (Kosmidis and Denning 2015). The severity of fungal infection depends mainly on two things fungal extension in tissues and immune response. After inhalation of spores, macrophages detect these spores in lungs alveoli and engulf and destroy them and if the human is immunocompromised then severe allergy with systemic disorder may occur (Brakhage et al. 2010; Milos et al. 2011). Immunocompromised humans are more susceptible to these infections with severe clinical picture and mortality rate (Bassetti et al. 2015). COVID associated pulmonary aspergillosis, invasive aspergillosis, influenza associated pulmonary aspergillosis and chronic pulmonary aspergillosis have been reported.

3. OCCUPATIONAL EXPOSURE

Aspergillosis is an infection caused by various *Aspergillus* species like *Aspergillus Fumigatus*. As aspergillosis can be developed by anyone, due to higher exposure of *Aspergillus* spores' certain occupations are there to be considered at high risk (Malik et al. 2003, Wild, 2010). However, here are the commonly associated occupations with higher risk of aspergillosis such as farmers and agricultural

workers, veterinarians and animal handlers, construction workers, woodworkers, gardeners and horticulturist, waste handlers and compost workers (Malik et al. 2003). Organic materials such as moldy hay, straw, grain and compost harbour *Aspergillus* spores which can lead to respiratory infection upon inhalation of these spores (Malik et al. 2003). Therefore, farmers are at high risk or aspergillosis due to exposure to these organic materials. Farmers with weakened immune system or pre-existing lung conditions are more susceptible to aspergillosis (Wild 2010). Veterinarians and other animal handlers are found at increased risk of aspergillosis due to their close contact with animals especially birds as in bird droppings, decaying organic matter and contaminated bedding material *Aspergillus* spores can be found (Williams et al. 2004). There are two veterinary procedures which can increase the risk of exposure such as handling contaminated materials or performing surgeries because during these procedures inhaled spores can cause respiratory aspergillosis (Malik et al. 2003, Williams et al. 2004). Buildings with dampness, water damage or poor ventilation can provide a favourable environment to molds for their growth as spores need high humidity to grow and demolition of renovation of such type of buildings provide chance to these spores to be released in the air increasing the risk of inhalation (Reddy et al. 2009, Viegas et al. 2013). So, working in molds contaminated environments may increase the risk of exposure to construction workers which can lead to aspergillosis (Reddy et al. 2009). Wood can be moldy when stored in damp conditions or exposed to water damage may encounter *Aspergillus* spore for woodworkers such as carpenters and furniture makers working with wood contaminated with molds (Malik et al. 2003, Siruguri et al. 2012) Manipulation of such type of wood by cutting, sanding or any other way can lead to release of spore increasing the risk of inhalation (Siruguri et al. 2012). *Aspergillus* spores are also present in soil, compost, decaying plant matter or rotting wood. So, professional gardeners and horticulturists mag be exposed to *Aspergillus* spores working in gardening, landscaping and horticulture (Malik et al. 2003, Williams et al. 2004, Wild and Gong 2010) During gardening activities or handling plants when these materials are disturbed there is a chances to spores release into the air leading to inhalation causing respiratory aspergillosis (Wild 2010, Siruguri et al. 2012). The most important source of *Aspergillus* spores is waste. So, waste handlers and compost workers involved in waste management may face increased exposure to *Aspergillus* spores from moldy organic waste such as rotting vegetation, food waste or compost piles having high concentration of *Aspergillus* fungi (Reddy et al. 2009, Viegas et al. 2013). Working in proximity to contaminated materials can result in respiratory infections.

It is important to note that not only the above discussed occupations are at the risk of aspergillosis. Instead of these, mill workers, textile workers, bakers and those working in laboratories are also at the high risk of aspergillosis depending upon the specific tasks and presence of *Aspergillus* in their working environment. Along with the presence of *Aspergillus* infection, there is a need to prevent this infection. Preventive measures can be the use of personal protective equipments, proper ventilation, regular cleaning, and maintenance of work environments can reduce the risk of exposing the people to the *Aspergillus* spores in occupational areas. People working in high risk occupations should take special measures like potential hazards, maintain good respiratory hygiene and if they experience any type of respiratory symptom or suspect exposure to *Aspergillus* they should take medical attention.

4. SOURCES OF EXPOSURE

Most of the nosocomial aspergillosis outbreaks have been reported due to air contamination (Fig. 2). In 1970s and 1980s, the outbreaks were due to the sources existing outside the hospitals with inadequate ventilation systems (Maschmeyer et al. 2007, Warnock 2007). A study has shown that pigeon excreta has been the source in two outbreaks (Maschmeyer et al. 2007, Warnock 2007).

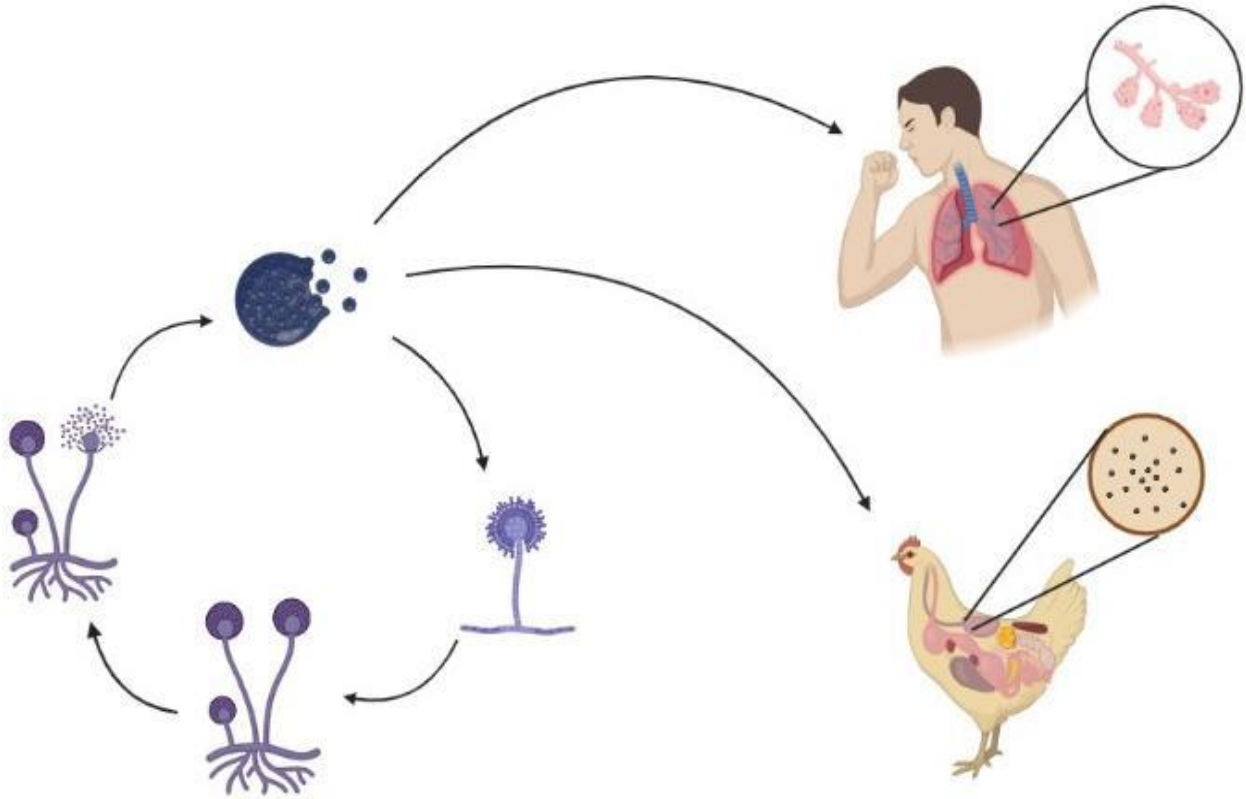

Fig. 2: Sources of exposure to *Aspergillus* for humans.

Internal construction or renovation with failure to control spread of contaminated dust or debris has been the main source of most outbreaks of nosocomial aspergillosis (Almyroudis et al. 2005, Ballard et al. 2008). Especially the main point of outbreaks was the renovation or construction of that floor where infected patients were housed (Almyroudis et al. 2005, Ballard et al. 2008). Hospital location's renovations have been a great source of infection especially where ancillary procedures were performed such as radiology (Meerssemam et al. 2004). Contaminated air vents or filters have been a great source of aspergillosis (Maschmeyer et al. 2007, Warnock 2007). Other objects like syringes and spinal needles, a liquid nitrogen tank near the operating room (Kronman et al. 2007), gauze used to cover venepuncture sites (Laarkin et al. 1996), dressing supplies, latex finger stalls (Menotti et al. 2005), and electronic equipment in the operating room (Heinemann et al. 2004) can be the source of infection. Water exposure has been a great source of infection (Laarkin et al. 1996). Now at this time dust above acoustical ceiling tiles has been a source of infection when acoustical ceiling tiles have been removed or damaged, allowing airborne dissemination of fungal spores (Almyroudis et al. 2005). Aspergillosis is also caused by inhalation of fungal spores resulting in pulmonary disease. Dissemination from pulmonary site is well described with more than 500 cases of post-operative aspergillosis (Pasquolotto 2006). From these, most cases are due to airborne infection during surgical procedure such as cardiac surgery, ophthalmological surgery, and dental surgery. Cutaneous aspergillosis has been reported due to contaminated dressing materials (Laarkin et al. 1996). Besides above mentioned sources, animals have also been a source of aspergillosis for veterinarians and farmers as well such as poultry birds, cat, dog, etc.

5. PATHOGENESIS AND CLINICAL MANIFESTATIONS

The life cycle of *Aspergillus* infection begins with conidia production which are asexual spores that are dispersed to maintain the indoor and outdoor environment ubiquity (Falvey 2007). Humans get infected with these conidia spores via inhalation of these spores which then deposit in the bronchioles or alveolar spaces. Conidia are not removed by the primary resident phagocytes (mucociliary clearance encounter epithelial cells or alveolar macrophages) of the lungs as alveolar macrophages are responsible for conidia phagocytosis and neutrophils initiation to the site of infection. Conidia that survive from macrophage killing, germinate and become the target of infiltrating neutrophils that are responsible to destroy hyphae and infection occurs due to dysfunction in these host defences and *Aspergillus Fumigatus* growth in pulmonary environment (Morris et al. 2000).

Humans can be infected from different *Aspergillus* species from transmitted from animals. Clinical manifestations of aspergillosis in humans vary with site of infection and immune status. Respiratory aspergillosis is common when transmitted zoonotically. The manifestations range from mild allergic reactions to invasive infections like allergic bronchopulmonary aspergillosis, aspergilloma, invasive pulmonary aspergillosis (Barnes 2006). Allergic Bronchopulmonary Aspergillosis is an allergic hypersensitivity response noticed by wheezing, shortness of breath, coughing, recurrent episodes of bronchitis or asthma which leads to the lung tissue damage if not treated well (Barnes 2006). Aspergilloma is a fungal ball develops in lung cavities or damaged lung tissue and discovered on chest imaging asymptomatically (Singh 2005). Symptomatically, it appears with chest pain, chronic cough, hemoptysis and respiratory distress (Singh 2005, Barnes 2006). Invasive pulmonary aspergillosis mostly affects immunocompromised individual that's why this is called life threatening infection which can lead to pleuric chest pain, cough, fever, shortness of breath.

Cutaneous aspergillosis occurs due to direct contact of spores into the skin through burns, wounds. It results in local skin infections like ulcers, abscesses, bruises (Lee et al. 2004). In immunocompromised individuals, infection spreads from the initial site. Ocular aspergillosis occurs due to either direct inoculation of spores or dissemination from the infected site with the manifestation of keratinitis, endophthalmitis, uveitis, or orbital cellulitis with symptoms like eye redness, pain etc (Fig. 3) (Lee et al. 2004, Barnes 2006). If *Aspergillus* infection spreads from primary infection site to other organs, it is called disseminated aspergillosis. It commonly affects liver, heart, brain, kidneys, bones and it can be life threatening.

6. DIAGNOSIS AND TREATMENT

Diagnosis of every disease depends upon history, signs and lesions. The history like poor environmental conditions, severe respiratory signs or bird carcasses are indications for aspergillosis. Clinically Aspergillosis is not specific as the other infections (Dahlhausen et al. 2004). Affected birds may have white granulomatous nodules or cheesy plaques in the lungs, air sacs or other organs as diagnostic sign. Definitive diagnosis of aspergillosis is possible (Jones and Orosz 2000, Charlton et al. 2008, Beernaert et al. 2010). Different staining methods can be used to detect fungal hyphae such as Periodic Acid-Schiff, Bauer's and Gridley's, Methanamine Silver and Grocott's and Gomori stain. Furthermore, lactophenol cotton blue stain can be used to detect fungal hyphae if wet smear from specific nodules fixed with 20% potassium hydroxide stained with lactophenol cotton blue stain. Use of Parker's India ink is another way to detect fungal hyphae. Affected organs showed different structures under histopathological examination like granulomatous foci with central depressed coagulative necrosis surrounded by inflammatory cells and congestion of pulmonary and perialveolar blood vessels with perivascular edema was also diagnosed (Girma et al 2016). Samples for diagnostic purpose can be

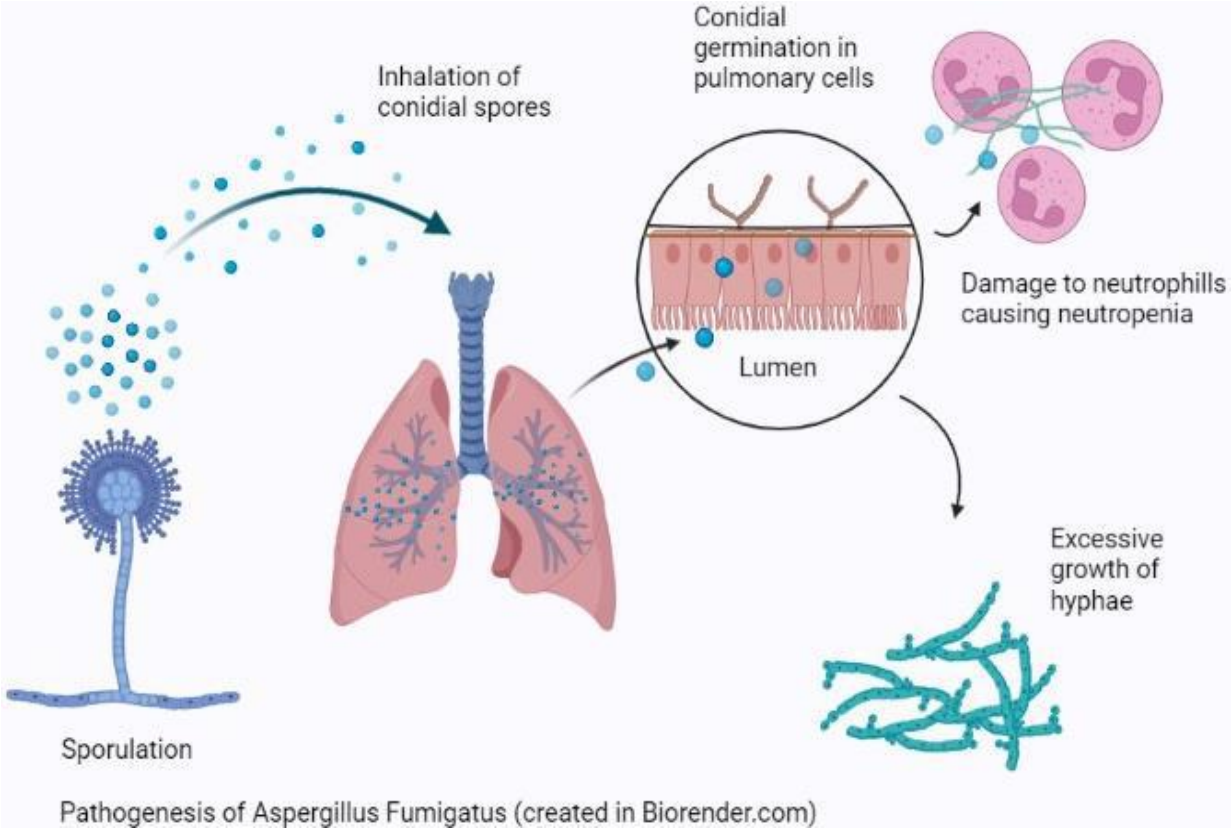

Fig. 3: Pathogenesis of *Aspergillus* infection.

selected from different affected parts of the body such as larynx, trachea, lungs, air sacs and brain and cultured on Sabouraud's dextrose agar or malt agar which are selective specific media for fungal growth and incubated at 37°C for 24 hours. By this method, characteristic conidial head and colony of fungal species can be identified (Dahlhausen et al. 2004). However, the main point should be noted that *Aspergillus* infection still appear in negative culture. Biochemical and haematological parameters are other methods to diagnose *Aspergillus* infection (Jones and Orosz 2000). Serological test can also be performed to diagnose *Aspergillus* infection such as enzyme-linked immunosorbent assay (Le Loc'h et al. 2005, Arca-Ruibal et al. 2006), immunohistochemistry (Beytut et al. 2004, Beytut 2007), galactomannan assay and plasma protein electrophoresis (Cray et al. 2006, 2009a, b). Confirmatory methods to diagnose Aspergillosis are use of monoclonal or polyclonal antibodies. Radiographically, lateral and ventrodorsal views of suspected birds can indicate the aspergillosis (Jones and Orosz 2000). If suspected birds are diagnosed endoscopically then yellowish-white plaques covered with green or grey hyphae of fungal growth can be observed in abdominal air sacs (Dahlhausen et al. 2004). Molecular techniques can also be used to diagnose aspergillosis like molecular beacon technology, polymerase chain reaction (PCR) and nucleic acid sequencing based amplification (Dahlhausen et al. 2004, Balajee et al. 2009, Saleemi et al. 2012, Zhao and Perlin 2013).

7. PREVENTION AND CONTROL

The only way to protect chickens is prevention (Arné et al. 2011). There should be strict hygienic and sanitary conditions at setters, brooders and hatcheries (Beernaert et al. 2010). It is recommended that

formaldehyde or antifungal compounds as thiabendazole (120-360 g/m3) should be used through cleaning, disinfection and fumigation (Pattisson et al. 2008). Using azoles for environmental disinfection and decontamination of bedding is common (Nawrot et al. 2019). The litter and feed should be free of contamination by controlling environmental conditions. The litter should be free from molds and replaced by clean one. However, antifungal preparations as copper sulphate or nystatin can be used to treat moldy litter. Feeders and drinkers should be cleaned and disinfected (Kunkle et al. 2003). There should be no morbid bird in the flock. In such cases there is no effect of antifungal drugs on fungus as treatment is recommended in mild and early stages of infection. Effective dose of copper sulphate is 1:2000 through water or should be given through feed for 6 days treatment along with one or more antifungal drugs such as itraconazole, miconazole, eniconazole, clotrimazole, ketoconazole, fluconazole, amphotericin B and fungicidin (Dhama et al. 2013). Due to the most of fungistatic drugs, it has been noted that there is an increase in the resistance to the classical antifungal drugs. New trends are being applied to cover such infections such as use of essential oils of some plants that have showed broad spectrum potential antifungal activities in poultry (Pinto et al. 2009, Radwan et al. 2018b, Abed et al. 2021). Different studies have shown the antifungal properties or plants essential oils (Chuang et al. 2007, Kędzia and Holerna-Kedzia 2007, Yang and Clausen 2007, Pinto et al. 2009). Complete inhibition of fungal spores has been shown by a mixture of cinnamon, lavender, rosemary and sage oil at 1% concentration (Cvek et al. 2010). However, strong suppressive activity against *Aspergillus* species have been shown by cinnamon or cinnamon fortified with cinnamaldehyde at low concentration of 0.1% (Abed et al. 2021), or a concentration of 4% (López et al. 2005). There is a study on antifungal activity of thyme essential oil and thymol against molds (Klarić et al. 2007) and the results showed the vaporous phase having long lasting antifungal activity on molds from damp dwelling. It is important to control the conditions in a proper way as wearing gloves and masks during bird handling, ventilation should be proper, and feed should be free from molds (Mubarak 2017). If the infection has been detected at farm, there should be rapid diagnosis, treatment and disposal of dead birds to reduce infection spread (Mubarak 2017).

8. OCCUPATIONAL HEALTH POLICIES

There is a great role of occupational health policies and regulations in securing the health and wellbeing of workers along with reducing the incidence of aspergillosis and other occupational diseases (Azie et al. 2012, Blot et al. 2012). There are few policies and regulations in which employers play an important role in promoting a safe working environment such as risk assessment and prevention, education and training, implementation of control measures, provision of personal protective equipments, health surveillance, and compliance and enforcement (Blot et al. 2012). Occupational health policies need employers to conduct comprehensive risk assessments and identify potential hazards in the workplace especially those related to aspergillosis (Blot et al. 2012). This procedure involves the assessment of certain factors such as ventilation systems, exposure to molds contaminated materials and personal protective equipment requirements (Blot et al. 2012, Azie et al. 2012). When employers identify these risks then they can apply preventive measures in order to minimize workers exposure to *Aspergillus* spores such as improved ventilations, proper waste management, regular cleaning and use of appropriate personal protective equipment (Becker et al. 2003) Along with identifying these risks, employers also have a great responsibility regarding provision of proper education and training to workers about aspergillosis associated risks and necessary preventive measures (Becker et al. 2003, Leeflang et al. 2008). This training includes instructions on proper use of personal protective equipments as well as proper handling, storage and disposal of molds contaminated materials

(Montagna et al. 2012). When workers are well educated then employers tell them to protect themselves from these potential exposures and promote a safe environment in the workplace. Employers are responsible to apply control measures in order to decrease the risks of aspergillosis such as repair of water leaks or damage, regular monitoring of air quality and humidity levels, proper ventilation and proper work areas (Azie et al. 2012, Blot et al. 2012, Montagna et al. 2012). By applying these control measures results in decrease in molds growth ultimate decrease in risk of aspergillosis (Becker et al. 2003, Leeflang et al. 2008, Montagna et al. 2012) . Besides these all steps, employers are also responsible to provide personal protective equipments such as respiratory masks, gloves, protective clothing to workers who exposed to *Aspergillus* spores (Becker et al. 2003, Azie et al. 2012). Personal protective equipments play an important role to create a barrier between workers and harmful materials and reduce the risk of spores inhalation. Employers should make sure the availability of properly maintained personal protective equipments for workers. Besides the implementations of control measures, occupational health policies also need employers to apply health surveillance programs to check health of workers exposed to potential hazards including *Aspergillus* spores by doing regular medical check-ups and identifying early signs or symptoms of aspergillosis for timely treatment (Leeflang et al. 2008, Montagna et al. 2012) Health surveillance also collect the data of occupational diseases for future prevention strategies. Along with other opportunities, occupational health policies and regulations also provide a platform for compliance and enforcement (Becker et al. 2003, Blot et al. 2012). Employers are responsible to follow these regulations making sure the protection of workers from occupational hazards including aspergillosis. Compliance and occupational health policies emerge a safe working environment and decreases the risk of aspergillosis.

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Campylobacteriosis: A One Health Perspective on Abortion and Zoonosis

30

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ABSTRACT

All infectious diseases that can transfer from vertebrate animals to humans are referred to as zoonotic diseases, or zoonosis. Contact with animals or their bodily fluids, ingestion of contaminated animal products, and exposure to contaminated environments are the ways in which these diseases can be acquired. Human infections with Campylobacter fetus can cause a variety of clinical symptoms, such as acute diarrhea, septicemia, and severe neurological problems. These infections can cause a variety of problems during pregnancy, including placentitis, abortion, and neonatal sepsis, highlighting the broad and catastrophic effects of C. fetal infection in both adults and infants. Efficient management and treatment of the disease depend on the timely and accurate detection of Campylobacter infection. Every diagnostic strategy discussed in this chapter, including molecular, immunological, serological, culture-based, and next-generation sequencing approaches, has advantages and disadvantages. The availability of laboratory resources, test duration, and other factors all play a role in the diagnostic approach that is selected. Antibiotics and supportive care are often used throughout treatment after a diagnosis has been made by laboratory analysis of stool samples. Good food hygiene practices, risk factor education, a single health approach, hand hygiene, and reporting to better understand and enhance preventative actions are all important components in preventing and controlling the illness. New campylobacteriosis diagnostic tests and treatment alternatives are being studied in ongoing research. The One Health concept, which recognizes the connection of human, animal, and environmental health, has proven critical in addressing the importance of campylobacteriosis for public health. Experts in environmental health, animal health, and human well-being must work together and coordinate in order to prevent, detect, and treat zoonotic illnesses. Overall, the chapter provides useful information for understanding, preventive and managing this infectious disease.

Keywords: Campylobacter, Zoonotic nature, Abortion, Reproduction, Public health

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1. INTRODUCTION

Zoonosis or zoonotic disease, is the term used to describe any infectious disease that can spread from vertebrate animals to human. These diseases can be contracted through direct contact with animals or their body fluid, eating contaminated animal products, or being exposed to polluted environment. Various pathogens, including bacteria, viruses, parasites, and fungi can bring them on. Zoonotic diseases are a serious public health problem because they can cause outbreaks and epidemics. These diseases affect both humans and animals, causing a variety of signs and symptoms such as fever, gastrointestinal problems, respiratory issues, and abortion in mammals. One zoonotic disease caused by bacteria is Campylobacteriosis. Gram-negative bacteria with a distinctive curved or spiral-shaped rod appearance are called *Campylobacter*. Despite being fastidious, certain organisms can flourish in anaerobic circumstances, while others favor micro-aerobic ones (Kaakoush et al. 2015). In unfavorable growing conditions, *Campylobacter* species can produce viable but nonculturable cells (VBNC). Due to the organism's ability to establish colonization in hosts while remaining hidden by standard culture methods, this trait makes it difficult to undertake etiological research on the organism (Portner et al. 2007). Animals, such as cattle, birds, sheep, and pigs, have alimentary canals frequently colonised by commensal microbes called *Campylobacter* species. Because of avian relatively high body temperature, which creates an excellent development environment for this temperature-tolerant genus, avian species are recognized as a major reservoir of *Campylobacter* (Skirrow 1977). Therefore, it has been determined that poultry products, such as chicken meat, which is widely consumed by humans are the primary cause of gastroenteritis in people who are exposed to *Campylobacter* (Humphrey et al. 2007).

According to (Horrocks et al. 2009), specific bird species can spread the infection to broiler flocks horizontally and ultimately to people, particularly those who deal with or consume poultry (Humphrey et al. 2007). The three types of *Campylobacter—Campylobacter fetus ssp*. *fetus, Campylobacter coli*, and *Campylobacter jejuni*, are mainly present in the intestines and can be spread through fecal-oral contact, oral fluids, contaminated placenta, and fluids through the skin. The microorganisms can continue to spread across the flock from an aborting sheep to an uninfected one during outbreaks, which can result in pregnancy failure. A uterine infection in pregnant ewes can cause an abortion in the third trimester or the birth of a live infected lamb. Ewes often gain immunity to these bacteria after the initial infection. They can cause placentitis, which results in chorioallantois, yellow, friable cotyledons, and swelling tissue between the cotyledons. *C. jejuni* infections may cause serious consequences, such as Miller Fisher syndrome, arthritis, and Guillain-Barre syndrome (GBS) (Man 2011).

Certain nonzoonotic *Campylobacter* species, such as *C. concisus*, prevalent in the human oral cavity's microflora community, are of growing concern for non-zoonotic species. *C. concisus* is primarily found in healthy dental cavities, even though it was initially isolated from persons with periodontitis and gingivitis. Additional *Campylobacter* species have also been discovered in oral cavities, including *C. ureolyticus* and *C. curvus*. It has been proposed that a few species, namely *C. gracilis*, *C. rectus*, and *C. showae*, are the primary agents of periodontal diseases. Although some studies concentrated on their possible role in periodontal disease, more recent studies have mostly examined these species' ability to cause intestinal disorders such as inflammatory bowel disease (IBD) and gastroenteritis.

This chapter focuses mainly on the zoonotic species of *Campylobacter*, including their pathogenesis, symptoms, one health perspective, impact on reproduction, diagnostics, and preventative with therapeutic options (Man 2011).

2. CAUSES OF CAMPYLOBACTERIOSIS

Campylobacteriosis is caused by the genus *Campylobacter*. There are 24 species in the Campylobacter genus, and the *Campylobacter fetus* is significant due to its zoonotic significance. The *Campylobacter* bacteria frequently bring on a bacterial infection known as campylobacteriosis. The infection is typically contracted by consuming contaminated food and water or touching contaminated animals or their feces (Rukambile et al. 2019). The bacteria can infect meat during slaughtering and processing and are frequently detected in the intestines of animals, particularly poultry. Infections can also come via unpasteurized milk, uncleaned water, and contaminated vegetables while *C. fetus subspecies venerealis* mostly affects the reproductive systems of cattle and can cause infertility and abortion, *C. fetus subspecies fetus* is frequently found in the intestinal tracts of numerous animals, including cattle and sheep (Mahlangu et al. 2022).
Overall, understanding the cause, unique characteristics and habitats of *Campylobacter* bacteria is important for diagnosing, treating, and preventing infections caused by this bacterium.

3. PATHOGENESIS

According to studies on the pathogenesis of *Campylobacter* bacteria, the disease is mostly caused by the virulence of the infecting strain as well as the sensitivity of the host. It takes at least 800 organisms to cause the infection by consuming contaminated food or water (Lopes et al. 2021). The main events involved in the pathogenesis of campylobacteriosis are motility, chemotaxis, translocation, adhesion, invasion, and toxin generation. High motility and a spiral form allow the *Campylobacter* bacteria to get through the gastrointestinal mucus and attach to enterocytes, which are gut cells that release toxins to cause diarrhea. Depending on the strain, bacteria secrete different toxins, such as cytotoxins and enterotoxins, that vary in form and potency. The sort of discharged toxin, which might be minor to severe, determines how serious will be the enteritis. Immunoglobulin levels rise during the infection, with IgA being the most crucial because it may pass the gut barrier. IgA renders organisms immobile by causing aggregation and complement activation, granting temporary immunity against the pathogenic strain. Other types of immunoglobulins operate to stop bacteremia by concentrating on bacteria that enter the bloodstream. Bacteria can activate the cellular immune system. To infect healthy animals, the sick animal releases bacteria through body secretions, aborted fetus, and placenta (Fritz and Byers 2023). Pathogenesis of bacteria is shown in Fig. 1:

3.1. Pathogenesis of abortion

Bacteria enter into the body of animal by oral route from where they go to blood circulation. After entering the bloodstream, the organism induces a brief period of bacteremia lasting for approximately 1 to 2 weeks before it localizes in the chorionic epithelial cells and ultimately enters the fetus, where it causes abortion. Histopathological examination of placenta from an aborted ewe showed severe neutrophilic and fibrinonecrotizing placentitis (Dorsch et al. 2022). The placenta has a strong infiltration of inflammatory cells, as shown in the first image of Fig. 2. The second one has been highly magnified to demonstrate placentitis. The third one displays the arteriolitis of arterioles of chorion (Dorsch et al. 2022).

4. SYMPTOMS AND CLINICAL MANIFESTATIONS OF CAMPYLOBACTERIOSIS

Human Campylobacteriosis infection can cause a variety of clinical signs, including acute diarrheal illness and systemic illness (Man 2011). The location of the scattered pathogen affects the later appearances.

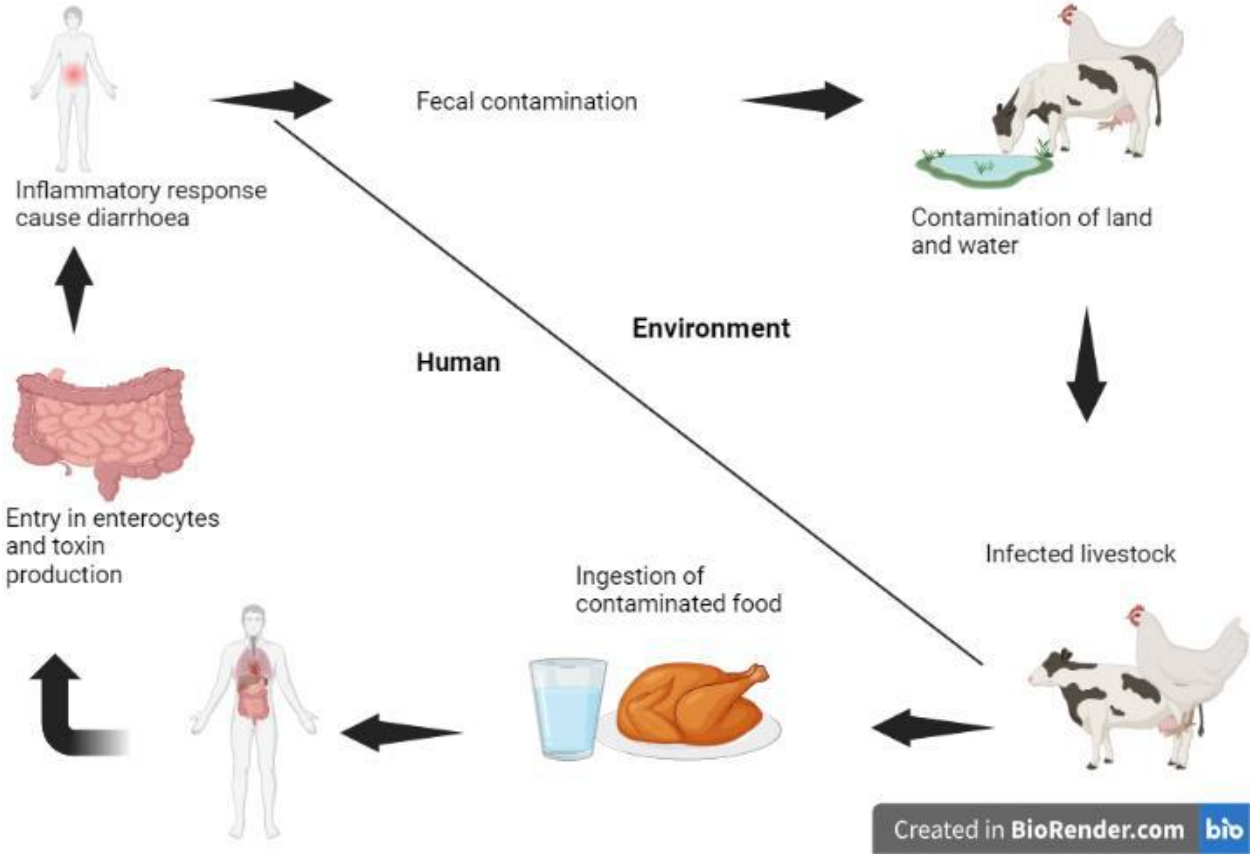

Fig. 1: Lifecycle and pathogenesis of *Campylobacter.*

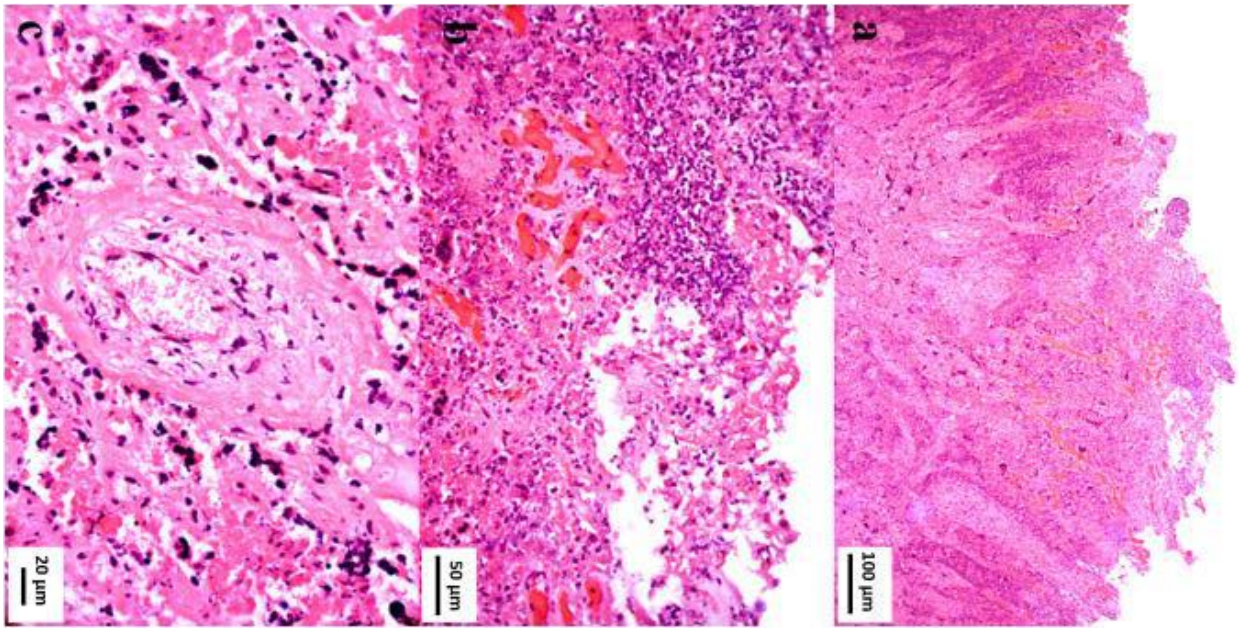

Fig. 2: The pathological changes in placenta (Dorsch et al. 2022).

Patients may occasionally display septicemia, a condition marked by fever without any obvious localized infection (Gazaigne et al. 2008). Neurological disorders such as meningitis, meningoencephalitis, brain abscesses, or subdural empyema may manifest as a consequence of *C.fetal* infection. Additionally, manifestations of this infection can include osteomyelitis, arthritis, lung abscesses, and prenatal illnesses such as endometritis, placentitis, and abortion (Man 2011). Additionally, vascular consequences from this kind of infection can include endocarditis, mycotic aneurysms, vasculitis, pericarditis, or thrombophlebitis. Any stage of pregnancy might experience *Campylobacter fetus* infections, which can cause a variety of clinical symptoms including fever, loss of appetite, placentitis, abortion, irregular estrus, prolonged breeding seasons and diarrhea. Infected pregnant women occasionally go through spontaneous miscarriages without exhibiting any other clinical symptoms (Fujihara et al. 2006). Additionally, babies with *C. fetus* infections are more likely to experience *C. fetus* sepsis, which can result in meningitis and have potentially fatal consequences. Nine of the 14 infants in the study who had *C. fetus* sepsis died, underscoring the seriousness of neonatal diseases (Fujihara et al. 2006). A *C. fetus* infection in the mother is typically linked to perinatal infections. Numerous research investigations on the subject have validated these conclusions. According to the data, the majority of *C. fetus* infections in people are brought on by *C. fetus subsp. fetus*, while *C. fetus subsp. venerealis* is identified from vaginal secretions (Holst et al. 1987). The pattern of subspecies distribution in bovine infections, where the reproductive system is colonized by *C. fetus subsp. venerealis*, which is also replicated in human infections. The ratio of *C. fetus subsp. fetus* to *C. fetus* sub spp. *venerealis*. Despite this, *venerealis* in human isolates is not well known, and subspecies identification is not frequently done in human diagnostic laboratories. In order to better understand the epidemiology of these diseases, it is advised that subspecies identification to be used in the investigation of these infections (Kalka-Moll et al. 2005).

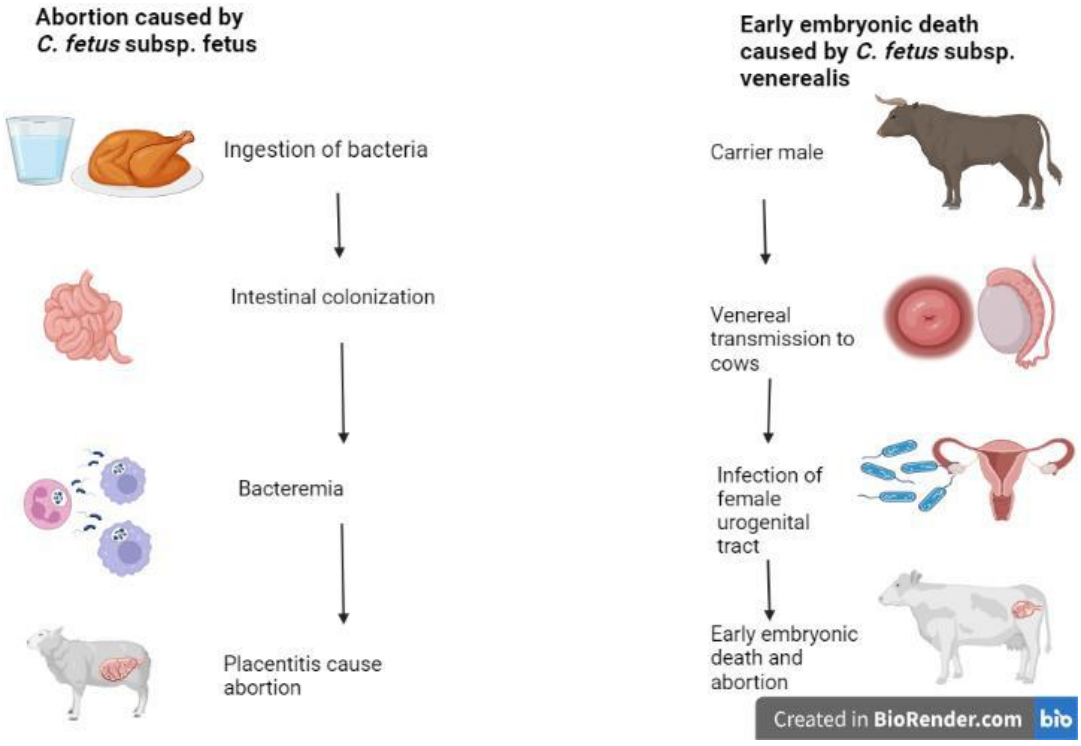

Fig. 3: Reproductive clinical manifestation of Campylobacteriosis.

Here we discuss briefly about the other complications caused by *Campylobacter* bacteria.

4.1. TERMINATION OF PREGNANCY (ABORTION)

According to researchers, *Campylobacter* can cause sporadic abortions in humans (Sahin et al. 2012) and termination of pregnancy in both large and small ruminants worldwide. Although *C. jejuni* outbreaks are becoming more frequent, *C. fetus* still accounts for most abortions in animals (Sahin et al. 2017). Septic abortion caused by *C. fetus* infection in the placenta was documented in early-pregnant women (Simor et al. 1986), and the first clinical instance of C. fetus-induced abortion in humans was testified in 1947 (Hannah et al. 2016).

Campylobacter-related abortion is more frequently seen in animals, although in America, the rate of *Campylobacter*-related abortion in bovine ranges from 1.78 to 15.99%. Additionally, a highly pathogenic *C. jejuni* clone known as clone SA that causes sheep abortions in the United States has been discovered by researchers (Sahin et al. 2012). The ability of clone SA to cause abortions was studied in a guinea pig model (Plummer et al. 2012), though there is currently no proof that it causes abortions in humans. However, it is impossible to completely rule out the possibility that clone SA will impact human abortions, thus more research is necessary.

4.2. GASTROENTERITIS

Globally, *Campylobacter* can induce gastrointestinal tract inflammation. This disease, which affects domestic animals, was first discovered in the 20th century. According to (Acheson and Allos 2001), the bacteria *Campylobacter* can lead to septic abortions in animals, especially sheep, cattle, and pigs. Since it is a zoonotic bacterium, it is also a frequent cause of gastroenteritis in humans, primarily through ingesting contaminated food, particularly poultry. Diarrhea, fever, and abdominal pain are some of the symptoms and signs of gastroenteritis brought on by *Campylobacter*, and they are comparable to those brought on by other pathogens including *Shigella* and *Salmonella* (Acheson and Allos 2001). To avoid misinterpretation based simply on clinical symptoms, diagnosis requires the isolation of *Campylobacter* from stool samples (Galanis 2007).

C. jejuni, which invades the gastrointestinal system and colonizes the colon and reduces the capacity of intestinal cell to absorb nutrients, is the primary cause of gastroenteritis brought on by *Campylobacter* (Konkel et al. 2001). Although *Campylobacter* gastroenteritis usually cures on its own within a few days, serious cases may necessitate the administration of antibiotics like azithromycin, erythromycin, or amoxicillin (Galanis 2007). Early detection is crucial because Guillain-Barre syndrome (GBS), a severe neurological condition that can develop after a *C. jejuni* infection, is frequently seen after the onset of *Campylobacter* gastroenteritis (Kuroki et al. 1993).

Campylobacter gastroenteritis can affect people of all ages, it is more frequently observed in infants and young adults. In order to stop the infection from spreading, it is crucial to take the essential precautions, such as proper handling and boiling food properly.

4.3. SEPSIS

Septicemia, also known as sepsis, is a health condition characterized by a bacterial pathogen that infects the bloodstream (Singer et al. 2016). Peritonitis, pancreatitis, sepsis, meningitis, and septic arthritis are some serious problems that can result from *Campylobacter* sepsis (Acheson and Allos 2001). A major cause of septicemia is *C. fetus*, especially in individuals who are extremely elderly or young, drink alcohol, have weakened immune systems, have had gastrointestinal surgery in the past, or have HIV infection (Nagy and Hla 2013). Even when infected with *Campylobacter* spp., healthy persons with a strong immune system can also get recurrent septicemia (Krause et al. 2002). The most frequent causes of gastrointestinal

illnesses in humans are *C. jejuni* and *C. coli*, however, infrequently (less than 1% of cases) lead to septicemia (Krause et al. 2002). To treat septicemia brought on by *Campylobacter* spp., various antibiotics including erythromycin, imipenem, carbapenem, and gentamicin can be administered (Krause et al. 2002). According to a study by the researcher, carbapenem effectively treated newborn sepsis caused by *C. fetus* (Fujihara et al. 2006).

4.4. INFLAMMATORY BOWEL DISEASE (IBD)

The two main types of inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease (CD), both cause chronic inflammation of the gastrointestinal tract and exhibit symptoms like extreme abdominal pain, diarrhea, weight loss, and fatigue (Lee and Chang 2003).IBD has become more prevalent all over the world (Molodecky et al. 2012), and it is thought that the gut microbiota, which contains bacterial pathogens such as invasive *Escherichia coli, Fusobacteria, Campylobacter,* and *Mycobacteria*, are responsible. IBD is likely to be influenced by environmental and genetic predisposition factors, despite its primary etiology being unknown (Sartor 2006).

Campylobacter spp. has been linked to IBD in several studies, with IBD patients having a greater prevalence of the bacteria in their intestinal samples. Eight different *Campylobacter* species, including *C. concisus*, *C. showae*, *C. hominis*, *C. rectus*, *C. gracilis*, *C. jejuni*, and C. ureolyticus, were isolated from patients. *C. concisus* was the most often seen species. (Zhang et al. 2009).

Numerous studies have been conducted on the connection between specific *Campylobacter* species strains and inflammatory bowel disease (IBD) emergence. According to studies, the prevalence of human oral *Campylobacter* species, including as *C. concisus* and *C. showae*, is higher in IBD patients' biopsy samples of the gut than in healthy people. Despite being mostly present in the mouth cavity, *C. concisus* has been found in the intestine of IBD patients and may be spread via saliva and food. Additionally discovered in the oral cavity, *C. showae* is strongly linked to an increased risk of IBD (Zhang 2015). Other *Campylobacter* species, including *C. gracilis, C. hominis, C. rectus, C. curvus, C. jejuni,* and *C. ureolyticus*, have also been noticed more frequently in IBD patients. However, the specific main specie of bacteria that cause IBD is not yet clear. *Campylobacter* species may be linked to the development of IBD, however additional research is required to prove this.

In conclusion, although evidence points to a potential connection between the development of IBD and certain *Campylobacter* bacteria, further research is required to validate this association. However, the discovery of human oral *Campylobacter* species in IBD patients' guts emphasizes the need for additional research into how these bacteria contribute to the progression of this disease.

4.5. PERIODONTITIS

Periodontitis is a common chronic inflammatory disorder brought on by the oral pathogenic bacterial species found in dental plaque. It weakens the connective tissue and other tissues that support the teeth and eventually results in tooth loss (Pihlstrom et al. 2005). *Campylobacter* species are one of the major bacteria in human periodontal spaces and have a significant influence on periodontitis development at various stages. Different *Campylobacter* species found in the oral cavity are intimately associated to various phases of periodontitis development in individuals with active damaging periodontal disease, *C. rectus*, for example, is a common *Campylobacter* species discovered in deeper subgingival pockets and plaque (Gmur and Guggenheim 1994). However, in patients with refractory periodontitis, *C. gracilis* and *C. concisus* are more common than health-associated bacteria. However, there is still a dearth of information regarding *C. concisus* involvement in oral infections.

While *C. rectus* was once believed to have a connection to periodontitis, the validity of many other *Campylobacter* species, including *C. gracilis*, C. curvus, and *C. concisus*, is still debatable (Henne et al. 2014). Therefore, further study is required to determine how these *Campylobacter* species affect tooth infections.

5. DIAGNOSTIC TECHNIQUES FOR CAMPYLOBACTERIOSIS

Due to the high prevalence of Campylobacteriosis, accurate and timely diagnosis is crucial for effective treatment and management of the disease. So here we will discuss the various diagnostic techniques that are used to identify and diagnose *Campylobacter* infection.

5.1. CULTURE-BASED METHODS

Culture-based methods are the gold standard for diagnosing *Campylobacter* infection. These methods involve isolating the bacteria from clinical samples such as stool, blood, or tissue, and then growing them in a laboratory setting (Özcan et al. 2022). The most commonly used culture media for *Campylobacter* isolation are selective media, such as *Campylobacter* blood-free selective agar (CCDA), and non-selective media, such as blood agar. The culture-based methods require specific laboratory conditions, such as microaerophilic conditions, and can take 3-4 days to obtain a positive result.

Table 1: Diseases caused by *Campylobacter* species and symptoms.

Diseases	Symptoms and risk factors	Zoonotic spp.	Reference
Abortion	One highly virulent clone of *C. jejuni*, known as SA, is primarily associated with sheep rather than humans.	C. fetus, *C. jejuni*,	(Sahin et al. 2012)
Gastroenteritis	*Campylobacter* spp. colonizes the intestinal epithelium. This colonization leads to a reduction in intestinal absorption capacity. *Campylobacter* spp. destroys cell structures and relocates across cells. The bacteria use several proteins to aid in their relocation. Biofilm formation is another mechanism used by *Campylobacter* spp. for relocation.	*C. coli* *C. jejuni*,	(Konkel et al. 2001)
Sepsis	Bacteria in blood. Low immunity level, Age, consumption of alcohol, History of gastric and intestinal surgery, and HIV infection .	*C. jejuni* C. fetus, *C. coli* C. lari,	(Nagy and Hla 2013)
IBD	Inflammation of GIT, pain, diarrhea. Presence of *Campylobacter* in the oral cavity may impact inflammatory bowel disease (IBD), although a direct correlation has not been established.	*C. jejuni* *C. hominis*,	(Mukhopadhya et al. 2011)
Periodontitis	The severity and stages of periodontitis are influenced by various *Campylobacter* species present in the oral cavity.	Spp. that cause periodontitis are not zoonotic	(Henne et al. 2014)

5.2. MOLECULAR METHODS

Molecular methods have gained popularity recently due to their high sensitivity and specificity. These methods involve detecting the genetic material of the bacteria, such as DNA or RNA, in clinical samples. The most commonly used molecular methods for *Campylobacter* detection are polymerase chain reaction

(PCR) and real-time PCR (RT-PCR) (Bodie 2022). These methods are faster and more sensitive than culture-based methods, with results available within hours. However, molecular methods require specialized laboratory equipment and expertise, which may not be available in all settings.

5.3. SEROLOGICAL METHODS

Serological methods involve detecting the presence of antibodies against *Campylobacter* in the blood of infected individuals (Borovikov et al. 2023). These methods are not used for diagnosing acute infections but can be useful for identifying past infections or for epidemiological studies. The most commonly used serological method is the enzyme-linked immunosorbent assay (ELISA), which detects antibodies against *Campylobacter* in blood samples (Borovikov et al. 2023). However, serological methods have limitations, such as low sensitivity and specificity, and cross-reactivity with other bacteria.

5.4. IMMUNOLOGICAL METHODS

Immunological methods involve detecting the presence of antigens, or proteins, produced by *Campylobacter* in clinical samples. These methods are less commonly used for *Campylobacter* detection, but have the advantage of being rapid and easy to perform. The lateral flow assay is the most commonly used immunological method, which detects *Campylobacter* antigens in stool samples. However, immunological methods also have limitations, such as low sensitivity and specificity, and may require culture-based or molecular methods confirmation.

5.5. NEXT-GENERATION SEQUENCING

According to researchers, next-generation sequencing (NGS) is a high-through technology that enables the quick sequencing of substantial volumes of DNA or RNA (Tong et al. 2021). Because NGS offers a thorough examination of the bacterial genome, it has the potential to completely change how *Campylobacter* infections are diagnosed. This can assist in identifying certain genes linked to virulence or antibiotic resistance, which can inform management and treatment plans. However, NGS needs specialized laboratory tools and training, which can restrict its applicability in environments with little resources.

In conclusion, prompt and precise identification of *Campylobacter* infection is essential for efficient management and treatment of the disease. There are benefits and drawbacks to each diagnostic approach covered in this chapter, including culture-based techniques, molecular methods, serological methods, immunological methods, and next-generation sequencing. The choice of diagnostic technique is based on several variables, including the availability of laboratory resources and the length of the test, etc.

6. TREATMENT, PREVENTION AND CONTROL OF CAMPYLOBACTERIOSIS

Effective treatment, prevention, and control methods are crucial to decrease the prevalence and effects of campylobacteriosis.

6.1. TREATMENT OF CAMPYLOBACTERIOSIS

The majority of campylobacteriosis cases are self-limiting and disappear in a few days. Antibiotics, however, may occasionally be recommended in order to shorten the illness length and lessen the intensity of its

symptoms. Antibiotics like erythromycin and azithromycin are frequently used to treat Campylobacteriosis (Dai et al. 2020). Hospitalization might be necessary in serious situations to deliver intravenous fluids and electrolytes to prevent dehydration. Inflammation can be decreased with the use of steroids. NSAIDs are administered in case of fever. In hospitals, patients receive symptomatic treatment.

6.2. PREVENTION AND CONTROL OF CAMPYLOBACTERIOSIS

By practicing appropriate cleanliness habits, campylobacteriosis can be avoided most successfully. This entails using separate cutting boards for raw and cooked meat, washing hands with soap and warm water before and after handling food, and cooking chicken to an internal temperature of 165°F (74°C). Additionally, it's crucial to avoid unpasteurized milk, untreated water, and raw or undercooked meat. Additionally, until they are completely well, those ill with diarrhea should refrain from cooking for others. Reduced animal-to-human transmission of the germs is the main goal of treatment for Campylobacteriosis. This can be accomplished by taking steps like enhancing biosecurity and hygiene on farms and in processing facilities and administering vaccines and antibiotics to animals. Additionally, a thorough food safety programme that monitors food production and processing, tests food products, and conducts public education campaigns can assist to lower the frequency of Campylobacteriosis. Probiotics can also help children's intestines build a healthy microbiota so that harmful microbes cannot thrive there (Dai et al. 2020).

6.3. VACCINATION

An efficient strategy to stop the spread of the disease is to utilize vaccines to prevent campylobacteriosis in animals (Jeon et al. 2022). The risk of contaminating the environment and food products can be decreased by vaccination since it reduces the amount of bacteria that animals shed in their faeces. The genetic variety of the bacterium has made it difficult to produce vaccinations that are effective against *Campylobacter*.
In healthy cows and heifers, VIBRIN is a vaccine that can be used to avoid campylobacteriosis (vibriosis) brought on by *Campylobacter fetus* (Rush and Edmondson 2021).

6.4. ANTIBIOTICS

Another strategy for preventing the spread of Campylobacteriosis in animals is the administration of antibiotics. Infected animals can be treated with antibiotics, which also stop the bacteria from spreading to other animals. However, the rise of antibiotic-resistant *Campylobacter* strains in animals has been linked to the use of antibiotics, which poses a serious concern to public health (Yang et al. 2019).

6.5. BIOSECURITY

Enhancing biosecurity protocols in hospitals, farms, and processing facilities is crucial for lowering the prevalence of Campylobacteriosis (Abd El-Hack et al. 2021). This entails actions including maintaining stringent worker hygiene practices, routinely cleaning and disinfecting tools and facilities, and minimizing the movement of animals between farms and patients.

7. ONE HEALTH APPROACH TO CAMPYLOBACTERIOSIS AND PUBLIC HEALTH SIGNIFICANCE

Due to *Campylobacter*'s widespread, high prevalence and the possibility that it could result in serious illnesses like gastroenteritis, sepsis, and abortion, it is a significant public health problem. In addition to

direct contact with infected animals and contaminated food or water, the disease can also spread from person to person. Millions of individuals are thought to be affected each year by Campylobacteriosis, which is thought to be the most frequent bacterial cause of foodborne sickness worldwide (Holland et al. 2023).

The One Health concept, which acknowledges the interdependence of human, animal, and environmental health, has been crucial in addressing the importance of Campylobacteriosis for public health. To prevent, identify, and respond to zoonotic infections, the technique entails collaboration and coordination among professionals in environmental health, animal health, and human health Campylobacteriosis has a considerable impact on morbidity and mortality in public health. 400–500 million incidents of bacterial gastroenteritis are thought to be caused by the illness each year, making it the primary cause worldwide. Even while most Campylobacteriosis episodes are self-limiting and go away on their own, more serious cases can result in hospitalization, sepsis, and even fatality. Long-term consequences of the illness can also include reactive arthritis, Guillain-Barré syndrome, and irritable bowel syndrome (Endtz 2020).

Campylobacteriosis has a large financial impact. Global estimates place the annual cost of the disease's medical care, lost productivity, and diminished quality of life in the billions of dollars' range. Additionally, the economic damage spreads to the agriculture industry because diseased animals produce less and it costs money to put preventive measures in place.

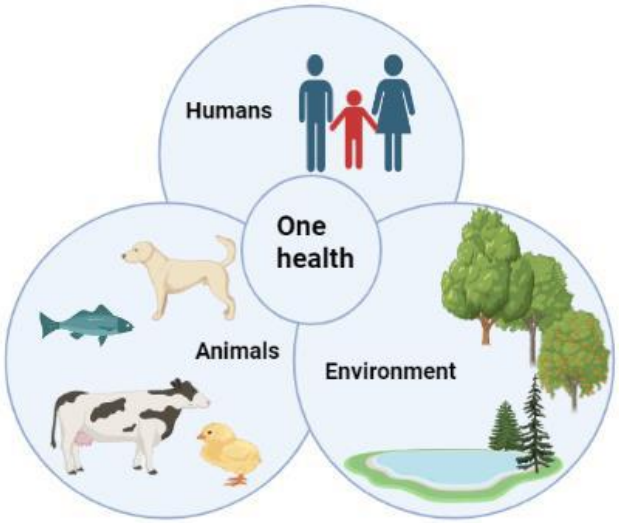

Fig. 4: One Health concept.

A One Health approach is required to address the importance of Campylobacteriosis for public health (Igwaran and Okoh 2019). This strategy acknowledges that Campylobacteriosis is a zoonotic illness that calls for a collaborative effort from environmental, animal, and human health experts. Effective preventative and control measures must be implemented throughout the whole food production chain, including on the farm, during transit, and at the processing level.

Vaccines, biosecurity precautions, and good hygiene practices are examples of prevention and control measures. The prevalence of the disease is decreased by immunizing animals against Campylobacteriosis. In healthy cows and heifers, vaccinations have been shown to be successful in avoiding the disease (Igwaran and Okoh 2019). Additionally, biosecurity measures, such as limiting access to farms and implementing sanitation protocols, can aid in preventing the disease's spread among animals.

To eradicate *Campylobacter* bacteria, the World Health Organization advises boiling poultry to an internal temperature of at least 165°F (Thames and Theradiyil Sukumaran 2020).

Campylobacteriosis must be prevented and controlled through early detection and surveillance. Modernized diagnostic procedures, such molecular techniques, can help quickly and accurately diagnose the illness, resulting in fast treatment and control measures. In addition, monitoring programs for both humans and animals can be used to spot illness outbreaks and keep track of their prevalence.

The One Health approach has successfully addressed the public health significance of Campylobacteriosis (Igwaran and Okoh 2019).Professionals in human health, animal health, and environmental health have been collaborating more recently, which has enhanced prevention and control methods. The disease still needs to be controlled and prevented, though, and much effort needs to be done. In conclusion, Campylobacteriosis is a serious public health concern because of its widespread occurrence and propensity to result in life-threatening illness. To combat Campylobacteriosis and secure the safety of both humans and animals, and lessen the strain on healthcare systems and the economy, it is crucial to use a One Health approach.

8. FUTURE DIRECTIONS IN RESEARCH AND CONTROL OF CAMPYLOBACTERIOSIS

The creation of efficient vaccinations is one of the future directions in the management of Campylobacteriosis. Although *Salmonella* and other bacterial diseases have been successfully treated with vaccines, creating one for *Campylobacter* has proven difficult (Frost et al. 2022). Outer membrane Surface proteins (OMPs), CmeC (a component of outer member), lipooligosaccharides, and flagellin are a few possible vaccine targets that researchers have found (Zeng et al. 2010). However, extensive preclinical and clinical investigations are required to fully evaluate the efficacy of these targets. Successful vaccine development would be a major step towards controlling Campylobacteriosis (Quintel et al. 2020).

The use of bacteriophages is a potential future strategy for combating Campylobacteriosis. Bacteriophages are viruses that target and destroy bacteria only. Both humans and animals have been used to successfully treat bacterial infections (D'Accolti et al. 2021). According to studies, bacteriophages can lower the amount of *Campylobacter* in chickens and other animals. Bacteriophage use in the food business may offer an antibiotic substitute and lessen the threat of antibiotic resistance.

In addition, a One Health strategy is required to lessen the impact of Campylobacteriosis. A concept called "One Health" acknowledges the connections between the health of people, animals, and the environment. As a result, managing the condition in animals can aid in lowering its prevalence in humans. Collaboration between the medical and veterinary professions and other fields like environmental health and food safety is necessary for the One Health concept.

Better monitoring mechanisms are also required to track the spread of Campylobacteriosis. The epidemiology and prevalence of the disease can be learned by surveillance systems, which can assist guide management measures. Advanced molecular typing techniques like whole genome sequencing can deliver more precise and in-depth data on the disease's transmission and spread.

Finally, there is a need for improved education and awareness programs to reduce the risk of infection (Bowler and Evans). Programmes for education can teach the general public and the food sector about

the dangers of Campylobacteriosis and how to stop the disease from spreading. These programmes can also offer guidance on handling, preparing, and storing food correctly to lower the risk of infection.
Finally, to lessen the disease's impact on public health and the food business, it is essential to create Campylobacteriosis control strategies and therapies. The creation of efficient vaccinations, the use of bacteriophages, a One Health strategy, better surveillance systems, and enhanced education and awareness campaigns are some potential directions in the control of Campylobacteriosis. If these tactics are successfully applied, Campylobacteriosis incidence might be greatly decreased, and both human and animal health and welfare could be enhanced.

9. CONCLUSION

As a result of *Campylobacter* species, Campylobacteriosis is a typical bacterial infection that can spread by contaminated water, food, or contact with sick animals. Fever, stomach- ache and diarrhea are just a few symptoms; this condition can produce. In some situations, it can also result in more serious problems. Antibiotics and supportive care are typically used during treatment after a diagnosis has been made through laboratory analysis of stool samples. Good food hygiene practices, risk factor education, one health approach, hand hygiene, and reporting to better understand and improve preventive measures are all part of preventing and controlling the illness. New Campylobacteriosis diagnostic methods and treatment options are being investigated in ongoing research. Overall, most cases of Campylobacteriosis can be effectively controlled with suitable preventative measures and quick treatment.

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Fungal Zoonosis and One Health

31

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ABSTRACT

Opportunistic fungi pose health risks, particularly for immunocompromised individuals. Agricultural azoles, widely used fungicides, may contribute to antifungal resistance in human populations. Climate change expands the geographical scope of fungal diseases, impacting both humans and animals. Animal-associated fungal infections, including zoonotic agents and environmental pathogens, present diverse challenges. Emerging zoonotic fungal diseases, with varying clinical manifestations, constitute a significant public health concern. The diminishing efficacy of antifungal medications necessitates innovative solutions to combat fungal infections. The intersection of agroecosystems and human health underscores the need for a comprehensive One Health approach. Collaborative efforts among medical professionals, veterinarians, policymakers, and other stakeholders are vital to address fungal infections and antifungal resistance, ensuring a holistic defense against the evolving challenges posed by these pathogens. The proactive implementation of One Health strategies at local, regional, national, and global levels is indispensable for effective prevention and control of fungal diseases.

Keywords: Opportunistic fungi, agricultural azoles, climate change, zoonotic fungal diseases, antifungal resistance, One Health approach.

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1. INTRODUCTION

The term "One Health" encompasses a strategic approach that embodies collaboration and synergistic efforts across the realms of animal health, environmental health, and human health, coupled with their associated domains of expertise. Idea of One Health was originally introduced in 2003 by conservationists, this concept sought to counteract the emergence of infectious diseases stemming from wildlife. Over time, the One Health concept has gained considerable traction, especially within the discourse on pandemic preparedness. Subsequently, the Food and Agriculture Organization (FAO), and the World Health Organization (WHO) have jointly embraced the One Health approach, recognizing its potential to galvanize concerted action. This initiative has further expanded the horizons of One Health, encompassing enzootic infections and the formidable challenge of antimicrobial resistance (AMR), thereby embracing the intricate interconnectedness of health concerns that traverse diverse domains (Abbas et al. 2022).

Central to the One Health strategy is the profound recognition of the interdependence of human, animal, and environmental well-being. This holistic approach to health underscores the undeniable fact that the state of one facet profoundly reverberates across the others. Importantly, it acknowledges the potential for diseases to transcend species barriers, thereby traversing seamlessly between animal and human populations. This holistic engagement of experts hailing from multifarious disciplines assumes an instrumental role in adeptly addressing the complex health challenges that confront us and charting a course toward comprehensive solutions (Gebreyes 2014; Häsler et al. 2020).

Fungi, occupying a pivotal ecological niche, function as primary decomposers within specific ecosystems and also establish symbiotic relationships with numerous organisms. These remarkable organisms contribute enzymes and medicinal compounds and serve as invaluable subjects for scientific experimentation. A monumental milestone was achieved in 1991 through a seminal research paper projecting the existence of approximately 1.5 million fungal species on our planet. Remarkably, this estimation was based on a mere 70,000 fungal species that had been formally documented until that juncture. This projection ignited a fervent quest to unearth hitherto undiscovered fungal species. Indeed, fungi traverse diverse ecosystems, permeating the natural environment. They execute a myriad of functions, many of which are intrinsic to the seamless functioning of ecosystems. However, it is important to acknowledge that certain fungal species can pose potential threats to human and animal health, potentially culminating in various infections and diseases.

Amid the complexity of fungal diversity, accurate differentiation between pathogenic and non-pathogenic fungal species emerges as a pivotal tenet. This distinction assumes paramount importance, enabling precise diagnosis and effective management of fungal infections. More recent estimations, grounded in advanced high-throughput sequencing methodologies, propose that the actual number of fungal species might surge to a staggering 5.1 million (Blackwell 2011). Such revelations underscore the imperative of robust interdisciplinary collaborations within the framework of One Health, as this approach holds the key to deciphering the intricate symbiosis between fungi, animals, humans, and the environment to mitigate risks, harness benefits, and propel a holistic paradigm of health and well-being.

2. FUNGI BASICS

Fungi stand as pivotal protagonists in the unfolding narrative of the emerging bioeconomy, effectively addressing the urgent global challenges at hand. They assume the role of indispensable agents, helping in resource productivity, generating renewable alternatives to fossil-derived products, transmuting waste streams into valuable constituents for sustenance and animal feed, combating lifestyle-related ailments, countering antibiotic resistance by reinforcing the gut biome, reinforcing crop resilience in the face of climatic variations, and serving as fertile hosts for the genesis of innovative biological drugs. The genesis

of these transformative applications of fungi is indelibly linked to the devoted efforts of mycologists spanning generations. The realm of mycology has engendered a profound comprehension of fungal biodiversity, evolution, genetics, physiology, ecology, pathogenesis, and nutritional dynamics – elemental knowledge base that forms the essential for the advancement of applied mycology. To unlock the power of fungi for the environment and people, we need a worldwide effort to boost the field of mycology.

The present juncture offers a fitting occasion to spotlight the monumental significance of fungi and the seminal role that mycology assumes in propelling sustainable global progress. Through heightened cognizance, we can harness the latent potential harbored within fungi and champion the ascendance of mycology. This undertaking will magnetize fresh talents into the discipline, gathering mycologists across the globe to secure imperative funding for fundamental research, and fortify the complex of the global mycology network. The progress of the bioeconomy, as propelled by the unparalleled attributes of fungi, rests on the basis of these pivotal steps. The fungal realm stands as an embodiment of inspiration, poised to render even more prodigious contributions (Lange 2014).

3. ZOONOTIC FUNGAL DISEASES: A NEXUS OF CONCERN

Zoonotic fungal diseases have emerged as a profound and intricate challenge to public health, traversing the intricate boundary between animals and humans. One exemplary illustration of this interplay is the occurrence of ringworm, which is caused by dermatophytes. This zoonotic fungal infection accentuates the remarkable capacity of these diseases to effortlessly transcend species barriers. The remarkably diverse transmission modalities encompass direct contact with infected animals, their body excretions, or environments contaminated with fungal spores (Chowdhary et al. 2017).

Similarly, the pathology of Penicilliosis exemplifies the intriguing connection between the human population and specific domestic companions like dogs and cats. In human, particularly those who are not immunosuppressed, this disorder shows a complex of symptoms, including generalized lymphadenopathy, febrile episodes, unintended weight loss, anemia, and an unproductive cough. Nonetheless, in the context of individuals infected with HIV, Penicilliosis assumes a far more aggressive character, extending its grasp to encompass a myriad of organs and tissues such as the skin, reticuloendothelial system, lungs, and intestines.

In a clear difference, the occurrence of penicilliosis (Penicilliosis is a fungal infection caused by the fungus *Penicillium marneffei*) in domestic animals like dogs and cats is rare. Among canines and felines, clinical manifestations are evident in various forms, ranging from dermatitis to rhinitis and *Otitis externa*. Closer examination reveals a spectrum of symptoms, including nasal discharge, external nasal ulcerations, and intriguingly, epistaxis. A curious observation lies in the fact that seemingly healthy canines can serve as asymptomatic carriers. Interestingly, up to 40% of dogs exhibit positive indications of *Talaromyces marneffei* in nasal swabs. There are also instances where dogs show disseminated infections, characterized by lymphadenopathy and bronchopneumonia (Seyedmousavi et al. 2015).

The complex connection between fungal diseases that can spread between animals and humans makes us think deeply. We need to explore how these diseases spread and affect different species, it's clear that we must fully understand them. This calls for teamwork among experts in medicine, veterinary science, and ecology to work together to solve these health challenges.

In these collaborative efforts, the search of knowledge shows importance, unreveal the effective strategies for prevention, intervention, and mitigation. Such efforts rest on a foundation of an accurate research that open up the complex mechanisms of zoonotic fungal diseases and their interactions with hosts. As we separate the underlying principles governing the emergence, transmission, and virulence of these fungal infections, we unearth insights that can guide the development of targeted interventions and therapeutics.

Moreover, fostering a shared understanding among medical professionals, veterinarians, ecologists, and researchers is pivotal. This symbiotic relationship facilitates the exchange of insights and experiences, enriching our collective knowledge reservoir. It paves the way for the identification of risk factors, the development of early detection methods, and the implementation of robust surveillance systems.

So, zoonotic fungal diseases highlight the complex interaction between animals and humans, transcending species boundaries with ease. The complex manifestations of these diseases in various hosts including humans, dogs, and cats have highlighted the need for comprehensive research and collaborative efforts across disciplines. The pursuit of knowledge, coupled with interdisciplinary cooperation, lays the foundation for effective management strategies. As we unravel the mysteries of these diseases, we illuminate pathways toward safeguarding both human and animal health.

4. FUNGAL PATHOGENS AND THEIR IMPLICATIONS FOR PUBLIC HEALTH

Fungi, often eclipsed by their more overt infectious counterparts, possess the latent potential to provoke a wide-range of diseases within human and animals. As our comprehension of these infections attains depth, the realization dawns that fungal pathogens, far from their seemingly superficial identity, wield the capacity to induce profound and intricate systemic illnesses. Moreover, the a lot of factors such as increased international travel, the increased use of immunosuppressive therapies, and the distresses in global climate has collectively created a breeding ground for the emergence and re-emergence of fungal diseases, magnifying their resonance within the domain of public health impact (Warnock 2006).

Fungal diseases affect human health in many ways, from simple skin infections to more severe and complex diseases that can harm the whole body. It is the vulnerable strata of our population, characterized by compromised immune systems as in case of HIV infection or passing through chemotherapy, who stands highly susceptibility to opportunistic fungis. A comprehensive grasp of the intricate epidemiology and weighty implications of these diseases assumes a pivotal role within the landscape of effective public health management (Brown et al., 2012; Benedict et al., 2016).

Foremost among these catalysts is the alarming increase in opportunistic infections, for example cryptococcosis and aspergillosis, which have high impact on immune-deficient hosts. This defenseless stratum includes people fighting with cancer, having organ transplants, people using immune-modulating drugs, and those having HIV/AIDS.

Among nosocomial infections, candidemia emerges as a major contributor to blood infections in the United States. The evolution of medical practice and advances in medicine are opening the gates to the invasion of new types of fungus, reinforced by challenging resistance to therapeutic intervention, within the field of medicine.

Concurrently, infections acquired within the community's shows a distinctive nomenclature such as coccidioidomycosis (also known as Valley Fever), blastomycosis, and histoplasmosis, trace their origins to fungi that have staked their ecological claim within specific geographic regions. These fungal organisms are characterized by their extraordinary sensitivity to fluctuations in temperature and humidity and have complex dynamics between climate and the course of disease spread. This delicate interaction lends an air of uncertainty when considering the potential effects of ongoing climate change on their distribution patterns and ecological behavior.

In summary, the debate about fungal diseases that intersects with public health resonates with an urgency that is both urgent and neglected. Fungal diseases may be overshadowed, but their influence is still undeniable. The convergence of their medical acumen, scientific research, and global awareness comes into focus as these diseases unravel the complex pathogenicity and pathways of disease control. Through these coordinated efforts, we strive to not only understand but also reduce the pressing threats posed by often-underestimated pathogens (Center for Disease Control and Prevention 2022). In

this way we can safeguard the well-being of human and animals, setting the stage for a more comprehensive and resilient paradigm of global health.

5. ANTIFUNGAL RESISTANCE AND MECHANISM

An increase in the fungal infections could be due to the emergence of pathogenic variants that exhibit resistance to conventional antifungal therapies. This challenge is compounded by restricted access to novel pharmacological interventions. The resistance phenomenon can be classified into intrinsic (primary) forms, genetically determined and associated with fungal species independently of drug exposure, and acquired (secondary) forms, which arise due to specific factors, often linked to antifungal medications or their analogs (Ben-Ami and Kontoyiannis 2021). It's noteworthy that resistance-conferring transposons or plasmids in fungi do not readily traverse between isolates. Nonetheless, the extensive array of antifungal agents deployed for two decades or more heightens the vulnerability to resistance evolution. Over the past decade, systemic antifungal agents have witnessed substantial usage, particularly in High-Income Countries (HIC) (Pathadka et al. 2022). Prolonged therapeutic regimens can lead to compromising adherence and escalating the potential for medication-related toxicity and resistance. Moreover, fungi's rapid adaptability to shifti environmental dynamics fosters the emergence of strains resistant to antifungal interventions, engendering a predictable escalation in minimal inhibitory concentration (MIC) values during treatment protocols.

Candida spp. stands as conspicuous examples of the most challenging pathogenic fungi on a global scale (Rabaan et al. 2023; Kaur and Nobile 2023). While the molecular substrates of Candida's resistance to antifungal treatment remain partly enigmatic, evidence interlinks mutations in ERG11 and TAC1B with fluconazole resistance, and mutations in the FKS gene with echinocandin resistance, characterized by upregulated multidrug efflux transporters and diminished glucan synthase sensitivity. Importantly, a significant proportion of *C. auris* isolates demonstrate fluconazole resistance, with approximately 30-50% showcasing resistance to amphotericin B, and a smaller fraction displaying resistance to echinocandins (Rybak et al. 2020; Izadi et al. 2022). Modern science has helped in understanding the high prevalence of Aspergillus strains resistant to azole antifungals, culminating in heightened morbidity, mortality, and resistance even against amphotericin B in specific Aspergillus lineages (Khojasteh et al. 2023). The ERG6 gene, governing the sterol-methyltransferase enzyme responsible for altering amphotericin B's molecular target, emerges as a point of implication. Although multiple studies have showcased in vitro resistance among Aspergillus spp. (Sen et al. 2022), a comprehensive comprehension of the correlation between amphotericin B's MIC values and clinical outcomes within distinct patient cohorts remains inchoate.

The intricate molecular underpinnings of resistance to triazoles predominantly encompass augmented expression of lanosterol 14α-demethylase, modifications in the binding locale, and intensified activity of transmembrane transport proteins facilitating drug efflux and curbing intracellular accumulation (Sen et al. 2022). This evolving landscape of antifungal resistance beckons for steadfast research, coordinated surveillance, and innovative therapeutic approaches. In the face of these mounting challenges, the preservation of effective antifungal armamentariums remains imperative for safeguarding human health. Fig. 1 shows antifungal drug resistance, its evolution, mechanism and impact.

Opportunistic pathogenic fungi are making their place in our surroundings, often disseminating an abundant spore payload into the atmosphere, subsequently exposing humans to these environmental fungal pathogens, manifesting as bioaerosols. Although these fungi commonly pose minimal peril to individuals in robust health, those grappling with compromised well-being or attenuated immunity are rendered susceptible to a spectrum of ailments . This spectrum encompasses superficial, allergic,

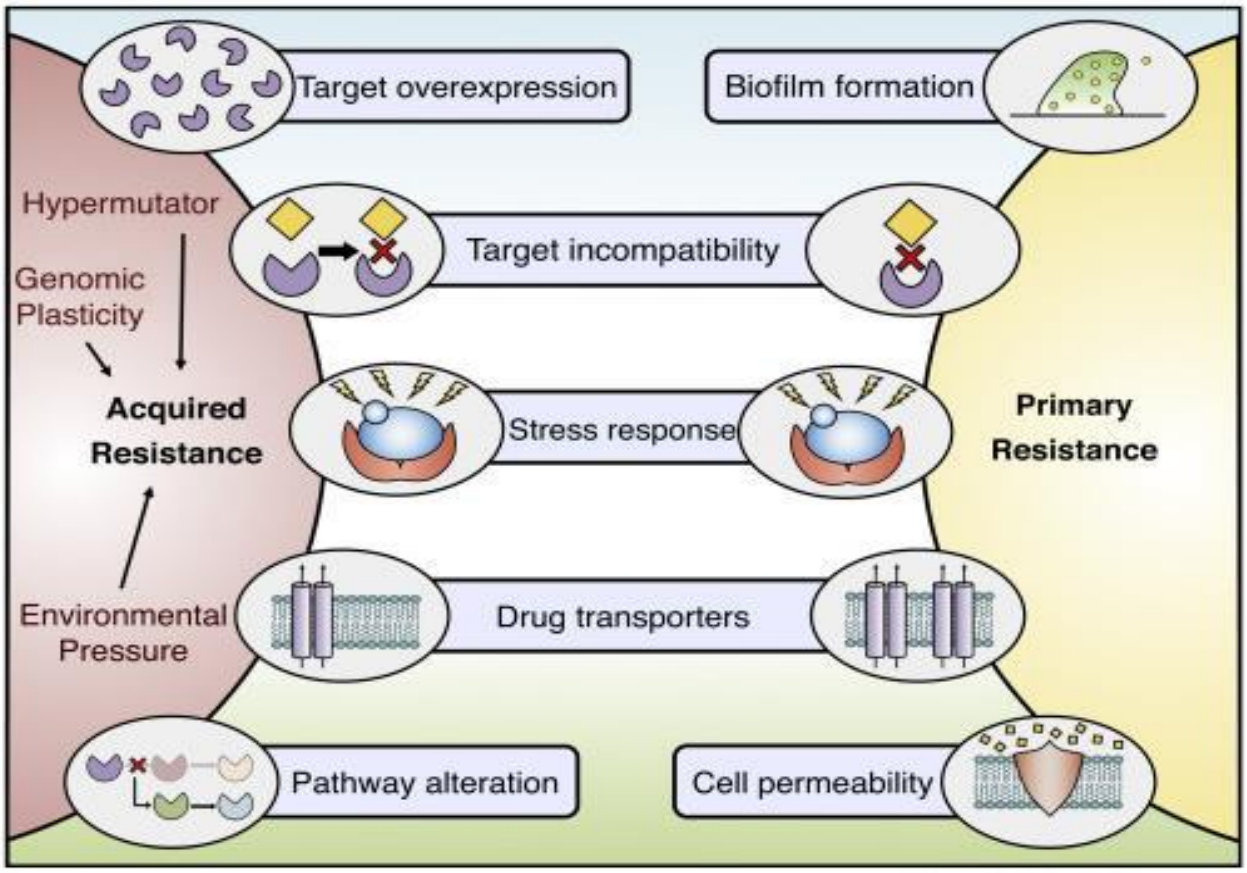

Fig. 1: Antifungal drug resistance: evolution, mechanism and impact

chronic, and in the gravest instances, potentially life-threatening invasive fungal diseases (IFDs). Notably, molecular epidemiological studies underscore the premise that the genesis of numerous fungal diseases is intricately interwoven with our environment (Fisher et al. 2022).

The links between environmental fungal populations and the subsequent human exposure to antifungal agents delineates a complexity where increasing environmental resistance may have effects on the clinical management of fungal infections. It highlights the constant need for innovation and adaptation to combat the ever-changing threats to crop health. Phytopathogenic fungi, which cause diseases in plants, can develop resistance to fungicides over time. This phenomenon is similar to the evolutionary arms race seen in other organisms, where the constant pressure to survive leads to the development of new strategies and defenses. The armamentarium of fungicides refers to the diverse range of fungicides available to farmers and growers to control fungal diseases. These fungicides work by targeting specific metabolic pathways or cellular processes in the fungi, inhibiting their growth and replication. However, over time, some fungi can develop mechanisms to overcome the effects of these fungicides, rendering them ineffective. This constant battle between fungicides and phytopathogenic fungi highlights the importance of integrated pest management strategies in agriculture. Instead of relying solely on fungicides, farmers need to adopt a holistic approach that includes crop rotation, genetic resistance, biological control agents, and cultural practices to reduce the reliance on fungicides and minimize the development of resistance. Furthermore, this evolutionary ballet serves as a reminder that the agricultural industry must continuously invest in research and development to stay ahead of the

evolving fungal pathogens. It is crucial to explore new modes of action for fungicides, develop innovative formulations, and enhance surveillance and monitoring systems to detect and respond to emerging resistance. By understanding and learning from the evolutionary ballet of phytopathogenic fungi, the agricultural industry can better protect its crops, ensure sustainable production, and mitigate the impact of fungicide resistance. It serves as a crucial lesson in the ongoing battle against plant diseases and the importance of staying one step ahead in the fight to protect our food supply. The constant evolution and adaptability of fungi in agriculture require agribusinesses to continually innovate and create modified versions of existing fungicides. They may also need to explore new chemical compositions to prevent the continuous development of resistance in fungi. This ongoing cycle of innovation is crucial to safeguarding crops from the relentless spread of resistance. (Steinberg et al. 2020).

A central point of concern revolves around the widespread application of broad-spectrum agricultural fungicides, particularly azoles, notable for their structural resonance with medical triazoles employed in the treatment of fungal infections. The global escalation in the utilization of agricultural azoles, coupled with their enduring presence in the environment, creates a milieu conducive to the potential incubation of resistance among opportunistic fungi. The emergence of azole-resistant fungal pathogens in human populations exhibits a strong correlation with the deployment of agricultural fungicides, casting the spotlight on potential eco-evolutionary linkages that seamlessly traverse the boundary between the environmental and clinical domains. This evokes valid concerns about the potential ramifications of agricultural practices in fostering the germination of antifungal resistance within the precincts of clinical settings (Fisher et al. 2022; Schoustra et al. 2018).

In the intricate orchestration of human health, the harmonious synchronization of efforts across diverse disciplines, spanning from the realm of medicine to the expanse of agriculture, assumes an imperative mantle in the endeavor to safeguard effective antifungal therapeutic options while concurrently preserving the delicate equilibrium of our ecosystems. The burgeoning realm of antifungal resistance serves as a poignant reminder of the intricate interplay between our actions and the broader milieu, underscoring the paramount importance of informed and collective stewardship of both our environment and our health.

6. THE INFLUENCE OF CLIMATE CHANGE ON FUNGAL DISEASES

The growing recognition of the environment's role in the emergence and resurgence of infectious diseases has been steadily advancing (Wu et al. 2016; El-Sayed and Kamel 2020). According to the United Nations Framework Convention on Climate Change, climate change encompasses alterations in the global atmosphere attributed to human activities, either directly or indirectly. These modifications surpass the inherent variability in climate observed over comparable periods (Farber and Carlarne 2017). This phenomenon has the potential to induce environmental pressures that engender the emergence of novel diseases caused by fungi (Garcia-Solache and Casadevall 2010). Despite the predominant focus on viral and bacterial diseases as potential founts of plagues and pandemics, fungi present equally substantial, if not greater, threats. It is worth noting that there are presently no available vaccines for fungal pathogens, the repository of antifungal agents remains exceedingly constricted, and fungi exhibit the ability to flourish as saprotrophs, generating copious infectious spores without necessitating host-to-host contact to initiate infection (Casadevall 2019). This unique capacity of fungi holds the potential to lead to the complete eradication of host populations (Fisher et al. 2012).

For most fungal species, their capacity to infect and establish themselves within mammals is restricted by their inability to thrive at elevated temperatures. Nevertheless, as climate change

precipitates incremental temperature elevations, fungi can adapt and develop thermotolerance, leading to an augmentation in the number of organisms capable of inducing disease (De Crecy et al. 2009; Casadevall 2020). Moreover, climate change has the propensity to broaden the geographical scope of pathogenic species or their vectors, consequently fostering the emergence of diseases in previously unaffected regions (De Crecy et al. 2009). Furthermore, the environmental perturbations brought about by climate change, encompassing events such as floods, storms, and hurricanes, can serve to disseminate and aerosolize fungi or introduce them through traumatic wounds, thereby potentially giving rise to infections by previously uncommon or unidentified fungal species.

7. ANIMAL-ASSOCIATED FUNGAL INFECTION AND THEIR ROLE IN ZOONOSIS

Various terms are used to describe infectious diseases associated with animals. Notably, expressions like "zoonosis" and "sapronosis" have been employed by diverse authors, occasionally causing overlap in their implications (Fisher 2018; Schaefer 2009). The World Health Organization (WHO) furnishes an official elucidation of zoonoses as infections that naturally transfer between vertebrate animals and humans. This characterization does not discriminate between the involvement of "true" pathogens or opportunistic agents (Schaefer 2009; De Hoog et al 2018; Hubálek and Rudolf 2010). Pathogens exhibit specialization in thriving within a mammalian host, effectively navigating their life cycle. In contrast, opportunistic agents inhabit specific environmental niches yet retain the capacity to endure within animal hosts. Instances where an opportunist, originating from inanimate sources like soil, decomposing plant matter, or excrement, triggers an infection and/or an epidemic, are termed sapronosis (De Hoog et al. 2018). None of these fungi solely depend on vertebrate hosts, yet their overall fitness is augmented when they incorporate a mammal into any phase of their life cycle. From time to time, sapronotic agents infect humans or other animals without displaying specific adaptations tailored to their host. Fungi that exhibit dual life cycles, encompassing phases both in the natural environment and within an animal host, are designated as environmental pathogens. These organisms lead a saprobic existence while also manifesting a distinct invasive phase uniquely suited to the warm-blooded vertebrate host once they establish themselves within (De Hoog et al. 2018; Gauthier 2015). Post-infection, environmental pathogens can be disseminated in the environment through defecation e.g., *Histoplasma capsulatum* or potentially released from the host's body upon death (e.g., *Coccidia immitis*) (Da Silva et al. 2021). Conversely, opportunistic fungi cannot transmit between hosts, and their survival is intricately linked with the lifespan of their host. In the event of the host's demise, these fungi also perish. Environmental pathogens, zoonotic agents, and agronomic agents exhibit marked differences in their life cycles, intended host populations, and clinical manifestations. It is imperative to establish a precise demarcation between these categories for a comprehensive understanding (De Hoog et al. 2018).

8. EMERGING ZOONOTIC FUNGAL DISEASES

Reports indicate that a substantial 75% of emerging infectious disease pathogens are of zoonotic origin, primarily originating from wildlife. Undoubtedly, zoonotic infections have become a significant challenge for millions of individuals in recent times. This is attributed to the resurgence or appearance of new pathogens, frequently leading to outbreaks in developing nations where public health infrastructure is insufficient. (Adebowale et al. 2018). Some emerging zoonotic fungal diseases are mentioned in Table 1 (Carpouron et al. 2022).

Table 1: Fungal diseases with their clinical manifestation and impact

Diseases	Clinical manifestation and Impact
Dermatophytoses	○ Increase in zoonotic infections due to new species emerging from animals
	○ Ongoing large-scale outbreak in India attributed to novel species
Sporotrichosis	○ Increasingly diagnosed in immunocompetent individuals
	○ S. brasiliensis more virulent and less sensitive to antifungals
Histoplasmosis	○ Varies from asymptomatic to fatal in immune-compromised individuals
	○ Wide host range and association with bats
Cryptococcosis	○ *Cryptococcus* (*C.*) *neoformans* and *C. gattii* cause different clinical manifestations
	○ The scarcity of antifungal treatments and antifungal resistance pose challenges
Emergomycosis	○ Potentially zoonotic, with rodents as potential vectors
	○ Molecular methods provide accurate identification
Talaromycosis	○ The rapid development of clinical manifestations and high mortality in non-HIV cases
	○ Little is known about natural environmental niches and transmission mechanisms

9. THE IMPERATIVE FOR COMBATING FUNGAL INFECTIONS

The diminishing availability of effective medications casts a profound shadow, not only on human health but also restricts the therapeutic arsenal accessible to patients. Established antifungal agents have been gradually integrated into agricultural practices over time (Azevedo et al. 2015). However, this integration introduces a complex conundrum: while these agents have proven valuable in controlling agricultural fungal pathogens, the propensity of numerous human pathogens to coexist within agroecosystems introduces the inherent risk of fostering drug resistance (Verweij et al. 2009; Zavrel and White 2015). The resultant emergence of resistance not only curtails treatment options significantly but also precipitates grave repercussions for patient outcomes. Thus, the imperatives of our time necessitate the innovative exploration and development of novel antifungal compounds, envisaging solutions that not only fortify human well-being but also safeguard agricultural productivity (Perlin et al. 2017).

The amplifying impacts of global warming usher in an environment uniquely conducive to the proliferation of fungal diseases. Fungi, previously constrained by physiological temperature boundaries, are poised to adapt to these shifting thermal landscapes, potentially acquiring the capability to induce infections (Garcia-Solache and Casadevall 2010). This apprehension gains further traction from the observations that certain fungal species exhibit a remarkable propensity to acclimate to altered thermal regimes through the mechanisms of natural selection (De Crecy et al. 2009).

As we navigate these intricate dynamics at the crossroads of human health, agriculture, and the environment, the imperative for rigorous research and multifaceted collaboration becomes all the more evident. The multifarious challenges posed by fungal diseases necessitate a holistic approach that bridges the realms of medical science, agriculture, and ecological understanding. Vigilance is of the essence, particularly in tracking and managing the emergence of antifungal resistance, both within clinical contexts and agrarian settings. Moreover, the pursuit of novel antifungal compounds demands interdisciplinary synergy, pooling the insights and expertise of chemists, biologists, pharmacologists, and agricultural specialists.

The diminishing efficacy of existing antifungal medications, coupled with the intersection of agroecosystems and human health, propels us into uncharted territory rife with challenges and opportunities. The growing specter of antifungal resistance, intertwined with the evolving landscape of global warming, necessitates proactive measures that span the boundaries of research, policy, and practice. By fostering a concerted effort to innovate, collaborate, and adapt, we can aspire to mitigate the impact of fungal diseases, ensuring the well-being of both humans and the ecosystems we inhabit.

The effective detection and containment of fungal epidemics hinge upon collaborative endeavors bridging diverse disciplines. The foundational concept of One Health, which inherently embraces synergistic collaboration and communication amongst seasoned professionals, veterinary experts, and custodians of food safety, has demonstrated commendable success. This model warrants universal embrace as a cornerstone approach to effectively combat communicable diseases. Within the realm of our scholarly inquiry, we have meticulously distilled intricate insights into the distribution and etiological underpinnings of fungal infections in the human populace, artfully captured within a tabular expose.

In sum, the exigency of addressing fungal infections reverberates through interconnected realms–from human health, where treatment modalities dwindle, to agriculture, where the specter of drug resistance looms. The intertwining effects of global warming, harboring potential shifts in fungal behavior, accentuate the significance of proactive measures. Collaborative synergies, epitomized by the One Health ethos, hold the potential to proactively detect, counteract, and mitigate the ramifications of fungal epidemics. This nuanced understanding paves the path for strategic interventions, arming us with knowledge to tackle the dynamic and evolving challenges posed by fungal infections.

10. ONE HEALTH APPROACHES TO FUNGAL DISEASE PREVENTION AND CONTROL

The global burden presented by fungal diseases constitutes a significant and intricate threat affecting the well-being of humans, animals, and the environment. This perilous scenario not only places human and livestock populations in jeopardy but also introduces vulnerabilities to the global food supply chain. Crucial therapeutic measures to combat fungal infections encompass antifungal drugs tailored for both human and animal use. Concurrently, fungicides play a pivotal role in safeguarding agricultural pursuits. Regrettably, the limited array of available antifungal agents has led to their concurrent application in both agricultural and medical spheres. This practice accelerates the emergence of resistance, thereby severely compromising our defenses against a spectrum of diseases.

Of heightened concern is the wide scale distribution of antifungal-resistant strains across the natural environment. These strains manifest resistance to the same categories of antifungal agents employed for treating infections in humans and animals, effectively obstructing effective therapeutic interventions in clinical settings. The intricate interplay between these domains underscores the imperative of adopting a comprehensive One Health approach to combat fungal diseases and effectively address the challenge of antifungal resistance. This approach ensures that the pursuit of treatment and protection for a specific group does not inadvertently compromise the well-being of other plant species, animals, or humans.

Within the purview of this comprehensive review, an exploration is undertaken into the origins of antifungal resistance. A thorough examination of the synergistic amalgamation of environmental and clinical resources is conducted, aimed at efficaciously managing disease. Furthermore, an in-depth investigation is conducted into the potential of leveraging drug synergy and repurposing strategies. This effort sheds illuminative insights into ongoing research endeavors focused on identifying fungal targets as a means to overcome resistance. Additionally, innovative technological methodologies are proposed for unearthing novel fungal targets, thereby contributing substantially to the collective endeavor aimed at addressing this urgent challenge.

To ensure effective prevention and control of fungal diseases, the implementation of One Health strategies at local, regional, national, and global tiers is indispensable. Collaborative endeavors among medical professionals, veterinarians, environmental scientists, policymakers, and other pertinent stakeholders are requisite to confront the intricate intricacies posed by fungal zoonotic diseases (Gebreyes 2014; Häsler et al. 2020).

11. CONCLUSION

The One Health paradigm has risen as a pivotal strategy to confront the intricate complexities that fungal diseases pose, impacting the well-being of humans, animals, and the environment. This encompassing framework acknowledges the intricate interplay among these components and underscores the criticality of collaborative endeavors and synergies that span diverse sectors and disciplines. Zoonotic fungal infections, with the capability to traverse between animals and humans, represent a significant concern in the realm of public health. Shifting climatic patterns, heightened global mobility, and the escalation of antimicrobial resistance have collectively contributed to the emergence and resurgence of fungal ailments. This underscores the urgency of adopting comprehensive and forward-looking strategies.

A profound comprehension of the epidemiology, burden, and ramifications of these diseases remains pivotal for the implementation of effective prevention and control measures. In the battle against fungal infections, a comprehensive approach necessitates the amalgamation of environmental and clinical resources, innovative strides in antifungal methodologies, and the exploration of avenues for synergizing drug effects and repurposing. The successful execution of One Health methodologies, fostering collaboration across a diverse spectrum of expertise, holds the key to safeguarding global health and mitigating the risks that fungal diseases pose to human populations and wildlife alike.

By embracing a One Health perspective, we fortify our readiness to address intricate health quandaries and achieve comprehensive solutions that guarantee the protection of all life forms and the surrounding ecosystem. As we navigate the complexities of fungal diseases, it becomes evident that a proactive and unified stance is paramount to preserving the integrity of our global health landscape. Through synergistic collaborations, innovative strategies, and a steadfast commitment to holistic well-being, we pave the way forward in managing and curtailing the impact of fungal diseases on humanity, animals, and the environment.

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Bovine Brucellosis in Pakistan: Epidemiological Investigations of a Zoonotic Disease

32

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ABSTRACT

Brucellosis is a major bacterial zoonotic disease with a global distribution. It is mainly a reproductive disease and infected animals are lifelong carriers within the herd. Humans become infected through close contact with livestock and consumption of milk products of infected animals. There is a lack of general awareness of this disease in Pakistan, with little information about the prevalence and disease epidemiology in smallholder settings. Further, dairy animals are critical to the livelihoods of smallholders, and the impact of the disease can seriously affect their economic security as well as their own health. The purpose of this chapter is to examine what is currently known about the epidemiology of brucellosis in Pakistan. After a review of current literature, it describes several studies undertaken as part of a PhD. The first study described is an assessment of diagnostic tools used to detect brucellosis in cattle and buffalos. This information is then used on the next study described, together with a Bayesian statistical method, to estimate prevalence of brucellosis in dairy animals in several districts in Punjab and Sindh provinces. To understand risk factors that may lead to brucellosis in livestock and humans, a 'knowledge attitude practices' (KAP) study is described, coupled with a participatory epidemiology study, which delved into the decision-making processes of male and female farmers in relation to practices which might affect brucellosis transmission. Finally, some implications of these findings are considered, and how an intervention program might be implemented in Pakistan.

Key words: intervention; KAP; prevalence; risk factor; smallholder

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1. INTRODUCTION

Brucellosis is one of the world's major zoonotic diseases. It is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and The World Organisation for Animal Health (WOAH, formerly the Office International des Epizooties, OIE) as one of the most widespread zoonoses in the world. Brucellosis in animals is predominantly a reproductive disease and causes abortion storms during the breeding season. These bacteria can spread within the herd via contaminated material, such as aborted foetus material and vaginal secretions and urine. *Brucella* pathogens are intercellular and persist within an individual animal, resulting in lifetime carrier status (Ficht 2003). The disease mainly affects sexually mature animals, and causes late-trimester abortions, weak calves, and infertility characterized by placentitis and epididymitis. Infected animals shed the pathogen in uterine discharge and milk (England et al. 2004). Brucellosis transmission typically occurs to other animals through oral contact with aborted foetal material (Bercovich 1998). Brucellosis can be considered to be a disease of animals; however, humans are accidental hosts. The disease in human's results from ingestion or inhalation of the pathogen or direct entrance via skin abrasions. It is also acquired through the consumption of raw milk and its products (Dasari et al. 2013).

Brucellosis is a neglected disease in Pakistan because of a lack of awareness of the disease and the absence of a control program, and its exact prevalence is unknown. Previous studies in Pakistan have focused on determining the prevalence of the disease on large commercial farms (Abubakkar et al. 2011). However, these estimates do not apply to the smallholder system in Pakistan, which comprises 95% of cattle in the country (Afzal 2009). Consequently, there is a need to address this important disease at the smallholder farmer level. It has also been stated that the data about brucellosis in Pakistan are sparse and inconsistent and therefore need to be investigated thoroughly (Munir et al. 2011). In developing countries, dairy animals are critical to the livelihoods of smallholder farmers and the rural poor, hence a great proportion of the Pakistani population may be affected in some way by the disease. Furthermore, in rural areas, the literacy rate is very low (UNESCO 2003) and most farming families have little knowledge about animal diseases. This, coupled with unhygienic practices (Asif et al. 2014), puts them at an even higher risk of contracting the disease if the pathogen is present in animals (WHO 2006). Housing and population density are key factors that have been linked to the progression of diseases and are likely to play a role in the Pakistani system.

This chapter explores various aspects of the epidemiology of bovine brucellosis in Pakistan. After reviewing some information published in the literature, it summarizes some of the key results published as a series of papers from a Ph.D. at Charles Sturt University, Australia, by the first author (Arif et al. 2017; Arif et al. 2018a; Arif et al. 2018b; Arif et al. 2019). In addition, the implications of these results are discussed, together with how the information may be used for an intervention program to control bovine brucellosis in Pakistan.

2. RISK FACTORS FOR BRUCELLOSIS IDENTIFIED IN THE LITERATURE

Brucellosis is an important zoonosis for both developed and developing countries. However, the disease is of more concern in developing countries where there are numerous socio-economic limitations, and factors that contribute towards disease spread, both in animals and humans. Risk factors for animal brucellosis are well documented in the literature, which includes animal age, species, breeding status, herd size, purchasing of new animals, abortion history as well as the herd management practices that contribute to disease transmission between animals. In particular, risk factors specific to developed- and developing-country contexts have also been identified (Hirsh et al. 2004; Lindahl et al. 2014). Recently, a few reports also confirmed these risk factors for bovine brucellosis in Pakistan (Ali et al. 2017).

Human brucellosis is an occupational disease, as people who have close interactions with animals are likely to have a higher risk of contracting the disease, including farmers, butchers, animal health service providers, slaughterhouse workers, and laboratory technicians (Al Shamahy and Wright 2001). Risk factors for human brucellosis are also established and have been described earlier (Lindahl et al. 2015). However, human brucellosis is strongly linked to the environment where people live and the routine practices they use to manage animals. The herd management practices, for example handling animal abortions, consumption of raw milk and its products, assisting animal parturition, and living in a shared place with animals, are known to increase the risk of human brucellosis (Lulu et al. 1988; Corbel 2006; Sofian et al. 2008). Engagement in risky practices for disease transmission varies in different countries and production systems according to the awareness level of the farming communities, animal production systems, and the culture of the region. The association of these practices with disease and with disease awareness among farmers has been investigated in other developing countries (Lindahl et al. 2015). However, risky practices and their association with brucellosis have not been studied in smallholder farming communities in Pakistan. In conclusion, there is a great need to investigate the disease epidemiology in the smallholder production system, to investigate farmers' awareness levels, their perceptions towards risk, disease burden in the study region as well as the best diagnostic approach for the local field conditions.

3. BACKGROUND AND OVERVIEW OF THE APPROACH USED IN THE PRESENT STUDY

Brucellosis is a neglected disease in Pakistan and remains an endemic challenge due to a lack of public awareness and consistent preventive measures. While limited studies have been carried out on large and commercial farms, smallholder dairies, which account for 90% of the dairy industry in Pakistan (Afzal 2009), are neglected. To address this important disease at the smallholder farmer level, the studies described here report on several critical components that are required to enhance understanding of the issue. The first of these is developing a method for the estimation of the prevalence of bovine brucellosis in smallholder farms and the second is the identification of household management practices and herd management practices on smallholder farms that may present a risk for the acquisition of brucellosis from cattle and/or buffalo in humans. Finally, information about the potential for uptake of biosecurity measures by these farmers and their families was obtained, along with the knowledge, perception, and communication networks of farming communities regarding zoonotic diseases. This work provides direction to develop a targeted intervention program that will contribute to the control of brucellosis at the smallholder level. A graphical summary of the approach used in this study is shown in Fig. 1.

4. DIAGNOSTIC TESTS FOR THE DETECTION OF BRUCELLOSIS

Three different diagnostic tests were used in this study, namely the Rose-Bengal test (RBT), competitive ELISA (C-ELISA), and indirect ELISA (I-ELISA). In the absence of a 'gold standard' to detect Brucella infection, a Bayesian latent class analysis (LCA) method (Hui and Walter 1980) was used to evaluate diagnostic test performance in terms of sensitivity (Se) and specificity (Sp), as well as prevalence estimates. However, the evaluation of diagnostic tests reported by Arif et al. (2018a) revealed some discrepancies in the published literature in terms of Se and Sp of RBT. RBT is considered to have a high Se (OIE 2009) and this assumption has been the basis of the use of this test as a screening test rather than a confirmatory test. For example, studies carried out in Zambia and Zimbabwe (Muma et al. 2007; Matope et al. 2011) reported a very high Se (84-99%) but, in contrast, two recent studies (Rahman et al. 2013; Ahasan et al. 2017) reported a very low Se (58-80%) of RBT when used in field conditions in Bangladesh. The research reported in the current study supports these findings with laboratory analyses from several samples testing positive based on the

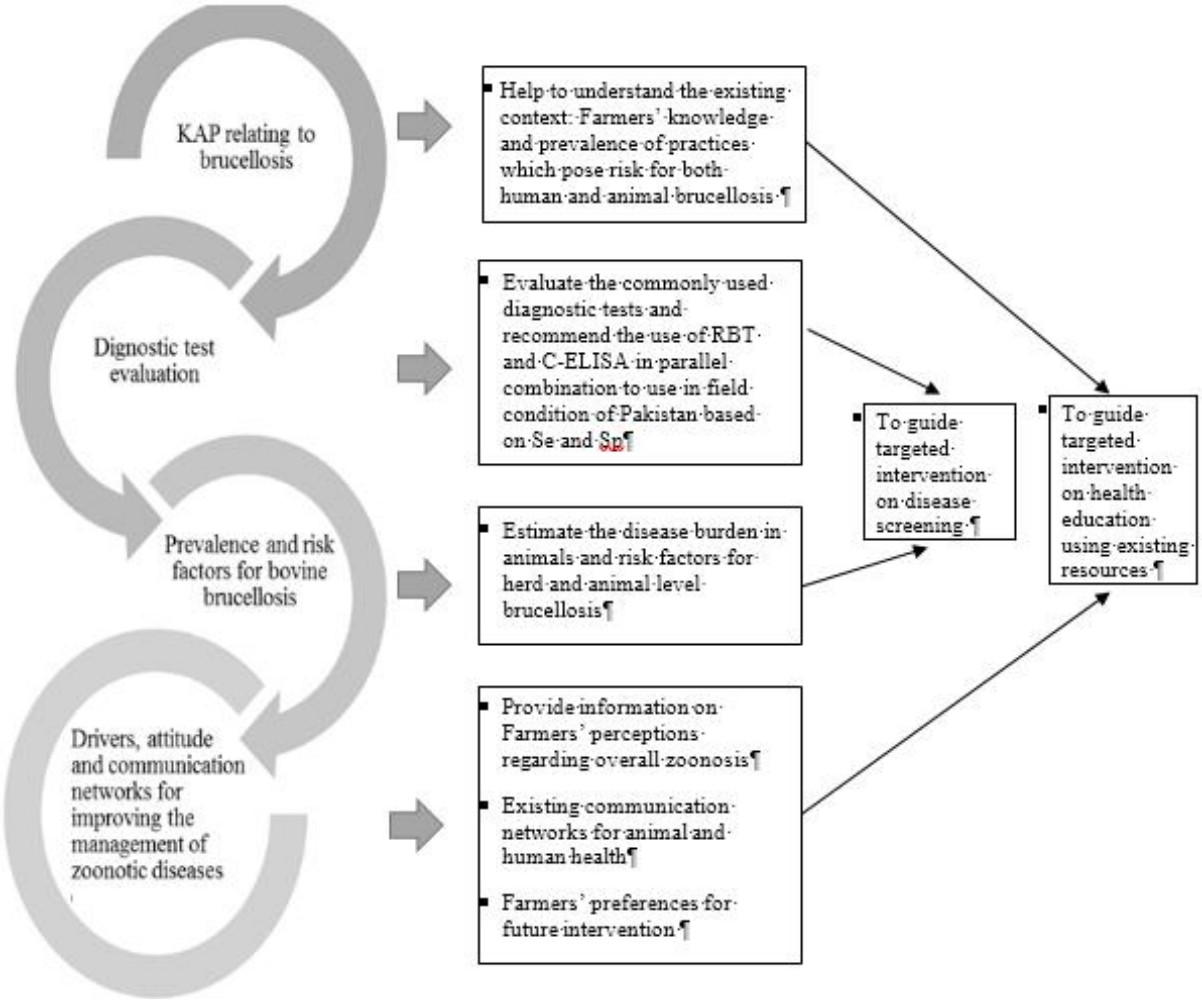

Fig. 1*:* Overarching aims of the project and the potential implications of the work. KAP is 'knowledge, attitudes and practices'

results of ELISA and negative on RBT. Therefore, this evidence raises concerns for the use of RBT as a screening test in field situations in Pakistan, and, to our knowledge, this is the first time that diagnostic tests for brucellosis have been comprehensively evaluated in Pakistani field conditions. The results of the current work indicate that C-ELISA has a higher Se compared to RBT and I-ELISA using LCA. The study also found that the diagnostic tests perform differently in cattle and buffalo and, in general, the Se of all three tests were higher in buffalo compared to cattle. In isolation, C-ELISA performed better than RBT and I-ELISA. A comprehensive sensitivity analysis was also performed using different prior information on Se and Sp from the literature and it was found that the use of minimally informative priors in the LCA produces unbiased results. This approach has the advantage that it allows the tests to be developed purely for the local context, rather than being influenced by test performance in other contexts.

In the smallholder setting, there is a greater cost of a false negative result, leading to undetected cases of brucellosis with health impacts for both animals and humans. Therefore, in this setting, we would prefer to increase the Se at the cost of Sp. Considering this scenario, we also evaluated the Se and Sp of applying the three tests in different serial and parallel combinations. Based on this analysis, RBT and C-ELISA in parallel combination produce the highest negative predictive value (NPV) and reasonable positive

predictive value (PPV). This combination is cost-effective as only two tests are required, and not the additional I-ELISA test, and it also provides a better option for herd screening according to the local context. Therefore, this research suggests that none of the three tests evaluated in the current study should be used as a single test in naturally infected animals in Pakistan, as they are not sensitive enough to screen the herd. In the smallholder context, two or more tests are required to screen the herd, with the optimal choice being RBT and C-ELISA in parallel combination.

5. PREVALENCE ESTIMATES OF BOVINE BRUCELLOSIS

The seroprevalence of bovine brucellosis was investigated in seven districts of Pakistan, namely Kasur, Okara, Pakpattan, Jhelum, Bhakkar in Punjab province, and Thatta and Badin in Sindh province, Pakistan. These were obtained using the RBT and C-ELISA in parallel as reported in Arif et al. (2019), in line with the recommendation on diagnostic test results in Arif et al. (2018a). The overall herd-level prevalence was 16.2% but this varied widely between districts (Table 1). The districts Jhelum and Pakpattan; Okara and Kasur; and Bhakkar, Thatta, and Badin, were found to have high (48%), medium (11%), and (effectively) no (<1%) disease prevalence respectively. This finding indicates that there is variability of Brucella in different geographical locations. It was found that the disease is present in the northern irrigated agro-ecological zone which is also an arid zone by agro-climatic classification. However, while the reasons behind the variation in prevalence are not known with certainty, it could be due to unfavorable climate conditions resulting in reduced survival and transmission of the organism, or it is also possible that the disease has not yet been introduced in those districts where it was not detected. However, to evaluate these possibilities, information on the movements of animals between districts is required as this may suggest geographic patterns of disease transmission. Although this is not possible in Pakistan currently, due to a lack of accurate record keeping that tracks animal movements between the districts, it is recommended that such capacity is prioritized in future development. Some reports from other countries identify an association between the disease and climate variables, for example, humidity, but in the current context/production system, further investigation is required, using finer-level climate data and larger numbers of sites, to explore this putative association.

Table 1: Herd-level prevalence estimates (%) of bovine brucellosis in seven districts of Pakistan. LPCI and UPCI are the lower and upper bounds of the 95% posterior credibility intervals. Values were obtained from Arif et al. (2018a).

Province	District	Prevalence	LPCI	UPCI
Punjab	Jhelum	45.1	31.7	58.8
	Kasur	4.8	1.0	12.8
	Okara	11.8	4.8	22.6
	Pakpattan	41.1	26.8	54.8
	Bhakkar	1.4	0.1	7.0
Sindh	Badin	1.6	0.1	7.9
	Thatta	1.1	0.0	6.0

6. KNOWLEDGE, ATTITUDES, PRACTICES (KAP) STUDY

The knowledge, attitudes, practices (KAP) study (Arif et al. 2017) assessed the extent of existing knowledge and understanding relating to brucellosis and investigated the occurrence of practices at the farm and household level that pose a risk for humans contracting brucellosis. The results of this study identified that, while smallholder dairy farmers had usually heard about animal brucellosis, there was little awareness regarding human brucellosis. In addition, almost all farmers reported that they performed at

least one practice at both the farm and household level which poses a risk of Brucella transmission to other animals and humans. The results also revealed that the level of formal education of farmers is associated with their knowledge and understanding of the disease. The smallholder farmers with no formal education were less likely to be aware of the disease or perform good hygienic practices at their homes, compared with farmers with at least a middle level of formal education. This study was also carried out in seven districts and it was found that, although there was some variation, the prevalence of risky practices at both herd management and household level were high in all districts. Importantly this high level of risky practices occurred regardless of the disease prevalence, which was found to vary significantly between districts (Arif et al. 2019). This indicates that the presence of risky practices is likely to contribute to the spread of the disease where it is present. However, in the districts where the disease is not present, these practices still present a risk because if the disease is introduced in these regions it will spread quickly due favorable conditions and practices both in animals and humans.

This KAP study also comprehensively analyzed 'risk practice scores' (Farm cleaning risk score, Brucellosis herd transmission risk score, Household risk score) which are the total number of practices undertaken by farmers in each of their respective categories. These risky practice scores indicated that the knowledge of disease is an important predictor of the behavior as an increase in knowledge was associated with lower risk scores. None of the districts were risk-free in terms of practices undertaken by the majority of farmers and their families. Given the varied presence of Brucella between districts we can assert that there is no evidence of an association between risky behavior and presence or absence of Brucella at a district level, which is to be expected with a geographically-varied pathogen distribution. Therefore, if the disease is present (which is true for four of the seven districts investigated), a reduction in risky practices scores will eventually lead to a reduction in the prevalence of brucellosis. In addition, in areas where the disease is currently not present, a reduction in risky practices scores would be expected to reduce the chance of the disease spreading if it was introduced. Notably, the risky practices perceptions were also guided by cultural and religious beliefs which indicates that support to improve farmers' knowledge would not necessarily lead to practice change (Kansiime et al. 2014). The results of this research are useful to identify that not only will customization of the educational aspects of an intervention program be required, according to the risk profile of each region, but that customization without taking into account the cultural and religious sensitivities will result in limited change.

7. RISK FACTORS FOR BRUCELLOSIS

Based on the results reported by Arif et al. (2019), it was also found that last-trimester abortion, history of retained placenta, and the number of buffalo at farms were herd-level risk factors for the on-farm presence of brucellosis. The association with last-trimester abortion is in agreement with the biology of Brucella (McDermott et al. 1987) and similar findings have been reported in other studies (Boukary et al. 2013; Lindahl et al. 2014). The results of this study also suggest that larger numbers of buffalo on farms may be a risk factor for bovine brucellosis. However, this may be because there are a greater number of buffalo in the districts, as noted by Arif et al. (2019), with high disease prevalence and this association may be because the number of buffalo may be acting as a confounder or even an intervening variable, and consequently further investigations are required to determine the likely causal pathway, to adequately assess associations in future. The sampling approach used in this study resulted in a similar number of animals being sampled in each district, so this possible explanation could not be assessed from the data at hand. However, obtaining data on livestock density across geographical districts may help to resolve this issue. In conclusion, this research identified herd and animal risk factors associated with disease prevalence. This information can be used to design a targeted disease control program for the

local field conditions of Pakistan, and the results can also be used to prioritize the districts for intervention, according to the disease status.

8. PARTICIPATORY EPIDEMIOLOGY: QUALITATIVE ON-FARM RESEARCH

To inform future interventions, the drivers, attitudes, and communication networks for improving the management of zoonotic diseases, with a focus on human brucellosis, among smallholder farmers in Pakistan was explored using a participatory epidemiology (PE) approach (Arif et al. 2018). The PE approach involves focus groups and individual in-depth interviews to understand how individuals and communities view health-related and other issues (Catley et al. 2012). Collectively, this work helps to understand brucellosis within the current smallholder settings and also provides direction to develop disease control programs for the smallholder production system which is the predominant system in Pakistan.

This study was carried out in the districts where the disease is present (Arif et al. 2019), i.e., Jhelum, Kasur, Okara and Pakpattan, and provides an insight into farmers' perception and knowledge. In particular, it shows that the farmers are not concerned about zoonotic disease, and this attitude is guided by either the economic cost or experience in terms of exposure to the disease or to any awareness program. Similarly, there was a marked difference or disconnect between farmers' perception of risky practices and the likelihood of performing these practices. Some of the practices are a part of the culture and traditional knowledge, for example, consumption of raw milk and its products, which makes practice change difficult, regardless of knowledge. These aspects need to be addressed by a culturally appropriate strategy when mechanisms for reduction are discussed and implemented. In addition, some risky practices are undertaken out of necessity as there are no viable alternative approaches, for example, animals are housed within homes as alternative space is not available for housing animals. These practices will be difficult to modify unless farmers can access support, and in some cases, additional resources (for example, land/space for housing). In addition, the analysis of communication networks in this study indicates that the farmers often use several unreliable or poorly informed sources either for information about or treatment of both animal and human health. There are several stakeholders that farmers should be sensitively counseled against using to seek information regarding disease prevention measures or treatment, for example senior farmers or religious leaders, unless it is known that they are well trained and knowledgeable in animal and human health aspects. The results revealed that farmers have more trust in senior farmers than veterinarians and that they would only contact a veterinarian or human health service providers in the case of an emergency. These findings indicate that there is a trust gap between farmers and health service providers (human and animal). Therefore, animal and human health providers should identify the trusted farmers within the village and work together to transfer important information about zoonotic diseases.

In conclusion, the results of this qualitative research, in conjunction with the KAP study (Arif et al. 2017), provide insight into farmer knowledge, attitudes, and practices that is imperative to guide a targeted educational intervention. We believe this intervention holds great importance in a smallholder context as testing and slaughtering of infected animals is not an economically and socially viable option in these settings. Typically, smallholders have between five to eight animals and their day-to-day livelihood depends on these animals. These communities are reported to have closer contact between animals and farming families (WHO 2006) than large and commercial farmers. Therefore, these smallholder farmers warrant a higher priority to receive health education regarding preventive measures for zoonotic disease, given that important zoonoses such as human brucellosis can be controlled very effectively by adopting risk-free practices with careful planning and implementation.

9. LIMITATIONS AND CONTEXTUALIZATION OF THE RESEARCH WORK

The findings of this study should be interpreted considering the context and the production system of Pakistan. Although we made every effort to reduce the biases, some limitations of the work are listed below.

Estimation of disease prevalence (Arif et al. 2017) was carried out on smallholder farms in seven districts of Pakistan, and so it follows that this will not be a perfect representation of the whole country. However, this is the first study carried out on smallholder farms in Pakistan with such a large number of samples and covering different agroecological zones. The findings indicate there is substantial variation in disease prevalence in the sampled districts which is very important information for designing a disease control program. This variation suggests there is no “one size fits all” or one intervention program that can be effective for the whole smallholder system across the country in terms of limiting the disease. However, despite this local variation, the results of disease burden might have more importance at the regional level, i.e. across the subcontinent, as these issues are equally applicable to other countries with smallholder production systems. Indeed, smallholder farms dominate the farming systems of most developing countries, many of which also have endemic brucellosis. Without having the disease burden information in smallholder settings, we cannot estimate the risk for human brucellosis.

Another potential limitation of this research could be the sampling approach for herd and animal-level prevalence estimation. It is understood that different sampling approaches are required for the selection of animals in small herds compared to large ones. For this study, we sampled a maximum of three animals per herd, but the herd size should not be overlooked when interpreting the results. The herds studied here are small: fewer than 10 animals in each and often only two or three. Indeed, just less than half of the farms sampled had herds comprised of three or fewer animals. So, overall, a sizeable proportion of animals were sampled from the farms, and it is considered that the sampling approach used here provides a good representation of mixed cattle and buffalo farms.

In this study, participants were selected from villages that were included as part of an Australian-funded project (ASLP Dairy Project) (Warriach et al. 2019). This could be another limiting factor as selected participants were smallholder farmers who were already directly or indirectly working with the project and had exposure to an extension program addressing the whole dairy farm system. Prior agreement to participate in this extension program may indicate that these farmers are somewhat more progressive, especially because the program involved engaging both men and women from farming families. In the same village, there is another group of farmers who have a traditional mindset and are less willing to participate in any developmental program. In some situations, such farmers also do not allow female farmers to participate in any program. Therefore, the results of this chapter should also be interpreted considering these factors. Nonetheless, we found a lack of knowledge and understanding regarding the disease and risky practices even in the group of smallholder farmers who have had exposure to some kind of extension program. Therefore, it is anticipated that the traditional group of smallholder farmers would have even less knowledge and understanding, and perhaps greater levels of risky practices. Further, this anticipated difference between the two groups of smallholder farmers within the same village might affect the way a future educational training program is implemented. We believe this is an important issue and it will be necessary to find a way to involve all farmers if a control program starts. This is beyond the scope of the current project but this gap needs to be considered for future work. Unless an intervention program can be implemented across the entire farming community in a village, this would leave a big proportion of the population at risk and the disease will persist, particularly in villages that practice common grazing because of the greater risk of the disease spreading in this scenario.

Future work also needs to consider the concept of what should be considered a herd, as this research shows that there are several farmers who send their animals for common grazing with other animals of the village. This would suggest that in those villages, the whole village may be considered a herd as animals are mixing. Further studies to understand the nature of common grazing and disease transmission across village livestock may be valuable to identify the likely effect of this mixing pattern on disease transmission. This may indicate if villages with common grazing need a different intervention program.

10. OUTLINE OF ISSUES FOR AN INTERVENTION PROGRAM

This research shows the need for a targeted intervention program in Pakistan, both to guide an educational program and for bovine brucellosis screening and control programs. The intervention program must provide a longer-term solution rather than a 'quick fix' to the problem. No single solution can solve this problem, and a holistic approach is required which includes realistic and achievable objectives. It must be very broadly based and take into consideration societal and community issues, educational aspects, as well as the epidemiology of the zoonotic disease. Below is a brief outline of what needs to be considered, based on the results of this research, if we would proceed to start an intervention program.

For an effective intervention program, categorization of the districts into low or high prevalence regions is imperative, to implement effective targeted intervention. This essentially represents a 'risk-based intervention' program. Categorization of districts can be done by carrying out a small cross-sectional study in each region to estimate seroprevalence using the approach and tests described by Arif et al. (2017), Arif et al. (2019). For example, from the current study, a district with high disease prevalence (Jhelum) and one with low prevalence (Bhakkar) would be selected in the initial phase of an intervention program. If the prevalence in an intervention area is high, then we may adopt an intense educational intervention via farmer discussion groups using trusted individuals. However, for areas where the disease is absent or has very low prevalence, then we could adopt a less intensive mass communication program to convey health information to the farming communities.

The development of training material on disease preventive measures with support from social scientists who have extensive knowledge of the culture and religion within each region would be beneficial. Ideally, this training material would include fact sheets and short videos in the local language on different topics. Highlighting certain disease scenarios or short case studies of the risky practice both at the farm and household level would allow farmers to personally identify with these issues and allow interpretation by educated and non-educated members of the community. Educational awareness should also be provided through trusted sources of animal and human health providers as highlighted in the PE study (Arif et al. 2018). Educational sessions should be carried out in the form of focus groups both for men and women, conducted separately because of cultural sensitivities and different risk perceptions. Furthermore, this educational intervention should be carried out in districts with both high and low disease prevalence (for prevention of the spread of disease in the future) but if fewer resources are available then priority will be given to districts with high disease prevalence.

In addition, if there are sufficient resources, this would allow an intervention team to also quality vaccinate the animals in high prevalence areas. However, this would require prior training of veterinary assistants about vaccination handling since the Brucella vaccine is live and itself carries a risk for disease transmission if it is not handled properly. Both disease screening and vaccination can significantly reduce the prevalence of the disease in animals which will also result in a reduction in the risk of human brucellosis and increase animal production. While vaccination is often used as an important step in disease control, within a smallholder context such as that studied here, likely, this will not represent a

valid option. The cost of vaccination, given the lack of local production, along with the difficulty in handling and the lack of appropriately trained administrators, are all rate-limiting issues at this stage. As such, awareness of disease and behavior change are more appropriate interventions in this environment in the short term.

To enhance the effectiveness of an intervention program, a phased introduction is recommended. For this, at least one village should be selected as an intervention village, and a corresponding number of villages without intervention. An impact assessment of the program can be performed by carrying out a small KAP study, perhaps supported by focus group interviews. This will be helpful to assess any improvement in the practices and also to make any modifications if required. This model can be replicated or rolled out to other villages or districts, but again its impacts need to be assessed.

To run a control program there will be an absolute need to involve government livestock and human health departments 'on the ground' and other stakeholders. In Pakistan, zoonotic diseases are currently not addressed by either of these government agencies, however, a synergistic One Health approach is required by both departments to guarantee both dimensions are covered in an integrated and cooperative way. The control program would equip the field staff of both departments to disseminate the program at a 'grassroots' level.

Before implementing a control program there is also a need to conduct a cost-benefit analysis. For example, a cost-benefit analysis was recently conducted in India to assess the viability of a brucellosis intervention program for cattle and buffalo (Singh et al. 2018). Such a program can be more cost-effective if it is rolled out with other intervention programs, for example, a tuberculosis control program. Also, a risk assessment study could be performed to assess if there is any risk involved in implementing such an intervention program. Critically, this evaluation (costs, benefits, risks) must be broadly based, with input from the government stakeholders, but also involve local rural communities, as without their commitment, it is difficult to consider that a program could be successful.

11. CONCLUSION

The findings outlined in this chapter can be explained in the form of a complex network involving the interactions between farmers, livestock, and Brucella organisms, together with the environments they all operate in. Addressing this complex network has required the use of a range of epidemiological tools, involving both quantitative and qualitative approaches, to evaluate the disease burden over seven districts of Pakistan and to identify the best combination of diagnostic procedures to be used in field conditions in this country (RBT and C-ELISA in parallel combination). Using these methods, it was found that Brucella infection is present in cattle and buffalo in four out of the seven districts studied, some with high disease prevalence, and this can constitute a substantial public health risk for rural smallholder communities as well as resulting in production losses for this system. In addition, a range of practices were identified that pose a risk of brucellosis, not only to livestock but also to humans, given the intimate contact between livestock and their owners in rural communities. Further insights about brucellosis and other zoonotic diseases in terms of farmers' understanding, risk perception, and sources of information were obtained from a series of in-depth farmer interviews and textual analyses, in particular exploring gender differences. These findings work together to increase our understanding of brucellosis in the smallholder systems of Pakistan, particularly through a 'One Health' perspective. This information provides the foundations on which to build an intervention program to reduce the impact of this disease on animals and humans. In conclusion, the findings and recommendations presented in this chapter can help to guide future intervention programs that will result in marked added value to the smallholder communities in Pakistan.

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Brucellosis: A Global Challenge

33

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ABSTRACT

Brucellosis (Malta fever, Mediterranean fever, or undulant fever) is a zoonotic infectious disease caused by bacteria of the genus Brucella. This disease affects both humans and animals, posing significant public health and economic concerns worldwide. Brucellosis remains a prevalent global issue, particularly in regions with inadequate veterinary control and surveillance systems. The transmission of brucellosis occurs primarily through direct contact with infected animals or consumption of contaminated products such as unpasteurized milk, cheese, and meat. The disease can spread through inhalation of infected aerosols or contaminated environmental sources. Human-to-human transmission is rare but possible, mainly through sexual intercourse, vertical transmission from mother to child, or laboratory. Clinically brucellosis in humans varies widely showing a flu-like illness, with symptoms including fever, chills, sweats, fatigue, myalgia, and joint pain. In some cases, brucellosis can become chronic and lead to more severe complications, such as arthritis, endocarditis, neurologic disorders, and reproductive. The management of brucellosis involves a multidisciplinary approach, i.e. accurate diagnosis, appropriate treatment, and comprehensive surveillance and control measures. Antibiotics are the mainstay of therapy, typically administered for several weeks or months, depending on the clinical presentation and severity of the disease. Preventive measures include the implementation of vaccination programs for livestock, strict hygiene practices in animal husbandry, pasteurization of dairy products, and public education regarding the risks associated with consuming unpasteurized animal products. Control of brucellosis requires collaboration between veterinary and human health sectors, as well as active participation from governments, international organizations, and communities. Improved diagnostic methods, surveillance systems, and public awareness are crucial to reducing the burden of brucellosis and preventing its spread.

Keywords: Brucellosis, Malta fever, global issue, public health, zoonosis.

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1. INTRODUCTION

Brucellosis (Malta fever, Mediterranean fever, or undulant fever) is a widespread zoonotic disease caused by the bacterial genus Brucella (B.) (Khurana et al. 2021). This infectious disease affects animal species, including livestock such as cattle, goats, pigs, and wildlife populations (Yagupsky et al. 2019). Brucellosis poses significant threats to both animal and human health, leading to substantial economic losses, public health concerns, and challenges in international trade (Bagheri Nejad et al. 2020). Brucella species, including *Brucella abortus*, *Brucella melitensis*, and *Brucella suis*, are responsible for the diverse outcomes of brucellosis in various host species (O'callaghan 2020). For instance, *B. abortus* primarily affects cattle, leading to reproductive disorders such as abortion and decreased fertility (Wang and Jiang 2020). *B. melitensis* is commonly associated with goats and sheep, causing significant economic losses in the form of reduced milk production and abortions (Alim et al. 2020). *B. suis*, affects pigs and can infect humans, leading to chronic debilitating symptoms (Di Bonaventura et al. 2021). *B. canis* mainly affects dogs, and rarely transmits to humans (Bosilkovski et al. 2021). Some least common species that can lead to brucellosis *B. neotomae* (infects desert wood rats) and *B. ovis* (affects sheep) (Zhou et al. 2020).

The significance of brucellosis extends beyond its impact on animal health (Bendrey et al. 2020). Economically, brucellosis can result in substantial losses in livestock production due to reduced productivity, culling of infected animals, and trade restrictions imposed by importing countries (Unuvar et al. 2019). Moreover, the disease's zoonotic potential is of great concern. Humans can contract acquire brucellosis through direct contact with infected animals, consumption of contaminated animal products, or occupational exposure, leading to a wide range of clinical symptoms (Lianou et al. 2022). The zoonotic transmission of brucellosis emphasizes the need for effective control measures to safeguard public health (Sibhat et al. 2022). An accurate diagnosis of brucellosis is crucial for effective disease management (Khan et al. 2021) Various laboratory methods, including serological tests, culture, and polymerase chain reaction (PCR) assays, are employed to detect Brucella species (Bakheet and Alnakhli 2019). However, diagnostic challenges persist due to the bacteria's slow growth rate, low bacteremia levels, and antigenic variability (Deka et al. 2020). The development of reliable and rapid diagnostic tools is essential to facilitate early detection and appropriate treatment, both in animals and humans (Barreto-Argilagos and Rodríguez-Torrens 2022). To combat the spread of brucellosis, comprehensive prevention and control strategies are necessary (Al-Sherida et al. 2020). Vaccination programs have proven effective in reducing the incidence of brucellosis in animal populations (Ghanbari et al. 2020). Vaccines, such as the *B. abortus* strain 19 vaccine for cattle and the *B. melitensis* Rev. 1 vaccine for small ruminants, have played a vital role in disease control (Berhanu and Pal 2020). Furthermore, a one health approach, which integrates human and animal health sectors, is crucial for addressing brucellosis comprehensively. Public health measures, such as education campaigns promoting proper hygiene practices and the promotion of safe food handling, are essential in reducing human exposure to Brucella species (Tialla 2022). By adopting a multidisciplinary and collaborative approach, we can effectively combat this disease and mitigate its impact on both animal and human populations (Zhang et al. 2019).

2. EPIDEMIOLOGICAL SURVEILLANCE, GLOBAL DISTRIBUTION AND PREVALENCE OF HUMAN BRUCELLOSIS

Conducting surveillance studies to monitor the prevalence, geographical distribution, and risk factors of brucellosis provides valuable data for targeted control interventions (Iqbal et al. 2020.) (Aragón-Aranda et al. 2020). Human brucellosis cases are distributed worldwide, with varying prevalence rates across regions of Asia and Africa (Siengsanan-Lamont et al. 2021). The disease is endemic in countries such as India and Pakistan, particularly in regions where livestock farming and close interactions with animals are common (Esmaeili et al. 2019). It affects both developed and developing countries but its occurrence is more pronounced in low- and middle-income countries, where livestock farming is less regulated (Sun et al. 2020). In Africa, human brucellosis is a significant public health concern, particularly in sub-Saharan countries. The disease is prevalent in regions with extensive livestock farming, such as Ethiopia, Nigeria, and Sudan (Recht et al. 2020). Lack of awareness, limited access to healthcare facilities, and poor veterinary control programs contribute to the high burden of brucellosis in these areas (Madut et al. 2019).
Moreover, nomadic lifestyles and the consumption of raw animal products perpetuate the spread of the disease (Dadar et al. 2022). Asia experiences a significant burden of human brucellosis. High disease prevalence in countries like India, China, and Pakistan is primarily due to large-scale livestock farming and consumption of unpasteurized dairy products (Hussain et al. 2021). Occupational exposure among farmers, veterinarians, and abattoir workers also contributes to the transmission of disease (Jadav and Raval 2019). Additionally, weak surveillance systems and inadequate diagnostic facilities hinder effective disease control and prevention strategies in many Asian countries (Ntivuguruzwa et al. 2021). Human brucellosis remains a concern in America Mexico, Peru, and Argentina reporting numbers of cases. In these regions, transmission occurs through the consumption of unpasteurized dairy products and contact with infected animals, such as goats, cattle, and pigs (Dadar et al. 2020). Inadequate veterinary control measures, limited access to healthcare, and challenges in diagnosis contribute to the persistence of brucellosis in these regions (Adel 2022). In Europe, human brucellosis is less common. However, some countries still face a considerable burden of the disease, including Greece, Italy, and Spain (Kefaloudi et al. 2022). Transmission occurs through the consumption of contaminated dairy products and contact with infected livestock. Control measures, such as vaccination campaigns and enhanced surveillance, have led to a decline in reported cases in recent years (Jamil et al. 2020).

3. BRUCELLOSIS-ASSOCIATED SOCIO-ECONOMIC BURDEN

The impact of brucellosis is not limited to the direct losses incurred by infected animals (production and reproduction losses) but also includes indirect costs associated with control measures, reduced productivity, trade restrictions, and human health implications (Mengele et al. 2023). Brucellosis requires prompt diagnosis and treatment, which adds to the economic burden on livestock producers (Machelart et al. 2020). Diagnostic tests, medication, and veterinary services contribute to the cost of managing infected animals. Additionally, implementing preventive measures and control strategies, such as vaccination campaigns and culling of infected animals, further increases the expenses for farmers and livestock producers (Baroncelli et al. 2022). The disease has significant public health implications, including debilitating symptoms such as fever, fatigue, joint pain, and prolonged illness (Uzunović et al. 2020). In some cases, it can lead to serious complications, affecting various organs, such as the heart, liver, and spleen. Infected individuals experience a loss of productivity due to the prolonged illness, leading to decreased work efficiency and absenteeism. The diagnosis, treatment, and follow-up care place a significant financial burden on individuals, families, and healthcare systems (Hussain et al. 2020). Brucellosis has a significant impact on international trade and food safety. The presence of brucellosis in animal population has great impact for international trade (Erkyihun et al. 2022). Countries such as

Australia, America and New Zealand, have strict regulations regarding the import and export of animals and animal products to prevent the spread of infectious diseases (About et al. 2023). The presence of brucellosis can result in trade restrictions on livestock and animal products (Troupin et al. 2022). Countries affected by brucellosis showed great economic loss, reduced market access, and decreased competitiveness in the international trade of livestock and animal-derived products (Bodenham et al. 2020). Food safety has great impact in brucellosis control. Consumption of unpasteurized dairy products, such as milk and cheese, derived from infected animals can lead to human brucellosis. Therefore, ensuring safe food production and implementing effective control measures, such as pasteurization and strict hygiene practices, is essential (Mol et al. 2020).

Meeting international standards for food safety is vital to prevent the transmission of brucellosis through contaminated animal products and maintain consumer confidence in global trade (Pinn-Woodcock et al. 2023). Brucellosis exerts a negative impact on wildlife populations, particularly in areas where domestic animals and wildlife come into close contact (Kucuk et al. 2021). Wildlife reservoirs, such as elk, bison, and feral swine, can perpetuate the infection cycle, leading the transmission to livestock and vice versa. The disease has detrimental effects on the population dynamics of wildlife species by reduced fertility and increased mortality (Grützke et al. 2021).

4. BRUCELLOSIS AND PUBLIC HEALTH

Brucellosis poses significant diagnostic challenges due to its nonspecific symptoms, leading to delayed diagnoses. This results in prolonged illness, increased morbidity, and the potential for secondary transmission (Zhang et al. 2021). Brucellosis requires prolonged antibiotic treatment, which can be expensive with side effects (Mortola et al. 2019). Inadequate access to healthcare facilities and medications exacerbates the burden on affected individuals (Jamil et al. 2021). Brucellosis can impact occupational health, particularly among farmers, veterinarians, and abattoir workers, who are at higher risk of exposure to infected animals (Lozano-López et al. 2022).

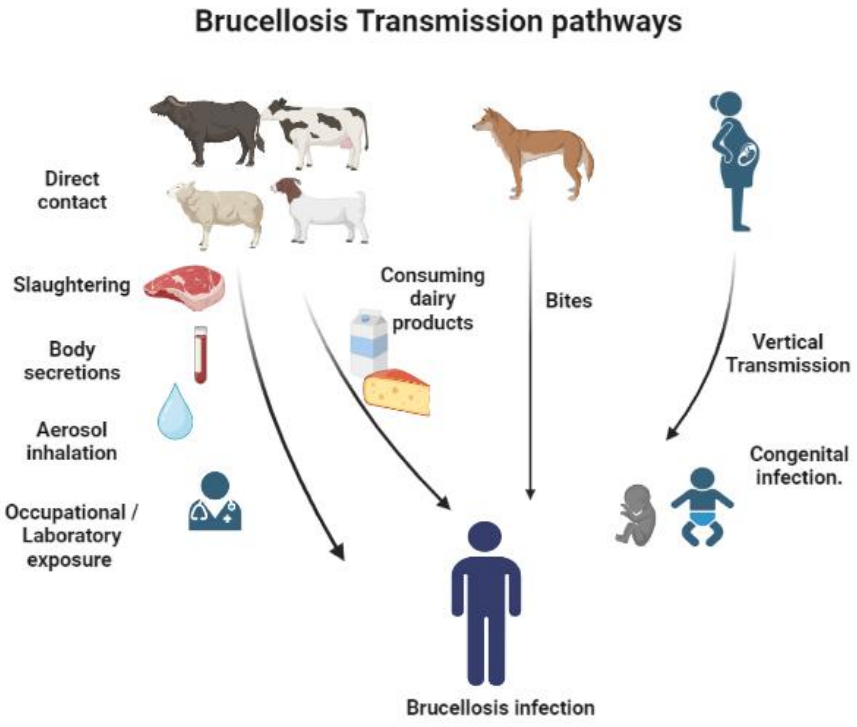

Fig. 1: Brucellosis transmission cycle from animals to humans

5. TRANSMISSION PATHWAY OF BRUCELLOSIS

Brucellosis is a zoonotic disease, and can be transmitted between animals and humans (Dafale et al. 2020). Domestic livestock, including cattle, sheep, goats, and pigs, are the primary sources of infection for humans (Santos et al. 2021). Other animals, such as dogs, camels, and wild ungulates, can carry and transmit the disease. The zoonotic potential of brucellosis have a significant impact on one health (Getahun et al. 2022).

6. BRUCELLOSIS CLINICAL SIGNS, DIAGNOSIS AND TREATMENT

Brucellosis causes a wide range of symptoms, often resembling a flu-like illness (Dadar et al. 2022). The incubation period of the disease varies from 5 days to several months. The most common symptoms include: Prolonged intermittent Fever with chills and sweating (Disease hallmark) lasting for weeks or months. Generalized body aches, joint pain, and muscle soreness, fatigue, night sweats, persistent headache, gastrointestinal symptoms (nausea, vomiting, abdominal pain, and diarrhea), respiratory Symptoms (cough, chest pain, and difficulty breathing) , and neurological complications (Avila-Granados et al. 2019). Diagnosis of brucellosis can be challenging due to its nonspecific symptoms (Bendrey and Fournié 2021). Healthcare providers should maintain a high “Index of Suspicion”, especially in individuals with a history of exposure to livestock or consumption of unpasteurized dairy products (Alhazmi et al. 2022). The following are the diagnostic tools for Brucellosis:
Treatment of brucellosis requires a combination of antibiotics for an extended period to ensure complete eradication of the bacteria and to prevent relapse (Waldrop and Sriranganathan 2019). The choice of antibiotics depends on the individual patient, disease severity, and drug susceptibility testing. Commonly used antibiotics include: Doxycycline (prescribed in combination with another antibiotic) (Tialla 2021) Rifampin (used with doxycycline to increase treatment efficacy) and Trimethoprim-Sulfamethoxazole (used as an alternative therapy) (Nthiwa et al. 2019). The treatment duration is usually 6 weeks and can extend up to several months, depending on the clinical response. Patients must complete the full course of antibiotics to ensure complete eradication of the bacteria (Kelly et al. 2021).

7. BRUCELLOSIS UNDER ONE HEALTH APPROACH

The one health approach emphasizes the importance of collaboration between sectors dealing with human, animal, and environmental health (Galarce et al. 2021). In the context of brucellosis prevention and control, the important measures are:
a) Effective collaboration between veterinary services, public health agencies, agriculture, and other relevant stakeholders. It includes Joint-efforts information sharing, mutual coordination, and the development of integrated strategies to tackle the disease (Mia et al. 2022).
b) Encouraging interdisciplinary research leads to a better understanding of the disease dynamics, transmission routes, and risk factors. This knowledge can guide the development of targeted interventions and policies for brucellosis prevention and control (Kauffman and Petersen 2019).
c) International cooperation and collaboration by sharing best practices, expertise, and resources to help countries with limited resources in enhancing their prevention and control efforts (Asante et al. 2019).
d) Launching public health measures and awareness campaigns by using strategies such as; Provision of health education and public awareness through different channels, ensuring the adoption of occupational safety practices by people with high-risk jobs (use of personal protective equipment, good hygiene practices, and regular health screenings) to minimize infection risk and implementing strict food safety regulations and standards (Milk pasteurization and proper handling of animal products), to reduces the risk of food-borne brucellosis transmission (Kim et al. 2019).

Table 1: Tests for diagnosis of brucellosis.

Serial No.	Diagnostic test	Use	Limitation	References
1	Blood Culture	Gold standard for diagnosis Cultured on specific media (Farrell's or Castañeda's medium) under enhanced safety conditions to avoid laboratory-acquired infections	❖ Significant risk to laboratory personnel ❖ Time consuming (requires several weeks)	(Pascual et al. 2022)
2	Serological Tests	❖ Rose Bengal test ❖ Standard Agglutination Test (SAT) ❖ Complement Fixation Test (CFT) and enzyme-linked immunosorbent assay (ELISA) ❖ Widely used due to their simplicity and high sensitivity ❖ Detect specific antibodies produced by the host in response to Brucella infection	❖ False-positive results due to cross-reactivity (with Yersinia spp. and Francisella tularensis) ❖ False-negative results can occur during the acute phase of the disease. before antibody production or when immunosuppression ❖ Cannot distinguish between active and past infections	(Buhmann et al. 2019)
3	Polymerase Chain Reaction (PCR).	❖ Can directly detect Brucella DNA in clinical samples ❖ Provides a rapid and specific diagnosis ❖ Various PCR formats (conventional PCR, real-time PCR, and multiplex PCR) ❖ Offer high specificity, even at low bacterial loads ❖ Can differentiate between different Brucella species and strains ❖ Significantly time-saving ❖ Facilitates timely treatment initiation	❖ Requires well-equipped laboratories, skilled personnel, and expensive equipment ❖ False-negative results if the absence of the target DNA in the specimen or if the presence of inhibitors	(Saddique et al. 2019)

e) Ensuring better surveillance and monitoring system for early detection, rapid response, and ongoing assessment of brucellosis prevalence and trends (Dhand et al. 2021). Essential strategies include: establishing a quick and effective disease-reporting system for veterinarians and farmers to prevent disease spread. Strengthening the laboratory infrastructure and diagnostic capabilities for accurate diagnosis of brucellosis (Khatibi et al. 2021).

8. PREVENTION AND CONTROL STRATEGIES: A COMPREHENSIVE APPROACH

Preventing brucellosis primarily focuses on reducing exposure to infected animals and their products (Shome et al. 2020). To alleviate the public health concerns and socio-economic burden of brucellosis, comprehensive prevention and control strategies are required. Following measures should be adopted to control Brucellosis:

1) Public education and awareness about brucellosis transmission, symptoms, and preventive measures to facilitate early detection and treatment of brucellosis (Ferreira et al. 2023).

2) Implementing vaccination programs for livestock to reduce the prevalence of brucellosis in animal populations, subsequently decreasing the risk of human infection.

3) Improving food safety practices by promoting pasteurization of dairy products, and ensuring proper meat-handling and cooking to minimize the risk of bacterial transmission (Al Jindan 2021).

4) Adapting personal protective measures by individuals at high risk of exposure, such as farmers, veterinarians, and slaughterhouse workers, through the use of protective clothing and gloves when handling animals or their tissues (Moreno 2022).
5) Vaccination programs for animals play a crucial role in preventing and controlling brucellosis in animals, primarily cattle, goats, and pigs, which are known reservoirs of the bacteria (Ma et al. 2022). The main aspects vital in effective vaccination programs are the use of live attenuated vaccines (RB51 strain for cattle and Rev 1 strain for small ruminants) which have been proven successful to control brucellosis. These vaccines provide long-lasting immunity and reduce the bacterial shedding of in animals, thus minimizing the risk of transmission to humans (Elrashedy et al. 2022). Early vaccination of animals is essential to prevent the infection within flocks. Timely vaccination of young animals, preferably prior to sexual maturity, is important for reducing the risk of brucellosis transmission (Nyerere et al. 2020). Implementing comprehensive herd vaccination programs help achieve higher vaccination coverage and enhance overall disease control. Regular monitoring of vaccination status, coverage rates, and revaccination are critical for the success of these programs (Bahmani and Bahmani 2022). Strong surveillance and reporting should be done to ensure effective control (Tao et al. 2021).

9. BRUCELLOSIS CONTROL

Control of brucellosis plays an important role in minimizing the disease spread and the economic loses. Switzerland successfully eradicated bovine brucellosis through a comprehensive control program involving strict movement controls, test-and-slaughter strategies, and vaccination. The country achieved disease-free status in 1999, highlighting the effectiveness of integrated control measures (Pal et al. 2020). Spain implemented a nationwide control program that included systematic surveillance, test-and-slaughter strategies, and vaccination campaigns (Tian et al. 2020; Almashhadany 2021). Similarly, Mongolia successfully reduced the incidence of brucellosis in both humans and animals through a one health approach (Machavarapu et al. 2019). Collaborative efforts involving veterinary services, public health agencies, and communities led to increased awareness, improved diagnostics, and enhanced surveillance and control measures (Rahman et al. 2019).

10. CONCLUSION

Brucellosis prevention and control requires a comprehensive approach that combines vaccination programs for animals, the One Health approach, public health measures, and surveillance systems. By implementing these strategies, we can reduce the incidence of brucellosis in animals, minimize the risk of transmission to humans, and mitigate the socio-economic impacts associated with this significant public health threat. To effectively combat brucellosis globally, collaborative efforts and continuous implementation of prevention and control measures are required.

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Brucella Zoonosis: Treatment and Prevention Guide

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ABSTRACT

David Bruce, identified Brucella melitensis in 1886, initially known as Malta Fever. Themistocles Zammit later revealed its zoonotic origin in goats. Brucella is a zoonotic disease transmitted from animals to humans. Brucella is a non-spore-forming gram-negative coccobacilli, lack capsules and virulence genes. Despite being non-motile, they possess genes for flagellum construction. The Brucella genus comprised of nine species shows host-specific genomic similarities, challenging understanding of survival mechanisms and intracellular growth. In animals, Brucella enters via mucous membranes or skin, bypassing immune defenses in organs, causing persistent infections, especially in the reproductive tract, leading to abortion. Infected animals shed the bacteria in fluids. In humans, Brucellosis enters through contaminated products or direct contact with animals, inducing systemic symptoms. Chronic cases may result in skeletal issues or rare neurological complications. Brucellosis, a geographically dynamic disease, in which cases are prevalent in Central Asia and escalating across the Middle East. Despite successful eradication in certain regions, brucellosis persists globally, impacting animal production and public health. Brucella strains exhibit zoonotic potential, with B. melitensis posing the highest risk. Effective eradication efforts have reduced human cases in certain countries. Human brucellosis presents diagnostic challenges, relying on laboratory tests due to varied clinical manifestations. Culture isolation remains the gold standard, while serological tests like the Brucella agglutination test and PCR-based methods are essential. In cattle, the Brucella ring test and blood tests are key for monitoring and eradication efforts. Swine brucellosis lacks a reliable serological test, but buffered plate Brucella antigen tests are practical. Ovine and caprine brucellosis screening relies on tests like the Rose Bengal plate agglutination, complement fixation, and indirect ELISA tests. Brucellosis treatment challenges arise from intracellular adaptation of the bacteria. Combining doxycycline and streptomycin (DS) is the most effective, although parenteral administration poses challenges. The rifampicin-doxycycline (DR) oral regimen is an alternative, but less potent, requiring individualized consideration, monitoring, and follow-up for optimal outcomes. In high-prevalence areas, controlling and eradicating brucellosis involves vaccination and the removal of infected animals. Key vaccines include B. abortus strains 19 and RB51 for cattle and B. melitensis strain Rev1 for goats and sheep. Despite vaccination, total Brucella eradication requires additional measures and sound husbandry practices due to vaccines providing partial protection, especially in regions with elevated infection rates.

Keywords: Brucellosis, Zoonotic disease, Epidemiology, Diagnosis, Treatment, Vaccination

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1. INTRODUCTION

David Bruce (1855-1931), a British army physician, identified "Micrococcus melitensis" from the spleen of a man who died of "Malta Fever" in 1886. This condition was prevalent, although it was sometimes mistaken with other ailments, particularly malaria. However, with crucial discoveries and advancements in the late nineteenth and early twentieth century, a substantial knowledge of this mysterious disease emerged much later. However, for about two decades after the isolation of M. melitensis, the real nature of Malta fever remained a mystery, with it being misdiagnosed as a vector-borne disease (Godfroid et al. 2005). It wasn't until 1905 that Themistocles Zammit, a Maltese physician, accidentally revealed the disease's zoonotic origin. He isolated B. melitensis from milk of goat, which was a pivotal finding. It was thought that goats are immune to infection since they showed no indications of sickness after being injected with Brucella cultures (Wyatt 2005). The startling findings that healthy goats may function as disease carriers was heralded as one of the most significant advances in epidemiology. Benhard Bang, a Danish veterinarian, discovered B. abortus, as the causal agent responsible for cow abortion, or Bang's disease, in 1897 (Khurana et al. 2021). Alice Evans, a distinguished American scientist known for her important study on harmful microorganism in milk and dairy related products, eventually affirmed the link between Malta fever and Bang's disease. Following these discovery, the genus was renamed Brucella in honor of David Bruce (Spink 1956). Evans' pioneering study on Brucella was essential in arguing for pasteurization procedures to protect against human brucellosis in the United States (Garcell et al. 2016). This discovery challenged prior conceptions of the disease's spread and demanded a reevaluation of management methods related with its incidence on land and in the water. Brucellosis is a zoonotic illness, which means it may spread from animals to people and vice versa.

Domestic animals, such as cattle, goat, sheep, pigs, and different wildlife species, play a role as key reservoirs for Brucella bacteria (Alton and Forsyth 1996). Humans generally get the infection by directly coming into contact with infected animals or their products, including unpasteurized milk and dairy products, or through exposure to contaminated animal tissues or fluids. In both animals and humans, the sickness appears differently. Brucella infection in animals can cause reproductive difficulties, such as abortions and decreased fertility, which can have serious economic ramifications for the cattle business (Gwida et al. 2010). One of the difficulties in controlling brucellosis is that it can persist in animal populations even in the absence of obvious clinical indications (Potter, 2013). Infected animals can become asymptomatic carriers, occasionally releasing the germs and creating a continual danger of transmission to humans and other vulnerable animals. Control strategies for brucellosis include immunization of animals, culling of sick animals, and stringent cleanliness techniques, particularly in the dairy sector (Dadar et al. 2021). Advances in diagnostic tools and molecular biology have increased our understanding of the variety and epidemiology of Brucella strains in recent years. Brucella species and strains have been found, each with variable degrees of virulence and host specificity (Christopher 2010). This understanding has aided in the creation of focused control measures for various settings and locations. Furthermore, research efforts have been aimed on producing effective vaccines for both animal

and human brucellosis. Vaccination of animals has shown encouraging benefits in lowering the occurrence of brucellosis in some areas (Dadar et al. 2021). Human vaccine development, on the other hand, remains a problem due to the disease's complexity and the need to balance safety and efficacy. Moreover, Brucellosis has been the subject of countless important discoveries throughout history, owing to its ancient beginnings. From David Bruce's early isolation of the causal agent through Themistocles Zammit's observation of its zoonotic character and Bang and Evans' identification of distinct Brucella species, each contribution has played an important part in developing our understanding of this complicated illness (Edwards and Jawad 2006; Wyatt 2016 ; Ghanbari et al. 2020).

2. CAUSATIVE AGENTS/ETIOLOGY

Brucella spp. are intriguing facultative intracellular gram-negative coccobacilli that are not spore-forming or capsulated (Alton and Forsyth 1996). Despite being categorized as non-motile, they have all of the genes required to build a functioning flagellum, with the exception of the chemotactic system. These adaptable bacteria are classified as Proteobacteria alpha-2, together with Ochrobactrum, Rhizobium, Rhodobacter, Agrobacterium, Bartonella, and Rickettsia (Fretin et al. 2005). The Brucella genus now has nine identified species, seven of which harm domestic animals: B. abortus, B. suis, B. canis, B. ovis, B. neotomae, B. microti and B. melitensis. Furthermore, two species, B. ceti and B. pinnipedialis, prey on marine animals (Liu 2015). The first three terrestrial species are known as classical Brucella, with seven biovars reported for B. abortus, three for B. melitensis, and five for B. suis. The other species have not yet been classified as biovars (Liu 2015). Surprisingly, Brucella strains are classified according to the host species they primarily infect. Because of advances in genomics, ten genomic sequences encompassing five Brucella species have been sequenced: B. abortus, B. melitensis, B. ovis, B. suis, and B. canis (Halling et al. 2005). Furthermore, around 25 additional Brucella strains/species are being sequenced. According to these genome investigations, Brucella members have surprisingly comparable genome sizes and gene compositions. Each species has two circular chromosomes and an average genome size of roughly 3.29 Mb. Chromosome I is roughly 2.11 Mb in length, whereas Chromosome II is around 1.18 Mb in length. All Brucella genomes have a G+C content of around 57.2% for Chromosome I and 57.3% for Chromosome II (Bohlin et al. 2010). Surprisingly, despite their mostly intracellular lifestyle, a study of 10 published Brucella genomes reveals similar aberrant areas in both chromosomes, suggesting the effect of horizontal gene transfer (Wattam et al. 2009). Brucella does not have any traditional virulence genes that encode capsules, plasmids, pili, or exotoxins. As a result, our understanding of the variables influencing their survival in the host and growth inside phagocytic cells is restricted in comparison to other bacterial pathogens (Głowacka et al. 2018). Furthermore, the complexities of Brucella's interactions with its host continue to provide considerable problems, necessitating continued study to uncover the underlying processes.

3. PATHOGENESIS

3.1. ANIMALS

Brucella primarily infiltrates the animal host through mucous membranes or skin abrasions, and it can also spread through the respiratory tract or contaminated feed and water. Within the host, it evades the immune system by residing in macrophages and dendritic cells. Upon initial penetration, localized infections arise in lymph nodes, spleen, and other organs. Bacteria proliferate and form granulomas, providing protection against immune responses and antimicrobial treatments. Systemic dissemination occurs through the circulation, leading to persistent granulomatous lesions in organs like the liver, reproductive organs, and mammary glands. The disease in animals is characterized by long-lasting

infection, with a particular affinity for the reproductive system, leading to abortion, stillbirth, or low birth weight. Infected females experience persistent endometritis, placentitis, and pregnancy loss as the bacteria colonize the placenta and uterine lining. Infected animals shed Brucella in bodily fluids like milk, urine, and reproductive secretions, serving as a source of infection for other vulnerable species and perpetuating the transmission cycle (Alton and Forsyth 1996 ; López-Santiago et al. 2019).

4. HUMANS

Brucellosis, typically contracted through the ingestion of contaminated animal products like unpasteurized milk, cheese, and meat, or direct contact with sick animals, can enter the body through mucous membranes or skin abrasions. Within the host, Brucella infiltrates monocytes and macrophages, establishing residence and multiplying. It induces granulomas in various organs, including the spleen, liver, and bone marrow. The infection spreads systemically through the circulation. Common non-specific symptoms include fever, fatigue, headache, joint discomfort, and sweating. In humans, Brucellosis may become chronic, leading to recurrent fever episodes and associated symptoms. The bacteria can persist in the body for months or even years, resulting in relapses and long-term complications. Brucella exhibits a predilection for the skeletal system, frequently causing osteoarticular issues like arthritis and spondylitis in humans. In rare cases, the bacteria can invade the central nervous system, leading to neurological symptoms such as meningitis or encephalitis (Alton and Forsyth 1996 ; de Figueiredo et al. 2015).

5. IMMUNOBIOLOGY

The stealthy nature of Brucella is primarily attributed to the unique nature of its smooth lipopolysaccharide (LPS) on the cell surface. The elongated fatty acid molecules on the lipid A portion of Brucella LPS reduce its toxicity and immunogenicity, making it a weak TLR4 agonist. This property allows Brucella to attack host cells with less activity. Moreover, the rough brucellae lacks the O-polysaccharide component of LPS, exhibit cytotoxicity to macrophage cells (Paul de Figueiredo et al. 2015). Although a comparative analysis of the lipid A from smooth and rough organisms have not been conducted. The lack of cytotoxic activity in rough LPS suggests that the O-polysaccharide is essential for the stealthy behavior of the organism (Stranahan and Arenas-Gamboa 2021).

In addition to the weak Brucella LPS agonist activity, the organism expresses novel immune regulatory factors that suppress the innate immune response. One such factor is the TIR-containing protein, TcpB/BtpA, which interacts with cytoplasmic MyD88 adaptor like/TIRAP. TcpB prevents MyD88 binding to TIRAP, accelerating its degradation and impairing TLR signaling, leading to reduced proinflammatory cytokine production. Another protein, BtpB, interferes with TLR signaling through MyD88, inhibiting dendritic cell maturation. The redundant factors of TcpB/BtpA and BtpB functions may explain the failure to identify these immunoregulatory genes through simple transposon screens (Jiao et al. 2021). Lack of expression of tcpB resulting in increased immune activation, resulting in reduced overall lifespan of microorganism. TcpB may act through protein kinase B to inhibit the NF-κB–mediated proinflammatory response and induce IL-10 production, ultimately contributing to the Brucella stealthy behavior (Smith et al. 2013).

Protection against Brucella has been studied in a variety of animal models, including mice, guinea pigs, ruminants, nonhuman primates, and humans. A T helper cell type 1 (Th1) response, including CD4+ and CD8+ T cells, is essential for protection. Antibodies to LPS, particularly the O-polysaccharide, may contribute to protection, but the role of the T helper cell type 2 (Th2) humoral immune response is unclear (Silva et al. 2011). Cytokines, such as IL-12, interferon-γ, tumor necrosis factor-α, IL-1, and IL-6, play important roles in mediating both innate and adaptive immune responses against Brucella. Reports

suggest that IL-1–dependent induction of colony-stimulating factor increases neutrophil and macrophage influx into the spleen, contributing to protection.

A variety of antigen of innate immune system, including complement, opsonins, phagocytes, innate lymphocytes, and cytokines, confer passive resistance to intracellular killing mechanisms. However, the importance of the type IV secretion system (T4SS) in the long-term Brucella infection is becoming clearer. Brucellae, like other intracellular pathogens, modifies the innate immune response to create recurrent adhesion and ensure long-term persistence (Paul de Figueiredo et al. 2015). The organism avoids the innate immune response by stealthy infiltration into host cells and controls protein secretion, cellular trafficking, and bacterial replication to alter the course of both innate and adaptive immune response.

The failure of immunization against Brucella infection is associated with a weak immune response, partly controlled by the attenuated innate immune response. As a stealth invader, Brucella enters host cells through TLR ligand interaction without apparent activation of the innate immune response (Pellegrini et al. 2022). Knockout mice deficient in either TLR2 or TLR4 do not significantly affect the ability to control the pathogen. However, cells deficient in MyD88 maintain a two-log increase in bacterial infection, indicating redundancy in host functions (Fang et al. 2010). By Evading the innate immune response of the host, Brucella can gain a foothold, while subsequent stimulation contributes to the spread of infection.

6. WORLDWIDE SPREAD AND ECONOMIC SIGNIFICANCE

Brucellosis, a disease with a continually fluctuating geographical distribution, is undergoing epidemiological changes as a result of a variety of variables including hygienic, economical, and political issues, as well as increased international travel (Khoshnood et al. 2022). Cases of human brucellosis have been observed, especially in Central Asia, and there is a significant surge in its spread across numerous Middle Eastern nations (Seleem et al 2010). Except in places where bovine brucellosis (B. abortus) has been successfully eliminated (no documented cases for at least five years), this illness is common (Godfroid et al. 2010). Certain countries have achieved brucellosis-free status, including Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden, and the United Kingdom, as well as Mediterranean European nations, northern and eastern African countries, Near Eastern countries, India, Central Asia, Mexico, and Central and South America. In contrast, these areas are currently dealing with brucellosis and have not yet eliminated the disease. (Khurana et al. 2021). Although B. melitensis has not been found in certain places, there are no convincing reports of its eradication from small ruminants elsewhere in the world (Blasco 1997). Human brucellosis, while being a nationally notifiable and reportable illness in the majority of nations, is severely underreported, with official figures reflecting only a fraction of the real incidence. As a result, the real worldwide burden of human brucellosis remains unknown, with estimates ranging from 0 to 160 cases per 100,000 people (Lai et al. 2021). The economic consequences of brucellosis are significant over the world, impacting both animal production (by lower milk, abortion, and delayed conception) and population health (via treatment expenses and productivity loss). Official estimates in Latin America, for example, show yearly losses of about $600 million due to bovine brucellosis (Angara et al. 2016). Though brucellosis eradication initiatives can be costly, they are thought to be cost-effective, with estimates indicating that every dollar put in eradication efforts save cost of treatment.

7. ZOONOSIS

Five of the nine identified Brucella species may infect people, with B. melitensis being the most virulent and invasive, followed in descending order of severity by B. suis, B. abortus, and B. canis. The zoonotic potential of marine brucellae (B. ceti) is well known (Liu 2015). Notably, in the United States B. melitensis,

B. suis, and B. abortus are categorized as possible bio-weapons due to their high infectivity, particularly through aerosolization (Khurana et al. 2021). Early signs of brucellosis are fever, joint pain and fatigue which make epidemic diagnosis difficult (Jiang et al. 2019). Infected animals directly transmit the disease to humans, or humans contract it by consuming their products, particularly unpasteurized milk and dairy products like cheese made from sheep and goat milk (Abdali et al. 2020). Specific occupational groups, such as veterinarians, agricultural laborers, meat-packing workers and ranchers are more vulnerable (Mobo et al. 2010). While B. suis and B. abortus infections mostly afflict workers, B. melitensis infections are more common in the general population (Alton and Forsyth 1996). Sheep or goat milk with B. melitensis is an important source of human brucellosis globally, causing multiple outbreaks; in certain regions, B. melitensis is responsible for 99% of human brucellosis cases (Rossetti et al. 2017). Human infections have decreased significantly as a result of brucellosis eradication efforts in animal reservoirs. For instance, in the United States, as a result of national bovine brucellosis eradication programme significant decline in human cases over time was reported. Denmark and France had comparable success in eradicating human brucellosis through eradication campaigns (Meyer 1956). Brucellosis typically affects people who come into direct contact with infected animals and consume milk and dairy products that are not pasteurize. Despite the fact that Brucella is extremely contagious when inhaled, inhaling the germs is not a common mechanism of infection (Głowacka et al. 2018). Certain occupational groups, however, such as laboratory and slaughterhouse workers, face severe risks in this respect. Brucella spp. account for up to 2 percent of all laboratory-related infections, making them the most prevalent pathogens found in laboratories (Madut et al. 2019). In 1999, 11.9% of clinical microbiology laboratory employees in Spain had laboratory-acquired brucellosis, according to a study (Bouza et al. 2005).

8. IMPACT OF BRUCELLOSIS ON HUMAN

Undulant fever, marked by temperature variations from 37.8°C in the morning to 40.8°C in the afternoon, as well as nocturnal sweats generating a unique odor, chills, and weakness, are the most prevalent signs of brucellosis. Anorexia, malaise, headache, sleeplessness, arthralgia, sexual dysfunction, constipation and anxiousness are other common symptoms. Furthermore, human brucellosis is recognized for its complications, which can damage numerous internal organs and cause a variety of symptoms depending on the site of infection. Encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis are some of the possible consequences (Dadar et al. 2021). Pregnant women infected with Brucella may have spontaneous abortions, which occur most frequently in the first and second trimesters of pregnancy (Bosilkovski et al. 2020). Brucella endocarditis is an uncommon but severe complication that accounts for at least 80% of brucellosis fatalities (Raju et al. 2013). Due to a lack of proper medication during the acute phase, brucellae might become localized in various tissues and organs, resulting in a difficult-to-treat subacute or chronic condition (Khan and Zahoor 2018). Brucellosis symptoms and signs can be confused with those of other diseases such as enteric fever, rheumatic fever malaria, thrombophlebitis, TB, fungal infections, autoimmune disorders, tumors and cholecystitis. However, with vaccine strains, the illness course is frequently shorter and less severe. Direct transmission of brucellosis from person to person is extremely rare. Breastfeeding women, on the other hand, may pass the virus to their newborns, and sexual transmission has also been recorded.

9. IMPACT OF BRUCELLOSIS ON ANIMALS

Abortion is the most prevalent clinical indication of Brucella infection in numerous livestock species, including cattle, sheep, goats, pigs, and camels (Garin-Bastuji et al. 1998). B. abortus is the most common strain responsible for infection in cattle. They can, however, become transiently infected with B. suis and,

more often, B. melitensis when they share pasture or facilities with diseased pigs, goats, and sheep (Gumaa et al. 2020). Because B. melitensis and B. suis may be spread through cow's milk, they can constitute a major public health risk. Symptoms are observed in pregnant animals, including abortion (weak calves or premature or full-term delivery of dead calves), during the second half of gestation, particularly in the third trimester, as well as placental retention and metritis (Bosilkovski et al. 2020). Infected cows may see a 20-30% decrease in milk output (Dadar et al. 2021). Brucellae reside in the supra-mammary lymph nodes and mammary glands of 80% of infected animals, secreting the infection into their milk constantly throughout their lives (Meador et al. 1989). Although most infected cows only have one abortion, the placenta can be highly contaminated during successive seemingly normal calvings. B. melitensis is the major causative agent of brucellosis in goats. Goats can become infected with B. abortus in places where B. melitensis is absent. Late abortion, stillbirths, reduced fertility, and low milk production are all symptoms of Brucellosis in goats. Sheep brucellosis is divided into two types: ram epididymitis and classical brucellosis. The non-zoonotic agent B. ovis causes ram epididymitis, whereas classical brucellosis is produced by B. melitensis and, like goat brucellosis, poses a substantial public health danger. Aside from miscarriage, pigs might suffer from lameness, hind limb paralysis, orchitis, spondylitis, and, on rare occasions, metritis or abscesses. Camels can become infected with B. melitensis and B. abortus if they graze among infected sheep, goats, and cattle. Infected camel milk is a major cause of infection, particularly in the Middle East, where its importance is sometimes overlooked. B. canis is the major etiologic agent of brucellosis in dogs (Khurana et al. 2021). There have been isolated occurrences of brucellosis in dogs caused by B. abortus, B. suis, and B. melitensis. Dogs infected with B. canis may develop reproductive issues such as miscarriages in the third trimester, conception failures or still births, as well as other issues such as ophthalmic, musculoskeletal, or dermatologic diseases (Santos et al. 2021). Although people are vulnerable to B. canis, the danger is smaller than with traditional brucella.

10. BRUCELLOSIS TESTING AND DETECTION METHODS

Human brucellosis has a complicated clinical picture, making a diagnosis based merely on symptoms problematic (Yagupsky et al. 2019). In endemic areas, every episode of fever with an unknown etiology is frequently considered to be brucellosis, emphasizing the significance of establishing the diagnosis by laboratory tests. It is crucial to make an accurate and timely diagnosis since delays or misdiagnosis can lead to treatment failures, relapses, chronic disease courses, localized complications, and even high case fatality rates. A correct diagnosis requires a complete case history, especially in non-endemic locations, to rule out travel-associated brucellosis or the ingestion of contaminated milk products imported from endemic regions (Yagupsky and Baron 2005). The isolation of Brucella from blood, bone marrow, lymph nodes, or cerebrospinal fluids is the gold standard for diagnosing brucellosis in people (Yagupsky et al. 2019). However, due to its sluggish development and limited sensitivity, culture cannot serve as a screening test. The sensitivity of Brucella isolation is influenced by individual laboratory procedures, the amount of pathogen in clinical samples, the stage of illness, the use of antibiotics prior to diagnosis, the culture methods used, and the specific Brucella strain involved (Yagupsky et al. 2019). The detection sensitivity varies greatly, ranging from 15 to 70 percent in individuals with acute infection and being considerably less in people with chronic illness (Yagupsky et al. 2019). The lysis centrifugation approach has recently demonstrated excellent results, with higher percentages of positive blood cultures (91% in acute brucellosis and 74% in chronic brucellosis) (Mantur and Mangalgi 2004). The existence of antibodies against the O-side chain of Brucella lipopolysaccharide is revealed by serological studies, which assess the serum's capacity to agglutinate a standardized quantity of dead B. abortus (Monreal et al. 2003). Brucella-specific IgM antibodies, followed by IgG antibodies, often develop in the last days of first week of the

disease and remain the most common and relevant markers for brucellosis diagnosis in the laboratory (Al Jindan et al. 2019). Furthermore, as compared to handling Brucella cultures, these agglutination tests are quicker and lower the risk of laboratory-acquired infections. It is important to note, however, that these serological assays are ineffective for identifying infections caused by B. canis, a strain that is inherently O-side chain lacking (Mol et al. 2020). The standard tube Brucella agglutination test is routinely used to diagnose acute brucellosis (Seleem et al. 2010). In chronic brucellosis, however, the 2-mercaptoethanol test and complement fixation tests are employed to detect current infection even when agglutination titers revert to low levels (Buchanan and Faber 1980). The 2ME test is identical to the standard tube Brucella agglutination test, except that leaving IgM antibodies inactive, 2ME is added to destroy disulfide bonds (Seleem et al. 2010). Other helpful diagnostic procedures for human brucellosis exist in addition to the aforementioned tests. The Rose Bengal test, counter immune-electrophoresis, Coombs test, immunocapture agglutination test, latex agglutination, and the indirect enzyme-linked immunosorbent assay are examples of these (Seleem et al. 2010). The use of polymerase chain reaction (PCR)-based tests for molecular diagnosis of human brucellosis has been recommended as a more helpful and sensitive method (Navarro et al. 2004). Such approaches, however, have not yet been completely verified for normal laboratory usage. Brucellosis testing in livestock is often done as part of monitoring efforts and disease eradication except for diagnostic purposes. Each country has its own policy regarding livestock testing. The Brucella ring test, which identifies antibodies in pooled milk samples from dairy herds, and the market cattle identification blood test, which analyzes serum antibodies in blood samples, are the two principal procedures for evaluating brucellosis in cattle in the United States (Godfroid et al. 2010). No serological test for swine brucellosis has been proved to be reliable for routine diagnosis. In contrast, buffered Brucella antigen tests, including the Rose Bengal plate agglutination test and the buffered plate agglutination test, are more accurate in practice in comparison with other tests for identifying infected herds (Lucero and Bolpe 1998). Rose Bengal plate agglutination, complement fixation, and indirect ELISA tests are commonly suggested for screening flocks and individual animals in the diagnosis of ovine and caprine brucellosis (Blasco et al. 1994). To summarize, human brucellosis is a difficult illness to identify clinically due to its wide range of clinical manifestations. Laboratory testing are required to confirm the diagnosis and distinguish it from other febrile diseases. While Brucella culture remains the gold standard, its sluggish development and poor sensitivity make it unsuitable as a screening test. Serological tests are often employed for diagnosis, such as the standard tube Brucella agglutination test for acute cases and the 2-mercaptoethanol and complement fixation tests for chronic infections. Molecular diagnostics employing PCR-based tests shows promise, but further research is needed. Testing is essential in cattle for disease eradication and monitoring programs, with different tests recommended depending on the species.

11. TREATMENT GUIDE

Treatment failures and relapses in brucellosis are prevalent due to Brucella's capacity to adapt inside its intracellular habitat, such as macrophages, and can be impacted by the medication combination utilized and patient compliance (Alavi and Alavi 2013 ; Mode et al. 2022). Because monotherapies with single antibiotics have been associated with significant rates of recurrence, the most successful way to treating brucellosis entails combining two medications. Due to its fewer side effects and lower recurrence rates, the use of streptomycin with doxycycline has emerged as the current effective treatment choice for instances of localized and acute brucellosis (Yousefi-Nooraie et al. 2012; Alavi and Alavi 2013). However, neither streptomycin nor doxycycline can effectively limit intracellular brucella proliferation. Despite its success, the DS regimen has disadvantages, most notably the requirement for streptomycin administration through parenteral route for three weeks, making it less practicable and less favored by patients. A

combination of parenteral gentamicin (5 mg/kg) and doxycycline therapy (6 weeks) for seven days has been deemed an appropriate replacement, since it provides reasonable effectiveness and enhanced convenience (Roushan et al. 2006). The World Health Organization (WHO) has long regarded DS combinations as the gold standard for brucellosis treatment (Alavi and Alavi 2013). However, in 1986, revision of recommendations by the Joint FAO/WHO Expert Committee on Brucellosis for treating adult acute brucellosis and introduced the use of rifampicin (600-900 mg/day orally) in combination with doxycycline (200 mg/day orally) as the regimen of choice, commonly known as the DR regimen (Falagas and Bliziotis 2006). Concerns regarding streptomycin's parenteral administration and the need for more accessible treatment choices motivated the decision to revise the guidelines. Nonetheless, studies comparing the efficacy of the DR regimen to the classic DS combination have found that the DS regimen is still more successful, particularly in acute brucellosis patients (Solera et al. 1995). The greater effectiveness of the DS combination may be attributable to streptomycin's powerful bactericidal action against Brucella, particularly in its acute form. While the DR regimen provides an oral option, it may not achieve the same level of bacterial eradication, increasing the likelihood of recurrence, particularly in acute patients. When deciding on the most effective treatment plan, it is critical to evaluate the individual circumstances of each patient as well as the strain of Brucella involved. Drug resistance trends, patient compliance, and the severity of the disease should all be considered. Close monitoring and follow-up assessments of patients during therapy are required to achieve effective results and limit the chance of recurrence. Because of Brucella's intracellular localization and capacity to adapt within host cells, brucellosis is a difficult infectious illness to cure. Combining two antibiotics, such as doxycycline and streptomycin (DS), has been shown to be the most effective treatment method, especially in localized and acute brucellosis. Among the treatment regimens available, the DS regimen stands as the gold standard, parenteral administration can be inconvenient and unpopular with patients. The DR regimen (rifampicin with doxycycline) gives an oral option as well, but it may be less effective, particularly in acute instances. Individual patient features and bacterial characteristics must be carefully considered when choosing the best treatment plan, with continuous monitoring and follow-up to guarantee effective results and limit the chance of recurrence.

12. VACCINATION AND IMMUNITY

Vaccination of vulnerable hosts and the eradication of diseased animals are critical techniques for controlling and eradicating this zoonosis in high-prevalence areas. B. abortus strain 19 and the USDA-approved strain RB51 are the most often used vaccinations for bovine brucellosis (CDC 1998 ; Stevens et al. 1997). Unlike strain 19, strain RB51 does not interfere with serological diagnosis. The persistence of antibodies while employing the B. abortus strain 19 vaccine is mostly determined by the age of the animals at the time of inoculation (Simpson et al. 2018 ; Seleem et al. 2010). Successful eradication programs must strictly limit the age at which strain 19 immunization is permitted, as testing and killing, in conjunction with vaccination, are critical components of such efforts. B. melitensis strain Rev1 is regarded the best vaccine for brucellosis control in goat and sheep, especially when delivered through conjunctival route in normal doses (Blasco 1997 ; Goodwin and Pascual 2016). However, the Rev1 vaccination is extremely virulent and can cause abortions when administered during pregnancy (Hensel et al. 2020). Furthermore, the immunity response after vaccination is same as reaction reported following acquired infection, limiting the effectiveness of control measures. Efforts have been undertaken to generate novel live attenuated rough B. melitensis vaccines without the O-side chain, which have yet to be tested in the field (Yang et al. 2013). It is critical to emphasize that total eradication of Brucella cannot be based exclusively on vaccination, because Brucella vaccines only provide partial protection, which may be undermined in the

face of increased infection rates (Seleem et al. 2010). As a result, a successful vaccination program must be accompanied with sound husbandry practices. Live vaccines, such as strains 19-BA and 104M of B. abortus are presently exclusively utilized in the Russia and China (Heidary et al. 2022). These vaccinations are intended to protect humans against brucellosis, however they are only available in certain areas. Brucella infections in animals have serious economic and public health consequences, especially in underdeveloped nations. Vaccination, in conjunction with control methods and sound husbandry techniques, is critical for disease control and eradication success. Vaccines for individual animal species are available, each with its own set of benefits and drawbacks. While vaccinations are important, their effectiveness must be supplemented by comprehensive control tactics in order to effectively combat brucellosis.

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Significance of Nanoparticles as Prophylactic and Treatment Option for Bacterial and Reverse Zoonosis

35

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ABSTRACT

A zoonotic disease spread spontaneously from vertebrate animals to people or from humans to vertebrate animals. Over 60% of human pathogens are zoonotic in nature. Bacteria, viruses, fungus, protozoa, parasites, and other pathogens may cause zoonosis. Climate change, urbanization, animal movement and trade, travel and tourism, vector biology, human and natural causes have all had a significant impact on the emergence, re-emergence, distribution, and patterns of zoonoses. Due to the emergence of resistant strains of zoonotic pathogens, nanotechnology can be very helpful in combating such pathogens. Nanotechnology has a wide range of applications in disease diagnostics, preventative and therapeutic fields. Nanoparticles (NPs) are well-established components of various successful targeted drug delivery systems, and the characteristic physicochemical features of several nano-formulations have demonstrated excellent bactericidal effects. Aside from its therapeutic potential, nano-vaccines and theragnostic uses of nano-formulations have received considerable interest as an alternate way of combating certain microbial pathogens. This book chapter focuses on the latest applications of nanomedicine in battling key bacterial zoonotic and reverse zoonotic illnesses, as well as their potential benefits, limits, and future possibilities for developing successful eradication tactics.

Key words: Zoonosis, nanoparticles, bacterial zoonosis, reverse zoonosis, one health.

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1. INTRODUCTION

Natural transmission of zoonotic diseases from animals to humans results in more than 60% of infectious diseases. A variety of transmission modes, including direct transmission (infected organisms, contamination of food, vector bite, direct touch with a contaminated entity), Indirect or passive transmission (fomite), can result in the transmission of zoonotic disease (O'Brien et al. 2017). The word "zoonoses" originated from the Greek word "zoon" meaning animal and "noses" meaning disease. A zoonosis can be referred to any disease or infection that can be transmitted impulsively from vertebrate animals to humans or from people to animals. As compared with "anthropozoonosis" (diseases that are transmitted from animals to people) and "zooanthroponosis" (diseases that are transmitted from people to animals), which study the main mode of pathogen transmission between animals and other people, 'zoonosis' is seen to be the most suitable (Chomel and Sun 2011). In addition, the term "amphixenoses" has been developed to characterize illnesses that may spread both ways and are kept in humans and lower vertebrate species.

The emergence and reemergence of zoonotic infectious disease is aided by socio-cultural practices like farming, hunting, and tourism, as well as growing ecological changes that favor the development of pathogenic vectors. Urbanization has also increased human contact with wildlife, which serves as a significant reservoir for zoonotic infectious diseases (Jones et al. 2013). Numerous domestic and wild animal species serve as reservoirs for newly developing and reemerging human diseases. They are brought on by diseases that come from animals or products derived from animals, such as viruses, bacteria, fungi, Rickettsia, and parasites (Pal 2005).

Although it is believed that improvements in prophylactic, diagnostic, and therapeutic measures have reduced the number of deaths from infectious diseases, rapid anthropozoonotic/zoonotic (animal to human) and zooanthroponotic/reverse zoonotic (human to other vertebrates) transmission of the pathogens and the worldwide origination of resistant pathogenic strains are casting doubt on this optimistic scenario (Prasad et al. 2021). The WHO lists the emergence of extensively drug-resistant (XDR) and multidrug-resistant (MDR) bacterial populations very among the top three challenges that public health is facing in the twenty-first century. According to the microbial populations' acquired non-susceptibility to various antimicrobial groups, the resistant pathogenic strains can be categorized and characterized as MDR, XDR, and PDR (pandrug-resistant) (Magiorakos et al. 2012). The acquisition of evolutionary mutations in various genes involved in survival and their exchange among various microbial populations are the main causes of the development of resistance. This is primarily due to the inequitable and improper use of antibiotics in humans and animals, which enforces the need for new antibiotics for their therapeutic management (Meier et al. 2022).

About 6KT out of the 9KT of total antibacterial consumption in Europe is given as growth promoters to animals, accounting for nearly 90% of all antibiotics used in veterinary applications are administered orally, exceeding the recommended effective dose range far too frequently (Cantas and Suer 2014). This may stimulate resistance development by the commensal microflora of the animal gastrointestinal tract serving as a major source of resistance genes for harmful microorganisms. Furthermore, antibiotic residues enter the intestinal flora or food chain, accelerating the development of antibiotic resistance in humans (Prasad et al. 2021).

Currently, a variety of nanoparticles have been proposed for use in medical science due to the rapid development of nanotechnology. Nanomaterials will soon be employed to treat a variety of severe or chronic ailments due to their distinctive chemical and physical features (Angeli et al. 2008). First-line zoonotic bacteria treatments have included chloramphenicol, ampicillin, and sulphamethoxazole (Tollefson et al. 1998; Arshad et al. 2021). Because of the greater toxic effects, resistance of the drug, and protracted pharmacodynamics, amphotericin B, which is frequently used to treat parasite infections, has limited

utilization (Cronenberg et al. 2021). In order to prevent the spread of zoonotic diseases, MDR and antibiotic-resistant bacterial populations have been declared to be the main public health concern (Zhou et al. 2021). Due to numerous drug resistance, a lack of oral bioavailability, reduced permeability, toxicity, and high cost, current chemotherapy cannot cure infection. Considering that virus particles are similar in size to those of bacteria, nanotechnology is beneficial in the fight against both zoonotic diseases and bacteria (Wang et al. 2021).

In this context, nanomedicine can be a useful option for developing cost-effective preventative, diagnostic, and therapeutic solutions to handle communicable diseases in the age of microbial resistance. Nanoparticles (NPs) are well-established components consisting of various successful targeted drug delivery systems, and the characteristic physicochemical features of several nano-formulations have demonstrated excellent bactericidal effects. Besides its therapeutic potential, nano-vaccines and theragnostic uses of nano-formulations have received considerable interest as an alternate way of combating certain microbial pathogens. This book chapter focuses on the latest applications of nanomedicine in battling key bacterial zoonotic and reverse zoonotic diseases, as well as their potential benefits, limits, and future possibilities for developing successful eradication tactics.

2. NANOTECHNOLOGY IN MEDICINE

Nanomedicine is a discipline of medicine that uses nanotechnology data and techniques in order to prevent and cure diseases. Nanomedicine is the use of very small size particles for diagnosis, drug delivery, sensing, or impelling in a live body as shown in Fig. 1 (Prasad et al. 2021).

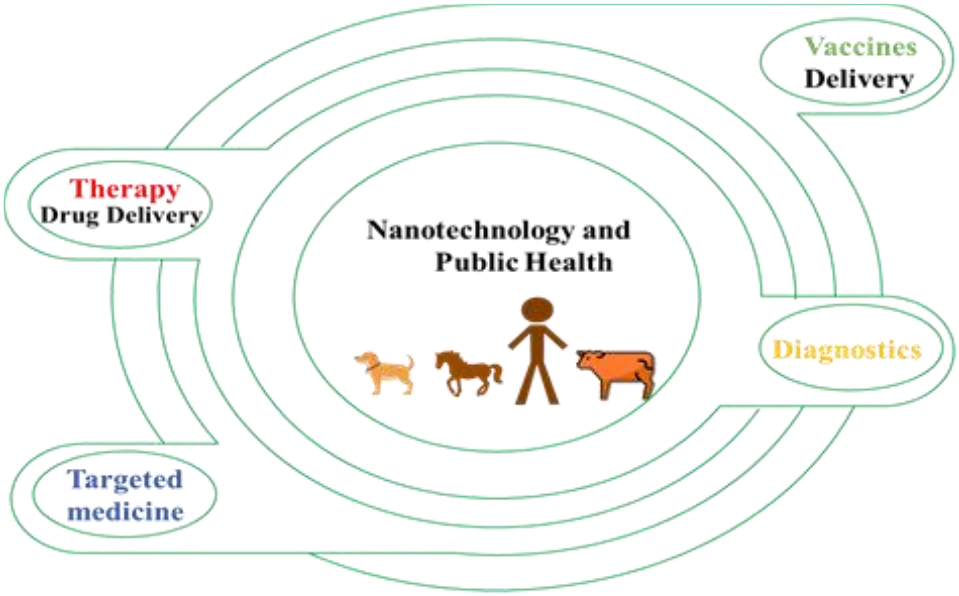

Fig. 1: Application areas of nanotechnology in public health

2.1. NANOTECHNOLOGY FOR USE IN PREVENTIVE MEDICINE

A strict preventative approach is critical for avoiding the fast spread of infectious diseases. Continuous efforts have resulted in a number of advancements in both conventional and new-generation vaccines. Various vaccines based on sub-units as well as DNA against various infectious like tuberculosis and brucellosis etc and transmissible diseases, such as the MVA85A, VPM-1002, and Vaccae TM (M. vaccae) vaccines for tuberculosis (Tameris et al. 2013), Ty21A, and Vi polysaccharide

vaccines for salmonella, Escherichia coli (E. coli) O157 vaccine for cows (Matthews 2013) "Brucella chemical vaccine" (BCV) for human and strains have been developed. However, these developments still leave room for improvement because recent sub-unit vaccines have weak immunogenicity and poor intrinsic in-vivo stability.

Additionally, these vaccines frequently face problems with limited targeting effectiveness, solubility, controlled release deficiency, fast clearance, toxic effects, and need for booster doses that must be dealt with better adjuvants and delivery of antigen through a carrier-mediated mechanism (Kim et al. 2014). Human and animal vaccines have many comparable problems, such as the necessity for protective immunity, safety, and efficient manufacture (Şenel 2021). Traditionally, many of the vaccinations approved for use on animals contain live as well as live-atrophied pathogens having antigenic activity. Subunit vaccines, which are synthesized from one or more specific components obtained from microorganisms acting as an antigen rather than a complete pathogen, are now being developed in response to safety concerns.

Adjuvants are necessary for these vaccinations to generate immunity against pathogens and prolonged safety against infections in animals. Liposomes, nanospheres, nanoemulsions, synthetic and natural polymer-based complexes, dendrimers, carbon nanoparticle-based complexes, inorganic and metal oxide nanoparticles, polypeptide nanoparticles, VLPs (Virus-Like Particles), immune-stimulating complexes (ISCOMs), and other commonly used nanocarriers for vaccine delivery have recently revolutionized the prophylactic management of several communicable diseases (Pati et al. 2018). Vaccine delivery mechanisms, such as liposomes, ISCOMs, virus-like particles, and polymeric particles, can be broadly categorized into two classes: immunostimulatory adjuvants (synthesized from microbes) that frequently show molecular patterns that are linked with pathogens and vaccine delivery systems (Singh and O'Hagan, 2003; Arca et al., 2009; Schwendener 2014).

An established framework for addressing complex needs, like M72+ AS01E, is provided by nanotechnology. It is a liposome-conjugated tuberculosis vaccine that is synthesized by the “M72’’ fusion protein and it is conjugated with the "AS01E" adjuvant complex. This enhances the antigenic ability of “M72’’ that produces a strong immune response. This ensures that it is delivered specifically to the antigen-presenting cells (macrophages and dendritic cells) (Kim et al. 2014). Vaccines are now prepared that penetrates the mucosa of animal and are applied with spray or drinking water. Mass vaccination has been evaluated in feed production, poultry, and other livestock areas. It has proven to provide adequate protection against diseases that could result in a significant financial loss on the farm and to ensure that the antigen remains stable (Scheerlinck and Greenwood, 2006; Calderon-Nieva et al. 2017).

Gregoriadis and Allison (Year) were the first to discover that liposomes can trigger immunological responses to integrated or linked antigens in the early 1970s (Allison and Gregoriadis, 1974). By altering the composition of lipids and factors required in synthesis including charge, size of distribution, catching, and where antigens or adjuvants are included, liposomes can be made to target antigen-presenting cells more effectively (Perrie et al. 2016). Due to these qualities, liposomes have received a lot of interest in the administration of vaccines against animal diseases (Sadozai and Saeidi, 2013). Using liposomes to transfer DNA complexes expressing the Toxoplasma gondii MIC3 gene of sheep was also studied. It has been demonstrated that administering a liposomal vaccination intramuscularly to sheep causes an immunological response against T. gondii (Hiszczyńska-Sawicka et al. 2012).

The immune-stimulating complexes (ISCOMs), which are also being researched for the administration of vaccines in animals, are cage-like particles with an average diameter of 40nm. These are made of Quillaja saponins, cholesterol, and phospholipids (Morein et al. 2004). ISCOMs without antigen is marketed under the brand name ISCOMATRIX and have been investigated as a possible adjuvant for vaccines (Sjölander et

al. 2001). Although the safety of these systems has been shown, it is noteworthy that there aren't many clinical investigations on ISCOMs-based vaccinations in animals, which might be explained by poor local tolerance (Sun et al. 2009; Bigaeva et al. 2016).

VLPs are non-infectious multiprotein structures that are designed to self-assemble from viral structural proteins. They range in size from 20nm to 100nm (Cimica and Galarza 2017). VLPs can be used as effective stand-alone vaccines or vaccination platforms because they have physical properties that are highly immunostimulatory, are structurally comparable to the virus from which they were produced, and have antigenic characteristics with real virions (Mohsen et al. 2017). Although their promise is not fully realized compared to human VLP vaccines there are still difficulties to be resolved with manufacturing procedures or the creation of chimeric VLPs. VLPs are increasingly being evaluated as veterinary vaccinations (Crisci et al. 2012 and Liu et al. 2012).

In the past few years, polymeric nanoparticles (PNPs) have gained a high interest due to their tiny size which gives them distinctive characteristics and behaviors (Farokhzad and Langer 2009). Controlled release, the possibility to integrate therapy and imaging, the preservation of drug molecules and their precise targeting, and the facilitation of improvements in the therapeutic index are benefits of PNPs as carriers (Crucho et al. 2017). Table I shows some recent vaccine development that is based on nanoparticles against zoonotic bacteria.

2.2. NANOTECHNOLOGY IN DIAGNOSTIC FIELD

The identification of pathogens is a crucial step in the diagnosis, effective management, and control of infectious animal diseases. When an animal come in contact with a pathogen, it may take several days, weeks, or even months until whole-organism signs indicate the existence of the disease. By then, the disease may be rampant, necessitating the eradication of whole herds (Şenel 2021). In order to diagnose infections in cattle and poultry, traditional biochemical approaches including enzyme - linked immunosorbent assays (ELISA) and plate-based techniques are being used (Vidic et al. 2017). Animal infectious illness diagnostics has also employed molecular methods like real-time PCR (RT-PCR) and polymerase chain reaction (PCR). However, the application of these techniques is not ideal for field study as they are time-consuming and usually unable to discriminate between low and highly pathogenic strains (Zarlenga and Higgins 2001; Hoffmann et al. 2009).

The inherent drawbacks of conventional diagnostic approaches, which typically rely on culture-based pathogen identification. Serological techniques, and PCR based detection techniques that are typically much time, money, and resource intensive, and are frequently overcame by nanomaterial-based platforms using laser technology, nuclear magnetic resonance (NMR), fluorescence labelling, microfluidics or lab-on-a-chip devices, flow cytometry, or biosensors for diagnosis and imaging (Tallury et al. 2010; Wang et al. 2017; Xu et al. 2018).

Gold nanoparticle (AuNP) based diagnostics have become a popular option due to their distinctive optical features, which include resonant light scattering and surface plasmon resonance (SPR) absorption. In order to help in the diagnosis, the complimentary oligonucleotide conjugated AuNPs can connect with the pathogen's target DNA to produce aggregates that change color visibly. This method of detection has been used to diagnose TB with success (Baptista et al. 2006, Soo et al. 2009). Nanocrystals called quantum dots are created from semiconductor materials. Quantum dots may be created in two ways: top-down (the dimensionality of solid matter is gradually decreased), and bottom-up (quantum dots are formed by chemical synthesis or epitaxial growth). These techniques have been successful in creating quantum dots with diameters of a few nanometers, which are sufficiently tiny to exhibit quantum mechanical features. Quantum dots (QDs) are remarkable for having very composition-and size-dependent optical and electrical characteristics (Pisanic Ii et al. 2014).

Table 1: Vaccine developed on nanoparticles against zoonotic bacteria

Sr. No.	Antigen	Nanoparticles	Bacteria	Application	Action	Model	References
1	Protective antigen (rPA) and lethal factor (rLF)	Nanoemulsion conjugation	*Bacillus anthracis*	Nasal Immunization	Start T helper 1 and 2 response	Mice	(Bielinska et al., 2007)
2	Recombinant BLSOmp31	Poloxamer 407-Chitosan (P407-Ch)	*Brucella ovis*	Intranasal	Starts IgA response	Rams	(Díaz et al. 2019)
3	*Yersinia pestis* V immunogen fused with protein anchor (V-PA)	Near-filed scanning optical microscopy (NSOM), atomic force microscopy (AFM)	*Yersinia pestis*	Intranasal mucosal vaccination	Start T helper 1 and 2 response	Mice	(Huang et al. 2014)
4	Outer membrane protein (Omp31)	Calcium phosphate, Aluminum hydroxide and chitosan NPs	*Brucella melitensis*	Subcutaneous injection	Th17 response	Mice	(Abkar et al., 2019)
5	Glycoconjugate vaccine	Gold nanoparticles (AuNPs)	*Burkholderia pseudomallei*	Intranasal	High antibody titer	Mice	(Gregory et al. 2015)
6	Clostridium perfringens ε-toxin	Membrane-camouflaged nanoparticles (MNPs) Poly (DL-lactide-co-glycolide) Carboxylate End Group (PLGA)	*C. perfringens*	Intravenous	3-day protection	Mice	(Xu et al., 2023)
7	Listeriolysin peptide 91-99 (LLO91-99), glyceraldehyde-3-phosphate dehydrogenase 1-22 peptide (GAPDH1-22)	Gold glyconanoparticles (GNP)	*Listeria monocytogenes*	Intravenous	Vaccinated mothers gave birth to new born free of bacteria	Mice	(Calderón-Gonzalez et al. 2016)

The special qualities of QDs have been used in a variety of ways during the past 15 years to create more accurate, quick, and practical bioassays. The safety of QD components, such as Cd, as well as problems with test repeatability, are of particular concern. Due to these problems, it has been suggested that QDs have been restricted to specialized investigations (Resch-Genger 2008). For the identification of Mycobacteria, *E. coli*, Salmonella, Cholera toxin, etc., the prescribed method has been effectively used (Gazouli et al. 2010; Yang and Li 2006; Goldman et al. 2004).

Numerous medicinal applications, including cancer treatment, nano diagnostics, and bioimaging, have effectively used magnetic nanoparticles (Wang et al. 2017). The increased separation and detection of aligned magnetic nanoparticles bound to targeted drugs in the presence of an applied magnetic field forms the basis for this diagnostic (Shinde et al. 2012). Iron oxide nanoparticles with magnetite or maghemite cores, which are frequently used magnetic nanoparticles, have been employed as contrast to magnetic resonance imaging. In order to identify variety of pathogens including viruses, bacteria, and parasites, the surface of iron oxide nanoparticles can typically be changed and coupled with antibodies, proteins, and nucleic acids. The effective and early detection of the infectious disease (malaria) has been proven using magnetic nanoparticles with an iron oxide core and a silver shell (Yuen and Liu 2012).

For the sensitive and reliable diagnosis of M. tuberculosis for TB, nanodevice-based diagnostic systems have been created. But for now, it's still quite difficult to quickly identify TB patients in underdeveloped nations (Liong et al. 2013). A schematic diagram showing the procedure of M. tuberculosis detection through magnetic barcode assay is shown in Fig. 2. This ligand attached magnetic NP-based probes have

been used to specifically identify a variety of pathogens, including Mycobacteria, Listeria, Staphylococcus, E. coli, and others, to the single cell level (Prasad et al. 2021).

For the detection of different infections, silver nanoparticles, metallic nanowires, Detonation Nanodiamond (DND) Detection Systems, silica nanoparticles, etc. are also utilized; nevertheless, the majority of these revolutionary nano diagnostics are too expensive for widespread usage, especially in developing countries (Tallury et al. 2010; Soo et al. 2012). Table 2 lists the numerous zoonotic and reverse zoonotic bacterial illnesses for which nano-diagnostic uses have been reported.

2.3. NANOTECHNOLOGY FOR USE IN THERAPEUTICS

Currently, drug delivery systems dominate nanomedicine, making up more than 75% of all sales (Wagner et al. 2006). The size range of nanomaterials is comparable to that of proteins and other macromolecular structures found inside live cells. As a result, nanoparticles are prepared to use the cellular machinery already in place to assist the transport of pharmaceuticals. The special properties of nanoparticles (NPs) that include medications are encapsulated, disseminated, absorbed, or conjugated which might improve performance in a range of dosage forms. When properly designed, drug particles can have greater adherence to biological surfaces, higher saturation solubility, quick dissolution, and resistance to settling, all of which contribute to a faster beginning of therapeutic action and higher bioavailability. Furthermore, the bulk of molecules inside a nanostructure are found on the particle surface, maximizing the loading and delivery of cargoes including medicines, proteins, and polynucleotides to selected cells and tissues (Bamrungsap et al. 2012).

Nano formulations are proven bactericidal agents that can be used alone or in conjunction with current antibiotics to treat microbiological diseases. These properties have been skillfully exploited in nanotherapeutics to avoid drug resistance and microbes that produce biofilms (Razei et al. 2017). In order to create nanotherapeutic approaches against microbial superbugs, a wide range of metallic or bimetallic nanomaterials, including iron oxide (Fe3O4), zinc oxide (ZnO), titanium oxide (TiO) NPs, gold and silver NPs, or their combination in a single NP, are widely used (Kulshreshtha 2017; Baptista 2018). In addition to acting as a direct bactericidal agent, nanocarriers offer excellent therapeutic control through targeted delivery of existing antibiotics to the infection sites and sustained drug release to maintain the minimum inhibitory concentration (MIC) for an extended period of time while minimizing potential side effects (Bermudez et al. 2017). This may also help shorten the duration of certain intracellular, antibiotic-resistant microorganisms treatment regimens, such Mycobacteria (Xu et al. 2018).

A variety of nanoforms, including solid metal-containing NPs and polymers as well as biological materials including albumin, gelatin, and phospholipids for liposomes, have been tested as drug delivery systems. High size variation polymer-drug conjugates are often not regarded as NPs. However, they are also included into these nano delivery systems since their size can still be adjusted to within 100nm. These nano delivery systems can be made to have medications dissolved inside the particle matrix, encapsulated inside lipids, or absorbed onto the particle surface. In addition, the increased permeability and retention (EPR) effect allows nanoparticles to aggregate preferentially at tumor, inflammatory, and infectious sites. The EPR effect involves site-specific properties that are not connected to healthy tissues or organs, leading to more precise targeting (Bamrungsap et al. 2012).

2.4. NANOTECHNOLOGY FOR TREATMENT OF BACTERIAL AND REVERSE ZOONOSES

Infectious diseases, particularly bacterial diseases, are huge burden on public health, killing about 14 million people each year (Nii-Trebi 2017; Ali et al. 2017). Bacterial infections molecular components, such as their genetic material, ribosomes, cell membranes, cell wall, and biosynthetic pathways, differ greatly

Table 2: Some recent nano diagnostic tools for detection of zoonotic bacteria

Sr. No.	Detection technique	Nanoparticle	Pathogen	Application	Model	Reference
1	Magnetic bead-based DNA detection assay	QDs associated through biotin–streptavidin conjugate	*E. coli* O157:H7	might be used for quick illness diagnosis	*In-vitro*	(Liu et al. 2008)
2	Surface-Enhanced Raman Scattering (SERS)	Nano silver associates	*E.coli*	Helpful in pathogen detection	*In-vitro*	(Zeiri and Efrima, 2006)
3	Genus-specific anti-lipopolysaccharide (LPS) monoclonal antibody (mAb)	Gold nanoparticle	*Salmonella* spp.	Novel competitive strip sensor for fast detection	*In-vitro*	(Wang et al., 2016)
4	Bio-barcoded electrochemical biosensor	Gold nanoparticles (AuNPs), magnetic nanoparticles (MNPs)	*B. anthracis*	Potential applications in multiple detection of bioterrorism threat agents	*In-vitro*	(Zhang et al., 2010)
5	Aptamer	Fe3O4@Au magnetic nanoparticles	*S. aureus*	High specificity can be achieved within 50 min	*In-vitro*	(Pang et al., 2019)
6	Magnetic separation and lateral flow immunoassay (LFIA)	Fe3O4	*L. monocytogenes*	Low cost, good selectivity and convenience	*In-vitro*	(Du et al., 2022)
7	Loop-mediated isothermal amplification (m-LAMP) amplicons	Colloidal gold solution	*Leptospira*	Fast detection	Urine	(Bamrungsap et al., 2012)

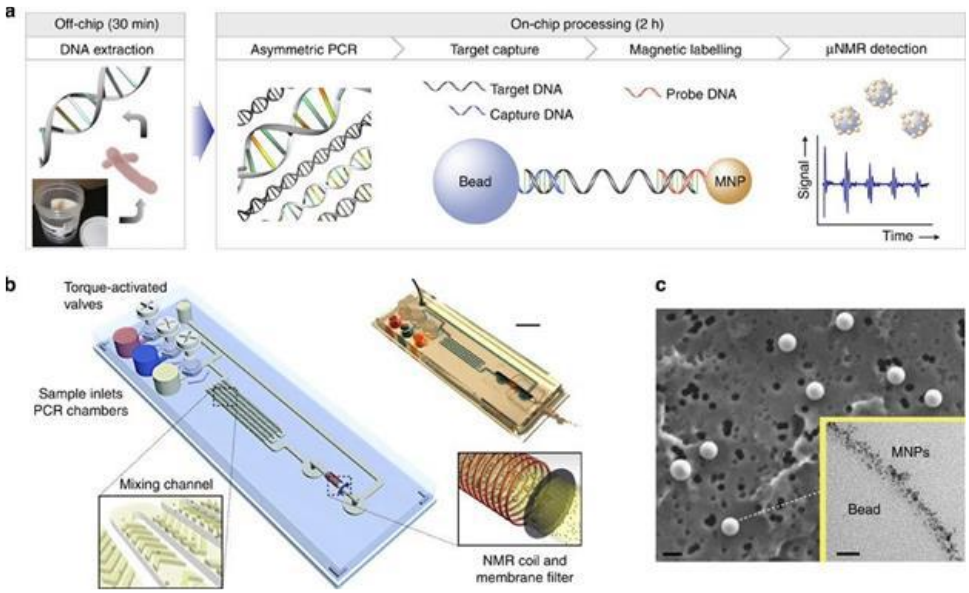

Fig. 2: Magnetic barcode assay: Amplified isolated DNA is labbled with MNPs beads and varifiation is done under electron microscope (Liong et al. 2013)

from human cells, these elements are typically used to develop antimicrobial drugs (Ganewatta and Tang 2015). Efflux pumps, enzymatic suppression by hydrolytic degradation or chemical alterations like the addition of a phosphate group, acetylation, hydrolysis, changing target, reprogramming biological synthesis, and accelerated evolution of acquired resistance in microorganisms are some of the methods

used by bacteria to resist antimicrobials (Van et al. 2018). Furthermore, several antibiotics, including fluoroquinolone and aminoglycosides, have detrimental side effects (Poulikakos and Falagas 2013). The majority of multidrug resistance (MDR) infections need extended antibiotic therapy that are accompanied by significant health-care costs (Masri et al. 2019). Some recent nano antibiotics have been listed in Table 3.

Several of the most common infectious diseases are bacterial in origin and have the potential to spread through zoonotic and reverse zoonotic mechanisms, including tuberculosis, salmonellosis, shigellosis, pneumococcal disease, campylobacteriosis, listeriosis, and E. coli infection. These diseases pose a serious risk to the health of both humans and animals worldwide (Cassini et al. 2018). The rapid development of antibiotic resistance among these microbial groups is also posing a significant threat, as evidenced by the fact that Salmonellae and Staphylococcus aureus were designated as high priority bacterial pathogens that cause disease in the first report by the WHO on high priority list of resistance bacteria while Enterobacteriaceae group bacteria were documented as critical (Zaragoza-Bastida et al. 2020).

With the conjugation of oral amikacin administration and lipid-crystal NP delivery method, MatinasBioPharma (MTNB) has disclosed significant preclinical effectiveness of MAT2501 in the in-vitro model of Mycobacterium abscesses infection (Da-Silva et al. 2012). Since liposomal NP core has a large drug loading capacity, it is possible to prevent hydrophilic antimicrobial medicines from being degraded in-vivo by encasing them within the liposomal NP core. Aerosolized liposomal antibiotics (ciprofloxacin, tobramycin, amphotericin B, and amikacin) have been shown to have a considerable curative impact in the prevention and treatment of acute and chronic respiratory tract infections (Bassetti 2020).

Combining AgNPs with Simvastatin led to a synergistic impact of bactericidal antibacterial activity against a number of resistant species, including extended spectrum beta lactamase producing E. coli and methicillin resistant S. aureus (Figueiredo et al. 2019). Silver nanoparticles due to their antibacterial qualities, are also applied in the medical industry to treat skin wounds and dermatitis (Owusu et al. 2016). Since S. aureus and K. pneumoniae have been shown to be efficiently controlled by Cryson nano sliver antibacterial and deodorant agent, it may be useful to manage bacterial zoonoses. A copper oxide-nanorod-based artificial enzyme called "NanoZymes" has been shown to fight against E. coli and Golden Staph infections by photo modulated reactive oxygen species formation, which is efficient in controlling nosocomial and aerosol infections (Jansson et al. 2014). The development of novel nano-antimicrobials using a wide range of molecules against numerous resistant and biofilm-producing microbes is ongoing, but them in-vivo efficacy, toxicity, cost effectiveness, and economic viability need to be properly assessed before common applications of these formulations can be made for greater public health benefit (Hadad et al. 1995).

3. CONCLUSION

The scientific field of nanotechnology works with particles with a size range of a few nanometers. According to the article, nanotechnology has uses in the detection, diagnosis, and management of industries, including pharmaceuticals, health sciences, and livestock etc. The creation of nanoparticles involves different metals, including nickel, gold, silver, and casein micelles etc. Nanotechnology also makes it feasible to administer drugs. Past predictions of nanomedicine as a cure-all have generated more enthusiasm than reality, and the underlying difficulties are frequently disregarded until they are suddenly noticed during clinical translation. The potential for nanomedicine is unquestionably great, but the understanding of how these nano-formulations behave in-vivo for therapeutic use is limited. These problems are now being addressed more precisely for practical application and are anticipated to produce more practical answers soon.

Table 3: Recent nano antibiotics against zoonotic bacteria

Sr. No.	Nano antibiotic	Deliver system	Pathogen	Action	Model	Reference
1	AgNP with Colistin, penicillin G and Amoxicillin	Adjuvants	*Salmonella enterica, Staphylococcus aureus, Escherichia coli, Actinobacillus pleuropneumoniae, Streptococcus uberis, Pasteurella multocida*	Antibacterial actions against resistant strains	*In-vitro*	(Smekalova et al. 2016)
2	Gentamicin-loaded magnetite block ionomer complexes (MBICs)	Magnetite block ionomer complexes	*B. melitensis*	High clearance of pathogen	*In-vitro*	(Jain-Gupta et al. 2013)
3	Doxycycline hydrochloride and rifampicin	Polymeric nanoparticles	*B. abortus*	Effective	*In-vitro* and mice	(Dawre et al. 2022)
4	Zinc oxide nanoparticles (ZnO-NPs), copper oxide nanoparticles (CuO-NPs)	Food borne zoonosis control	*L. monocytogenes*	Effective antibiotic action	*In-vitro*	(Osaili et al. 2019)
5	Rifabutin	Encapsulated in liposomes	*Tuberculosis*	Increased therapeutic activity	Rat	(Gaspar et al. 2008)
6	Iron oxide nanoparticles (α-Fe2O3)	Anti-bacterial	*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Escherichia coli*	Strong Antibiotic activity	*In-vitro*	(Buarki et al., 2022)
7	Metal-based dendrimeric nanoclusters with isoniazid	Dendrimer complexed with copper	*M. tuberculosis*	Alternative and an innovative therapy in the treatment of tuberculosis	*In-vitro*	(Rodrigues and Shende, 2020)

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Vibrionaceae and Fish Zoonosis

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ABSTRACT

Fish carry a wide variety of illnesses, some of which may be transmitted to humans (known as zoonotic diseases). There are primarily two sources of human illness included consuming raw fish (can be undercooked) or by the water contaminated with mucus or feces of already infected fish. The transmission of fish illnesses to people is complicated by a number of factors, including microorganisms (bacteria, viruses, parasites, fungus), host state (open sores on the body, spine penetration, immunocompromised), and environmental variables (unclean water). Gram-negative and Gram-positive bacteria are two broad types of zoonotic bacteria but the gram-negative is main agents of fish zoonosis. Members of the family Vibrionaceae are Gram-negative filaments causing various human, fish and shellfish infections come from a variety of their species. Humans may get diarrhoea, lesion infections, and sometimes extraintestinal infections as a result of certain vibrios. Vibrio (V.) cholerae (including V. cholerae O1/O139 strains responsible for cholera and additional V. cholerae strains associated with diarrhoea, wound infections, and septicemia) is responsible for the majority of the world's most severe diseases, followed by V. parahaemolyticus and V. vulnificus. Vibrios have been also linked to causing the condition known as vibriosis. Molecular identification is a key tool in clinical diagnostics. For species-level confirmation, real-time PCR and conventional PCR are both effective methods for detecting all major Vibrio spp. pathogens. To make disease management quick and efficient and to gives the knowledge required to stop and treat aquatic zoonotic pathogens, it is necessary to regularly examine and manage the quality of fish ingested. Thus, a multidisciplinary approach that takes into account the potential fish pathogens, features of fish biology, and a full understanding of environmental factors is necessary for the implementation of successful disease prevention and control approaches. The One Health (OH) concept should be enhanced and extended as it has become relevant in the treatment of zoonotic fish infections.

Keywords: Fish Zoonosis, Vibrionaceae, Zoonotic Agents, Zoonotic Detection, Antibiotic Resistance, Diagnosis, Prevention and Control

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1. INTRODUCTION

1.1. FISH ZOONOSIS

Increases in global population and per capita fish consumption have contributed to a surge in seafood demand in recent years. While the aquaculture sectors have demonstrated global expansion sustainably, but these are not without their share of risks (Shamsi 2019). Due to the importance of seafood as a protein source for people, there is always the chance of contracting a waterborne disease in addition to getting seafood poisoning. Environmental Health and Safety (EHS)/Occupational Health (2016) and Raissy (2017) report that many crucial components of fish and water may transfer illness to people. The immune system plays a significant role in determining the severity of aquatic zoonotic illnesses.
However, there are primarily two sources of human illness.
1- Consuming raw fish (can be undercooked) or by the water contaminated with mucus or feces of already infected fish is the primary risk factor for acquiring a fish-borne illness.
2- Transmission occurs when the infectious agent comes into contact with an open wound or abrasion/scratch on the skin (Raissy 2017).
3- Raissy (2017) reports that 46% of fish-derived zoonotic diseases are transmitted orally, while 15% of zoonotic illnesses originate in fish and may spread via more than one route. 24% of transmission occurs via direct skin contact with fish, and 19% occurs by ingestion of water containing infected organisms.

2. FISH ZOONOSIS CASE STUDY

Research found that eating contaminated fish in Americans causes around 260,000 illnesses each year. From the food groups causing outbreaks, most commonly reported is the fish meat. The Foodborne Disease Outbreak Surveillance System (FDOSS) of the Centres for Disease Control (CDC) gathers information on foodborne illness outbreaks. Additionally, according to Barrett et al. (2017), fish was implicated in about 857 outbreaks that resulted in 4,815 illnesses, 359 hospitalisations, and 59 deaths. These perilous fish-borne zoonosis outbreaks that have been recorded throughout the years highlight the need of keeping an eye on these diseases.
Emerging diseases are widespread in aquatic species. According to World Health Organization (WHO) "an emerging disease has appeared in a population for the first time, or may have existed before but is rapidly increasing in incidence or geographic range." The possibility for transmission from animals to humans is one aspect of emerging illnesses that is not well understood. The transmission of fish illnesses to people is complicated by a number of factors, including microorganisms (bacteria, viruses, parasites, fungus), host state (open sores on the body, spine penetration, immunocompromised), and environmental variables (unclean water). Bacteria, parasites, and viruses are the most significant infectious agents associated with fish (Meurens et al. 2021).

3. BACTERIAL ZOONOTIC AGENTS

Gram-negative and Gram-positive bacteria are two broad types of bacteria but the gram-negative is main zoonotic agents of fish. Even fish that seem healthy may have germs, especially in the kidneys and intestines (Meron et al. 2020).

4. THE FAMILY VIBRIONACEAE

Members of the family Vibrionaceae are Gram-negative filaments that may be straight or curled. They used polar flagella for the movement. Respiratory and fermentative metabolic processes are both used

by facultative anaerobes. Some species including few strains of some species are bioluminescent, generally aquatic, often found alongside aquatic creatures and flora, and may be found in fresh, brackish, and saline water. Human infections come from a variety of species. Fish, eels, and other aquatic animals are harmed by some species. The Vibrionaceae family currently has 143 described species, which are grouped under the phylum Proteobacteria. The six genera *Aliivibrio*, *Enterovibrio*, *Grimontia*, *Photobacterium, Salinivibrio* and *Vibrio* make up the class Gammaproteobacteria (Farmer and Janda 2015).

5. HABITATS

According to Campbell et al. (1957), Baumann and Baumann (1981), Sakazaki and Balows (1981), Simidu and Tsukamoto (1985), members of the Vibrionaceae family occupy specific ecological niches. Humans may get diarrhoea, lesion infections, and sometimes extraintestinal infections as a result of certain vibrios. Infected wounds and widespread illnesses are caused by bacteria in aquatic animals. Aquatic settings are rich in vibrios and similar species. The distribution of these organisms is influenced by many variables, but the most important ones are probably specific human, animal, or plant hosts, readily accessible inorganic nutrients and carbon sources, temperature, salinity, dissolved oxygen, and, for aquatic species, depth below the surface.

6. REPRESENTATIVES OF VIBRIONACEAE

Members of the family Vibrionaceae are now a major concern to fish and shellfish infections (Fig. 1, 2, and 3). The resurgence of interest has led to the description of new species and a deeper knowledge of the biology of long-known taxa. Several species have been discovered so far as potential fish pathogens. Numerous species of vibrio have now been recognized. Ten of these creatures have been isolated from humans. *Vibrio (V.) cholerae* (including *V. cholerae* O1/O139 strains responsible for cholera and additional *V. cholerae* strains associated with diarrhoea, wound infections, and septicemia) is responsible for the majority of the world's most severe diseases, followed by *V. parahaemolyticus* and *V. vulnificus*. There are four more species of *Vibrio* that may be harmful to humans but often only cause less severe illness including *V. mimicus*, *V. fluvialis*, *V. furnissii*, and *V. alginolyticus*. As a consequence of recent taxonomic work, two closely related species that were formerly classified as members of the genus *Vibrio* have been reclassified as separate entities, *Grimontia hollisae* (previously *Vibrio hollisae*) and *Photobacterium damselae* subspecies *damselae* (previously *Vibrio damselae*). *V. metschnikovii, V. cincinnatiensis*, and *V. carchariae* have been the primary focus of case reporting, however it is yet unknown how these infections are significant to people. Their potential as human pathogens has been called into question (Morris 2013).

7. GENUS *VIBRIO*

Most commonly members of the genus *Vibrio* are capable of imitating widespread fish and human diseases. *Vibrio* is ubiquitous in estuarine and coastal marine environments and displays seasonal population changes. The pace at which organic matter is broken down in these habitats influences the amount of dissolved organic carbon at higher trophic levels in the marine food web. However, certain strains of Vibrio are opportunistic bacteria that may make humans and marine animals infectious (Austin 2010).

There is a lot of genetic and biological similarity amongst Vibrio species. Horizontal gene transfer and recombination, sometimes known as the "borrowing of genes from other species," shaped their two chromosomes, or genomes. There is genetic diversity among these illnesses, but they all originate in marine and aquatic environments. They thrive in somewhat salty, moderate water, and their numbers in

Fig. 1: Vibriosis-affected turbot with extensive surface bleeding (Photograph courtesy of Professor X.-H. Zhang)

Fig. 2: On the surface of olive flounder is an ulcer caused by *Vibrio sp*. (Photograph courtesy of Dr. D.-H. Kim)

Fig. 3: Hemorrhaging on the fins and around the opercula of a sea bass. The etiological agent was *V. anguillarum* (Photograph courtesy of Dr. V. Jencic)

the wild tend to increase as the temperature rises (Baker-Austin et al. 2017). *Vibrio spp*. are the most common pathogens in aquatic environments and seafood, contributing to human sickness. *V. cholerae, V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus* are some of the most often seen pathogenic species. There is a distinct seasonal trend to infections caused by *Vibrio spp.,* with the majority of cases occurring in the winter. Infections caused by *Vibrio spp.* often manifest in people when they come into contact with contaminated water or consume seafood that has not been properly prepared (Table 1) (Oliver 2005).

8. CHOLERA AND NON-CHOLERA INFECTIONS

Cholera and other illnesses caused by the dangerous *Vibrio* bacteria may be roughly divided into two categories, which are cholera and non-cholera infectious groups. Cholera is a potentially fatal diarrheal sickness caused by ingesting contaminated food or water. Even purified water may harbor the cholera virus. Non-cholera *Vibrio spp*., such as *V. parahaemolyticus* and *V. vulnificus*, are the etiological agents of vibriosis, a group of disorders whose clinical manifestations vary based on the pathogen species, route of infection, and host susceptibility. Mild gastroenteritis or primary septicaemia (septicaemia caused by eating infected food that is raw or undercooked) may be caused by non-cholera bacteria, whereas wound

Table 1: Most important vibrio members behaving as human pathogens (Baker-Austin et al. 2018).

vibrio member	Infectious sources			Infectious route		Clinical demonstrations
	Sea food	Sea water	Fresh water	Oral contact	Wound contact	
V. cholera (O1 and O139 strains)	Rare	Rare	Yes	Yes	Rare	Cholera, gastroenteritis and rare wound infections
V. cholera (Remaining Strains)	Yes	Yes	None	Yes	Yes	Wound as well as ear infection and gastroenteritis with rare septicemia
V. vulnificus	Yes	Yes	None	Yes	Yes	Sepsis with gastroenteritis and wound infections
V. parahaemolyticus	Yes	Rare	None	Yes	Yes	gastroenteritis as well as wound infections with rarely sepsis occurrence
V. mimicus	Rare	Yes	None	Yes	Yes	Rare wound, ear as well as ear infection with gastroenteritis occurrence
V. fluvialis	None	Yes	None	Yes	Yes	Rare wound, ear as well as ear infection with gastroenteritis occurrence
V. alginolyticus	None	Yes	None	None	Yes	Common ear and wound infection with rare sepsis
V. hollisae	Yes	Yes	None	Yes	None	Gastroenteritis as well as wound infections in common and rare sepsis
V. metschnikovii	None	Yes	None	Probable	None	Common sepsis as well as gastroenteritis

infection and secondary septicaemia can be caused by exposure to contaminated water. Non-cholera *Vibrio spp*., found in seawater and shellfish, prefer moderate to high salinities. The most significant environmental human illnesses originating in aquatic and marine environments are caused by these bacteria (Thompson and Swings 2006).

9. *VIBRIO* IN MARINE FISH

The most prevalent *Vibrio* species in marine fish are *V. vulnificus* and *V. parahaemolyticus*, whereas *V. cholerae, V. vulnificus*, and *V. parahaemolyticus* are responsible for the bulk of human diseases. Fish infected with *Vibrio* often exhibit listlessness, skin lesions, exophthalmia, and ultimately death as clinical manifestations. Splenic enlargement, abdominal dropsy, intestinal inflammation, epidermal haemorrhage, scale exfoliation, pop-eye, and tail decay are among other signs that have been reported (Smith 2011).

10. *VIBRIO* SPECIES IN HUMANS

In humans, *V. cholerae, V. vulnificus,* and *V. parahaemolyticus* provide the most risk since they may cause gastroenteritis, wound infections, and septicemia, respectively. Other *Vibrio* species, such as *V. mimicus, V. fluvialis, V. furnissii,* and *V. alginolyticus*, have also been linked to human infections; however, their associated disorders tend to be milder (Baker-Austin et al. 2018). Regarding the environmental prevalence, the reports of the isolation of three species*, V. metschnikovii, V. cincinnatiensis,* and *V. carchariae,* may have been more indicative of asymptomatic colonization than infection (Morris Jr et al. 2003).

11. VIBRIOSIS

Several species of *Vibrio* and *Photobacterium* cause major infections in fish, crustaceans, mollusks, corals, and rotifers. These bacteria have been linked to causing the condition known as vibriosis (Gomez-Gil et al.

2014). The pathogen *Aliivibrio salmonicida*, formerly known as *V. salmonicida,* causes the Hitra disease or cold water vibriosis in Atlantic salmon (*Salmo salar*). These reclassifications are the result of recent developments in *Vibrio* taxonomy. Broadly speaking, vibriosis refers to any kind of septicemia brought on by these tiny organisms. According to the research Red limb illness was also called penaeid vibriosis, luminous vibriosis, and penaeid bacterial septicemia until it was renamed. An epizootic is a brief worldwide pandemic of a communicable illness in a constrained geographical region. Since then, it has been discovered in a wide variety of marine organisms and invertebrates around the globe. It was first seen in eels. Vibriosis has become a substantial economic influence on marine fish culture, significantly impacting many fisheries and other farmed animals, since all marine fish are vulnerable to at least one *Vibrio* species. Cell morphology, physiology, and biochemistry of a specific *Vibrio* species are commonly determined using biochemical tests and molecular approaches (Austin et al. 2012).

12. VIBRIOSIS THREE PRINCIPAL PHASES

Invasion (through skin, appendages, gills, or anus), tissue and host cell damage, and outflow (perhaps resulting in death) are the three primary phases of vibriosis. Siderophores, extracellular products (ECPs), hydrolytic enzymes, and poisons are all examples of the types of virulence factors that *Vibrio* may create. Although specific *Vibrio* species have been linked to a 100% fatality rate, resistance to vibriosis relies on how the infection, host, and environment interact (Hernández-Cabanyero and Amaro 2020).

13. HIGH-RISK *VIBRIO* SPECIES

- ***V. parahaemolyticus***

V. parahaemolyticus is the pathogen most often associated with food-borne gastroenteritis in a variety of countries, accounting for around 25% of cases. Rarely, eating infected raw or undercooked fish may be lethal, leading to invasive septicemia or acute gastroenteritis (Zarei et al. 2012).

- ***V. alginolyticus***

V. alginolyticus has the potential to greatly raise human morbidity and mortality rates. Exposure to saltwater has been linked to cases of gastroenteritis and significant extraintestinal infections such as otitis externa and traumatic wound infections. *V. alginolyticus,* which was formerly the third most prevalent *Vibrio* species to cause human illness, is now the second most common (Gomez et al. 2003).

- ***V. vulnificus***

Human septicemia, necrotizing wound infections, and gastroenteritis are largely brought on by *V. vulnificus*. Contrary to *V. parahemolyticus, V. vulnificus* produces septicemia with severe symptoms and a mortality rate of more than 50%. It is also very invasive (Tao et al. 2012).

14. ZOONOTIC DETECTION

Numerous facultative diseases with an environmental niche, which are usually difficult to differentiate between infections in common and strict zoonosis, are generally classified as fish zoonosis. It is essential to establish if human and fish illnesses are caused by the same organism before making any inferences

given what is known about the characteristics of diseases produced by different bacterial agents in people and fishers, as well as their transmission mechanisms (Neogi et al. 2010). There is little data on whether illnesses in animals and humans are brought on by the same bacterial strains, serotypes, or in certain cases, species. Our capacity to identify whether human diseases have come from infectious fish, the environment, or briefly colonized or contaminated fish products has improved and thanks to the use of molecular tools (Di Pinto et al. 2005).

15. *VIBRIO* DETECTION IN DIETARY SAMPLES

Selective media, such as thiosulfate citrate bile salts sucrose (TCBS), are often used in conventional microbiological techniques for the detection of *Vibrio* in food samples. Isolating organisms from seafood and marine habitats may be challenging, time demanding, and less sensitive when utilizing standard phenotyping and biochemical testing procedures. New molecular techniques have developed as a response to these problems. *V. parahaemolyticus* was previously identified in seafood using the gyrB and toxR loci. *V. alginolyticus, V. vulnificus,* and *V. parahaemolyticus* collagenase gene sequences are genetic markers (Di Pinto et al. 2005; Neogi et al. 2010).

16. TRANSMISSION OF *VIBRIO* FROM FISH TO HUMAN

Food contamination continues to be an issue on a global scale. Recent changes in food consumption habits and improvements in food production and processing methods have brought up new risks. Summertime ingestion of unclean water and undercooked seafood is another epidemiological sign of *V. cholerae* spread (You et al. 2021).

Consequently, isolated species cannot be detected using traditional biochemical techniques. Molecular identification is a key tool in clinical diagnostics. PCR-based detection focuses on specific DNA sections to identify bacterial strains. Additionally, PCR amplified the 16S rRNA gene, generated positive findings, and allowed the identification of live but uncultivable isolates in the sample. Its usage among academics is expanding since it is less labor-intensive and considerably quicker than traditional approaches (Teh et al. 2010). *Vibrio* may be used to spread illnesses including lesions, septicemia, erythema, and tissue necrosis from fish to people. Increased customer preference for prepared seafood, such as fresh fish flesh segments, may result in diseases connected to *V. parahaemolyticus* (You et al. 2021). A crucial zoonotic pathogen that endangers the public's health is *V. vulnificus.* Consuming raw shellfish has been shown to cause primary septicemia in people. It may also cause secondary septicemia when exposed to saltwater (Carmona-Salido et al. 2021).

17. MAJOR INFLUENCE OF ANTIBIOTIC RESISTANCE ON ZOONOSIS

For more than 60 years, antibiotics have been regarded as a successful therapy for bacterial infections. Microorganisms, conversely, have evolved diverse resistance mechanisms to fight against the innovative drugs that are employed to kill them. Over 50,000 people die each year in Europe and the US alone as a consequence of infections brought on by resistant germs, which has rapidly grown in recent years. The mortality rate from this illness is noticeably greater in poor and impoverished countries (Mackey et al. 2014). Due to the massive use of various antibiotics in aquaculture to promote development and prevent bacterial infections, antimicrobial resistance has grown to be a severe threat to both human and veterinary health worldwide. Infected food may directly transfer antimicrobial-resistance genes to people, particularly those linked to mobile genetic elements (Shakerian et al. 2018).

The AMR pattern differs from nation to nation depending on the use of antimicrobial drugs. Between 2000 and 2015, the worldwide consumption of antibiotics rose by 36%, with notable regional variances. Gram-negative AMR pathogens are the most often used therapy for *Vibrio* species, notably *V. cholerae* (Wibisono et al. 2020). Antibiotic usage in India was 12.9×10^9 units per person per year on average in 2010. Antimicrobial drugs usually enable efficient surveillance of harmful microorganisms that cause infectious diseases. The incorrect use of antimicrobial treatments in society causes the emergence of bacteria that are resistant to the drugs, which might endanger human health (Riwu et al. 2020; Widodo et al. 2020).

18. DIAGNOSIS AND SCREENING

18.1. DIAGNOSIS OF CHOLERA

Cholera is a severe form of diarrhoea that causes fast fluid loss (dehydration) and is characterized by rice-water stools that must be forcibly evacuated (purged) at a speed of roughly 1 liter per hour. Nausea and vomiting are common symptoms of this severe diarrhoea. Most instances of *V. cholerae* infection are asymptomatic, accounting for around 75% (WHO 2016), whereas 5% are mild, 35% are moderate, and 60% are severe (Qadri et al. 2005; Harris et al. 2008). The incubation period for *V. cholerae* is normally five days, but may range from a few hours to several days. A person may be infectious (i.e., release live microbes in their faeces) for up to fourteen days, as stated by the World Health Organization (2017). When a patient checks into a healthcare facility, they often have a stool or blood sample obtained for microbiological identification (Azman et al. 2013).

18.2. DIAGNOSIS OF VIBRIOSIS

If a patient presents with watery diarrhoea and has recently consumed raw or undercooked seafood, especially oysters, or if a wound infection develops after being exposed to sea water, the doctor may suspect vibriosis (CDC 2017). The vast majority of *V. parahaemolyticus* infections are short-lived and not very severe. The incubation time for *V. parahaemolyticus* infections typically lasts between 12 to 24 hours after ingestion. Stomachache, diarrhoea, nausea, headache, fever, and chills are all common clinical manifestations. In situations of severe gastrointestinal vibriosis (such as *V. vulnificus* infection, where 90% of patients need hospitalization), obtaining the patient's exposure history is critical. Those with preexisting conditions like diabetes or liver disease are at a higher risk. The typical incubation period for *V. vulnificus* infections is 48 hours from ingestion to the onset of symptoms, and 16 hours when open wounds are involved. This highlights the need of prompt diagnosis. In extreme cases of *V. vulnificus* infection, severe necrotizing fasciitis might occurs. Microbiological confirmation of the diagnosis is performed by collecting the necessary clinical samples (faeces, blood, lesions, or ear secretions) (Baker-Austin et al. 2017).

18.3. MICROBIOLOGICAL DIAGNOSIS

Vibrio spp. are often easily cultured from clinical samples. Using TCBS agar, which consists of thiosulfate citrate, bile salts, and sucrose, is the gold standard for isolating and subculturing *Vibrio spp*. *V. cholerae* and *V. alginolyticus*, use sucrose for energy and produce yellow colonies on TCBS agar medium, whereas *V. parahaemolyticus, V. mimicus*, and *V. vulnificus*, utilize other sugars and produce green colonies. Several other media may be used to develop colonies that appear green on TCBS agar; for example, blood agar and CHROM agar can be used to isolate *V. parahaemolyticus*, while cellobiose-polymyxin B-colistin (CPC) medium can be used to isolate *V. vulnificus* (Croci et al. 2007). In the United States, *Vibrio spp.* are

often initially isolated from clinical samples using blood agar. Samples that provide positive culture findings may be sent to specialist labs for confirmation testing, which often entails species-specific PCR methods. For species-level confirmation, real-time PCR and conventional PCR are both effective methods for detecting all major *Vibrio spp*. pathogens (Nordstrom et al. 2007).
Vibrio spp. are routinely identified by biochemical assays; however, these techniques have drawbacks. For example, the right serological testing to further classify isolates has constraints. These techniques, for instance, are expensive, time-consuming, labor-intensive, and need highly skilled employees to interpret the data (Martinez et al. 2006; Croci et al. 2007).

19. PREVENTION AND CONTROL

Microbial agents in fish may increase public health problems, hence it is crucial to inform the public about germs and the dangers of eating raw or undercooked fish. It is necessary to regularly examine and manage the quality of fish ingested. This makes disease management quick and efficient and gives the knowledge required to stop and treat aquatic zoonotic pathogens (Bibi et al. 2015).
As fish are raised in a system that depends on natural environmental conditions for production, it is challenging to control fish zoonotic agents. The deterioration of the aquatic environment, which is also a key element in determining fish health, is the primary cause of the majority of fish illnesses. Thus, a multidisciplinary approach that takes into account potential fish pathogens, features of fish biology, and a full understanding of environmental factors is necessary for the implementation of successful disease prevention and control approaches (Toranzo et al. 2005). Cleaning and maintaining a pond effectively reduces the amount of host species that might disrupt ecosystems and animal populations. Several studies have shown that ponds that have not been cleaned and sanitized prior to restocking increase the likelihood that pathogens would be retained (Clausen et al. 2012; Tran et al. 2019).
A community's risk for contracting a fish-borne disease may be affected by a number of variables, such as its location, access to raw seafood, diet, level of hygiene, and fishing practices. Behavior in one's personal and social life is equally crucial. Diseases caused by eating contaminated fish are prevalent not only in the developing countries but around the world (Chai et al. 2005). Growing international markets, increased consumer demand, improved transportation infrastructure, and demographic shifts all contribute to the prevalence of fish-related disorders in developed nations. There are steps that may be done to lessen the danger presented by zoonotic viruses throughout the harvest, storage, processing, and post-processing phases. The seafood sector and government agencies may help to reduce the hazards presented by zoonotic fish-derived diseases by implementing different initiatives, such as good manufacturing practices (GMP) systems. Antibiotic treatment is common in bacterial zoonotic infections, thus certain zoonotic factors may be controlled by the use of antibiotics (Shin and Park 2018). As a result, people who work with fish need to be aware of zoonotic illnesses and preventive measures. Although avoiding all interaction with water and fish in aquaculture systems is unrealistic, prophylaxis may be the best way to lower the risk of these zoonotic illnesses (Smith 2011).
Wearing disposable gloves and avoiding contact with fish fluids are vital. It is critical to see a doctor even if general symptoms appear. The best strategy is frequent hand washing, especially after coming into touch with fish or water directly. It's also important to avoid doing anything that can contaminate your clean hands, such eating or drinking. Vectors, insects, and other pathogens may spread zoonotic diseases to humans via a variety of routes, including ingestion, inhalation, contact with inanimate objects, and direct or indirect contact. Preventing the spread of fish parasites requires regular maintenance of fishing infrastructure and technological equipment. Parasites may be killed by frying fish for 15 seconds at 62 °C (however this may not be adequate to destroy all bacterial toxins) (Shamsi 2016). Methods for managing, preventing, and monitoring zoonotic pathogens are shown in Fig. 4.

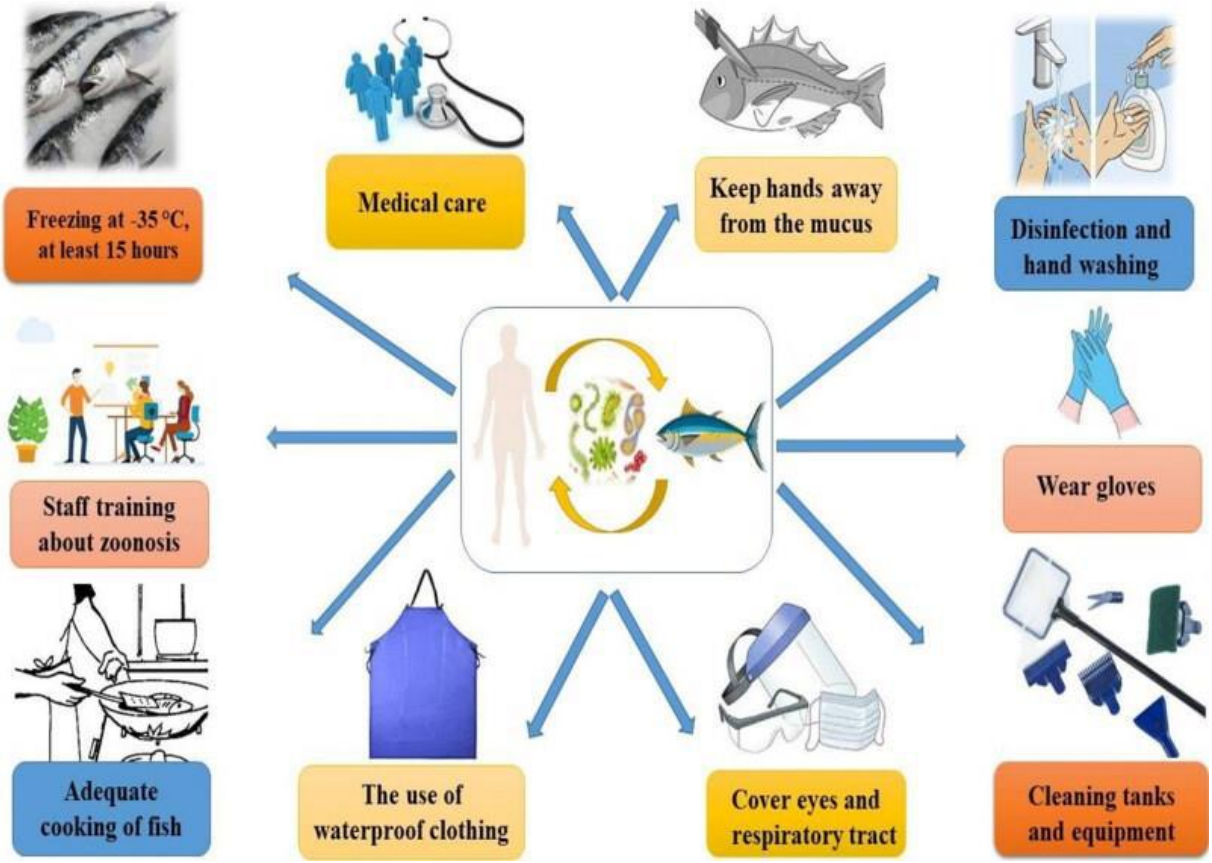

Fig. 4: Possible strategies for preventing and controlling zoonotic diseases.

20. ONE HEALTH (OH) APPROACH

The One Health (OH) concept should be enhanced and extended as it has become relevant in the treatment of zoonotic fish infections. Fish zoonosis epidemics can be stopped by having healthy fish, a healthy environment, healthy people, and a strong health care system. In order to solve One Health issues connected to seafood safety, it is advantageous to develop stakeholder relationships and involvement (Shamsi 2019).

The World Health Organization (WHO) asserts that international travel and trade have facilitated the spread of zoonotic illnesses. Inadequate sample transportation techniques and a lack of lab facilities for early sickness detection have also contributed to the spread of the illnesses in rural regions, where public health resources are few. Improving early sickness and pathogen detection, boosting infection treatment, and regulating vectors are among the most important suggestions in this area, as outlined by the World Health Organization (WHO) in 2021. A multidisciplinary and cross-sectoral approach is needed to manage and prevent zoonoses. It's important to keep an eye on the "One Health" strategy as you teach students, institutions, teams, and international organizations about it (Aggarwal and Ramachandran 2020).

21. CONCLUSION AND FUTURE PROSPECTS

Fish carry a wide variety of illnesses, some of which may be transmitted to humans (known as zoonotic diseases). Research into marine zoonotics has expanded in response to rising concerns about the spread

of disease caused by zoonotic agents in the worldwide health and fishing industries. However, there is still a lack of variety in the ecology, incidence, and spread of fish-borne illnesses.

The medical community, food business, and biosecurity would all benefit from a heightened knowledge of disease morphological identification and environmental prevalence.

In order to accurately identify fish-borne zoonotic illnesses, novel molecular diagnostic approaches need to be developed. This will make it easier and cheaper to monitor fish for zoonotic diseases in freshwater, agricultural, marine, and ornamental settings. Aquatic diseases are unfortunately widespread among human's despite of the fact that eating fish may be economically beneficial. Therefore, it is crucial for public health and should be regarded a fundamental part of human civilization to have appropriate information about these and to educate control and preventative techniques. Any epidemic or possible breakout of a zoonotic disease in fish might be managed effectively and sustainably via the implementation of the One Health concept through the improvement of multiple control mechanisms.

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Dermatophytosis in One-Health Perspective

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ABSTRACT

Dermatophytosis is associated with a unique group of fungi commonly known as dermatophytes and usually infect keratinous tissue, stratum corneum of the skin, invade the hair, nails of the host, feathers, horns and hooves. It is a contagious cutaneous infection usually prefer a hot and humid environment for their growth and infectivity, therefore, commonly occur in tropical regions worldwide. Phylogenetically, dermatophytes are closely related to monophylectic keratinophilic filamentous fungi. These are categorized into three genera i.e., Epidermophyton, Microsporum and Trichophyton. Dermatophytosis is commonly named after the body part get infected with the dermatophytes i.e., T. capitis if scalp and hair are involved, T. unguium if nails are affected, T. corporis if non-hairy skin become affected etc. Most of the dermatophytes resides in soil for years hence named geophilic. The dermatophytes adapted to humans are called anthropophilic, whereas some dermatophytes are adapted to animals known as zoophilic. Both geophilic and zoophilic are capable to transmit to the humans directly through arthroconidia shed by infected skin and/or hair of the infected host and deposited on fomites such as brushes and clippers. Dermatophytosis (tinea capitis) is more common in children (up to 60%), while tinea pedis is prevalent more than 50% in adults. Tinea cruris is more common in population lives in hot climates. Mostly, dermatophytosis can be serious in immunosuppressed individuals. The infection usually resolves within 2-4 weeks with topical application of the drugs like eficonazole and tavaborole and/or oral administration of the drugs i.e., itraconazole, fluconazole, griseofulvin, terbinafine etc. In order to control dermatophytosis effectively, One-Health approach highlights the significance of cooperation between medical professionals, veterinarians, environmental scientists, and public health specialists.

Keywords: Dermatophytes, Skin infection, Zoonosis, One-Health approach, Prevention

CITATION

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1. INTRODUCTION

Dermatophytosis is a common contagious mycotic infection associated with a mold group of fungi known as dermatophytes and cause cutaneous infection (Chanyachailert et al. 2023). Dermatophytes are unique group of fungi and possess alike antigenicity, infectivity and usually prefer a hot and humid environment for their growth; therefore, dermatophytosis commonly occur in tropical regions. Dermatophytes infect keratinous tissue and are able to break keratin in tissues and invade the hair, the stratum corneum of skin, nails of a living host, feathers, horns and hooves (Tachibana et al. 2017; Jartarkar et al. 2020). Dermatophytes are usually classified on the basis of the body parts they affect and referred to as ringworm or tinea derived from Latin word for "larva" or "worm" i.e., Tinea capitis (T. capitis) if dermatophytes affect scalp and hair, T. corporis if affect non-hairy skin, T. unguium if affect nails, also called onchomyosis, T. manuum if dermatophytes involved hands, T. cruris if involved groin, T. pedis if feet are involved etc. (AL-Khikani 2020; Jartarkar et al. 2020). Most of the dermatophytes reside in soil (geophilic) for years. Some dermatophytes are adapted to humans (anthropophilic), whereas few are adapted to animals (zoophilic). The zoophilic and geophilic are capable to transmit to the humans and are of zoonotic importance (Jartarkar et al. 2020; Chanyachailert et al. 2023). Dermatophytes usually remain in stratum corneum of the skin, thereby can be self-limiting disease. In livestock, it is of economic importance because it damages the hides (Chanyachailert et al. 2023).

2. ETIOLOGY

Phylogenetically, dermatophytes are closely related to monophylectic keratinophilic filamentous fungi. These are categorized into three genera i.e., *Epidermophyton*, *Microsporum* and *Trichophyton*. The species of these genera which cannot invade and infect keratinous tissue are not included in the dermatophytes. The life cycle of the dermatophytes usually has two phases: the anamorph state (also known as imperfect state) which is the asexual phase, and the teleomorph state (also known as perfect state) that is the sexual phase. Although the teleomorphs state has not yet been identified but the generic name given to the dermatophytes that accommodates the known sexual forms is *Arthroderma* and refers to both *Microsporum* and *Trichophyton* (Ghannoum and Nancy 2009; Smith and McGinnis 2011; Jartarkar et al. 2020).

3. EPIDEMIOLOGY

3.1. CLASSIFICATION

Dermatophytes are also classified as under on the basis of principal ecologic niches as (Moskaluk and VandeWoude 2022);

- Anthropophilic commonly named as human loving
- Zoophilic also known as animal loving
- Geophilic commonly known as soil loving

It is worth mention here that many dermatophytes possess characteristics of two or more ecological niche. This classification helps to determine the source of infection caused by dermatophyte. Among these three niches, zoophilic and geophilic dermatophytes are of clinical importance and causes severe inflammation with self-limiting lesions. However, anthropophilic dermatophytes cause less inflammation but chronic lesions (Segal and Elad 2021; Chanyachailert et al. 2023).

3.2. TRANSMISSION

Dermatophytes infect humans and animals after contact with spores (conidia) growing in a vertebrate host normally form only arthrospores (arthroconidia). The dermatophytes are usually transmitted directly through arthroconidia shed by infected skin and/or hair. These infected hyphal fragments and arthroconidia can easily disseminate from infected host and deposited on fomites such as brushes and clippers and transmitted to the others. Some studies had reported that these arthroconidia are the primary cause of new infections and remain viable in the environment for several years (Smith and McGinnis 2011; Segal and Elad 2021).

3.3. GEOGRAPHICAL DISTRIBUTION

Distribution of dermatophytes is worldwide with variability to the individual species. As the hot and humid environment favors the growth of dermatophytes, therefore, these are more prevalent in tropical and subtropical regions. The prevalence of the species in a geographic zone is dependent on certain factors i.e., migration pattern of the population, primary and secondary host range, lifestyle standards and climate preferences. Some species are restricted to a particular geographic zone, e.g., *Trichophyton concentricum* (*T. concentricum*) is endemic to the South Pacific and some area of South America and not reported in Europe (Ghannoum and Nancy 2009). Contrarily, *Microsporum canis* (*M. canis*)*, T. tonsurans* and *T. violaceum* are common in Europe, Middle East and Africa (Farag et al. 2018; Chanyachailert et al. 2023). Whereas prevalence of *M. canis*, *M. nanum, T. verrucosum, T. mentagrophytes*, *M. gypseum* and *T. equinum* is established worldwide. In Asia especially Indian Subcontinent, *T. simii* is thought to be endemic (Beguin et al. 2013; Farag et al. 2018; Segal and Elad 2021).

Dermatophytosis occurs at different sites of the body. Many scientific reports are published in this regard which indicate the occurrence of dermatophytosis at different sites and parts of the body. Dermatophytosis is the infection caused by a dermatophyte but it is observed that all dermatophytes are not involved in causing the infection. The dermatophyte (fungus) colonizes in the stratum corneum of the skin but it does not penetrate the viable tissues because it grows in a radial manner in the stratum corneum (Sultan et al. 2020; Martinez-Rossi et al. 2021). Invasion of the dermatophyte into the hair is an example of colonization of the dermatophytes in the non-living tissue. The colonization of the fungus in the stratum corneum may result in the development of the disease. Infection of the skin and other body parts also depend on the region. In Africa, tinea capitis is common which is also called as with a prevalence of 14-86% in children (Farag et al. 2018). Reports based on the data of two-decades have shown that *Trichophyton tonsurans* is the major causative agent that infect children with tinea capitis and achieved near exclusionary proportions in the USA (Sultan et al. 2020; Segal and Elad 2021; Chanyachailert et al. 2023). Similarly, the same dermatophyte infects the scalp and hair of the children in Canada (Petrucelli et al. 2020; Chanyachailert et al. 2023). Whereas *T. violaceum* is a major causative agent associated with tinea capitis in the children of India and Nepal (Beguin et al. 2013). Prevalence of dermatophytosis of the skin is higher in tropical regions (Andrews and Burns 2008; Smith and McGinnis 2011). The rate of infection is higher in non-natives to that of the indigenous population. It is essential to study different genera of the dermatophytes, their management, prevention and control of infection to understand the epidemiology of dermatophytosis (Martinez-Rossi et al. 2021).

3.4. MORBIDITY AND MORTALITY

It is observed that exposure of individuals to the spores of dermatophytes does not always lead to the infection. However, prevalence of dermatophyte infections varies with risk factors like the

climate, skin injuries, hot and humid environment and the animal contact because dermatophytosis is a common disease of puppies and kittens, however, there is no evidence of increased risk of the infection in elderly pets (Moriello 2019). Dermatophytosis (tinea capitis) is more common in children (up to 60%), while tinea pedis is prevalent more than 50% in adults. Tinea cruris is more common in population lives in hot climates. Mostly, dermatophytosis is not serious in healthy individuals; however, it can be serious in immunosuppressed individuals. The infection usually resolves within 2-4 weeks with good treatment (Andrews and Burns 2008; Smith and McGinnis 2011; Martinez-Rossi et al. 2021).

4. CLINICAL MANIFESTATIONS AND TREATMENT

Clinical manifestations of the dermatophytosis depend on the involvement of body area. The term tinea (Latin word for "larva" or "worm") is used to describe the area of body involved. The most common body sites infected with dermatophytosis are termed as: T. capitis (scalp and hair), T. corporis (non-hairy skin), T. unguium (nails, also called onchomyosis), T. manuum (hand), T. cruris (groin), T. pedis (feet) etc. (Ghannoum and Nancy 2009; Smith and McGinnis 2011; Martinez-Rossi et al. 2021). Below are the clinical manifestations of some of the most common types of dermatophytosis:

4.1. TINEA CAPITIS

4.1.1. SYNONYMS

Tinea tonsurans, Ringworm of the eyebrows, eyelashes and scalp

Tinea capitis is the common disease primarily found in the infants, children and young adolescents all over the world (Andrews and Burns 2008) which involve infection of the hair shaft and scalp. It causes stratum corneum's scaling associated with inflammation and sometime inflammation may not be there. It may be one of the two types of infection: ectothrix or endothrix. In ectothrix, the arthroconidia of the dermatophytes cover the surface of hair shaft and could be easily recognized by the destruction of hair follicle, while in endothrix, hyphae form arthrospores within the hair shaft. Invasion of dermatophytes in scalp hair follicles results in alopecia. The causative agents for this form of dermatophytosis (tinea capitis) is either *Microsporum* or *Trichophyton*. *M. canis* and *M. audouinii* are the species which cause pronounced inflammatory response and is of ectothrix type, whereas *T. tonsurans* invades in the hair shaft and causes endothrix type of infection (Ghannoum and Nancy 2009; Smith and McGinnis 2011; Seema et al. 2017). This disease usually spread from humans to humans (anthropophilic), however it is also transmitted from soil to the humans (geophilic) and from animal to humans (zoophilic) (Petrucelli et al. 2020).

Tinea capitis occurs on eyebrows, eyelashes and scalp. At early stages, lesions are present in the form of papules, pustules or nodules with alopecia. As disease progresses, itching becomes intense and there is development of erythematous papules or kerion on the scalp. Kerion is an inflammatory pus-filled folliculitis. Kerion is different form favus (Latin word means "honeycomb"). Favus is a special form of endothrix in which a crust is formed along the hair shaft due to accumulation of pus of hair follicle which looks like crusted-honeycomb (also termed as scutula) on the scalp and lead to hair loss. Favus is associated with *T. schoenleinii*. Dermatophytes of two endothrix species *T. tonsurans* and *T. violaceum* tend to induce severe and chronic infection than those of ectothrix and causes "black-dot ringworm" that is characterized by a "black-dot" on the scalp due to breakage of infected hair (Smith and McGinnis 2011; Gupta and Drummond-Main 2013; Seema et al. 2017).

The treatment regimen for tinea capitis is as follow (Gupta and Drummond-Main, 2013):

- Oral administration of the antifungal agents is recommended for eradication of fungal infection present in hair or hair follicles. For that, Griseofulvin is used @ 10 mg/kg/day continuous for 10 weeks (Gupta and Drummond-Main 2013).
- For reduction of treatment periods, itraconazole @ 5 mg/kg/day is recommended for continuous up to 4 weeks.
- Another effective antifungal agent fluconazole is used @ 3-6 mg/kg/day for 6 weeks.
- Terbinafine is considered more effective and can be recommended for 4 weeks at the dose rate as follow:
  - The children weigh <20 kg is 62.5 mg/day
  - Patients weigh 20-40 kg @ 125 mg/day
  - Adults weigh >40 kg @ 250 mg/day
- To reduce the risk of disease progression, topical treatment should be applied along with oral therapy.

For that purpose, creams and shampoos can be used which contain azoles and selenium sulfide (Seema et al. 2017).

4.2. TINEA CORPORIS

4.2.1. SYNONYM

Ringworm of the body

Tinea corporis refers to the colonization of arthroconidia of dermatophytes on glabrous skin. It is superficial fungal infection and limit itself in the superficial layer radially without involving the viable tissues. It excludes the hand, feet, face, scalp, nails and groin region. All species of *Epidermatophyton*, *Trichophyton* and *Microsporum* are able to produce lesions of tinea corporis, however, most prevalent etiological species are *M. canis*, *T. rubrum* and *T. mentagophytes*. Lesions produced by these species are ring-shaped and may be single, multiple or confluent. The fugus invades and colonize in the stratum corneum for 1-3 weeks. After colonization, it exhibits two basic types of lesions as clinical signs i.e., annular and vesicular. Lesions show inflammatory responses from low range causing small, dry scaling and erythematic annular patches to a high inflammatory lesions (vesicular type) like vesicles, pustules and marked erythema with subsequent crust formation. Zoophilic dermatophytes such as *T. mentagrophytes* usually exhibit the characteristic of vesicular lesions. Infection of hair follicles can lead to pustular, reddened, psoriasiform plaques, elevated or verrucous lesions with a severe inflammatory response as exhibited by kerion is termed Majocchi's granuloma (Smith and McGinnis 2011; Seema et al. 2017; Martinez-Rossi et al. 2021). Due to this severe inflammatory lesions, secondary bacterial infection may occur due to involvement of opportunistic bacteria. A special form of T. corporis named T. imbricata that is reported in the population of Southeast Asia, South America and Pacific Islands of Oceania. This form is associated with *T. concentricum*. The lesions of this disease spread over 70% of the body and characterized by polycyclic rings of papulo-squamous scales. Transmission of this disease is through direct contact and genetic susceptibility inheritance in an autosomal recessive pattern is also reported (Ghannoum and Nancy 2009; Smith and McGinnis 2011).

Therapeutic regimen recommended for the treatment of tinea corporis is as under (Ghannoum and Nancy 2009; Smith and McGinnis 2011):

- Topical application of creams contains antifungals agents like ketoconazole, econazole, terbinafine and clotrimazole for drying the involved skin.
- For oral treatment, any antifungal drug from the following can be used:
  - Griseofulvin @ 500 mg/day continuous for up to 6 weeks
  - Itraconazole @ 100 mg/day for 2 weeks

- Fluconazole @ 150 mg/day continuous for 6 weeks
- Terbinafine @ 250 mg/day continuous for 4 weeks

4.3. TINEA UNGUIUM

4.3.1. SYNONYMS

Nail's Ringworm, Onychomycosis, Dermatophytic onychomycosis

Fungal infection of nail is called tinea unguium associated with dermatophytes. The other term which makes a confusion is onychomycosis which is refers to infection caused by any non-dermatophytic fungus. However, onychomycosis is the term that commonly used to describe all fungal infections of the nail. In this chapter, we will discuss onychomycosis due to dermatophytes. The prevalence of onychomycosis is 2-13% of the general population. The perquisites of this condition are already existence of the tinea pedis in the same individual, immunosuppression, age, hot and humid environment and genetic predisposition. Onychomycosis occurs after invasion of the dermatophytes into the nail. It starts from the lateral distal subungual surface of the nail associated with *T. rubrum* and sporadically by *T. tonsurans*, *T. mentagrophytes* or *E. floccosum*. The condition is characterized by white, crumbly appearance of the nail due to accumulation of crumbling subungual debris, the nail become thickened due to subungual hyperkeratosis. The superficial white nail appears due to white patches on the nail's surface and is associated with *T. mentagrophytes*. Severe cases involve entire nail surface which causes dystrophy of the nails and involves the cornified layer of the nail bed and hyponychium (Ghannoum and Nancy 2009; Smith and McGinnis 2011).

The success of treatment depends on identification of the causative agent.

- Topical application of creams containing antifungal agents has low success rate because of poor penetration. However, new agents eficonazole and tavaborole are known for good penetration in the nail and are more effective than older agents (Jinna and Finch 2015; Tachibana et al. 2017).
- Oral administration of antifungal agents is recommended for the treatment of tinea unguium. Following agents can be used:
- Itraconazole @ 400 mg/kg daily for a week. Must be repeated after a month and continued for 3-4 months for toenail involvement.
- Fluconazole @ 150-300 mg once a week for 6-12 months. Treatment may be continued till normal growth replace the infected nail
- Terbinafine is given @ 250 mg/day daily for 5-6 weeks for fingernails and 10-12 weeks for toenails. Meta-analysis of the worldwide published data reported that terbinafine has more success rate than other agents (Gupta et al. 2013).

4.4. TINEA PEDIS

4.4.1. SYNONYMS

Foot Ringworm, Athlete's foot

Tinea pedis or foot ringworm is the term designated for dermatophytosis of the feet. It is thought to be the most common dermatophytosis with prevalence rate of 30-70%. The typical sites of infection are interdigital spaces and soles. If infection involves entire foot, then the term used is moccasin foot. The infection varies from mild to severe, acute to chronic and may have inflammation, vesicles and pustules (Canavan and Elewski 2015; Turkistani et al. 2022). Predisposing factors of the infection are hot and

humid environment, age, gender (common in males than females), absence of sebaceous glands in feet, wearing of shoes. The most common clinical form of T. pedis is interdigital clefts of the toe and characterized by maceration, shedding, and fissuring of the skin having dead skin (white epidermis) and odor. It may spread to the sole, heel and dorsal surface of the foot. The principal etiologic agents of T. pedis are *T. rubrum* and *T. mentagrophytes var. interdigitale*. It is thought that if T. pedis remained untreated the it may lead to onychomycosis (Canavan and Elewski 2015; Turkistani et al. 2022). The infection caused by *T. mentagrophytes var. interdigitale* exhibits vesicular form of the disease in which ulcerative eczematoid vesiculo-pustules or bullae occurs which spread to the heel, anterior area and dorsal surface of the foot. Involvement of opportunistic bacteria exaggerates the condition and lead to cellulitis, lymphangitis and lymphadenitis (Canavan and Elewski 2015; Jartarkar et al. 2020; Turkistani et al. 2022).

Tinea pedis is successfully treated with combination of topical and oral antifungal agents. Following regimens are recommended for the treatment (Subissi et al. 2010; Scorzoni et al. 2017):

- For topical application, preparation of luliconazole, benzylamine, ciclopirox olamine, haloprogin, and naftifine 2% is recommended for 1-2 weeks.
- Oral administration of itraconazole @ 400 mg/kg daily for one week.

5. NEW AGENTS FOR THE TREATMENT OF DERMATOPHYTOSIS

Following are the new agents discovered by the scientists for the treatment of dermatophytosis and must be checked in humans:

- Apigenin, a flavone, has shown effective against certain dermatophytes in mice and should be tested in humans (Singh et al. 2014).
- Tavaborole is a broad spectrum oxaborole which showed effective in humans against certain dermatophytes (Elewski 2014).

6. DIAGNOSIS

There are three major methods for diagnosis of dermatophytosis (Aboul-Ella et al. 2020; Moskaluk and VandeWoude 2022; Chanyachailert et al. 2023).

6.1. CLINICAL EXAMINATION

The suspected case of fungus can be initially screen out by using Wood's light (filtered ultraviolet light). Under this light, hair or skin infected with *Microsporum* will exhibits a bright blue-green fluorescence which will help to go for further examination of that animal (Aboul-Ella et al. 2020; Moskaluk and VandeWoude 2022; Chanyachailert et al. 2023).

6.2. LABORATORY EXAMINATION

In this method, clinical sample of skin is added to 10% potassium hydroxide (KOH). A drop of that sample is then placed on glass slide and covered with a cover slip. After 3 minutes of preparation of glass slide to allow for digestion of host cells, it is examined under microscope at 400x under phase-contrast microscopy. We can observe the presence of septate hyphae or fungal conidia under phase contrast microscopy. This procedure will confirm the presence of fungal disease in that sample (Aboul-Ella et al. 2020; Moskaluk and VandeWoude 2022; Chanyachailert et al. 2023).

6.3. CULTURE METHOD

For identification of species of the fungus, sample should be cultured on selective, such as Mycosel and Mycobiotic agar, and non-selective fungal media, such as Sabouraud dextrose agar and potato dextrose agar. These cultures are recommended to grow in Petri plates sealed with gas-permeable tape or parafilm or screw-capped tubes. These cultures for dermatophyte isolation should be then incubated at 30°C for 1-2 weeks before examination (Aboul-Ella et al. 2020; Moskaluk and VandeWoude 2022; Chanyachailert et al. 2023).

7. PREVENTION AND CONTROL

Prevention and control of the disease is possible by adopting following measures (Jartarkar et al. 2020; Chanyachailert et al. 2023):

- One of the major preventions is to control the disease in animals to avoid human dermatophytosis caused by zoophilic.
- Animals suffering from dermatophytosis should be kept isolated and properly treated. Other animals of that area should be examined with Wood's light.
- Area and fomites of the affected animal must be disinfection properly and remnants (hair, wool, nails etc.) of the diseased animal should be disposed.
- Vaccine should be developed and used for prevention and control of the disease.
- Good hygiene of the individual and environment is necessary to reduce the occurrence of the disease.
- Skin should be kept clean and dry especially the feet and groin skin (Chanyachailert et al. 2023.

8. CONCLUSION

In summary, considering dermatophytosis from the standpoint of One Health emphasizes the intricate interactions that occur between environmental, animal, and human variables in the development and treatment of this fungal illness. Humans and animals are both impacted by the frequent dermatological illness known as "dermatophytosis," which highlights the connection between animal and human health. In order to effectively treat dermatophytosis, this One Health approach highlights the significance of cooperation between medical professionals, veterinarians, environmental scientists, and public health specialists. Through comprehending the common risk factors, pathways of transmission, and reservoirs associated with this fungus, we may create more potent preventive and management plans

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Zoonotic Importance of Bartonellosis

38

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ABSTRACT

History has witnessed several vector-borne zoonotic diseases which have taken millions of precious human lives. Bartonellosis is one of the most important vector-borne diseases that caused 7,000 causalities in 1870, and these causalities continued even after centuries. Bartonellosis is a broad term that describes the diseases caused by a gram-negative bacterium of a genus Bartonella (Oroya fever, Carrison's disease) and spp. Bartonella. Members of the Bartonella genus are short, pleomorphic, gram-negative, aerobic, and oxidase-negative organisms within the α2 subgroup of the Proteobacteria class. Hosts for Bartonellosis vary from rodents to domestic dogs and cats. B. henselae, B. clarridgeiae, and B. koehlerae mainly infect cats. B. vinsonii subsp. berkhoffii causes infection in dogs and coyotes (Canis latrans), B. alsatica, causes infection in wild rabbits. Similarly, B. bacilliformis and B. quintana B. bacilliformis and B. quintana infect humans. The bacteria enter the host's bloodstream directly or via lymphatic system and colonize within the erythrocytes. The signs of infections vary from species to species, but Bartonellosis is mostly characterized by fever, hemolytic anemia, myalgia, paler, and arthralgia. In cats, Bartonellosis is associated with lymphadenitis, endocarditis, gingivitis, and stomatitis. Endocarditis is the most important feature of Bartonellosis in dogs. Human activities are affecting the global environment and the rise in temperature, is increasing the interaction of arthropods with human or mammalian species. Thus, to control the disease effectively, reduction in animal-vector interaction, improving diagnostic techniques, and updating treatment regimens are essential.

Key words: Bartonellosis; Vector-borne disease; Oroya fever; Carrison's disease; Zoonotic diseases

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1. INTRODUCTION

Many vector-borne pathogens have emerged in the past few decades; nearly all have zoonotic potential, creating a new challenge for global health. Arthropods such as ticks and mosquitoes have been the most important cause of vector-borne diseases for centuries. These arthropods provide a space for several bacterial, viral, and parasitic agents and act as transmission sources from animal to human. Arthropods having a pathogen with zoonotic potential mainly affect pets or domestic animals, ultimately spreading infection to humans when these animals are consumed or in contact with humans (Telfer et al. 2007). Some of the most compelling bacterial zoonotic and vector-borne diseases throughout the globe include Lyme disease, Bartonellosis, Ehrlichiosis, etc. These diseases have caused severe infections in the past and still exist as an endemic in several countries. An increase in the different vector-borne diseases in the past few decades provides a chance for One Health professionals to collaborate to understand the complex pathophysiology of vector-borne zoonotic diseases (Walker et al. 1996; Mai 2022).

Genus *Bartonella* is a clade of alphaproteobacteria that contain gram-negative, pleomorphic, and fastidious aerobic bacteria. Until 1993, it was thought that the genus *Bartonella* consists of only one species. However, with the advancement in microbiology and the interest of One health professionals due to their high morbidity, several other species of *Bartonella* have been identified. *Bartonella spp.* gained the attention of veterinarians in the late 1990s when an increase in animal reservoir hosts was recorded. For example, *Bartonella henselae* was found in cats, *Bartonella vinsonii* was isolated from wild canids, and *Bartonella bovis* was isolated from domestic cattle (Boulouis et al. 2005). *Bartonella* causes bacteremia in mammalian reservoir hosts with few or no symptoms. Thus, contact with a healthy dog, cat, or other reservoir host of *Bartonella* can cause the transmission of bacteria from one animal to another or human as well. Bartonellosis is a term used to describe the infection caused by *Bartonella spp.* The most common infection caused by Bartonella spp. include peliosis hepatitis, Carrison's disease, Oroya fever, and trench fever. Arthropod vectors, such as ticks, fleas, and lice, are also considered the most important source of Bartonellosis transmission within animals and humans (Jacomo et al. 2002).

2. BACTERIOLOGY

Genus *Bartonella* consists of more than 20 bacterial species, and most of these bacteria have been reclassified from the genus *Rochalimeae* and *Grahamella.* All *Bartonella spp.* are very closely related to each other and have 98% homology in the sequences of their 16S rRNA genes. Members of the Bartonella genus are short, pleomorphic, gram-negative, aerobic, and oxidase-negative organisms within the α2 subgroup of the *Proteobacteria* class. They have very close evolutionary relations with bacteria of the genera *Rhizobium* and *Brucella.* Bartonella spp. can be easily grown at 37°C with 5% carbon dioxide on an axenic medium. However, fetal bovine serum and tissue culture also provide a suitable environment for Bartonella for its growth (La Scola and Raoult 1999).

Some species of *Bartonella,* such as *Bartonella bacilliformis* and *B. clarridgeiae* have a unique structure called flagella, facilitating the bacterium in erythrocyte invasion. Other species of Bartonella use the actin-dependent invasion-mediated mechanism of cellular invasion (Dehio et al. 1997).

3. HISTORY OF BARTONELLOSIS

In 1870, a severe fever outbreak occurred among the Railway workers in Oroya, leading to 7,000 causalities. The disease was named "Oroya fever" and was a headache for all medical professionals and local communities at that time. In 1885, Daniel Carrison, a medical student and native of Peru, tried to solve the problem by vaccinating himself from the verruca tumor. But, twenty-three days later, Daniel Carrison experienced the signs of Oroya fever and died. In honor of Carrison's attempt, the disease was

later named "Carrison's disease." In 1902 and 1903, Barton performed a bacteriological investigation and performed a necropsy of the people who died from Carrison's disease. Healthcare professionals studied the different characteristics of the causative agent of Carrison's disease, the pathogen associated with this disease, and the pathogen *Bartonia* (Medicine 1915).

Until 1990, before the discovery of the AIDS virus, most of the *Bartonella* spp. were not identified. However, during the early epidemic of AIDS, the bacteria responsible for the transmission of Carrison's disease was histopathologically visualized with silver stains within bacillary angiomatosis and peliosis hepatis lesions. With the effort of Dr. David Relman and colleagues, a new species of *Bartonella* was identified. It was concluded that the identified species cause cat scratch disease, which highlights the zoonitic spread of Bartonellosis. Later, it was confirmed that most of the *Bartonella* spp. transmit to cats through fleas (Yore et al. 2014).

In 1993, Brenner studied the characteristics of the *Rochalimaea* species and provided a proposal to remove the Family *Bartonellaceae* from the Order *Rickettsiales* and unify the different species of genus *Rochalimaea* with *Bartonellaceae.* This led to the addition of four new members in the genus *Bartonellaceae.* These members include B. .*quintana*, *B.vinsonii*, *B.henselae*, and *B. elizabethae* (Brenner et al. 1993).

Similarly, in 1995, the genus *Grahamella,* an arthropod transmitted gram-negative bacterium, was unified with the genus *Bartonellaceae* and led to the addition of five new species, including *Bartonella talpae*, *B. peromysci*, *B. grahamii*, *B. taylorii*, and *B. doshiae* (Birtles et al. 1995). Later, more species of Bartonella were isolated from dogs, domestic cats, and wild rats and *Bartonella washoensis* was isolated from a patient with cardiac disease. The investigation revealed that the patient had contact with rodents, confirming the zoonotic potential of the genus *Bartonella.*

4. LIFE CYCLE

The infection cycle of Bartonellosis starts with the inoculation of the bacteria in specific mammalian hosts. Inoculation is mainly performed by the blood-sucking arthropods or contact with the infected animals or rodents. When a bacterium containing arthropods sucks blood from a mammal, the affected area experiences irritation and scratching, leading to the inoculation of bacterium-containing insect feces into the dermis. Upon inoculation, the Bartonella resides in the primary niche (dermis) of the host for a specific time and then seeded in the bloodstream, where it colonizes in erythrocytes. But, during the invasion of the dermal niche, several immune cells, including macrophages, phagocytosed the bacteria to prevent entry into the blood. But, sometimes, *Bartonella* also gains entry to the bloodstream via lymphatic vessels. Mainly, research has proved that *Bartonella* invades the blood-containing niches by affecting the endothelial cells (Shown in the fig-1). Colonization of the bacteria within the erythrocytes is performed in several steps, including adhesion with erythrocytes, invasion, and intracellular persistence that enable the continuous vector transmission of the infection (Fig. 1). During the whole course of the infection, the weak immune response of the patient and moderate inflammatory profile is highly beneficial for *Bartonella.* The bacterium also affects the host's immune response via passive immune evasion and immunomodulation (Harms and Dehio 2012; Jin et al. 2023).

5. EPIDEMIOLOGY

5.1 HOST

Many research studies have proved a species-specific association between the *Bartonella spp.* and their hosts or vectors. Most of the Bartonella species infect the mammalian hosts, where a primary infection occurs, followed by chronic bacteremia, which might be asymptomatic. Hosts for Bartonellosis vary from

rodents to domestic dogs and cats. *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* mainly infect cats. *B. vinsonii* subsp. *berkhoffii* causes infection in dogs and coyotes (*Canis latrans*), *B. alsatica,* causes infection in wild rabbits. Similarly, *B. bacilliformis* and *B. quintana B. bacilliformis* and *B. quintana* infect humans (Jacomo et al. 2002).

5.2 VECTORS

Bartonellosis can be transmitted by several vectors, including sand flies, human louse, cat flea, the mite, and the vole ear mite; still, the data is considered incomplete. Research conducted in Los Angeles confirmed that 61% of the fleas found on rats are infected with *B. elizabethae* and other species. Contact of these bacterium-containing rodents with a human or other animal causes endocarditis and febrile illness (Breitschwerdt and Kordick 2000).
Table 1 explains the different reservoir hosts and vectors for important *Bartonella* species.

5.3 TRANSMISSION

5.3.1 TRANSMISSION BETWEEN NATURAL HOSTS

As previously discussed, vectors play an important role in transmitting Bartonellosis from one infected animal to another. Furthermore, there is a specific association between the vector, natural host, and *Bartonella* species. Research has proved that the vector may inoculate *Bartonella* species through the bite and scratch of the reservoir hosts or direct contact with the blood of infected animals.
Bats also play an essential role in the transmission of Bartonellosis. A study conducted in the USA confirmed the presence of *Bartonella spp*. in bat flies. These significant findings suggest that bats as a reservoir host can cause spillover of the infection, leading to the transmission in animals and humans (Morse et al. 2012).

Table 1: Species of *Bartonella* spp. in various animals with their vectors

Specie	Reservoir host	Vector involved.
B. bacilliformis	Human	Phlebotomines
B. quintana	Human	Human Body Lice
B. henselae	Cats	*C. felis (fleas)*
B. clarridgeiae	Cats	*C. felis*
B. vinsonii subsp. *berkhoffii*	Dogs	Fleas and ticks
B. alsatica	Rabbits	Fleas or ticks.

In 2018, Corduneanu et al. (2018) detected the presence of *Bartonella spp.* DNA in bats' heart tissues led to the addition of four new species in the genus Bartonella. The presence of Bartonella species in bats opens a new debate and area of research for veterinarians and One health professionals. Interaction of Bartonella-infected bats or bat flies with animals can lead to the transmission of Bartonellosis within the animals and humans.
Rats are another important reservoir hosts for several Bartonella species. In an extensive analysis of rats from 13 sites in the United States and Portugal, *Bartonella spp.* were isolated from the blood of 19% *Rattus norvegicus* and 112% *Rattus* (Ellis et al. 1999).

5.3.2 ZOONOTIC TRANSMISSION

The Bartonella species present in the bloodstream of an infected animal can be transmitted to humans by biting the vector or close contact with the bacterium-containing secretion, including blood of the infected

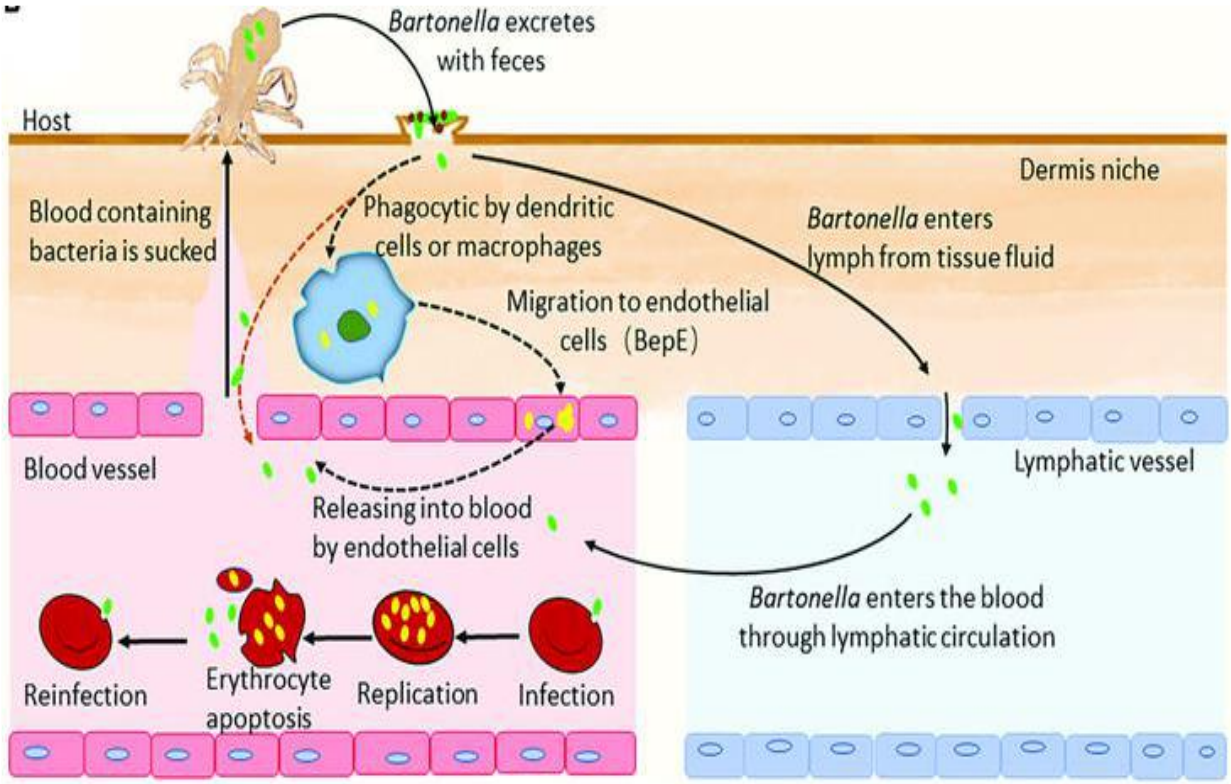

Fig. 1: Life cycle of Bartonella in the mammalian specie

dogs, cats, or other animals. Humans can be a primary or accidental host to Bartonellosis. In humans, vector transmission of *Bartonella* can be possible in two ways:

1. When contaminated, arthropod feces are inoculated through animal bites or scratches. When the host causes wound contamination by scratching irritated arthropod bites, cats, dogs, and people are essential incidental hosts and key reservoirs for these forms of transmission (Fig. 2).
2. Direct transmission of bacteria by the bite of vector. For example, sand flies, such as *Lutzomyia verrucarum,* can transmit the *B. bacilliformis* among humans. Similarly, *Ixodes ricinus* ticks can cause the transmission of *B. henselae* (Battisti et al. 2015; Cotté et al. 2008).

5.4 GEOGRAPHICAL DISTRIBUTION OF BARTONELLA SPECIES

Bartonellosis is a significant zoonotic disease that is a potential threat for the whole globe, but some specific species are endemic and limited to specific geographical regions. Bartonella infection is a recognized public health threat in the United States, where it causes CSD at a rate of 4.7 per 100,000 people aged 65 and older and 500 hospital admissions annually (Sepulveda-Garcia et al. 2023). *B. henselae* is an important species that affects domestic cats and has zoonotic potential. The population of *B. henselae* varies and is high, mainly in warm and humid environments (68% in the Philippines). There are two major genotypes of *B. henselae,* i.e., Houston-1 and Marseille. According to a study, *B. henselae* type Marseille is most commonly found in Western Europe, Western U.S.A, and Australia cats. On the other hand, Houston-1 is dominant in Asian countries, especially in Japan and the Philippines. The prevalence of Houston-1 in Australia and the U.S.A. indicates that this genotype has more zoonotic potential than Marseille (Chomel and Kasten, 2010). Cat Scratch Disease (CSD) is one of the most common

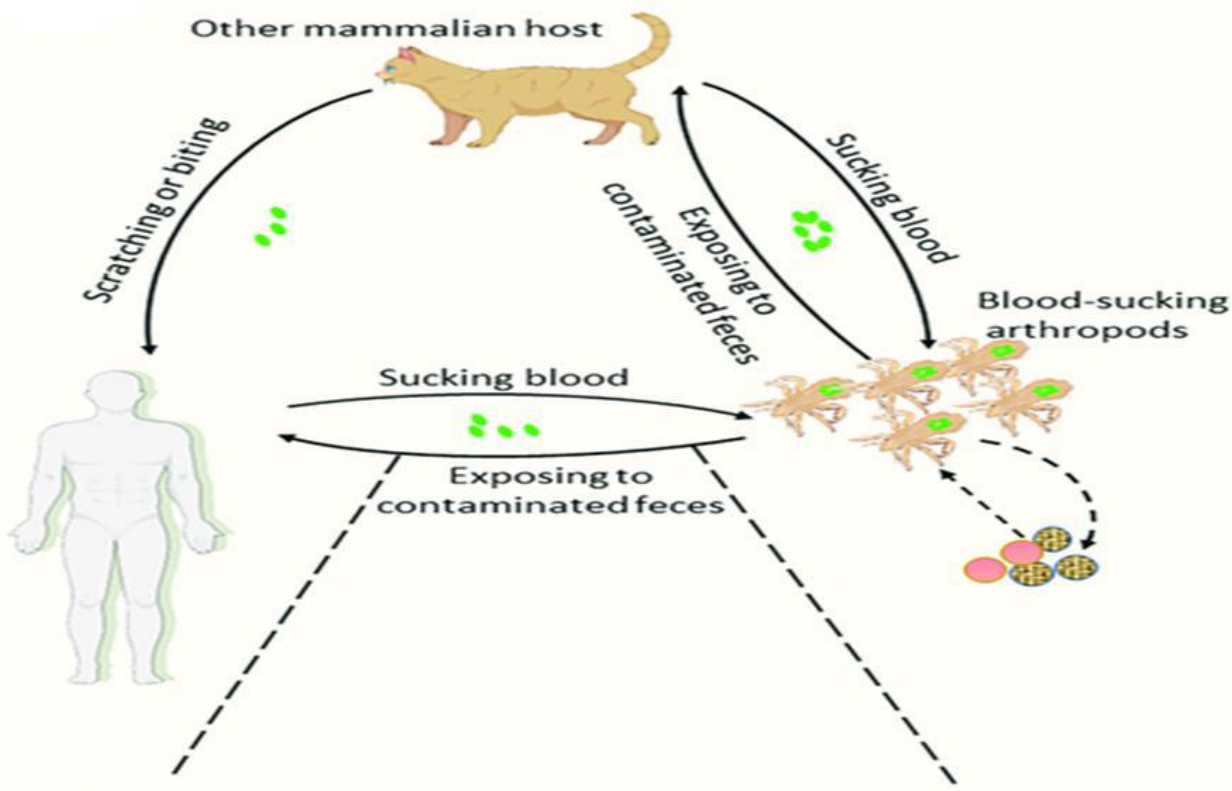

Fig. 2: Pattern of transmission of *Bartonella*

forms of Bartonellosis. The disease was first reported in France in 1950, but the causative agent was identified in 1993. A report published in 2005 showed that the total number of CSD in the U.S.A. is 22,000 and 24,000, along with 2,000 severe cases that require hospitalization (Boulouis et al. 2005).

Bartonella clarridgeiae is another important bacterium that belongs to the genus *Bartonella*. The bacterium was first isolated from the cat of an HIV patient in 1995. This species of Bartonella is more prevalent in Thailand, the Philippines, France, and the Netherlands. However, few studies have also reported its isolation from cats of other countries in the U.S.A, Australia, Japan, and Taiwan (Maruyama et al. 2001).

Bartonella koehlerae is one of the fastidious bacteria to grow and rarely infects cats. Major cases of this bacterium are mostly reported in warmer regions; for example, the prevalence of this bacterium in cats is 80% in California and 0% in the Netherlands. Similarly, the seroprevalence of *Bartonella koehlerae* in the Middle East is 15% (Chomel et al. 2003; Switzer et al. 2013; Alanazi et al. 2020).

B. quintana, B. bovis, and *B. vinsonii berkhoffii* also cause Bartonellosis in pets and some domestic animals, including cattle. *B. quintana* was first isolated during World War I and II, when their association was found with trench fever. *B. quintana* is most commonly found in regions with more head lice (Sangaré et al. 2014). *B. bovis* was first isolated in cattle that were suffering from endocarditis. *B. bovis* is most prevalent in French Guyanna (70%), U.S.A (50 to 89%), France (36%), Italy (24%), and West Africa (20%) (Bai et al. 2013). *Bartonella* spp. has also been identified in the cattle of Pakistan (Ghafar et al. 2020).

6. CLINICAL MANIFESTATION

The clinical manifestation of Bartonellosis varies from species to species. The below points highlight the important clinical features of Bartonellosis in different animals and humans.

6.1 CLINICAL FEATURES OF BARTONELLOSIS IN HUMANS

As discussed, humans can be accidental and primary hosts for several *Bartonella* species. The clinical feature of Bartonellosis depends on the *Bartonella* species involved in infection. *Bartonella bacilliformis,* an agent responsible for Oroya fever, only infects humans and is characterized mainly by fever, hemolytic anemia, myalgia, paler, and arthralgia (Kosek et al. 2000). The disease can be lethal if left untreated. In most cases, chronic bacteremia develops after a few weeks or even years of the acute infection, primarily associated with the eruption of nodular skin lesions. Many researchers have proved that *Bartonella bacilliformis* is endemic in several regions, including Peru, where infection is present in asymptomatic forms (Chomel and Kasten 2010).

B. quintana is responsible for trench fever, transmitted by body lice, and only infects humans. After the incubation period, the infection is characterized by fever, headache, leg pain, and sometimes thrombocytopenia. *B. quintana* also causes endocarditis, chronic lymphadenopathy, and angiomatosis. Several asymptomatic carriers of the infection had also been identified in 1940 (Swift 1920).

Some species of the *Bartonella*, such as *B. henselae,* are zoonotic and transmitted from infected cats to humans. Infection with *B. henselae* is characterized by benign regional lymphadenopathy. After scratches from infected cats, papules that turn into pustules develop within 7 to 8 days at the inoculation site. In some patients, encephalitis, endocarditis, hemolytic anemia, hepatosplenomegaly, osteomyelitis, and pneumonia also develop. Most patients with CSD recover within one year without any sequelae. In children, infection with *B. henselae* also causes arthritis and skin nodules (Margileth et al. 1987; Chomel and Kasten 2010).

In immunocompromised patients, bacillary angiomatosis is one of the most common clinical manifestations of Bartonellosis. Bacillary angiomatosis is characterized by chronic vascular lesions histopathologically and clinically similar to verruga peruana caused by *B. bacilliformis*. These lesions are most common in HIV patients with CD 4+ cell counts below 50/mm3 (Koehler 2000).

6.2 CLINICAL FEATURES OF BARTONELLOSIS IN ANIMALS

6.2.1 CATS

Domestic and wild cats are reserve hosts for several species of *Bartonella*, especially *B. henselae.* Mostly, Bartonellosis is asymptomatic, but healthy-looking cats can transmit infections to other animals and humans. However, in case of severe infection, lymphadenitis, gingivitis, and stomatitis are the most important clinical features in felines. Bartonella-associated endocarditis has also been reported. However, lymphadenitis and endocarditis in asymptomatic cats are difficult to diagnose until the causative agent is identified in lesions (Ueno et al. 1996; Chomel et al. 2003).

Local inflammation, anorexia, lethargy, and lymphadenopathy are the most common clinical signs of Bartonellosis in cats. Neurological abnormalities, including behavioral and vestibular dysfunction, have also been reported in experimentally infected cats. Histopathological changes in Bartonellosis include focal monocytic myocardial inflammation and lymphocytic interstitial nephritis (Guptill et al. 1997).

In *B. koehlerae,* no clinical signs appear in infected cats.

6.2.2 DOGS

B. vinsonii subsp. *berkhoffii* is the most common cause of Bartonellosis in dogs worldwide, including in California and Arizona (Honadel et al. 2001). In dogs, *B. vinsonii* occurs as a co-infection with other tick-borne diseases, such as *Ehrlichia* and *Babesia.* Endocarditis is one of the most common clinical manifestations of *B. vinsonii* in dogs and is highly zoonotic. Other clinical signs associated with this

Bartonellosis include neurological disorders, lameness, lethargy, myocarditis, and cardiac arrhythmias. Endocarditis includes lesions associated with the aortic valve and the presence of vegetative lesions. Sometimes, *B. vinsonii-associated* Bartonellosis is asymptomatic in dogs and bacteremia results in the transmission of the bacterium to humans. However, in most cases, Bartonellosis causes immunosuppression in dogs, leading to monocytic phagocytosis, CD8 lymphocytopenia, impaired CD8+ T lymphocyte function, and impaired B cell antigen presentation within lymph nodes (Breitschwerdt et al. 1999).

6.2.3 WILD ANIMALS

Many Bartonella species have been isolated from several free-ranging and captive wild animals, including wild felids, domestic mammals, and wild rodents. For example, *B. vinsonii* subsp. *berkhoffii* has been isolated from coyotes. Similarly, *B. bovis* has also been isolated from domestic cattle, where it is responsible for endocarditis. But, in most mammalian species, Bartonellosis causes silent bacteremia and promotes bacterium transmission from one species to another (Kosoy and Goodrich, 2019).

7. ENVIRONMENTAL CHANGE: A POTENTIAL THREAT FOR BARTONELLOSIS TRANSMISSION

Due to the increase in industrialization, environmental pollution, and the trend of environmental toxic products in daily life, our earth's climate is changing rapidly. The earth's temperature is increasing daily, and glaciers are melting, leading to floods, and spreading diseases worldwide. Climate change affects the re-emergence of several vector-borne diseases, such as Bartonellosis, in several ways (Rocklöv and Dubrow 2020). Vectors are ectothermic, i.e., they change their body temperature according to the environment and perform better in warm environments. Vectors' interaction with the host for feeding and survival increases with an increase in temperature. For example, *Ixodes ricinus* is a potential vector for transmitting *B. henselae.* The lifecycle of *Ixodes ricinus* depends upon several factors, including humidity and temperature. However, climate change, such as rise in temperature, has increased the interaction of this tick with the mammalian host, leading to the transmission of *B. henselae* among wild and domestic cats (Caminade et al. 2019).

8. DIAGNOSIS OF BARTONELLOSIS

Serology, PCR, ELISA, culture, and IFA tests can diagnose Bartonellosis in humans and other animals.

8.1. CULTURE

Most species of *Bartonella* are gram-negative bacilli that are oxidase and catalase negative. Bacterium requires CO_2 -rich media for growth and can be easily obtained in 12 to 14 days when grown on blood media. Many pieces of research have proved that cell coculture media allow the more rapid growth of *Bartonella* compared to agar-rich media. Freezing the EDTA collected specimen for 24 hours results in a colony count compared to an isolator tube (Agan and Dolan 2002).

8.2. POLYMERASE CHAIN REACTION

Polymerase Chain Reaction (PCR) has revolutionized the diagnosis of several important infectious agents, including viruses and bacteria. PCR detects the pathological agent's DNA or RNA by using various clinical samples. DNA can be collected in significant quantities by using nucleic acid amplification. The collected

DNA is then analyzed by a variety of techniques. Microbiologists have successfully identified different species of Bartonella by using the PCR technique, which requires little starting time. However, PCR sample preparation to avoid contamination as an unknown inhibitor can affect the results. The most common PCR techniques for detecting *Bartonella* in a sample include ERIC-PCR, AP-PCR, PCR-EIA, and REP-PCR (Johnson et al. 2003).

8.3. ELECTROPHORESIS, SOUTHERN BLOT, AND RFLP

For an efficient detection of different Bartonella species, different molecular analysis procedures, such as Electrophoresis, southern blot, and RFLP, are very significant. These procedures are most commonly available and used throughout the world. However, these procedures require a large time for the sample preparation, more labor, and a large quantity of nucleic acid to perform these tests. These tests are often used in conjunction with PCR (Pérez et al. 2011).

9. TREATMENT

The ability of the Bartonella species to invade the erythrocytes provides them with protection against the host's immune system and from antibiotics used to treat infection. Any remaining bacteria after treatment can replicate the infection again, even after a year. Many studies have proved that Rifampicin, Gentamicin, and Ciprofloxacin are the best choices for treating Bartonellosis in humans (Angelakis and Raoult 2014). However, there is no standard protocol for treating dogs and cats. But Doxycycline, Amoxicillin, Enrofloxacin, and Rifampin can be effective. These antibiotics should be used carefully, as irrational use can lead to antibiotic resistance (Álvarez-Fernández et al. 2018). Table 2 shows list of effective antibiotics used against Bartonella species in different animals.

10. FUTURE INTERVENTIONS

Due to rapid environmental changes throughout the globe, illegal transport of animals, and increase in contact with wildlife, pets, and humans with each other, the number of Bartonellosis is increasing day by day. Animal bites, scrapes, arthropods, and even needle sticks can spread this zoonotic infection.

Table 2: Effective antibiotics of Bartonella species in different animals.

Host	*Bartonella* specie and clinical manifestation	Antibiotic
Cats	Bacteremia due to any specie of *Bartonella*	Doxycycline, Azithromycin
Cats	Endocarditis due to *B. henselae*	Marbofloxacin + Azithromycin
Cats	*B. vinsonii berkhoffii induced* Osteomyelitis and polyarthritis	Amoxicillin-clavulanate+ Azithromycin
Dog	Splenic vasculitis, thrombosis, and infarction/*B. henselae*	Doxycycline + Trimethoprim-sulfamethoxazole
Dog	Endocarditis/*B. koehlerae*	Ampicillin + Enrofloxacin
Human	*B. bacilliformis* induced Oroya fever	Chloramphenicol

Furthermore, diagnosing and treating *Bartonella* transmission has become more difficult due to the discovery of novel species and subspecies, as well as the wide variety of animal reservoir hosts and arthropod vectors that can transmit these bacteria. Bartonella bacilliformis infections in the Peruvian Andes were historically referred to as "Bartonallosis" and were spread by sandflies. However, it currently covers infections brought on by any Bartonella species wherever in the world. Numerous cell types are susceptible to *Bartonella* infection, which can result in a variety of clinical and pathological symptoms in

both animals and people (Brook et al. 2017). Thus, there is more need to take strict control measures to prevent the rapid spread of infection among animals and humans.

Due to the unavailability of the vaccine against Bartonellosis, controlling the vectors and reservoir host from direct contact with the human is the best solution. Strict control measures should be taken to avoid the trans-boundary transmission of the infection. Transport of animals from one city to another and export from one country should be done with strict measures to control ticks and flies. In dogs and cats, acaricides should be used as collars to avoid their contact with ticks and transmission of infection. Furthermore, dogs, cats, and humans should avoid contact with rodents, bats, and other stray animals. Veterinarians and pet owners must continue to use caution while treating pets for fleas to lower the risk of disease transmission. When recommending pets or pet care to immunocompromised patients, veterinarians should consider the cat's place of origin because *B. henselae* bacteremia appears more common among cats from shelters. Veterinarians are susceptible to collecting Bartonella from bites, scratches, and needlestick wounds. Veterinarians should make efforts to prevent such injuries, such as using anesthesia when necessary, cleansing wounds properly, and seeking medical attention right after, given the high prevalence of Bartonella bacteremia in young and stray cats. When expecting to come into touch with animal blood or saliva, gloves should be worn. While cats and dogs are significant Bartonella carriers, people can also contract the illness from other sources, such as arthropods or even blood transfusions. Particularly among homeless populations, human body lice can spread B. quintana, which causes relapsing fever and endocarditis (Sykes and Chomel 2014).

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Anthrax and its Impact on Public Health

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ABSTRACT

Anthrax is an acute, febrile, infectious disease of animals and humans associated with Bacillus anthracis, a gram-positive, rod-shaped bacterium. This bacterium is capable to form highly resistant spores under appropriate condition and proficient of persisting their virulence efficacy for many years. Bacillus anthracis is a complex organism that has three protein parts referred to as components I, II, and III. All vertebrates are susceptible to this disease, however, sheep and cattle are the most frequently affected, whereas horses and goats are less frequently affected. Humans can also develop the disease by eating the meat or handling the hair, bones, wool or carcasses of affected animals. It occurs as a cutaneous, pulmonary or intestinal infection in humans. Severe outbreaks of the disease are usually encountered in the region having tropical and sub-tropical climates, where there is high annual rainfall. The clinical signs include skin lesions (90-95%), cough, chest pain, malaise, and fever. Early symptoms show similarity with flu-like illness. Within 3-6 days, rapid hypoxia, elevated temperature, and mediastinal widening might occur. Meningitis also develops in later stages. The mortality rate is nearly 100% if left untreated. Mortality can be decreased by commencing an appropriate protocol of antibiotics. Antimicrobial treatment is often useless in the case of acute Anthrax, however, treatment should be initiated within 24 hours of the first sign of the infection. The treatment can be done with streptomycin, penicillin, tetracycline, chloramphenicol, and erythromycin. The line of treatment should be followed for five days.

Key words: Cutaneous infection, Zoonosis, Public Health, treatment, antimicrobials.

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1. INTRODUCTION

Anthrax, a lethal infection, is also infectious and highly transmissible (Fulako 2004). The gram-positive bacteria *Bacillus anthracis*, which typically exists in the soil in endemic zones and is capable of spore formation, is what causes anthrax. Non motile by locomotion, *B. anthracis* is anaerobic and produces centrally located spores. Taxonomically, the causative organism belongs to the Bacillaceae family (Shafazand et al. 2001).

Generally, anthrax affects a wide range of animals, including domestic and wild animals worldwide. *B. anthracis* can infect food animals, most commonly sheep, cattle, goats, and horses. Some species show resistance to the organism, such as birds are very resistant. Similarly, pigs are more resistant than horses and sheep. Whereas in pets, such as dogs and cats, are also resistant. The majority of anthrax cases occur in non-vaccinated animals. Humans are also at risk of developing Anthrax when they come into contact with the remains of the infected animals or the infected animals themselves. It is mainly an industrial hazard for workers that deal with bone products, processed hides, wool, goat hairs, and infected wildlife. Contact with infected meat is also a source of it (Baron et al. 1994). Humans can acquire anthrax from an infected animal or animal which has been expired due to this disease or by exposure with by-products contaminated with this pathogen (Sidwa et al. 2020).

A human can be infected with this bacterium through the gastrointestinal tract (gastrointestinal Anthrax), skin lesions (cutaneous Anthrax), or respiratory routes (pulmonary Anthrax) (Dixon et al. 1999). The two forms in which *B. anthracis* occurs are vegetative cells found inside the body and spores for persistence in the environment or soil (Santelli et al. 2004). It is primarily found in the endospore form in the soil, which allows it to continue to function in this form for many years. The potential and lethal use of *B. anthracis* is a bioterrorism agent in which spores formed by this bacterium can be sprayed and aerosolized to remote areas to spread disease. The events of 2001, however, have verified and made clear that bioterrorism linked to this bacterium is not merely a danger, but rather a reality (Jernigan 2001). *B. anthracis* has a tripartite toxin and a poly-D- glutamic acid capsule as its two main virulence factors (Jernigan 2001). In order to hide from the body's macrophages, Pathogenic *B. anthracis* forms a capsule that resembles the host's immune system (Mock and Mignot 2003). The tripartite toxin of this bacterium is composed of three separately secreted proteins: lethal factor (LF), edema factor (EF), and protective antigen (PA) (Mock and Mignot 2003; Turk 2007). A binary A-B toxin, edema toxin (ETx) and lethal toxin (LTx), respectively, are created when anthrax toxin functions as the binding/linking (B) domain and EF and LF act as the active (A) domains (Singh et al. 1999). The bacterium multiplies significantly following contact with skin lesions or ingestion and kills the infected host, whether human or animal, within a few days or weeks. Anthrax disease is not a problem in developed nations because only a minimal number of cases have been documented. However, in countries where agriculture is the only source of income, cutaneous anthrax poses a serious threat to public health. Although anthrax has been linked to humans for a long time, it only recently gained public attention following incidents in the United States in September 2001.

2. EPIDEMIOLOGY

2.1. OCCURRENCE

Historically, this disease originated from sub–Saharan Africa and later spread to various countries worldwide. The prevalence of this illness varies depending on the environment, the soil, and the activities that promote the spread of *B. anthracis* globally. As a result, just because they are isolated from one atmosphere does not necessarily mean that that environment is in their habitation (Foster and

Slonczewski 2017). The more frequent origins of this disease are inadvertently consuming filthy bone meal or pasture by tanner effluent. However, there are few outbreaks, and the affected animals are small. In most countries, compared to satirical incidences, there is a significant decline in its occurrence by developing an active live stokes vaccine coupled with penicillin and implementing the quarantine protocol and regulations (Read 2003).

The contaminated soil is the definitive reservoir of the causative agent, and spores of the bacterium remain viable for decades. The major host of the disease, herbivores, become infected more quickly when they forage in a polluted area. Because the causative organism does not solely rely on a host reservoir, it is difficult to remove from an area, which explains why it is still endemic in many nations (Carter and Wise 2004). Products from infected animals and contact with infected animals are the exclusive sources of human infection. Classification of human Anthrax varies widely depending on the direct contact or handling. Suppose a human acquired the disease directly from the infected animal. In that case, it is considered nonindustrial, whereas it is industrial if a human gets this disease while handling an infected animal's contaminated products. Veterinarians, butchers, and farmers are usually affected by nonindustrial Anthrax as they work with animal carcasses or their byproducts. On the other hand, management of contaminated hides, wool, hair, bone meal, or byproducts led to the development of industrial anthrax. Due to dust inhalation that contains spores, industrial anthrax is more likely to cause lung disease (Constable et al. 2016).

2.2. RISK FACTORS

2.2.1. HOST FACTOR

All vertebrates are susceptible to the disease, however sheep and cattle are the most frequently affected, whereas horses and goats are less frequently affected. Humans dominate this group, whereas pigs, cats, and dogs are relatively resistant. In farm animals, Anthrax is invariably lethal, except in resistant animals like pigs, and in these species, the case ratio and fatality are also high (Read 2003).

2.3. AGENT FACTOR

The lethal factor (LF) and edema factor (EF) sections both contain virulence factors that contribute to the virulence of the virulent strains. Toxins associated with spore-forming units are termed protective antigens. *Bacillus anthracis*'s primary virulence factors are its capsule and toxins. *Bacillus anthracis* is a complex organism that has three protein parts referred to as components I, II, and III. Edema factor (EF), lethal factor (LF), and protective factor (PF) make up their first, second, and third components, respectively.

Each basic component also has one mobile protein. The elements that enter the host cells include LF and EF, which compete for binding with the protection factor (PF), which has a role in membrane translocation (Paccani et al. 2007). These three crucial elements combine their effects to give *Bacillus anthracis* its poisonous properties. There is relatively low mortality when the infection is caused by component one and two, and lethality reaches a maximum when component three also act with the first two components. Only those virulent strains encapsulated toxigenic (Carter and Wise 2004).

2.4. ENVIRONMENTAL FACTOR

In climates including tropical and sub-tropical, where there are high rainfalls annually, the infection persists more in the soil, and severe outbreaks of the disease are usually encountered. Every summer, the outbreak

spreads over African nations, and in certain years, when there has been a lot of rain, it has intensified to a terrible rate. During this time, wild species including hippos, elephants, and cape buffalo experience large mortality rates. Predators may act like disease carriers, which could be the cause (Constable et al. 2016). A soil-borne illness can cause periodic outbreaks in areas with cool, temperate climates.
For instance, strong rain following a protracted drought, a dry summer following a protracted heavy rain, and always in warm weather with a constant temperature of 15°C. There is improbability in the hypothesis that these conditions result in vegetative proliferation and sporulation with the production of Anthrax. However, spores consist of a greater buoyant density and become concentrated in the wet soil, which helps them to remain suspended in the still water and allows further concentration on the soil as the water evaporates. The climate relationship has made the prediction of anthrax life expectancy easy in the soil (Van Ness 2008).

2.5. TRANSMISSION

Infectious agents enter the host body by various means, including inhalation, ingestion, or through the host's skin. Organisms spread in the body's area by insects, streams, carnivores, dogs, wild birds, and contaminated feces from the infected animals. In the new areas, contamination usually occurs through contaminated animal products, including fertilizers, hides, wool, bone meal, or contaminated forage, concentrates, or other feeds. Infection through inhalation is of minor importance, but the possibility of infection through dust should always be in mind. In wool and hair industries, inhalation of spores by workers is the primary source of Wool Sorter's disease (Constable et al. 2016).

3. PATHOGENESIS

After inhalation through the pulmonary route, the bacterium needs a lesion that helps it to enter the host's body, following germination of the spores and is carried to the lymphatic system, where multiplication occurs. During the incubation period, bacteria are filtrated by the reticuloendothelial system and spleen. Last but not least, the toxin's impact causes the system to breakdown in the final hours of life. Toxins cause the endothelial cell lining of the veins to break down, which ultimately leads to internal bleeding. Moreover, in systemic disease, they induce lethality in target tissues (Moayeri 2015). After ingestion of the spores, there is the development of infection through the mucous membrane, in the epithelium surrounding the erupting teeth, fibrous foodstuff, and through scratches. The causative organism is resistant to systemic phagocytosis due to the presence of factor D-glutamic acid capsule, which stimulates its proliferation in draining lymph nodes and eventually reaches into the bloodstream, passing through the lymphatic vessels and causing septicemia. This septicemia results in a massive invasion of body tissues, and a lethal toxin causes tissue damage and edema. Ultimately, there is cell death from the cumulative effect of shock, terminal anoxia, and acute renal failure (Foster and Slonczewski 2017).

4. CLINICAL FINDINGS

4.1. CLINICAL FINDINGS IN ANIMAL

Obligate causative agent by nature, the typical incubation period for this virus is 3-7 days, while it can occasionally last up to 14 days. The disease's progression in herbivores, however, ranges from chronic to acute (Hungerford 1990).
Some of the clinical findings in per acute form of the disease in sheep, cattle, or goat that has no previous history of ill are a few convulsions, collapse, sudden onset, dyspnea, trembling, and staggering (ss

(Carter and Wise 2004). There might be complete or partial absence of rigor mortis in affected animals. At the mouth, nostrils, anus, or vulva, black, tarry-like dark blood does not clot in these animals (Collins and Huey 2015).

A sudden increase in body temperature, excitement, cardiac, pulmonary distress, depression, convulsion, and death are present clinical findings that can be seen in acute form. Due to the 41.5°c rise in temperature, there may be abortion or lack of rumination. Bleeding may occur from several natural orifices of the body, mainly lasting about 36-48 hours (Hungerford 1990). In relatively resistant animals like pigs and horses, fever, listlessness, edema of body tissues, petechial hemorrhage, and anorexia are common clinical findings. At the nostrils, bloody froth may be seen with dysentery (Collins and Huey 2015). On the other hand, subcutaneous edematous swelling present locally at the ventral neck, thorax, and shoulders characterized the chronic form of the disease.

4.2. CLINICAL FINDINGS IN HUMAN

More than 90–95 percent of human cases manifest the illness on the skin. Areas of exposed skin become itchy and develop sores. These lesions develop and pass-through various stages, including vesicular, with a blister becoming hemorrhagic, a popular eschar that develops 2-6 days after the hemorrhagic stage. These vesicle dries and ultimately transform into a depressed dark black scab. This black scab is a malignant pustule with widespread swelling (edema) and redness. The disease lesions are usually without pain, but surrounding edema results in pain in the body. If left untreated, lesions can reach the lymph nodes (regional). In severe cases, septicemia can be seen. Overall, untreated cases have rare death events if early treatment occurs, but the case fatality rate varies from 5% to 20% (Nijm and Hugh-Jones 2001).

The inhalation form of the disease is rare and can be seen with little and non-specific clinical signs such as cough, chest pain, malaise, and fever. Early symptoms show similarity with flu-like illness. Within 3-6 days, rapid hypoxia, elevated temperature, and mediastinal widening might occur. In this case, the mortality rate is nearly 100% if left untreated. Meningitis also presents. Mortality can be decreased by commencing an appropriate protocol of antibiotics (Collins and Huey 2015).

In other forms, the oropharyngeal or intestinal form is rare, and there is no outbreak in developed countries, but there are massive outbreaks of these forms in developing countries. The main reason for these outbreaks is ingesting contaminated meat from infected animals. There may be GIT symptoms associated with septicemia, fever, and ultimately death of the host. In this form, the case fatality rate reaches 25-75%. However, in oropharyngeal Anthrax, lymphadenopathy leads to neck swelling, throat pain, oral ulcers, fever, and dysphagia, ultimately resulting in death dur severe swelling and septicemia. Reports showed it has similar fatality rates to the intestinal form (Collins and Huey 2015).

5. TREATMENT

The causative agent shows susceptibility to several drugs, including streptomycin, penicillin, tetracycline, chloramphenicol, and erythromycin. The line of treatment should be followed for five days. Antimicrobial treatment is often useless in the case of acute Anthrax (Hirsh and Zee 2003). If treatment is initiated 24 hours after the infection, then the above-listed antibiotics can act as a life-saving drug during treatment. Multiple animal deaths may be seen after the treatment is stopped. The degree of protection to the animal through antibiotics varies from 10-90%. After the end of treatment, the combination of antibiotics and protective antigen vaccine can fully protect all the animals. Numerous animals whose handling was postponed after 24 hours post-infection were found to have varying degrees of toxemia and bacteremia (Schlomovitz et al. 2011).

6. CONTROL AND PREVENTION

Whenever an outbreak of the disease occurs, health authorities related to animals must be informed to ensure control measures such as for carcass disposal, the carcass should be properly burned or buried deep. Control methods, such as the treatment and isolation of sick animals, the immunization of sensitive stocks, and the longer-than-three-week-long quarantine of the sites, must be closely monitored. The milk of sick animals should be discarded using the proper procedures. It is required to use 10% NaOH (sodium hydroxide) to disinfect burns and fences. If treated with 3% acetic acid at a rate of 8 Liters/square meter, boiling utensils for 30 minutes will aid in the death of all types of spores (Hirsh and Zee 2003).

The control and elimination of the disease rely heavily on vaccination. Although the vaccines act as protective, they sometimes initiate several reactions in the host's body. One of the vaccines derived from an encapsulated strain has proved to help control the disease. This vaccine provides immunity for at least one year, whereas vaccines obtained from living antigens do not provide immunity (Sharma and Adlakha 1996).

For the control of Anthrax, the control of milk and meat-producing animals play a vital role in avoiding the risk to the human population. Avoid unnecessary waste. During an outbreak, quarantining the farm, diverting attention to cadavers and discharge, and immunizing survivors indirectly reduce human exposure to animal diseases. Moving milk and meat from the farm is prohibited during the quarantine period to stop the disease from getting into the food supply. Putting an end to the infection cycle begins with stopping the infection source. Getting other animals out of the afflicted area as soon as possible is vital. Therefore, fly control should be considered if flies are suspected of being significant vectors. For imported animal products from specific regions, formaldehyde must be used to disinfect materials like hair and wool. Sterilize bio-endemic foods using steam for 15 minutes at 115°C or dry heat for 3 hours at 150°C (Hirsh and Zee 2003).

7. IMPACT ON PUBLIC HEALTH

Anthrax predominantly impacts herbivore animals. Contact with sick animals or their waste products is the most common way humans become ill. People who work with hides, goat hair, bone products, wool, and contaminated wildlife are most at risk of contracting anthrax. Additionally, it can be caught by contact with infected meat, such as from workers at an abattoir. New infection sites in animals may emerge as a result of the introduction of animal feed containing bone meal. When handling pet meat or working in a knackery, people can suffer cutaneous rashes. Anthrax can also be employed as a bio-warfare or bio-terrorism agent and is most likely dispersed as an aerosol; therefore, any new case should be evaluated with this possibility in mind, particularly but not primarily in cases of pulmonary Anthrax (Nijm and Hugh-Jones 2001).

In some nations, anthrax epidemics still have a high risk, occasionally affecting people. According to estimates, each anthrax-infected cow in Africa may cause up to ten human cases. However, in wealthy countries, anthrax cases have significantly decreased. In the early 1900s, there were over 130 human cases per year in the U.S., but today, there are often only one or two cases of cutaneous Anthrax per year. Anthrax cases are rare and intermittent in many nations, mostly among veterinarians, agricultural workers, and people who produce items made of hides, hair, wool, and bones. At least 90–95 percent of all naturally occurring anthrax infections occur on the skin. Although it appears less common, outbreaks linked to tainted meat can also involve the gastrointestinal form. Although inhalational Anthrax is uncommon, aerosolized biological weapons should create a significant amount of this type. 11 instances of cutaneous and 11 cases of inhalation anthrax were associated with a bioterrorist attack in 2001 that used mail that was infected with anthrax. The death rate is impacted by the disease's form. According to estimates, 5-

20% of cutaneous anthrax cases that go untreated result in fatalities, but less than 1% of those who receive antibiotic therapy do. In contrast, even with the proper care, inhalational Anthrax has a high fatality rate. Older, more extensive treatment regimens may reduce the mortality rate, although earlier estimates estimated that the case-fatality rate for this variety was close to 90% to 100% (Nijm and Hugh-Jones 2001).

8. PUBLIC HEALTH ACTION RECOMMENDED

- With the proper legislation for meat handling and efficient animal immunization, anthrax disease can be avoided—regulations governing the sale of meat and hygienic procedures at slaughterhouses. Before being killed and sold, animals intended for human consumption should be inspected.
- Coordination with the veterinary and animal husbandry departments, surveillance, and a cow vaccination drive.
- The hamlets provide the locals with highly critical one-line health education input as part of a behavioral change communication campaign for anthrax avoidance. Avoid raw meat and thoroughly prepare it before eating to reduce your risk of getting Anthrax.
- The inoculation of animals against Anthrax is crucial. Breaking the cycle of poverty and infection is one of the most effective ways to provide health security to the great majority of disadvantaged groups and keep them from further falling into poverty.

9. CONCLUSION

Anthrax is a zoonotic disease and is of major concern for both humans and animals. This disease can be transmitted from affected animals or from the carcass of affected animals or their by-products. The spores of this bacterium can tolerate environmental conditions and can persist in soil for several years. In this way, soil acts as a main reservoir for domestic and wild mammals. Outbreaks of anthrax in different species of animals have been reported. Suspected cases should be confirmed by performing laboratory tests. As this disease is zoonotic, so we can eliminate the risk for humans by controlling and managing this disease in animals. This can be done by proper disease surveillance, outbreaks investigation, quarantine of affected animal, awareness among animal owners and persons working at slaughter house, implementing effective vaccination programmes, proper disposal of infected animal carcass and by adopting effective disinfection procedures.

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Cat Scratch Disease

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ABSTRACT

The transmission and prevalence of contagious diseases are intricately connected to interactions among humans, animals, and the broader ecosystem. Many infectious diseases affecting humans have their origins in animals, with a global escalation in both incidence rates and geographical distribution. The proximity and contact between humans and animals, particularly those serving as reservoirs for emerging zoonotic diseases, pose a significant risk for disease transmission. The contemporary surge in pet ownership amplifies concerns regarding potential infections transmitted from animals to humans. Among the spectrum of zoonotic diseases, Cat Scratch Disease (CSD) stands out as a global Anthropozoonosis associated with Bartonella henselae, a Gram-negative bacterium. This disease was discovered in 1983. The transmission occurs anywhere cats and their fleas are present, leading to serious public health implications. Although CSD typically manifests as a self-limiting bacterial infection of regional lymph nodes, approximately 4% to 9.6% of cases develop severe symptoms necessitating hospitalization. These symptoms encompass retinitis/neuro-retinitis, brain or spinal cord inflammation, hepatosplenic disease, osteomyelitis, and skin rashes. The therapeutic approach to Bartonella infection is tailored based on the patient's clinical presentation and immune status, with a favorable prognosis observed in the majority of CSD cases, characterized by spontaneous resolution. The use of antibiotics for the treatment of CSD always remained a topic of debate, however, antibiotics can be used to shorten the course of CSD. Azithromycin, rifampicin, ciprofloxacin or trimethoprim-sulfamethoxazole can be used for the treatment of CSD. Scratches, bites and licks from kittens or stray cats should be avoided to minimize the risk of CSD. Moreover, A Comprehensive flea control system/treatment for cats can help to lower the risk of human infection.

Keywords: Zoonotic Diseases, Cat Scratch Disease, Bartonella henselae, Disease transmission, Public Health.

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1. INTRODUCTION

Humans, animals, and the ecosystem all have a substantial impact on the onset and spread of various contagious illnesses (Thompson and Kutz, 2019). The majority of diseases that afflict humans have their roots in animals. According to the "2010 Asia Pacific Strategy for Emerging Diseases" report, it was estimated that roughly 60% of emerging human infections are zoonotic (WHO, 2020). While in recent years, the newly emerged human diseases have been predominantly of animal origin (Rahman 2020).

Emerging zoonosis refers to zoonotic diseases that are either recently identified, newly evolved, or previously known but are now experiencing a surge in incidence or expanding into new geographical areas, hosts, or vectors (Rahman et al. 2020). Over the past seven decades, at least 250 zoonosis have been identified as emerging or re-emerging zoonotic diseases. These diseases have been spreading rapidly on a global scale, both in terms of incidence and geographical distribution. Humans are at risk of contracting these diseases due to close contact with animals, which often serve as reservoirs for these emerging and re-emerging zoonotic diseases (Woolhouse and Gowtage-Sequerua 2005).

1.1. ZOONOTIC DISEASES OF PETS AND COMPANION ANIMALS

The presence of pets in bedrooms, allowed by approximately 14% to 62% of pet owners, poses an increased risk of zoonotic diseases (Chomel and Sun 2016). The population of companion and pet animals has significantly grown in recent decades, but it's important to recognize that these animals can also serve as a substantial reservoir for disease-causing agents. This surge in pet ownership has raised concerns about the potential transmission of infections to humans. In many contemporary households, animals are cherished as pets, placing a significant portion of the population at risk of contracting zoonotic diseases from their pets, companion animals, and even exotic birds and animals. Various types of infectious diseases, including viral, bacterial, parasitic, and fungal, are associated with these animals (Halsby KD et al. 2014).

Pets and companion animals are frequently associated with various zoonotic diseases, including but not limited to brucellosis, campylobacteriosis, chlamydiosis, cat scratch fever (caused by Bartonella henselae), ehrlichiosis, giardiasis, hantavirus, hookworm infections, influenza, rabies, Lyme disease, rocky mountain spotted fever, leptospirosis, monkeypox, pasteurellosis, Q fever, plague, roundworm infections, salmonellosis, methicillin-resistant Staphylococcus aureus (MRSA) infections, streptococcal infections, toxoplasmosis, and tularemia etc. These zoonotic diseases, including salmonellosis, staphylococcosis, and rabies, can be found across a broad spectrum of pets and companion animals (Halsby et al 2014; Day 2016; Jacob and Lorber 2016).

2. INTRODUCTION TO CAT SCRATCH DISEASE

Cat Scratch Disease (CSD) occurs worldwide, anywhere cats and their fleas are found. It is a self-limiting bacterial infection of the regional lymph nodes associated with a Gram-negative bacterium of Bartonella species. In humans, the disease usually becomes clinical after one to two weeks, following a feline scratch with primary signs of inflammatory nodules at the site of the scratch. Different Bartonella species cause diseases termed as bartonellosis in humans. Bartonella bacteria can infect humans through fleas, body lice, sand flies, or flea-infested animals.

2.1. HISTORICAL NOTES

The genus Bartonella belongs to the Alphaproteo bacteria class and Bartonellaceae family. It shares a close genetic relationship with Brucella and Agrobacterium while being less closely related to members

of the Rickettsiaceae family (Rose and Koehler 2020). Debre et al. (1905) first reported the clinical syndrome of cat scratch disease in 1950 but until 1983, the causative agent was not recognized spite several studies. In the same year, Wear et al (1883) used Warthin-Starry silver stain and found small, gram-negative, pleomorphic bacillus in infected lymph nodes of CSD patients. What originally was known as “Cat Scratch Bacillus” was named as Afipia felis by Brenner et al. (1991). In 1993, there was a proposal to merge the genera Bartonella and Rochalimaea into a single genus and to exclude the Bartonellaceae family from the Rickettsiales order, based on genetic similarities (Rose and Koehler 2020).

2.2. ETIOLOGY

Cat scratch disease, an Anthropozoonosis with a global spread, is associated with Bartonella henselae and poses serious public health issues (Alonso et al, 2021). The causative agent of CSD was discovered in 1983, after 50 years of being unknown. At first, *Afipia felis* was suspected as the causative agent, but later studies did not support this hypothesis. In the 1990s, it was proven beyond doubt that Rochalimaea henselae, which was later renamed as Bartonella henselae, is associated with CSD. Bartonella henselae (B. henselae) can be found in the blood of cats and can infect other cats through fleas. However, it is unlikely that fleas directly transmit the bacterium to humans, but they may help increase the number of infected cats (Windsor 2001).

2.3. IDENTIFICATION OF BARTONELLA SPECIE

The initial isolated colonies of a specific Bartonella species can exhibit morphologies. For instance, in the case of B. henselae, it may appear as either (Koehler et al. 1883; Rose and Koehler 2020):

- Irregular, elevated, whitish, rough, and dry in texture, often described as "cauliflower", "molar tooth," or "verrucous." These colonies exhibit agar pitting and adhere strongly to the agar. or
- Flat, circular, with a moist appearance, and less adherent. These colonies are sometimes present alongside the rough ones within the same primary culture. The extent of colony heterogeneity varies depending on the species. B. henselae typically displays a higher proportion of rough colonies, whereas primary cultures of B. quintana colonies are almost always uniformly smooth, albeit varying in size. Subsequent subcultures of B. henselae on agar tend to show an increasing proportion of smooth colonies over time (Koehler et al. 1883; Rose and Koehler 2020).

Preliminary identification of either B. henselae or B. quintana can be established if the following characteristics are observed:

- Prolonged incubation time exceeding 7 days before colonies become visible.
- The appearance of small, curved, gram-negative bacilli in Gram stain.
- Negative reactions for catalase and oxidase (Dumler et al. 2019; Welch et al. 1993).

2.4. GEOGRAPHICAL DISTRIBUTION

Cat-scratch disease often exhibits a seasonal pattern, with the highest incidence during the autumn and winter months. This phenomenon could be attributed to cat breeding cycles or the tendency for people to acquire new young pets during these specific times of the year. (Windsor 2001). B. henselae is found all across the globe (Yehudina and Trypilka 2021). After the identification of the bacterial source of Cat Scratch Disease (CSD), the connection between cats and human infections caused by B. henselae was confirmed. Over 90% of patients had a documented history of contact with cats. In a case-control study, the highest correlation with CSD incidence was found in individuals who owned kittens aged 12 months

or younger, had been licked on the face, scratched or bitten by a kitten, or possessed a kitten with fleas. (Allizond et al. 2019).

2.4.1. HUMANS

Cat scratch disease, an infection caused by B. henselae, is prevalent across the globe (Pal 2018a). However, it is important to mention that in most countries, cat scratch disease is not subject to mandatory reporting in human cases. (Sutu et al., 2020) Reports of this have been documented in numerous countries including the United States, Canada, Germany, The Netherlands, Switzerland, France, Spain, Italy, the UK, Japan, and Australia (Carithers 1985; Windsor 2001; Tsukahara, 2002; Willams et al. 2002; Pal 2018a). Following the late 1970s, the UK recorded a notably reduced number of cases, a change likely linked to the removal of the skin antigen test due to safety concerns. In regions with temperate climates, there is a heightened risk of CSD cases during the autumn and winter seasons. (Yehudina and Trypilka 2021). In the United States, cat scratch disease leads to approximately 22,000 reported cases each year, resulting in over 2,000 hospitalizations and causing an annual economic impact of $12 million. (Pal 2007; Pal 2018a).

2.4.2. CATS

Serological studies have revealed that B. henselae infection is widespread among domestic cats, with antibodies ranging from 4% to 80%. Bacteremia in domestic cats can persist for varying periods, ranging from a few weeks to several years. (Theel and Ross 2019). Bacteremia is more frequently observed in young cats, particularly those under a year old, compared to older cats. Across domestic cat populations, significant regional differences have been noted in the prevalence of B. henselae type I (Houston I) and type II (Marseille). While in most parts of Europe, B. henselae type II is the predominant strain, in eastern Asia, type I accounts for the majority, comprising 70% to 80% of B. henselae isolates. (Nguyen 1952).

2.5. ROUTE OF TRANSMISSION AND CYCLE

Bartonellosis follows a typical pattern of vector-borne diseases. The reservoir host usually has intra erythrocyte bacteria. Ctenocephalidis felis is the arthropod vector responsible for horizontal transmission among cats (Baranowski 2022). The arthropod vector feeds on blood, picks up the infection and passes it on to another reservoir or accidental host (Regier et al. 2016). Recent investigations have revealed that arthropod vectors, like ticks, lice, chiggers, and mosquitoes are responsible for transmission (Regier et al. 2016), however, transmission of the disease to man depends upon environmental factors and type of contact (Greer and Keefer 1951).

The primary route of entry for B. henselae into the body is typically through a scratch that has been contaminated with flea feces. Furthermore, cat saliva carries microorganisms that can be transmitted to humans through either cat bites or through areas of the skin that the cat licks and causes abrasions. (Pal 2018a). The infection happens more often in colder seasons, which might be related to the way cats breed (Windsor 2001).

There is no supporting evidence to support the transmission of zoonotic Bartonella from one individual to another through casual contact. (Menezes et al. 2020). On the contrary, (Konstantinou et al. 2020) *Bartonella henselae* was successfully cultured from human red blood cell (RBC) units that were contaminated with the bacteria and incubated at 40°C for 35 days, indicating a potential risk of transmission through blood transfusions (Fig. 1).

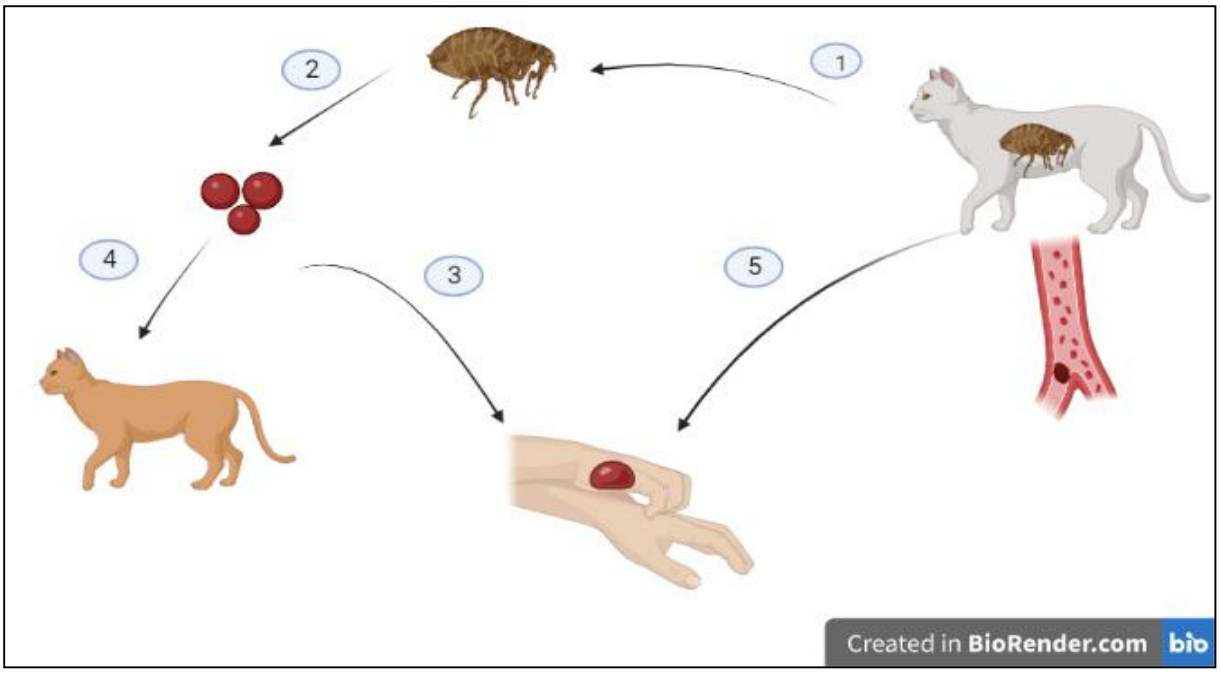

Fig. 1: Pattern of Transmission of Bordetellosis

2.6. PATHOGENESIS

The reaction to *Bartonella henselae* infection varies based on the immune system's condition in the infected individual. In those with a strong immune system, the response tends to be characterized by the formation of granulomas and the presence of pus, whereas immunocompromised patients typically exhibit a vasoproliferative response (Bass et al. 1997; Blagova et al. 2021), along with arteriolar proliferations and dilatations and lymphoid hyperplasia. Bartonella infection triggers a T-helper-cellular response mediated by interferon-ã, resulting in the mobilization and activation of macrophages, leading ultimately to development of the granulomatous disease (Schweyer et al. 2002; Blagova et al. 2021).

2.7. DISEASE IN HUMANS

Cat-scratch disease is usually a mild illness that goes away on its own. However, about 4% to 9.6% of people with CSD develop more serious symptoms that require hospitalization. These symptoms can include retinitis/ neuro-retinitis, inflammation of the brain or spinal cord, hepatosplenic disease, osteomyelitis, and skin rashes etc. (Mazur-Melewska et al. 2015).

CSD primarily impacts the lymph nodes that receive lymphatic drainage from the site of introduction, typically resulting from a scratch or bite inflicted by a young feline. Cat scratch disease has been clinically documented for more than five decades; however, the specific bacterium responsible for the disease remained unidentified for a considerable period. Dr. Douglas Wear, a pathologist, successfully isolated a novel bacterium from the lymph nodes of individuals suffering from cat scratch disease. Subsequently, extensive research efforts spanning several decades were dedicated to uncovering the precise bacteriologic agent involved in this condition (Baranowski 2022).

The onset of the disease is marked by the presence of a reddish raised lesion (erythema), either single or in clusters, at the site of infection. The diagnosis becomes easier if the doctor has access to the patient's medical

history and information regarding cat scratches or identifies visible signs of animal aggression. The lesion typically appears 3 to 10 days after the initial infection and progresses through stages of erythema, vesicles, papules, and crusting. In cases of typical cat-scratch disease (CSD), localized swelling of the lymph nodes occurs 1 to 3 weeks after the infection and can persist for several months (Mazur-Melewska et al. 2015).

2.8. CLINICAL SIGNS

The infection often seems to be asymptomatic therefore, the importance of Bartonella spp. as animal infections remains largely unclear. The complexity of investigations is largely contributed by the prevalence of infections in healthy animals, uncertainties in organism-specific diagnostic tests, and the potential for co-infection with other bacteria.

2.8.1. CATS

Cats that naturally carry *B. henselae* bacteremia typically do not show any noticeable symptoms. In controlled experiments where cats were intentionally exposed to it, the majority remained asymptomatic or displayed only mild clinical indications, such as localized responses at the injection site, minor non-specific fevers, brief and mild behavioral or neurological issues, temporary and mild anemia, eosinophilia, or reproductive abnormalities. An exception was observed in one cat heavily infested with fleas, which may not have developed an effective immune response, and as a result, became seriously ill. A post-mortem examination of this cat revealed myocarditis. Similarly, establishing conclusive evidence that *bartonella* causes illness in naturally infected cats has been a challenging task. (Johnson et al. 2020).

2.8.2. HUMANS

Bartonella henselae has the potential to disseminate and affect various organs like the liver, spleen, eye, or central nervous system in specific individuals. When the infection is localized, patients typically experience a condition that resolves on its own, but those with a systemic infection can face severe, potentially life-threatening complications. The most common clinical sign of Cat Scratch Disease (CSD) is persistent swollen lymph nodes. (Keret et al. 1998).
Common manifestations include warm, painful, and red nodes. Patients may also experience minor symptoms such as fever, as well as systemic issues like fatigue, general discomfort, loss of appetite, and headaches (Fig. 2). Typically, the majority of patients (around 50-85%) have only one affected node, with the most frequently involved nodes being in the axillary and epitrochlear regions, as well as the head and neck, and inguinal areas (Theel and Ross 2019).

2.9. DIAGNOSIS

Despite the fact that the CSD syndrome was originally described in 1950, the causative agent and diagnostic procedures weren't identified until the middle of the 1980s and later. Therefore, due to advancements in diagnostic procedures and other illnesses that resemble CSD, the epidemiology of CSD may have changed in recent decades (Mesher et al. 2016).
Diagnosing CSD can be difficult because the symptoms are often not specific to the disease (Amin et al. 2022). Additionally, it is difficult to culture the bacterium in the lab and there is no single test that is always accurate. However, the following tests can aid in the diagnosis of CSD (Pennisi et al. 2013);

- CSD may be diagnosed clinically in patients with typical signs and symptoms along with history.
- Blood tests can be used to look for antibodies to *B. henselae*, but these tests are not always reliable.

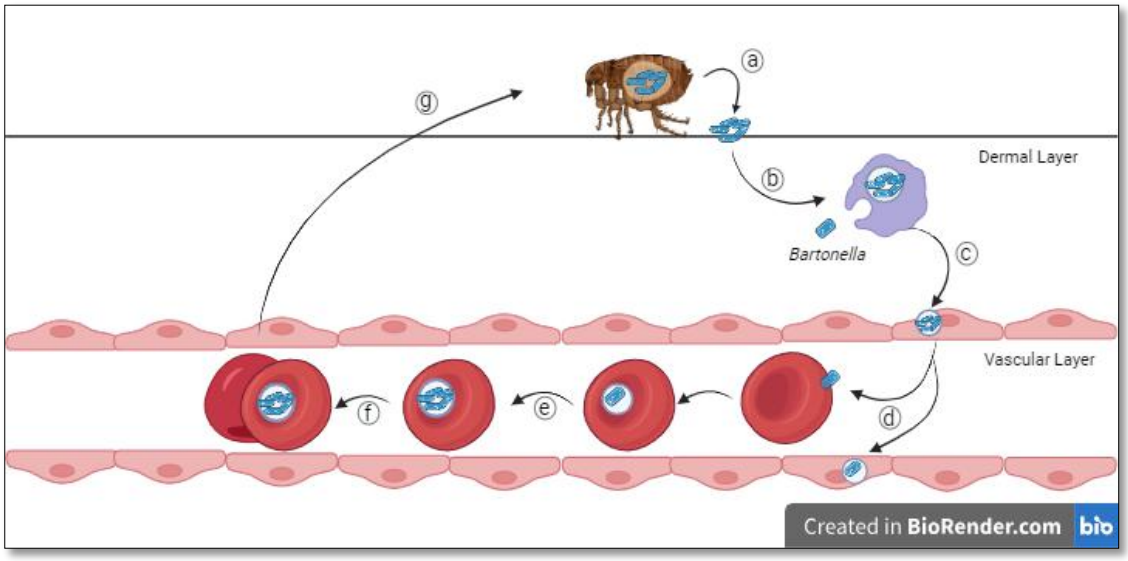

Fig. 2: The typical infection strategy employed by Bartonellae involves several stages, as depicted in the illustration. It begins with transmission through an arthropod vector (a). The bartonellae initially establish themselves in a primary location, likely by entering migratory cells (b) and eventually reaching the vascular endothelium (c), where they maintain an intracellular presence. Subsequently, these bacteria move into the bloodstream (d), where they infect erythrocytes and circle back to re-infect the primary site. Following limited replication within red blood cells (e), they persist within the intraerythrocytic environment (f), which is conducive to transmission by bloodsucking arthropods (g).

- Polymerase chain reaction (PCR) testing can be used to look for *B. henselae* DNA in tissue samples. To reduce false-negative results, repeated blood cultures are required, or PCR should be performed on multiple biological samples i.e., blood, lymph nodes, oral swab, etc.).
- Serology (ELISA) can be more useful for exclusion than for confirmation of the infection. Although cross-reactivity with other *Bartonella* species may limit the interpretation.

In the conventional approach, the identification of CSD used to necessitates meeting three out of four specific conditions (Barajas et al. 2017):

- Having had contact with a cat and exhibiting a primary inoculation site.
- Receiving a positive result in the CSD skin test.
- Obtaining negative findings in investigations ruling out other potential causes of subacute lymphadenopathy.
- Displaying distinct histopathological characteristics in the biopsy results.

However, when patients do not present with a systemic illness, the isolation of *B. henselae* is often unsuccessful. In such cases, the most commonly employed diagnostic test involves serological examination for the presence of antibodies to *B. henselae*.

However, when patients do not present with a systemic illness, the isolation of *B. henselae* is often unsuccessful. In such cases, the most commonly employed diagnostic test involves serological examination for the presence of antibodies to *B. henselae*. (Annoura et al. 2020).

2.9.1. DIFFERENTIAL DIAGNOSIS

The differential diagnosis for typical Cat Scratch Disease (CSD) encompasses various causes of unilateral lymphadenopathy, including typical or atypical mycobacterial infections, tularemia, plague, brucellosis,

syphilis, sporotrichosis, histoplasmosis, coccidioidomycosis, toxoplasmosis, infectious mononucleosis syndromes, lymphoma, and other types of neoplasms (Koehler and Duncan 2005). It is important to note that the diagnosis of CSD can be missed if the healthcare provider fails to obtain a comprehensive medical history, especially in cases of atypical CSD syndromes. This oversight can also occur in adult patients with typical CSD when internists without experience in diagnosing CSD are involved, as opposed to pediatricians. Additionally, in elderly adults over 60 years of age, manifestations of CSD can be less typical, further complicating diagnosis (Ben-Ami et al. 2005). Given that domestic cats are the most common type of companion animals in the United States, it is crucial to gather a thorough history of animal exposure when evaluating a patient exhibiting signs consistent with CSD. Fortunately, in the majority of CSD cases, typical or atypical, typically a spontaneous resolution occurs.

2.10. TREATMENT

The therapeutic approach for Bartonella infection is determined by the patient's clinical symptoms and immune status (Mazur-Melewska et al. 2015). Given the natural progression of uncomplicated CSD, antibiotics are not recommended for localized CSD (Klotz et al. 2011). In immunocompetent patients with mild-to-moderate infections, management involves reassurance, regular follow-up, and pain relievers (Mazur-Melewska et al. 2015; Zangwill 2021). If the lymph nodes become suppurative, aspiration is advised to alleviate discomfort, while caution is advised against incision and drainage due to the potential development of chronic sinus tracts. During aspiration, it is recommended to target multiple locations since multiple septate pockets containing coalesced microabscesses are often present (Mazur-Melewska et al. 2015).

The use of antibiotics for the treatment of CSD always remained a topic of debate, however, antibiotics can be used to shorten the course of CSD (Mazur-Melewska et al. 2015). In cases of significant lymphadenopathy, azithromycin can be used @ 10 mg/kg on the first day, followed by 5 mg/kg per day on days 2 to 5. Other options include rifampicin @ 20 mg/kg/day divided into two doses for 2-3 weeks, ciprofloxacin @ 20-30 mg/kg/day in two doses for 2-3 weeks, or trimethoprim-sulfamethoxazole (trimethoprim @ 8 mg/kg/day and sulfamethoxazole @ 40 mg/kg/day, divided into two doses).

2.11. PROGNOSIS

Immunocompetent patients with CSD typically have an excellent prognosis, with a high likelihood of complete recovery. However, in about 5-10% of cases, notable morbidity may arise, typically resulting from the involvement of the central or peripheral nervous system or the manifestation of disseminated disease affecting multiple body systems. It should be emphasized that experiencing a single episode of cat-scratch disease confers lifelong immunity to all affected individuals (Mazur-Melewska et al. 2015).

2.12. PREVENTION AND CONTROL

It is not advised to remove cats from households just because they are able to transmit *B. hensalea* (Konstantinou et al. 2020). Cat scratch disease can be prevented and controlled by the following measures (Mesher et al. 2016):

- Scratches, bites and licks from kittens or stray cats should be avoided to minimize the risk of CSD. Immunocompromised people should take special care about that.
- A Comprehensive flea control system/treatment for cats can help to lower the risk of human infection as CSD is a zoonotic infection that is sustained and transmitted among cats by fleas.

Washing hands after handling them might help lessen the risk by removing possibly contagious flea feces that could get inside the skin through cuts or abrasions.

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Advanced Diagnostic Techniques for Listeriosis

41

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ABSTRACT

Listeria monocytogenes is the significant food-borne microbe causing a yearly flare-up of food contamination on the planet. Babies, pregnant moms, and immune-compromised individuals are at high hazard. Due to the epithelial grasp (by E-cadherin restricting), it can smother safe cells and flourish in the gastrointestinal lot till the cerebrum through blood stream. Identification generally elaborate culture techniques in view of specific advancement and plating followed by the portrayal of Listeria spp. in view of morphology, sugar aging and haemolytic properties. These techniques are the highest quality level; yet they are extended and may not be appropriate for testing of food varieties with short time spans of usability. Thus more quick tests were created in light of antibodies (ELISA) or molecular techniques (PCR or DNA hybridization). While these tests have equivalent responsiveness, they are quick and permit testing to be finished within 48 h. All the more as of late, molecular techniques were formed that target RNA instead of DNA, like RT-PCR, or nucleic acid based sequence amplification (NASBA). These tests give a proportion of cell feasibility as well as be utilized for quantitative examination. Furthermore, different tests are accessible for sub-species characterization, which are especially valuable in epidemiological studies. Differential test used were phenotypic markers comprised of multilocus enzyme electrophoresis and serotyping. At present phenotyping techniques are replaced by molecular methods which are more precise and rapid. These new techniques are presently principally utilized in research however their extensive potential for routine testing in the future can't be disregarded.

Key words: Listeriosis, Serology, Polymerase Chain Reaction, Electrophoresis, phenotypic techniques

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1. INTRODUCTION

Listeria is a Gram-positive bacterium that proliferates within cells. It causes food-originated diseases in humans and animals. Spoiled silage frequently leads to this disease in animals, but it is very difficult to identify the root cause of the disease because it is prevalent in the natural environment and farm premises (Orsi et al. 2016). Carrier animals may continuously shed Listeria in their milk and feces (Dehkordi et al. 2013). Listeriosis is an emerging food-borne zoonotic disease that is transmitted through contaminated milk, meat, its products, water, ready-to-eat food, salads, etc. (Meyer-Broseta et al. 2003). Due to changes in life style of human beings, people prefer to take ready-to-eat food so there is a high chance of carrying the Listeria spp. Especially, the immune-compromised people such as old aged, neonates, and pregnant women are affected by this disease. The significant symptoms include septicemia, encephalitis, and abortion (Hunt et al. 2012).

L. monocytogenes is the causative agent of Listeriosis that can persist and reproduce in different climatic circumstances i.e. decreased temperature, high saline concentrations, and low pH (Sleator et al. 2003). The main source of contagion is spoiled fodder, meat, milk, etc. with Listeria spp. Refrigerated products are more susceptible to listeria contamination. With the recent advancement in science, it has been revealed that the mortality due to Listeriosis is higher than all the other food-borne diseases such as Salmonella, Campylobacter, and Vibrio (Behravesh et al. 2011).

Various pathogenic factors are linked with the *L. monocytogenes*. Among these Haemolysin and Lysin O are the most important factors and assist in the escape from the phagocytic defense mechanism of the mammalian cell. Haemolysin factor is encoded by the hlyA gene (Camejo et al. 2011). The iap gene is also essential for invasion into the intestine of the host and this gene is specific for the host to target. For the molecular affirmation of pathogenic factors of *L. monocytogenes* it is essential to target both these genes (hlyA and iap). There is a huge number of *L. monocytogenes* in clinical samples, but it is very difficult to detect in food samples owing to the limited numbers in food items. The food authority in the US has devised a zero-tolerance for Listeria in ready-to-eat (RTE) foods. Therefore, a single bacterium in the RTE is critical and dangerous for consumers and only the PCR techniques can detect this very low level of pathogen in food items (Luber et al. 2011).

Accurate diagnosis of Listeriosis can be made by isolation and identification of bacterium, but it is tiresome and laborious for cultural growth and biochemical characterizations. Different sero-diagnostic tests have been devised to detect listeriosis, but the chances of false positive results are higher. Consequently, now-a-days, ELISA and advanced molecular techniques are preferred as compared to conventional cultural methods so it is the need of the hour to appraise different advanced diagnostic tools for the identification and detection of Listeria spp. in food items and other clinical samples, and precautionary measures should be adopted to prevent the spread of this disease globally. The detail of detection and typing methods mostly used for listeriosis is described in Fig. 1.

A 100 CFU per gram of Listeria in foodstuff is required to be infectious for animals or humans. Because of non-indicated side effects, it is hard to distinguish at the beginning phase. It was observed that the 10 CFU in 25 grams of packed food items caused this disease and 100 CFU per mL led to the reappearance. In this way, researchers fostered a few methods to satisfy the requirement for a vigorous and delicate strategy to distinguish *L. monocytogenes*. The pertinent and accessible techniques for the detection of Listeriosis are mentioned below:

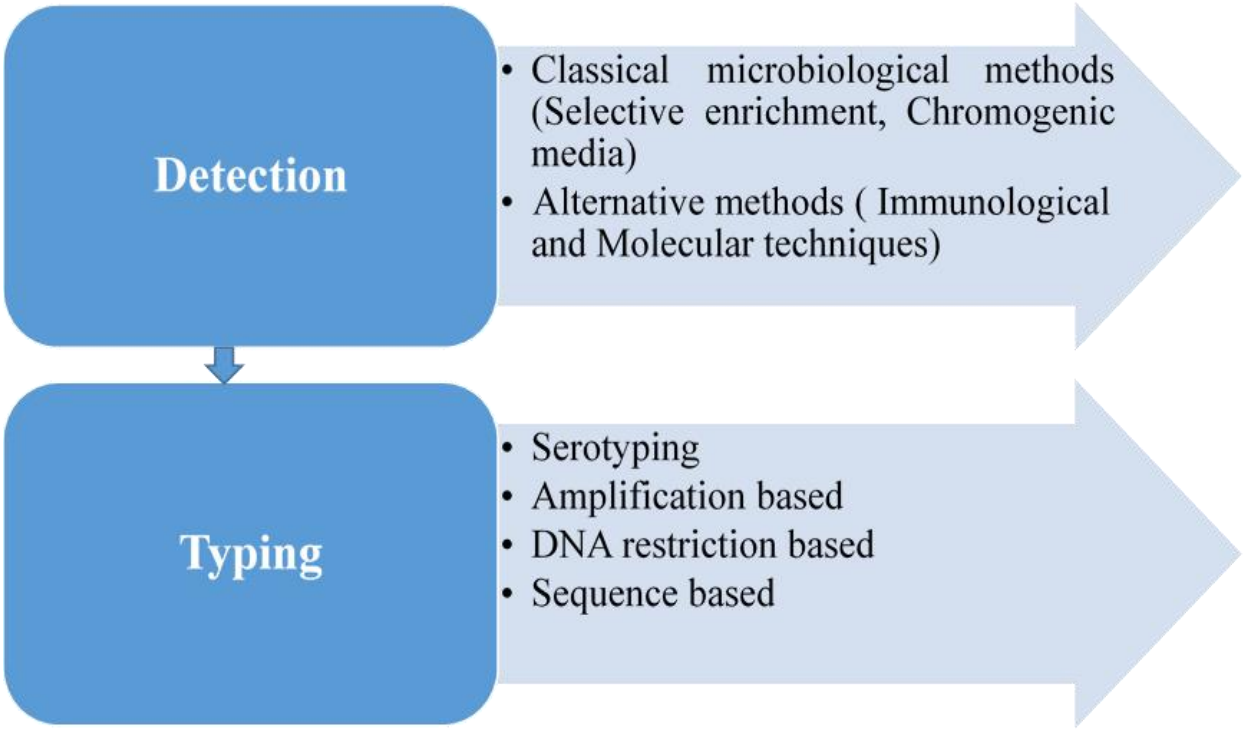

Fig. 1: The detection and typing methods for Listeriosis

1. CULTURE-BASED TECHNIQUES

The tedious yet exact cold advancement technique was made during the 1990s (Lorber 2007). The Food and drug authority (FDA) proposes to use chromogenic medium for the distinguishing proof of Listeria species (Janzten et al. 2006). Lecithin was hydrolyzed, and the blue/green settlements showed up because of the separation of the substrate by a compound β-D-glucosidase. Subsequent to the affirmation of Listeria, it was re-culture in non-particular agar and ready for 5 days in length biochemical analysis. Furthermore, there may be chances of false positive results, a requirement for a few synthetic compounds, media, and reagents, as well as a necessity of time and energy (Jadhav et al. 2012). According to the FDA procedure for isolation of Listeria in milk and fish samples, the Limit of detection (LOD) should be below 1 CFU/mL (Hitchins and Jinneman 2013; Valimaa et al. 2015). The results of Valimaa et al. were identical to ISO 11290-1 technique created in 2004. Afterward, it was revealed that the LOD was 1 CFU per gram through the USDA-FSIS technique (Valimaa et al. 2013). The MPN method was more delicate than a chromogenic media (Dwivedi and Jaykus 2011). To distinguish proof of Listeria, the PCR method was more reassuring than past methodologies i.e., cultural and chromogenic methods (Law et al. 2015).

2. IMMUNOLOGICAL TECHNIQUES

Antigen-neutralizer test was revealed to be capable of identification of listeriosis. As reported the immunological method is more sensitive as compared to conventional techniques, which is 105 cells/mL. However, the preparation of antibody for the immunological reaction is time time-consuming process (Diaz-Amigo et al. 2013).

3. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

In this assay, the antibody is immobilize to a microtitre plate for capturing antigen, and then a secondary antibody labeled with enzyme was added to identify the antigen. This is a very rapid method for the detection of Listeria spp. mostly for food samples. A recent advancement of the Listeria test facility from food and environment samples within 30 h of receipt. This test is very accurate and delicate, and equally sensitive to cultural methods.

Traditionally, serological methods have been used for the detection of listeriosis but they have been mostly untrustworthy and deficient in precision. Substantial cross-reactivity with other Gram-positive bacteria has been noticed. *L. monocytogenes* is widespread in the environment, and humans and animals are frequently exposed to this bacterium. In humans about 53% of serum antibody against *L. monocytogenes* have been described. A similar pattern has been stated in animals but with minor alterations in different species (Dhama et al. 2015).

Haemolysin and listeriolysin O are the main pathogenic factors. These factors can stimulate an antibody response. Indirect ELISA was used for the detection of anti-LLO in listeriosis but cross-reactivity of LLO with cytolysins has been reported. This is the hurdle in the development of a dependable ELISA test for the detection of listeriosis (Hara et al. 2008).

The Sandwich ELISA technique was better than traditional methods to distinguish Listeria in foodstuff tests (Bell and Kyriakides 2005). The LOD was 105-106 CFU per mL while the counter antigen was utilized to focus on Listeria. Enzyme-linked fluorescence assay (ELFA) is used for the detection of listeria spp. in food samples (Ueda and Kuwabara 2010). Depending upon the sample's acidity and alkalinity, an LOD of 105–106 CFU/mL is established to be precise. For detection of listeriosis, the sera samples were diluted at 1 ratio 200 and used in indirect ELISA. The positive negative ratio was fixed to more than 2 (Malla et al. 2021).

4. IMMUNO-MAGNETIC SEPARATION

In this method concentrated bacterial cells are combined with a magnetic field by using nanoparticles. This technique was used to increase the reliability of detection (Amagliani et al. 2006). The immune-based technique uses anti-Listeria with immune-magnetic nanoparticle coated beads for identification of gene (hlyA) in milk sample. The LOD was found to be less than 102 CFU/0.5 mL (Yang et al. 2007). Additionally, magnetic beads coated with endolysin were used for the detection of Listeria from contaminated raw milk. The LOD range is 102 - 103 CFU/mL (Walcher et al. 2010).

5. MOLECULAR METHODS OF DETECTION

5.1. DNA MICROARRAYS

The bacterium genes plcA, plcB, clpE and inlB can be manipulated for DNA microarray (Volokhov et al. 2002). Volokhov and colleagues described that the detection of Listeriosis was positive through this technique. The scientists explored serotype-explicit test separation by consolidating 585 genomic DNA (10 samples) blended tests and observed that it was effective for 29 tests (Borucki and Call 2003). From that point forward, it was used as a corroborative strategy to take a look at the particularity of polymorphism and PCR enhancement. With an identified breaking point of 8 logs CFU/mL (Brehm-Stecher and Johnson 2007), it was revealed that 9/16 of microarray practiced to experiment falsely tainted milk were disease positive. This method is precise and authentic. However, it needs tolerance and can cross-hybridize, which may lead to a false result (Bang et al. 2013).

5.2. DNA HYBRIDIZATION

It is the simplest technique to identify Listeria spp. in foodstuff. The occurrence of an objective succession is recognized using an oligonucleotide test of correlative grouping to the objective DNA arrangement which encompasses a name for identification. Radioactive elements integrated into an oligonucleotide arrangement were recently used as marks for recognition. biotinylated tests, tests integrating digoxygenin permit identification of target arrangements with identical aversions to radioactive tests, lacking the dangers related to radioactivity. Hybridization in a microtitre plate is a helpful and exceptionally delicate and explicit methodology for the location of Listeriosis in a high quantity (Paniel, N et al. 2013). This test point fundamentally for the separation of different Listeria species by focusing on the qualities of the degenerative factors. Industrially accessible hybridization tests are regularly utilized for the testing of food sources and are widely established for their responsiveness and exactness. Accuprobe is a hybridization of labeled probes to pathogenic factor mRNA, therein only viable cells are identified. This test was established based on in-situ hybridization of labeled probes to target RNA (Umesha et al. 2018). This test has been used so far just for the recognizable proof of Listeria in sewage (Stephan et al. 2003).

5.3. RIBOTYPING

It is based on diversities in ribosomal proteins. This strategy was primarily practiced to lay out phylogenetic relations to organize prokaryotes. Relations of organic entities can be matched by determining how closely DNA sequences resemble a particular feature. The utmost valuable feature for the valuation of phylogenetic relation is the feature coding for ribosomal RNA on the point that ribosomal qualities exists in all organic entities. Frequent copies across the genome and ribosome capability have been dared to be stable over lengthy transformative spans. Ribotyping of Listeria isolates comprises the constrained chemical processing of chromosomal DNA followed by hybridization using an rRNA test. Regarding banding designs, these are utilized to sort Listeria into ribotypes and lay out the likeness of secludes. Ribotyping has been broadly dispensed in epidemiological investigations and computerization has permitted the variation of this strategy for routine investigation. Although this strategy is helpful and provides great reproducibility with the force of separation for *L. monocytogenes*, it does not exactly match with other molecular procedures (Louie et al. 1996).

5.4. RESTRICTION ENZYME ANALYSIS

In this technique, the specific DNA components were sliced through enzymes. The separation of DNA creates pieces of bands with different sizes. These DNA band sizes and quantities were analyzed and visualized through gel electrophoresis. The chromosomal bacterial DNA is referred as restriction enzyme and the exhibition of this technique is fundamentally upgraded by blending with Pulse Field Gel Electrophoresis. Consequently, DNA particles flows under the electrical field. DNA components can be isolated by their sizes. The larger body mass particle travel less whereas the less weight particles move faster in the electric field. Utilizing regular electrophoresis, DNA particles of up to 20 k base may also be isolated by Pulse Field Gel Electrophoresis (Maule et al. 1998). With just slight changes, this procedure can be useful to specify any bacterium. This method is more specific as compared to other composing techniques (Jadhav et al. 2012).

5.5. PCR METHODS

This molecular technique is used for the detection of microbes in samples of the tiniest quantity. Intensity cycles in PCR requisite a bunch of particular introductions for amplification of target region/ gene of interest. The different stages in PCR reaction include denaturation, annealing and extension of DNA (Fig. 2).

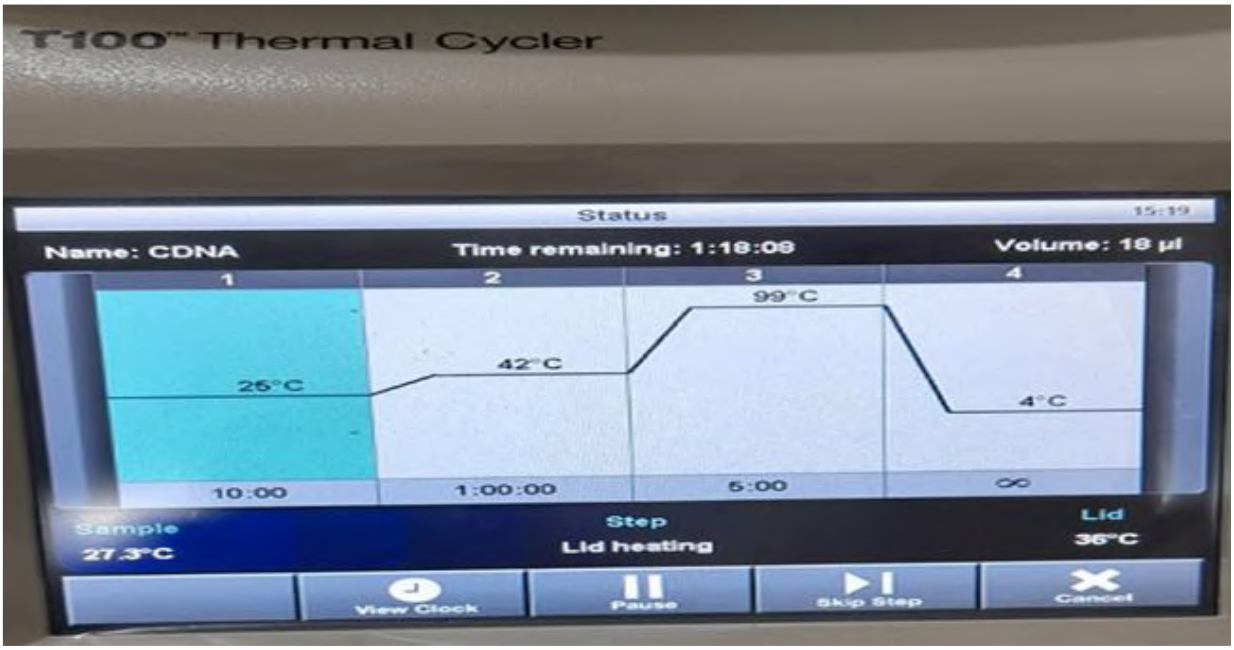

Fig. 2: Thermocycler indicated different cyclic conditions of a PCR reaction

The outcomes are then separated by electrophoresis. The different PCR techniques are used to distinguish Listeria as under:

5.5.1. CONVENTIONAL PCR

Polymerase chain reaction (PCR) is used to amplify the segment of DNA and a very minute amount of target DNA is required for detection. Recently PCR technique is considered to be the most sensitive and reliable for the detection of Listeria spp. The differences among different Listeria *spp* and the primers targeting the specific genes of pathogenic factors have been established. Before proceeding to PCR, it is recommended to selectively enrich the food items as the foodstuff contains the inhibitors.

The result obtained from PCR was affirmative for 56 out of 217 cases in normally spoiled samples. The scientists used a basis intended to focus on the genes coding for pathogenic and different proteins of Listeria for the detection (Aznar and Alarcón 2003).

In non-reasonable DNA enhancement, the PCR strategy proved a misleading positive value (Klein and Juneja 1997). Invert transcriptase PCR was used as mRNA that has a short lifetime and quickly breaks down after cell demise, it also focuses on the feature (hly and PrfA) records as opposed to DNA. To approve the procedure, they utilized cooked meat that was deliberately contaminated. They observed that the diagnosis was delicate to 1 CFU per gram. Skillet and Breidt used constant PCR and made progress with ethidium monoazide to enhance lifeless cells, contending contrary to it as a proficient technique for distinguishing microscopic organisms in low quantities (Pan and Breidt 2007).

5.5.2. DNA SEQUENCING

Sequences are defined as the method for determining the sequence of nucleotide bases in DNA. The nucleotide sequence codes the genetic information that cells use to grow and function. It is essential for assessing the function of genes.

DNA sequencing is the most precise technique for assessing genetic relationships of Listeria spp. Multilocus sequence typing (MLST) has been engaged for sequencing the other genes. This technique is developed for targeting the genes (fla, hly, actA, iap, inl, mpl and prfA) and typing of *L. monocytogenes*. In Navsari Gujarat, a total of 200 samples of food were analyzed and 18 samples were found positive for Listeria spp. The highest prevalence was observed in milk samples (8 Nos.). *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. monocytogenes* were detected in food items of animal origin. Further, *L. monocytogenes* was tested for pathogenic factors (iap, actA and hly) which showed that high chance of transmission of listeriosis through the consumption of raw milk (Nayak et al. 2015). Various *L. monocytogenes* qualities and their capabilities were clarified and there was truly expanding succession information aggregated in data sets like the data set of the Public Community for Biotechnology which is accessible for observations. The data that the total sequence of *L. monocytogenes* genome is essential and the effect of which is enormous. A business sequencing pack focusing on 16S RNA qualities is accessible from Applied Biosystems (Allerberger 2003).

Epidemiological studies on a worldwide level are significant to research the hazards linked with *L. monocytogenes* genes in food samples. Then, the World Health Organization has found a method to evaluate the hazards that are connected with Listeria in food items. The pattern of epidemiological testing is focused on molecular strategies and in this manner measures should be taken to optimize these tests. In light of the fact that numerous labs use different response conditions or limitation catalysts as well as various test boundaries (Rocourt et al. 2003).

5.5.2.1. MULTIPLEX PCR

Multiplex PCR is used for the detection of many pathogens in the same isolate. This technique is frequently used for food samples as it reduces costs and labor. In the Nested PCR technique, many primers target the same gene. This increases the reliability and sensitivity for the detection of Listeria spp. in clinical isolates of milk and environment.

Multiplex PCR is as a solid, productive, and efficient strategy to diagnose disease in suspected samples (Alarcón et al. 2004). This technique is mostly used to recognize six normal food-originated microbes in ready-to-eat food (Lei et al. 2008). The Multiplex PCR (MPCR) technique focuses on the haemolysin gene of *L. monocytogenes*, the nuc gene of *S. aureus* and the invA gene of *S. enterica*, with a breaking point of 1 CFU per mL (Zhang et al. 2009). The MPCR is vague for the comparative estimated amplicon and advancement (Mustapha and Li 2006).

5.5.3. RT-PCR

In this technique, in the first step mRNA is converted to cDNA through reverse transcriptase enzyme. In the second step, the cDNA is amplified by using target-specific primers and DNA polymerase. Listeria spp. can be detected in meat and waste samples via PT-PCR.

The SYBR green is used for binding dye with DNA. The light is emitted on excitement. The light enhances with the intensity of PCR products. SYBR green is the simplest and most cost-effective dye for use in RT-PCR. The PCR-based examination was created with recognition the breaking point of Colony-forming Unit per 25 grams of food, which is comparable to the ISO procedure (11290-1) for Listeria identification. LOD acquired was 1 × 104 CFU/mL (Kaclíková et al. 2003). It was revealed that the absolute viable count was identified in broccoli (Bhagwat 2003). A hly, PCR examination to identify Listeria was made and used various fixations to spike the sample, and as far as still up in the air to be 8 (Rodriguez-Lazaro et al. 2005). To grow the extent of the procedure, Reverse transcriptase PCR to evaluate the fluorescence transmitted by samples. The got Limit of detection was 10-105 CFU per mL (Berrada et al. 2006). An identification

S. no.	Techniques	Samples	Description	LOD	References
.	Cultural	Foodstuff	Blue/Green colonies of *Listeria*	4 days	Ottaviani et al. 1997
.	BAM	Dairy	Specific for *Listeria*	1 Colony forming unit per mL	Valimaa et al. 2015
.	ELISA	Foodstuff	*Listeria* and other microbes	10^5–10^6 CFU per mL	Bell and Kyriakides 2005
.	Immuno-magnetic tech	Dairy samples	For the detect hlyA gene of Listeria	10^4 CFU per milliliter	Yang et al. 2007
.	Microarrays	Dairy samples	An antigen based probe was used	10^8 CFU per milliliter	Brehm-Stecher and Johnson 2007
.	PCR	Clinical Sample	Primers to target *specific genes*	101 CFU per milliliter	Aznar and Alarcón 2003
.	MP- PCR	Human	Food originated pathogens	7.9×10^1 CFU per milliliter of *Listeria*	Alarcón et al. 2004
.	T- PCR	Foodstuff	For detect *Listeria* from food.	10^4 CFU per milliliter	Kaclíková et al. 2003
.	RT-PCR	Foodstuff	*ssrA* gene amplication	One to five CFU per milliliter	O'Grady et al. 2008
.	qRT-PCR	Meat samples	Reverse transcriptase-PCR	10^2 CFU per milliliter	Suo et al. 2010
.	Biosensor	Antigen of bacteria	immobilize to polyclonal antibodies	tiny detection	Leonard et al. 2004
.	Plasmon resonance	Antigen of bacteria	immobilizing Au-labeled secondary antibodies	10^2 CFU per milliliter	Poltronieri et al. 2009
.	Immuno- sensor	B-lymphocyte	B-lymphocyte cell fused in collagen.	10^2–10^4 CFU per mL	Banerjee and Bhunia 2010
.	Paper multi-biocatalyst	Bacteria	For identification *L. monocytogenes* by using various biomarkers	10^4 CFU per mL	Zhang et al. 2022

technique utilizing SYBR indicates in occurrence of non-specific DNA and dimer development (Fairchild et al. 2006). The focus on the ssrA quality in normally and misleadingly polluted food varieties (milk items, meat, and veggies) brought about an identification cutoff of 1-5 CFU per 25 grams (O'Grady et al. 2008). Subsequently, he decided that it was a shrewd procedure for the specific sample. It was revealed the consequence of a PCR examination with the discovery of furthest reaches of 18 CFU per g on normally and misleadingly defiled ground hamburger and chicken (Suo et al. 2010).

6. BIOSENSOR-BASED TECHNIQUES:

It is a natural sample analyzer utilizing a sample as an item and an electrochemical setup creating comprehensible information. An antibody passed over a chip (biosensor) halted on polyclonal anti-rabbit antibodies (Fab) to identify *L. monocytogenes* (Leonard et al. 2004). For the affirmation of *L. monocytogenes*, the surface plasmon resonance is used and it has LOD is 102 CFU/mL (Poltronieri et al. 2009). In this stage, Au-marked optional antibodies were utilized. On additional progression, it was revealed the utilization of B-lymphocyte converged in collagen lattice as a detecting stage to identify lysin O of Listeria from spoiled foodstuff with a detection cutoff value of 102-104 CFU per gram (Banerjee and Bhunia 2010).

A miniature fluidic gadget that identifies DNA amplicons based on hybridization responses with a immobilized test and biotin signal DNA strands, and catalyzed by a horseradish peroxidase (Lui et al. 2015). In this technique, CL signs created utilizing HRP-luminol framework were uplifted with p-iodine and recognized with CCD framework. The incorporating location of 3 markers was effectively made by two

altered changed working cathodes on a multi-biocatalyst stage. The touchy and solid recognizable proof of *L. monocytogenes* was accomplished by utilizing the versatile multi-biocatalyst stage with a more extensive identification sort and lower limit (Du et al. 2022). Likewise, in 2022 to additional development, the detecting innovation Du et al. fostered a fluorescence-based double acknowledgment gathering utilizing Fe3O4. The direct scope of the discovery of unadulterated culture went from 1.4 × 101 to 107 CFU per mL. Among previously described techniques, the culture methods are normally favored owning to their accessibility, responsiveness, practicality and the highest quality levels contrasted and different techniques that are approved. To sum up, the accessibility and advance of Listeria identification techniques are introduced in Table 1.

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Molecular Pathology of Campylobacter

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ABSTRACT

Campylobacter spp. are globally prevalent zoonotic pathogens causing bacterial diarrheal diseases. Found in warm-blooded animals and diverse environments, they transmit to humans through contaminated water, food, or contact with diseased animals. Human campylobacteriosis, caused primarily by Campylobacter coli (C. coli) and Campylobacter jejuni (C. jejuni), manifests as gastroenteritis and ranks among the leading causes of global diarrheal diseases. These infections can lead to severe complications, including autoimmune disorders like Guillain-Barre syndrome (GBS). In animals, infections can result in clinical effects like abortions, liver disease, and infertility. Campylobacter spp. lack typical human disease virulence factors, suggesting that clinical symptoms in campylobacteriosis are primarily triggered by the host immune response. This chapter explores the intricate interactions between C. jejuni and host tissues, focusing on the molecular pathology and inflammatory responses elicited, with an emphasis on the involvement of immune cells. The gastrointestinal epithelial cells play a crucial role in the initial stage of responding to C. jejuni infections through adhesion and extracellular sensing. Toll-like receptors (TLRs) are involved in detecting invasive infections, triggering proinflammatory responses. Upon invasion, C. jejuni uses Campylobacter invasion antigens (Cia) to penetrate intestinal cells, leading to increased IL-8 secretion and neutrophil chemotaxis. The genotoxin cytolethal distending toxin (CDT) and CRISPR-associated gene 9 (CjeCas9) contribute to host DNA disruption, apoptosis, and inflammation. Neutrophils, eosinophils, and mast cells play roles in tissue damage, with neutrophils restricting C. jejuni growth and eosinophils exhibiting activation responses. The adaptive immune response involves B and T lymphocytes generating antibodies and cytotoxic T cells respectively. Monocytes/macrophages, dendritic cells, and natural killer (NK) cells act as key players bridging innate and adaptive immunity, with various roles in inflammation, tissue repair, and modulating immune responses. NK cells interact with C. jejuni components to suppress inflammation and coordinate T lymphocyte responses. Understanding these complex interactions is crucial for unraveling the mechanisms underpinning Campylobacter-induced tissue pathology and inflammation, paving the way for advancements in disease management and prevention.

Keywords: Campylobacter jejuni (C. jejuni), Gastroenteritis, Immune response, Neutrophil extracellular traps (NETs), Macrophages, Inflammation

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1. INTRODUCTION

Campylobacter spp. are important zoonotic pathogens and are one of the most prevalent causes of bacterial diarrheal diseases around the globe (Olvera-Ramírez et al. 2023). *Campylobacter* spp. inhabits a wide variety of environments, and the *Campylobacter* genus is frequently found in the intestine of warm-blooded animals, such as ruminants, poultry, and pigs. Its transmission to humans can occur by consuming tainted water or food or by coming into close contact with diseased animals (Chlebicz and Śliżewska 2018; Bunduruș et al. 2023). Wildlife can also have high pathogen-shedding potential and may play a crucial role in the spread of these zoonotic pathogens (Olvera-Ramírez et al. 2023). Even though there is a modest danger of zoonotic agents in wild birds infecting humans, this issue is thought to be a developing concern (Wei et al. 2019). The *Campylobacter* genus species have been classified using studies based on their prevalence in a range of animals and environmental reservoirs (Soto-Beltrán et al. 2023). The infection brought on by members of the genus *Campylobacter* in humans is known as human campylobacteriosis. Human campylobacteriosis exhibits gastroenteritis and is among the four main causes of diarrheal diseases around the globe (WHO 2020). Although the primary causes of human campylobacteriosis are *Campylobacter coli* (*C. coli*) and *Campylobacter jejuni* (*C. jejuni*) (Man, 2011) but a wide range of other *Campylobacter* species such as *Campylobacter fetus* (*C. fetus*), *Campylobacter mucosalis* (C. *mucosalis), Campylobacter concisus* (*C. concisus*), *Campylobacter upsaliensis* (C. *upsaliensis*), *Campylobacter rectus* (C. *rectus*), and *Campylobacter lari* (C. *lari*) have also been recovered from human clinical samples (Sheppard et al. 2009; Igwaran and Okoh 2019).

Abdominal pain, diarrhea, malaise, and fever are clinical outcomes of *Campylobacter* infections. Even though symptoms are typically self-limiting and may last for up to two weeks, the illness can occasionally be more severe and can have post-infection sequelae (Tegtmeyer et al. 2021). Certain other gastrointestinal conditions, like esophageal diseases, inflammatory bowel disease, colon cancer, cholecystitis, celiac disease, and periodontitis, can also be caused by *Campylobacter* species (Verdu et al. 2007; Kaakoush et al. 2015). The *Campylobacter* infections can be followed by fatal, life-threating autoimmune disorders such as Guillain-Barre syndrome (GBS), reactive arthritis (ReA), Miller Fisher syndrome, and irritable bowel syndrome (IBS) (Callahan et al. 2021; Soto-Beltrán et al. 2023). *C. concisus*, a member of the other emerging group of *Campylobacter*s spp. that are typical in human oral commensal flora, has lately been associated with non-oral conditions (Kato et al. 2023). Campylobacteriosis can develop at doses as minimal as 800 colony-forming units (CFU), while *C. jejuni* infections can develop at doses as minimal as 360 CFU (Hara-Kudo and Takatori 2011).

In 1909, *Campylobacter* spp. was first recognized as a source of animal disease, yet until 1980, it was not identified as a cause of infection in humans (Galate and Bangde 2015). *Campylobacter* species are frequently cited as a prominent source of bacterial gastroenteritis in both developed and developing nations (EFSA 2021). The *Campylobacter* genus belongs to the family *Campylobacteraceae*, the order *Campylobacterales*, and the class *Epsilonproteobacteria* (Vandamme et al. 2015). The *Campylobacter* genus currently has 32 officially recognized species, along with 9 subspecies and 4 biovars (ITIS 2020). *Campylobacters* are Gram-negative, microaerophilic, corkscrew-shaped bacteria with a size range of 0.5 to 5 µm in length and 0.2 to 0.9 microns in width (Wassenaar and Newell 2006; Vandamme et al. 2015). Majority of the *Campylobacter* species are fastidious organisms that often demand a microaerophilic environment for growth (Soto-Beltrán et al. 2023). The ideal temperature for the growth of

thermotolerant *Campylobacter* species is between 37 and 42°C, and the thermotolerant *Campylobacter* species include *C. coli, Campylobacter insulaenigrae* (*C. insulaenigrae*), *C. upsaliensis, Campylobacter helveticus* (*C. helveticus*), *C. lari,* and *C. jejuni* (Wassenaar and Newell 2006; Vandamme et al. 2015). While other *Campylobacter* species except of these thermotolerant *Campylobacter* are thought to be non-thermotolerant, having an optimum temperature of growth, i.e., 37°C (Soto-Beltrán et al. 2023). The environmental abundance of thermophilic *Campylobacter* species eventually acts as a bridge for the spread of this bacterial pathogen between various hosts and habitats (Dearlove et al. 2016; Gölz et al. 2018).

Complex gastroenteritis may develop as a result of the *Campylobacter* bacterium's unusual capacity to adapt to various settings; in certain situations, this condition may be difficult to treat due to increased resistance to various medications (Bunduruş et al. 2023). The pathogenic *Campylobacter* spp. have the ability of long-term survival in food products, regardless of their inability to flourish outside the homeotherms' digestive tracts. These bacteria are typically vulnerable to environmental stress, yet they have evolved a variety of survival strategies for the environment and the food chain, which can result in human infections (Chlebicz and Śliżewska 2018). A wide range of virulence factors are encoded by the *Campylobacter* genome, giving the bacterium capacity to affect host immunological defenses, make biofilms, and withstand antimicrobials, which ultimately increase its infection-inducing potential (Bunduruș et al. 2023). *Campylobacter* spp. can contaminate both dairy products and meat; however raw milk is particularly prone to infection (Newell et al. 2017; Chlebicz and Śliżewska 2018). Chicken meat can get contaminated with *Campylobacter* at slaughterhouses due to *Campylobacter*-infected chickens' gut content coming into contact with chicken carcasses (Newell et al. 2011).

Campylobacter spp. infections can also occur in animals and can make them experience a range of clinical effects. For example, *C. fetus* subsp. fetus causes abortions in cattle, goats, and sheep; *C. hepaticus* induces spotty liver disease in layer hens; and *C. fetus* subsp. venerealis causes infertility in cattle (Courtice et al. 2018; Crawshaw 2019). *Campylobacter* colonization in chicks typically occurs at 2-3 weeks of age, but they are usually asymptomatic after colonization (Newell and Fearnley 2003; Awad et al. 2015; Connerton et al. 2018). In infected chickens, *Campylobacter* spp. colonizes the mucosa of the cloaca crypts and cecum, and chickens may also have these bacteria in their liver and spleen (Chlebicz and Śliżewska 2018). Wildlife can also serve as a reservoir, amplifying hosts, and even a source of *Campylobacter* (Becker et al. 2015). Particular emphasis has been placed on the origin of these strains, and it has been suggested that chicken's *C. hepaticus* could have an environmental origin (Phung et al. 2020; Wu et al. 2022).

Most of the investigations are centered around *C. jejuni*, as it is the most common cause of diarrheal illnesses even in the industrialized world. *Campylobacter* spp., in contrast to other bacteria that cause gastrointestinal tract diseases, lacks some of the traditional virulence factors that are frequently linked to cause disease in humans. Therefore, it is thought that the host immunological response to the bacteria is principally responsible for the clinical symptoms of human campylobacteriosis and the gastrointestinal disease. Since gastrointestinal disease is typically caused by the host's immunological response, the onset of postinfectious disorders may come from the misdirection or dysregulation of the same inflammatory response (Callahan et al. 2021). Therefore, it is crucial for human health and the disease diagnostic fields to understand the molecular pathology, mainly including the cellular immune responses to *Campylobacter* and the immunological events crucial for the disease onset and the post-infectious disorders (Callahan 2023).

Molecular pathology is a branch of the biomedical sciences that concentrates on the development, progression, and evolution of diseases on the molecular level. Molecular pathology is typically treated as a subgroup of the pathology. In traditional pathology, the morphological manifestations of disease are focused. However, molecular pathology also incorporates molecular biology tools in order to: isolate and identify the infectious disease-causing agents; comprehend differential gene expression role in disease etiology; provide more precise methods of disease diagnosis; and offer more individualized therapy options. Molecular pathology can be approached from a variety of perspectives, and it also incorporates

immunology, genetics, and other medical field aspects. Cell culture and cell isolation are the main approaches utilized in molecular pathology to determine links between gene alterations and disease. The other methods used in molecular pathology involve tissue microdissection methods, gel electrophoresis methods, amplification methods, hybridization methods, and nucleic acid sequencing. Nucleic acid sequencing further consists of proteomics, and DNA microarrays. Along with being used in biomedical research to understand specific disorders, molecular pathology also has practical applications for patients. The development of molecular diagnostics is a result of biological breakthroughs that have led to an improved understanding of the molecular mechanisms. Prior to this comprehension, morphologic observations were used for the diagnosis of different states of disease (Kaoud 2012).
An insight into *Campylobacter* host tissue pathology and inflammatory responses, along with the aspects of the host's immune cells involved, is given below.

2. EPITHELIAL CELLS

There are two processes that happen within epithelial cells. These are;

2.1. ADHESION AND EXTRACELLULAR SENSING

Gastrointestinal epithelial cells, along with acting as a physical barrier, are also fitted with intracellular and extracellular receptors that may, respectively, detect invasive infections and sample the lumen of the gut (Tang et al. 2016). *C. jejuni* can penetrate the distal intestine and proximal colon mucus layer to make it to the intestinal epithelial cells (IECs) apical surface after being ingested in fairly small infectious doses via contaminated drinking water or food (Chang and Miller 2006; Teunis et al. 2018). *C. jejuni* can attach to IECs and infiltrate them once it has passed through the mucus layer (Hendrixson and DiRita, 2004; Lugert et al. 2015). Toll-like receptor (TLR) reporter HeLa cells have been reported to be triggered by lysed *C. jejuni* via the sensing activities of different TLRs used to sense the bacterium. These TLRs include TLR1/2/6 and TLR4, which recognize bacterial lipoproteins and lipopolysaccharides, respectively. NF-κB is activated by these TLRs being stimulated, which is transduced via the MyD88 signaling cascade. IL-1β, IL-8, IL-12p42, GRO-α, tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein 1 (MCP-1) are all produced and secreted as a result of NF-κB activation (Konkel et al. 2020). TLR4 activation also activates the Toll/IL-1R domain-having adaptor-inducing IFN-β (TRIF) signaling cascade, culminating in IFN-β production (Hu and Hickey 2005; de Zoete et al. 2010; Yu and Gao 2015). Human IECs release IL-8 after being stimulated by *C. jejuni*, which then encourages chemoattraction along with numerous neutrophils recruitment to the infection site (Hickey et al. 2000). Along with IL-8, a proinflammatory cytokine called IL-6, required for mounting an adaptive immune response, is released when IEC TLR1/2/6 are stimulated (Friis et al. 2009). Beta-defensins 2 and 3 are also produced by IECs in response to *C. jejuni* stimulation, although the stimulus necessary for induction is yet undefined (Zilbauer et al. 2005). Beta-defensins are secreted cationic antimicrobial peptides that can attach to the bacterial membranes, which are negatively charged, prompting leukocyte chemoattraction and bacterial cell death (Cobo and Chadee 2013).

2.2. INVASION AND INTRACELLULAR RESPONSES

C. jejuni enters IECs once it has reached the apical surface, and this invasion is reliant on the *Campylobacter* invasion antigen (Cia) protein secretion (Buelow et al. 2011). Cia proteins, along with encouraging cellular invasion, can also activate the extracellular signal-regulated kinases (ERK) and p38 mitogen-activated protein (MAP) kinase pathway to increase IL-8 secretion from IECs. This increased IL-8 production from IECs causes robust neutrophil chemotaxis to the infection site. Eventually, *C. jejuni*

invades IECs by remodeling host microtubules and actin, even though it doesn't seem to create actin tails for intracellular trafficking. This indicates that *C. jejuni* continues to retain itself within a *Campylobacter*-containing vesicle (CCV) (Watson and Galán 2008; Samuelson et al. 2013). Some strains of *C. jejuni* produce cytolethal distending toxin (CDT), a genotoxin, once they are intracellular. CDT can induce cell cycle arrest, cell swelling, and cell distension (Lara-Tejero and Galán 2000; Scuron et al. 2016). Epithelial barrier disruption and impairment of signaling pathways, which change the immune response of the host, are predicted outcomes of this cellular response (Scuron et al. 2016). The formation of the CCV in IECs may be significantly influenced by CDT. Furthermore, the bacterium may use alternative strategies to target the DNA of the host, as *C. jejuni* strains without CDT nonetheless cause disease and DNA damage. For instance, it was recently shown that *C. jejuni*, while in IECs, explains clustered regularly interspaced palindromic repeat (CRISPR)-associated gene 9 (CjeCas9) linked with the outer membrane vesicle. The CjeCas9 gene can target the DNA of the host, causing epithelial cell death right after being released, along with the proinflammatory gene expression's upregulation (Saha et al. 2020; Saha et al. 2020). Furthermore, several investigations have shown that *C. jejuni* triggers IECs' caspase-3-dependent apoptosis, although the behind mechanism of this reaction is yet undefined (Butkevych et al. 2020). Since it seems that *C. jejuni* uses a variety of mechanisms to disrupt the DNA of the host and those responses could induce inflammation. Therefore, more studies should be done to fully characterize these systems and understand how they affect tissue pathology and inflammation (Callahan et al. 2021).

IECs have the ability to sense intracellular *C. jejuni* along with responding to extracellular bacteria. Intracellular *C. jejuni* can activate TLR9, which further recognizes intracellular DNA (de Zoete et al. 2010). Furthermore, nucleotide-binding oligomerization protein (NOD) receptors seem to be involved in the recognition of intracellular *C. jejuni.* The lack of NOD2 in colonocytes may inhibit the host immunological response, leading to an increase in the bacterial burden; however, other immune cells, such as macrophages and dendritic cells (DCs), express NOD2 (Moreira and Zamboni 2012). In fact, NOD2 activates the antibacterial function in IECs, particularly against *C. jejuni* (Barnich et al. 2005). Additionally, in response to *C. jejuni*, NOD1 is also activated, which causes a decrease in intracellular *C. jejuni* and an increase in hBD2 and IL-8 (Zilbauer et al. 2007). Since NOD activation and cytotoxicity are closely related, it is possible to hypothesize that epithelial NOD signaling causes tissue pathology in infected people (Heim et al. 2019). This bacterium can travel to the colonocyte's basolateral side while inside the CCV and exocytose to the colon's underlying tissue to come into contact with chemoattracted leukocytes (Kopecko et al. 2001; Callahan 2023). It has been found that tight junction disruption brought on by *C. jejuni* causes barrier dysfunction, which in turn signals the production of pro-inflammatory cytokines. Proinflammatory cytokines include IL-1β, IL-6, IL-13, TNF-α, IFN-γ, and MCP-1 (Schmidt et al. 2019). Further research is required to determine how this virulence factor affects inflammation during campylobacteriosis because tight junction proteins are crucial for controlling intestinal inflammation following damage (Slifer and Blikslager 2020).

Neutrophils, eosinophils, and mast cells are also involved in *Campylobacter*-induced tissue damage and pathology, along with generating innate immune cell responses (Callahan et al. 2021).

3. NEUTROPHILS

Neutrophils are the first innate immune cells drawn to the infection site after *Campylobacter* effectively penetrates the epithelial barrier (Kolaczkowska and Kubes 2013). The three primary antibacterial functions of neutrophils include microbe degradation, phagocytosis, antimicrobial proteins release via degranulation, and the exclusion of neutrophil extracellular traps (NETs) (Callahan et al. 2021). Due to their high proinflammatory activity and abundance in colonic tissue during *C. jejuni* infection, neutrophils must be taken into account as a possible cause for acute and chronic illnesses as well as tissue pathology.

Neutrophils move from the basolateral to the apical side of the epithelium within colonic crypts, which are reliant on 12-lipoxygenase (12-LOX), a host-derived enzyme, and n-formyl peptides from bacterial sources (Murphy et al. 2011). The interaction between *C. jejuni* and neutrophils causes complement-opsonized cells to be phagocytosed, leading to the generation of reactive oxygen species (ROS), which directly kills the bacterium and causes localized tissue damage (WALAN et al. 1992; Heimesaat et al. 2023). Along with phagocytosis and direct cell death, a large number of neutrophil-derived antimicrobial proteins are released into the surrounding tissue, and they build up in the feces of individuals with a *C. jejuni* infection. These antimicrobial proteins include neutrophil elastase (Ela2), lipocalin-2 (Lcn2), calgranulin C (S100A12), and myeloperoxidase (MPO). These antimicrobial proteins' activities indicate that during an infection, their release is a possible factor in the growth restriction of *C. jejuni*, and these proteins are probably released as a consequence of degranulation. Ela2 and MPO were also observed to colocalize with NETs in the infection brought on by *C. jejui* (Shank et al. 2018; Callahan et al. 2020). It has been hypothesized that NETs play part in the intestinal pathology and formation of crypt abscess during campylobacteriosis because of their cytotoxic nature. These NETs may have a significant impact on the emergence of the postinfectious disorders outlined in the introduction, as these structures are linked to a variety of autoimmune diseases (Li et al. 2020). More investigation into *C. jejuni*-neutrophil interactions is required because of the link between pathology, inflammation, neutrophil activity, and the emergence of autoimmune diseases (Callahan 2023).

4. EOSINOPHILS

Eosinophils are effectively activated in vitro by *C. jejuni*, which causes degranulation, chemotaxis, a respiratory burst, and eosinophil cationic proteins (ECPs) release. Although, the involvement of eosinophils in campylobacteriosis has received scant direct evidence (Svensson and Wennerås 2005; Hogan et al. 2013). Despite the eosinophil's rarity, their response to *C. jejuni* and their function in gastrointestinal inflammation have led to hypothesis that they may play a role in the emergence of post-infectious disorders as well as in inflammation during infections (Callahan et al. 2021).

5. MAST CELLS

Mast cells are recognized as inflammatory granulocytes, and they release a number of cytokines and histamine (Krystel-Whittemore et al. 2016). It is thought that mast cells have a small role in infection, despite the fact that they have been identified in the stools of individuals infected with *Campylobacter* (Hendrixson and DiRita 2004). Mast cell closeness to enteric nerves was observed to be correlated with stomach pain during IBS, so even though mast cells do not seem to be directly implicated in campylobacteriosis, their participation in gastroenteritis cannot be completely dismissed (Callahan et al. 2021).

Both B and T lymphocytes are also engaged in *Campylobacter*-generated infection. B cell responses occur along with antibody production, while T cell responses occur alongside subtype switching. Both together constitute the adaptive immune response (Callahan et al. 2021; Al-Naenaeey et al. 2022).

6. B Lymphocytes

In order for humoral immune responses to begin, antigen-reactive B cells must be exposed to antigens. Titers of serum IgM, IgA, and IgG antibodies relevant to bacterial epitopes peak approximately 11 days following infection with *C. jejuni* in humans (Black et al. 1988). Autoreactive IgG1 antibodies are the most prevalent subtype of antibodies produced after campylobacteriosis (Malik et al. 2014). As there is a significant link between IgG1 levels and GBS severity, it has been proposed that this reaction is crucial to

the GBS development following infection with *C. jejuni*. On average, GBS can affect 1/900 people. This proposition is largely supported by the finding that a number of the IgG and IgA antibodies generated during infection may also cross-react with the human GM1 gangliosides found in the neurons (Masanta et al. 2013). Also, this reaction is probably brought on by some LOS core oligosaccharides of *C. jejuni* that mimic human ganglioside GM1 structures (Yuki et al. 2004). However, to comprehend the biochemical, genetic, and molecular underpinnings of these responses, additional research must be undertaken (Callahan et al. 2021).

7. T Lymphocytes

Studies have shown that there is likely a connection between tissue pathology and inflammatory T lymphocyte activities (Malik et al. 2014). IL-12, which is secreted by mature dendritic cells during the later stages of infection, encourages naive T cells to develop into T helper 1 (Th1) cells, which then generate IFN-γ (Hu et al. 2006; Rathinam et al. 2009). Th1-derived cytokines, once they had undergone differentiation into Th1 lymphocytes, peak 7 to 14 days after infection, with IFN-γ^+ $CD4^+$ T cells being the most prevalent lymphocyte in humans infected with *C. jejuni* (Fimlaid et al. 2014). These findings lead to the hypothesis that campylobacteriosis is predominantly a Th1 lymphocyte illness with a secondary development of Th17 cells. Patients may have a higher proportion of Vδ1 γδ (Vδ 1) $CD8^+$ T cells among the T cells generated during human *Campylobacter* infection, which is particularly intriguing given that these cells are linked to autoimmunity and cytotoxicity (Scelsa et al. 2004; Presti et al. 2021). Proinflammatory cytokines can activate the Vδ 1 T cell receptor (TCR) in the colon and intestines, and DCs can also activate Vδ 1 cells by utilizing microbial antigens, particularly lipid extracts from Gram-ve bacteria. The effective immunoregulation and host defense linked to Vδ 1 T cells depend on this recognition (Das et al. 2004). TLR4, an antigen linked to the previously described GM1 ganglioside, may also help T lymphocytes identify *C. jejuni* LOS (Cutillo et al. 2020). T cells may therefore be extremely important in the tissue pathology and the emergence of autoantibodies subsequent to campylobacteriosis (Callahan et al. 2021). Fig. 1 shows the body cells evoked in response to *Campylobacter* infection. Monocytes/macrophages, natural killer cells (NK cells), and dendritic cells are also produced in response to infection brought on by *Campylobacter*. These cells bridge the gap between the innate and adaptive immune responses (Callahan et al. 2021; Callahan 2023).

8. MONOCYTES/MACROPHAGES

Monocytes play a role in pathogen identification and inflammation, and monocyte-derived tissue-resident memory macrophages perform essential immunological tasks. These immunological tasks include anti-inflammatory signaling pathway promotion and tissue repair (Ginhoux and Jung 2014). Tissue-resident macrophages particularly ingest and degrade foreign material, debris, and dead cells, along with performing the functions of coordinators of the tissue's inflammatory immune response and expert antigen presenters (Varol et al. 2015). The human peripheral blood mononuclear cells (PBMC) were discovered to release an increased amount of IL-6 and IL-8 in the wake of infection (Hamza et al. 2017). By utilizing macrophage-like differentiated THP-1 cells, IL-8 secretion was also observed, demonstrating the importance of neutrophil chemotaxis during infection (Jones et al. 2003). Differentiated macrophages are effective at eliminating intracellular bacteria because *C. jejuni* is unable to evade being delivered to lysosomes; however, some strains of the bacterium can survive intracellularly inside monocytes and can cause apoptosis (Hickey et al. 2005). More investigation is required to comprehend the molecular mechanisms behind the proinflammatory switches that occur in macrophages and monocytes infected with *C. jejuni* (Callahan et al. 2021).

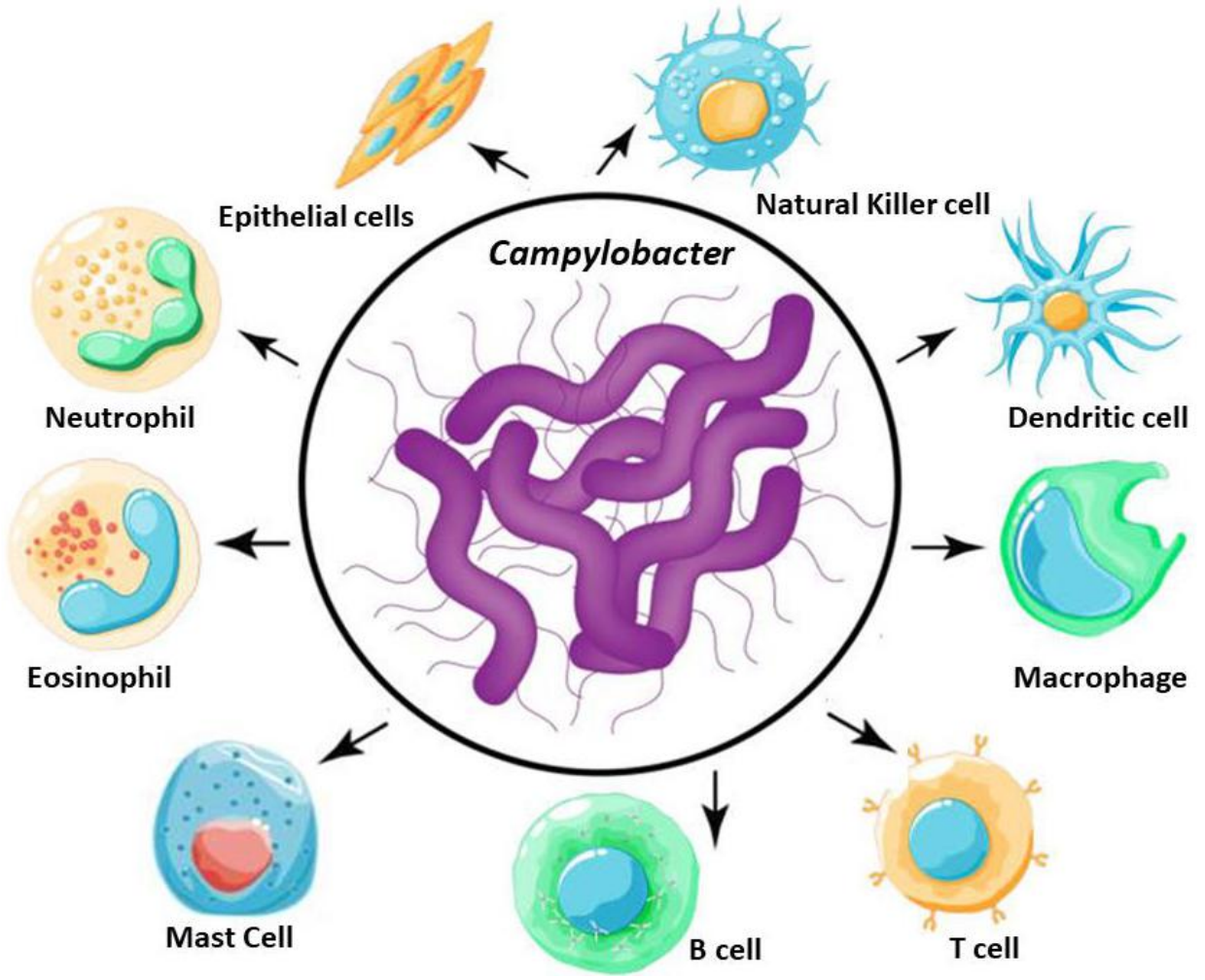

Fig. 1: Major body cells engaged in *Campylobacter*-induced infection

9. DENDRITIC CELLS

Dendritic cells (DCs), which serve as professional antigen-presenting cells activating the adaptive immune response, can also originate from monocytes (Patente et al. 2019). As DCs sample the intestinal lumen and transcytose during infection, they most likely come into contact with *Campylobacter* in the lamina propria intraluminally (Niess et al. 2005). Siglec-10-expressing DCs may contribute to *C. jejuni* mucosal immunity by acting as anti-inflammatory cells, in contrast to the crucial function that IL-10 plays in reducing intestinal inflammation. However, it has not yet been determined how these cells contribute to campylobacteriosis (Stephenson et al. 2014). Additionally, DCs triggered by *C. jejuni* release NF-κB-dependent chemokines, which further include growth-related oncogene α (GRO-α), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, monokine induced by gamma interferon (MIG), RANTES, and IP-10 (Hu et al. 2012). *C. jejuni* causes the phosphorylation of stress-activated protein kinase/Jun N-terminal protein kinase (SAPK/JNK), mitogen-activated protein kinases (MAPKs), P44/42, and P38 to induce chemokines and cytokines secretion. CD40, CD80, CD86, and mature phenotype cell surface major histocompatibility complex class II (MHC-II) are significantly upregulated after DCs are activated. DCs then

effectively internalize and eliminate *C. jejuni* (Hu et al. 2006). While campylobacteriosis appears to have anti-inflammatory effects from DCs, proinflammatory DCs in response to pathogen-associated molecular patterns within injured colonic tissue have also been shown in an increased amount (Stagg 2018). Therefore, it can be concluded that DCs are critical for campylobacteriosis, shaping and laying the groundwork for post-infection activity via the release of both anti-inflammatory and inflammatory cytokines, as well as antigen presentation (Hu et al. 2006; Callahan et al. 2021).

10. NK CELLS

NK cells react with the antigens of commensal and pathogenic bacteria, as well as with other various host cell types within the stroma and epithelium (Poggi et al. 2019). Siglec-7 molecules are used by NK cells to attach to the *C. jejuni* LOS, which promotes host inflammatory response and immunity (Avril et al. 2006). NK cells' cytotoxicity and activation pathways are diminished by Siglec-7, which ultimately reduces inflammation (Daly et al. 2019). The killer cell immunoglobulin-like receptor KIR2DS4 gets highly bound by conserved *C. jejuni* RecA epitopes provided by HLA-C*05:01 alongside LOS binding, which ultimately stimulates KIR2DS41 NK cells (Sim et al. 2019). Together, the aforementioned responses show that in the wake of *C. jejuni* infection, NK cells suppress the immune system for the host's advantage and coordinate T lymphocyte responses by antigen presentation (Callahan et al. 2021).

11. CONCLUSION

Campylobacter is the most common bacterium that causes gastroenteritis in people, although little is known about its host molecular pathology. Even though *C. jejuni* lacks the classical virulence factors that more thoroughly researched gastrointestinal pathogens have, it still invades the human GIT system and triggers a strong immunological response that seems to be the cause of significant immunopathology at the extraintestinal sites and colon. There is a significant knowledge gap in the host's molecular pathology in response to the infection brought on by *C. jejuni*, as it colonizes several mammals with a variety of clinical signs. Although this factor can help us understand each host's response to *Campylobacter* and it might also give an understanding of the divergent or shared evolution of immune mechanisms among various hosts. Therefore, the field of *C. jejuni* is an excellent spot to start comprehending the bacterial and host components that cause both systemic and colonic inflammation, along with the treatments and methods that might be useful for minimizing these effects. For instance, the current finding of innate memory may shed light on the autoimmunity that characterizes the postinfectious disorders of *Campylobacter* infections. The field of molecular pathology has made such great strides in recent times that these impacts can be comprehended in both in vivo and in vitro settings. By enhancing our knowledge of molecular pathology during and after infection, this discipline can commence devising strategies that might enable better understanding, diagnostics, and treatment of the disease, which will ultimately help to decrease *Campylobacter* prevalence across the globe.

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Shigellosis; A Clinical Perspective

43

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ABSTRACT

The complexities of Shigellosis and a highly transmissible gastrointestinal illness brought on by pathogenic Shigella spp., are examined in this book chapter. This disease is extremely important worldwide, especially in nations of low economy and poor sanitation conditions. Shigellosis is a serious risk to a wide range of age groups, and it is more severe in susceptible groups. The chapter explores medical intervention, personal cleanliness, and sanitation habits as preventive measures, highlighting the critical role that oral dehydration therapy plays in managing dehydration and lowering mortality. A critical analysis of public health interventions such as handwashing and cleanliness is prompted by the growing problem of antibiotic resistance in severe instances. The ongoing efforts to create vaccines are emphasised, and it is acknowledged that further study is necessary to improve our knowledge of Shigella species and shigellosis.

Keywords: Shigellosis, Shigella spp., Gastrointestinal, Preventive measures, Vaccines

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1. INTRODUCTION

Shigellosis, commonly known as bacillary dysentery, is a gastrointestinal disorder caused by pathogenic Shigella spp. Shigellosis is a widespread public health concern that affects millions of people globally, particularly in regions where clean water, sanitation, and medical treatment are scarce (Rehman et al. 2011; Moxley 2022). This extremely infectious disease develops on poor hygiene, making it difficult to manage and prevent its propagation. It can affect people of all ages, but small children, the elderly, and those with compromised immune systems are more vulnerable (Sharif et al. 2018). Shigella are relatively resistant to stomach acid and only a small number of bacteria is capable of causing infection (Bavishi and Dupont 2011). After ingestion, bacteria replicate in the small intestine and moving to the large intestine, where it releases Shigella enterotoxin and serotoxin type 1, resulting in watery or bloody faeces (Navaneethan and Giannella 2008). Clinical signs typically develop 12 hours to 3 days following consumption of the organism, with a 3-day mean incubation time. High fever, vomiting, and cramping pain in the stomach are common symptoms, followed by bloody mucous diarrhea. The condition normally resolves itself after 5 to 7 days of the onset of symptoms (Niyogi 2005). However, the condition may lead to problems and even death in susceptible individuals (Aslam and Okafor 2018). The goal of this chapter is to provide an overview over shigellosis in depth, explore its numerous clinical perspectives, and emphasize its worldwide significance.

2. ETIOLOGICAL AGENTS

The main etiological agents of shigellosis are

- Shigella (S.) dysenteriae (12 serotypes) Serotype A
- Shigella (S.) flexneri (6 serotypes) Serotype B
- Shigella (S.) boydii (23 serotypes) Serotype C
- Shigella (S.) sonnei (a serotype)

The S. flexneri and S. dysenteriae result in bloody diarrhea, whereas S. sonnei causes moderate illness that may be limited to lung abscesses (Niyogi 2005; Yang et al. 2005).

3. SHIGELLA DISCOVERY: A HISTORY

Kiyoshi Shiga isolated and found the first Shigella species, S. dysenteriae type 1, in 1896. Shiga worked as a research associate at the Institute of Infectious Diseases, directed by Kitasato. Shiga was first assigned to the Department of Tuberculosis and Diphtheria, but in late 1897 Kitasto switched his focus to the microbiological investigation of a Skiri (dysentery) epidemic (Yang et al. 2007; Lampel et al. 2018). The Japanese word "skiri" means "red diarrhea" and is derived from the Chinese character "skiri". Epidemics of dysentery were common in Japan in the last decade of the 19th century, affecting thousands of people and developing a significant number of fatalities (Niyogi 2005; Shaw-Taylor 2020). The 1897 sekiri outbreak killed almost 91,000 people, with a death rate of more than 20%. At the Institute of Infectious Diseases, Shiga examined 36 dysentery cases. He isolated a bacillus from bowel that fermented dextrose, was negative in the indole reaction, and did not produce acid from mannitol. When fed to dogs, the organism's subculture induced diarrhea (Trofa et al. 1999; Lampel et al. 2018). However, a simple agglutination procedure was the key to his incredible discovery. Shiga found that when exposed to the serum of convalescent dysentery patients, the organism usually aggregated. He gratefully acknowledged Dr. Kitasato's help in his publication of his findings (Bartholomew 1998; Lampel et al. 2018). Shiga continued to characterize the microbe, which was first known as Bacillus dysenterie. He specifically discussed the organism's generation of harmful substances. Shiga toxin, one of these components, has recently been assessed in historical perspective. In the period right after Shiga's discovery of the dysentery bacillus, similar microbes were reported by other researchers

over the next 40 years. Three more species of related microbes were identified and taxonomically grouped into the genus Shigella. These species were named Shigella dysenteriae, Shigella flexneri, Shigella boydii, and Shigella sonnei in honor of their discoverer Shiga, Flexner, Boyd, and Sonei (Niyogi 2005). Shigella was named for the first time according to the 1930 edition of Bergey's Manual of Determinative Bacteriology (Trofa et al. 1999; Lampel et al. 2018).

4. CHARACTERISTICS OF SHIGELLA

The genus Shigella belongs to Enterobacteriaceae family. They are small, non-enveloped, non-motile, gram-negative and facultative anaerobic bacteria (Ashkenazi 2004; Bintsis 2017). In DNA hybridization tests, Escherichia coli and Shigella spp. could not be differentiated at the polynucleotide level; nevertheless, the latter species' virulence phenotype was distinct (Khot and Fisher 2013). Enteroinvasive Escherichia coli (EIEC), which has a similar biochemistry to Shigella, can also cause diarrhea and/or dysentery (Ud-Din and Wahid 2014; Belotserkovsky and Sansonetti 2018). Shigella is also serologically linked to several EIECs. EIEC serotype 124, for example, binds S. dysenteriae type 3 in antisera (Belotserkovsky and Sansonetti 2018). Shigella are classified into four distinct groups based on biochemical and serological differences: S. dysenteriae (serogroup A, with 13 serotypes); S. flexneri (serogroup B, with 15 serotypes including subtypes); S. boydii (serogroup C, with 18 serotypes); and S. sonnei (serogroup D, with a single serotype) (Niyogi 2005; Nataro et al. 2011). It is based on the O-antigen component of the lipopolysaccharide that makes up the cell wall's outermost membrane. Serogroups A, B, and C are physiologically quite similar, although S. sonnei may be differentiated from other serogroups by D-galactosidase and ornithine decarboxylase biochemical responses (Hale and Keusch 1996; Niyogi 2005; 2013). The major cause of epidemic dysentery is thought to be S. dysenteriae serotype 1, commonly known as Shiga bacillus (Opintan and Newman 2007). Shigella plaque outbreaks have expanded around the world during the last 40 years (Jun et al. 2016; Lampel et al. 2018).

5. Epidemiology

Shigellosis is a serious health issue globally even after more than 100 years it was first identified. This is especially true in developing nations with poor hygiene and contaminated water supplies (Girma 2015; Mama and Alemu 2016). Humans are the sole natural host of Shigellathis bacterium (Lampel 2013). Shigellosis is most common in children aged 1 to 4 years, however outbreaks of S. dysenteriae type 1 affect people of all ages (Niyogi 2005; Passwell et al. 2010). Shigella typically causes a recurrent looping process of increased nutritional issues, repeated infections, and stunted growth in underprivileged children (Giannattasio et al. 2016). Children in daycares, migrant workers, travelers to underdeveloped nations, prisoners, and homosexual men are the most often infected in the United States and Europe (Hargro and Ferrante 2009; Taneja and Mewara 2016).

The most common mode of transmission is faeco-oral contact. Shigella is very virulent despite having a low infectivity inoculum (almost 10 bacteria) (Sansonetti et al. 1982). People with diarrhea are the primary source of transmission. More infrequently, transmission is associated with contaminated water and food or wastes; yet, the organism's survival in the environment is frequently challenging (Bryan 1977; Kotloff et al. 2018). Flies, notably the common housefly, can act as vectors for the transmission of shigellosis in specific circumstances, where human faeces are not well handled (Levine and Levine 1991; Issa 2019). Shigellosis affects everyone; however, some people are more vulnerable than others. Shigella has been linked to 5 to 15% of cases of diarrhea and 30 to 50% of cases of dysentery (Das et al. 2012; Pons et al. 2013; Taneja and Mewara 2016). Epidemic Shigellosis is the most common form of Shigella infection in underdeveloped countries, but the majority of Shigella infections are endemic (Bennish and Ahmed 2020). In developing countries, endemic Shigellosis accounts for around 10% of all diarrheal cases in children under the age of

five, and it is responsible for up to 75% of diarrheal deaths (Podewils et al. 2004; O'Ryan et al. 2005). S. flexneri is a frequent pathogen in underdeveloped nations, accounting for around 10% of all incidents of diarrhea in children under the age of five (Alam and Ashraf 2003; Reither et al. 2007). Shigella type 1 causes epidemiological and endemic disorders, whereas rare outbreaks of S. sonnei, transmitted by raw food or polluted water, account for more than 75% of cases in developed countries per year (Girard et al. 2006). S. sonnei-caused illnesses are generally less severe (Ashkenazi 2004). S. boydii, a fourth species discovered in India, is now rarely found outside the Indian subcontinent. Surprisingly, although being isolated three times more frequently than S. flexneri in the United States, the latter is most common in homosexual males (Niyogi 2005; Aggarwal et al. 2016).

The HIV epidemic is linked to the spread of shigellosis in many parts of the world. HIV-associated immunodeficiency causes more severe clinical symptoms of Shigella infection, such as chronic or recurrent intestinal illness and bacteremia (Logan et al. 2016). Several reports indicate that wild-type Shigella infection conferring protective immunity. Long-term exposure to high-risk situations reduces disease incidence. The finding that this immunity is serotype-specific (e.g., against the organism's LPS-O antigen) is closely related to vaccine development. Shigella N-somatic antigen antibody responses emerge early after infection and follow the typical course of anti-LPS antibodies, with IgM responses peaking within a few weeks and decreasing after one to two years (Bonilla 2000).

6. STATUS OF SHIGELLOSIS IN PAKISTAN

Shigellosis epidemics have previously occurred in Pakistan, especially in regions with low hygienic conditions and insufficient access to clean water. The disease is frequently linked to unhygienic surroundings, tainted food, and lack of hygiene habits. Shigellosis is particularly dangerous for young children, especially those under the age of five. In order to prevent and control shigellosis, the Pakistani government has worked with international health organizations to upgrade the nation's sanitation system, encourage good hygiene habits, and increase public knowledge of the condition. These initiatives include giving people access to clean water, encouraging handwashing, and putting public health measures in place to stop the disease from spreading. It is recommended to contact reputable organizations like the Ministry of Health or the World Health Organization (WHO) to receive the most recent details on the state of shigellosis in Pakistan. They can provide access to the most recent data, epidemic updates, and preventative and treatment advice (Ahmed et al. 2003; Von Seidlein et al. 2006; Khan et al. 2009; Nisa et al. 2020).

7. PATHOGENESIS OF SHIGELLA AND VIRULENCE FACTORS

Shigella infections are usually restricted to the mucosa of the intestine. It's ability to penetrate and colonies the intestinal epithelium is a critical factor in the sickness (Alamdary et al. 2018). Shigellosis pathophysiology is complex, involving antecedent enterotoxic and/or potentially cytotoxic diarrhea, cytokine-mediated colitis, and colonic epithelial necrosis (Gascón 2006; Thompson 2019). Shigella invasion of the colonic epithelium and lamina propria causes the underlying physiological damage that initiates this inflammatory cascade (Phalipon and Sansonetti 2007). Colitis and mucosal ulcers result in bloody mucous stools and/or febrile diarrhea. The illness process is aided by the host's acute inflammatory response to Shigella infection and subsequent generation of cytokines. In recent years, much has been discovered about the complicated virulence mechanisms used by bacterium to enter epithelial cells and disseminate to neighboring cells (Philpott et al. 2000). Cell invasion and infection dissemination are complex processes that demonstrate various genetic involvement. The process can be split into at least four stages:

(1) Cell invasion;
(2) Intracellular proliferation;
(3) Intracellular and intercellular proliferation; and
(4) Host cell death (Guichon et al. 2001; Parsot 2005).
The organism can infiltrate the intestinal epithelium and M-cells, which are lymphoid follicles that line the mucosal specialized epithelial cells. Several phases are involved in the infection process, including micropinocytosis, escape into the cytoplasm, and subsequent spread and invasion of neighboring cells. A 'virulence plasmid' encodes the IpaJ protein, which facilitates micropinocytosis. Viral genes of Shigella are part of a complicated regulatory cascade that is still being investigated. Shigella penetrates epithelial cells through reorganizing the cytoskeleton, starting with a type III secretion system that appears to be GTPase-controlled. Other investigations discovered many plasmids and chromosomal locations. Interactions with membrane lipoproteins, Shigella nitric oxide-independent clearance, and interferon-dependence of drug resistance are all essential factors in Shigella virulence and invasiveness (Guichon et al. 2001; Parsot 2005; Burnaevskiy et al. 2013; Mattock and Blocker 2017).

8. TOXIN

Flexner discovered that parenteral injection of deceased Shigella cultures into mice resulted in death, only two years after Shigella completely defined as type 1 Shigella, and concluded that the disease was caused by "a toxic substance and not the infection itself" (Lampel et al. 2018). Conradi discovered three years later that a culture autolysis of S. dysenteriae type 1 produced diarrhea, paralysis, and mortality in young rabbits 48 to 72 hours after intravenous injection (Niyogi 2013; Lampel et al. 2018). As a result of these discoveries, the active substance is known as Shiga neurotoxin or simply Shiga toxin. Todd soon observed that injecting S. flexneri filtrate induced diarrhea but not paralysis, indicating that S. dysenteriae type 1 produces particular neurotoxins, which was later validated with the finding of the gene (Niyogi 2013; Lampel et al. 2018). These early researchers clearly observed a combination action of the endotoxin lipopolysaccharide (LPS) and the toxin Shiga protein. Shigella strains produce three types of enterotoxins: (a) Shigella enterotoxin 1 (SHET1), which is found in all S. flexneri strains. Shigella enterotoxin 2 (SHET2) was discovered on a big plasmid linked to Shigella virulence. SHET2 is found in many (but not all) Shigella and entero-invasive Escherichia coli (EIEC) serotypes. When evaluated in rabbit ileal loops, the soluble toxins SHET1 and SHET2 demonstrated considerable enterotoxic action in vitro. In addition, genetic engineering can be employed to attenuate novel vaccination candidates against Shigella and phage-transmitted Shiga toxins generated by S. dysenteriae (Noriega et al. 1995; Vargas et al. 1999; Gray et al. 2015; Kotloff et al. 2018).
Shiga toxins are neurotoxic, cytotoxic, and enterotoxic. They are encoded by chromosomal genes and have two domain structures, 1-A and 5-B, which are identical to Shiga-like toxins seen in an enterohemorrhagic E. coli infection (O'Loughlin and Robins-Browne 2001; CR et al. 2020).

9. EFFECT ON THE INTESTINE

Shiga toxins attach to receptors in the small intestine, blocking electrolytes, glucose, and amino acids from being absorbed into the intestinal lumen (Field 2003).

10. CYTOTOXICITY

The Shiga toxin B subunit binds to host cell glycolipids in the colon, and the A1 domain is internalized via receptor-mediated endocytosis, resulting in irreversible inactivation of the 60S ribosomal subunit, inhibiting protein synthesis, and cell death (Lee et al. 2016).

11. NEUROTOXICITY

Fever and stomach cramping are symptoms of neurotoxicity. Shiga toxin is not required for S. dysenteriae type 1 pathogenicity in primates, but it does add to the severity of clinical symptoms, especially bloody diarrhea/dysentery. Shigella infection normally has numerous stages, and the manifestations vary based on the infecting species, the host's age, the existence of risk factors, and the host's unique immunological status. In S. dysenteriae, the incubation period is 1-4 days but can last up to 8 days. Shigellosis, often known as acute bacterial dysentery, is an invasive infection of the human colon that causes symptoms ranging from brief diarrhea to inflammatory bowel disease (O'Loughlin and Robins-Browne 2001; Stearns-Kurosawa et al. 2010).

12. SIGNS AND SYMPTOMS

Clinical symptoms often manifest itself within 24 to 48 hours of ingestion of infectious dose, and is accompanied by systemic symptoms such as fever, tiredness, malaise, and anorexia. Watery diarrhea is often the only clinical sign of a moderate infection and usually precedes dysentery (Niyogi 2005). Dysentery can manifest itself over hours or days and is frequently accompanied by tiny volumes of bloody bowl, mucous, stomach pains, and tenesmus. The distal colon is primarily impacted in most individuals with dysentery, and the resulting inflammatory colitis is evidenced by loose and frequent stools due to ileal fluid leakage. Patients with severe dysentery can produce more than 20 dysentery stools each day (Lampel 2013). The daily loss of 200 to 300 ml of serum protein through the stool is another sign of dysentery. Serum protein loss depletes nitrogen stores and exacerbate starvation and retardation. Immune factor depletion also increases the risk of associated infectious illnesses and leads to mass mortality (Niyogi 2005; 2013). It is frequently observed in cases of shigellosis that anorexia may persist even as the patient is recovering, potentially leading to a decline in their nutritional well-being.. Shigellosis is rarely linked with severe dehydration and substantial fluid loss (Niyogi 2005; Lampel et al. 2018).

A variety of strange occurrences are also possible. Seizures are the most prevalent, usually occurring during a febrile condition with no associated encephalopathy. Microangiopathic hemolytic anemia may worsen Shiga toxin-produced infection and appear as uremic syndrome. Most cases of shigellosis in otherwise healthy people are self-limiting and recover without complications within 5 to 7 days. Acute life-threatening consequences in malnourished newborns and young children are especially common in underdeveloped nations (Lampel 2013; Lampel et al. 2018). Metabolic abnormalities i.e., dehydration, hyponatremia, and hypoglycemia, intestinal consequences i.e., toxic megacolon, rectal prolapse, and intestinal perforation, and, in rare cases, sepsis are other symptoms. Shigella bacteria have also been found in HIV patients and other immunocompromised individuals (Lampel 2013; Niyogi 2013). The most prevalent chronic symptoms are persistent diarrhea and malnutrition which is a rare post-infectious condition that primarily affects adults following infection. S. flexneri serotypes induce reactive inflammatory arthritis alone or as part of the Reiter's syndrome group comprising arthritis, conjunctivitis, and urethritis. A realistic approach to reduce shigellosis mortality must continue to emphasize prevention and early antimicrobial therapy over treating developed problems (Niyogi 2005; Lampel 2013; Niyogi 2013; Lampel et al. 2018).

13. CLINICAL DIAGNOSIS

Patients with watery diarrhea and fever should be evaluated for Shigellosis. Clinically, the diarrheal stage of illness is indistinguishable from other bacterial, viral, and protozoal infections. Shigella diarrhea can cause nausea and vomiting, however these symptoms can also be caused by atypical Salmonella spp. and enterotoxigenic E. coli infections. Shigellosis has been identified by bloody and mucous stool; however, the

differential diagnosis should include EIEC, Salmonella enteritidis, Yersinia enterocolitica, Campylobacter spp., and Entamoeba histolytica. Although blood is common in amebic faeces, it is usually dark brown rather than the vivid red as seen with Shigella infection. Shigellosis is characterized by diffuse erythema with small ulcers on the mucosal surface on sigmoidoscopy, whereas amebiasis is characterized by distinct ulcers without systemic inflammation (Hale and Keusch 1996; Niyogi 2005; Keusch 2009; Lampel 2013; Niyogi 2013; Lampel et al. 2018).

14. DIAGNOSIS IN LABORATORY

Although clinical indications of shigellosis can raise suspicion, the diagnosis is dependent on the isolation and identification of Shigella in the stool. Shigella can only live for a short time outside the human body; therefore, stool samples should be processed within a few hours of collection. Faecal samples should be collected early in the disease, when there are usually a high number of pathogens in the faeces, preferably before beginning antibiotic treatment (Niyogi 2005). Positive cultures are typically obtained from blood-stained mucus plugs in fresh stool specimens collected during the disease's acute phase. Rectal swabs can also be utilized for Shigella culture if the specimen will be processed swiftly or if the swab can be stored and transported in Cary-Blair transport medium. Shigella samples can also be transported on buffered glycerol medium (BGS). Although BGS remains basic (as seen by a continuous pink color after feces addition), it is thought to be superior to Carey-Blair medium (Lampel 2013).

In microbiology laboratories, Shigella is commonly isolated by primary passage cultures in differential/selective media with aerobic incubation to prevent the growth of normal anaerobic flora. MacConkey Agar, Hektoen Enteric, Salmonella-Shigella Agar, Xylose Lysine Deoxycholate, and Deoxycholate Citrate media are all common principal isolation medium. S. dysenteriae type 1 and S. sonnei do not grow well on Salmonella-Shigella Agar. These media contain bile salts, which hinder the growth of other gram-negative bacteria, as well as a pH indicator, which distinguishes lactose-fermenting bacteria (coliforms) from non-lactose-fermenting bacteria like Shigella. After a brief growth period, liquid enrichment medium can be seeded with faeces samples and cultivated on selective/differential agar media. After incubating the first batch of isolation medium overnight at 37 °C, the colorless, non-lactose-fermenting colonies can be inoculated with trisaccharide agar (TSI). Shigella generates a base-poor end and an acidic end in this media, and there are no air bubbles in the agar. This reaction indicated a possible identify, which was validated by slide agglutination tests using commercially available antisera against serogroup and serotype. Some normal gut flora E. coli biotypes are remarkably similar to Shigella. The capacity of these E. coli bacteria to decarboxylate lysine distinguishes them from Shigella. However, some coliform bacteria cause invasive intestinal disease because they carry virulent Shigella-like plasmids, and these pathogens are frequently discovered through extensive serological testing for EIEC serotypes (Lampel 2013; Lampel et al. 2018).

Shigella detection techniques that are sensitive and fast have been developed. Genetic probes or polymerase chain reaction (PCR) primers are used in these approaches. Virulence genes that have been specifically targeted, such as the plasmid locus (ipl) or the locus encoding the antigenic virulence factor IpaH, play a critical role in understanding and combating the pathogenic mechanisms of the associated microorganisms. Although more sensitive than traditional diagnostic methods, these procedures necessitate sophisticated facilities that may be too specialized for common clinical laboratory use (Theron et al. 2001; Niyogi 2005; Gómez-Duarte et al. 2009).

According to the National Committee for Clinical Laboratory Standards, all confirmed Shigella isolates should be evaluated for antibiotic susceptibility using the agar diffusion or Broth dilution procedure. To properly calculate the minimum inhibitory concentration (MIC), newer procedures such as the Epsilometer test (E-test) can be applied. Its biggest downside is its exorbitant price. A commercial PC-based geographic information system (GIS) was recently deployed in a S. sonnei infection outbreak in Fort Bragg, North

Carolina. GIS allows for the direct visualization of infectious disease transmission dynamics linked with community outbreaks (Wilson et al. 2006; Mirnejad et al. 2013).

15. PATIENT CARE

Rehydration therapy is an essential initial intervention that can be done to correct dehydration caused by any type of diarrhea and greatly reduce diarrheal fatalities. Oral dehydration therapy, developed by the World Health Organization, has proven to be effective and safe. It is a critical component of the life-saving treatment of acute watery and desiccant diarrhea, as well as a key component of worldwide diarrheal disease control program (Victora et al. 2000). Although severe dehydration is uncommon in shigellosis, it is usually self-limiting with proper fluid intake, and the choice to administer antibiotics is based on the severity of the ailment, the patient's age, and the possibility of further infection spread. Strong antimicrobials can considerably reduce shigellosis symptoms in 48 hours while minimizing the usual disease duration from 5-7 days to 3 days (Farthing et al. 2013). These additionally shorten the amount of time it takes to shed once symptoms disappear. Relapses of shigellosis can extend from 2 days to 10 days or more without antibiotic therapy, and the risk of serious complications or mortality is extensively raised, particularly in infections caused by S. dysenteriae type 1 or S. flexneri. Inadequate shigellosis treatment is a major cause of chronic diarrhea (Victora et al. 2000; Farthing et al. 2013).

If shigellosis is diagnosed, all patients with this kind should be treated with antibiotics, with the choice of drugs based on the antibiotic susceptibility pattern of the shigellosis strains prevalent in the region (Sack et al. 2001). If the patient improves after two days of treatment, a five-day course should be completed. If the patient's condition does not improve, the antibiotic should be changed. If the patient does not improve after a second antibiotic dose, the diagnosis should be reconsidered, and a stool microscopy, culture, and sensitivity tests should be conducted. However, due to widespread drug resistance difficulties, following WHO standards for shigellosis treatment can be problematic (Niyogi 2005; 2007; 2013).

A range of antimicrobial medicines are useful in the treatment of shigellosis. Options are restricted due to the emergence of resistance worldwide. Shigella resistance to sulfonamides, tetracyclines, ampicillin, and TMP-SMX is widespread, hence these medications are not indicated as first-line therapy. Quinolones were the medicine of choice for treating Shigella in the 1990s. All current quinolones show excellent in vitro action, and numerous trials show therapeutic efficacy. For known or suspected shigellosis, most authorities now prescribe oral quinolones (ciprofloxacin, levofloxacin, or norfloxacin). One or two doses are sufficient for mild to moderate dysentery, whereas a 3- to 5-day course of treatment is recommended for complex bacterial dysentery or confirmed S. dysenteriae type 1 infection. Although single doses of 800 mg norfloxacin and 1 g cypra-ofloxacin have been demonstrated to be beneficial against S. dysenteriae type 1 infections, but these are currently less effective. None of the newer fluoroquinolones are safe for youngsters or pregnant women to use. The use of fluoroquinolones in children is restricted due to the possibility of chondrotoxicity. However, there is mounting evidence that these are not dangerous. Although shorter treatment cycles have been explored, all previous research used a 5-day treatment plan. With quinolone resistance developing and concerns regarding its safety in youngsters, the search for alternative drugs begins. Although first and second generation cephalosporins are active in vitro, clinical trials have been unsatisfactory. Cefixime has been studied in the treatment of shigellosis in adults, with a success rate of just 53% (Spence 2004). Clinical trials in Israel, however, have demonstrated that cefixime and ceftriaxone have higher bacteriological and clinical cure rates and are safe for usage in children (Diniz-Santos et al. 2006).

Recently, it was discovered that azithromycin, a macrolide medication with good intracellular permeability and modest anti-Shigella action in vitro, is useful in the treatment of shigellosis. Non-absorbable antibacterial rifaximin is another option worth investigating further. In vitro antibiotic susceptibility and clinical efficacy did not have a perfect link. Although the antibiotics employed must be effective against pathogenic germs,

several medicines used in vitro are clinically useless. The use of inefficient antibiotics, that is, antibiotics to which the organism has developed resistance or that are clinically ineffective, can pose dangers. In addition to the drug's potential systemic side effects, it also disrupts the normal intestinal flora. Furthermore, changes in the frequency of serogroups in Shigella isolates, as well as changes in antimicrobial susceptibility patterns, make it challenging to discover effective medications to treat Shigellosis. As a result, the deployment of surveillance systems is critical for effective shigellosis treatment and control. Seizures, encephalopathy, and intestinal perforation are required to specialized therapy in addition to antibiotics and fluids (Niyogi 2005; 2007; 2013; Kotloff et al. 2018; Lampel et al. 2018).

16. ANTIBIOTIC'S RESISTANCE

The history of the genus Shigella indicates that it is subjected to acquire drug resistance. Shigella strains have been increasingly becoming resistant to the most widely used and affordable antimicrobial drugs over the past few decades, which has resulted in treatment failure and higher mortality rates (Lampel et al. 2018). Shigellosis, especially that brought on by S. dysenteriae type 1, is difficult to treat due to multidrug resistance. Sulfa medicines were quite effective in the 1940s, but by the 1950s, they were of little use. Tetracycline later proved to be very effective in the 1960s, followed by ampicillin and cotrimoxazole. From the late 1960s to the 1980s, there was an increase in resistance to these antibiotics. Ciprofloxacin and other fluoroquinolones, ceftriaxone, and azithromycin are antimicrobials that remain proven effective. Even within a single site spread across two different areas, antimicrobial resistance trends to vary from site to site. This might be a result of the emergence and distribution of clones that are resistant to antibiotics (Niyogi 2005; Lampel 2013; Chung et al. 2021).

17. CONTROL STRATEGIES

Ensuring an ample supply of clean water and practicing proper hygiene in managing feacal matter are crucial tactics in combating shigellosis, as is the case with various intestinal illnesses. At best, these public health measures are long-term approaches to prevent enteric illnesses in impoverished nations. Comprehensive media and personal awareness campaigns that feature the following components are among the most successful approaches for reducing mortality and morbidity;

1. Encourage handwashing after faeces and educate all households on how to actively prevent faecal contamination of food and water;
2. Promote the practice of breastfeeding; and
3. Encourage the use of oral treatment to treat acute diarrhea (Ahmed et al. 2003; Niyogi 2005; Kotloff et al. 2018).

18. VACCINATION

Despite the fact that S. dysenteriae type 1 was found to be the source of epidemic dysentery in Japan in 1898, no vaccine is currently authorized and there is no agreement on the mechanisms behind host immunity to Shigella (Kotloff et al. 2018). The creation of a new generation of vaccine candidates has been made possible by developments in biotechnology and significant improvements in our knowledge of the molecular processes underlying Shigella pathogenicity. Current Shigella vaccine choices have been proven to be safe and immunological in animal models and are based on attenuated S. flexneri or S. sonnei strains, dead S. flexneri strains, or certain synthetic polysaccharides (Niyogi 2005). Polysaccharide conjugates that are specific for S. freundii and S. sonnei have been proven to be kid-safe and immunogenic. In clinical trials, several of these vaccinations have previously been tried and have demonstrated promise in avoiding

diarrhea. Shigella immunity is serotype-specific, therefore the effectiveness of a Shigella vaccination in a given situation depends partially on how the serotypes are presented in the vaccine (Mani et al. 2016). Therefore, it is essential to comprehend the serotype distribution of clinical isolates in order to create novel vaccines and determine if these are appropriate for use in projects related to public health. There is a critical need for type 1 and type 2a Shigella vaccine development. Shigellosis still has a serious impact on the world and is not sufficiently managed by current preventive and treatment strategies. Innovative approaches, such as creating vaccinations against the most prevalent serotypes, might be very advantageous (Niyogi 2005; 2013; Mani et al. 2016; Lampel et al. 2018).

19. FUTURE PERSPECTIVES

To learn more about the Shigella species much more research is required. There is still a gap between the real origin of Shigella spp. and many elements of its pathophysiology and virulence mechanisms that have been revealed by investigations. The migration path of Shigella spp. from underdeveloped to developed nations remains in need of attention and explanation. The finding of intricate underlying processes required investigation of the structural and molecular aspects of Shigella spp. In order to comprehend the rising burden and changing environment globally, it was necessary to conduct proper surveillance and monitoring of its epidemiology in connection to other closely related species. The obligation of healthcare professionals and scientists to seek out better solutions and devise methods for the benefit of mankind has increased more than ever in recent years as a result of technological innovation, new lab instruments, equipment, protocols, and the decreasing cost of sequencing.

20. CONCLUSION

Shigellosis or bacillary dysentery is a highly contagious gastrointestinal disease caused by pathogenic Shigella spp. It constitutes a global health problem, especially in areas with poor sanitation, limited access to clean water and medical care. Shigellosis affects all ages, but is more dangerous for vulnerable groups. Prevention includes proper sanitation, personal hygiene and medical care. Oral dehydration therapy is essential to control dehydration and reduce mortality. In severe cases, antibiotic resistance is a challenge. Public health measures such as hand washing and hygiene are important. Vaccine development is ongoing. Further research is needed to better understand Shigella spp. and shigellosis.

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Zoonotic Diseases Caused by Mastitic Milk

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ABSTRACT

Mastitis is inflammation of animals udder or mammary gland occur due to bacterial invasion followed by injury and it is the infectious zoonotic disease caused by the consumption of raw milk. Consumption of this raw milk led to many zoonotic diseases like bovine tuberculosis, listeriosis, brucellosis, Q fever, salmonellosis, leptospirosis and mycoplasma infections. Bovine tuberculosis in mammals caused by Mycobacterium bovis and it primarily affect upper and lower respiratory tract. Listeriosis is caused by listeria monocytogenes and it mainly cause damage to the CNS of animal. Q fever caused by coxialla burnetti which is highly resistant to environment and more dangerous for pregnant animals and cause pre mature birth or abortion and human get infection by respiratory route. Salmonellosis is caused by bacteria of the genus Salmonella belongs to the family Enterobacteriaceae and affect broad host spectrum. Salmonella is the major cause of intestinal infection in many hosts. Raw milk and milk products and uncooked meat is the main cause of these infections. These results in great economical loses and preventer by taking hygienic measures and consumption of properly cooked meat and pasteurized milk.

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1. INTRODUCTION

Mastitis is the inflammation of the udder or mammary gland of animals caused by the invasion of pathogenic bacteria followed by injury (Jiang et al. 2023). Unhygienic practices adopt during milking at farms lead to the spread and proliferation of pathogenic bacteria (Pal et al. 2023). Mastitis is the infectious zoonotic disease that causes infections through milking machines or milker's hands. In contrast, zoonosis in humans is caused by the consumption of raw, apparently hygienic, or unpasteurized milk (Schoder et al. 2023). Physical, chemical, cellular, and microbiological changes in mastitic milk cause economic losses worldwide and promote reduced reproductive life span of suffered animals (Joy et al. 2023). Many outbreaks have occurred due to unpasteurized mastitic milk (Dendani and Arcangioli 2023). This milk led to many zoonotic diseases like Brucellosis, Mycoplasma infections, tuberculosis and leptospirosis. Mastitic milk is the major cause of livestock diseases. *E.coli*, *Staphylococcus (S.) agalactiae*, *S. dysgalactiae*, *S. uberis*, and *S. aureus*, constitute 95% of all infectious diseases (Nobrega et al. 2023). Bovine sources cause approximately 65000 deaths of people suffering from tuberculosis in Wales and England between 1912 and 1937. In 1938, prior to World War II, milk borne outbreaks consisted of 25% of all outbreaks (Loddenkemper and Konietzko 2018). The prevalence level of *Listeria monocytogenese* and *Campylobacter jejuni* in milk is 13% (Jayarao et al. 2006).

1.1. BOVINE TUBERCULOSIS

Mycobacterium bovis complex contains the mycobacteria that cause tuberculosis with the wide range of host. The upper and lower respiratory tract and lymph nodes are affected with gross lesions. Transmission occurs via different routes of dairy products. The disease can be diagnosed by clinical signs and tuberculin skin tests in live animals and postmortem after death. It is of great public health significance to the people closely associated with animals (Al-Asady and Ali 2023).

1.2. ETIOLOGY

The *Mycobacterium (M.) bovis* comprises all of the mycobacteria that cause tuberculosis in mammals except the *M. avium* that caused the disease in birds (Javed et al. 2023). *M. bovis* is primarily responsible for infection in cattle (Parija 2023a). A zoonotic aspect of tuberculosis in humans is also related to the *M. bovis* that is responsible for bovine tuberculosis, causing chronic, progressive disease and primary disease in the respiratory system. It has a wide range of hosts, including humans, domesticated animals and wild animals. *M. bovis* is a considerable risk for human tuberculosis but does not establish as readily as *M. tuberculosis* in humans.

1.3. HOST SPECIFICITY

Bovine TB is one of the zoonotic diseases that impact livestock and humans and results in great production and economic losses in livestock (Bezos et al. 2023). M. bovis has a broad range of hosts than M. tuberculosis (Guimaraes et al. 2023). Table 1 highlights the hosts for *M. bovis.*
If amplifier hosts are infected, these can cause disease among animals and humans (Labruna 2009).

1.4. LESION DEVELOPMENT

M. bovis grows intracellularly in macrophages, the primary host cell (Verbeke et al. 2023). Experimentally, gross lesions can be seen in the respiratory tract and associated lymph nodes 14 days after infection. The caseous core lesions were small, light yellowish in color , and macrophage giant cells and neutrophilic

Table 1: Hosts of *M. bovis*

Natural host	Domestic cattle's.
Maintenance host	Captive deer and goats. European wild boar in Spain, New Zealand Brush-tailed possum, the European badger in the United Kingdom, Cape buffalo, and antelope in southern Africa are also the maintenance hosts for *M. bovis* (de Lisle et al. 2001).
Dead end host	Horses and sheep are among domestic animals (Coleman and Cooke 2001).
Amplifier host	Pigs, camelids, dogs, cats, goats, and farmed wild boars (Broughan et al. 2013).

debris were in evidence. Some mineralization and fibrosis were seen with developing lesions and more extensive necrosis consisting of intact and degenerative neutrophils, macrophages, and lymphocytes. Respiratory tract is infected in both direct and indirect contact. A very small dose can cause the infection through respiration that can be 1000 times less than the intestinal route (Hope et al. 2023).

1.5. TRANSMISSION

Milk is a route of transmission from a diseased cow to humans. Other common routes are ingesting raw milk and milk products and professional contact with infected animals. Routes of transmission are:

1.5.1. INGESTION (MILK AND MILK PRODUCTS)

Consumption of raw milk is the main source of infection from infected cattle to humans as mycobacteria can persist in unpasteurized milk (Islam et al. 2023). But once pasteurized, it becomes inactive. Many bacteria are shed in tuberculosis mastitis milk that is enough to infect the milk pool of 100 milking cows. Milk product like cheese from unpasteurized milk is also a risk for the population. Bacteria persist in unpasteurized milk and are less likely to be affected by pH, acid, alkali, and chemical disinfectants. *M. bovis* can persist for longer times in different varieties of products obtained from unpasteurized milk (Zeineldin et al. 2023).

1.5.2. INGESTION (MEAT AND MEAT PRODUCTS)

Eating the undercooked meat of affected animal can be a source of infection in humans. It is not an affective route as tuberculous lesions are not present in skeletal muscles. It's only possible in case of very advanced infection. During the meat inspection, the affected parts are removed, and if more than 1 organ or carcass is affected the whole carcass and offal are condemned. *M. bovis* is very sensitive to heat and cooking the meat at more than 60^0C temperature can result in no viable unit of *M. bovis* (Ahmad et al. 2023).

1.5.3. RESPIRATORY ROUTE

Respiratory route is common for animal-to-animal transmission. Professional zoonosis in those occupations and being exposed to aerosols is the route for human infection (Anderson et al. 2023).

1.5.4. CUTANEOUS/MUCOSAL TRANSMISSION

It is a rare route of transmission and has historical interest where it was an occasional source of localized skin, tendon and lymph node lesions, otitis, and conjunctivitis in milkers and veterinarians during surgical interventions (Phillips et al. 2003).

1.6. SING AND SYMPTOMS

The organ systems affected determines the sign and symptoms:

- In early stages, Tuberculous animals are clinically normal and no clinical evidence can be seen (Dinh-Hung et al. 2023).
- Progressive weakness, debility and mild fluctuating fever are the gradual onsetting signs.
- When there is lungs involvement, reduced exercise tolerance, dyspnea, and chronic moist cough more noticeable in morning and cold weather. Lymph nodes of head may swell and signs of obstruction of affected organs are seen when internal lymph nodes swell.
- Gastrointestinal involvement causes diarrhea or constipation.
- Persistent mastitis and hypertrophy may result from mammary tuberculosis that seen in varying populations of animals.
- Tuberculosis metritis may result in infertility and abortion, and chronic purulent vaginal discharge can be seen.
- Military tuberculosis caused by a hematogenous route can result in acute or subacute death from primary or secondary lesions (Nguyen et al. 2023).

1.7. PUBLIC HEALTH SIGNIFICANCE

Zoonotic tuberculosis is of public health concern in the whole world. More seen in developing countries (10-15%). Risk factors in developing countries include

- Human immunodeficiency virus (HIV).
- Poverty.
- Raw or undercooked dairy products.
- Social and cultural factors such as consuming raw blood.

Veterinarians, farmers, milkers, and abattoir workers are at risk from any route like inhalational, ingestion or cutaneous. Milk of affected mastitic cows can contaminate whole milk of 100 cows (Du et al. 2023).

1.8. DIAGNOSIS

Different methods are used in live and dead animals for the diagnosis of tuberculosis. The disease is manifested by clinical signs in live animals, and a Tuberculin skin test is also used (Gomez-Buendia et al. 2023).

1.9. TUBERCULIN SKIN TEST

This test measures delayed responsiveness to hypersensitivity**.** Tuberculin, purified protein derivative (PPD), is injected intradermally and recognized by World Health Organization. Single intradermal test (SITT) and comparative intradermal test (CITT) are two variations of this test. In the SITT method, PPD-B is injected at the neck region. A positive test is considered in case of selling at the injection site. It is measured by pairs of calipers before and after 72 hours of injection. More than 4mm thickness change indicates a positive for *M. bovis* infection. CITT is used to differentiate the animals affected with nontuberculous mycobacteria. This test addresses the cross-reaction between *M. bovis* and *M. avium*. PPD-B and PPD-A are injected side by side at 12cm difference and skin swelling is measured after 72 hours. The test is considered positive if swelling at PPD-B is more than 4mm than at PPD-A site (Shukla et al. 2016).

1.10. CONTROL

Adapting the control measures has lowered the number of infections. Effective disinfectants such as phenol, glutaraldehyde, iodine, and formaldehyde are used. Also, a heat of 250^0 F is effective. Due to infectious nature of the disease, no treatment is recommended. Long-term therapies are used to treat the condition. Pyrazinamide, an anti-tuberculosis drug, is ineffective against *M. bovis*, but rifampicin and isoniazid are effective (Zhang and Mitchison 2003).
A vaccine could be the preventive option, but it only reduces the severity of the disease, not prevent it entirely. The vaccine was developed in the 1920s by Calmette and Guerin. Another way is to inhibit direct contact of deer with cattle. Avoid offering deer saliva mixed fodder to cattle.

1.11. LISTERIOSIS

Clinical Signs of listeriosis and prevalence and incidence in dairy cattle in England in 1500 dairy farms were investigated with the help of a postal questionnaire survey. 64.1% was the response rate. The emerging zoonotic infection of ruminants is listeriosis, and the causative agent is *Listeria (L.) monocytogenes* (LM). Listeria affects CNS pathology (neuropathology), including rhombencephalitis. Lineage 3 of the Listeria monocytogenes serotype 4b strain is the cause of Listeria (Bundrant et al. 2011). Continued checking of animal listeriosis cases or outbreaks is necessary to improve animal health.

1.12. ETIOLOGY

Most of the animal listeriosis cases are caused by lineage 3 and 4. Contaminated silage is the number one cause of listeriosis. Contamination of the raw materials, wildlife or bird feces, or manure is also a cause of listeriosis. pH more significant than 5 of improperly fermented silage is a source for the growth of Listeria monocytogenes.0.1 billion colony forming units (CFU) /g is the number for Listeria monocytogenes in the poorly fermented silage. Morbidity is 8-10 percent, and mortality is 15 percent. Animals showing no clinical signs may also be a source of *L. monocytogenes*. *L. monocytogenes* is known as a harmful bacterial pathogen and has been a major case of food-borne diseases (Meurer et al. 2023).

1.13. HOST SPECTRUM

In immunocompromised adults, Listeria monocytogenes is a virulent bacterium that causes a number of food-borne diseases with very high mortality rates. Food-borne infection is a case for miscarriage (abortions) in pregnant women. Immunocompetent individuals cause localized gastrointestinal symptoms (Allahverdy and Rashid 2023).

1.14. EPIDEMIOLOGY

L. monocytogenes have different serotypes usually isolated from foods, large number of clinical cases in the world are due to set of serotypes (e.g., 1/2 a, 1/2 b, 4 b). Listeriosis related subsets of serotypes are 1/2a and 4 b (Brown et al. 2023).

1.15. ECONOMIC IMPACT

So, if marginal benefit is equal to marginal cost, then the level of food safety measurement is optimum. Benefits and costs of L. monocytogenes are estimated from published literature, different methods of

economic analysis. Annually benefits for Listeria monocytogenes estimated measures range between 2.3 to 22 billion dollars (Koopmans et al. 2023).

1.16. TEST FOR LISTERIA MONOCYTOGENES DETECTION

16. 1. PROCEDURE

For virulence of genes DNA sequencing data actA and inlA were used for phylogeny of Listeria monocytogenes and to test for positive result selection. For the absence or presence of genes, isolates were screened and assigned an internalin profile. To find out each isolate's relative cytopathology, plaquing assays should be performed (Wang et al. 2023b).

16.2. RESULTS

Listeria monocytogenes represents 2 separated evolutionary lineages results confirmed that. Under positive selection genes actA and inlA consist of amino acid sites. At some places, specific residues are associated by lineage and manifested of listeriosis. Predominantly and clonal composition of isolates from case of encephalitis was lineage 1. Just Genetically more modified and equally represented by isolates from case of encephalitis versus septicemia and fetus infection is lineage 2. Lineage 2 has lessee cytopathology in vitro as compared to lineage 1isolates (Hou 2023).

1.17. SUBTYPING OF UNUSUAL STRAIN

Main subtyping methods are of 2 types:

1. Bases of DNA sequencing on a 660 base pairs_ sigB allelic typing.
2. Used partial sequencing of sigB, addB, Idh, pbpA, lmo0490, lmao 2763, polC, prs, rarA, lmo1555 _subtyping by a 10-locus MLSA scheme.

Polymerase chain reaction of target genes by dideoxynucleosides a cause for obtaining sequence data for these genes (Hou 2023).

1.18. CLINICAL SIGNS

Broad range of clinical signs are linked to Listeria monocytogenes starting from a healthy fecal carrier state and non- invasive disease to invasive systemic disease infections. Abortions and stillbirths in sheep are also caused by Listeria spp. Tissue specificity in mammalian hosts is caused by Listeria monocytogenes. Virulence of pathogens varies with different factors. By bacteriology and histopathological test of fixed CNS tissues from single disease animal clinical diagnose of encephalitic listeriosis (circling disease). As the abscesses are mostly linked with encephalitis listeriosis (Subramaniyan et al. 2023).

1.19. PREVENTION AND CONTROL

Natural environment includes sea water, and fresh water of these areas are widely distributed by Listeria monocytogenes. During processing pollution and contamination of food present on sea may happen to occur and on seafoods very less levels (less than 100 CFU /g) for *L. monocytogenes* are frequently present. There are some options for prevention of Listeria monocytogenes from equipment or foods, other than heat treatment, that is also very effective. To minimize the multiplication of Listeria

monocytogenes in the final product is therefore essential. Cleaning and sanitizing program are included in the preventive measures, designed for decreasing the number of *L. monocytogenes* in the factories environment (Gómez-Galindo et al. 2023).

1.20. TREATMENT

- Ampicillin (Seki et al. 2023)
- Gentamicin (Li et al. 2023)
- Trimethoprim- sulfamethoxazole (Wang et al. 2023a)

2. Q FEVER

The zoonotic disorder Q fever is a disease that occurs by a gram-negative type bacterium, *Coxiella (C.) burnetti* that is present in the environment, all around (Navaei 2023). Transmission to the humans is mainly by the respiratory route by inhalation of aerosols, and the consumption of the contaminated products of the animals the reservoirs include the cattle and other pets. The Q Fever is exceedingly asymptomatic. However, in humans it may show symptoms from acute to chronic. The chronic symptoms include endocarditis mainly and are observed in patients with previous valvopathy and immunocompromised hists as well as pregnant. The treatment is effective but should be chosen according to the acute or chronic status of disease. Vaccination of animals can prevent the shedding of bacteria as well as abortions in the animals (Statham 2023).

2.1. HISTORY AND BACKGROUND

Different researchers have worked on Q Fever in different eras. Edward Holbrook Derrick 1937 described the febrile disease in Queensland, Brisbane, and Australia slaughterhouse workers. He was invited to investigate the outbreak in Brisbane and tried to isolate the causative agent of febrile disease by inoculating the guinea pigs, but failed. The etiological agent of the disease was first named as *Rickettsia burnetii* (Zhang et al. 2023) but in 1938 a new genus Coxeilla proposed by D. Phillip suggested the name *Coxiella burnetti* (Bell and Philip 1952), a name honoring the cox and burnetti who identified Q fever agent the new Rickettsial specie.

2.2. HOST SPECTRUM

The hosts for Q fever includes the humans, ruminants, pets but the common host cattle sheep and goat and rarely reptiles and birds. The causative agent is shed in the urine, feaces, milk and birth products (Van den Brom and Vellema 2009).

2.3. EPIDEMIOLOGY

The respiratory route is the basic route of contamination in humans, contamination by aerosols occurs directly from the birth products of the animals. The *C. burnetti* is very resistant to the environmental factors and may survive for weeks in the places where animals are present. Ingestion is small less important factor previously but is now a controversial topic these days. *C. burnetti* has been found in the arthropods as well, specifically in ticks but arthropods caused disease is not significant in the humans. Two cases were reported in the France caused by Rickettsia conorii and *C. burnetii*. Sexual transmission remains confined to the humans and animals. Sexual Experiments on infected mice were failed (Pires et al. 2023).

2.4. BACTERIOLOGY

It is an obligate intra-cellular, Gram negative type of bacteria from Legionellae's order, was observed in the rickettsia-like organism in liver and spleen of mice and was inoculated in their urines, first time. The major target cells are those located in the body tissues liver, lungs, spleen and lymph nodes and the monocytes circulating in the blood stream. Two different Antigenic forms have been found of *C. burnetti* and are distinguished on the basis of surface lipopolysaccharides. Phase I is the virulent type that completes the LPS on their surface, while the phase II is non virulent, having incomplete LPS and is non virulent (Metters et al. 2023).

2.5. ANIMALS

2.5.1. CATTLE

Q fever is widespread in cattle but is asymptomatic. Clinical manifestations include the premature delivery, Birth of weak off-spring and Abortion. *C. burnetti* is significantly associated with the Placentitis. Unlike humans, Cattle do not show the respiratory signs. Study by Guatteo show that milk is the 45% shedder and is the more positive for the samples as compared to the feaces or vaginal samples. Pregnant animals are high at risk as compared to non-pregnant animals. A combined shedding of virulent micro-organism in vaginal secretions and in feaces is 14.6% and 10% of cases, respectively (Porter et al. 2011).

2. 6. SHEEP AND GOAT

pneumonia, still births, Abortions and delivery of weak offspring are the results of the Q fever in Goats. In many countries, Goats cause the zoonotic effect to humans as their close contact and raising. Similar to cattle, Pregnant animals are high at risk as compared to non-pregnant animals. Animals may acquire the infection in the uterus and the mammary Gland (Lang 1990).

2.7. SHEEPS

Show the chronic infections due to *C. burnetii* caused Q fever. It results in the abortions similar to goats, and shed the microorganism in the vaginal secretions, feaces and urine but to a lesser stretch in milk (Gilsdorf et al. 2008).

2.8. CATS AND DOGS

Q fever is prevalent with the pets so is associated with the humans, in developed and under developed areas.

In the Feline family, the Q fever does not show any symptoms so remains undiagnosed, but the infected organism sheds the *C. burnetti* in the environment and plays the major role in zoonosis. Studies show that seroprevalence was high in the street cats as compared to the domestic cats (Kilic et al. 2008).

In Dogs, infection occurs potentially by the inhalation of spores of *C. burnetti*, bite of ticks, consumption of infected placentas and milk from the infected ruminants. The parturient dogs show the highly affected rate, puppies mostly die within 24 hours of birth, since it is associated with the early death of the puppies (Paris and Day 2023).

2.9. HORSES

Equine family shows the positive results for the presence of *C. burnetti*. But there is no zoonotic linkage is found between human and equines (Özcelik et al. 2023).

2.10. WILD ANIMALS

Wild life is considered less important for the Q fever zoonosis. Many wild birds and other mammals have been found to be the hosts of the infectious organisms but do not show the disease symptoms.

2.11. HUMANS

In humans, the Q fever shows the different types. It causes the acute to chronic disease in humans. The incubation period of 1-3 weeks is required to cause the disease.
In acute q fever, infection is totally asymptomatic in half of the cases, but if show, the symptoms include the fatigue, fever, headache, and influenza like symptoms. Pneumonia is the important symptom shown by the humans. It causes the abortions, intra uterine fetal death, premature birth and uterine growth retardation. The death rates in Q fever are only 1-2% while myocarditis occurs in less than 1% cases (Magdalini et al. 2023).

2.12. CHRONIC Q FEVER

The persistent infection for 6 months results in the chronic case of Q fever. Inflammation of internal lining of heart walls and chambers is observed and which occurs in 60-70% of cases, in chronic Q fever. The main symptoms observed are neurological. Headache, confusion, behavioral problems, and convulsions can be seen. In the pregnant mothers, future abortions are expected if infected with the Q fever. Antibiotic treatment is less effective and morality rates hit more than 50% (Debowski et al. 2023). Table 2 shows the affected species, symptoms and clinical manifestations.

Table 2: Species affected, disease symptoms and clinical manifestation

Specie	Symptoms	Clinical manifestations
Cattle	Asymptomatic (mostly)	Problems with the newborn
Sheep & Goat	Uterus & mammary gland	Pneumonia, New born issues
Cats & Dogs	Asymptomatic	Puppies died 24 hrs.
Wild Animals	No disease symptoms	
Humans	Polymorphic	Fatigue, Fever, Endocarditis

2.13. PREVENTION AND PREDISPOSING FACTORS

The general preventions include the avoiding contact with the livestock, not getting contaminated with the birth fluids of the animals, consuming pasteurized milk and milk products. Moreover, Q VAX vaccines are also in practice since 1989. After the use of vaccines, the positive cases of Q fever have markedly dropped (Chow et al. 2023).

2.14. TREATMENT

Doxycycline is used for 14 to 21 days in non-gestating females and other patients. Hydrochloroquines are also in practice, phagolysosome pH is increased and the association with the doxycycline bactericidal effect.

Fluoroquinolones, as their ability to penetrate the central nervous system, are suggested in the cases of meningoencephalitis. In pregnant females, long term bacteriostatic are advises till delivery. After delivery, doxycycline with hydroxychloroquine for 1 year is advised to given to the patients (Peng et al. 2023).

2.15. SALMONELLOSIS

Salmonellosis is zoonotic disease caused by bacteria of the genus Salmonella belongs to the family Enterobacteriaceae. Salmonella is the major cause of intestinal infection and it is present worldwide. It is the major problem in public and animal health. Salmonellosis is mainly characterized by headache, fever, malaise, vomiting, nausea, diarrhea and cramps. Although the infection can be an asymptomatic in some cases. It can occur in many forms of syndrome like gastroenteritis, bacteremia, enteric fever etc. Salmonella has more than 2000 serotypes but only 10-12 serotypes are involved in disease. Severity of infection depends upon the serotype and can be severe when the patient is immune deficient. Contaminated food has a major role in spreading the disease (Kuria 2023).

2.16. HOST SPECTRUM

Salmonella has a broad range of spectrum and includes cattle, horses, sheep, goat, cat, dogs, pigs and humans (de Silva et al. 2023).

2.17. CHARACTERISTICS OF SALMONELLA

Non spore forming
Rod shape
Motile (peritrichous flagella)
Oxidase test -ve
Gram negative
Have 2000+ serotypes
Facultative anaerobes (Kuria 2023)
Table 3 highlights the clinical features due to various Salmonella species.

Table 3: Clinical features of various Salmonella species

Features	*S. typhi*	Other species of Salmonella
Diarrhea	Absent	Present
Chronic Carrier	Present	May be present
Production of H2S	Present	Present
Fermentation of lactose	Absent	Absent
Reservoir host	Humans	Animals
Availability of vaccine	Present	Absent

2.18. TYPES OF SALMONELLOSIS

Salmonellosis can be broadly classified into two categories:

2.18.1. NON TYPHOIDAL SALMONELLOSIS (NTS)

This type of salmonellosis is food-borne and is caused by many serotypes of Salmonella (excluding Salmonella typhi). It is the major cause of a gastroenteritis in animals. It also produces enterocolitis in

horses (Peter et al. 2023). Mainly spread by contaminated food. It is a self-limiting disease and animals are the main reservoir. Severity of infection depends on the serotype/serovar involved.

2.18.2. TYPHOIDAL SALMONELLOSIS

This type of salmonellosis is caused by bacteria Salmonella typhi. It is mainly transmitted by feco-oral route. It is the severe form of salmonellosis and mortality is high. It is characterized by fever, headache, nausea, cramps, vomiting and diarrhea. Generally, animals are not reservoir (Cho et al. 2023).

2.19. PATHOGENESIS

Asymptomatic carrier state, enteric fever, gastroenteritis, focal infection and septicemia are the several syndromes caused by Salmonella. The type of syndrome depends on the type of serovar involved, for example, enteric fever is produced by *S. typhi* and paratyphoid-A while septicemia is produced by Salmonella choleraesuis. But in rare cases any type of syndrome can be produced by any serovar being involved. Generally, infants, adult (over 50 years) and immunodeficient patients are at risk.
Salmonella can spread from person to person and is usually brought into the body through contaminated food (Dietrich et al. 2023). Pathogenesis depends on some virulence factors, which are:

- The capacity of bacteria to occupy cells
- A fully formed lipopolysaccharide coat
- The capacity of bacteria to divide intracellularly
- Production of toxins.

After being ingested, the bacteria settled in the colon and the ileum, occupy the epithelium and divide within the lymphoid follicles and epithelium. Bacteria binds to the specific receptors present on the epithelial cells and invasion started in which the ruffling of enterocyte membrane occurs resulting in pinocytosis of the bacteria. The bacteria divide intracellularly, multiply throughout the entire body via systemic circulation, then ascend through the reticuloendothelial system.
Most bacteria cause an acute inflammatory response after colonizing the intestines, which leads to ulceration. They may release cytotoxins that prevent protein synthesis. Due to inflammatory response, symptoms like diarrhea, abdominal pain, chills, fever and leukocytosis will produce. Feces may also contain blood, mucus and polymorphonuclear leukocytes (Parija 2023b).

2.20. EPIDEMIOLOGY

Non typhoidal salmonellosis is mainly transmitted by the food which is contaminated as a salmonellosis is a zoonotic disease and has a vast majority of a reservoir. Domestic and wild animals, pigs, turkeys and chickens are the common reservoir of NTS. Animal products are the main route of transmission which are not properly cooked and bacteria survivability increases in these products.
Typhoidal salmonellosis lack animal reservoir and mainly transmitted from person to person. Contaminated water and human faces are the major route of transmission. Plasmid DNA fingerprinting and Pulsed field gel electrophoresis are the main tools for studying and tracing the outbreaks of salmonellosis.
Asymptomatic carrier state and increasing antibiotic resistance are the two main factors which have epidemiological significance in both type of salmonellosis (de Silva et al. 2023).

2.21. DISEASE IN CATTLE

It is the major disease of cattle caused by serotype *S. Dublin* and *S. typhimurium*. These bacteria enter the body through ingestion and causes diarrhea and acute enteritis (*S. Typhimurium*) while systemic

infections and abortions are caused by *S. Dublin*. After getting entry into the body, there will be bacteremia and infection spread to lungs, liver, lymph nodes and spleen of the animal. Infection gets entry into the placentomes and causes abortion. Death may also occur during the infection.
The main clinical signs include severe diarrhea, fever (above 40 °C), dysentery, enteritis and abortion (in late pregnancy) (Senbeta 2023). Diagnosis is mainly based on the clinical signs and isolation of the organism from various discharges of the animal. Serological testing can also be performed.

2.22. DISEASE IN HORSES

The main causative agents of Salmonellosis are *S. bongori* and *S. enterica*. These species have more than 2400 serotypes which can be differentiated by O and H antigens. It is the main cause of colitis and diarrhea in horses. There are three forms of salmonellosis which are identified in horses:
1- The first type is subclinical carrier in which the animal may or may not be shedding the bacteria but has the ability to spread the infection to the other animals through feed, water or by direct contact. Given that the bacteria are intermittently and infrequently shed in the feaces, multiple cultures and PCR assays may be required to detect the carriers. If under stress, the carrier might get sick.
2- The second type is a mild clinical course which is characterized by fever, depression, soft feaces (not watery) and anorexia. CBC may show absolute neutropenia. This type is self-limiting and lasts for 4-5 days.
3- The third type is expressed as a severe clinical form which features abdominal pain, anorexia, depression and neutropenia. Diarrhea has a characteristic foul smell. Dehydration and electrolyte losses occur rapidly. Signs of hypovolemic shock and sepsis also develop. There may be colonic inflammation, gas distention, abdominal discomfort and infarction (Mair and Sherlock 2023).

2.23. DISEASE IN POULTRY

The main causative agents are:
- *S. Gallinarum*, *S. Pullorum* (non-motile)
- *S. Paratyphoid* (motile)
These agents have worldwide distribution. *S. Paratyphoid* is of significant importance because it can spread through contaminated poultry meat. Turkeys and chicken are the excellent hosts for *S. Gallinarum* and *S. Pullorum* while *S. Paratyphoid* can be transmitted to all the animals (Mair and Sherlock 2023).

2.23.1. PULLORUM DISEASE

The causative agent is *S. Pullorum*. In the incubator, you might see chicks that hatched from infected eggs, sick or even dead chicks. Disease goes undetected for 5-10 days. Excreta of infected birds may be greenish brown or white in color. Without any obvious signs, the infection persists within the flocks for a long time. Reduction in egg production, fertility and hatchability may occur. The main clinical signs are depression, inappetence, ruffled feathers, white diarrhea, closed eyes, loud chirping, gasping, rent pasting and lameness. Upon postmortem splenomegaly, urate crystals and grey nodules in heart and lungs may also be seen (Lublin and Farnoushi 2023).

2.23.2. FOWL TYPHOID

The causative agent of fowl typhoid is *S. Gallinarum*. Symptoms are same as that of pullorum disease. The main clinical signs are inappetence, dejection, yellow diarrhea and thirst etc. Upon postmortem there may be anemia, enlarged liver and enteritis (Nehra et al. 2023).

2.23.3. PARATYPHOID INFECTIONS

S. Montevideo, *S. Derby* and *S. anatum* are the common isolates found in Paratyphoid infections. Morbidity is high but mortality is low. Clinical signs are diarrhea, ruffled feathers, loss of appetite and dejection. Signs may be mild or absent. Upon post mortem there may be enteritis, pericarditis, unabsorbed yolk and dehydration (Nehra et al. 2023).

2.24. DISEASE IN HUMANS

In humans, three forms of disease have been recognized:
1- Septicemia
2- Enterocolitis
3- Typhoid fever (de Silva et al. 2023)

2.25. LABORATORY DIAGNOSIS

The organism can be isolated from the fecal sample in and enterocolitis while blood sample is required to isolate the bacteria in enteric fever. In case of bone marrow, the result of culture is often positive. On EMB and McConkey agar, the bacteria form colorless colonies. Salmonella forms gas and H2S on TSI with the exception of a *S. Typhi* which does not produce gas. Serological test can also be performed to identify these bacteria (Kuria 2023).

2.26. TREATMENT

Ciprofloxacin and ceftriaxone are the drugs of choice in salmonellosis. For the chronic carriers of S. Typhi, ampicillin cam be given.
Ampicillin and potentiated sulfonamides can be given in ruminants.
Oxytetracycline + neomycin (combined therapy) is given in poultry.
In horses' colloids, crystalloid fluids and various antimicrobials can be given.
In dog's chloramphenicol, fluoroquinolones and trimethoprim-sulphonamide is recommended.
Various vaccines are available for the prevention of salmonellosis (Kuria 2023).
Fig. 1 highlights various diseases along with clinical symptoms that are spread by mastitic milk of cattle.

3. CONCLUSION

Mastitis is the inflammation of udder or mammary glands caused by invasion of bacteria after any injury. Intake of this milk leads to the occurrence of many zoonotic diseases like brucellosis, mycoplasmas, tuberculosis, leptospirosis, listeriosis, salmonellosis and Q fever etc. Bovine tuberculosis is caused by *Mycobactrium bovis*. Clinical signs and symptoms include gross lesions in respiratory tract and associated lymph nodes. Listeriosis is cause by *Listeria monocytogenes*. It results in sepsis and meningitis, meningoencephalitis, rhombencephalitis in immunocompromised patients and fetal infection in pregnant women. It also causes gastroenteritis even in healthy individuals. This disease effect CNS of both animal and human. Q fever is caused by *Coxiella burnetti*. Human get Q fever by inhalation from birth products of affected animal. Clinical signs and symptoms in human in chronic cases include endocarditis and neurological signs especially in immune compromised person. In acute cases of Q fever in human fever, fatigue, headache, pneumonia and influenza like symptoms are observed. In pregnant female abortions, intrauterine fetal death and premature birth and low weight in newborn

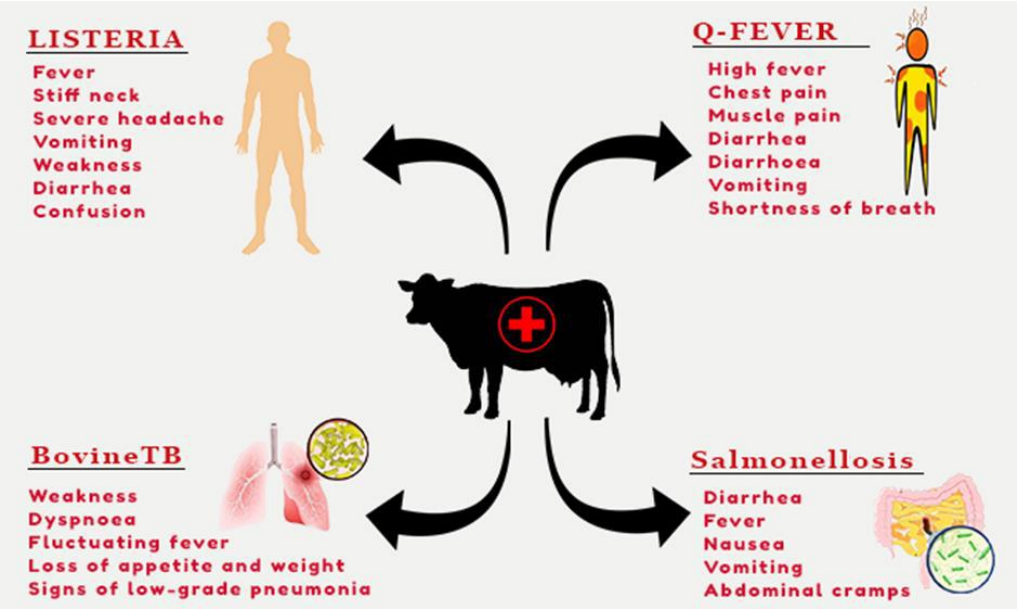

Fig. 1: Diseases spread by mastitic cattle milk

babies may result in Q fever. Salmonellosis mainly cause acute foodborne bacterial gastroenteritis. These diseases can be controlled by taking properly cooked meat and pasteurized milk. Various vaccines can also be used to control these diseases. Different drugs are also effective against these diseases.

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Rat Bite Fever Human Disease

45

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ABSTRACT

Rat Bite Fever is an emerging and re-emerging zoonotic disease that occurs in periodic, endemic, and epidemic forms. It is an acute, febrile, and systemic disease classically characterized by acute relapsing fever, rashes, migratory polyarthritis which affects the hands, wrists, and knees. In 1926, in Haverhill, Massachusetts, a large bacterial outbreak was reported by Place and Sutton, caused by the contamination of milk products with Streptobacillus moniliformis (S. moniliformis) bacteria. This outbreak was named Haverhill fever. It is an infrequent disease transmitted by rats and its causative agents are two specific types of bacteria that generate two different kinds of illnesses such as Spirillosis and Streptobacillosis. S. moniliformis bacteria are geographically present only in North America (Streptobacillary Rat Bite Fever) whereas Spirillum minus (S. minus) bacteria that is only reported in Asia and causing Spirillary Rat Bite Fever. It is also called Sodoku in Japanese which means rat poison (So= rat and doku= poison). Both bacterial species are common in rats and can be transmitted from rats to humans through urine, nasal passages, feces, or eye excretions of an infested rat. However, sometimes the infection is spread through food contaminated with excretions such as feces and urine. Specialized culture conditions or PCR tests are usually used for the diagnosis of Rat Bite Fever. Treatment with Tetracycline and Penicillin is commonly used for Rat Bite Fever. If not treated, its mortality rate is 10% to 13%, and a 53% mortality rate with endocarditis in some cases. In order to decrease the risk of infection, when an individual has been bitten by a rat, the affected area should be thoroughly washed and cleaned with disinfected as soon as possible.

Keywords: Spirillum minus, Streptobacillus moniliformis, Rat Bite Fever, Rat, zoonotic disease.

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1. INTRODUCTION

Rat Bite Fever is an emerging and re-emerging zoonotic disease that occurs in periodic, endemic, and epidemic forms (Pal 2005; Pal et al. 2021). It is an acute, febrile, and systemic disease classically characterized or manifested by acute relapsing fever, rashes, migratory polyarthralgia, or polyarthritis which affects the hands, wrists, elbows, shoulders, and knees (Hudsmith et al. 2001). In 1926, in Haverhill, Massachusetts, a large bacterial outbreak was reported by Place and Sutton, caused by the contamination of milk products with *Streptobacillus moniliformis* (*S. moniliformis*) bacteria. This outbreak was named Haverhill fever (Place et al. 1934). It is an infrequent disease transmitted by rats and its causative agents are two specific types of bacteria that generate two different kinds of illnesses such as Spirillosis and Streptobacillosis (Pal 2007). *S. moniliformis* bacteria are geographically present only in North America [Streptobacillary Rat Bite Fever] (Ogawa et al. 2018) whereas *Spirillum minus* (*S. minus*) bacteria that is only reported in Asia and causing Spirillary Rat Bite Fever, also called Sodok (Fukushima et al. 2018). The majority of the cases are reported in Japan, but specific strains have also been identified in the U.S., Europe, Australia, and Africa. Both bacterial species are common in rats and can be transmitted from rats to humans through urine, nasal passages, feces, or eye excretions of an infested rat. However, sometimes the infection is spread through food contaminated with excretions such as feces and urine. In addition, pets like dogs and cats that come into contact with rats can also be a source of transmission (Eisenberg et al. 2016).

Millions of people around the world are attacked by animals every year. Dogs and cats are being responsible for approximately 90% of these incidents. (Griego et al.1995). Rats are only 1% accountable for these bites (Glaser et al. 2000), and 2% of rat bites lead to Rat Bite Fever. People have long recognized that these bites can cause the disease. Indian physician Wagahbhat treated a cutaneous burn caused by a rat bite in the 2300s years ago (Row 1918). Many spectators consider it to be the first reported case of Rat Bite Fever in India.

For several years, there were significant misperceptions concerning the diagnosis of Rat Bite Fever. Tileston in 1916, Blake as well as Schottmuller 1914 and other researchers, isolated *Streptothrix muris ratti* (*Streptobacillus monliformis*) from the blood of patients bitten by rats with persistent fever, nearly about 100 years ago (Schottmuller 1914). Scientists in Japan believed that the only causative agent of Rat Bite Fever was *Spirochaeta morsis muris* or *S. minus* (Futaki et al. 1916). However, it is now assumed that *S. moniliformis* or *S. minus* are the causative agents of Rat Bite Fever. Rat bite fever is more frequently caused by *S. moniliformis* worldwide and *S. minus* infection is recounted less frequently and primarily this infection is reported in Asia. This infection is known as Sodoku in Japanese which means rat poison (So= rat and doku= poison) (Place et al. 1934; Pal 2007). The diagnosis of *S. monliformis* is challenging because it is a fussy microbe that makes ultimate analysis complicated (Shadrin et al. 2020). Specialized culture conditions or PCR tests are usually used for the diagnosis of Rat Bite Fever (Rovid 2021).

Treatment with Tetracycline and Penicillin is commonly used for Rat Bite Fever (DuBray et al. 2019). If not treated, its mortality rate is 10% to 13% (Zhang et al. 2019), and a 53% mortality rate with endocarditis in some cases (McKee et al. 2013). In order to decrease the risk of infection, when an individual has been bitten by a rat, the affected area should be thoroughly washed and cleaned with disinfected as soon as possible.

2. ETIOLOGY

S. monliformis and *S. minus* are the causative agents of Rat Bite Fever and they are also the source of two distinct types of illness. A comparison of key characteristics and clinical features of Rat-Borne Streptobacillus infection from *S. moniliformis* and *S. minus* has been mentioned in Table 1.

2.1. *STREPTOBACILLUS MONILIFORMIS*

In history, *S. moniliformis* was known by several names such as "*Multiforms*" and "*Asterococcus muris*" by Heilman in 1941 (Heilman 1941). "*Nocardia muris*", "*Actinomyces -muris ratti*" and "*Streptothrix muris ratti*" by Borgan and Gaustad in 1948 (Borgen et al. 1948). "*S. moniliformis* (Levaditi et al. 1925) "*Haverhillia moniliformis*" and "*Proactinomyces muris*" by Parker et al. 1926 (parker et al. 1926), *Actnobacillus muris* by Waterson et al. 1953 (waterson et al. 1953). *S. moniliformis* is most frequently capable of inducing Rat Bite Fever (Rosser et al. 2014). *Streptobacillus notomytis (S. notomytis)* is new species of Streptobacillus that can infrequently cause Rat Bite Fever in humans (Kusuda et al. 2020). *Streptobacillius felis* is another species that is also associated with rat bite infections in humans, in addition to *S. notomytis* (Matt et al. 2021).

2.2. MORPHOLOGY OF *S. MONILIFORMIS*

S. moniliformis can take various forms, including filaments, chains, or curved rods (Paegle et al. 1976). It is a gram-negative, non-motile, extremely pleomorphic, non-acid-fast organism with a rod-shaped appearance. When observed under a compound microscope, it typically appears as a straight line,

Table 1: Comparison of key Characteristics and Clinical Features of Rat-Borne Streptobacillus infection; *S. moniliformis* vs. *S. minus.*

Causative agents	*S. moniliformis*	*S. minus*
Morphology of organism.	Gram-negative, highly pleomorphic, filamentous, chains or curved rod-shaped bacteria with bulbul's swellings.	Gram-negative, tightly coiled, short and thick spiral rod-shaped bacteria.
Geographical distribution.	Worldwide.	Mainly Asia.
Route of Transmission.	Haverhill fever is caused by a rat bite, scrape, mucosal contact, or contaminated food.	Rat bite.
Onset of bite wound healing.	Rapid healing.	At the outset of symptoms, a chancre-like lesion developed but was quickly healing.
Incubation period.	3-10 days.	1-3 weeks.
Signs at the onset of a disease.	Headache, nausea, vomiting, and fever.	Fever, chills, and nausea.
Local signs.	A gentle lymphadenitis.	Localized lymphangitis as well as lymphadenopathy.
Type of Fever or nature.	An irregular or asymmetrical Relapsing fever.	A regular or consistent relapsing fever.
Arrival (Normal).	2-3 days.	2-4 weeks.
Polyarthritis.	Ordinary (reported in 49% of the patients).	Unusual.
Rashes (Eruptions).	Morbilli form to purpurie.	Molecular, often microscopy confluent.
Percentage of effectiveness.	75%.	50%.
Untreated mortality rate.	7-13%.	6.5%.
Complications.	Pneumonitis, prostatitis, pancreatitis, myocarditis, endocarditis, pericarditis, myocarditis, anemia, diarrhea, as well as abscesses in various organs.	Myocarditis, Endocarditis, nephritis, hepatitis, meningitis, and splenomegaly are rare but can occur.
Diagnosis.	Molecular methods, culture. Microscopy.	Diagnosis, molecular methods, culture, microscopy; vaccination of animals
Antibiotics (Drug of choice).	Penicillin.	Penicillin.

although it can sometimes be fusiform and contain several adjacent asymmetrical bulbous or Monilia-like swellings. The bacteria are often organized in chains and may appear somewhat roughly in clusters. Its colonies typically look like a "puff ball or cotton ball". *S. moniliformis* measures 0.3 to 0.7 micrometers in width and 1 to 5 micrometers in length. Filaments and bead-like chains can reach lengths of up to 150 micrometers and may include fusiform swellings that are 1 to 3 micrometers wide (Lambe et al. 1973). *S. moniliformis* exists in two different forms. It is usually present in the bacillary form, but it can also take on the inducible or spontaneous form. The Spontaneous form presents in L form which is cell wall-deficient and appears clustered with a morphological resemblance to "fried eggs". The L form is a nonpathogenic type (Freundt 1956).

2.3. *SPIRILLUM MINUS*

S. minus is another sporadic disease-causing agent related to Rat Bite Fever and is primarily found in the Middle East. It was discovered in the 19th century and initially known by various names, such as "*Leptospiramorsus minor*", "*Spirocheta morsus muris*" or "*Sporozoamuris*", "*Spirochaeta laverani*", "*Spironema minor*". In 1924, it was officially named *S. minus* (Washburn 2005). There is very limited knowledge about the taxonomic relation of *S. minus* (Kusuda et al. 2020).

2.4. MORPHOLOGY OF *S. MINUS*

S. minus is a tightly coiled, short, thick, gram-negative, spiral rod-shaped bacterium with two to six-helix spirals, each approximately 0.2 to 0.5-micrometer diameter (Washburn 1995; Washburn 2005). *S. minus* cannot be cultivated or grown in synthetic media. Dark-field microscopy, Wright stain, or Giemsa stain is used for examining the characteristic features and for the initial diagnosis of Spirochetes (Washburn 2005).

3. HOSTS

3.1. RATS

Rats belong to the primary reservoir of *S. moniliformis,* which is normally present in the commensal flora of the rat's respiratory tract (Eisenberg et al. 2016). Laboratory mice and household pet rats have demonstrated colonization rates ranging from 10% to 100% by *S. monliformis*, while wild rats often exhibit colonization rates between 50% and 100%. Infected rats occasionally show the symptoms of the disease but the majority of the rats are asymptomatic (Paegle et al. 1976).

3.2. MICE

S. monliformis is not commonly found in all strains of mice and in fact, many inbred strains of mice are significantly resistant to streptobacillosis disease. Infected laboratory mice might demonstrate the signs of disease such as polyarthritis, septic lymphadenitis, and multi-organ micro abscesses which can further lead to cachexia, septicemia, and death (Glaser et al. 2000).

3.3. OTHER ANIMALS

Infection and colonization of *S. moniliformis* have been reported in ferrets, pigs, gerbils, cats, and dogs (Torres et al. 2003). Streptobacillary disease has been demonstrated in turkeys and koalas and has also been reported in non-human primates, such as Titi Monkeys (Valverde et al. 2002).

3.4. HUMAN INFECTIVITY

Rat bite fever is a zoonotic disorder that can cause disease in humans (Fox et al. 2007). Rat bite fever, along with rat infestations involving *S. moniliformis* represents a significant public health concern with both physical and pathological implications that leftovers unknown.

4. EPIDEMIOLOGY

In the United States, *S. moniliformis* rarely causes Rat Bite Fever, and only a few cases are reported yearly. From 2000 to 2012, only 17 cases were documented in California (Gupta et al. 2017). *S. moniliformis* is responsible for only 10% of Rat Bite Fever cases in human beings (Etscorn et al. 1987; Hagelskjaer et al. 1998). But nowadays it has become common practice to keep rats as indoor pets in Europe (Royer 2015). As a result, approximately 20,000 rat bite cases occur annually in Europe (Julius et al. 2021). According to ancient studies, people living in poverty are more commonly infected with Rat Bite Fever and 50% of cases are reported in children (Hirschhorn et al. 1999; Josephson 2012). Additionally, pet store workers and laboratory technicians are also at risk because rats have gained popularity as pets and are used as research subjects (King et al. 2021). Pregnant women, immune-compromised individuals, and people over 65 years old are at a high risk. In Asia, the commonly existing bacteria responsible for causing Rat Bite Fever is *S. minus,* which is known as Sodoku (Fukushima et al. 2018). In Japan, in 1979, the *S. notomytis* species was initially secluded from spinifex hopping mice and was further hereditarily studied in 2015. In spite of the fact that neither the illness nor the causative organism is well understood by health officials, over two hundred instances of Rat Bite Fever in the nation. The oldest documented case of Rat Bite Fever was in an 87-year-old male (Torres et al. 2003), while the youngest reported case was a 2-month-old baby (Sens et al. 1989; Elliott et al. 2007).

5. TRANSMISSION

Rat Bite Fever is a zoonotic disease, its causative agent, *S. minus* is directly transmitted by vectors such as rats and mice to individuals primarily through bite or scrape and *S. moniliformis* can be spread through ingestion (Vanderpool et al. 2007). Rat Bite Fever causing bacteria are also observed in dogs, cats, ferrets, and especially in laboratory animals like *Rattus norvegicus* (Norwegian rat) and *Rattus rattus* (Black rat) which are recognized as potential reservoirs of Rat Bite Fever (Gaastra et al. 2009). *S. moniliforms* are commensal organisms normally found in the respiratory flora of rats, as well as in their oral, nasal, and conjunctival secretions and even in the urine of infected animals as shown in Fig. 1 (Elliott et al. 2007). It is estimated that 1 out of 10 rat bites lead to Rat Bite Fever (Hagelskjaer et al. 1998). An individual can acquire the infection through direct contact with a contaminated surface if they have an open wound or mucus membrane. Haverhill fever (epidemic arthritic erythema) can also be transmitted by contaminated food or water with rat's stools. Rat Bite Fever is not a contagious illness and cannot be transferred directly from person to person. Transmission from one person to another person has never been documented.

6. PATHOGENESIS

Rat Bite Fever has a low incidence and a low fatality rate when diagnosed and treated. Therefore, few details are known regarding the pathogenesis of *S. moniliformis*. Morphological abnormalities seen in Rat Bite Fever which are often linked to bacterial diseases include lymph node sinus hyperplasia, interstitial pneumonia, hepatosplenomegaly, endocarditis, and myocarditis as shown in Fig. 2. All of these abnormalities are visible in the autopsy of the Rat Bite Fever patient. Autopsies of Rat Bite Fever patients

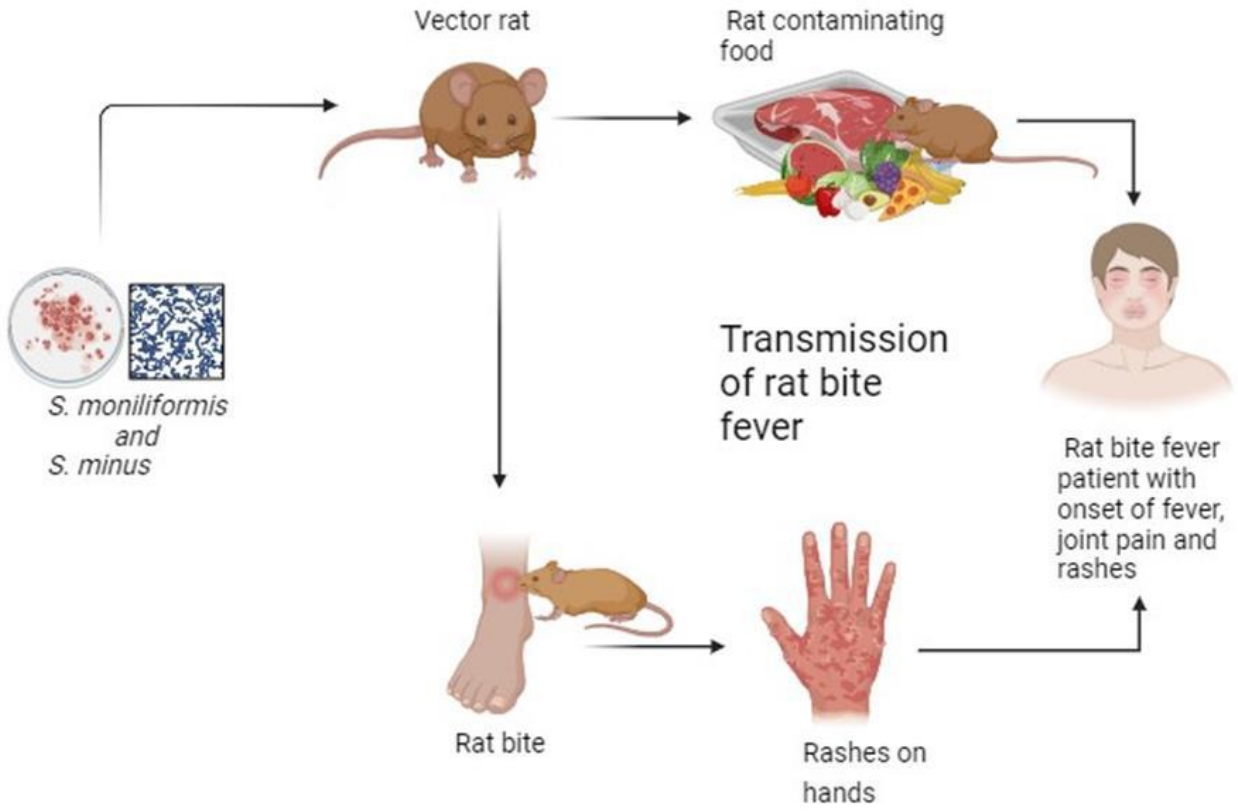

Fig. 1: Transmission of Rat Bite Fever.

have shown degenerative abnormalities in the kidneys and liver. Leukocytoclastic vasculitis was also observed during the skin biopsy of a previous Rat Bite Fever patient (Zhang et al. 2019). Experimental infection in mice causes progressive polyarthritis, which exhibits fibrin purulent exudate in the joint space and nearby periosteal tissue within the first 24 hours of the attack of an etiological agent. By day 4, this condition transforms into a predominantly periarticular abscess, and by day 7, necrosis occurs due to the presence of macrophages. After two weeks, periostitis starts to form, and three weeks later, fibrous connective tissue starts to proliferate. Although the organisms have been removed from the blood, liver, and spleen, it is concerning that they may still be present in joint spaces three months after infection (Elliott et al. 2007). Early attempts must be made to identify potential infections because the signs of Rat Bite Fever match with those of other diseases such as Post-infectious arthritis, Rheumatoid arthritis, Lyme disease, and Hemolytic uremic syndrome.

7. CLINICAL SIGNS

Rat Bite Fever is typically asymptomatic in carrier rats, but secondary bacterial pulmonary infections and abscesses can be absorbed sporadically. The pathogenicity of the disease changes depending on the strain of bacteria in rats, and affected rats may develop prolonged septicemia, leading to sudden death (Pongsuttiyakorn et al. 2021). The typical clinical signs of Rat Bite Fever include weight loss, hemoglobinuria, cyanosis, conjunctivitis, cervical lymphadenitis, and diarrhea. Acute signs of the disease include supportive polyarthritis, osteomyelitis, and abscesses as shown in Fig. 3 (Baker 2003). In humans, Rat Bite Fever caused by *S. monliformis* has been associated with two different clinical syndromes.

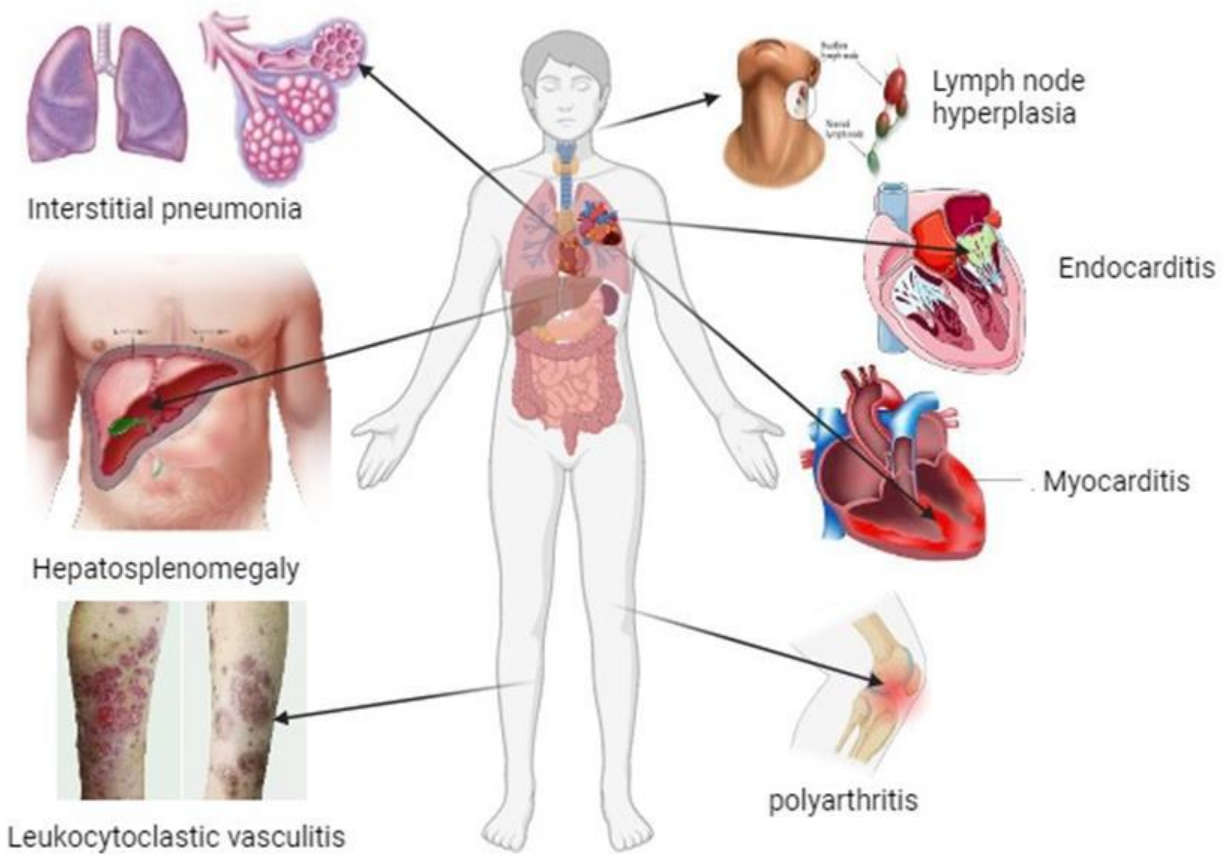

Fig. 2: Pathogenesis of Rat Bite Fever.

Haverhill fever syndrome is one of the outbreaks of epidemic disease that was first reported in 1926 and is contracted by humans as a result of the consumption of milk and food infected with *S. moniliformis*. Patients with Haverhill fever experience symptoms and indications that resemble Rat Bite Fever, but the disease is hallmarked by pronounced vomiting and pharyngitis, with no common temporal and geographic exposure to rats (Abusalameh et al. 2018). On the other hand, the Rat Bite Fever is the more typical syndrome caused by *S. monliformis* bacteria. The symptoms of the disease include a sudden onset of high fever (92%), severe migratory arthralgia (66%), rashes (61%), headaches (34%), sore throat (17%), vomiting (40%) and hepatitis which commonly appears 2-4 days after the onset of infection (Washburn 1995 and Mutters 1999). In some patients, meningitis, endocarditis, hepatitis, and localized abscesses have also been noticed (Elliott et al. 2007; Abusalameh et al. 2018).

The clinical signs of infections caused by *S. notomytis* are characterized by fever, rashes, polyarthritis, hepatitis, meningitis, and spondylodiscitis which are also common (Kusuda et al. 2020; Pongsuttiyakorn et al. 2021). *S. minus* bacteria cause rat bite infections in the Middle East which are referred to as Sodoku. In terms of medical characteristics and geographic distribution, this condition is distinct from Rat Bite Fever. The bite area becomes indurated and develops into an ulcer following a latent period of approximately 14-18 days, often associated with regional lymphadenopathy and fever. There are frequent relapses spaced by 3 to 7-day afebrile intervals. Red, brown, and even black macular rashes arise in around 50% of patients contain plagues. Joint manifestations are rarely seen (Adams et al. 1955; Freels et al. 2004). Cerebrospinal fever, mastoid bone inflammation, Hamman-Rich syndrome, polyarthritis nodosa, sarcoidosis, Idiopathic pericarditis, myocardial inflammation, liver inflammation,

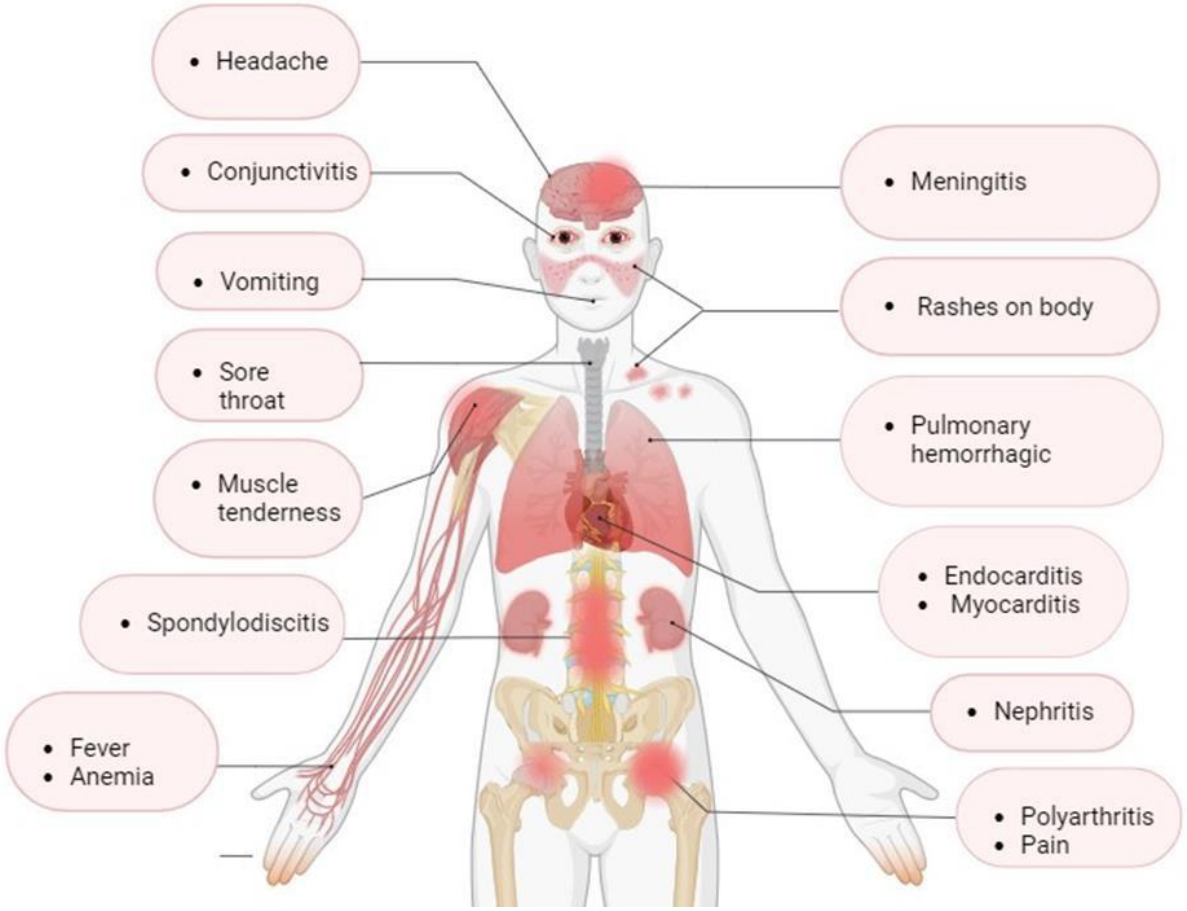

Fig. 3: Clinical Signs of Rat Bite Fever.

prostate pain syndrome, infectious arthritis as well as the development of abscesses in various organs are extensively reported complication of Rat Bite Fever (Abusalameh et al. 2018; DuBray et al. 2019). The Prognosis of infectious endocarditis in addition to Rat Bite Fever is particularly poor, with a 50% fatality rate (DuBray et al. 2019; Pena et al. 2020).

8. HISTOPATHOLOGIC FINDINGS

An L2-L3 spinal disc aspiration procedure, under CT guidance, was carried out for histopathology and culture analysis. Gram-negative rods become visible in gram-staining. The histopathological showed discitis-like fibrocartilage with degenerative alterations and acute inflammation. Histopathology of vertebral disc's fibro-elastic cartilage indicates that Rat Bite Fever may be the potential cause of osteomyelitis and discitis (Eisenberg et al. 2016; Abusalameh et al. 2018). A 9-year-old girl, who had received all of her vaccinations, was discovered to have Leukocytoclastic vasculitis, which is characterized by dense perivascular neutrophil infiltration, fibrinoid changes in the arterial wall, and localized epidermal necrosis. This was observed through a deep and superficial influx of dense perivascular neutrophils in the punch biopsy of her right thigh. *S. monliformis* extremely pleomorphic, filiform structures, were identified as gram-negative, rod-shaped, non-motile, non-acid-fast bacteria within a little vessel and identified using Gram staining (Elliott et al. 2007). Additional histochemical stains, include Grocott's and silver methylamine, acid-fast bacilli, and periodic acid Schiff) were negative for microorganisms.

9. HISTOLOGICAL LESIONS

There is a significant lack of knowledge about the histological lesions associated with Rat Bite Fever in humans. Observations have been conducted using animal models to better understand the disease process. In affected rats, the histological lesions associated with Rat Bite Fever vary depending on the stage of infection and the organ involved. Commonly affected organs include the skin, joints, heart, liver, lungs, and spleen. A few histological lesions that have been studied in animal models are given below.

9.1. SKIN

At the site of a rat bite or scratch, inflammatory changes can be noticed, which may include the infiltration of immune cells such as neutrophils and lymphocytes. These changes can be accompanied by necrosis and ulceration of the skin (Abusalameh et al. 2018).

9.2. JOINTS

Arthritis is a common feature of Rat Bite Fever. The affected joints show synovial hyperplasia, inflammatory cell infiltration, pannus formation (proliferation of granulation tissue), and destruction of articular cartilage (Abusalameh et al. 2018; DuBray et al. 2019).

9.3. HEART

Histological examination of the heart involved myocarditis which is characterized by infiltration of inflammatory cells into the myocardium. This condition can lead to the necrosis of cardiac muscle fibers and the presence of inflammatory cells such as lymphocytes and macrophages (Abusalameh et al. 2018; DuBray et al. 2019).

9.4. LIVER

Inflammation of the liver or hepatitis can also be absorbed in the case of Rat Bite Fever. Histologically, this condition is characterized by necrosis of the focal area, infiltration of inflammatory cells, and congestion of blood vessels (Abusalameh et al. 2018; DuBray et al. 2019).

9.5. SPLEEN

Inflammation of the spleen (splenitis) can also be absorbed in case of Rat Bite Fever. This condition includes the infiltration of immune cells, congestion of blood vessels, and destruction of splenic architecture (Abusalameh et al. 2018).

9.6. LUNGS

Pulmonary lesions in Rat Bite Fever can alter and may include intestinal pneumonia, bronchopneumonia, or abscess formation. Necrosis and congestion of blood vessels can be absorbed in the affected lungs (Abusalameh et al. 2018; DuBray et al. 2019).

10. DIFFERENTIAL DIAGNOSIS

In recent studies, the Rat Bite Fever infection has shown strong similarities to other infections that are detected during the incubation period. These similarities include a triplex pattern of fever, rheumatoid

symptoms, and rashes on various parts of the body, during the period of definitive analysis and in the period of symptom remission (Onodera et al. 2020; Shadrin et al. 2020. Note that, the distinction between these common symptoms of Rat Bite Fever is wide-ranging (Raffin et al. 1979; Raffin et al. 1979; Ojukwu et al. 2002; Freels et al. 2004). Rat bite fever can be differentially diagnosed from microbial sepsis caused by *streptococcus pyogenes* and *Staphylococcus aureus*, as well as from dispersed gonorrhea and meningococcemia. Furthermore, it can be differentiated from *Streptococcus pyogenes*-related diseases such as Lyme disease, ehrlichiosis, brucellosis, post-streptococcal reactive arthritis, rheumatic fever, scarlet fever, and rheumatic fever (Rordorf et al. 2000). In endemic areas, rickettsial infections like Rocky Mountain Spotted fever must be considered. Rat Bite Fever also shows a resemblance to spirochetal infections such as secondary syphilis and leptospirosis. Venereal disease laboratory tests (VDRL) are perceived to be false positive in 50% of the Rat Bite Fever patients. Some viral infections, such as *Parvovirus* B19 and *Espteinbarrvirus* are noticeable and confused with Rat Bite Fever. Relapsing fever can also confuse the Rat Bite Fever with malaria, typhoid fever, and Borrelia recurrentis. Non-infectious diseases that are somewhat similar to Rat Bite Fever are collagen vascular disease and drug reactions (Kimura et al. 2008).

11. DIAGNOSIS

The diagnosis of Rat Bite Fever can be complex due to several reasons, such as lack of knowledge, the involvement of multiple contributory agents, and a multitude of complications. To diagnose Streptobacillus infection, serology, organism isolation or molecular methods can be used. Among these methods, the molecular method, PCR is considered highly sensitive and more accurate for diagnosing *S. moniliformis* infection in Rattus species (Van Nood et al. 2005; Gaastra et al. 2009; Eisenberg et al. 2016). The confirmation of *S. moniliformis* by molecular method (PCR) has been more frequently reported (Chean et al. 2012). Medical confirmation in humans is typically attained through the culture of blood, joint puncture, or liquor cerebrospinal (Van Nood et al. 2005; Irvine et al. 2006; Gaastra et al. 2009). However, this method is not suitable due to the slow growth of the bacterium (Hagelskjaer et al. 1998). *Streptobacillus* bacteria are fastidious, making the culture of *S. moniliformis* or *S. notomytis* difficult (Madhubashini et al. 2013). Confirm Rat Bite Fever diagnosis can be quite challenging. If *S. moniliformis* or *S. notomytis* is suspected as the underlying cause, plasma, synovial fluid, or aspirates of abscesses should be injected into bacteriological media that lack sodium polyanethol sulfonate (SPS), such as anaerobic culture media. Sodium polyanethol sulfonate which is a common component of aerobic blood culture media may limit the growth of *S. moniliformis* or *S. notomytis* and thus provide indications of low negative prognostic values (Lambe et al. 1973; Washburn 1995). The gold standard diagnostic method used in the laboratory for isolating the causative agent from specimens like blood, synovial fluid, or abscess aspirates. However, even with the proper diagnostic tests, in cases of simple disease, synovial fluid cultures might be negative because the etiology of polyarthritis could be related to an immune-mediated response, which essentially means that it's a somewhat real disease involving the human body. The negative organic responses such as peroxidase, phosphate, fumarate, and amidohydrolase might be inadequate for detecting *S. moniliformis* in the majority of medical research laboratories (Joshi et al. 2010).

Reliable serological tests are not available for diagnosis of *S. moniliformis*. However, various alternative tests, such as gas-liquid chromatography, PCR, rRNA sequencing, and 16SDNA sequencing, have been found to be more sensitive than culture for detecting *S. moniliformis* (Miraflor et al. 2015; Eisenberg et al. 2016). It’s important to note that using regular blood samples for these kinds of tests is not permitted. The bacterium *S. minus* cannot be cultivated in artificial media. Dark-field, Phase contrast preparations, Wright, Giemsa, or silver staining are the principal techniques used for microbiological diagnosis and identifying the typical morphology of causative organisms. Using dark-field microscopy, spirochetes can be diagnosed in the blood of these animals after 4–15 days. However, there is no serological or molecular (PCR) testing

available for *S. minus* due to the inability to culture this bacterium. (Washburn 1995). Therefore, it is advisable to conduct further research to develop immunological and molecular techniques for the diagnosis of *S. minus* infection or Spirillosis.

12. TREATMENT

Rat Bite Fever is often treated with Penicillin G, which is also the drug of choice for this infection (DuBray et al. 2019). The recommended dose of Penicillin G for adults is 400,000–600,000 IU per day intravenously (IV) for 7–14 days. If there is no response to the antibiotic within 24 hours (two days), the dosage may be raised to 1.3 million IU orally. Ceftriaxone is also advised for the treatment of Rat Bite Fever infection, typically for a duration of two weeks. The adult dose rate for Ceftriaxone is 1.5 to 2 g per day (Zhang et al. 2019). In the case of children, treatment usually begins with Beromycin at a dose of approximately 3 grams per day, administered in four divided doses for the first seven days. Afterward, intravenous Penicillin G is recommended at a dosage of 20,000 to 50,000 IU/ kg of body weight per day (equivalent to 15 to 29 mg/kg/day), for the first five to seven days. Patients with Penicillin allergies can also be treated with Doxycycline and oral Tetracycline (Gaastra et al. 2009).

S. moniliformis is also exquisitely sensitive to multiple antibiotics, including Ceftriaxone, Clindamycin, Tetracycline, Erythromycin, Cephalosporin, and Vancomycin. It is also somewhat vulnerable to aminoglycosides, fluoroquinolones, and Chloramphenicol (Edwards et al. 1986; McKee et al. 2013; Shadrin et al. 2020). However*, S. moniliformis* shows resistance to Trimethoprim-Sulfamethoxazole, Polymyxin B, and Nalidixic acid (Rygg et al. 1992; Wullenweber 1995). In the case of Rat Bite Fever without any complications, treatment can typically be completed within two weeks. However, if Rat Bite Fever is complicated by endocarditis, a longer course of treatment is required. This may involve an overdose of Penicillin G in combination with either Gentamicin or an Aminoglycoside in a dual treatment approach (McCormack et al. 1967; Torres et al. 2003). If the isolate is sensitive to the concentration of 0.1 g/ml, the recommended dosage for adults is 4.7 million IU/day (equal to 4.8 grams) of Intramuscular (IM) Procaine Penicillin G. For adults, if the isolate is more resistant, they should receive 20 million IU/day (equal to13 grams) of intravenous (IV) Penicillin G (Rupp 1992). Children should be administered a dosage of 96 to 144 mg/kg (equal to 150,000 to 250,000 IU/kg/day).

Arthroscopy or Joint lavage and arthroplasty are recommended for managing the localization of disease inside the joints, especially in case of septic arthritis of large joints. Arthroscopy is preferred over arthrotomy because it is less intrusive than arthrotomy, this is especially important for pediatric patients because it allows for direct examination and observation of joint structure, as well as an assessment of the level of demolition or destruction that may have occurred. Additionally, it enables the evaluation of any potential disturbance in the ongoing development of children (Donatto 1998). Surgical intervention is significant in decreasing the bacterial burden within the joint and facilitating local drainage to eliminate any infections present in the affected joint.

13. PREVENTION AND CONTROL

The occurrence of Rat Bite Fever can be reduced by various measures. Firstly, the municipal or public environments should be kept free from rats, where accidental contact with rats is the most predominant source of transmission. The Public should be careful about consuming contaminated food and water such as unpasteurized milk (Graves et al. 2001). Individuals should properly sanitize and wash their hands after any direct contact with rats. Applying antiseptic and following prophylactic chemoprophylaxis after any scratch (Pal 2007). The protective measures should be followed by laboratory employees, pet store

workers, and those working with sewage. It's important to avoid handling wild rats, whether dead or living (Walker et al. 2019). Furthermore, if Rat Bite Fever is suspected in Lab workers, pet store employees, and owners of pets must obtain emergency medical attention (Shadrin et al. 2020). Antibiotic therapy should begin as soon as possible to reduce the illness's development and consequences (Onodera et al. 2020).

14. PROGNOSIS

Bacterial endocarditis due to Streptobacillary infection has a predominantly poor prognosis, with death rates of 60% (DuBray et al. 2019; Pena et al. 2020). The prognosis of Streptobacillary septic arthritis is superior when all patients are treated without experiencing long-term complications (Rupp 1992).

15. CONCLUSION

Rat Bite Fever is a relatively unappreciated disease that can have potentially serious consequences, including a 10% mortality rate. This disease typically presents with a sudden onset of fever, joint pain, and rashes. It is transmitted through a rat bite or contact with a rat's saliva, urine, and feces. However, humans can also ingest excreta through water and contaminated foods, such as raw milk. One significant challenge in diagnosing Rat Bite Fever is its nonspecific and variable symptoms, especially in patients with no prior history of animal exposure. Molecular diagnosis becomes necessary, particularly when there is no suspicion of exposure to rats or their excretions. Clinicians need to maintain awareness of this diagnosis when the patient's history of exposure is suggestive, as the symptoms are nonspecific and can be variable. Early diagnosis and rapid treatment with antibiotics are essential to prevent the disease from progressing to more severe stages and disease complications. Further investigation is needed to better understand the pathophysiology, and epidemiology of Rat Bite Fever.

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A One-health Approach to Combat Common Pet-associated Fungal Zoonosis

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ABSTRACT

Fungal zoonosis is an infectious disease that can spread from animals to humans. Most of the emerging and re-emerging infections caused by zoonosis. These can be transmitted directly or indirectly by fungi and can pose a serious threat to the world. The emerging infections are those that affect a population within a geographic area for the first time. In addition to posing a serious hazard to society, fungi can spread by sapronotic and zoonotic transmission. According to epidemiological studies, there is a rise in fungal infections in domestic animals. The most emerging cause of this rise in infections is climate change i.e., the fluctuation in temperature, humidity, change in human lifestyle, ecological disruptions and weak immune system. Exposure of zoonotic infection by direct interaction with pets, livestock animals, pet handler and importers. Transmission either directly via direct contact with secretions and excretions of animals, aerosol, faeco-oral route, skin abrasions, cuts and scratches. Fungi such as Dermatophytes, Aspergillus, Cryptococcus, Histoplasma can spread among pets and humans, leading to various diseases and infections such as Dermatophytosis, Histoplasmosis, Cryptococococsis, Paracoccidioimycosis and Aspergillosis. To properly address this issue a One Health strategy that emphasizes the connection of animal, human and environmental health is necessary. In developing countries, education and awareness are particularly necessary where the people lack even the most basic knowledge of numerous issues. In order to mitigate the impact of fungal zoonosis education, better veterinary procedures, cooperative research efforts are essential. By adopting a One Health approach, we can protect the health of both humans and animals.

Keywords: Fungal zoonosis, Dermatophytes, Aspergillosis, Histoplasmosis, Cryptococcosis, Paracoccidioimycosis

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1. INTRODUCTION

An infectious disease that can transmit from animals to humans, is known as zoonosis. A bacterium, virus, fungus, or protozoon parasite can cause it. These infections are transmitted directly or indirectly and become a threat globally. Most of the animals act as reservoirs of the infectious agent and then transmit this infection to humans or other animals. An infected host can also transfer the population's causative agent (Karesh et al. 2012). Most of the emerging and re-emerging infections are caused by zoonosis due to the close contact of animals and humans (Cutler et al. 2010). The infections appearing and affecting a population within a geographical location for the first time are called emerging infections. The infections that once were a global health concern but then declined notably and rise again in a particular geographical area or population called re-emerging infectious diseases. Climate change, import/export, changes in human lifestyle, and ecological disturbances are some of the top reasons behind the re-emergence of infectious diseases. Fungal infections are also a critical threat to society and can be transmitted by zoonotic and sapronotic transmission (Akritidis 2011). The most prevalent fungal infection groups are dermatophytosis, histoplasmosis, and sporotrichosis. Some of the fungal diseases lacked attention in world public health efforts, so having fewer strategies to treat these diseases (Barros et al. 2011; Moretti et al. 2013).

2. FUNGAL ZOONOSIS AND ONE HEALTH

Since fungus zoonosis is a complex and linked health problem based on interactions between animals, people, and the environment, it needs to be prevented on a global scale. The "One Health" method involves working with researchers, policymakers, and leaders locally, nationally, and internationally to enhance the health conditions of people, animals, and the environment. It is an effective approach to organize all stakeholders that can provide benefits to health sectors and arrange all government agencies (Erkyihun and Alemayehu 2022).

Epidemiological studies show an increase in fungal infection in domestic animals. The most emerging cause of this rise in infections is climate change i.e., the fluctuation in temperature and humidity. A rise is seen in the transmission from pet animals to humans. The expected reason for this is the change in human behaviour towards animals. Animals and humans are always living side by side, depending upon each other socially and economically but in recent years there is a rise in the trend of keeping animals as pets at house. There develops a close relationship between a pet and its owner hence close skin contact can easily transfer the fungal infection to humans (Vinke et al. 2020). Another alarming reason for this increase is immunity weakness. The drastic change in the immunity response is seen in the COVID-19 pandemic. The reason for this is still not certain and more research is needed to find the cause and effect of this issue (Azkur et al. 2020).

Pet-associated fungal zoonosis is a growing concern in public health due to its impact on both human and animal populations. Fungi such as *Dermatophytes, Aspergillus*, and *Cryptococcus* can spread among pets and humans, leading to various diseases and infections. A One Health strategy, which recognizes the interdependence of human, animal, and environmental health, is essential to effectively combat this issue.

3. ONE HEALTH APPROACH

The One Health philosophy acknowledges the interconnectedness and need to treat human, animal, and ecosystem health as one unit. By applying this approach to pet-associated fungal zoonosis, we can better understand the complex interactions that lead to the transmission and spread of fungal pathogens. It provides a framework for collaboration and integration of efforts between human and veterinary

medicine, microbiology, public health, environmental science, and other related fields. However, in low- and middle-income countries (LMICs) one health approach received little attention while persistence and emergence of zoonosis poorly understood (Gebreyes et al. 2014).

One health is a multifunctional approach that has an objective to provide ideal health conditions by recognising the connection between environment, humans, animals and plants at global, international, national and local levels. This integrated approach motivates different sectors and even communities to work together to solve the issue of environment and public health threats. It is a system to mitigate and prevent the threats to health and ecosystem (Erkyihun and Alemayehu 2022). The main objective of this approach is to act against climate change, provides clean water, energy, air, healthy and nutritious food. One health approach can enforce different sectors and communities working together to implement policies and legislations for the improvement of public health conditions (Kaswa et al. 2023).

Pet-associated fungal zoonosis is a growing concern in public health due to its impact on both human and animal populations. Fungi such as dermatophytes, Aspergillus, and Cryptococcus can spread among pets and humans, leading to various diseases and infections. To effectively combat this problem, a One Health approach, which acknowledges the interconnectedness between human, animal, and environmental health, is imperative.

The concept of one health strategy was given by Rudolf Virchow (1821-1902) in 19th century and then this idea grew globally in 21st century to prevent epidemic diseases and maintained the environmental integrity by collaborating all the departments related to health, food and environment to make new policies and laws for the healthy life of the society (Monath et al. 2010). World bank, United Nations system influenza coordinator, World Organization of animal health (OIE), United Nations Food and agriculture health (FAO) and United Nations Children Fund collaborate and worked on a strategic framework “Contributing One World, One Health”, to reduce the threat of infectious diseases at humans, animals and environment levels in 2008. World Medical Association (WMA) contributing this concept by educating veterinary professionals and in medical schools. The World Medical Association (WMA) and World Veterinary Association (WVA) recommended interdisciplinary collaboration between veterinary professionals and medical personnel to improve the health of both humans and animals at the Global Conference on One Health in 2015, which expanded the one health platform (Erkyihun et al., 2021; Buttigieg 2015).

4. IMPORTANCE OF ONE HEALTH APPROACH

Humans and animals share a common ecosystem so the drug resistant microbes can easily transmit from animal to humans and human to humans by close contact with them and the use of contaminated food. We can prevent the humans from most of the zoonotic diseases like rabies, brucellosis and anthrax by controlling the animals as they are causative agents. Antimicrobial resistance (AMR), environmental pollution, climate change due to anthropogenic activities (emission of greenhouse gases i.e., carbon dioxide, and methane by vehicles and industries) and the destruction of ecosystem are the main problems in the society that can be handle by the collaboration of all departments to eradicate and control these issues. Therefore, a well-coordinated approach to health in the human, animal, and environmental sectors is essential. (Day et al., 2012)

Through one health, collaboration and coordination improve disease investigation, stakeholder communication, diagnostic laboratory systems, and the network for early response and zoonosis detection. This strategy is useful in the prevention of zoonotic diseases by ensuring the collaborative work of all the public health departments. One Health approach deals with common health problems such as zoonosis, antimicrobial resistance (AMR) and food safety at the global level by collaboration, coordination and communication between the health-related departments (Garcia et al. 2004). Anyone can contribute

by promoting and implementing the One Health policies in Human-Animal-Environment health especially the professionals in veterinary and medical sectors can contribute by applying it in their regular practice (Erkyihun et al., 2021).

5. PET ASSOCIATED FUNGAL ZOONOTIC DISEASES

Zoonoses is considered as one of the major public health hazards worldwide, which lead to high mortality in recent times where infectious pathogen not only act as the main source of disease transfer from animal to humans but also as carrier for those pathogens' natural environment (Toma et al. 1999). Inappropriate public health policies mostly in underdeveloped and often in developed countries may result in reoccurrence and emergence of such zoonotic infections globally (Akritidis 2011). There is massive rise in population due to which increases in the food demand and more commercialization resulted in decrease invasion of wildlife habitats and more interaction between humans and animals become the leading cause of spread of different diseases (Satterthwaite et al. 2010). Moreover, exposure of zoonotic infection by direct interaction with pets, livestock animals and indirect connection with pet industry having animal breeders, pet handler, importer and pet distributor and people in close vicinity with animals may act as major infection source (Otero-Abad and Torgerson 2013).

Pets can serve as one of the most important risk factors associated with zoonotic infections, as infection can spread by direct contact with household and indirectly by general public interaction, where majority of people are unaware of zoonotic aspect of these pets especially in developed countries where pets may become important part and parcel of almost every home. There are various routes for transmission either directly via direct contact with secretions and excretions of animals, aerosol, faeco-oral route, skin abrasions, cuts and scratches through which people may develop pet related infections (Mani et al. 2009). A lt of work has been done on pet associated infection caused by bacteria, virus, and parasites but less research done on the fungal zoonotic infection which particularly associated with pets. Globally there are different animals and birds which can kept as pet like cat, dog, guinea pig, rodents, mice, fish etc. (Badyal and Desai 2014).

According to reports there is concept of ecological fitting which shows animal-pathogen interaction between infectious agent (fungi) and its host, where pathogen may adopt different strategies to multiply, colonize and complete its lifespan inside host (mammals) and disturb its immune system. If got a chance it releases and effect the surrounding environment as well (Wolfe et al. 2012).

Fungal diseases termed as silent killer due to its high mortality rate, causing 1.5 million deaths annually without showing signs and symptoms. These fungal infections can be cured but unfortunately very less research has been done in this field and most of these fungi responsible to boost up or increase fatality rate when combined with some other infections like respiratory problems(asthma), organ transplantation, AIDS and cancer (Toland et al., 2020). From last few decades' different epidemiological studies showed the strong connection and increase percentage of different fungal infections particularly associated with domestic and wild animals, but less data available regarding pet association with fungal groups (Wong et al. 2007). Some of important pet associated fungal zoonotic diseases are Dermatphytosis, Basidiobolomycosis, Histoplasmosis, Sporothricosis and Cryptococcosis (Friberg 2021).

5.1. DERMATOPHYTOSIS

Dermatophytosis, also known as ringworm or tinea, is a pet-associated fungal disease of the skin and hair which usually affect the keratinized superficial tissues of human (Fig. 1 2 and 3) usually caused by three important genera i.e., Microsporum, Trichophyton and Epidermophyton. Dermatophytosis is considered to be the most common infection of human skin transferred from pets (Hay, Johns et al. 2014). Regarding

animals, dermatophytosis get more attention due to its zoonotic aspect in dogs (*Trichophyton mentagrophytes*) along with domestic cats (*Microsporum canis*) which is responsible for its spread in humans directly and indirectly by farm workers (Moriello et al., 2017).
Cats may caught *M. canis* infection by staying inside as well as from outside (*A. vanbreuseghemii*) by interaction with hunters. So, there is a chance of transmission of such dermatophytes from soil or rodent during hunting (Drouot et al. 2009). The route of transmission is through the interaction of hair and scales, fungal arthrospores with formites in the infectious environment, and the host natural immune system plays a key role in avoiding dematophtic attack, as well as acting as a predisposing factor for host infection if it is accompanied by ectoparasites. The incidence of dermatophytosis increase due to rapid demand and placement of dog and cat as pets. Dermatophytosis particularly by *M. canis* is contagious and fatal disease which may serve as the main cause of nosocomial infections (Drusin et al. 2000). Table 1 enlisted common dermatophytes species along with the disease it causes and ecology (Bouchara et al. 2017).

5.2. SPOROTRICHOSIS

Sporotrichosis is one of the very important emerging zoonotic disease of the recent times causing severe health problems globally (Etchecopaz et al. 2021). In last two decades the incidence of sporotrichosis rise

Table 1: Common dermatophytes species, diseases and ecology

Species	Diseases	Ecology
Microsporum audouinii	Tinea capitis, mild inflammation	anthropophilic
Microsporum canis	Tinea capitis, severe inflammation	Zoophilic
Trichophyton tonsurans	Tinea. capitis, mild inflammation	Anthropophilic
Trichophyton mentagrophytes	Tinea pedis, tinea manum, tinea unguim, tinea barbae	anthropophilic and zoophilic strains
Trichophyton rubrum	Tinea pedis, tinea manum, tinea unguim, tinea barbae	Anthropophilic
Epidermophyton floccosum	tinea cruris, tinea pedis tinea manum	Anthropophilic

up from few hundred to 10,000 annually. The main culprit of this endemic was *Sporothrix (S.) brasiliensis* and *Sporothrix (S.) sckenckii*. According to study conducted in Brazil which showed the reason behind survival of *S. brasiliensis* in cats was due to structure modification and its survival at high temperature (Bongomin 2017). During cat fights, this fungus can transfer to other cat and humans as well by scratches and cuts (Inokuma 2010). *S. brasiliensis* is a contagious species for which cat act as the primary reservoir, transfering it to dogs and rats as well. The rapid spread of *S. sckenckii* was observed in adjacent states and to date no effective treatment is available. Previous research found that cats, together with dogs, rodents, and squirrels, were mostly responsible for zoonotic spread. Inhalation of conidial spores results in zoonotic transmission to humans (Toriello 2021).
There is evidence of transferring *Sporothrix spp.* through the respiratory droplets of cats. The transmission occurs by the sneezing of infected cat that expel the respiratory droplets having infectious microbes. Physicians should aware of this new transmitting route while treating the cases of *Sporothrix spp.* in humans (Rodrigues et al. 2022). The typical primary lesion of sporotrichosis shown in Fig. 4.

5.3. HISTOPLASMOSIS

Histoplasmosis, also termed as cave sickness, emerging as the serious zoonotic fungal disease responsible for high infection rate up to 5,00,000 people suffer from severe illness and if remain untreated lead to 25,000 deaths annually. Its prevalence increases rapidly to the Caribbean, Southeastern Asia and South and Central America (Almeida et al. 2019). The *Histoplasma (H.) capsulatum* is the etiological agent, firstly

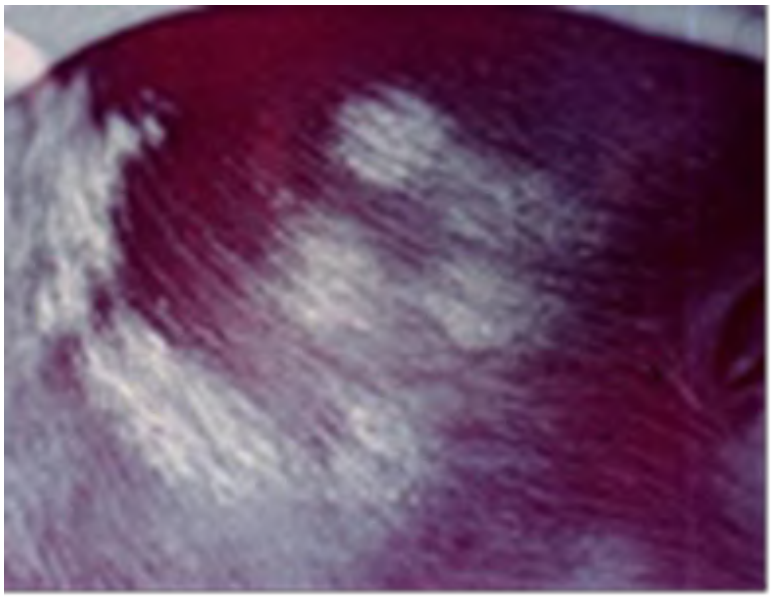

Fig. 1: Tinea capitis under Wood's Light

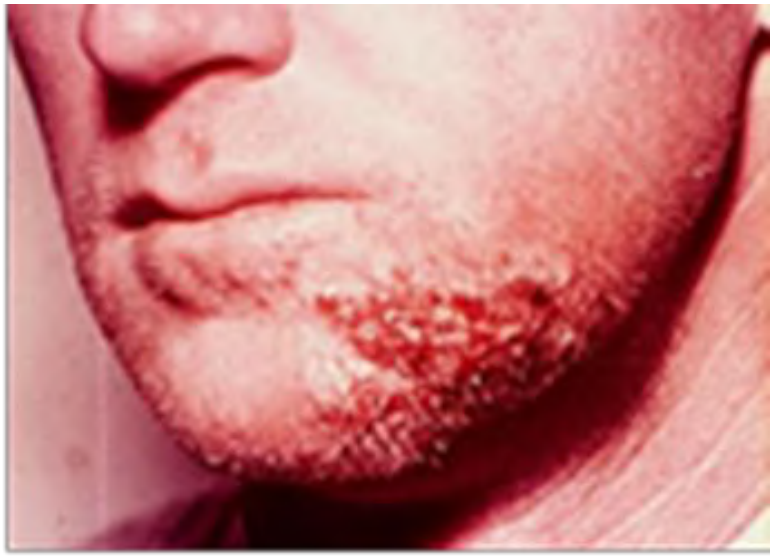

Fig. 2: Lesions of Tinea barbae

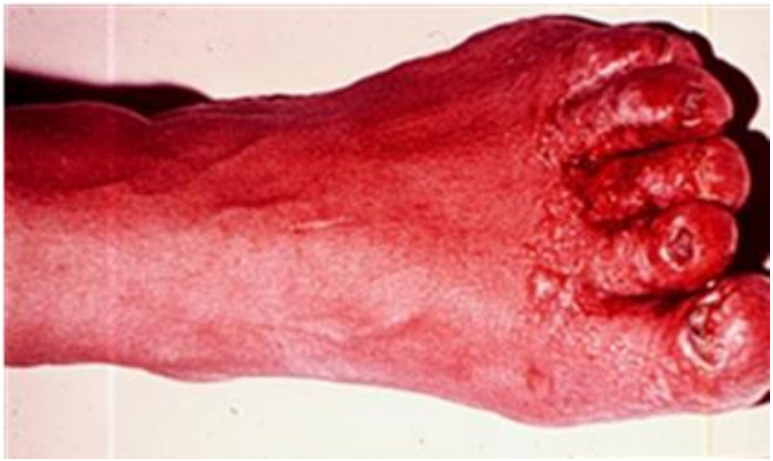

Fig. 3: Lesions of Tinea pedis

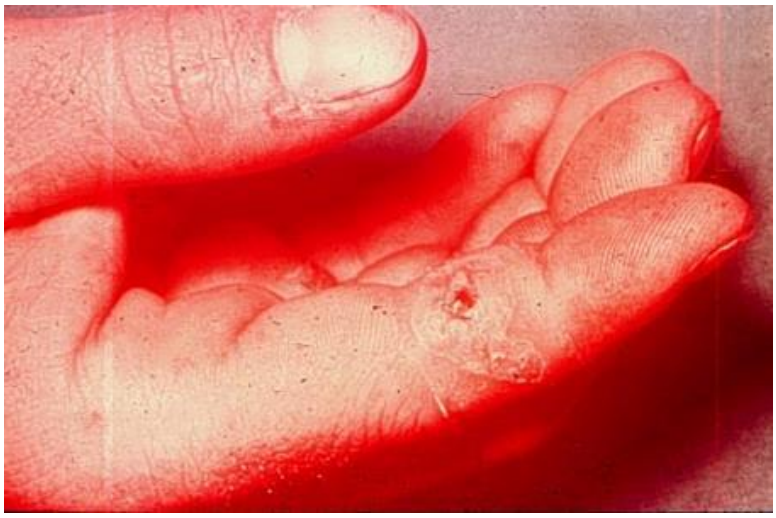

Fig. 4: Primary lesion of sporotrichosis

detected in Mexico later on accompanied by great genetic diversity having various virulent factors (Dias et al. 2019). *H. capsulatum* may survive in faecal material of bats and birds and soil of that area encroached with faeces (Antinori 2014). Regarding regional distribution, infected bats travelled great distances and disseminated the disease (Overgaauw et al. 2020). Different recreational activities at wildlife habitats like caving, hiking, bat and bird fighting may become the main culprit behind transfer of histoplasmosis (Diaz 2018). The aerosol transmission of histoplasmosis is done by inhalation of airborne conidia. More incidence is observed in pregnant women and immunocompromised individuals but it remain asymptomatic in immunocompetent persons. Histoplasmosis symptoms can range from being asymptomatic in immunocompetent patients to being lethal in those with impaired immune systems (Benedict et al. 2020).

5.4. CRYPTOCOCCOSIS

Cryptococcosis is recognized as the notorious, contagious and deadly zoonotic fungal disease. This infectious fungus can be fatal without showing any abnormalities in immune system causing more than 1 million deaths annually (Bongomin et al., 2017). Cryptococcosis, caused by *Cryptococcus (C.) neoformans,* is responsible for meningoencephalitis in HIV patients and *Cryptococcus (C.) gattii* may be asymptomatic and cause pulmonary infections in immunocompromised patients (Gushiken et al. 2021). The *Cryptococcus spp.* can be isolated from bird's waste materials such as pigeon or chicken droppings and from soil contaminated with birds' droppings. Some serotypes of fungi were isolated from eucalyptus trees. This fungus usually accompanied each other forming complexes and attack the respiratory system of mammals and farm animals and their companions (dogs and cat) resulting in various outbreaks (Refai 2017).

5.5. BASIDIOBOLOMYCOSIS

Basidiobolomycosis is rare but emerging zoonotic subcutaneous fungal disease which occur due to *Basidiobolus (B.) ranarum* present in decaying plant material, foodstuff, damage leaves and infected soil in the surrounding area (Shreef et al. 2017). Its presence can be observed in the GI tract of amphibians, reptiles, fish and mammals (dog, bat, humans) and in faecal material of kangaroos (El-Shabrawi and Kamal 2011). The fungus is more prevalent in Asia, Africa, South America and Europe and spread by inhaling in

area carrying plant decaying material. It can also be transmitted as a result of traumatic injury during implants. This fungal spores can enter in the body by cut/abrasion in skin and gradually resulted in lumpy growth under the skin, legs and arms. If remain untreated, it transfer to deeper tissues of vital organs like brain leading to death in severe cases. The ingestion of contaminated soil can also transfer and spread the disease (Ageel et al. 2017).

6. POTENTIAL ZOONOTIC FUNGAL DISEASES TRANSMITTED TO HUMANS

6.1. PARACOCCIDIOIDOMYCOSIS (PCM)

PCM is an airborne zoonotic fungal infection caused by *Paracoccidioides brasiliensis* resulted in acute to chronic illness. It is grown in the soil and is commonly found in Brazil, Latin America and Columbia. There are number of factors like human movement to different places, environmental and agriculture modifications, weather changes and expansion in land that may contribute to the spread of PCM. The PCM, which is common in dogs, cats, and other domestic and wild animal by inhaling its conidial spore in the surrounding environment and penetrates in humans and animals through the cutaneous and subcutaneous skin barriers. PCM may also distributed through residential and commercial dwelling areas (Martinez 2015)

6.2. PENICILLOSIS

Penicillosis is an emerging zoonotic fungal disease prevalent in south –East Asia and isolated from liver of bamboo rat. *Talaromyces (Penicillium) marneffei* is causative agent responsible for human infection, causing outbreaks in tropical areas. Dogs, acting as reservoir, are responsible for transmission of this fungus to humans (Hu et al. 2013), Furthermore, the Penicillosis marked as major opportunistic infection which is associated with HIV and AIDS resulted in severe complication. Genetic analysis confirmed the presence of same type of pathogen in rat as in humans which showed its zoonotic relatedness regarding fungal infection (Cao et al. 2011).

The close interaction of infected animals harboring different infectious pathogen may become the leading cause of public health hazard. Pet animals with special reference to dogs and cats serve as primary vector and more prone to transmit such infectious fungi to humans and cause sever life threating fungal infection in humans and animals (Toland et al., 2020).

7. PREVENTION AND CONTROL STRATEGIES

Prevention and control strategies have a long-lasting impact on the spread of fungal infection, its pathogenicity, and transmission from animal to humans. It helps in developing new trends in veterinary sciences as well as in human medical centres. This also provides awareness and education to general public in order to reduce the risk of developing the fungal infections in pet animals and becoming the emerging and re-emerging zoonoses (Rahman et al. 2020).

7.1. ENHANCED SURVEILLANCE SYSTEMS

Collaboration between human and veterinary healthcare providers is essential to establish effective surveillance systems. This would involve monitoring and reporting cases of pet-associated fungal zoonosis to track the prevalence, identify sources of infection, and implement appropriate preventive measures.

The World Medical and Veterinary association signed an agreement to improve the public health conditions by controlling zoonosis such as rabies and AMR (Erkyihun and Alemayehu 2022).
Pet owners and workers of pet shops should aware of causes, symptoms and required medical aid for a fungal infection in the pets. The knowledge of causative agent will help in prevention of infection by eliminating them before it causing the disease in animals. The information of symptoms can benefit to identify the infection on time and to get medical assistance before it become threatening to animal and transferred to humans. Owners and workers should know the implementation of surveillance and monitoring management system. In it not just include the preventive measures but also include the monitoring of all the activities, behaviour, preventive measures and treatments (Garcia et al., 2004).

7.2. EDUCATION AND AWARENESS

In order to prevent and control fungal zoonotic illnesses, it is essential to raise awareness among pet owners, medical experts, and the public. Educational campaigns should focus on proper hygiene practices, early detection of symptoms, and the importance of seeking timely veterinary and medical care. Emergency preparedness platforms must be generated to prioritize the disease risk assessment, simulation exercise and contingency planning (Wolfe et al., 2012).
Education and awareness are particularly necessary in the underdeveloped countries where it is lacking and people do not even have basic knowledge about many serious issues. In underdeveloped countries, there is no trend towards pet ownership, and only a tiny percentage of people maintain pets as pets. Because of this, they lack the fundamental information needed to properly care for feed, and maintain pets. This lack of knowledge leads to development of diseases specifically skin diseases, which include fungal infections in pets and their transmission from the pet animals to humans (Buttigieg , 2015).

7.3. IMPROVED VETERINARY PRACTICES

Implementing rigorous infection control measures in veterinary clinics and animal shelters is crucial. This includes routine screening of pets, isolating infected animals, practicing strict hygiene protocols, and appropriate treatment of infected pets to prevent further transmission. Laboratories are important to detect the pathogens so it's important to increase capacity and integration between them so that laboratories can share protocols and understand the outbreak of a zoonotic disease (Dias et al. 2019).
To improve the veterinary practices, a credible practice called clinical audit is used. Clinical audits done by systematically reviewing the current practices of treatments and handling the patient and bring improvement in the process. By implementing clinical audits in the veterinary literature will bring sustainable improvement in treating and handing of pet animals. In addition to this a pain scale must be implemented in veterinary clinics to assess the severity of pain in the pet animals specially dogs and cats (Rose and Pang 2021).
A mobile veterinary service is also helpful to provide the medical aid on the spot in emergency cases. This will also provide aid to the owners and workers of pets to get medical assistance for their animal on time (Bennett et al. 2019).

7.4. ENVIRONMENTAL MANAGEMENT

The transmission of fungal diseases is significantly influence by environmental conditions. In particular in pet habitats and animal housing facilities, proper waste management, ventilation, and routine disinfection can assist lessen contamination and fungal growth. To stop and manage zoonotic disease outbreaks, a skilled workforce of public health, wildlife, environmental, and domestic animal professionals must be

form on a global, regional, and local level. It should be back by one-health policies (Rocque et al. 2019). Environmental conditions such as temperature and humidity have a direct relation with the fungal growth and hence effect the spread of fungal diseases. Because of manmade activity, the climate is changing dramatically. The rise in greenhouse gases like carbon dioxide, nitrous oxide, and methane has an effect on everything from temperature and humidity to water and light quality.

Temperature and humidity variations, in particular, have an impact on the pathogenicity, survival, and life cycle of fungi. Management and maintenance of these environmental conditions in the pet-keeping areas may help in the reduction of fungal infections in pet animals and ultimately reduce the chance of zoonoses in humans. In this regard, it is important to keep pet animals' fur dry, especially those with long hair, such as some types of cats and dogs. Maintaining the temperature to optimum may help in the prevention of fungal infections (Ageel et al., 2017).

7.5. COLLABORATIVE RESEARCH

Research efforts should focus on studying the epidemiology, pathogenesis, and transmission dynamics of pet-associated fungal zoonosis. This interdisciplinary research would aid in the development of new diagnostic tools, effective treatment strategies, and the identification of potential reservoirs and vectors involved in the transmission. A data sharing platform can be created between all relevant organizations for timely integration and to understand the burden of diseases in the society (Hay et al., 2014).

Research provides benefits to researchers, medical practitioners, and to public in educating them about the health of pet animals and its direct and indirect link to the health of people related to these pet animals. It provides the awareness to the owners of pet animals about the importance of taking medical aid on time. Collaborative research is beneficial especially to the underdeveloped countries as it is economically helpful and provide much needed instructions to the people of these areas (Hosey and Melfi 2014).

8. CONCLUSION

Pet-associated fungal zoonosis poses a significant threat to both human and animal health as the health of pet is linked to the health of the people related to them. Embracing the One Health approach is crucial in tackling this issue comprehensively. The close interaction of infected animals harboring different infectious pathogen may become the leading cause of public health hazard. Pet animals with special reference to dogs and cats serve as primary vector and more prone to transmit such infectious fungi to humans and cause severe life threating fungal infection in humans and animals. By understanding the interconnectedness between humans, animals, and the environment, we can implement effective preventive and control strategies. Enhanced surveillance systems, education and awareness campaigns, improved veterinary practices, environmental management, and collaborative research efforts are key components in combating pet-associated fungal zoonosis. By adopting a One Health approach, we can protect the health of both humans and animals and reduce the burden of fungal zoonotic diseases in our communities.

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Use of Nutritional Components for the Control of Zoonotic Listeriosis

47

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ABSTRACT

Listeriosis is a disease of animal origin caused by L. monocytogenes. The disease affects humans, animals, poultry, and marine life also. Humans get infection with the consumption of contaminated foods mainly foods from animals such as milk and meat. The outbreaks of the disease are sporadic, but mortality rate is high in humans. The disease is controlled by antibiotics in humans, animals and poultry. But the L. monocytogenes have attained resistance against the antibiotics. The novel and alternative control strategies to overcome this problem are the nutritional and biological methods. The objective of this study was to review and conclude all the possible nutritional control methods for listeriosis. They consist of use of probiotics, bacteriophages, peptides, herbal use, essential oils and nanoparticles. The use of nutritional treatments is specific and safe for public health as they do not have any toxicity. These methods stop the growth of L. monocytogenes by causing cell death of bacteria through different mechanisms. Their important mechanism of action is the pore formation in cell membranes and outflow of components from bacterial cell. Most of the studies have been conducted to control L. monocytogenes by these methods in food industry. Further research needs to be conducted to control listeriosis in animals and humans.

Keywords: L. monocytogenes, animals, humans, treatments, foods, meat

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1. INTRODUCTION

Listeria (L.) monocytogenes, a gram-positive bacterium, causes the zoonotic disease known as listeriosis. The pathogen is cellular in nature and capable of intercellular movement, allowing it to traverse the blood-brain and placental barriers (Janakiraman 2008). According to Dhama et al. (2015), the condition is also known as meningoencephalitis, silage disease, and circular disease. This disease is transmissible to animals, livestock, fish, birds, crustaceans, and humans. Dhama et al. (2013) reported that *L. monocytogenes*-contaminated food can transmit the disease to humans. Direct contact with the environment and diseased animals can also infect humans (Matle et al. 2020). This disease is more likely to be contracted by pregnant women, young infants, old persons, and those with compromised immune systems. If animals consume contaminated silage, they may acquire this disease (Chen et al. 2020). The disease's signs and symptoms manifest infrequently but in extreme cases. The disease causes encephalitis, septicemia, meningitis, rhombencephalitis, meningoencephalitis, miscarriage, abortion, perinatal infections, and GIT infections in animals (Mateus et al. 2013).

Food handling and processing can lead to sporadic and epidemic outbreaks of listeriosis by contaminating commodities with the bacterium. The disease affects animals and humans globally (Dhama et al. 2013). According to Salama et al. (2018) and Jensen et al. (2014), the South African (2017) and Danish (2014) listeriosis epidemics resulted in 204 and 37 fatalities, respectively. In 2008, the disease caused 23 deaths in Canada, which is also a significant burden in industrialized nations such as the United States and Europe (Thomas et al. 2015). The disease has an incidence rate of 0.3% and a mortality rate of 21% in the United States (Tack et al. 2019). Between 2011 and 2017, China recorded 562 cases with a fatality rate of 32.68 % (Fan et al. 2019). It also causes substantial economic losses for the cattle industry, food contamination, and abortions in both humans and animals (Li et al. 2014).

A wide range of techniques are used for handling and caring for people, animals, and food. Standard interventions for both humans and animals include antibiotics and food-borne disinfectants (Guerrero-Navarro et al. 2019). This bacterium develops resistance to antibiotics and disinfectants due to their wide and frequent use. Second, because the bacteria are intracellular, the medications must enter the cells and accumulate there for the organism to be eradicated (Pagliano et al. 2017). Various remedies are required to prevent these complications and treat listeriosis (Dhama et al. 2015). Nutritional therapies are among the most cutting-edge and modern methods for controlling *L. monocytogenes*. The use of bacteriophages to control *L. monocytogenes* during the processing of meat, meat products, and poultry is an efficient method (Klumpp and Loessner 2013). As antibacterial agents, essential oils and plant extracts play a crucial role in the treatment of *L. monocytogenes*. Probiotics are essential dietary components that eliminate this pathogen and strengthen the immune system. To treat and manage *L. monocytogenes* in humans, animals, and food, cytokines, chicken eggs, prebiotics, enzybiotics, medicinal compounds, and nanoparticles are required (Dhama et al. 2015). The use of nutritional and biological control measures has increased over the past few years. In consideration of this, the objective of this book chapter is to identify and summarize all the prospective nutritional management strategies that have been used or may be used to control *L. monocytogenes*.

2. TRANSMISSION BY ANIMALS

Animal feed is a common source of *L. monocytogenes*. The majority of bacterial reservoirs consist of infected animals that excrete the pathogen via their feaces. Without adequate sanitation precautions during lactation, feaces are the primary source of pathogen contamination in milk (Rodriguez et al. 2021). Inadequately constructed silage serves as a source of bacteria for animals. *L. monocytogenes* is unable to flourish in silage with a pH between 3.7 and 4.7. In addition, it strengthens the animal's immune system

by providing microorganisms (Limin et al. 2018). 7.5% of silage samples tested positive for *L. monocytogenes*, according to research done by Nucera et al. (2016). Small ruminants, such as sheep, are more susceptible to listeriosis due to their fodder and grass forage consumption. 2.5% to 5.9% *L. monocytogenes* positive clamp silage samples were discovered (Rodriguez et al. 2021). Antibiotic-resistant microorganisms are posing a threat to public health and facilitating the development of novel management strategies. Consequently, there has been an increase in the adoption of nutritional and biological management techniques over the past few decades (Rothrock et al. 2017).

Other food sources such as crops, and pasture are also capable of transmitting bacteria to animals (Locatelli et al. 2013). Cats considerably contribute to the spread of bacteria through grazing animals' manure (Mohammed et al. 2010). According to Matto et al. (2017), the infection was acquired by a two-year-old heifer browsing on bovine manure-contaminated ground. According to a research, *L. monocytogenes* is spread by high-velocity winds and heavy precipitation, which contaminate pasture vegetation and infect animals (Pang et al. 2017). In agricultural contexts, water is the primary source of bacteria, and all sediment contaminations eventually contaminate water with this bacterium. It was reported that the prevalence of microorganisms in animal colonies was 6.5% (Mohammed et al. 2010). Farm surfaces are usually contaminated with the pathogen, so farm workers' and veterinarians' shoes can disseminate the bacterium (Schoder et al. 2011).

Listeria affects both domestic and wild animals, including sheep, goats, and livestock, with ewes being the most susceptible. Initial observations of the bacteria were made in guinea pigs and rabbits (Malakar et al. 2019). The intestines and milk of these animals contaminate humans. According to a study, 42.5 % of meat and meat products obtained by restaurants contained *L. monocytogenes* (Ng et al. 1995). In another study conducted in China, it was determined that the prevalence of microorganisms in beef and pork was 9.1% and 11.4%, respectively (Cavalcanti et al. 2022). According to Schoder et al. (2023), the prevalence of the bacteria in milk from cattle and small animals (sheep and goats) was 13% and 17%, respectively.

3. TRANSMISSION BY POULTRY AND SEAFOOD

Infectious variants of listeriosis in poultry are uncommon and frequently asymptomatic (Wesley 2007). In China (2014) and Washington (2013), sporadic infectious epidemics of listeriosis affected poultry which were raised at home (Crespo et al. 2013; Gu et al. 2015). Two of the primary risk factors for *L. monocytogenes* transmission in poultry are the hatchery and the development conditions of the farms (where live fowl are maintained). Incubation of fertilized eggs derived from reproductive progenitors precedes the hatching of birds in a hatchery. Consequently, the pathogen may spread to the egg surface, embryo, and neonatal birds as described in Fig. 1 (Rothrock et al. 2017). Only a few studies have demonstrated bacterial transmission at this time. Cox et al. (1997) of the United States discovered *L. monocytogenes* in only 1% of chicken napkins and 6% of eggshells. Another study conducted in Thailand discovered no evidence of *L. monocytogenes* in hatchery conditions (Kanarat et al. 2011).

The second place in which pathogens may be transmitted to poultry is agricultural and growing conditions. The chicks are relocated to the location of the farm's expansion. *L. monocytogenes* can be found in numerous environments and production sites, including vegetation, water, soil, enclosures, feed, and excretion (Dhama et al. 2013). Based on 2012 research conducted in the United States, the prevalence of the bacteria in ambient samples varied between 1.45% and 53.3% (Jones et al. 2012). *L. monocytogenes* was nevertheless more prevalent than anticipated in 2010 samples of broiler litters, nutrients, water, and soil from U.S. poultry farms (Milillo et al. 2012; Rothrock et al. 2017). Some poultry farms allow access to other animals, including dogs, goats, sheep, cattle, pigs, and other livestock that serve as bacterial reservoirs and can disseminate pathogens to fowl (Aury et al. 2011). Consequently, these are a few sites where *L. monocytogenes* could potentially spread to poultry.

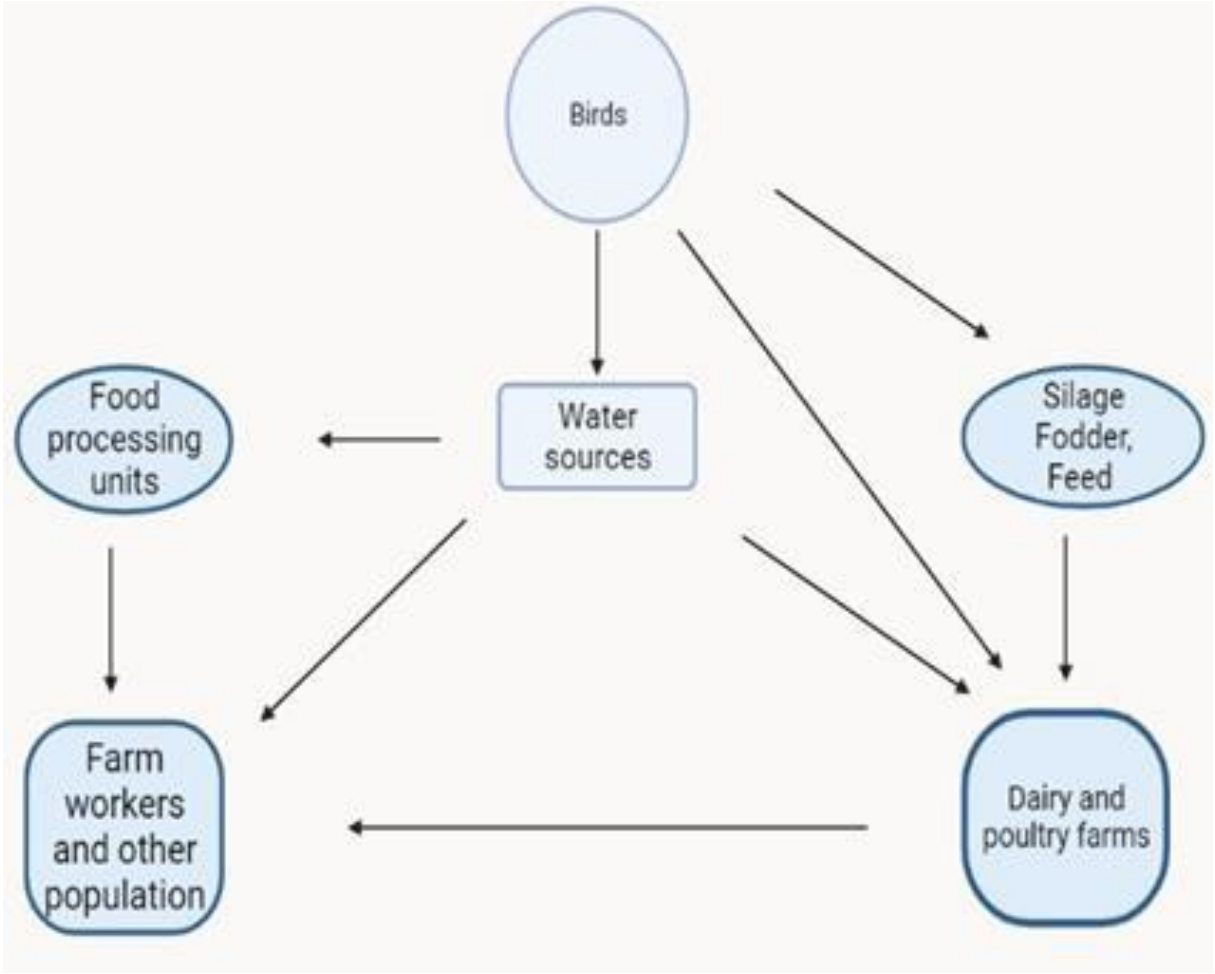

Fig. 1: Different possible routes for the transimission of L. monocytogenes.

4. NUTRITIONAL CONTROL OF LISTERIOSIS

4.1. *PROBIOTICS*

Probiotics are beneficial microorganisms that are given to the host in moderate amounts. Common sources of probiotics include milk and dairy products like yogurt and cheese (Zielinska et al. 2018). They are safe to use and can be used to eradicate pathogenic microorganisms in food to preserve it. Probiotics assist in food preservation by preventing the proliferation of *L. monocytogenes* in food. According to Rios-Covian et al. (2018), the most commonly used probiotics to treat *L. monocytogenes* are Bifidobacterium, lactic acid bacteria (LAB), and yeasts. Probiotics inhibit *L. monocytogenes* growth by preventing biofilm formation, reducing the availability of nutrients and energy for bacterial cells, interfering with quorum sensing mechanisms, and decreasing *L. monocytogenes'* environmental tolerance (Martín et al. 2022).
Bacteriocins such as nisin, which are generated by LAB and secreted by probiotics, inhibit the proliferation of *L. monocytogenes* in food. Three hours of nisin treatment inhibited *L. monocytogenes* growth by six log10 CFU/g (Zhao et al. 2020). Combining bacteriocins with other compounds increases their efficacy. Nisin and fatty acids were utilized in a study, and the outcomes demonstrated a 5 log10 CFU/ml reduction

in *L. monocytogenes* as well as an inhibition of biofilm formation (Zhou et al. 2020). Biofilms are bacterial microcolonies that adhere to surfaces and are encased in polymeric substances that are extraordinarily resistant to external stimuli (Nwaiwu et al. 2021).

L. monocytogenes and probiotics compete for the energy source (ATP). The overproduction of metabolites by LAB increases energy consumption and decreases the ability of *L. monocytogenes* to produce energy. Probiotics emit acetic and lactic acids, which prevent electron transfer and decrease energy generation similar to how probiotics and *L. monocytogenes* compete for essential nutrients (Aljewicz and Cichosz 2017). Due to their accelerated growth, probiotics deplete *L. monocytogenes* of nutrients, resulting in their eventual demise (Wu et al. 2022). In numerous foods, *L. monocytogenes* is inhibited by probiotics. *L. monocytogenes* in beef samples decreased by 2.57 log10 CFU/g when probiotics such as *Lactobacillus plantarum* and *Lactobacillus reuteri* were administered (Khalili Sadaghiani et al. 2019). LAB also decreased the amount of *L. monocytogenes* in chicken breast meat (Costa et al. 2018). When probiotics (LAB) are introduced to cheese, they decrease the temperature, pH, and water activities of *L. monocytogenes*, thus decreasing its concentration (Gonzalez-Fandos et al. 2020).

4.2. *PLANT EXTRACTS*

L. monocytogenes in various foods is now controlled and treated with plant extracts, such as essential oils (EOs) and herbal remedies. EOs are utilized for antiliteral purposes (Bajpai et al. 2019). EOs are derived from the leaves, roots, seeds, blossoms, blooming, and bark of various plants. Rosemary, thyme, and oregano are examples of essential oils employed for nonliteral purposes (Dhama et al. 2015). The oils of *Cinnamomum crassinervium* and *Cinnamomum cuspidatum* were utilized to limit the development and decrease the quantity of *L. monocytogenes* (Calo et al. 2015). EOs are used in foods as flavoring and preservation agents in addition to their antibacterial properties. The principal antibacterial components of EOs that eliminate pathogenic microorganisms are phenols and terpenes (Pietrysiak et al. 2019).

Because EOs are made up of a variety of chemical components, they have a variety of methods to eradicate pathogens. They can penetrate the bacterial cell wall and inhibit the functioning of the bacterial cell as shown in Fig. 2. Due to their hydrophobic nature, they cause the lipid bilayer of mitochondrial and bacterial cell membranes to rupture. Changes in the permeability of the cell membrane result in the loss of essential ions and other cell components, ultimately leading to cell death (Calo et al. 2015). Phenolic components of EOs alter intracellular proton transport, cell permeability, cytoplasmic membrane integrity, and energy synthesis, ultimately leading to cell death (Bajpai et al. 2019).

The impact of EOs on the growth of *L. monocytogenes* in a variety of foods has been analyzed. El Abed et al. (2014) discovered that beef treated with various concentrations of EOs derived from *Thymus capitata* enhanced the beef's activity against *L. monocytogenes*. According to another study by Giarratana et al. (2016), three genotypes of *L. monocytogenes* grew more slowly in the presence of rosemary and thyme essential oils at concentrations of 0.25% and 0.50%. When the effects of clove EOs (1% and 2%) on chicken were tested in the laboratory on seven strains of *L. monocytogenes*, a significant reduction in the number of bacteria was observed (Mytle et al. 2006). Likewise, steak containing 10% clove essential oil was completely delayed (Khaleque et al. 2016). Storage conditions, such as temperature and time, do not affect the proliferation of *L. monocytogenes*. The use of EOs from savory, cinnamon, *Satureja horvatii*, qysoom, nutmeg, and oregano inhibited the proliferation of *L. monocytogene*s (Yousefi et al. 2020). In contrast, it has been discovered that plant extracts may aid in the control of *L. monocytogenes*. It has been demonstrated that white tea, almond skin, and coffee extracts inhibit *L. monocytogenes* proliferation (Zamuz et al. 2021). It has also been determined that plant products (EOs) have no negative impact on fish or animals. There have been no reports of cancer-causing effects when consumed orally in large quantities.

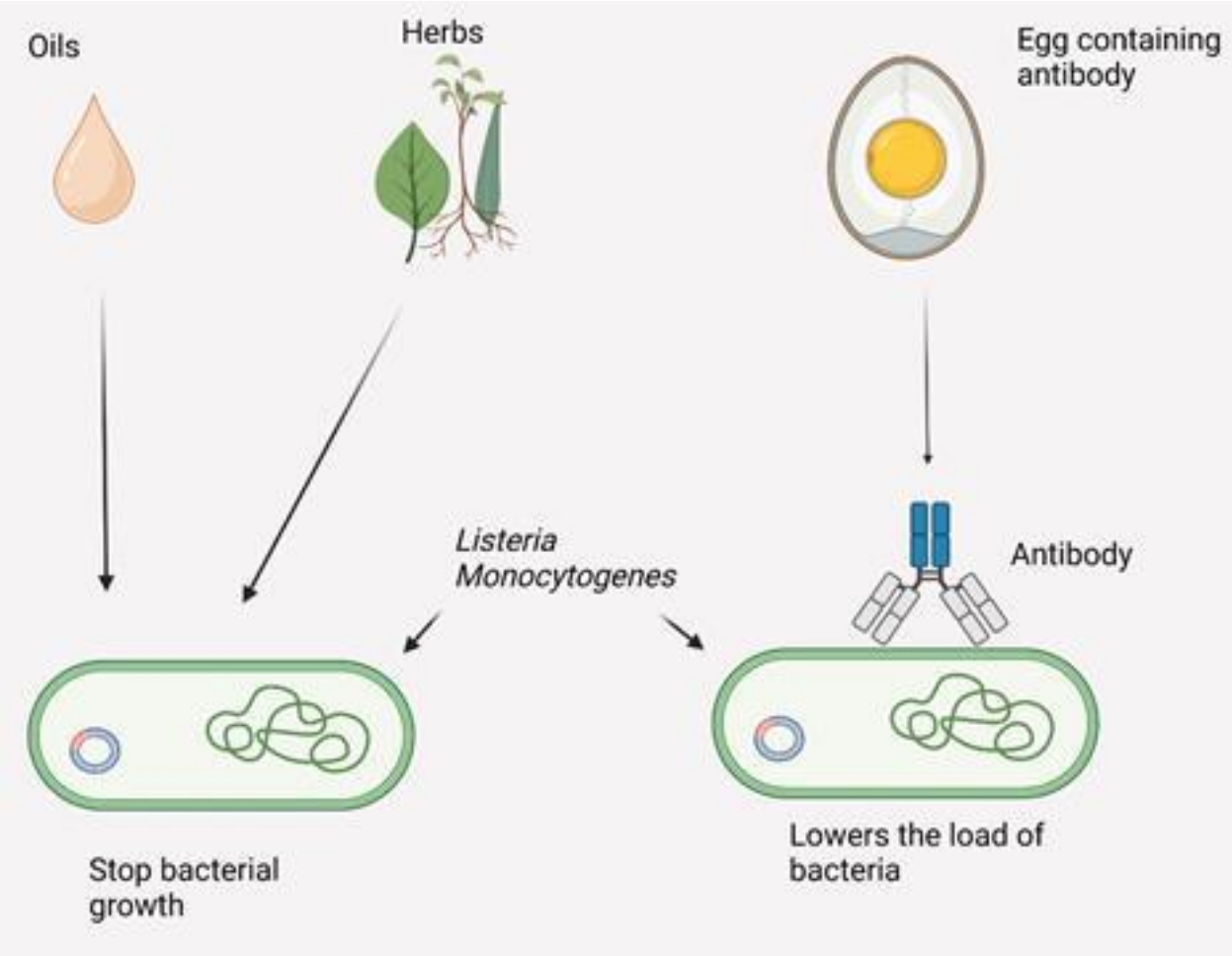

Fig. 2: Use of oils, herbs, and egg yolk antibodies to control *L. monocytogenes*.

However, they have disadvantages and, when used in large quantities, modify the flavor and aroma of food. Numerous strategies, such as the use of EOs in edible coatings, EO combinations, and microencapsulation, have been devised to address this problem (Yousefi et al. 2020).

4.3. *BACTERIOPHAGES*

Bacterial pathogens are responsible for the onset of severe and sometimes fatal illnesses, posing significant challenges in terms of their management and treatment. Bacteriophages have significant attention from researchers due to their potential to eradicate antibiotic-resistant bacteria while preserving the natural gut microbiota (Gandham 2015). Bacteriophages are viral agents that induce infection and undergo replication inside bacterial cells, ultimately leading to the lysis of the affected bacterial cell. Phages possess two fundamental mechanisms for bactericidal activity, namely lytic and lysogenic pathways. In the lytic mechanism, viral particles introduce their genetic material into the host bacterial cell and exert control over its metabolic processes. According to Batinovic et al. (2019), recently developed bacteriophages undergo replication inside the host cell and subsequently exit the cell, resulting in the demise of the bacterial cell. This process also has the potential to infect more cells. The lysogenic process entails the integration of phage genetic material into the chromosomal material of

the host bacterial cell, resulting in the formation of a prophage. Subsequently, the prophage undergoes reproduction inside the host cell (Wernicki et al. 2017). The conversion of this process into a lytic mechanism may occur at any given moment as a result of both internal and external cell-triggering signals (O'Sullivan et al. 2019).

A total of around 500 phages of the Listeria genus have been identified, with the majority exhibiting a lysogenic lifecycle. It is worth noting that these lysogenic phages have limited use in the field of biocontrol (Hagens and Loessner 2014). A limited number of listeria phages have been discovered as having virulent properties for managing listeria. The use of Listeria phages has been employed to manage *L. monocytogenes* in several contexts, including animals, milk, meat, fish, cheese, fruits, and vegetables (Kawacka et al. 2020). ListSheildTM (LMP-102) and ListexTM (P100) are the phages most often used to control *L. monocytogenes* in food products. In an experimental investigation, mice were administered a daily oral dose of 2x1012 phage concentrations per kg for five consecutive days. In this investigation, the researchers used a total of 100 P100 phages, which have been previously established as safe for usage and have shown a lack of adverse effects (Carlton et al. 2005). Soni et al. (2010) reported a significant decrease in the population of *L. monocytogenes* in catfish after the application of P100 phages. In a similar vein, the lytic effects of phages were examined by using LMP7 and LMP1 phages in soy broth and pasteurized milk samples obtained from commercial establishments. The use of these phages resulted in a notable decrease in the proliferation of *L. monocytogenes*, as seen in the study conducted by Lee et al. (2017). According to Bigot et al. (2011), the application of phages on packaged chicken meat resulted in a reduction in bacterial count and subsequent inhibition of bacterial growth over 21 days. An alternative strategy involves the use of a phage cocktail including many phages inside a unified assemblage. A solution consisting of a combination of six phages was administered to various food samples, resulting in a significant decrease in the proliferation of *L. monocytogenes* (Moye et al. 2018). Utilizing a cocktail of phages as opposed to a single phage is a potentially advantageous strategy, as it enables the targeting of a wider range of bacterial strains and mitigates the likelihood of bacterial resistance development towards therapy. This phenomenon might be attributed to the presence of many phages inside the cocktail, which ensures that if a particular bacterium develops resistance to one phage, it remains susceptible to other phages (Moye et al. 2018).

The effectiveness of phages might be modified by several circumstances. Multiple variables affect the functioning abilities of phages in complicated matrices of food and their interactions with phage-host systems. The elements included in this study are resistance, pH levels, phage concentrations, bonding properties, temperature, stability of phage form, and content of foods (Kawacka et al. 2020).

4.4. *PEPTIDES AND POLYPEPTIDES*

Peptides and polypeptides are becoming more common to be used in therapeutic contexts. Due to their high specificity and low toxicity, peptides are essential in medicine (Sato et al. 2006). Some dietary peptides are produced by the enzymatic proteolysis of proteins derived from other species, and they play a crucial role in the fight against microorganisms. Proline, arginine, and glycine are peptides that inhibit the development of *L. monocytogenes*. Moreover, barbel peptides derived from the enzymatic hydrolysis of barbel muscle proteins are employed to control *L. monocytogenes* (Falardeau et al. 2021). Bacterial ribosomes produce bacteriocins such as nisin, pediocins, enterocins, and lacticins, similar to peptides and polypeptides (Slozilova et al. 2014).

Peptides regulate the proliferation of *L. monocytogenes* in several distinct methods. Electrostatic forces adhere to the cell walls and membranes of microorganisms. The positively charged end of peptides interacts with mannose receptors and negatively charged lipids on the bacterial cell membrane (Kumariya

et al. 2019). As a result of the flux of potassium ions outside the bacterial cell, these interactions halt the production of peptidoglycans in the cell wall and modify polarization. As a result, the propelling force of the protons and the equilibrium of the water are disturbed, resulting in an energy deficit. According to Egan et al. (2016), the metabolites and nutrients of a bacterial cell exit through the cell's apertures and cause cell death. LAB produces peptides/bacteriocins via both pore-formation and peptidoglycan synthesis inhibition pathways (Bizani et al. 2008).

Numerous studies have investigated the effect of peptides on *L. monocytogenes* proliferation. The administration of Cerein 8A peptides at 4°C and 160 AU/ml concentrations slowed *L. monocytogenes* proliferation in milk by a factor of three (Kiran and Osmanagaglu 2014). When chicken flesh was stored at 4°C for 14 days, pediocin inhibited the growth of the target bacteria by 3.8 log (Renye et al. 2009). *L. monocytogenes* proliferation in salami was inhibited by 1.6 logs using Enterocin (Yap et al. 2021). Chicken and other avian eggs are an abundant source of peptides (antibodies) that inhibit *L. monocytogenes* development (Dhama et al. 2015). The interactions of peptides with dietary components, peptide-degrading enzymes, variations in the viscosity, fatty acid content, and fluid content of bacterial cell membranes, and peptide-degrading enzymes may all reduce their antibacterial effects. All of these factors reduce the ability of peptides to attach to the bacterial cell of interest and, consequently, their efficacy. To circumvent this issue, the peptides are microencapsulated and combined with additional preservatives (Chugh et al. 2021).

4.5. *NANOPARTICLES*

Nanoparticles (NPs) with medical significance are inorganic substances between 1 and 100 nm in size (Al-Shabib et al. 2020). Nanoparticles are increasingly used to inhibit the proliferation of microorganisms. Chemical synthesis and plant extraction are both viable options (Khezerlou et al. 2018). The use of NPs can be used to control *L. monocytogenes*. The most commonly used nanoparticles (NPs) to inhibit *L. monocytogenes* in various foods are ZnO, MgO, CuO, Ag, and sulfur (Ahmadi et al. 2016; Kumar et al. 2021; Priyadarshi et al. 2022). Different processes are used by NPs as part of their action mechanism to regulate *L. monocytogenes*. Due to their small size, they can more easily enter bacterial cells, where they can disrupt the respiratory system and genome, thereby eradicating the bacterium. Moreover, they impact the expression of the virulence gene in bacterial cells (Zakarienė et al. 2018). Other processes include the formation of oxygen-reactive species, the release of ions from nanoparticles, and the production of free radicals. When these particles adhere to the membranes of bacterial cells, they create holes that kill the bacteria (Rai et al. 2009). For NPs to effectively regulate microorganisms, their size is crucial. Research indicates that smaller NPs are more effective against *L. monocytogenes* than larger NPs (Firouzabadi et al. 2014). This is because smaller NPs have a greater surface area and a greater potential for cell interaction. Silver nanoparticles may inhibit the formation of listeria biofilm (Sani et al. 2022). Similar research demonstrated that Silver and CuO can eradicate *L. monocytogenes* (Milillo et al. 2012).

5. CONCLUSION

As a consequence of frequent and extensive use of antibiotics, the microorganisms that cause listeriosis have developed drug resistance. New approaches to control bacteria are being implemented to address this issue. Utilizing biological techniques and nutritional components is one of the most widespread new approaches. Probiotics, plant extracts, peptides, essential oils, bacteriophages, and nanoparticles are among the methods. Before destroying *L. monocytogenes,* bacteriophages modulate bacterial development by introducing their genetic material into bacterial cells. Before causing cell death, probiotics

release substances such as acids and bacteriocins that inhibit bacterial growth. Nanoparticles, peptides, and essential oils are all capable of permeating a cell and rupturing bacterial cell membranes, thereby interfering with normal cell activity and resulting in cell mortality. These are effective methods for preventing the spread of *L. monocytogenes*. To fully comprehend how these strategies function, additional research is necessary. The majority of labor is performed in the food industry, and data on listeria in humans and animals is scarce. Evaluating the effects of dietary approaches for managing *L. monocytogenes* in animals and, eventually, humans will necessitate future research.

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Fungal Zoonotic Infections in Fish an emerging threat to Aquatic and Terrestrial life

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ABSTRACT

Fish fungal infections represent an emerging threat with important implications for both animal and human health. Several primary fish pathogens have zoonotic potential, underscoring the relevance of fish mycoses for public health issues globally. Fungal pathogens of free-living and farmed fish are ubiquitous in aquatic environments. They cause superficial to disseminated infections and play a significant role in morbidity and mortality events in cultured and wild fish stocks. Although most published cases of fungal infections in fish refer to opportunistic pathogens that take advantage of compromised host immunity to invade the host, some fungi can behave as primary pathogens, able to cause disease even in apparently immunocompetent fish. The genera with the greatest number of pathogenic species for fish include Aphanomyces, Aspergillus, Candida, Chrysosporium, Exophiala, Fusarium, Ichthyophonus, Paecilomyces, Penicillium, Phoma, Saprolegnia, Trichophyton, and Trichosporon although zoonotic species are described mainly within genera Chrysosporium, Histoplasma, Paracoccidioides, Sporothrix or Cryptococcus. Yeasts play a role in algun pseudomycoses and mycoses. Saprolegniasis, epizootic ulcerative syndrome, branchiomycosis and dermal pseudomycoses are some examples of emerging fungal infectious diseases causing morbidity and mortality in farmed fish and amphibians and economic losses to aquaculture. This chapter examines the incidence and distribution of clinically relevant fungi in fish hosts, pathological findings associated with infection, and present knowledge on transmission routes. Challenges related to therapy and preventive strategies are discussed. Finally, the zoonotic potential and possible implications for public health will be addressed.

Keywords: Fish, Fungi, Zoonotic infections, Challenges, Preventive strategies

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1. INTRODUCTION

1.1. FUNGAL ZOONOTIC INFECTIONS IN FISH

A fungus-related zoonosis in fish, caused by several pathogenic fungi, is a very serious health problem for both aquaculture and humans. This chapter provides an in-depth analysis of common fungal pathogens affecting fish, their modes of transmission, clinical manifestations in humans, and strategies for the prevention and management of fungal pathogens. Fungal zoonosis in fish is complex and requires an understanding of its dynamics in order to implement effective control measures and protect aquatic ecosystems and human health.

1.2. FUNGAL ZOONOTIC DISEASES OF FISH

Non-photosynthetic microorganisms that live as saprophytes in dead organic material and soil are known as fungi. Only 300 out of 1.5 million identified fungal spp. are pathogenic to humans (Abara et al. 2017). The most commonly known fungal diseases with their etiological agent are presented in Table 1.

Table 1: The most commonly known fungal diseases of fish

Disease	Causative Agent	Common Name
Basidiobolomycosis	*Basidiobolus ranarum*	*Subcutaneous zygomycosis or subcutaneous phycomycosis*
Sporotrichosis	*Sporothrix schenckii*	Rose gardener's disease
Shrimp Mycosis	*Lagenidium spp.*	Larval mycosis
Aspergillosis	*Aspergillus spp*.	Common mold
Icthyophonosis	*Ichthyophonus hoferi*	White spot disease
Branchiomycosis	*Branchiomyces demigrans*	gill rot
Saprolegniosis	*Saprolegnia parasitica*	Cotton moulds
Dermocystidiosis	Dermocystidium	A gill disease
Exophialiasis	*Exophiala salmonis, E. psychrophila*	Black yeast like fungi

The epidemiology of fungal diseases in fishes is complex and varies depending on the type of fungus and the environmental conditions. Fungal spores can be found in many aquatic environments but infection typically occurs when fish are stressed or injured, allowing the fungus to penetrate the skin or gills. Factors that can increase the risk of infection include poor water quality, overcrowding, and poor nutrition. Saprolegniosis, caused by the fungus Saprolegnia spp. is one of the most common fungal diseases in fishes. This infection can affect a wide range of fish species, and outbreaks often occur during the spawning season when fish are most vulnerable. The fungus can be spread through contact with infected fish, water or equipment and it can survive in the environment for a long time period.
Achlya infections are caused by the fungus Achlya spp. and these are typically associated with freshwater environments (Chauhan et al. 2012; Chauhan et al. 2013). This infection can occur in both wild and farmed fish populations and outbreaks are often linked to poor water quality and

overcrowding. Fusarium infections are caused by the fungus Fusarium spp. and are less common in fishes than Saprolegnia and Achlya infections (Ke et al. 2016). These infections are typically associated with warmer water temperatures and can be transmitted through contact with infected fish or water.

Fungal infections in fish can be caused by a variety of different types of fungi. They can occur in both wild and farmed fish populations and can cause serious health problems, including mortality (Iqbal et al. 2012). Some of the common fungal diseases that affect fish include saprolegniasis, ichthyophthiriasis and cryptococcosis (Van Den Berg et al. 2013).

Saprolegniasis, also known as water mold disease, is caused by the fungus Saprolegnia. This fungus thrives in cool, oxygen-rich water and can infect fish that have weakened immune systems due to poor nutrition, overcrowding, or other stressors. Infected fish may develop grayish-white cotton like growths on their skin and fins (Barde et al. 2020).

Ichthyophthiriasis also known as white spot disease, is caused by the parasite *Ichthyophthirius multifiliis*. Although it is not a true fungus but often included in discussions of fungal diseases due to its similarities in presentation and treatment. Infected fish may develop white spots on their skin, fins, gills and may exhibit respiratory distress with certian behavioral changes (Von Gersdorff Jørgensen 2017).

Cryptococcosis is caused by the fungus Cryptococcus neoformans and can affect both freshwater and marine fish. This disease is less common than saprolegniasis and ichthyophthiriasis but it can be serious and difficult to treat. Infected fish may develop skin lesions, neurological symptoms and other health problems (Sato et al. 2015).

1.3. BASIDIOBOLOMYCOSIS

Basidiobolomycosis is a fungal infection caused by the fungus *Basidiobolus ranarum* (Al-Shanafey et al. 2012; Shreef et al. 2018). The mode of transmission of the fungus is not fully understood but it is believed to enter the body through minor cuts, abrasions or insect bites (El-Shabrawi and Kamal 2011). *Basidiobolus ranarum* is commonly found in soil, decaying vegetation and the digestive tracts of reptiles and amphibians. The fungus may be acquired by ingestion of contaminated soil or by direct inoculation of the fungus through the skin or mucous membranes (Shreef et al. 2018).

Fig. 1: Rainbow trout with symptoms of Basidiobolus infection. a) abdomen distension, b) Lesions on ventral part, c) pigmentation on skin, and d) necropsy (Shahi et al. 2023).

Risk factors for the infection include immunosuppression, diabetes mellitus and malnutrition. Basidiobolomycosis is more common in tropical and subtropical regions and is often associated with exposure to agricultural land or bodies of water (Geramizadeh et al. 2015).

The fungus enters the skin of the fish through cuts, abrasions or insect bites. It then spreads to the deeper tissues and forms a granulomatous lesion (Sackey et al. 2017). The fungi produce hyphae which release enzymes and cause tissue damage. The lesion may ulcerate, leading to secondary bacterial infections (Mendiratta et al. 2012; Anaparthy and Deepika 2014).

The clinical signs involved skin lesions with raised edges and central necrosis. Ulceration of skin with inflammation. Reddened and swollen areas with poor healing.

1.4. SPOROTRICHOSIS

Sporotrichosis is a fungal infection caused by the fungus *Sporothrix schenckii* (Barros et al. 2011). The fungus is found in soil and on plant matter such as sphagnum moss, rose bushes and hay (Barros et al. 2011). The mode of transmission is through direct inoculation of the fungus into the skin usually through a small cut or scratch. The infection is most commonly associated with activities that involve handling contaminated materials such as gardening or handling of animals (e.g., cats with sporotrichosis). The infection can also be acquired by inhalation of fungal spores or through the bites of infected animals (e.g. cats). Risk factors for sporotrichosis includes having a weakened immune system, working with plants or soil, and living in areas where the fungus is endemic. Sporotrichosis is not contagious and cannot be transmitted from person to person.

The fungus enters the skin of the fish through wounds or punctures. It then spreads to the lymphatic system and forms nodules in the skin and subcutaneous tissues (Valente et al. 2020). The fungi produce spores that are released from the nodules and can infect other areas of the body. In severe cases, the infection can spread to internal organs such as the liver, spleen and lungs.

The clinical signs involved small papules or nodules on the skin. Ulcers or abscesses with draining pus. Lymph node enlargement. Fever, anorexia, weight loss and lethargy.

1.5. SHRIMP MYCOSIS

Shrimp mycosis is caused by a fungus called *Lagenidium callinectes* (Uddin et al. 2013). The fungus is commonly found in marine and estuarine environments and infects a variety of shrimp species (Lee et al. 2016). The mode of transmission is through direct contact with infected water or another infected shrimp. The fungus can penetrate the exoskeleton of the shrimp and infect the gills, causing black discoloration, reduced oxygen uptake and ultimately death. Environmental factors such as high salinity, high temperature and low dissolved oxygen can increase the prevalence of shrimp mycosis. Shrimp mycosis is primarily a problem in shrimp aquaculture and can result in significant economic losses for the industry.

Control measures for shrimp mycosis include maintaining good water quality, avoiding overcrowding and using antifungal treatments. The fungus enters the shrimp through the cuticle or damaged exoskeleton. It then invades the underlying tissues and organs of the shrimp. The fungi produce hyphae that grow rapidly and cause tissue damage. The infection can spread throughout the shrimp's body and cause systemic disease (Czeczuga et al. 2012).

Clinical signs involved red or brown discoloration on the shell. Black spots or blotches on the carapace or appendages. Abnormal behavior such as lethargy, loss of appetite and reduced movement.

1.6. ASPERGILLOSIS

Aspergillosis is caused by the Aspergillus species of fungi, found in soil and indoor environments (Panackal et al. 2010; Hany et al. 2015). The infection is transmitted by inhaling fungal spores during activities like gardening or construction. Individuals with weakened immune systems are at higher risk for infection. Aspergillosis can present in various forms and symptoms depend on severity. The fungus enters the fish through the respiratory tract (Tsantes et al. 2022). It then grows and produces spores that can cause inflammation and necrosis of the lung tissue. The fungi can also invade other organs such as the liver and spleen. In severe cases, the infection can lead to sepsis and death (Arastehfar et al. 2021).
The clinical signs are respiratory distress and gasping (Bariteau et al. 2014). Coughing, sneezing, and nasal discharge weight loss anorexia, weakness, lethargy and depression.

1.7. ICHTHYOPHONOSIS

Ichthyophonosis is a fungal infection that affects fish caused by the fungus *Ichthyophonus hoferi* (Zuray et al. 2012; Hershberger et al. 2016; Gregg et al. 2016). The fungus is found in marine and freshwater environments and infects a variety of fish species (Jafarizadeh et al. 2014). The mode of transmission is through ingestion of infected tissue or through direct contact with infected fish. The infection can cause lethargy, weight loss and ultimately death in infected fish. Humans are not at risk of contracting ichthyophonosis from infected fish.
The fungus enters the fish through the skin or gills. It then invades the deeper tissues and causes necrosis and inflammation. The fungi produce spores that can spread to other parts of the body and cause secondary infections. The infection can lead to systemic disease and death in severe cases.
Clinical signs involved ulcerative skin lesions. Hyperemia and necrosis of the fins. Discoloration of skin and eyes. Loss of scales and skin shedding. Lethargy, anorexia, and weight loss (Jafarizadeh et al. 2014).

1.8. BRANCHIOMYCOSIS

Branchiomycosis is a fungal infection that affects fish, caused by the fungus *Branchiomyces sanguinis* (Sheikha and Mankodi 2021; Shinn et al. 2023). The fungus is commonly found in freshwater environments and infects a variety of fish species. The mode of transmission is through inhalation of fungal spores or through direct contact with infected fish or contaminated water. The infection can cause respiratory distress, skin lesions, and ultimately, death in infected fish. Humans are not at risk of contracting branchiomycosis from infected fish.
The fungus enters the fish through the gills (Pauland and Sahoo 2018). It then invades the gill tissue and causes inflammation and necrosis (Roberts 2012). The fungi produce spores that can spread to other parts of the body and cause secondary infections. The infection can lead to respiratory distress, anemia and death.
Clinical signs involved are respiratory distress and gasping. Increased mucus production and gill discoloration. Difficulty in feeding and lethargy. Anemia, weight loss, poor growth and mortality.

1.9. SAPROLEGNIOSIS

Saprolegniosis is a fungal infection that affects fish and other aquatic animals, caused by the fungus *Saprolegnia spp*. (Van Den Berg et al. 2013). The fungus is commonly found in freshwater environments and infects a variety of fish and amphibian species (Shinn et al. 2023).

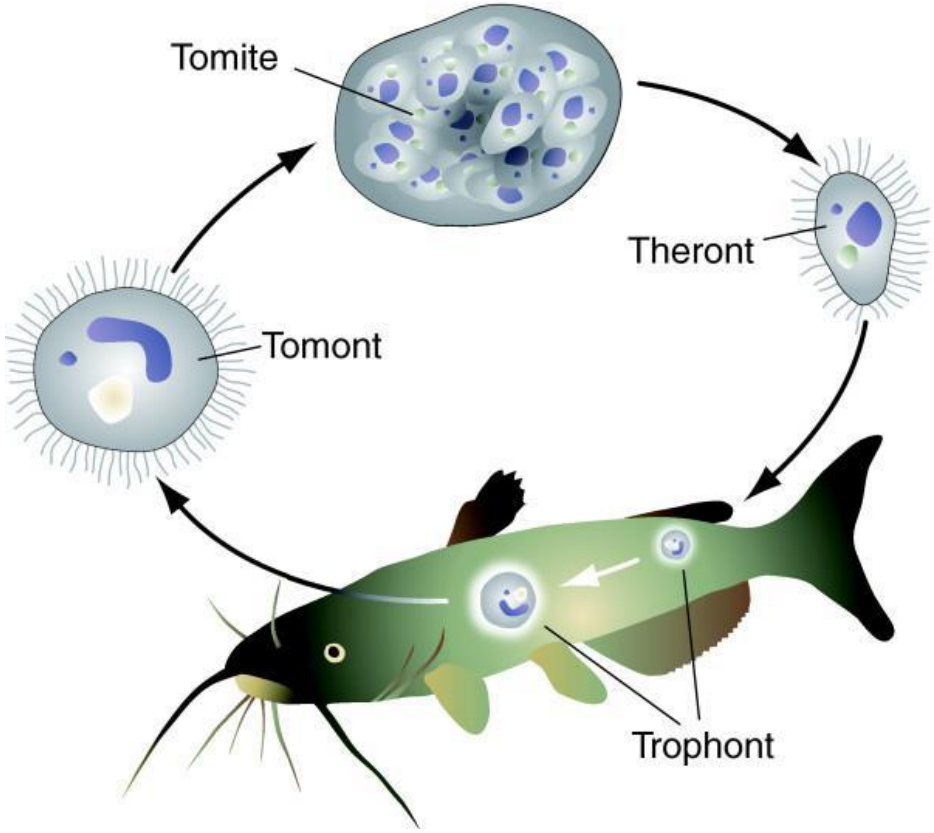

Fig. 2: Life cycle of Ichthyophonosis (Coyne et al. 2011).

Fig. 3: Branchiomycosis in fish (Abduhalilova et al. 2023).

The mode of transmission is through direct contact with infected animals or contaminated water. The infection can cause white cotton-like growths on the skin and fins of infected animals as well as systemic infections that can be fatal. Saprolegniosis can also affect eggs and larvae, causing reduced hatching and survival rates.

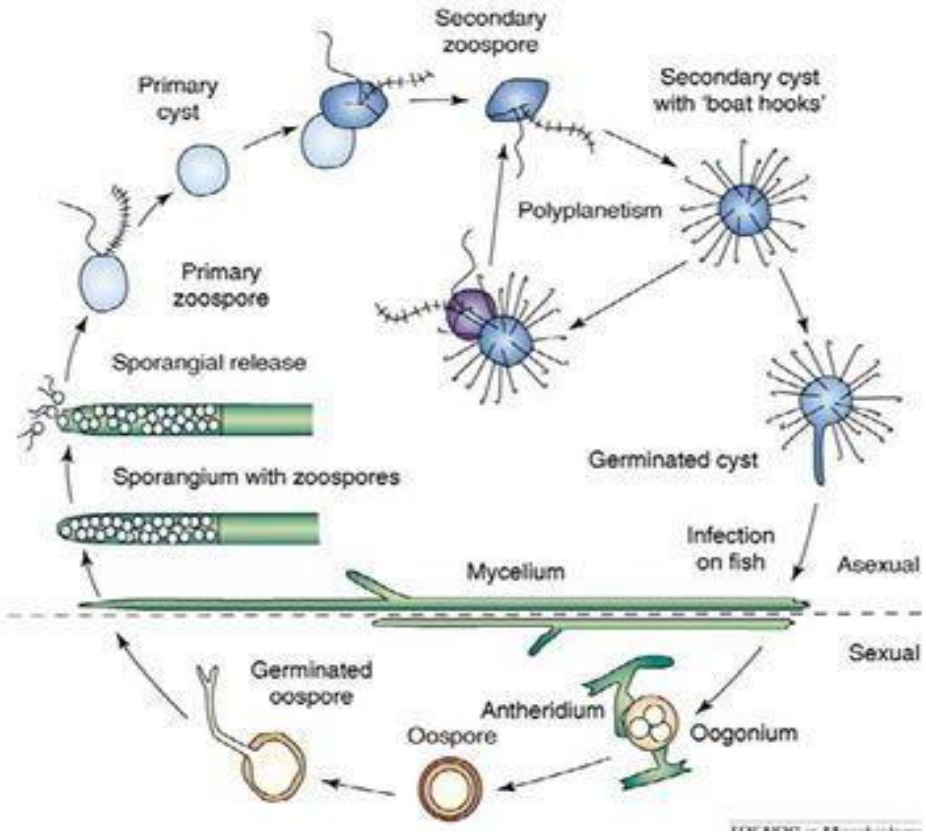

Fig. 4: Life cycle of Saprolegnia (Lone and Manohar 2018).

The fungus enters the fish through damaged skin or fins. It then invades the tissues and causes necrosis and inflammation. The fungi produce hyphae that grow rapidly and can cover the skin and fins with a cotton-like growth. The infection can lead to secondary bacterial infections and death in severe cases. Clinical signs include white or grayish cotton-like growth on skin and fins. Lesions with raised edges and central ulceration. Necrotic tissues with inflammation. Lethargy, anorexia, weight loss and mortality.
Use of potassium permanganate, formalin and provision of iodine solutions are common therapies for fungal illness. It is recommended to provide a bath therapy using NaOH (10–25 gm/L for 10–20 min), KmNO4 (1 gramme in 100 L of water for 30–90 min), or CUSO4 (5–10 gm in 100 L of water for 10–30 min). Overtreatment may result in fish tissues damage, which can lead to recurring infections. The proper control of the environment is of the utmost importance for an efficient treatment of chronic diseases. Don not transport the contaminated fish if an infection is present (Barde et al. 2020).

1.10. DERMOCYSTIDIOSIS

Dermocystidium spp. is a genus of fungus that infects fish and amphibians (Mahboub and Shaheen, 2020; Sellyei et al. 2020). The exact mode of transmission of dermocystidiosis is not fully understood, but it is thought to be through direct contact with infected animals or contaminated water. The fungus can infect a variety of fish and amphibian species but some are more susceptible than others. The infection can cause skin lesions, swelling, and ultimately death in infected animals. The fungus can also infect eggs and larvae, causing reduced hatching and survival rates. Control measures for dermocystidiosis include maintaining good water quality, avoiding overcrowding and using antifungal treatments. Humans are not at risk of contracting dermocystidiosis from infected animals (Plaul et al. 2018).

The fungus enters the fish through the skin or fins. It then invades the deeper tissues and forms cysts. The fungi can also cause inflammation and necrosis of the surrounding tissue. The infection can lead to systemic disease and death in severe cases.
Clinical signs involve multiple cysts on the skin and fins. Raised, nodular lesions on the body. Thickening and darkening of the skin. Lethargy and anorexia.

1.11. EXOPHIALIASIS

Pathogenic *Exophiala* spp. causing infection in cold-blooded animals generally belong to the 'salmonis clade' and the '*E. angulospora* complex (De Hoog et al. 2011; Thitla et al. 2022).
The fungus enters the fish through the skin or fins. It then invades the deeper tissues and causes necrosis and inflammation. The fungi produce melanin which can cause black or brown discoloration of the skin and fins. The infection can lead to systemic disease and death in severe cases.
Clinical signs include black or brown discoloration on skin and fins. Small nodules or papules on the skin. Lesions with necrosis and inflammation. Lethargy anorexia, weight loss and mortality.

2. CONTROL STRATEGIES OF FUNGAL ZOONOSIS IN FISH

- Averting is the most common control for branchiomycosis
- Effective management practices will create an environment that is unsuitable for the growth of fungus.
- Particular caution must be taken to prevent the spread of the disease to unaffected areas. All tanks, raceways, and aquaria must be sanitized and dried in order to prevent mortalities. Copper sulphate and formalin have been used in this case.
- Ponds need to be dried and treated with copper sulphate (2–3 kg/ha) and quicklime calcium oxide (Ganguly et al. 2016).
- An extended term bath in Acriflavine neutral or forma green for 7 days helps in reduction of this condition.
- It's best to bury dead fish.
- The greatest way to prevent Saprolegniasis is by skilled leadership practices
- Observation and correction of sanitation is required when Saprolegnia is detected in an aquatic system.
- Avoiding crowds to reduce injuries, especially during spawning, maintaining enough nutrition, and maintaining excellent water quality and circulation can all help avoid the spread of the disease.
- Fish infected with Icthyophonus hoferi will always harbour the infection; there is no treatment.

3. FACTORS OF FUNGAL ZOONOSIS

Fish-to-human transmission of infectious diseases caused by fungus is referred to as fungal zoonosis. Fish infections by fungi are rather common and can have a big impact on both the aquaculture sector and public health. There are several elements that influence the development and spread of fungal zoonosis in fish:

3.1. AQUATIC FUNGAL PATHOGENS

Aquatic environments, such as freshwater, marine and brackish water habitats are accessible to a variety of fungi. These fungi can infect fish through direct contact with polluted water or by entering their skin and mucous membranes (Zhang et al. 2022). Fish are frequently infected by organisms of the genera Saprolegnia, Achlya, Aphanomyces, and Fusarium.

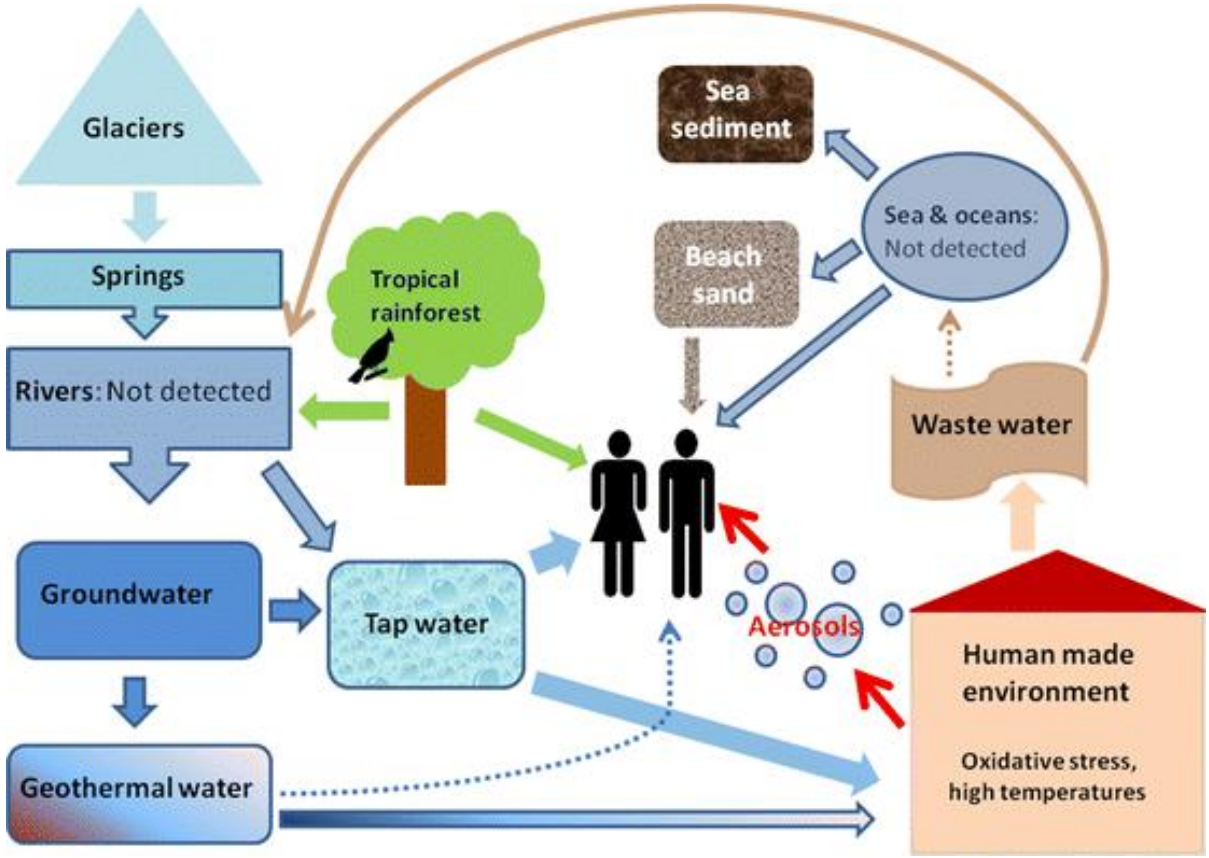

Fig. 5: Presence of Exophialaosis in natural and man-made environment (Babič et al. 2018).

3.2. POOR WATER QUALITY AND STRESS

Fish with fungal diseases are far more likely to experience stress. The fish immune system is weakened by stressors such as overpopulation, poor water quality, temperature changes, and inadequate diet which makes them more vulnerable to fungus-related infections. High fish density aquaculture environments can produce circumstances that favor the proliferation of fungi. (Tedesco et al. 2022).

3.3. WOUNDS AND INJURIES

Fish are susceptible to wounds and injuries brought on by a variety of things, including handling, predation, and environmental dangers. Fungal pathogens can enter the body through open wounds, causing isolated or systemic ailments. Injury during handling and transportation in fisheries and fish farms is a risk factor for zoonotic fungal disease (Beckmann et al. 2020).

3.4. IMMUNE SUPPRESSION

Fish may experience immunosuppression like other animals as a result of a variety of factors such as environmental stresses, inadequate nutrition, and exposure to toxins. Fish with weakened immune systems are more susceptible to fungal infections because they are less able to establish a robust immunological response to pathogens (Tedesco et al. 2022).

3.5. INTERSPECIES TRANSMISSION OF DISEASE

Fungal diseases can spread from diseased fish to human. Workers in fisheries, aquaculture or fish farms may come into direct contact with diseased fish or polluted water which might result in sickness (Li et al. 2019).

3.6. HANDLING AND PROCESSING PRACTICES

Due to inappropriate handling and processing practices, aquaculture employees, fish handlers, and fishermen may be at risk of developing a fungal zoonosis. Humans may get fungal diseases through contact with fish tissues and contaminated surfaces, particularly if they sustain wounds or cuts to the skin (de Silva et al. 2023).

3.7. IMMUNOCOMPROMISED INDIVIDUALS

Fungal zoonotic infections are more likely to affect immunocompromised individuals such as those with underlying medical issues, the elderly, and those receiving immunosuppressive medications. If these individuals are exposed to fungal infections from fish, they are more likely to experience serious consequences (Narayan et al. 2023).

4. PREVENTIVE MEASURES FOR FUNGAL ZOONOSIS

Fish can experience less stress and have stronger immune systems due to appropriate feeding and maintained water quality, which reduces the danger of fungus diseases. It is possible to stop the spread of fungal infections among fish populations and lower the danger of transmission to humans by implementing good hygiene and sanitation practices in fisheries and fish farms (Rahman et al. 2020).
In aquaculture settings, regular checkups and disease surveillance can aid in the early detection of fungal infections and speed up treatment and control procedures. The danger of direct contact with fungal diseases can be reduced by providing workers with protective clothing and equipment. Consumers and industry workers can become more aware of the dangers of fungal zoonosis and the value of safe fish handling and eating practices through public health education (Ziarati et al. 2022).
It is crucial to recognize and manage the causes of fish fungal zoonosis in order to improve both fish and human health. The danger of fungal infections can be decreased by applying preventative measures and upholding great aquaculture practices, resulting in safer fish consumption and a better aquatic ecosystem (Rossow et al. 2020)
To protect both human health and aquatic ecosystems, it is essential to prevent and manage fish fungal zoonosis. Fish may transmit zoonotic fungal infections to humans, and certain fungal pathogens can seriously harm aquaculture's bottom line. The risk of fungal infections can be reduced, and their effects can be minimized, by setting control measures into action. Here are some crucial methods for preventing and managing fish fungal zoonosis:

4.1. WATER QUALITY Management

Maintaining high water quality is essential for keeping fish free of fungus infection. Fish may become stressed and have their immune systems weakened by poor water quality which includes high levels of ammonia, nitrite or organic matter, leaving them more susceptible to fungus and its infections. Fungal outbreaks may be prevented by regular monitoring and effective water quality management

techniques such sufficient filtration and water exchange (Teshome and Addis 2019).

4.2. PROPER NUTRITION

Giving fish a healthy, balanced diet boosts their immune system and increases their resistance to fungus infections. The use of high-quality feeds and avoiding overfeeding are two feeding techniques that can improve fish health and lessen their sensitivity to fungi-related ailments.

4.3. BIOSECURITY AND QUARANTINE

Fungal infections can be prevented by using quarantine procedures for newly imported fish into aquaculture operations. The spread of illnesses can be controlled by properly monitoring and isolating new fish before reintroducing them to the main population. Additionally, the entry and spread of fungal diseases can be stopped by following strict biosecurity precautions such as limiting access to aquaculture facilities and sanitizing equipment (Mocho et al. 2022).

4.4. ENVIRONMENTAL HYGIENE

In order to prevent fungal infections, it's essential to keep fish farming facilities clean and hygienic. Reducing the fungal burden and lowering the risk of transmission can be accomplished by routinely cleaning and disinfecting surfaces, equipment, and tanks. Quick removal of dead or diseased fish also helps to stop the spread of fungi (Lõhmus and Björklund 2015).

4.5. WATER TREATMENT

Treatments like ultraviolet (UV) sterilization or ozone treatment can be used to control the fungal growth in water. These techniques can reduce the risk of new infections and limiting the spread of fungal diseases.

4.6. DISEASE SURVEILLANCE

Early diagnosis of fungal infections in fish populations depends on routine monitoring and disease surveillance. Initial detection of infection enables rapid intervention and control measures. Monitoring fish behaviour, appearance, and general health can aid in spotting any early-stage health problems (Shamsi 2016).

4.7. MEDICATION AND TREATMENTS

Fish with confirmed fungal infections can be treated with the right antifungal medicines. In order to prevent residues of drugs in fish intended for human consumption, it is essential to know about the dose limit and its half-life in water.

4.8. EDUCATION AND RESEARCH

Continued research practices and their epidemiology in fish is necessary to develop more effective prevention and control strategies for fungal zoonosis in fish. Providing knowledge to fish farmers, aquaculture managing workers and the general public about fungal zoonosis may lead to public

awareness from the protection of diseases caused by fungus (Zadoks et al. 2020).

5. CONCLUSION

In conclusion, a combination of effective management practices, strict biosecurity controls, early identification and suitable medication is required to prevent and control fungal zoonosis in fish. We can lessen the effect of fungal diseases on fish populations, maintain human health and encourage sustainable aquaculture practices through implementing these ideas into practices.

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Incidence, Transmission Mechanisms and Pathologic Implications of Bacterial Zoonotic Diseases of Fish

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ABSTRACT

Fish harbor a variety of bacterial pathogens, some of which can cause disease in humans. Zoonotic bacteria that may be present in asymptomatic fish hosts pose a public health risk when transmitted to humans through handling or consuming infected fish. Mycobacteriosis, caused by species in the *Mycobacterium marinum* group, is perhaps the most notorious zoonotic infection associated with fish and can produce serious skin infections in humans. Streptococcosis and staphylococcosis also occur with some frequency in fish and can result in human cases of septicemia, endocarditis or pneumonia if injured skin comes into contact with infected fish tissues and bacteria access wounds. Additionally, some Vibrio and Clostridium species found among fish may cause wound infections or gastrointestinal illness in humans, usually subsequent to exposure through handling or ingesting raw seafood. Clinical signs of bacterial zoonosis are variable and diagnosis in human cases can prove complicated by the vast diversity of potential pathogens involved. Preventative measures center on educating aquarists and fish handlers to avoid direct contact with ulcerated areas, lesions or feces from diseased fish. For consumer safety, good aquaculture practices that reduce bacterial loads in farmed fish stock are recommended. Moreover, thoroughly cooking fish to an internal temperature over 140°F destroys pathogens that may be present. Additional research priorities include better characterization of bacterial diversity among wild and farmed fish, investigating genetic and immunological aspects of disease resistance, developing improved diagnostics through genomic analysis, assessing efficacy of existing antibacterial treatments in clearing pathogens prior to human consumption and formulating integrated control strategies to mitigate risks. This book chapter examines the incidence, transmission mechanisms and pathologic implications of common bacterial zoonosis originating from fish hosts.

Keywords: Fish, Zoonotic diseases, Incidence, Transmission mechanisms, Pathologic implications, Bacterial zoonosis

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1. INTRODUCTION

The interplay between humans, animals and the environment has long been recognized as a significant factor in the emergence and spread of infectious diseases. Among zoonotic diseases, those transmitted from fish to humans hold a unique place due to the increasing popularity of fish as a vital food source worldwide (Ziarati et al. 2022). This chapter provides a comprehensive review of bacterial zoonotic diseases in fish, exploring the risks, transmission mechanisms, and control measures associated with these infections. By understanding the complexity of these diseases, we can devise effective strategies to safeguard public health and ensure the sustainability of the fishing industry.

The consumption of fish as a primary protein source has surged in recent decades, driven by the recognition of its nutritional value and health benefits. However, this growing demand has also given rise to concerns about the transmission of zoonotic diseases from fish to humans (Farzadnia and Naeemipour 2020). Bacterial pathogens are of particular concern due to their ability to cause severe illnesses and pose significant economic risks to the fishing industry. In this chapter, we will explore the most common bacterial zoonotic diseases associated with fish, their modes of transmission, and the preventive measures to mitigate the risks (Irshath et al. 2023). The most common bacterial zoonotic diseases of fish are presented in Table 1.

1. LISTERIOSIS

Listeria monocytogenes is another significant bacterial pathogen that can be transmitted to humans through contaminated fish. The mechanism of action of listeria is shown in Fig. 1. This bacterium is widely distributed in the environment and can survive under various conditions. Listeriosis, the disease caused by *L. monocytogenes*, is particularly dangerous for pregnant women, elderly individuals, and those with weakened immune systems (Lassen et al. 2016). Symptoms range from mild flu-like signs to severe invasive infections like meningitis and septicemia. Proper fish processing, hygiene, and refrigeration are vital in preventing L. monocytogenes contamination.

Listeria monocytogenes has the ability to invade several cell types, including macrophages. After getting confined inside phagosomes and being absorbed by macrophages, Listeria monocytogenes secrete virulence factors, such as listeriolysin O (LLO) that damage the phagosomal membrane and invade the cytoplasm. Due to this invasion, it will survive (Köster et al. 2014).

A complex immune response that includes both the innate and adaptive immune systems is necessary to achieve the best protection possible against infections. By regulating the production of proinflammatory cytokines, type I interferons (IFNs), and antimicrobial effectors, the innate immune system plays a critical role in starting and coordinating host defenses (Iwasaki and Medzhitov 2010). Pattern recognition receptors (PRRs) act as molecular defenders that continuously scan the cytoplasm and

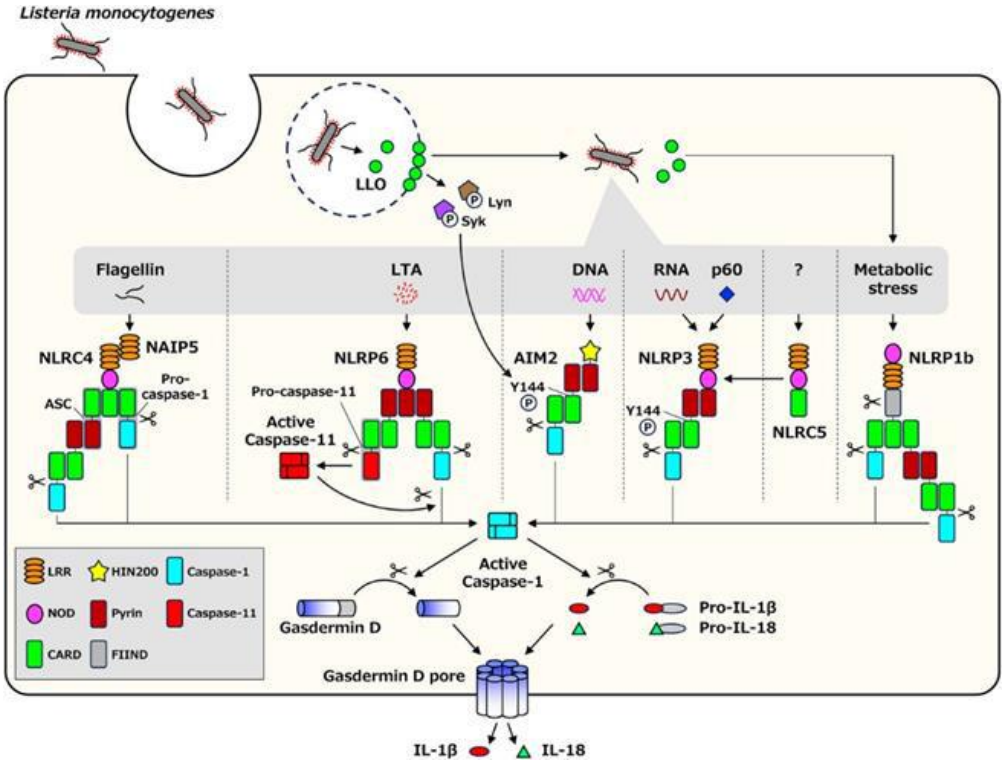

Fig. 1: The mechanism of action of Listeria (Matsuda et al. 2023).

Table 1: Bacterial zoonotic diseases of fish

S.No.	Name of Disease	Bacterial agent	Fish species effected	Human health impact
1	Listeriosis	*Listeria monocytogenes*	Various fish species	gastrointestinal symptoms and flu-like illness, poses more severe risk to pregnant women and newborns
2	Salmonellosis	*Salmonella spp.*	Smoked fish	Diarrhea, fever and abdominal cramps
3	Streptococcosis	*Streptococcus spp*.	Farmed fish	Skin infections
4	Erysipelothrix	*Erysipelothrix rhusiopathiae*	Whenever fish and shellfish handled	Joint pain and flu like symptoms
5	Campylobacter	*Legionella pneumophila*	fresh water fish	pneumonia
6	Vibrionaceae	*Vibrio spp.*	Marine and fresh water fish	Wound infections, GIT illness
7	Botulism	*Clostridium botulinum*	Various fish species	Muscle paralysis, respiratory disorder and even death
8	Pseudomonadaceae	*Pseudomonace spp.*	Various fish species	Abnormal breathing, skin infections
9	*Aeromoniasis*	*Aeromonas hydrophila*	Fresh water fish	GIT infection
10	Hafniaceae	*Hafnia alvei*	Various fish species	Skin lesions
11	Enterobacteriaceae	*Enterobacteriaceae spp*	Various fish species	Sepsis
12	*E. coli*	*E. coli*	Various fish species	Stomach cramps and vomitting
13	Salmonellosis	*Salmonella spp.*	Various fish species	Fever and intestine infection
14	Klebsiella	*Klebsiella spp.*	Various fish species	Wound infection and sepsis
15	Yersinia	*Yersinia spp.*	Various fish species	GIT infection

Several bacterial infections in fish species, including *Aeromonas septicemia* (Thirumalaikumar et al. 2021), Edwardsiellosis (Buján et al. 2018), Columnaris (Declercq et al. 2013), Streptococcosis (Luo et al. 2017), and vibriosis (Ji et al. 2020) have been reported in the aquaculture sector (Bhatnagar et al. 2023). There are several bacterial pathogens which transfer from fish to humans are explained below.

extracellular environment for potential dangers and danger by recognizing pathogen-associated molecular patterns (PAMPs) (Tartey and Takeuchi 2017). It could be feasible to regulate the immune response and improve the efficacy of existing treatments for drug-resistant strains by focusing on the inflammasome pathway. However, more research and clinical trials are needed to assess and confirm the efficacy and security of such an approach.

2. SALMONELLOSIS

Salmonella species are well-known pathogens responsible for foodborne infections worldwide. While commonly associated with poultry and eggs, salmonella can be transmitted to people through fish as well (Fig. 2). Fish act as a carrier for the transmission of the disease. Ingesting raw or undercooked fish contaminated with Salmonella can lead to salmonellosis, characterized by symptoms such as diarrhea, fever, and abdominal cramps (Bibi et al. 2015). Proper cooking, cross-contamination prevention, and maintaining good hygiene practices are essential in reducing the risk of Salmonella infections (Yacoub et al. 2023).

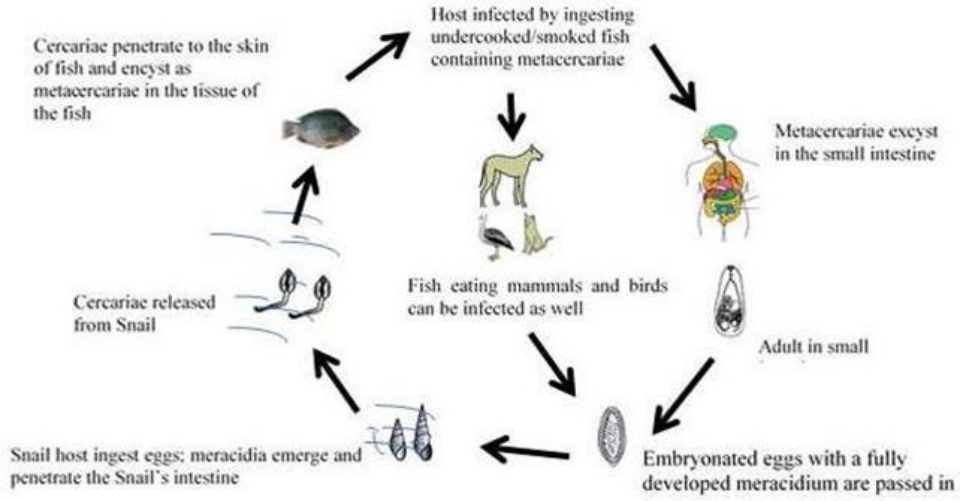

Fig. 2: Life cycle and transmission of salmonella (Bibi et al. 2015).

3. STREPTOCOCCOSIS

Streptococcosis is a neurological bacterial disease that mostly affects warm-water fish in tropical habitats, whether in freshwater or saltwater. The best circumstances for streptococcal epidemics include high stocking numbers, poor water quality, and high temperatures. Tilapia Fish can become infected with streptococcus as early as 5 grams old, and it persists throughout every stage of the tilapia life cycle. *Streptococcus agalactiae* and *Streptococcus iniae* are the two most common streptococcal infections in tilapia. Using specific health requirements MSD further divides *S. agalactiae* into two biotypes (Biotype 1 and Biotype 2). Serotype-specific strains of *S. agalactiae* produce each infection of a specific biotype (Li et al. 2014).

3. 1. *STREPTOCOCCUS INIAE*

Streptococcus iniae is an emerging zoonotic pathogen found in various aquatic environments, including fish farms (Berzak et al. 2019). It can cause invasive infections in humans who come into contact with infected fish, leading to bacteremia and meningitis as shown in Fig. 3. Fish handlers and those with open

wounds are particularly susceptible. Stringent biosecurity measures, prompt diagnosis, and appropriate antimicrobial treatment are crucial to prevent and manage *S. iniae* infections.

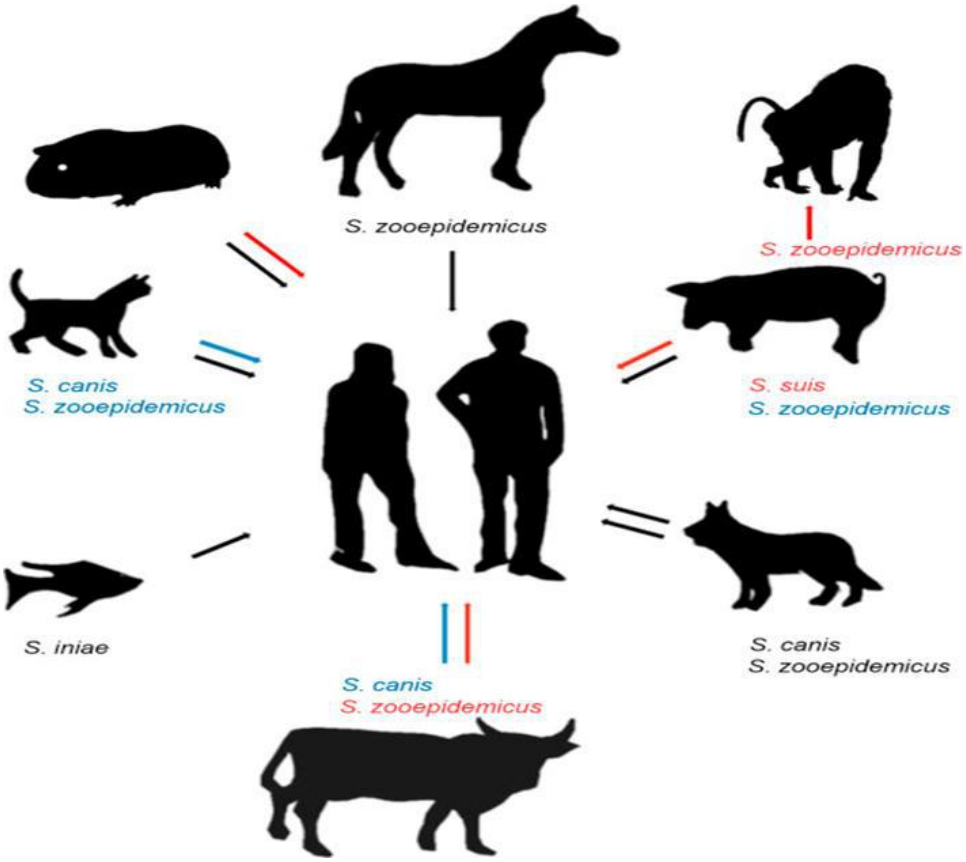

Fig. 3: Host pathogen relation of zoonotic streptococcus (Soares 2015).

External clinical symptoms of this fatal condition include exophthalmos (eye protrusion), conspicuous hemorrhages, corneal opacity, whirling at the water surface, and erosion of the caudal fin. 'C' or 'S'-shaped body posture, sluggish behavior, and bleeding abscesses around the mouth are further signs of unhealthy fish. A swollen spleen, abdominal distention, a pale liver, organ adhesion, and inflammation are examples of internal symptoms.

The chance of more fish acquiring an infection can be increased by overfeeding, stress and open cage culture. In order to further protect tilapia from streptococcus, MSD Animal Health suggests sanitizing equipment, separating diseased fish from healthy ones and vaccinating fish at the appropriate time.

4. ERYSIPELOTHRIX

Erysipelothrix is a Gram-positive bacterium which has a significant role in fish zoonoses (Boylan 2011). This bacterium causes Skin infections and acute sepsis and is related to Sea Mammals. *E. rhusiopathiae* (commanly known as *E. insidiosa*) is the most important specie, which is important from the zoonotic point of view as causes disease in humans and animals which affect vascular tissue, connective tissue, and Skin issues (Fig. 4).

Clinical symptoms include inflammation of muscle cells (myositisis), inflammation of skin necrotizing dermatis) and inflammation of other cells (cellultis). *E. rhusiopathiae* is a common fish bacterium but different countries have recorded mortality (Pomaranski et al. 2018; Pomaranski et al. 2020). A new type

of ornamental fish called Aeromonas hydrophila has just been discovered in fish (Hoseinifar et al. 2023). It leads to a great economic loss in aquaculture (Liu et al. 2020). Additionally, *E. rhusiopathiae* has zoonotic potential and has a definite occupational connection to the meat and fish sectors and it can cause erysipeloid in people (Opriessnig et al. 2020). Only contact with fish mucus the disease can occur in human beings. Although *E. rhusiopathiae* is not infectious to fish, it can infect humans due to its prolonged life in fish mucus and possible stability. Infections through dog scratching and bites have also been reported (Verma and Kumar 2018).

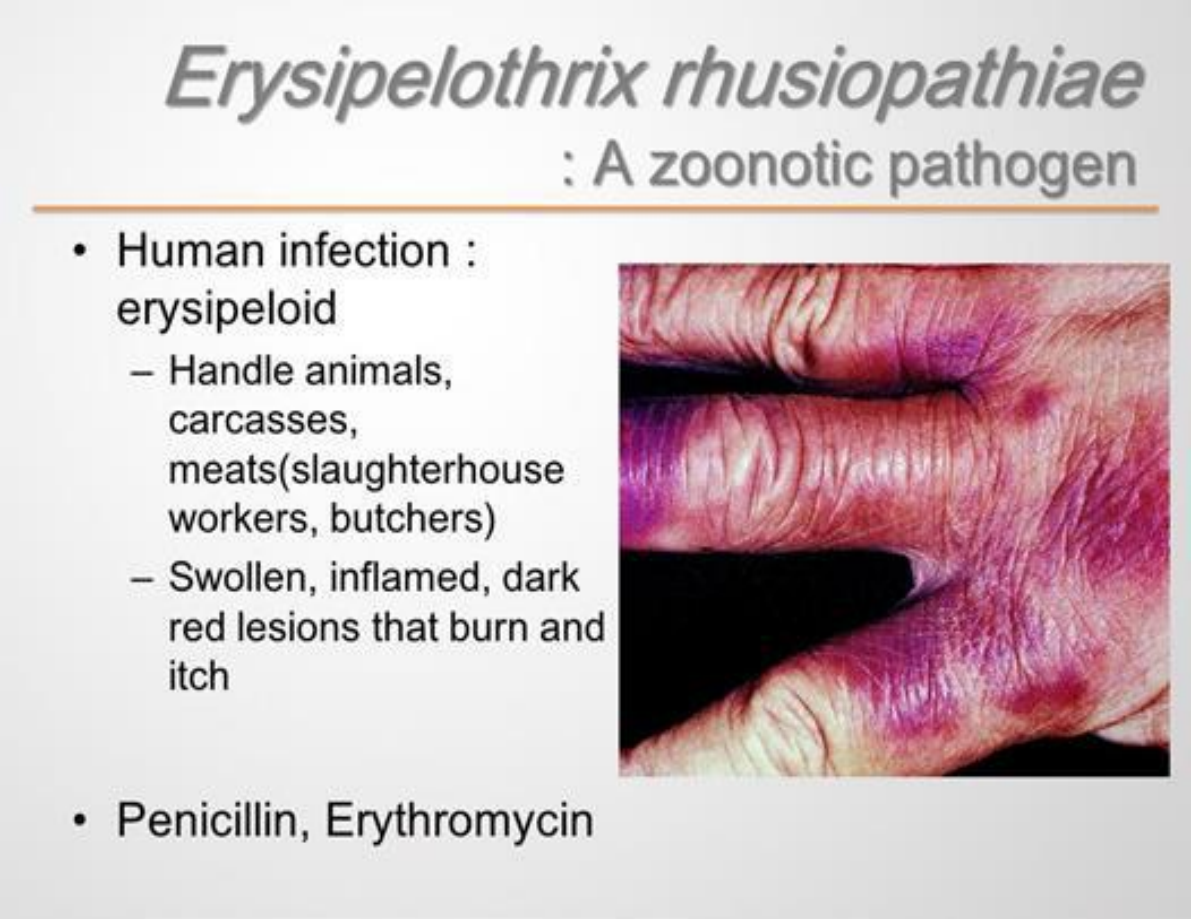

Fig. 4: Erysipelothrix infection in humans from fish (Slide player).

Erysipelothrix infections in humans are consequently brought on by contact with infected animals, their faeces, or products. Clinical symptoms of the illness include skin infections, particularly on the hands (Fig. 5), endocarditis, and sepsis. Among those who are at a high risk for Erysipelothrix infections are fishermen and veterinarians. This bacterium is linked to endocarditis (Wood and Steele 2019). Here are some more pictures of infection in humans (Fig. 6).

5. CAMPYLOBACTER

Campylobacter is a zoonotic agent that can be detected in the digestive system of various animals. (Facciolà et al. 2017). The most likely way that a food handler contracts campylobacter jejuni infection is

via touching their hands to a work surface or untreated water. The two enteropathogens of this genus that are of greatest importance are *Campylobacter jejuni* and *C. coli* (Deblais et al. 2023). Mode of transmission of campylobacter is presented in Fig. 7.

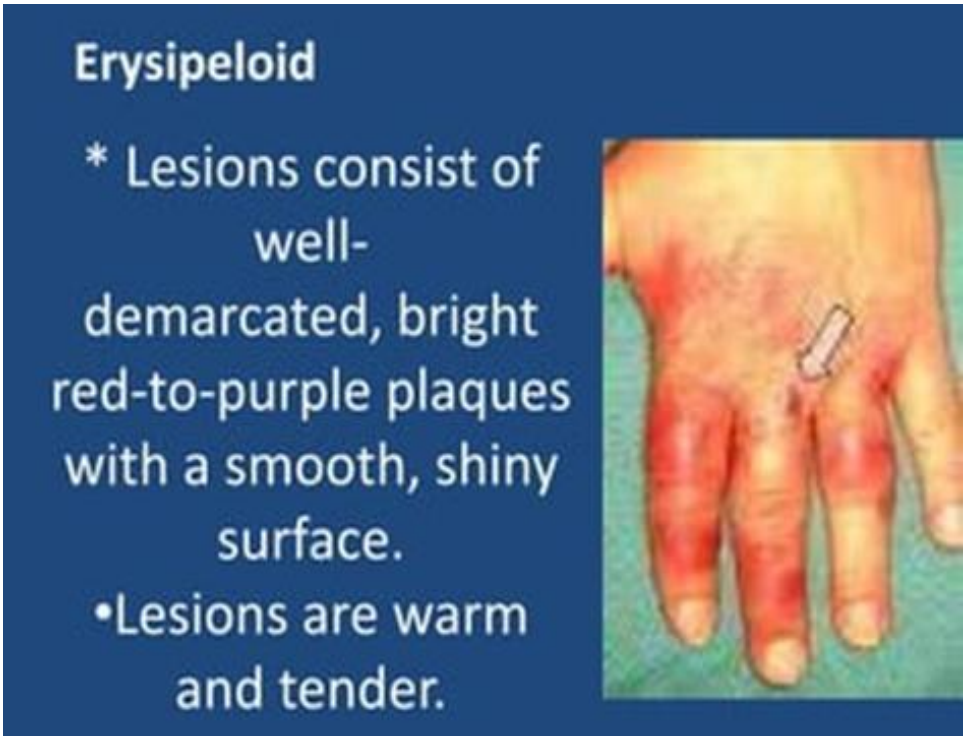

Fig. 5: Erysipeloid lesions (Slide.share).

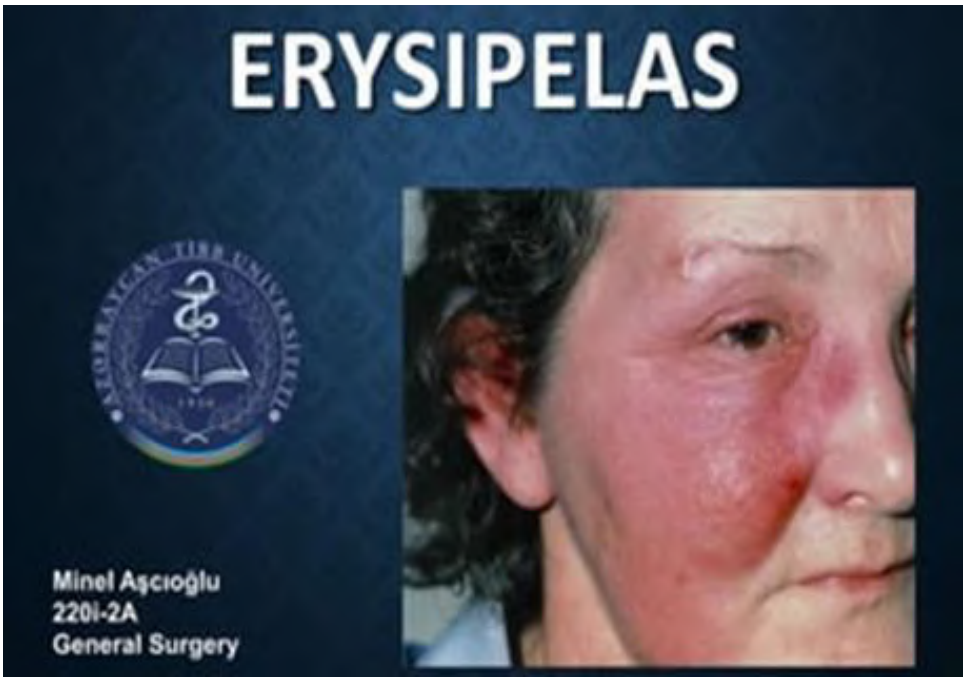

Fig. 6: Erysipelas (Erysipelas pptx. 2023).

In fish populations throughout Europe, North and South America, Australia and New Zealand, the bacteria are prevalent. By employing bacterial motility, disrupting intracellular signaling, intestinal cell

adhesion and invasion, causing cell death, dodging the host immune system and acquiring iron for their growth and survival, the bacteria that cause campylobacteriosis present as enteritis (Amin et al. 2023). Recently, a water-borne disease called *Plesiomonas shigelloides* has been reported in freshwater fish (Duman et al. 2023).

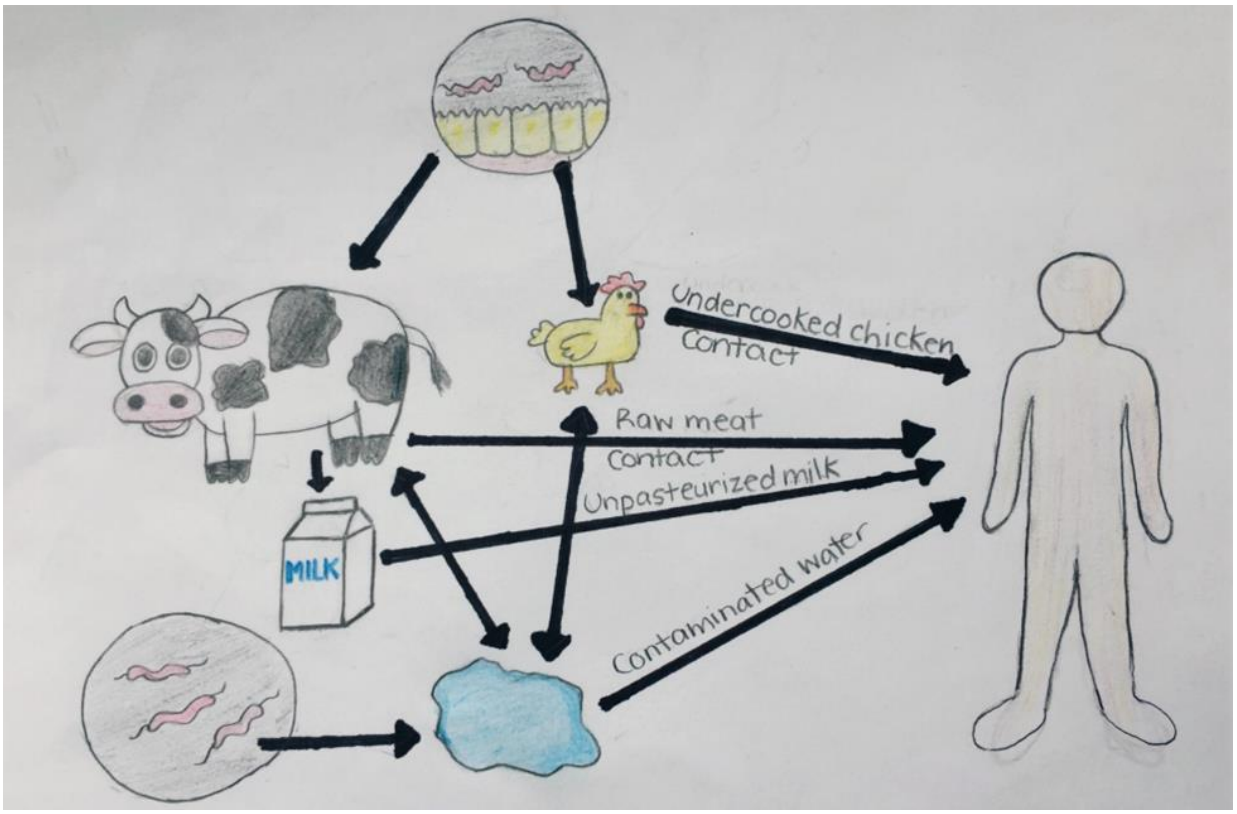

Fig. 7: Mode of Transmission of Campylobacter (Esson et al. 2016).

Legionella pneumophila which was also identified from a patient who worked at a fish market, is the bacteria that causes Legionnaires' disease/pneumonia. It spreads by aerosols and water. Salmonids, eels, goldfish, sole, sturgeon, trout, carps and turbot are all prone to yersiniosis, a contagious bacteremia also known as red mouth disease. Blood stains in the eye and exophthalmos are common symptoms of the disease (Yang et al. 2023).

The only way to completely eliminate Campylobacter from contaminated foods is by bactericidal treatment such as heating (such as cooking or pasteurisation) or irradiation. Despite the fact that Campylobacter infection tends to go away on its own, a recent assessment found that up to 80% of the population may have taken an oral antibiotic such a fluoroquinolone or macrolide for control (Dai et al. 2020).

6. VIBRIONACEAE

Vibrionaceae belongs to a family of gram-negative bacteria that can transmit disease in fish. Some of the species that commonly affect fish include *Vibrio anguillarum*, *Vibrio harveyi*, and Vibrio vulnificus (Helmi et al. 2020). Vibrionaceae infections in fish are often caused by the ingestion of contaminated food or water. Fish that are stressed or immunocompromised are more susceptible to infection.

The pathogenesis of Vibrionaceae infections in fish can vary depending on the species of bacteria involved. Some species produce toxins that can damage fish tissues, while others invade and multiply within the fish's cells. Vibrionaceae can be transmitted to fish through contaminated water, food, or equipment. Some species of Vibrionaceae can also be transmitted from infected fish to healthy fish (Takemura et al. 2014). The mode of transmission from aquatic environment to humans is shown in Fig. 8.

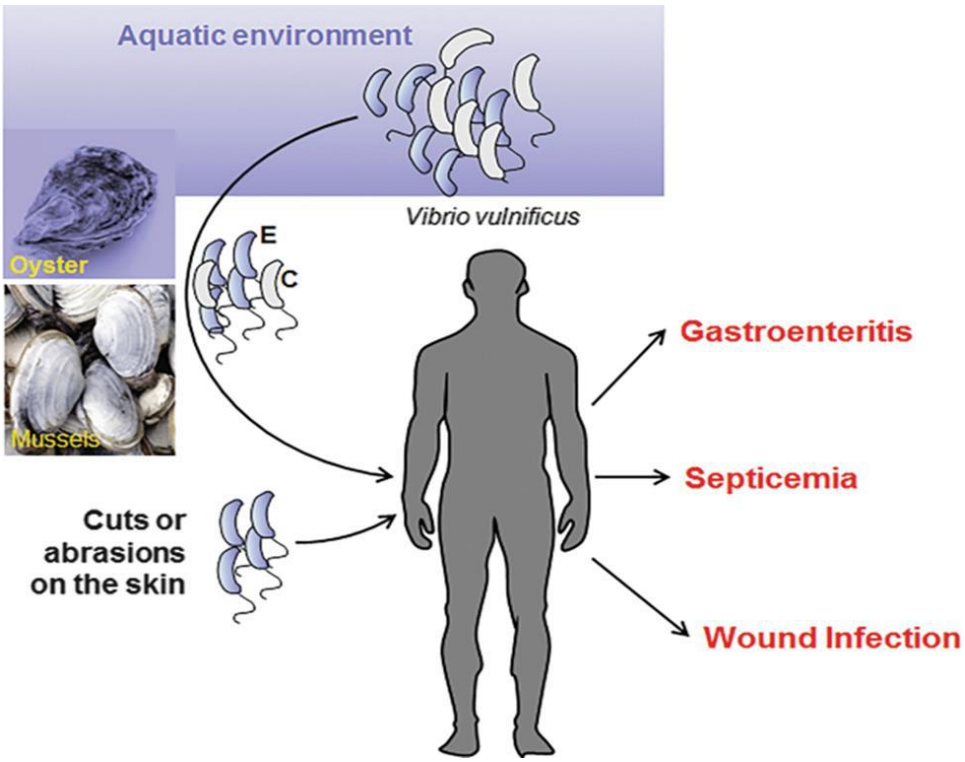

Fig. 8: Vibrio zoonosis (Bhunia and Bhunia 2018).

Vibrionaceae infections can occur in both wild and farmed fish populations. The incidence of these infections can vary depending on factors such as water temperature, salinity, and the presence of other pathogens. The clinical signs of Vibrionaceae infections in fish can include lethargy, loss of appetite, skin lesions, and hemorrhaging. In severe cases, the infection can be fatal (Ma et al. 2023).

Control measures for Vibrionaceae infections in fish include maintaining clean water and equipment, minimizing stress in fish populations, and implementing biosecurity measures to prevent the spread of infection. Antibiotics such as oxytetracycline and florfenicol can be used to treat Vibrionaceae infections in fish. However, however, excessive usage of antibiotics may result in the development of bacterial strains that are resistant to them (Banchi et al. 2022).

There are vaccines available for some species of Vibrionaceae that can be used to protect fish populations from infection. These vaccines are typically administered through injection or immersion in a vaccine solution. Postmortem lesions in fish infected with Vibrionaceae can include skin ulcers, hemorrhaging, and necrosis of internal organs such as the liver and spleen (Loo et al. 2023).

7. BOTULISM

Botulism in fish is caused by the bacterium *Clostridium botulinum*, which produces a potent neurotoxin that can cause paralysis in fish and other animals (Fig. 9). The spore-forming bacteria *Clostridium*

botulinum is responsible for the botulinum toxins production. The botulinum toxin is produced when *C. botulinum* spores germinate and grow in an anaerobic environment, such as a decomposing fish carcass or contaminated sediment (Novakova et al. 2023).

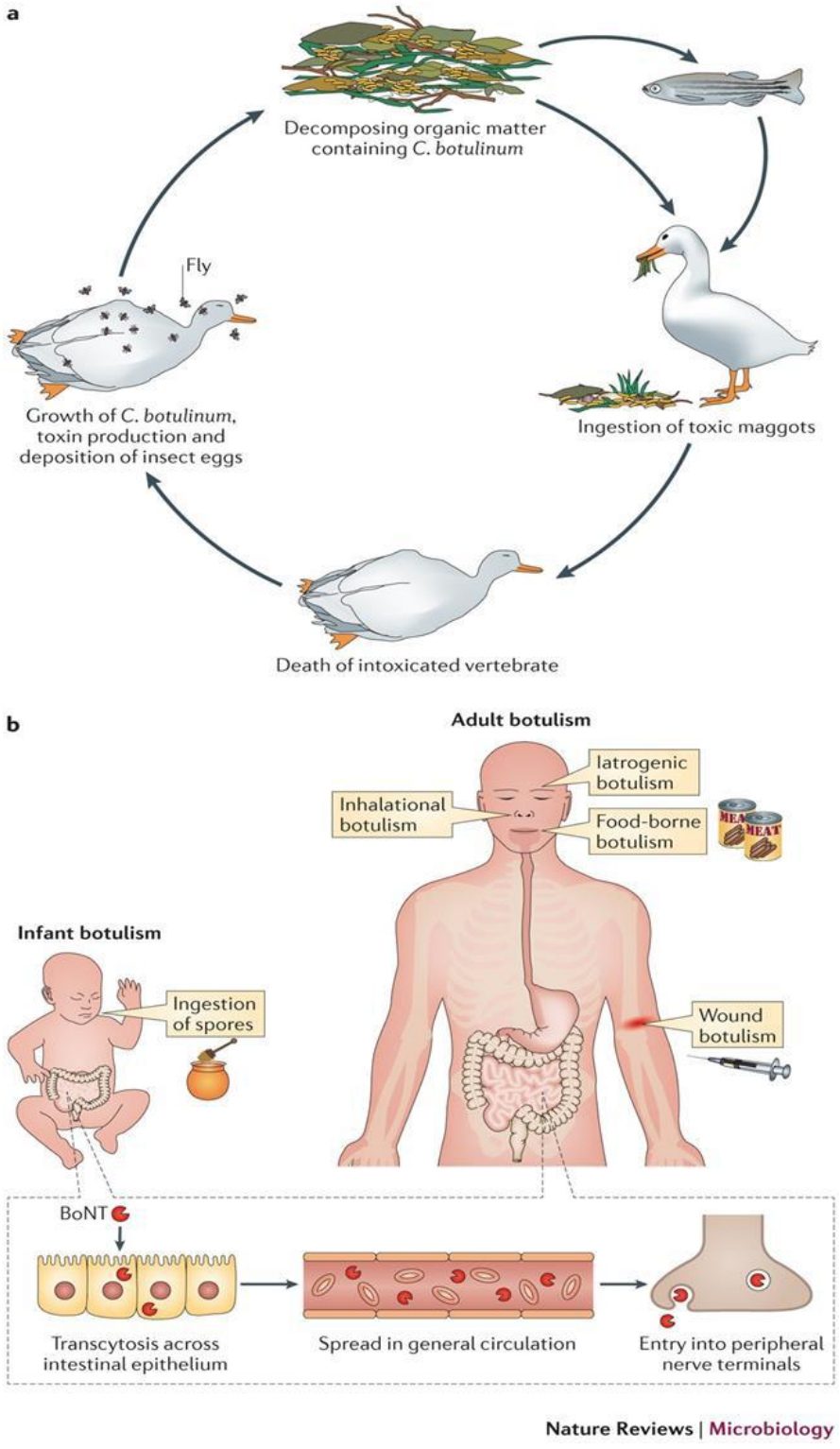

Fig. 9: Botulism toxicity (Rossetto et al. 2014).

The botulinum toxin is ingested by fish, which causes paralysis by blocking the release of acetylcholine, a neurotransmitter that stimulates muscle contractions. Fish can be exposed to botulinum toxin by eating contaminated feed or through contact with contaminated water or sediment. The toxin can also be transmitted from fish to fish through cannibalism. Botulism outbreaks in fish are most common in warm, stagnant waters with high organic loads. It can affect both wild and farmed fish populations (Peñuelas et al. 2022).

Fish with botulism may exhibit a variety of symptoms, including lethargy, loss of appetite, swimming in circles, and difficulty breathing. In severe cases, fish may be unable to swim or maintain buoyancy. Preventing the growth of *C. botulinum* spores is the key to controlling botulism in fish. This can be achieved by maintaining clean water quality and promptly removing dead or decaying fish from the environment (Goin et al. 2022).

There is no effective treatment for botulism in fish. Affected fish should be culled to prevent the spread of the toxin. There is no vaccine available for fish against botulism. Fish that have died from botulism may exhibit bloating, redness of the eyes and gills, and a lack of rigor mortis. The internal organs may also show signs of congestion and hemorrhage (Mirbehresi et al. 2022).

8. PSEUDOMONADACEAE

Pseudomonads is one of the most devastating fish infections that can cause hemorrhagic septicemia and ulcerative syndrome1. Numerous bacterial infections have an impact on a variety of aquatic species and cause significant economic losses on a global scale. *Pseudomonas aeruginosa* is a normal component of the fish microbiota but under stressful conditions like malnutrition and overcrowding, the bacteria have become highly opportunistic and pathogenic, causing illnesses like hemorrhagic septicemia, gill necrosis, abdominal distension, splenomegaly, friable liver and congested kidney (Holloway 2020). The various diseases caused by pseudomonads are listed in Fig. 10.

Frateuria, Pseudomonas, Xanthomonas and Zoogloea are the four genera that make up the family of gram-negative bacteria known as Pseudomonadaceae. These genera contain widespread saprophyte species that are harmful to people, animals, plants and soil microbes.

Pseudomonas aeruginosa is an environmental microorganism that can infect individuals in medical centers when they come into contact with polluted water or soil. The symptoms of pneumonia include an infection of the lungs, fever and chills, chest discomfort, fatigue and coughing up occasionally with yellow, green or dark mucus (Behzadi et al. 2021).

P. aeruginosa can cause infections in the human blood, lungs, or other parts of the body after surgery. Additionally, fish like tilapia are harmed by this bacterium. It can be found in short chains, pairs or even a single unit (Gajdács et al. 2021).

Following rigorous quarantine guidelines is the greatest way to stop Pseudomonas bacteria from spreading throughout your aquarium. In this way, the disease won't spread to the other fish in your tank if an anxious fish from capturing, transport and the new habitat begins to exhibit clinical symptoms (Duman et al. 2023).

An aminoglycoside plus an antipseudomonal beta-lactam (such as penicillin or cephalosporin) can be used in conjunction to treat pseudomonas infections. An aminoglycoside may be used with carbapenems (such as imipenem and meropenem) and antipseudomonal quinolones (Ali et al. 2023).

9. *AEROMONAS HYDROPHILA*

Fish that are infected with *Aeromonas hydrophila* develop "Motile Aeromonas Septicemia" (MAS), "Hemorrhagic Septicemia," "Ulcer Disease," or "Red-Sore Disease." There are various names for this

condition that refer to the lesions brought on by this bacterium, including ulcers on the fish skin and septicemia, in which the bacteria or bacterial toxins are present in many of the fish organs. A common gram-negative rod-shaped bacterium called *Aeromonas hydrophila* lives normally in the gastrointestinal system and is frequent exclusion from fresh water ponds. These bacteria cause a disease that mostly affects freshwater fish, including catfish, many types of bass and numerous tropical or ornamental fish (Nawaz et al. 2023). The of action of *Aeromonas hydrophila* is shown in Fig. 11.

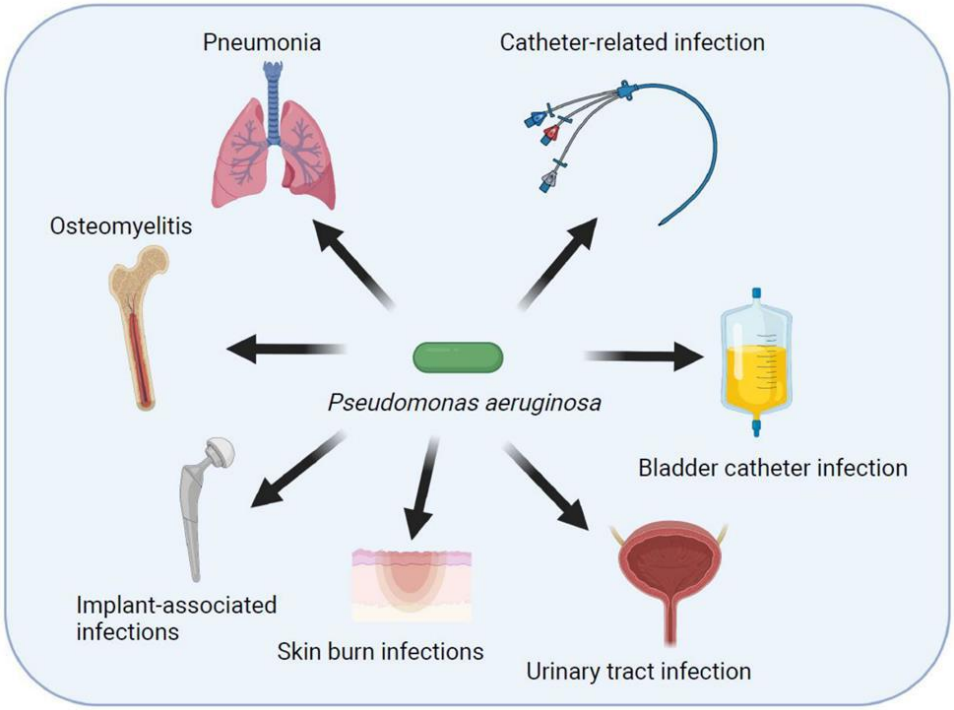

Fig. 10: Pseudomonads pathogenicity (Tuon et al. 2022).

Aeromonas hydrophila has frequently been referred to be an opportunistic pathogen. This seems to be a contradiction in terminology because most "opportunistic" bacteria often do not produce disease until other variables are present, but "pathogen" germs always cause disease. The term "opportunistic pathogen" indicates, however, that *Aeromonas hydrophila* is always capable of causing disease if given the chance. The organism is widespread in nature, as was previously mentioned, and is even present in fish intestines. when occurring naturally. *Aeromonas hydrophila* infections in fish are probably not a major issue. However, additional factors need to be taken into consideration when using intense fish-fanning systems, whether they be indoor aquariums or outdoor ponds. The existence of the disease is related to the fish stressed circumstances (Dorick et al. 2023).

Fish under adverse conditions due to poor water quality, such as high nitrite levels, low levels of dissolved oxygen (DO) or high levels of carbon dioxide (CO2) are more prone to become infected with *Aeromonas hydrophila*. Additionally, lower water temperatures are linked to a seasonal occurrence of more rep-ted fish mortalities in the spring.

Symptoms of *Aeromonas hydrophila* infection in fish might vary widely. These include skin ulcerations, lack of appetite, strange swimming patterns, pale gills, unexpected death in apparently healthy fish and pale gills. The skin ulcer may appear anywhere on the fish, and it frequently has a vivid red tissue border around it. The gills, kidneys, liver, spleen, pancreas and skeletal muscle are additional organs that are frequently impacted by this condition. The severity of the symptoms varies depending on the organism infectiousness, the fish susceptibility to infection, the presence or absence of bacteremia or

septicemia and the fish sensitivity to stress. The diagnosis of this disease based only upon symptoms is extremely inaccurate and may be financially devastating for the fish producer due to the diversity of these symptoms (Semwal et al. 2023).

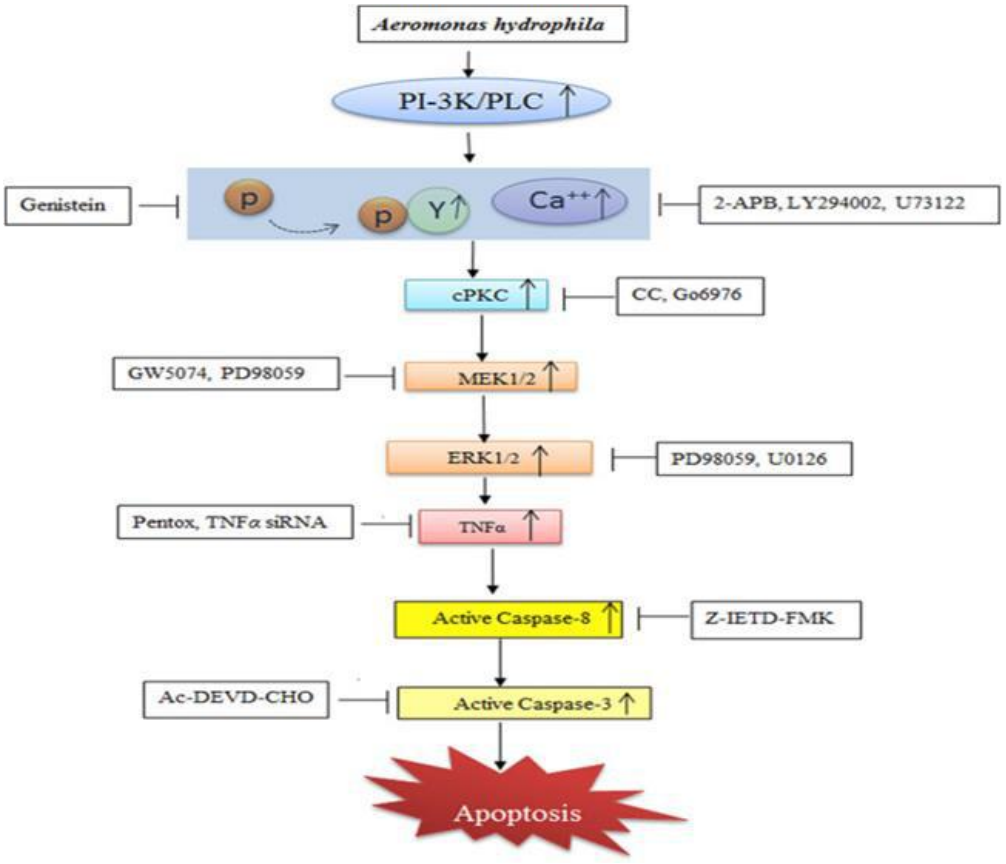

Fig. 11: *Aeromonas hydrophila* mode of action (Shelly et al. 2017).

Obviously, avoiding being infected with Aeromonas hydrophila is the best defence against infection. Although it might seem ridiculous, fish are far less likely to have this disease if stress factors are reduced by correct handling, stocking levels, nutrition, transportation and water that is not handled properly, is overcrowded, or is carried in inadequate circumstances. Poor sanitation, filtration and nutritional levels characterize the environment. Terramycin, an oxytetracycline and Remet-30 a potentiated sulfonamide is the only two antibiotics now used to treat this infection. A dip or bath is another approach for using antibiotics, though it is a bit of a contentious practice, and it is unclear if it is effective or successful. The indoor tank systems biofilters might be completely destroyed by this procedure, and it's conceivable that the fish may not get antibiotics (Ulzanah et al. 2023).

10. HAFNIACEAE

Hafniaceae is a family of flowering plants that belongs to the order Brassicales. The family consists of a single genus, Hafnia, which contains only one species, Hafnia alvei. This European native tiny annual herb may be found in a range of environments, including grasslands, meadows and cultivated fields (Cordovana et al. 2020).

Hafnia alvei is not typically used for any medicinal or culinary purposes, but it is known to produce a yellow pigment called hafnium. Hafnium is used in various industrial applications, including in the

production of nuclear reactor control rods, as a component in electronic devices, and as a coating for gas turbine blades (Ramos-Vivas 2020).

The etiology of Hafniaceae, as a family of plants, would focus on understanding the evolutionary history and genetic makeup of this group of plants. Hafnia alvei, the only species within the family Hafniaceae, is a small annual herb with limited economic importance or medicinal value. As such, research into its etiology has been limited. That being said, researchers have studied the distribution and ecological characteristics of Hafnia alvei, the only species within Hafniaceae, to gain a better understanding of its natural history and ecology. Hafnia alvei is widely distributed in Europe and has been found in a variety of habitats including grasslands, meadows and cultivated fields. It is considered a common and widespread plant species, but further research is needed to fully understand its ecological preferences and how it interacts with other plant and animal species in its environment (Cordovana et al. 2020).

In fish, Hafnia alvei infection has been associated with a range of clinical signs, including skin ulcers, fin rot, septicemia, and hemorrhagic septicemia. Other possible signs of infection may include lethargy, loss of appetite, and abnormal behavior. The severity of the clinical signs may depend on a variety of factors, including the species of fish, the strain of the bacterium, and the environment in which the fish is living. In some cases, infection with Hafnia alvei may be asymptomatic or may only cause mild clinical signs (Ramos and Dámaso 2000).

Maintaining good water quality, removing uneaten feed and waste, and ensuring proper disinfection of equipment and surfaces can help reduce the risk of bacterial infections in fish. Before introducing new fish to an existing population, quarantining them can help stop the spread of infections like Hafnia alvei. To stop the transmission of disease between fish populations, appropriate biosecurity measures should also be followed. In cases where infection is severe or widespread, the use of appropriate antimicrobial agents may be necessary to control the infection. However, it is important to use antimicrobials judiciously and in accordance with local regulations to prevent the development of antibiotic resistance (Zhu and Miller 2004).

11. ENTEROBACTERIACEAE

The Enterobacteriaceae family which is commonly found in aquatic environments and the digestive tract of fish can cause various human infections. Escherichia coli, Klebsiella, and Salmonella are one of the zoonotic fish agents that has been found in Iran, demonstrating human infection and transfer to others (Azimi et al. 2021). The life cycle of enterobacteria is shown in Fig. 12. These bacteria commonly infect humans through open wounds, scratches or contact with fish, which results in an infection and inflammation at the site of the bacterium's entrance or systemic illnesses. Human illnesses with various members of this family of bacteria have occasionally been connected to food sources, such as consuming imported dried fish contaminated with *S. Typhimurium* (Oliveira et al. 2017).

12. *E. COLI*

Fish are now a new vector for this bacterium in water sources. *E. coli* strains in many fish species have been identified. Different *E. coli* strains can be retained by fish and spread to other water sources. Although *E. coli* is not a part of the normal fish microbiota. It is commonly isolated from fish intestines and found in contaminated water. *E. coli* has been seen invading other fish tissues such the gills, kidneys, muscles, and bladder (Kusunur et al. 2022; Yohans et al. 2022).

13. SALMONELLA

Salmonella enterica subspecies enterica is effective in the development of intestinal sickness through fish, aquaculture products and water. Salmonella is not a common fish bacterium; however, its existence depends on the water quality and aquatic habitat. *Salmonella typhimurium* and *Salmonella enteritidis* which are naturally spread by contaminated seafood are the most common causes of salmonellosis in humans. Environmental contamination and bacterial dissemination are significantly facilitated by Salmonella's survival in fish digestion and its detection in human faeces. Salmonella-infected fish consumption can result in symptoms including diarrhea, cramping in the stomach, fever, and bacteremia. Smoked fish contaminated with salmonella can also spread germs to people through their gills and skin (Zhou et al. 2022).

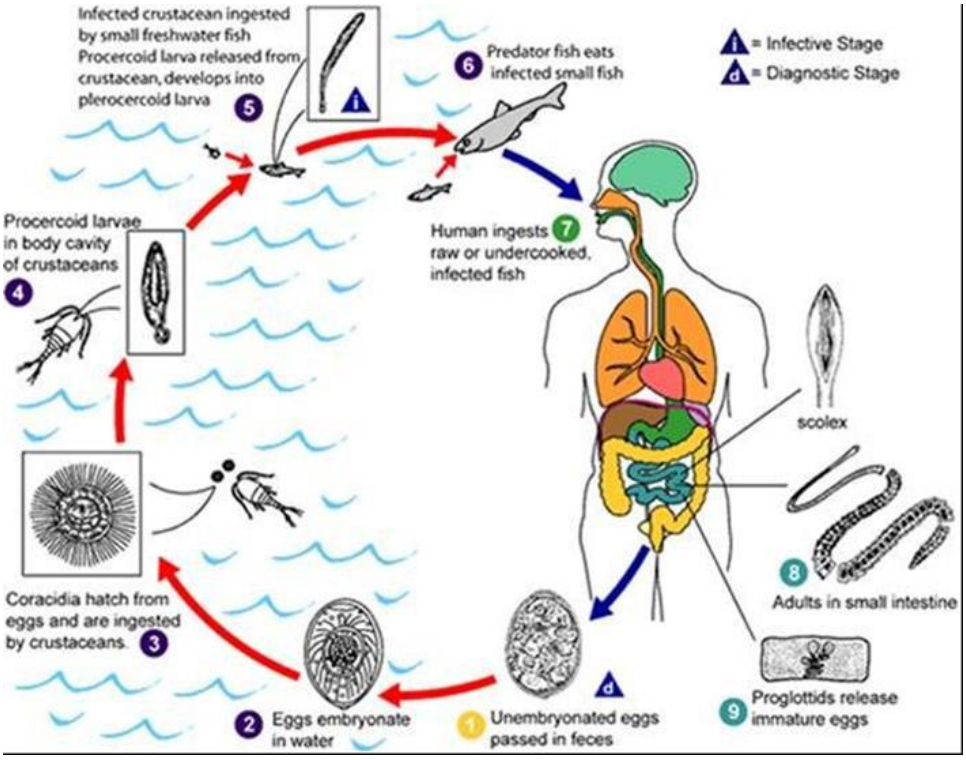

Fig. 12: Life cycle of enterobacteria (Shabani et al. 2019).

14. KLEBSIELLA

samples of water were taken from a dam, ocean, silt, and the intestinal contents of freshwater fish and prawns. There have been reports of K. pneumoniae isolation and diagnosis from farmed fish in India that had vacuolation and necrosis of hepatocytes in addition to clinical bleeding issues around the tail. Due to their zoonotic status and multi-drug resistance Klebsiella spp. (Klebsiella pneumoniae complex) is a threat to human transmission. Along with aberrant immune reactions, the direct effect of endotoxin also contributes to the appearance of Klebsiella infection in fish (Srinivasan et al. 2022).

15. YERSINIA

Yersinia is another gram-negative bacterium that affects both fresh and marine water fish. Over the past few decades, the incidence of *Y. ruckeri*-caused enteric red mouth (ERM) disease has significantly grown.

A few examples of the elements that influence a bacterium pathogenicity are the secretory system, pili, enzymes, toxins, outer membrane proteins, flagella, iron acquisition system, heat sensitivity factor and biofilm formation. The bacteria were isolated from a wound infection in a person who had come into contact with water, increasing the possibility that it was zoonotic but still requiring additional investigation (Wrobel et al. 2019). The mode of transmission is shown in Fig. 13.

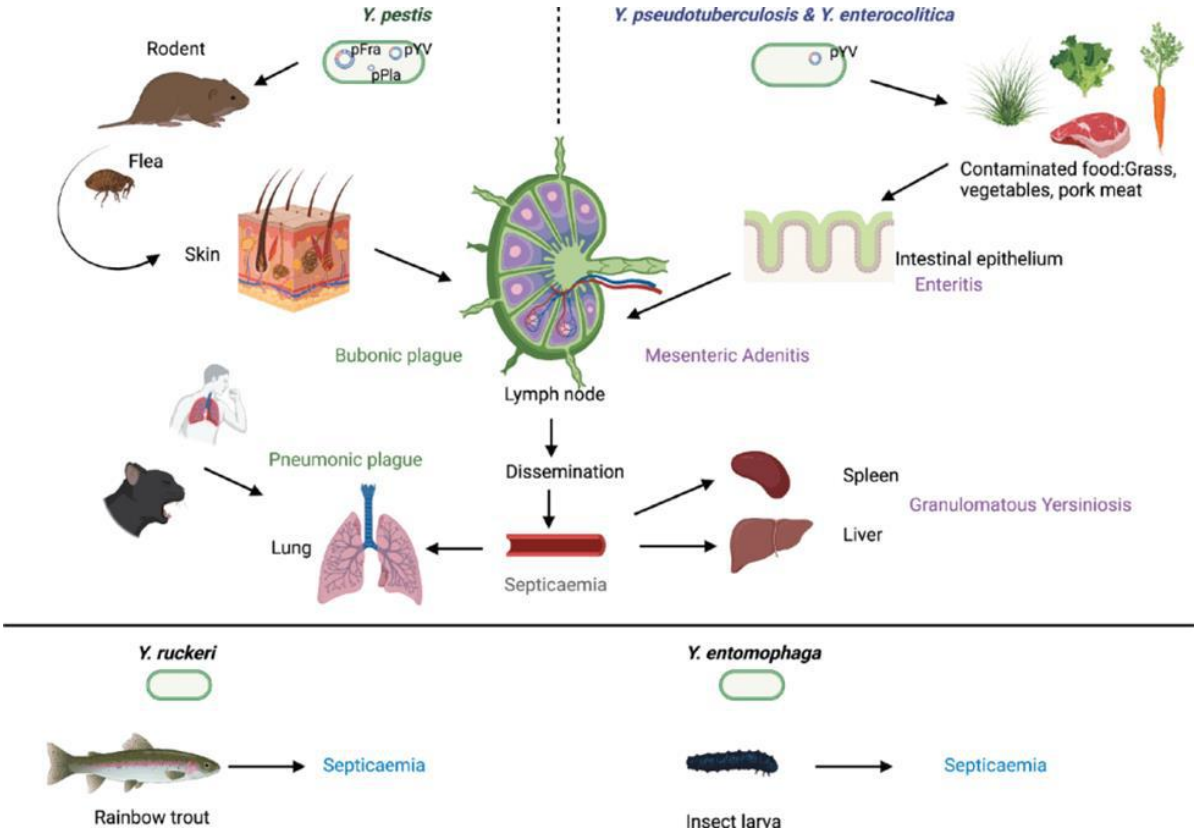

Fig. 13: Pathogenicity of Yersinia (Le Guern and Pizarro-Cerdá 2022).

16. TRANSMISSION AND RISK FACTORS

The transmission of bacterial zoonotic diseases from fish to humans involves various factors, including:

16.1 AQUACULTURE AND FISH FARMING PRACTICES

Intensive fish farming methods may lead to stressful and crowded circumstances which promote the spread of bacteria among fish populations. When animals are handled or processed by humans, high-density rearing can increase the danger of zoonotic infections.

16.2 TRADE AND GLOBALIZATION

The spread of zoonotic infections to other areas is facilitated by the global trading of fish and marine products. Contaminated fish from one part of the world transfer to other countries, contributed to disease outbreaks.

16.3 ENVIRONMENTAL AND CLIMATE CHANGE FACTORS

Aquatic ecosystems affected by climate change, which might influence the distribution and predominance of bacteria in fish populations. Pollution, fluctuating water salinity, and rising sea temperatures all have an impact on bacterial survival and proliferation, thereby raising the likelihood of zoonotic diseases.

17. TRANSMISSION OF BACTERIAL ZOONOSIS IN FISH

17.1 FISH TO HUMAN TRANSMISSION

- The primary route of transmission for bacterial zoonotic infections is the consumption of raw and uncooked fish.
- Contaminated water systems and the presence of bacterial pathogen in fish organs lead to infection in humans.

17.2 OCCUPATIONAL EXPOSURE

- During every day of their jobs, Fisherman, boaters, fish handlers are more likely to come into direct contact with diseased fish or polluted water which increases their risk of developing bacterial zoonosis.

17.3. RECREATION ACTIVITIES

- Recreation activities involves fishing, swimming, gardening, animals handling, ducks rearing might increase the risk of zoonotic infection.

18. HUMAN PATHOGENESIS AND CLINICAL SYMPTOMS

18.1 GASTROINTESTINAL INFECTION

- Many bacterial pathogens can cause common gastrointestinal symptoms like diarrhea, vomiting, and stomach discomfort.
- Salmonella spp., Vibrio parahaemolyticus, and other bacterial pathogens are frequently responsible for gastroenteritis caused by infected fish.

18.2 SKIN AND SOFT TISSUES INFECTION

- When handling fish, bacterial diseases like Streptococcus iniae and Vibrio vulnificus can enter the body through skin wounds or cuts, resulting in cellulitis and skin infections.

18.3 SYSTEMATIC INFECTIONS

- Several bacterial zoonotic pathogens can lead to serious systemic health issues specially in those who have weak immune systems.
- For instance, Vibrio vulnificus infections can result in septicemia which is potentially life-threatening.

19. CONTROL AND PREVENTION STRATEGIES

To minimize the risk of bacterial zoonotic diseases of fish several key control and preventive strategies can be implemented.

19.1 GOOF AQUACULTURE PRACTICES

Development of ethical and environmentally friendly aquaculture practices will help in lowering the risk of bacterial infections in fish populations. In order to prevent the development and propagation of zoonotic infections, it is essential to manage farms properly, check the quality of the water, and monitor disease outbreaks.

19.2 SAFETY OF FOOD AND HYGIENE

Implementing strict hygiene protocols must be used when handling, processing and transporting fish in order to avoid cross-contamination and reduce the possibility of zoonotic disease transmission.

19.3 REGULATORY MEASURES

Regulations for the farming of fish, processing, and trading can be established and enforced to ensure fidelity to food safety standards and lower the danger of zoonotic epidemics.

19.4 PUBLIC HEALTH EDUCATION

Promote awareness and early infection identification by educating consumers, fish handlers and medical experts about the dangers of bacterial zoonotic diseases due to fish.
Public health issues caused by bacterial zoonotic infections in fish must be successfully addressed, leading to the need for a multidisciplinary strategy. To protect both human health and the long-term viability of the fishing business, we can build focused preventative measures by understanding the biological mechanisms of zoonotic infections, their transmission methods, and risk factors. Public health organizations, policymakers, and stakeholders in aquaculture may work together to lower the risk of bacterial zoonotic diseases in fish and guarantee the security of seafood consumers across the world.

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Zoonotic Web of Tuberculosis

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ABSTRACT

Tuberculosis or TB is a disease of bacterial origin with an ancient history. The main causative agent of this disease in humans is *Mycobacterium Tuberculosis* or MTB. However recently it has been discovered that some other members of the genus *Mycobacterium* can also lead to TB after infection. If such agents are transmitted from animals to humans, then this type of TB is termed Zoonotic TB. This type of TB is usually more prevalent in people who come in contact with animals regularly under poor hygienic measures. Low quality of life and adaptation of poor hygienic measures are the main factors contributing to the spread of TB. In the present era, increased cases of zoonotic TB cases and the emergence of antimicrobial resistance among the *Mycobacterium* genus have caused a worldwide alarm about global health. This trend has alarmed researchers all across the world. They are now doing their level best to come up with alternatives for chemotherapy to prevent antimicrobial resistance. This situation calls for in-depth research about TB and the development of countermeasures for its control and eradication to remove this threat to global health.

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1. INTRODUCTION

TB is a pretty old disease with its history dating back to around 3 million years ago (Gutierrez et al. 2005). Zoonotic tuberculosis is the infection of the M. tuberculosis complex transmitted from an animal to a human or one human to the other (Biet et al. 2005; Quinn et al. 2011; Garcia-Jimenez et al. 2013). Zoonotic tuberculosis can be transferred from animals to humans through various sources. Raw or undercooked meat and milk are major mediums for the transfer of TB infection from animals to humans so TB thrives in the areas where milk pasteurization is rare. Similarly, inhalation of TB spores can also happen when cattle and humans come in close contact (Ashford et al. 2001).

Tb infection primarily begins spreading through the respiratory tract and leads to the production of its typical signs such as tubercles seen on the lungs during post-mortem. Although TB can affect any organ, mostly it is diagnosed as an active pulmonary infection (Pai et al. 2016; Al-Ghafli et al. 2019). Most of the time adult males are more affected by TB as compared to female adults (WHO 2021). Usually, younger patients have extra-pulmonary while older patients have pulmonary TB infections (Shannon et al. 2020).

This infection can occur in both animals and humans alike. Both wildlife and livestock animals are affected by TB. TB is not just a simple infectious disease it is a matter of global public health emergency that has re-emerged on the surface after decades of dormancy. Before the COVID-19 pandemic, TB was the single infectious disease with the maximum number of deaths on its credit (WHO 2021). These concerns were heightened as it was discovered that about half a million people were infected with rifampicin-resistant TB, making the issues of TB equally worrying for global battling TB and antibiotic resistance. Sustainable efforts will be needed to deal with this health threat and control its spread (WHO 2020).

Recently, TB has been going rampant in India due to its largest cattle population. As of 2017, there were around 21.8 million TB-infected cows in India (Srinivasan et al. 2018). The main culprit of cattle tuberculosis in India is suspected to be Mycobacterium orygis (Brites et al. 2018). The cattle population has been facing endemic tuberculosis in India leading to zoonotic infections of unknown burden. Some studies have stated that the prevalence of zoonotic TB could be up to 10 % in India (Prasad et al. 2006; Shah et al. 2006; Bapat et al. 2017). Although despite having the largest cattle populations the highest burden of animal TB is not from India but rather from Europe and the Americas. One logical explanation for these statistics might be the difference in the accuracy of studies conducted, sampling strategies and diagnostic facilities available in the Americas are much better than the ones in third-world countries (Ramos et al. 2020).

3. AETIOLOGY

Mycobacterium is a bacterial genus with a wide range of hosts and varying susceptibility and infectious pathophysiology for different hosts (Biet et al. 2005; Quinn et al. 2011; Garcia-Jimenez et al. 2013). The direct human-to-human infection is usually caused by the infection of *M. tuberculosis* which is also known as MTB. On the other hand, animal-to-human infections of TB or zoonotic TB are caused by another species known as *Mycobacterium bovis* (Fig. 1) (Morse et al. 2012; Muller et al. 2013). The *M. tuberculosis* and *M. bovis* are collectively known as the *M. tuberculosis* complex or MTBC (WHO 2020).

Another member of MTBC is *M. orygis* which was identified in 2012 however there is a lack of robust evidence regarding its zoonosis (Van Ingen et al. 2012; Lavender et al. 2013; Marcos et al. 2017; Rahim et al. 2017; Shannon et al. 2020). Some other bacteria linked to TB include *Mycobacterium caprae, Mycobacterium microti, Mycobacterium canetti, Mycobacterium mungi* and *Mycobacterium pinnipedii* (Richard et al. 2021). Still, *M. bovis* is most commonly diagnosed as the cause of zoonotic diagnosis (Duffy et al. 2020). *M. bovis* and *M. tuberculosis* cause almost the same symptoms when enter in human body. *M. bovis* causes extrapulmonary symptoms more than *M. tuberculosis*. They can be differentiated based on biochemical tests (Grange et al. 1996; Michel et al. 2010). The linkage between different TB pathogens and the symptoms caused by them is shown in Table 1.

4. GLOBAL TRENDS

Globally TB has affected 1/3 of the population (Getahun et al. 2015). The rise of COVID-19 has further increased the projection of TB and increased the expected case number to 6.3 million in the next five years with an additional death of 20% (Cilloni et al. 2020; Hogan et al. 2020; Stop TB Partnership 2020).

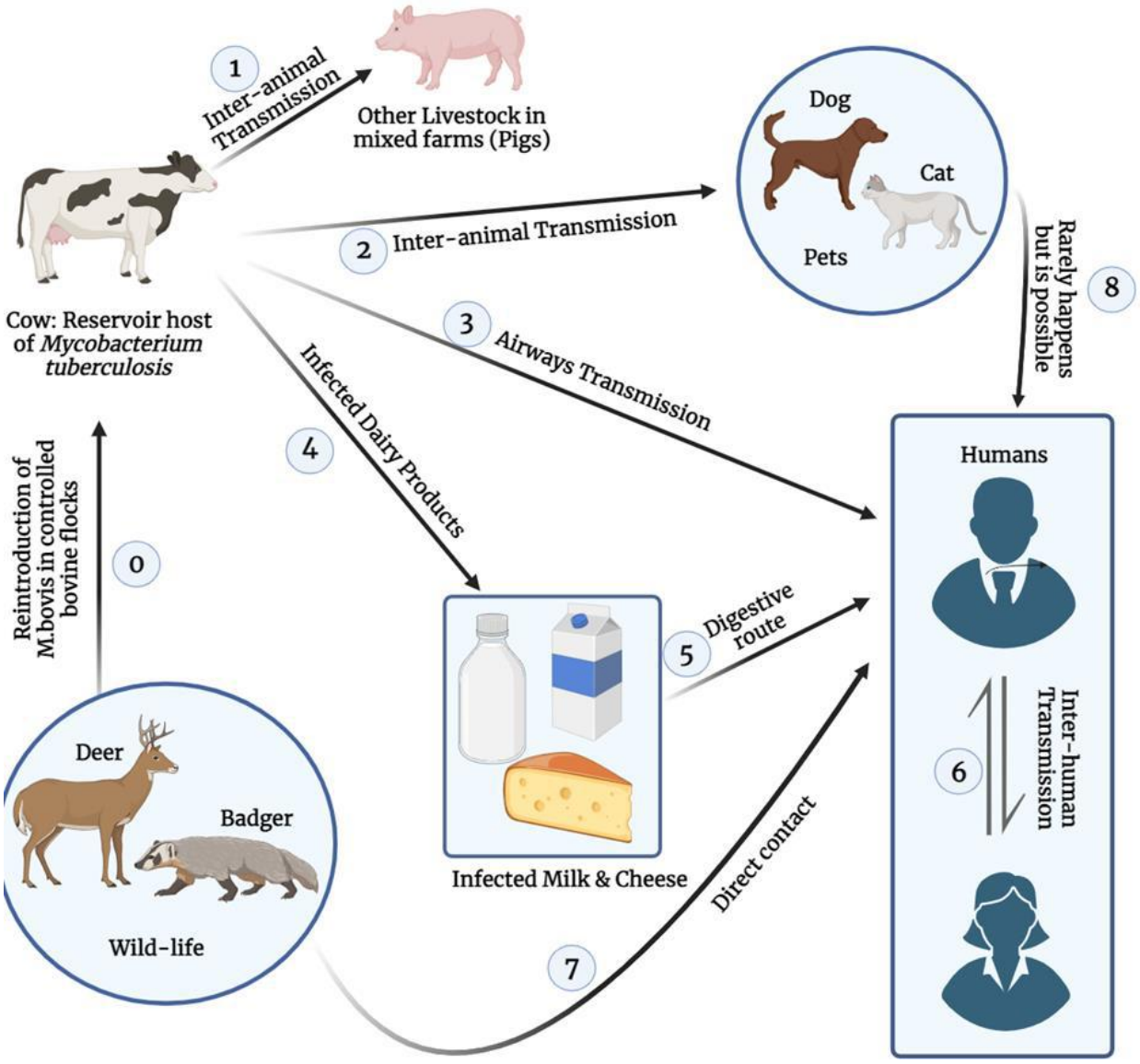

Fig. 1: Zoonotic Web of Tuberculosis.

India has the largest number of tuberculosis cases in the whole world (WHO 2021). Africa and Southeast Asia are the regions with the maximum number of cases affected by zoonotic tuberculosis (Ramos et al. 2020; WHO 2020). On the other hand TB especially, zoonotic TB is consistently declining in Europe with a prevalence of 10 cases out of 1000,000 population only. Similarly, the prevalence of zoonotic TB is less than 0.01% (Muller et al. 2013) with a few cases being caused by rare TB agents like *M. bovis* and *M. caprae* (Richard et al. 2021).

Pulmonary TB cases are mostly reported in rural regions linked with a lack of hygiene and awareness (O'Reilly and Daborn 1995). Additionally, bovine tuberculosis is also very common in cattle and so are the people working in close contact with cattle daily mostly rural people are into cattle farming. Consequently, the zoonotic ramifications of Tb result in a significant increase in the threat to the global public health of the human population (Shitaye et al. 2007; Legesse et al. 2011).

The main factors affecting the spread of TB are poor living standards, an unhygienic environment and many other factors that impair immunity and increase the risk of TB infection (Lonnroth et al. 2009). *M. bovis* tuberculosis is very rare in developed countries but common in developing countries because of using unpasteurized milk and having no hygienic veterinary measures (Michel et al. 2010).

Additionally, bad air quality and the prevalence of diabetes can also serve the factors ramping up the spread of TB in a region (Basnyat et al. 2018). Globally, Zoonotic TB has become a realistic concern for health security authorities as it was seen in 2019 that 140,000 cases out of 10 million TB cases were found to be zoonotic. Hence, global authorities have been trying their best to persuade country governments to make better TB control policies and accelerate development plans towards a tuberculosis-free world (WHO 2020). The global case ratio for Zoonotic TB might seem low but it is possibly due to a lack of facilities for the identification of *M. orygis* (Brites et al. 2018). It mostly happens that only *M. bovis* is detected as the cause of zoonotic TB globally along with attributed deaths and zoonotic TB burden. This methodology essentially ignores the contribution of other MTBC species in the spread of zoonotic TB (Duffy et al. 2020). That is why has been declared as a global public health emergency (Nathavitharana and Friedland 2015).

Table 1: Different types of TB pathogens leading to different symptoms.

No.	Pathogen	Origin	Symptoms	References
1.	*M. tuberculosis*	Human	Tuberculosis	(Morse et al. 2012; Muller et al. 2013)
2.	*M. bovis*	Animals	Tuberculosis with more inclination towards extrapulmonary symptoms	(Grange et al. 1996; Michel et al. 2010; Morse et al. 2012; Muller et al. 2013)
3.	*M. caprae, M. microti, M. canetti, M. mungi* and *M. pinnipedii*	Animals	Tuberculosis	(Richard et al. 2021)

5. EVIDENCE AND IMPACT OF ZOONOSIS

Zoonotic tuberculosis infection mostly happens when there is close contact between humans and animal species that have an abundant population around them such as food-based or companion animals (Johnson et al. 2020; Ramos et al. 2020). Close contact promotes unpasteurized milk consumption and aerosol spread. TB transmission also occurs from sheep and goats resulting in infection with *M. caprae*. Close contact with other non-domesticated species such as rodents, sea lions and seals, and banded mongooses consequently may lead to TB with infection from *M. microti*, *M. pinnipedii and M. mungi* respectively (Jagielski et al. 2016; Brites et al. 2018; Duffy et al. 2020). A comprehensive understanding of this vicious cycle of TB transmission can be gained from the schematic explanation provided in Fig. 2.

Tuberculosis in cattle is known as bovine tuberculosis. It is considered a major health problem of animals that is usually discovered when endemic in herds. Losses by TB are a major concern and cost up to US $ 3 billion annually worldwide (Waters et al. 2012).

Humans also get infected by TB through reverse zoonosis cycles. In reverse zoonosis, the disease spreads from animals to humans. This results in animals that are reservoirs for human disease-causing bacteria (Messenger 2014). The human infecting *M. tuberculosis* can infect a diverse range of hosts. Once infected an animal catches this infection it then begins acting as the new source for the spread of TB (Une and Mori 2007; BhanuRekha et al. 2015).

6. TREATMENT

Rifampicin, isoniazid and ethambutol are used to treat *M. bovis* tuberculosis according to the recommendation of the United States Centers for Disease Control and Prevention (American Thoracic Society 2003). This treatment regimen does not include pyrazinamide because several reviews in the past two decades, are investigated and it is concluded that all the strains of *M. bovis* are resistant to pyrazinamide. Hence, rifampicin, isoniazid and ethambutol once started, are continued for at least 9 months

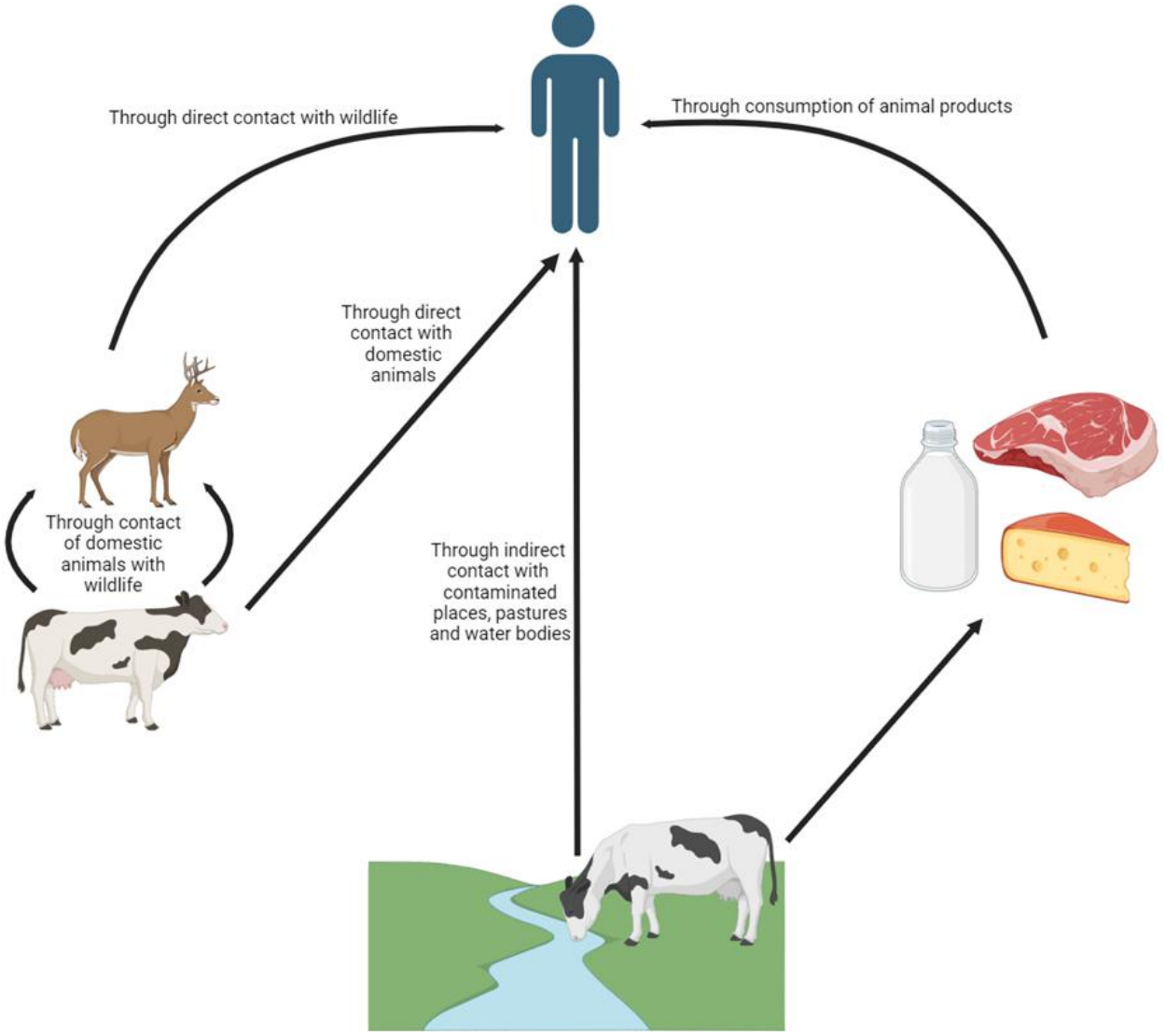

Fig. 2: Transmission Sources and Routes for Zoonotic TB Infection.

(de Kantor et al. 2008; Muller et al. 2013). No review has evaluated this treatment regimen and its outcomes in tuberculosis due to *M. bovis* (Lan et al. 2016).

Through initial database research, 985 worldwide publications are sorted for which 17 publications are selected for full-text review. The publications that did not report treatment are excluded and these are six in number. Some publications are also excluded because of different reasons as follows (Cicero et al. 2008;

Lan et al. 2016) instead of reporting 9 months of treatment, it only reported 6 months of treatment. Researchers provided the duration of treatment consisting of isoniazid-rifampicin-ethambutol varies from 4 to 12 months and it is without convincing results (Sauret et al. 1992). Researchers also include the treatment of patients who have multidrug-resistant strains of *M. bovis*, so it is also not included in our calculations (Esteban et al. 2005).

439 patients were reported with zoonotic tuberculosis caused by *M. bovis* in the United States of America, Argentina and the Netherlands from the three studies. Following are the reported facts. In LoBue studies, that were held in the United States of America for the period of 10 years from 1994-2003, the total patients were 167 out of which 7% patients were isoniazid-resistant and 1% patients were rifampicin-resistant, they were given isoniazid and rifampicin for the period of 9 months. 129 patients were cured of the disease, and 25 patients died. 12 patients lost follow-up, and in one patient there was a relapse of the disease by the same *M. bovis*. So, according to these statistics, the success rate (versus failed relapse) % is 99%. In the same way, success rate (versus fail + relapse + death + loss of follow-up) % is 77% (Grange 2001).

In the CORDOVA studies, that were held in Argentina for the period of 12 years from 1996-2008, a total of patients 23 out of which 3% patients were rifampicin-resistant and 3% patients were isoniazid-rifampicin resistant, they were given isoniazid, rifampicin and ethambutol for the period of 8 months to a year. 14 patients were cured completely from the disease. 1 patient failed to treat the disease, and 5 patients died of the disease despite taking this drug regimen. 3 patients lost to follow-up and no patient got relapse. So, according to this data, Success rate (versus fail + relapse) % is 93%. In the same way, the success rate (versus fail + relapse + death + loss of follow-up) % is 61% (Grange 2001).

In the MAJOOR studies, that were held in the Netherlands for the period of 14 years from 1993-2007, total patients were 231 out of which 5% patients were isoniazid-resistant, 1% patients were isoniazid and rifampicin resistant. Out of which 40 patients were given isoniazid and rifampicin. 25 patients were cured completely from the disease. 1 patient failed to treat the disease, and 12 patients died of the disease despite taking this drug regimen. 2 patients lost to follow-up and no patient got relapse. So, according to this data, the Success rate (versus fail + relapse) % is 96%. In the same way, the success rate (versus fail + relapse + death + loss of follow-up) % is 63%. 110 patients were given isoniazid, rifampicin and ethambutol. 91 patients were cured completely from the disease. 7 patients failed to treat the disease, and 9 patients died of the disease despite taking this drug regimen. 3 patients lost to follow-up and no patient got relapse. So, according to this data, Success rate (versus fail + relapse) % is 93%. In the same way, success rate (versus fail + relapse + death + loss of follow-up) % is 83%. 81 patients were given other and unknown drugs. 35 patients were cured completely from the disease. 6 patients failed to treat the disease, and 25 patients died of the disease despite taking this drug regimen. 15 patients lost to follow-up and no patient relapsed (Grange 2001).

7. ADVANCEMENTS

Despite advancement, zoonotic tuberculosis has remained an important health problem for both animals and humans over the past 20 years. With the arrival of tuberculin, basic control approaches included: the detection of disease via tuberculin skin test and then isolating the flock from other animals as well as from humans. There is also the isolation of animals within infected herds such as the Bang Method and the slaughtering of infected animals (Doyle and Stuart 1958).

There has been a tremendous decrease in zoonotic TB-infected cattle in New Zealand in the previous 18 years. In June 1993 there were 1694 infected flocks, these numbers decreased to only 79 infected cattle by June 2011. These good results were achieved by controlling the brushtail possum population in addition to the slaughtering of infected cattle and by strictly isolating the herd from other animals (Buddle et al. 2011). The cross-protective strategy is also used but it failed to convey an effective and secure vaccine for

zoonotic tuberculosis. This was stopped due to safety issues because sometimes there is dissemination of infective organisms and also shedding of organisms from the infected body. Human tuberculosis vaccine can be used against cattle *M. bovis* and it was established by Calmette and Guerin when they tried to make attenuated *mycobacterium bovis* for the treatment of human tuberculosis. This was done by serial propagation of bacillus on ox bile glycerine potato medium (Buddle et al. 2011).
Behring carried out trials on extra vaccines for immunization of cattle against zoonotic tuberculosis. These included: Taurin, a too-virulent mutated strain of bovine tuberculosis. Same as there is Tuberkulase, which consists of dead tuberculous bacillus that was given chloral hydrate sedative. These efforts are not fruitful because of specific etiological prophylaxis (Murphy et al. 2008). BCG can defend the host against natural *M. tuberculosis*. This has been proved by recent studies on cattle in Mexico and Ethiopia. Moreover, BCG shows defence in multiple host species in a large number of trials. The latest bovine vaccine contains live attenuated strains and it proved very efficient.

8. CONTROL AND PREVENTION

After decades of neglect and ignorance, serious efforts to control TB were initiated in 1991. It was the time when WHO declared TB a major public health issue globally (WHO 1991). Although WHO wasn't satisfied with the efforts of the countries, consequently it moved forward and declared TB as a global health emergency in the year 1993 (WHF 1993). A control regimen based on DOTS (directly observed therapy strategy) was released in 1994 in an attempt to limit the spread of TB (WHO 1994). Stop TB partnership between WHO and global advocacy organization launched the Global Plan to Stop TB 2001 (2001-2005) which was then succeeded by the Stop TB Strategy 2006-2015 in 2006. The Stop TB Strategy mainly focused on a patient-centred care approach to focus on TB patients (Raviglione and Uplekar 2006). Later on, the World Health Assembly devised the End TB Strategy in 2014. WHO launched this programme in 2015 (Uplekar et al. 2015).

9. RECENT TREND

Even in this age of modernization limited point of care (POC) diagnostics and insufficient reporting have reduced the reliability of data for determining the trend of incidence and prevalence of zoonotic tuberculosis in certain regions. Hence, it cannot be determined if TB prevalence and incidence are going up or down. However, one suggestion in this regard is to provide farmers and veterinarians with rapid test kits for quick diagnosis of TB, enabling them to make spot decisions about the fate of animals about quarantine or slaughtering. So there has been an increase in demand to educate the farmers and spend budget on research and development of quick diagnosis kits for TB (Duffy et al. 2020).
Recently another trend has emerged over the horizon of the medical industry to manufacture a vaccine against TB by Ag85 nanoparticles. Researchers and making tireless efforts to formulate a DNA-based vaccine against TB to put a preventive cure in the blood of people before this awful malady can reach them (Zhu et al. 2005).

10. UPCOMING TRENDS

More than a century ago, an approach was accepted worldwide for the diagnosis of bovine or zoonotic tuberculosis by checking the cell-mediated immune response of the host body against intradermal injection of tuberculin (de la Rua-Domenech et al. 2006). This test has weak results because the purified protein derivatives used in the tuberculin test are obtained from heat-killed specific strains of *mycobacterium bovis* on glycerol broth (Yang et al. 2012; Good et al. 2018). In certain regions where there

is high exposure of *M. bovis* in the environment, the tuberculin test case has larger induration which makes the test less sensitive (de la Rua-Domenech et al. 2006). Moreover, there are certain cross-reactive antigens are also present between pathogens and vaccines which also artificially increase the induration and make the test less sensitive (Yang et al. 2012; Good et al. 2018). According to modern studies, an in vitro interferon-gamma release assay (IGRA) is introduced as a secondary test to increase the overall sensitivity of the tuberculin test (Wood and Jones 2001; EFSA 2012).

In the past 20 years, a specific *M. bovis* antigen has been searched by comparative genomics and transcription that has the DIVA (Differentiating Infected from Vaccinated Animals) capability. This means it can identify or differentiate between BCG-vaccinated and not-vaccinated animals in a mixed flock. These antigens include ESAT-6, CFP-10 and Rv3615c. These are present in field strains of *M. bovis.* These are not present in BCG vaccines based on this it can differentiate between vaccinated and not vaccinated animals (Vordermeier et al. 1999; Young and Robertson 1999; Vordermeier et al. 2016).

11. IN VITRO SUSCEPTIBILITY AGAINST MOXIFLOXACIN

There is no available data that sheds light on moxifloxacin's effects on *M. bovis*. That's why a retrospective move was made for research by taking the cultures of sputum, pleural effusion, and nasal exudates from 33 patients for about 18 years from 1993-2011. The drug sensitivity test was performed by using *M. bovis*-BCG using MTBC genotype assay. The results were excellent, all 33 cultures showed susceptibility to moxifloxacin at less than 1 microgram per millilitre (Gumbo 2010).

12. VACCINES BASED ON NANOPARTICLES AGAINST *M. BOVIS* INFECTION

The latest vaccine is produced against *M. bovis* by genetically engineering the bacteria. This vaccine is formed by nanoparticle polyester inclusions. The control of tuberculosis is achieved by presenting, mycobacterial antigens, Ag85A and ESAT-6 on the surface of bio-beads. These bio-beads were extracted from host production bacteria, *E. coli* and GRAS bacterium. GRAS is generally accepted as a safe bacterium for removing bio-beads. Earlier published worldwide studies depicted that vaccination with Ag85A and ESAT-6 causes an increase in levels of antigen-specific interferon gamma, interleukin 17A, interleukin 6, tissue necrosis factor-alpha and interleukin 2 in the cells of the spleen. But there is no remarkable rise in interleukin 4, interleukin 5 or interleukin 1. However, the latest worldwide studies showed that CD4 and CD8 + T cells in mice which was vaccinated with the Ag85A and ESAT-6 bio-beads induced the release of antigen-specific interferon-gamma. These test mice had a remarkable decrease in bacterial count when treated with Ag85A and ESAT-6 bio-beads alone or given in combination with the BCG vaccination. These mice were previously exposed to aerosol *M. bovis* and these were compared with the control group which was not exposed to *M. bovis* (Zhu et al. 2005; Xi-Dan et al. 2009; Natalie et al. 2014). This nanoparticle-based vaccination has proven very cost-effective and efficient for the protection of cattle against *M. bovis*.

13. COMBINED DNA VACCINES

The immunological responses of diseased and healthy animals were calculated based on increased interferon-gamma in the whole blood. The interferon gamma is produced by T cells in response to the combined DNA vaccines including Ag85A, MPT64, and MPT83 or with PPD of the BCG vaccine (Xi-Dan et al. 2009).

A study is carried out to get the results that which vaccination method is more efficient. Experimental studies carried out over the previous 10 years of the BCG vaccine against *M. bovis* show that there is variation in the efficacy of this vaccine. Th1 response is the crucial step in the process of BCG vaccine because *M. bovis* is an intracellular organism. In DNA vaccine we changed the immune response of the affected organism from partially effective to absolutely effective because it can kill the bacteria (Zhu et al. 2005).

Vaccination in which plasmid DNA that expresses the HSP65 portion of *M. bovis* is introduced in mice followed by chemotherapy was proved very effective when the organism is introduced with *M. bovis* intravascularly (Xi-Dan et al. 2009).

In summary, it is concluded that combined DNA vaccines have better results than the traditional BCG vaccine for the prevention of *M. bovis* infection that causes zoonotic tuberculosis.

14. CONCLUSION

In summary, the present recommendations for the treatment of zoonotic tuberculosis caused by *M. bovis* have very little evidence. Still, it is a potential risk that needs attention for cure, treatment, prevention and eradication. So, thoughtful action plans should be implemented to counter and control it and prevent the emergence of drug-resistant TB.

According to the available data, although it is limited, the presently used regimen includes isoniazid-rifampicin or isoniazid, rifampicin and ethambutol are adequate and enough. The benefit we get by adding ethambutol to the regiment is not clear at all. For better results, this drug regimen should be continued for at least 9 months. Strict care and consistency should be maintained to get the best results out of this regimen while eliminating the risk of anti-microbial resistance at the same time.

HIV infection along with *M. bovis* infection has tremendously increased the mortality rate and causes limitations in the interpretation of results gained by these treatment regimens. Hence it proves that TB prevails among the immuno-compromised patients. So special care should be given to immuno-compromised people. They should be educated to follow proper dosing routines to prevent relapse of TB and control its spread.

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Public Policies for the Control of Zoonotic Tuberculosis

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ABSTRACT

Tuberculosis or TB is an infectious disease that has been rising rapidly in the world. Along with a rapid increase in regular TB cases zoonotic TB is also up surging. Several etiologic agents responsible for causing TB in animals have also been identified in human TB patients presenting strong evidence for laying the foundation of TB transmission through zoonosis. The more concerning portion is the lack and improper implementation of TB control policies made by local and international disease control bodies. Such situations have led to the emergence of increased TB prevalence while giving rise to antibiotic resistance among TB germs simultaneously.

Concerning zoonotic TB human-animal interaction in the case of pets and domestic animals is the only chance of direct contact for TB transmission and hence needs the application of proper hygiene measures for its control. Another possibility to come in contact with TB germs is by using unpasteurized milk products and being undercooked which may contain viable germs, leading to TB infection if consumed. Although the risk and load of TB through zoonosis have reduced a lot in economically developed regions after initiatives like the extermination of bovine termination programs middle and lower-income countries are still struggling to fend it off due to lack of awareness and resources. Still, there is a need for international health security institutes to collaborate with local governments to introduce changes in laws that can help in limiting TB transmission. This chapter focuses on different strategies that have been implemented on a larger scale as public policies to mitigate zoonotic tuberculosis and fruitful outcomes have been gained from them. The developing countries or the countries that are still fighting to eliminate tuberculosis must implement these policies on a national level to get rid of tuberculosis and set forth freedom from this malady.

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1. INTRODUCTION

We have seen an upsurge in the pace and frequency of the emergence and reemergence of infectious illnesses in recent decades (Morse et al. 2012). Zoonoses contribute to nearly 60% of all new infectious illnesses, demonstrating the importance of this aspect regarding the spread of diseases (Karesh et al. 2012). Human tuberculosis (TB) produced by Mycobacterium bovis, or zoonotic TB (zTB), is a specifically serious group of zoonosis. (Morse et al. 2012; Morand et al. 2014). In the past, zTB has widely been linked to extrapulmonary strains of TB infections and was believed to be typically contracted by consuming unpasteurized milk (Wedlock et al. 2002).

Humans infected with M. bovis showed signs of tuberculous cervical lymphadenitis in approximately 91% of patients and tuberculous meningitis in 28% of patients during the years 1901 to 1932 in Wales and England among children with less than 5 years of age (Grange and Yates 1994). Nevertheless, the extensive employment of pasteurization in milk over the twentieth century resulted in a significant decrease in its frequency in most regions of the world (de la Rua-Domenech 2006). This tells us how a small step of pasteurizing the milk can prevent such a notorious disease.

2. HISTORY

In the course of the past ten years, the World Health Organisation (WHO), World Organisation for Animal Health (OIE), and Food and Agriculture Organisation (FAO), have refocused their attention on zTB, listing it among the reappearing overlooked infectious diseases (WHO 2005; Maudlin et al. 2009; WHO 2014; El-Sayed et al. 2016). The expected number of fresh cases of zTB globally in 2016 was 147,000, with the majority occurring across Southeast Asia and Africa. (WHO 2017). These statistics, however, may have been underreported since the laboratory procedures for diagnosing zTB are not always available, particularly in countries with middle or low incomes (de la Rua-Domenech 2006; Olea-Popelka et al. 2017). This scenario guided the addition of zTB in WHO's End TB Strategy, which advocates for the early identification and treatment of all individuals with TB and represents one of the 17 Sustainable Development Goals established by the United Nations (United Nations General Assembly 2015; WHO 2017). Furthermore, zTB was featured for the very first time in the Stop TB Partnership's Global Plan to End TB 2016-2020—The Paradigm Shift (United Nations Office for Project Services 2015).

3. ETIOLOGICAL AGENT

Mycobacterium contains both obligatory infectious and saprophytic species in its genus. Approximately there are around 140 species, which have been divided into three major classifications: *Mycobacterium leprae* group, nontuberculous mycobacteria and *Mycobacterium tuberculosis* complex. The weakened strain of *M. bovis* utilised for the vaccine (bacille Calmette-Guérin, or BCG) and *M. bovis* itself, the causal agent of zTBvv and bovine TB (bTB) belong to the typical members of the *M. tuberculosis* complex. Other members include *Mycobacterium microti, Mycobacterium canettii, Mycobacterium caprae, Mycobacterium pinnipedii* and *Mycobacterium africanum* subtypes I and II (Smith et al. 2006; Wirth et al. 2008; Jagielski et al. 2014; El-Sayed et al. 2016). Throughout the past ten years, the following fresh variants of the complex have been outlined: *Mycobacterium orygis* (van Ingen et al. 2012), *Mycobacterium mungi* (Alexander et al. 2010), and chimpanzee bacillus (Coscolla et al. 2013).

4. TRANSMISSION

There are variable routes by which the transmission of *M. bovis* to humans can occur. When unpasteurized milk is consumed and the microbe enters the body through the digestive system, it can

cause the extrapulmonary type of zTB. This is the primary method for passing on the infection to human beings, particularly in nations without enough coverage or hygienic milk regulations (de la Rua-Domenech 2006). Since *M. bovis* is less sensitive to the pH of cheese compared to other pathological agents (de la Rua-Domenech 2006), the production process of cheese derived from raw milk does not ensure that the bacterium would be rendered inactive. As a result, conditions are created that suggest this food may be a means of transmission, even to people living in cities (Silva et al. 2013). Both high-income nations like the United States (Kinde et al. 2007; Gould et al. 2014) and middle-income nations like Brazil and Mexico (Harris et al. 2007; Cezar et al. 2016) have shown evidence of *M. bovis* contamination in cheese It is noteworthy to mention that in many different countries, raw milk cheeses are significant and customary products of family agriculture (Kinde et al. 2007). Except when properly cooked, livestock meat from animals with zTB is not considered a means of transmitting *M. bovis* since the bacillus rarely exists in muscle (de la Rua-Domenech 2006). People contract pulmonary bTB from cattle when they inhale droplets carrying mycobacteria. This is known as airborne transmission (Wedlock et al. 2002; LoBue et al. 2010). The development of molecular tools has yielded a body of data suggesting human-to-human transmission through the air, which was not much addressed until recently (Fritsche et al. 2004; de la Rua-Domenech 2006; Olea-Popelka et al. 2017). Some research indicates that *M. bovis* may spread directly from person to person and from person to cattle (reverse zoonosis), proposing individuals may behave as infectious agents (Fritsche et al. 2004; de la Rua-Domenech 2006). The discovery that *M. bovis* can be isolated from human sputum suggests that it may be a source of disease, particularly in enclosed spaces. Presumably as a result of the greater number of research published in high-income nations, the majority of reports of *M. bovis* transmission between humans originate from these nations. Dissemination has also been documented between humans and non-bovine household animals (Shrikrishna et al. 2009; Ramdas et al. 2015). *M. bovis* can be transmitted naturally between wild and domesticated animals (de la Rua-Domenech 2006). Percutaneous infection through wounds and scratches in the skin during the processing of carcasses from mammals with bTB (Shrikrishna et al. 2009), especially among personnel who do not employ sufficient equipment and clothes to reduce the risk of infection (Sa'idu et al. 2015), is another probable route of transmission. The incidence of zTB epidemics ought to be brought to light. Multidrug-resistant *M. bovis* forms have been transmitted nosocomially by Spanish hospitals (Blázquez et al. 1997; Guerrero et al. 1997; Rivero et al. 2001) which then later extended to Canada, the Netherlands (Samper et al. 1997), and other areas according to many investigators. There have also been reports of further outbreaks in Scotland (Hughes et al. 2003), the United States (Nitta et al. 2002), and, lately, Mexico (Vazquez-Chacon et al. 2015).

5. CONTROL STRATEGIES FOCUSED ON ANIMALS

Tuberculosis in cattle is a worldwide disease. In countries with higher incomes, the prevalence of TB has significantly declined since the start of bTB extermination initiatives in cattle (Amanfu 2006). Following 27 years of continuous eradication programmes Australia finally proclaimed itself to be a TB-free region (More et al. 2015). But despite a well-established campaign to eradicate the disease, cattle herds affected by various routes of transmission with bTB are still being found in other well-off countries like the United States (McCluskey et al. 2014). High bTB loads in herds are reported by low- and middle-income nations in Asia, South America and Africa (Amanfu 2006). Research reveals that the frequency is 13% in Uganda, 17% in Chad, and 39.6% in Mozambique (Ayele et al. 2004; Moiane et al. 2014). Some of the elements that lead to the loss of management of animal TB include the unregulated movement of cattle, the absence of mechanisms for tracking animals, and the shortage or lack of access to veterinary care (Amanfu 2006).

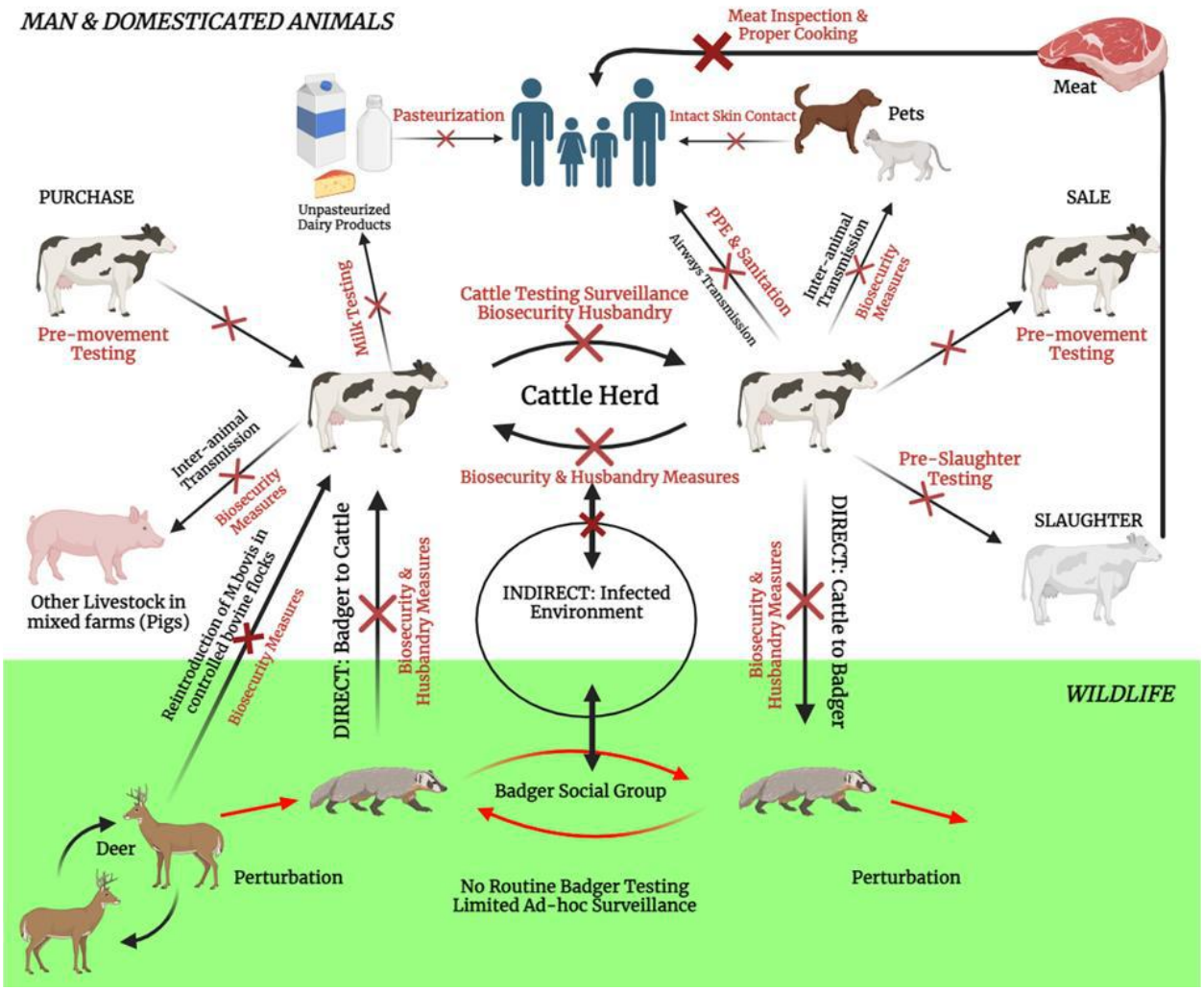

Fig. 1: Control Measures for Zoonotic Tuberculosis.

The risks imposed by this disease are affected a great deal by the management strategies implied to control it. Compared to beef cattle fed on pasture, dairy cows kept in confinement have a greater frequency of bTB. This research can be demonstrated by the longer lifespan of cattle intended for milk production and the fact that they are placed together for milking a minimum of one time a day, both of which are the elements that stimulate the growth of chronic illnesses like bTBPrograms to prevent bovine tuberculosis are often limited to domestic animals, which may limit their efficacy due to the potential for reintroduction of the disease through interaction with wild animals having *M. bovis* infections (Hlokwe et al. 2014).
Because wild animals can serve as significant reservoirs for *M. bovis* infections, prevention and control strategies should also be implemented for these infections (Fig. 1) (Musoke et al. 2015). It's crucial to remember that keeping wild or exotic animals in captivity, especially in wildlife parks and zoos encourages the spread of *M. bovis* and the potential for human transmission (Krajewska et al. 2015). Research carried out in South African parks has revealed that at least sixteen distinct wild animal species have been found to possess bTB (Hlokwe et al. 2014). Furthermore, there is a chance that animals that are shot or hunted for sport might carry the infection to people. According to some investigations on bTB in Spain, these animals had a five-fold greater incidence than cattle from the same area (Parra et al. 2006). Some writers

support the production of vaccinations for wildlife as a preventative step against this particular group (Buddle et al. 2013).

6. CONTROL STRATEGIES FOR THE ENVIRONMENT

M. bovis, like other mycobacteria, is immune to changes in the environment and can survive in soil for a period of 88 days and in water for approximately up to 58 days. However, throughout the warmer months of the year, this bacterium's resistance is significantly decreased (Fine et al. 2011; Barbier et al. 2016). Because of the potential to spread to healthy animals, the infection's existence in surroundings can jeopardize bTB control initiatives while clarifying why the illness persists in herds (Fine et al. 2011). The research has linked bTB to several environmental factors, including agricultural practices, weather-related aspects, and landscape characteristics. By optimizing these characteristics on farms, bTB can be lessened and, as a result, transmission among humans can be reduced. For example, extensive usage of hedgerows (especially along borders), along with having scattered water sources, could serve as a control measure to reduce the probability of bTB transmission (Winkler and Mathews 2015; Broughan et al. 2016).

7. HUMAN-FOCUSED CONTROL STRATEGIES

When it comes to preventive measures that lessen the likelihood that the general public will become infected with *M. bovis*, scientists and the World Health Organization agree that pasteurizing milk is the most successful approach even when weighed against inspecting slaughterhouses and testing animals for tuberculin (Roug et al. 2014; Vranjeˇs et al. 2015). Observing the carcasses of livestock in slaughterhouses to make sure that their flesh is fit for consumption by humans is known as inspection. Thus, slaughtered animals that may be *M. bovis* infected must be disposed of appropriately, and more research should be done for verification of *M. bovis* infection (Fig. 2). Take tainted items out of the food chain and implement proactive measures to manage their animal source. Conversely, the purpose of the intradermal tuberculin test is to discover as many infected animals as possible, hence emphasizing the necessity of eliminating all positive patients (Pritchard DG 1998). In well-developed countries, the pasteurization of milk is a common practice (Müller et al. 2013).

The federal regulation in the United States for this goal was established at the start of the previous century. Nonetheless, 25 of the 50 states allow the sale of raw milk, mostly in the states along the West Coast and the central region. States that allow raw milk have seen a higher frequency of outbreaks associated with it; nevertheless, there is little data linking these outbreaks to zTB (Lejeune et al. 2009; Langer et al. 2012). As long as certain hygienic requirements are fulfilled, some European Union nations, including Wales, Germany, Northern Ireland, England, and France allow the sale of milk and dairy products unpasteurized (Vranjeˇs et al. 2015). Low- and middle-income nations face somewhat distinct circumstances. Small farmers in Brazil continue to sell unpasteurized milk despite the country's prohibition on the purchase of unpasteurized milk for consumption by humans and an initiative to enhance the nutritional value of milk production (Nero et al. 2004).

Pastoral communities and low-grade dairy farms mostly sell about 80-90% of the total milk produced in several African states where pasteurisation of milk is not a common practice (Müller et al. 2013; Jans et al. 2017). In areas like Tanzania, approximately only 39% of the population has been reported to use boiling milk whereas approximately 90% of livestock farmers drink milk daily (Roug et al. 2014). In addition to that, in several well-developed countries like Belgium and America using less-processed dairy goods has become a trend and there is a frequent use of raw milk followed by the misconception that boiling the

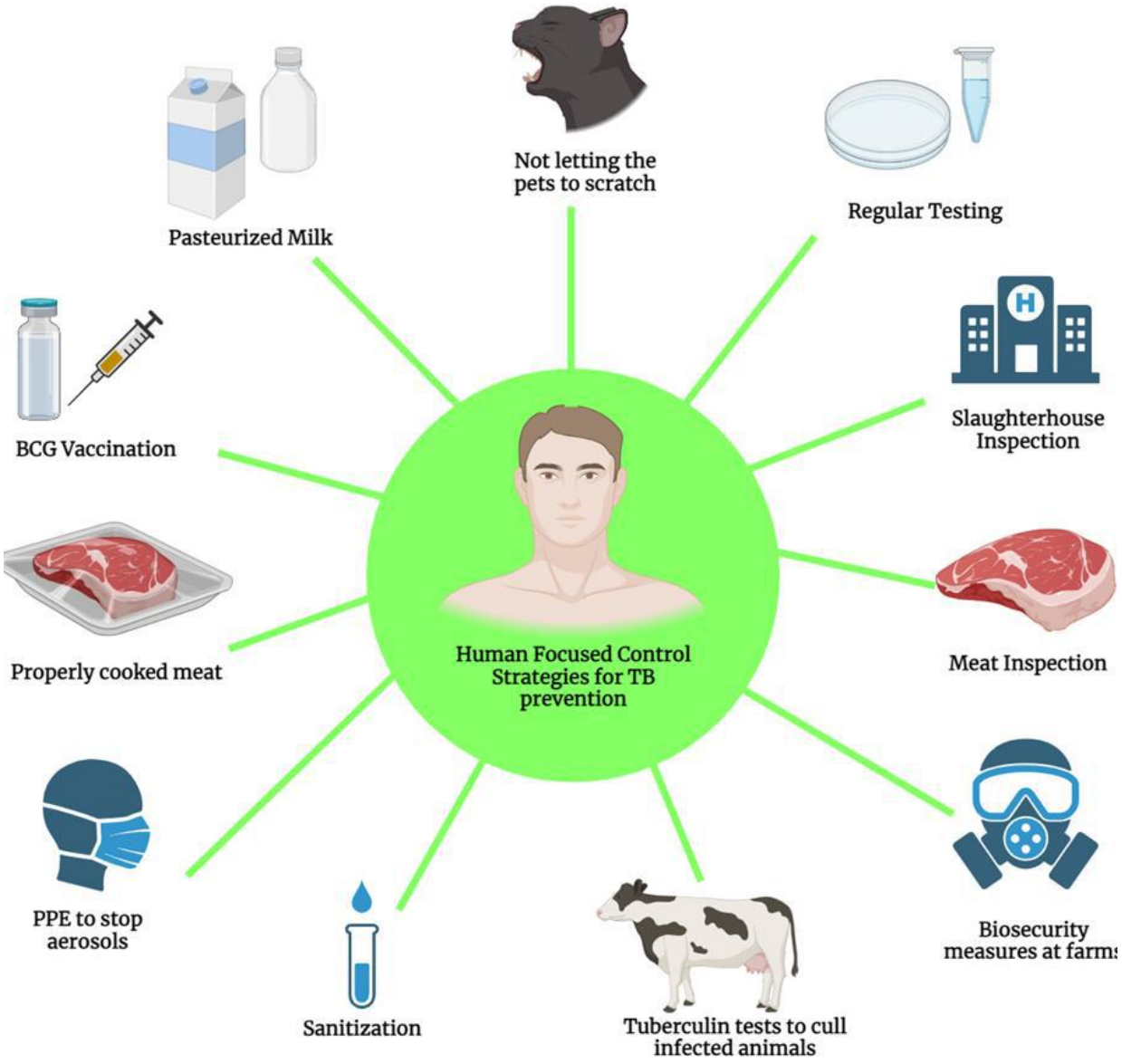

Fig. 2: Human Focused Control Strategies for TB Prevention.

milk ends its nutritional value (Oliver et al. 2009; Claeys et al. 2013). The contraction of *M. bovis* infection by humans can also be prevented by the use of the bacilli Calmette-Guérin (BCG) vaccine. Although the use of this vaccine has increased drastically, its quality and effectiveness are still debatable (Ottenhoff and Kaufmann 2012). This vaccine has proved to be more beneficial in children as it focuses on preventing the dissemination of the bacteria from the primary infection site thus preventing tuberculous meningitis and miliary disease which are more severe forms of TB (Grange and Yates 1994).

8. INTERNATIONAL EFFORTS

The World Organization for Animal Health (Office International des Epizooties; OIE) adopted a resolution in 1983 in response to the seriousness of the dangers posed by zoonotic tuberculosis to

public health. The decision called for the complete eradication of *M. bovis* for both economic and public health motives, the implementation of strict meat inspection regulations, the boiling or pasteurization of milk for consumption by humans, and ongoing investigations into BTB, with a focus on improving diagnostic tests (Kleeberg 1984). Other variations of BTB include cases that recur in older people who contracted the disease before BTB control measures were put in place; cases that are imported into developed nations from other parts of the world where BTB control measures are either nonexistent or useless; and cases linked to the ingestion of tainted animal-origin food items or contact with dead animals that were infected with bovine tuberculosis (Awah-Ndukum et al. 2011). Implementing a One Health strategy to manage zoonotic tuberculosis Control of zoonotic illnesses, such as tuberculosis (TB), is complicated due to the interaction of people, livestock, animals, and ecology in the epidemiology of these diseases (Palmer et al. 2012a). This makes the diseases a prime candidate for the use of the One Health strategy. Among the "deadly dozen," or possibly fatal zoonoses that might expand globally as a result of behavioural adjustments made to offset the consequences of global warming, is TB, according to the Wildlife Conservation Society (Singer 2009). The general decline in human and animal health (and immune systems) brought on by food and water scarcity can aid in the dissemination of zoonotic illness (Lamy et al. 2012). A test-and-cull approach is used in industrialized nations to control bovine tuberculosis (BTB) in cattle. For cattle owners in underdeveloped nations, the socioeconomic costs of this strategy may be unaffordable, which might lead to their unwillingness to take part in BTB control initiatives. (Katale et al. 2012). To control BTB in wildlife reservoirs, more shooting, capturing, or poisoning of the population has been done (Nugent et al. 2012), along with vaccination (Palmer et al. 2012b).

9. CONCLUSION

Tuberculosis is a very problematic disease for mankind. Although curable, its treatment is long, exhaustive and expensive. Additionally, zoonotic TB cases have also started rising making it a global threat to the human population. These circumstances along with the emergence of antibiotic resistance in etiologic agents of TB have necessitated the formulation of proper guideline-based policy-making efforts to prevent TB transmission. Local governments and international health-related bodies are now becoming more and more concerned about policy-making efforts regarding the control of TB and limiting its transmission.

International organizations and local governments are now concerned about stopping TB transmission both among animals and humans. Policies are now being introduced to control TB and reduce its spread to a minimum. These policies focus on both humans and animals to cover both human-to-human and animal-to-human transmission aspects of the spread of TB. Such policies include control of border movement and elimination of zoonosis through initiatives like bovine tuberculosis eradication. Some innovative efforts in this respect are being my international bodies. These efforts include the formulation of a TB vaccine and the development of cheap rapid test kits to prevent and identify TB right from the start. Additionally, there is a need to start awareness campaigns, especially in lower and middle-income countries to help people know more about the steps they need to take for participating in TB control efforts. Base-level efforts by local health security entities will primarily result in the reduction of TB transmission and ultimately its eradication.

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Herbal Treatment of Tuberculosis

52

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ABSTRACT

TB is a major threat to public health in the present era. TB as a problem has now become so severe for public health that it has now become a topic of constant debate on international health platforms. Now international health-related organizations have started emphasizing the importance of proper treatment for TB to control and eradicate TB. These programs started getting special attention after the emergence of drug resistance in etiologic agents of TB along with the revelation of the fact the rigorous administration routines of anti-TB drugs are leading to increased cases of hepatotoxicity in patients. This happens due to long dosing regimens of the TB drugs along with high doses being administered to counter drug resistance and eradicate the disease completely.

All these issues call for a new perspective on TB medication. Such an alternative is a medication manufactured from herbal extracts. This option kills two birds with one stone. It battles antibiotic resistance while minimizing the chances of liver damage and hepato-toxicity. The herbal extracts have a low cost of manufacturing and do not contain lethal amounts of toxins rendering them safe for consumption even in large amounts. Such useful characteristics of herbal medicine extracts necessitate thorough research to identify useful plants and the parts required to manufacture effective remedies.

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1. INTRODUCTION

TB or Tuberculosis is one of the most irresistible seasoned diseases to befall mankind (Sharma and Mohan 2013). It is a disease spreading from poor hygiene practices that affects the pulmonary system of the affected individual (Pereira et al. 2005). TB affects a devastating number of almost 8 million individuals annually. This *Mycobacterium tuberculosis* infection then leads to the demise of around 2-3 million patient's unfortunate patients (Dimayuga and Garcia 1991). The old sacred Indian text Vedas referred to TB as wasting illness or Yakshma. Robert Koch was the first scientist who discovered and announced the presence of tubercle bacillus during the monthly evening meeting of the Berlin Physiological Society on the 24th of March 1882. This date of March 24th was later selected to be the 'World TB Day' for crediting its revelation. Every year this day is celebrated to raise awareness among people regarding the control of TB (Sharma and Mohan 2013).

2. NEED FOR USE OF HERBAL MEDICINE

A study revealed that about 5 to 20%of people, undertaking the anti-TB medication were reported to have liver problems. These hepatic complications become severe due to the regimes of drug combinations (Arbex et al. 2010). INH, PZA, ethionamide (EMB), para-aminosalicylic acid (PAS) and RIF were discovered to be the cause of hepatic issues (Saukkonen et al. 2006). Hepatotoxicity is a common occurrence post-use of anti-TB drugs worldwide (Sharma et al. 2004). Clinically affected people display symptoms like abdominal pain, nausea, jaundice and vomiting. The laboratory testing of blood may also reveal increased levels of hepatic transaminases and bilirubin (Sharma and Sharma 2015). The anti-TB drug-related hepatotoxicity is a major reason for increased morbidity and mortality in TB patients undergoing treatment. Under these circumstances, the mortality rate jumps up to 6%- 12% after liver disease onset in TB patients who continue the use of hepatotoxic anti-TB drugs (Ali et al. 2013). The liver is a crucial organ that modulates several physiological mechanisms. It helps in the synthesis of several important molecules such as cholesterol, albumin, bile acid and fibrinogen. The liver is a major producer of proficient foreign molecules transforming enzymes. These enzymes in turn enable the liver to detoxify and metabolize several types of autochthonous (steroids, fatty acids etc.) and heterochthonous chemicals (drugs, insecticides, etc.) (Bedi et al. 2016).

Detoxification of drugs in the body by administration of Cytochrome P450 happens in three phases. These phases include transportation into bile, conjugation and transformation. Any anomaly in either of these phases can lead to hepatotoxicity. The covalent binding of the metabolite or drugs as a whole with host proteins produces oxidative stress that is the leading cause of liver injury (Saukkonen et al. 2006; Ramappa and Aithal 2013). Stress by oxidative agents can lead to damaged intracellular macromolecules. The macromolecules that usually face damage due to oxidative stress include DNA, RNA, lipids, glutathione, ATP and proteins (Bhattacharyya et al. 2014). Liver toxins also lead to the induction of an inflammatory response in the liver. Inflammation of the liver in turn causes activation of STAT3, MAPKs and NFkB signaling pathways (Ambade and Mandrekar 2012). The metabolism of anti-TB drugs leads to the production of several intermediate products that cause liver damage. Such an example is the metabolism of INH which produces acetyl diazine. This is then further processed into acetyl onium ion, ketene and acetyl radical. The conversion enzyme N-acetyltransferase 2 (NAT2) changes acetyl hydrazine to diacetyl hydrazine (DAH) through acetylation. Diacetyl hydrazine is non non-toxic product for cells however, in the presence of INH the process of acetylation is slowed down. A slowed-down acetylation results in an increased amount of non-acetylated hydrazines. These intermediate products can be toxic to the liver and cause hepatic injury (Scales and Timbrell 1982; Ramappa and Aithal 2013). Just like INH, PZA also induces oxidative stress and liver toxicity through the production of 5-hydroxypyrazinoic acid. PZA is converted to

pyrazinoic acid by the activation of the amidase enzyme. The pyrazinoic acid product is then hydroxylated to form 5-hydroxy pyrazinoic acid through a xanthine oxidase reaction ultimately leading to hepatic distress (Shih et al. 2013). Similarly, RIF can also cause toxicity when administered in combination with other drugs. RIF induces enzymatic pathways like (CYP3A4) Cytochrome P450 through (PXR) or hepatocyte Xeno Sensing Pregnane X Receptor. The time duration of the anti-TB drug combination regime is a crucial factor in determining the amount of RIF that could increase the metabolism of INH in turn leading to the formation of toxic metabolic intermediate products. The induction of isoniazid hydrolases in this process leads to increased hydrazine production. Increased hydrazine consequently alters the expression of proteins controlling the process of lipid metabolism (Guldberg Klenø et al. 2004). Prolonged exposure to RIF can produce hepatic distress through altered membrane permeability. This situation leads to considerably reduced activity of glucose-6-phosphate. Reduction in the activity of glucose-6-phosphate is one of the causes of the heightened lipid peroxidation in the liver (Koster and Slee 1980; Saraswathy and Devi 2001; Santhosh et al. 2006; Singh et al. 2016). Hence it can be established collectively that antiTB drugs lead to the production of toxic intermediate metabolites, free radicals and reactive oxygen species (ROS). These agents are the main sources of hepatic injuries. The drugs of anti-TB nature also interfere with normal lipid deposition. The interference produced by these drugs leads to activating CYP2E1, LDL uptake and fatty acid accumulation ultimately disturbing the lipid deposition. (Anundi et al. 1993; Upadhyay et al. 2007; Singh et al. 2016).

3. PREVIOUS ATTEMPTS AT TREATMENT

TB is an exceptionally contagious ailment and around 40% of people from India carry TB germs in their bodies making it the largest reservoir country of the disease (Agarwal 2004). The WHO indicated in 1998 that tuberculosis (TB) is a contagious disease caused by the bacillus *M. tuberculosis* (WHO 1998). Lungs are predominantly influenced by *M. tuberculosis*, this is the main reason leading to lung tuberculosis also known as pulmonary tuberculosis, after infection (Gangadharam 1993). In other instances, various parts and portions of the body can be found affected by TB likewise resulting in extra-pulmonary form of tuberculosis (Sharma and Mohan 2004). TB spreads more effectively in jam-packed environments where there is a lack of healthy practices, hygienic sustenance and poverty (Pereira et al. 2005). The primary source for the transmission of TB is likely the aerosol route through droplets from coughing or sneezing of a TB-affected person resulting in the release of Tubercle bacilli in the surrounding environment (Narwadiya 2011). Some typical symptoms of TB include chest pain, hemoptysis, coughing, fatigue, reduction in weight, and fever (Stanhope and Lancaster 1996). Even though high rates of curability can be achieved through treatment against TB by using antibiotics it is still spreading rapidly (Loddenkemper and Hauer 2010; Sloan et al. 2013). The marvelous weapons of medicine are antibiotics, starting with streptomycin (Schatz et al. 1994) and penicillin (Kardos and Demain 2011), which changed the system of medication after their discovery during the 1940s. These antibiotics provided mankind with suitable remedies for the most commonly occurring diseases of that time. However, hindrance in the advancement of antibiotics has confined the helpful life expectancy of antitoxins. This resulted in the necessity of a constant struggle to present new mixes (Spellberg and Shlaes 2014).

4. TREATMENT OF ZOONOTIC TUBERCULOSIS

The main threat prompted by Tuberculosis as a disease is its potential to rapidly burgeon into a significantly epidemic situation due to its exponentially high rate of transmission. This leads to the classification of TB as a reportable contagious disease. The characteristic variation in clinical appearance and status of TB

requires a thorough understanding of the underlying mechanisms related to the activation of diseases. This knowledge helps in the accurate diagnosis of TB along with helping us to identify the need for further tests (Lee 2018). The severity of tuberculosis is often underscored due to the appearance of secondary lesions. A few of these could even prove to be fatal. The potentially life-threatening situation arising from these lesions can lead to prolonged courses of antibiotic administration. Under certain circumstances of severe nature surgical intervention may also be needed. The severity of the impact made by TB on individual affected is thus linked in a sophisticated manner to the condition of secondary lesions. In an extended attempt to reduce the spread of TB, the main emphasis is placed on adopting a preventive approach. The preventive approach should encompass strict hygiene practices, vaccination of livestock should be enforced, awareness should be raised among the public and the consumption of unpasteurized dairy products should be discouraged (Abe et al. 2003).

Djibouti is rich with medicinal herbs boasting an extraordinary reservoir of biodiversity in terms of plant population. Djibouti houses a broad range of plant species. It holds promise for a stockpile of medically applicable herbs as an alternative herbal medicine. Researchers and scientists are working hard to come up with an extensive exploratory explanation for the options related to the use of these botanical resources. They are also trying to uncover the potential of Djibouti for finding the herbal treatments of diverse diseases and issues (Abdoul-latif et al. 2020; Ainane et al. 2020; Abdoul-Latif et al. 2022A; Abdoul-Latif et al. 2022B; Mohamed Abdoul-Latif et al. 2022A). These exploratory efforts have resulted in the discovery of several plant-based bioreactors as a potential avenue for the production of recombinant therapeutic agents of a protein nature targeted at animal health improvement. This innovative strategy emphasizes the collection of traditional knowledge and bleeding-edge biotechnology to resolve current challenges (Ainane et al. 2021; Mohamed Abdoul-Latif et al. 2022B; Mohamed Abdoul-Latif et al. 2023). The increasing interest in herbal medicine in the realm of veterinary medicinal practice is driven by several aspects. One important driving force out of these factors is the emergence of the idea among people that medicinal herbs have better efficacy and safety in contrast to chemical compounds. This strong belief exists among both practitioners and the general people alike. The existence of this idea led to the growing use of herbal medicine in veterinary practice. The major shift towards herbal remedies reflects an evolution of the veterinary medicine paradigm, aligning this one with holistic aspects and giving it a deeper connection with mother nature (Shin and Park 2018).

At the core of the epidemiological studies related to bovine tuberculosis, lies an enigmatic confusion as it shows disparity in prevalence levels. The disparity becomes evident when contrasting prevalence levels appear at the individual level which is low and an increased level of TB prevalence is seen within herds. This surprising disparity between individual and communal dynamics of TB provides it with a unique disease profile. There are also numerous instances of TB being reported at below-threshold levels across several herds. This intriguing pattern of TB bases itself upon the complex infection dynamics and management practices related to the control of disease within infected people. The nature of these complex dynamics is based upon the complexity integrated into comprehending the spread of bovine tuberculosis. It is further supported by the crucial need for multi-dimensional tactics to limit the spread of TB and for its prevention (Ntampaka et al. 2022).

In the context of the herbal medicinal herbs Djibouti has prime importance for holding most of the medicinal herb species. The effects of bovine tuberculosis on both public health and the productivity of livestock remain somewhat contained in such regions because of these effective plants. The limited number of virulent TB strains that can pose a direct threat to human health via raw milk consumption, is one of the mitigating factors controlling the spread of bovine TB. On the other hand, the existence of even a small number of instances provides the basis for the latent potential of TB transmission and demands

vigilance for its control. As farming methods intensify for better production with the simultaneous evolution of environmental and anthropogenic factors, the latent risks related to bovine tuberculosis's emergence into human populations can be expected. The complex relationship between TB and the wide-spectrum biodiversity of Djibouti presents a multi-dimensional mix of issues and opportunities. The potential of tuberculosis to transform into a proportionally significant epidemic necessitates vigilance of the utmost level. Early detection of disease and the implication of stringent preventive measures are the first steps for its control. Simultaneously Djibouti's abundant plant species should be explored to comprehend how these can offer novel therapeutic remedies crucial to battle TB. Formation of such remedies requires a homogenous mixture of modern science and traditional wisdom. The journey of Djibouti as a remedy calls for collaborative efforts between different domains of knowledge while transcending boundaries to protect the well-being of both animals and humans (Abdoul-Latif et al. 2023; Beyene et al. 2023; Mohamed Abdoul-Latif et al. 2023).

5. MODERN ATTEMPTS FOR TREATMENT OF TB

The latest and most advanced treatment technique for curing TB is dependent on drugs like rifampicin, isoniazid, pyrazinamide and ethambutol. These techniques and the drugs they depend upon are unable to achieve the desired effectiveness (Brigden et al. 2014). Additionally, these drugs are very expensive. Another serious downside of using these drugs is the life-altering side effects that are produced after their usage (Mohan and Sharma 2004; Zazueta-Beltran et al. 2011; Bhatcha 2013). Even if we criminally ignore all those serious side effects the rise of drug-resistant (Gupta et al. 2010; Zazueta-Beltran et al. 2011) and TB etiologic agents, along with the development of geographic-dependent strains (Firdessa et al. 2013) adds fuel to the already fiery situation of TB as an emerging threat in under-development countries of Africa that are already burdened heavy loads of TB. Such a situation calls for a mandatory search to develop new treatment regimens targeting medicinal plants. Hence encouraging the formation of a herbal product that can counter TB while minimizing the chances of developing further antimicrobial resistance (Kloos 1976; Kloos et al. 1978; Hostettmann et al. 2000; Askun et al. 2013; Bhatcha 2013; Andualem et al. 2014).

6. USE OF PLANT ADJUNCTS

WHO has recommended 6 to 9 months of DOTs or Directly Observed Therapy Short as a treatment course for curing TB. In many instances, the drugs used against *M. tuberculosis* infection may produce side effects such as hepatic damage even with the regimen of DOTs combination. The main reason for the liver damage induced by anti-TB is the distinct reaction of metabolism for dealing with these drugs (Rivers and Mancera 2008; Sonika and Kar 2012). The latest approach being followed, to reduce the thereafter side effects and up-surged efficacy of medicinal agents is the basic controlled intake planning. In combination or standalone, the herbs and medicinal plants are effective in reducing the adverse effects of drugs to a minimum. The ethno-medicinal utilization of medicinal herbs against hepatic disease has been documented well in the past. Though along with documentation awareness regarding their uses is also important for effectively treating the disease (Amadi and Orisakwe 2018). Plants and medicinal herbs are major reservoirs of several types of secondary metabolites with broad-spectrum effectiveness. This property enables these plants to play an important role towards the revelation of novel drug moiety against target diseases (Cragg and Newman 2013). The liver protection activities of several herbs against the liver damage induced by anti-TB drugs have been studied in various types of animal models.

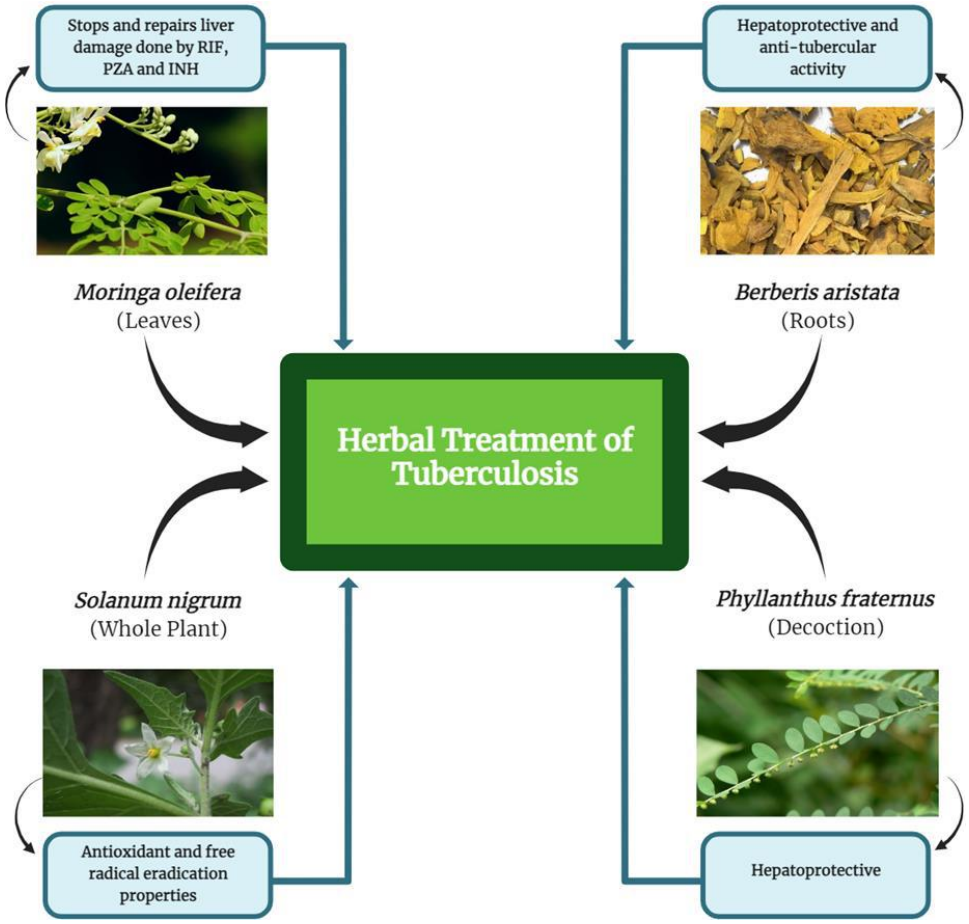

Fig. 1: Different parts of various plants used in the treatment of Tuberculosis. RIF(Rifampin); PZA(Pyrazinamide); INH(Isoniazid).

A study by (Sharma et al. 2004) was based on testing the impact of herbal and anti-TB drug combinations on the livers of the patients ingesting the medicine. Patients were divided into three groups and were studied for 12 weeks. The first group of patients was the one receiving capsules of Aloe vera extracts from the whole plant, whole plant *Solanum nigrum* and roots of *Berberis aristata*. The patients in the second and third groups were given a decoction of *Phyllanthus fraternus* and placebo starch capsules in the same order. At the end of the trial, the activity of liver enzymes and their serum levels were within the normal range in the first and second groups of patients. On the other hand, the third group was found to have a rise in serum levels of AST and ALT. Both the enzymes AST and ALT were used as marker enzymes to identify hepatotoxicity in that study (Sharma et al. 2004). An active source of phytochemicals with both hepatoprotective and antitubercular properties is *B. aristata* (Potdar et al. 2012; Unkeshwar et al. 2013; Mahapatra et al. 2014). Similarly, *S. nigrum* is a plant with potent antioxidant properties that can help it regulate the function of detoxification enzymes involved in the removal of toxic chemicals. It also exhibits free radical eradication characteristics (Lin et al. 2008). *Phyllanthus fraternu* also possesses hepatoprotective properties just like *S. nigrum* (Bera et al. 2011). All these plant extracts act through various mechanisms to produce desired effects (Fig. 2).

Another study by (Debnath et al. 2012) provided information regarding the use of adjunct Ayurvedic therapy with Ashwagandha to manage the pulmonary tuberculosis situation in a patient already ingesting anti-TB drugs. This study claimed that the use of Ashwagandha led to the modulation of the immune system, restoring SGPT and SGOT to their normal levels in the body, while increasing the

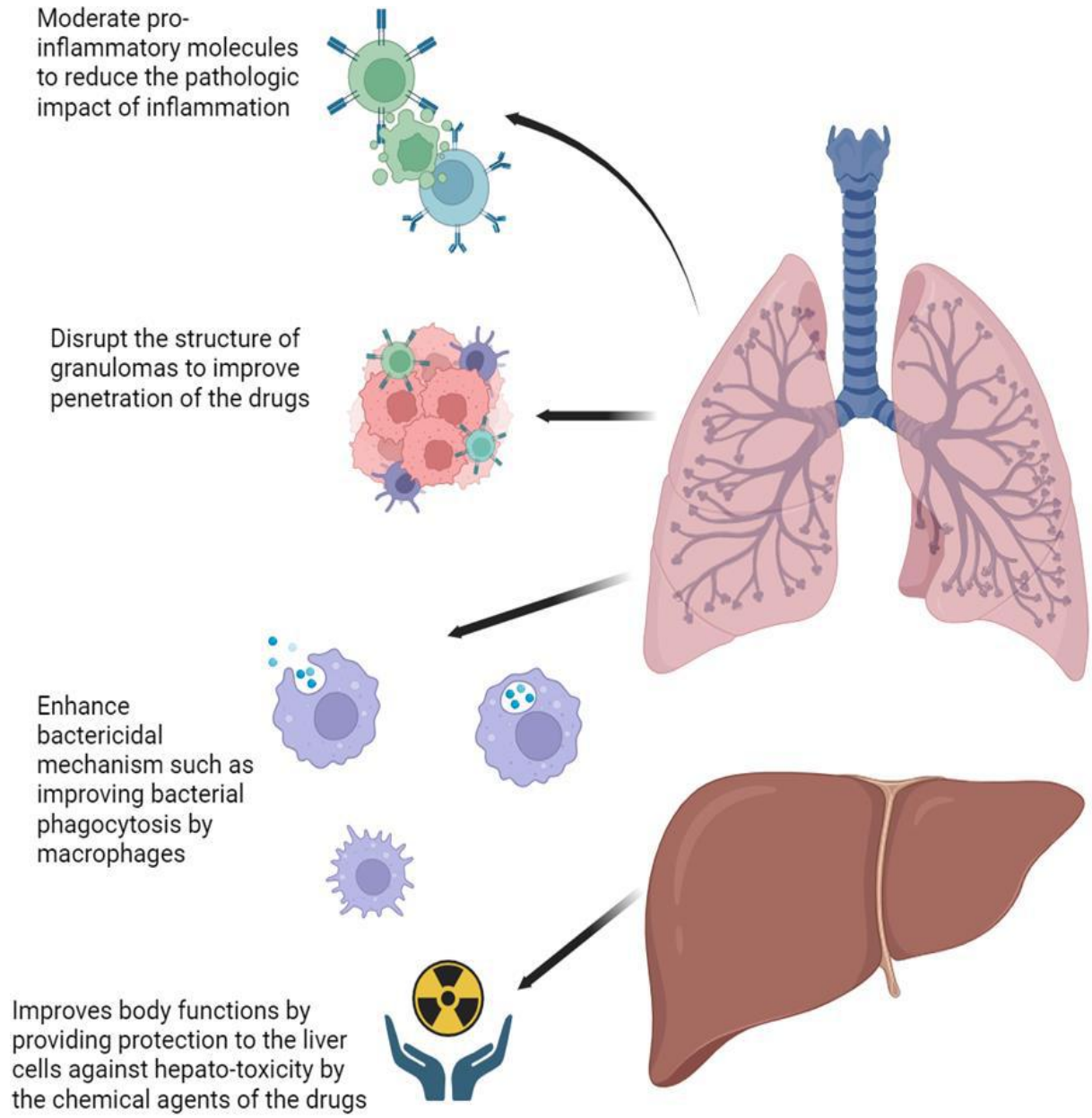

Fig. 2: Different modes of action adopted by anti-TB medicinal herbs to counter the infection and work synergistically with anti-TB drugs.

bioavailability of INH and PYZ at the same time and all this happened within 28 days of treatment against TB. Several in-vitro studies were conducted on animal models using medicinal herbs such as *Ficus religiosa, M. oleifera, Lawsonia inermis, T. chebula, W. somnifera, Tinospora cordifolia, C. auriculata* etc. also support the beneficial effects of these herbs in reduction of liver damage caused by anti-TB drugs. The combined regimen of Ayurvedic and Anti TB drugs can ensure an increase in the chances of survival for patients with pulmonary TB. Various clinical studies have revealed that the patients who were only receiving TB drugs for treatment had a cure and death rate of 11.42 % and 40.9 % respectively. On the other hand, when the patients were given a combined mix of TB drugs and

Table 1: Herbal treatment options for Tuberculosis.

No.	Plant	Part used	Effect	References
1.	*M. oleifera*	Leaf	Stops and repairs liver damage done by RIF, PZA and INH	(Pari and Kumar 2002)
2.	*B. aristata*	Roots	Hepatoprotective and anti-tubercular activity	(Potdar et al. 2012; Unkeshwar et al. 2013; Mahapatra et al. 2014)
3.	*S. nigrum*	Whole plant	Antioxidant and free radical eradication properties	(Lin et al. 2008)
4.	*P. fraternus*	Decoction	Hepatoprotective	(Bera et al. 2011)

Ayurvedic drugs the respective cure and death rates improved to 41.3% and 3.8% (Debnath et al. 2012). A smaller number of systematic studies also supported the effective use of Ayurveda drugs for managing pulmonary tuberculosis disease (Samal 2015). The plant-based preparations can effectively prevent hepatotoxicity and increase the viability of treatment outcomes (Table 1). Additionally, the use of these herbal drugs is free from of any toxicity or side effects that usually accompany the anti-TB drugs (Adhvaryu et al. 2008).

8. CONCLUSION

The main agenda of today's TB researchers is to provide mankind with a fulfilling remedy that can eradicate the disease in the patient while keeping the body of the host safe from the adverse effects of the drugs. The two main aspects of introducing plant-based medicine to treat TB are the use of plant extracts and the challenges faced in applying these remedies. The methodology of using herbal medicine for tuberculosis is being researched thoroughly across the world.

Herbal medicine has been explored as a potential treatment for tuberculosis due to the need for alternative treatment approaches. Several studies have investigated the use of plant adjuncts for the treatment of tuberculosis. Many challenges being faced in implementing herbal treatments are being resolved and new opportunities are being created in the herbal treatment section of the battle against TB. Challenges in the herbal treatment of tuberculosis include the need for further research and clinical validation. Opportunities exist for the development of new herbal-based therapies for tuberculosis treatment.

The leaf of *Moringa oleifera* is a major source of phytochemicals including terpenoids, flavonoids, saponins, alkaloids, carbohydrates, tannins, and glycosides. A study on these leaves reported that oral intake of *M. oleifera* leaf extracts has wondrous effects as it repairs normal liver activity in rats countering the hepatic damage done by RIF, PZA and INH (Fig. 1). These leaf extracts also appear to enhance the recovery of the liver from hepatic damage and restore the functioning of enzymes to normal including normality of factors like AST, lipid peroxidation, ALS, bilirubin and alkaline phosphatase in blood serum (Pari and Kumar 2002). The root extracts of *Cassia auriculata* have a significant impact in lowering the above-normal serum levels of ALT, AST, ALP, cholesterol, protein and total bilirubin found in the blood as a side effect of using anti-TB drugs that lead to hepatotoxicity. These root extracts also maintain the normal levels of the marker of oxidative stress; Malondialdehyde (MDA) and enzymatic antioxidants (Jaydeokar et al. 2014). One of the most highly valued medicinal herbs in Ayurvedic pharmacopoeia is *Terminalia chebula*. It has antioxidant properties along with activities of cell membrane stabilization. The fruits of this plant can prevent hepatotoxicity induced by the intake of anti-TB drug combinations (Tasduq et al. 2006). Plant-based formulations made of *T. chebula* also tend to be hepatoprotective. A study by Sankar et al. (Sankar et al. 2015) revealed that the multi-herbal formulation comprised of *Phyllanthus amarus, Tephrosia purpurea, Cycas circinalis, Pinius succinifera, Curcuma longa, Eclipta alba, Pistacia lentiscus, Orchis mascula, Withania somnifera* and *Picrrohiza kurooa* show supreme effectiveness against the oxidative injuries caused to the liver by INH and RIF in rats. The combination regime of medicinal plants

and anti-TB drugs for enhancing the treatment efficacy with minimal side effects also got supportive results from several clinical trials undertaken in the past.

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Use of Nanotechnology to Mitigate Tuberculosis

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ABSTRACT

Tuberculosis is a disastrous malady spreading exponentially throughout the world. TB has been long identified as a threat to global health by several international bodies but this threat was amplified upon the identification of multi-drug-resistant germs of TB. Such a scenario arose from several factors regarding TB. Some important factors in this aspect include lack of awareness as TB is prevalent in low-income, low-education countries and exhaustive treatment regimens for TB. Similarly, the adverse effects of drugs are also a factor leading to hampering proper dose implementation. Liver damage caused by anti-TB drugs often leads to a pause in TB medication in turn contributing to increased antibiotic resistance to etiologic agents of TB. Such problems can be easily overcome by developing antibiotic alternatives that can battle antibiotic resistance while reducing the length and adverse effects of the TB medication. Such an option regarding antibiotic alternatives is the use of nanotechnology and nanomaterials. These substances can help us battle anti-microbial resistance by bypassing the defense mechanism of TB bacteria while simultaneously preventing side effects of the drugs through reduced doses.

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1. INTRODUCTION

Tuberculosis is a disastrous malady spreading exponentially throughout the world. TB has been long identified as a threat to global health by several international bodies but this threat was amplified upon the identification of multi-drug-resistant germs of TB. Such a scenario arose from several factors regarding TB. Some important factors in this aspect include lack of awareness as TB is prevalent in low-income, low-education countries and exhaustive treatment regimens for TB. Similarly, the adverse effects of drugs are also a factor leading to hampering proper dose implementation. Liver damage caused by anti-TB drugs often leads to a pause in TB medication in turn contributing to increased antibiotic resistance to etiologic agents of TB. Such problems can be easily overcome by developing antibiotic alternatives that can battle antibiotic resistance while reducing the length and adverse effects of the TB medication. Such an option regarding antibiotic alternatives is the use of nanotechnology and nanomaterials. These substances can help us battle anti-microbial resistance by bypassing the defense mechanism of TB bacteria while simultaneously preventing side effects of the drugs through reduced doses.

2. WHY THE USE OF NANOTECHNOLOGY?

The bacteria *Mycobacterium tuberculosis* (Mtb) is the etiologic agent of tuberculosis (TB) and has dawned on mankind as bad news since antiquity. Despite its long record in past time, TB is still one of the 10 major causes producing death toll in the whole world both developed and under-developed countries included. It is the deadliest infectious disease. It is more serious than other life-threatening illnesses like acquired immune deficiency syndrome and human immunodeficiency virus infection (HIV/AIDS) (WHO 2020). The number of TB cases in 2018 was projected to be 10 million. In the same year, this illness claimed the lives of around 1.5 million people, 251,000 of whom were also HIV-positive. This amount sums up to a staggering number of 4000 fatalities per day (WHO 2020). One of the latest reports from the World Health Organization (WHO) has claimed the existence of infection in 23% of the global population with the latent phase of Mtb. This means that 23% of the global population cannot transmit the infection and are asymptomatic (WHO 2020). TB patients with latent infections however have a chance range starting from 5% up to 15% of developing active TB disease for a lifetime. The rate of infection re-emergence is higher in individuals with immunocompromising diseases such as Human Immuno-Deficiency Virus infection, undernutrition, and diabetes (Getahun et al. 2015). This is the reason for the higher incidence rates of TB in areas where many of these conditions are prevalent among the population. Among these regions are Africa and Southeast Asia, which together account for 68% of newly diagnosed cases of tuberculosis (WHO 2020). For drug-sensitive TB, a 2-month regimen of isoniazid (INH), ethambutol (ETB), pyrazinamide (PZA), and rifampicin (RIF) is now advised. After completing this course, a 4-month RIF plus INH phase should be implemented (Nahid et al. 2016; WHO 2018). WHO's 194 Member States routinely report drug-sensitive tuberculosis cases to it with cureability rates of at least 85% (WHO 2020). The major reason for a combined drug prescription of anti-TB drugs is to minimize the chances of drug-resistance development in Mtb strains, by attacking them with different modes of action all at once. However, the growth of drug-resistant TB strains has been caused by the inconsistent medication supply, patients' noncompliance with the treatment protocol, and the wrong execution of drug intake regimens due to a lack of competent monitoring (Aziz et al. 2004). Multidrug-resistant (MDR) strains are Mtb bacteria that are resistant to the preferred medications, RIF and INH. Conversely, MDR Mtb bacteria that contain an extra resistance to a fluoroquinolone medication (such as levofloxacin or moxifloxacin) in addition to a second-line injectable medication (like kanamycin, amikacin (AMK), or capreomycin) are referred to as extensively drug-resistant (XDR) strains. Additionally, during the past several years, numerous reports of cases involving all first- and second-line drug-resistant strains of tuberculosis have surfaced (Dheda et al. 2014).

3. PATHOGENESIS

Being an obligate intracellular pathogen, Mtb has unique human reservoirs. Mtb infection is initiated by the touch down of its bacilli in the lungs after inhalation of contaminated droplets expired by an individual affected with the active form of pulmonary TB (droplets can have expelled by inhalation at a great speed as in case when a patient coughs or sneezes). Once the bacilli reach the space of alveoli, they become internalized by the phagocytosis of alveolar macrophages (AMs). The innate immune system's primary action cells are alveolar macrophages. Pathogen-associated molecular patterns, or PAMPs, are recognized by the host cell's PPRs, or pattern recognition receptors. PAMPs are typically seen on the bacilli's outermost membrane during invasion (Figure 1). PAMPs cause the release of cytokines and chemokines, which in turn causes other phagocytic cell types, such as neutrophils, dendritic cells, and interstitial macrophages, to assault the infection site (Akira et al. 2006; Philips and Ernst 2012). In several cases ranging from 20 to 25% of the cases, macrophages can thoroughly quarantine the infection by eliminating the invading agent through phagocytosis (Verrall et al. 2014). However, the majority of invading Mtb bacilli manage to escape elimination by immunity cells (Gengenbacher and Kaufmann 2012). The Mtb saves itself by residing in the phagosome cells by the mechanism of the ESX1 secretion activation system. This system releases several other molecules along with proteins that target the internal systems of the phagosome. These attacks disrupt the membrane of the phagosome subsequently leading to the release of mycobacterial components. These components are released into the cytosol of the macrophage.

These molecules and proteins enable Mtb to arrest the maturation of the phagosome by hindering the fusion of lysosomes. Then Mtb moves on to transform the highly hostile environment inside a phagosome into a somewhat milder and survivable condition for itself. This enables it to replicate to replicate inside the macrophage once it survives through harsh conditions (Pai et al. 2016; Cheung et al. 2019). As the infected phagosome cells migrate to the lymph nodes of the pulmonary system they are processed and presented with antigens for priming of T cell priming (both CD4+ and CD8+). The T cells are produced by the adaptive immune response system, 15–18 days post-Mtb infection. These T cells then migrate to the site of the Mtb infection guided by the chemokines produced by infected cells (Zuñiga et al. 2012). Consequently, the pathogen can:

- Face elimination by the immune response cells of the host
- It may progress to active disease conditions (this situation mainly pops up in immunocompromised hosts)
- It may sustain itself in specific structures called granulomas– the typical pathological sign of tuberculosis (Pai et al. 2016).

4. CHALLENGES IN THE TREATMENT OF TUBERCULOSIS

Collectively, the occurrence of TB infection happens as a dynamic process comprising several pathological phases of granulomas coexisting (solid, caseous, and cavitary granuloma) simultaneously. This forces the bacilli to adapt to an ever-changing array of microenvironments for the sake of survival. The sub-optimal drug concentration levels achieved in the caseum of necrotic granuloma along with the resistance capabilities of dormant bacteria against anti-TB drugs make their eradication challenging. Resultantly, the non-replicating bacteria, which may seem harmless due to the asymptomatic nature of the infection, serve as TB re-activation reservoirs and drug-resistance gene pools. Additionally, a situation like an LTBI may emerge after a drug-based treatment of active infection disease. Such circumstances may arise if the bacteria are not fully eradicated during the treatment. The remaining few that are still present in the body can maintain themselves in a latent phase which later leads to relapse of the disease after a certain amount of time has passed (Behinaein and Cirillo 2019; WHO 2020). These contributing factors also help us to

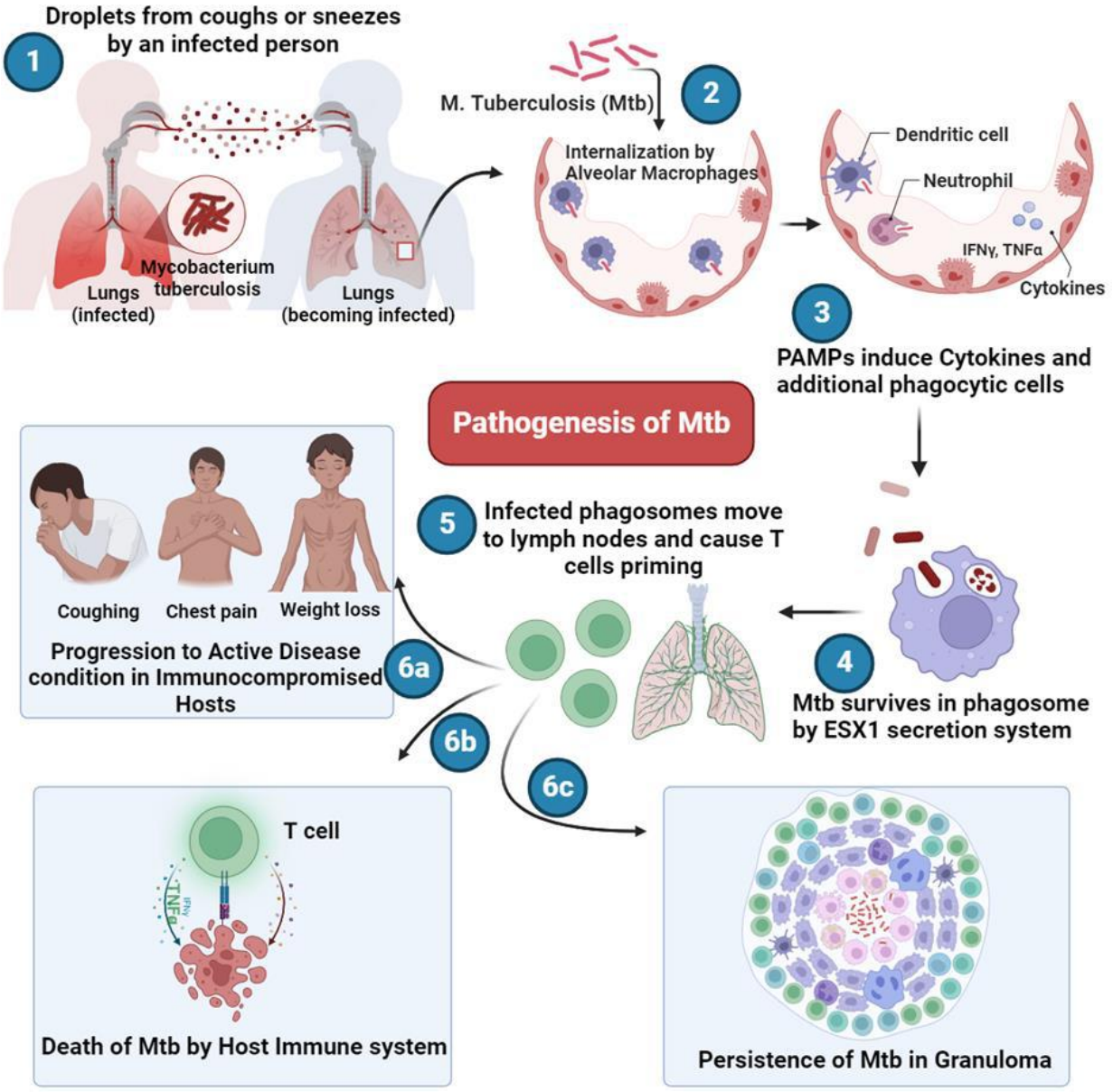

Fig. 1: Pathogenesis of *M. tuberculosis* infection.

comprehend the long duration of TB treatments mandatory for curing active TB infections. This much time is implemented for anti-TB drug administration to eradicate the infectious agent thoroughly. The drugs have high priority and more time to target the bacteria in the granulomas inside host cells.

TB or Tuberculosis has been a major killer disease around the globe. Even its treatment is arduous as it requires several first-line anti-TB drugs to be administered regularly for at least 6 months to get rid of the disease. Still, this is a tricky and arduous move to try and control TB with the administration of antimycobacterial drugs for several reasons including:

- Treatment through chemotherapy requires drug administration for a long duration,
- The effectiveness of the antimycobacterial drugs to reach targets and produce antimycobacterial effects upon them is sub-par

- The effectiveness of TB drugs is reduced due to various reasons such as poor stability and permeability
- All antimycobacterial agents are usually toxic to normal body cells too
- A large number of TB patients show non-compliance to prescribed medication protocol this factor can have attributed to the fact that TB therapy is very lengthy and has severe life-altering side effects.

Liver damage and hepatotoxicity are highly common and sometimes deadly adverse effects seen when these anti-TB medicines are used. Doses have an impact on these side effects. In addition, using INH also results in neurotoxicity. When EMB is used, eye toxicity results. In a similar way, STR results in permanent nephrotoxicity and ototoxicity even if it does not harm the liver. Since most of these issues are related to insufficient drug administration techniques, drug delivery technology and the scientific community, particularly formulation scientists, face significant challenges (Du Toit et al. 2006).

5. USE OF NANODELIVERY SYSTEMS

The use of nanocarriers-based drug delivery systems is an innovative and modern strategy denoting the future of medicine in the war against several types of diseases. The major advantages of drug delivery systems based on nanocarriers over free drugs are improved drug bioavailability and controlled drug release with desired dosage over a timeline according to the need of the treatment regimen. It also protects the drug agent by preventing it from inactivating through the entrapment of the chemical agent. The controlled drug release system keeps the drug dose maintained at the desired level. This reduces the number of doses administered to the patient. Reduced doses in turn minimize the adverse effects of the drug administration and intake frequency (Costa-Gouveia et al. 2017). For effectively targeting *M. tuberculosis* reservoirs, nanocarriers of various several types have been developed. Some of the many common examples of nanocarriers include liposomes, polymeric nanoparticles, nanocapsules, solid lipid nanoparticles, micelles, nanogels, inorganic nanocarriers, dendrimers etc. There are various methods of integrating chemotherapeutic agents into nanocarrier systems. Some common examples of these integration methods include but are not limited to adsorption, physical encapsulation, chemical conjugation etc. The most significant aspect regarding the utilization of nanocarrier systems is their potential ability to finely target the host cells either through passive accumulation or active targeting (Costa-Gouveia et al. 2017; Ladavière and Gref 2015).

6. IMPORTANT FACTORS TO BE CONSIDERED FOR USING NANO-DELIVERY

To produce maximum therapeutic benefits from a drug it must be made carefully. This extreme care system implemented in drug formulation practices forms the core concept behind an efficient drug delivery system (Fig. 2). The four "D's" stand for the four qualities that a drug delivery system should possess. These consist of disease, drug, delivery, and destination. The disease is the sole changeable factor among these four (Jiang et al. 2007). When the pace, location, or both of a medication release are altered, a modified-release system is created. Most often, encapsulation methods are utilized to create customized release systems. This is a widely used technique for creating controlled drug release systems that are heavily utilized in the pharmaceutical sector.

While other non-polymeric drug carriers, such as lipids, can also be utilized in the form of solid lipid nanoparticles (SLNs), chitosan, alginic acid, and poly(lactide-co-glycolide) (PLG) are among the polymers that have been shown to provide good results. Researchers from all over the world are becoming more interested in liposomes because of their promising applications in the field of medication delivery systems. Unrelated to the carrier system, a drug delivery system's main objective is improving the drug's bioavailability. Increased drug bioavailability can be achieved by bypassing the potential factors that can influence it hindering its maximum effectiveness. Various nano-particle approaches have been formulated

and presented to the world for improvement in delivering chemotherapeutic drugs to their exclusive target sites. By aggressively localizing the chemotherapeutic agent's chemical and pharmacological action on the intended location or organ, this strategy serves to increase the therapeutic index value of the drug. This method also reduces the unfavorable side effects of the medications and non-target assaults. Therefore, it can be concluded that drug delivery systems based on nanoparticulates can also contribute in increasing tolerance to toxic chemotherapy by lowering the lethal index value of the medication while concurrently increasing its bioavailability (Shegokar et al. 2011). It can be noted from the current knowledge that the application of nanocarrier systems for anti-TB drugs is suspected to produce various benefits for TB patients including, thorough treatment through utilization of smaller doses, it will be further complemented by the overwhelming response of first-pass metabolism, bypassing the gastrointestinal tract and the large number of efflux systems associated with it and dodging the pH-dependent or enzymatic degradation.

7. TYPES OF NANO-DELIVERY SYSTEMS

Polymeric nanoparticles or NPs are drug carrier systems. They are included in the nanoparticles category as they have a diameter of less than 1 m. The nanoparticle agents like nanospheres (NSs) and nanocapsules (NCs) differ according to their structural and compositional organization (28). Nanoparticles consist of a wide variety of polymeric shells encompassed by an oily core. The desired drug may be dissolved in this core of the nanoparticle agent. Another option is the adsorption of the drug to the polymeric wall. In contrast to this system, there is another formulation for nanoparticles that do not have oil in their ingredients. They are formed by a polymeric drug-entrapping matrix that entraps or adsorbs the drugs (Vauthler-Holtzscherer et al. 1991; Allémann et al. 1993; Puisieux et al. 1994; Bhardwaj et al. 2005; Jones et al. 2008). These nanoparticle systems have been developed by scientists for several types of therapeutic applications in the pharmaceutical industry. The main applications of these systems include the delivery of drugs administered through parenteral, ophthalmic or oral administration (Brasseur et al. 1991; Puisieux et al. 1994; Couvreur et al. 1995; Yoo et al. 2000). Nanoparticles can improve the solubility of comprising agents. Nanoparticles also reduce the therapeutic dose of a drug by improving its absorption of active ingredients. Additionally, the nanoparticles have several advantages when administered in the blood vessels as they lack thrombogenic properties, inert, non-toxic, non-inflammatory (they do not activate neutrophils), and non-immunogenic, all while avoiding the invasion of the reticuloendothelial system of vessels. Occasionally, PNs are used to reach target tissues or work at the surface of the cell (Schaffazick et al. 2003; Alexis et al. 2008; Saraf 2010; Kumari et al. 2010) of information gained by the characterization of these parameters can direct the proposition of models depicting the organization of the nanoparticles on a molecular level, which will be dependent on the qualitative and quantitative composition of the formulations. Moxifloxacin (MX), an antibiotic belonging to the fluoroquinolone class of drugs, has been discovered to be effective against *M. tuberculosis* with its potential reach close to RIF (Gosling et al. 2003). Yet, its intracellular activity against *M. tuberculosis* in macrophages is low. MX-poly (butyl cyanoacrylate) (PBCA) nanoparticles were created by (Kisich et al. 2007) to enhance the efficacy of MX against intracellular M. tuberculosis in macrophages. Moxifloxacin (MX-NP)-loaded PBCA nanoparticles were created by anionically polymerizing n-butyl-2-cyanoacrylate in the presence of a chemical agent. By measuring the particle size and polydispersity of the size distribution, MX-NP was successfully characterized. Compared to pharmacological agents without encapsulation, MX that had been encapsulated quickly and roughly three times more efficiently digested in macrophages. It was discovered to stay in the extracellular matrix of the macrophages for six times longer than the free drug did. When M. tuberculosis was positioned intracellularly, encapsulated MX was able to limit development at a concentration of 0.1 gmL−1, whereas free MX required a concentration of 1 gmL−1 to provide the same result. The process of encapsulating MX in PBCA nanoparticles improved its intake and half in the macrophages and resulted in an elevated drug

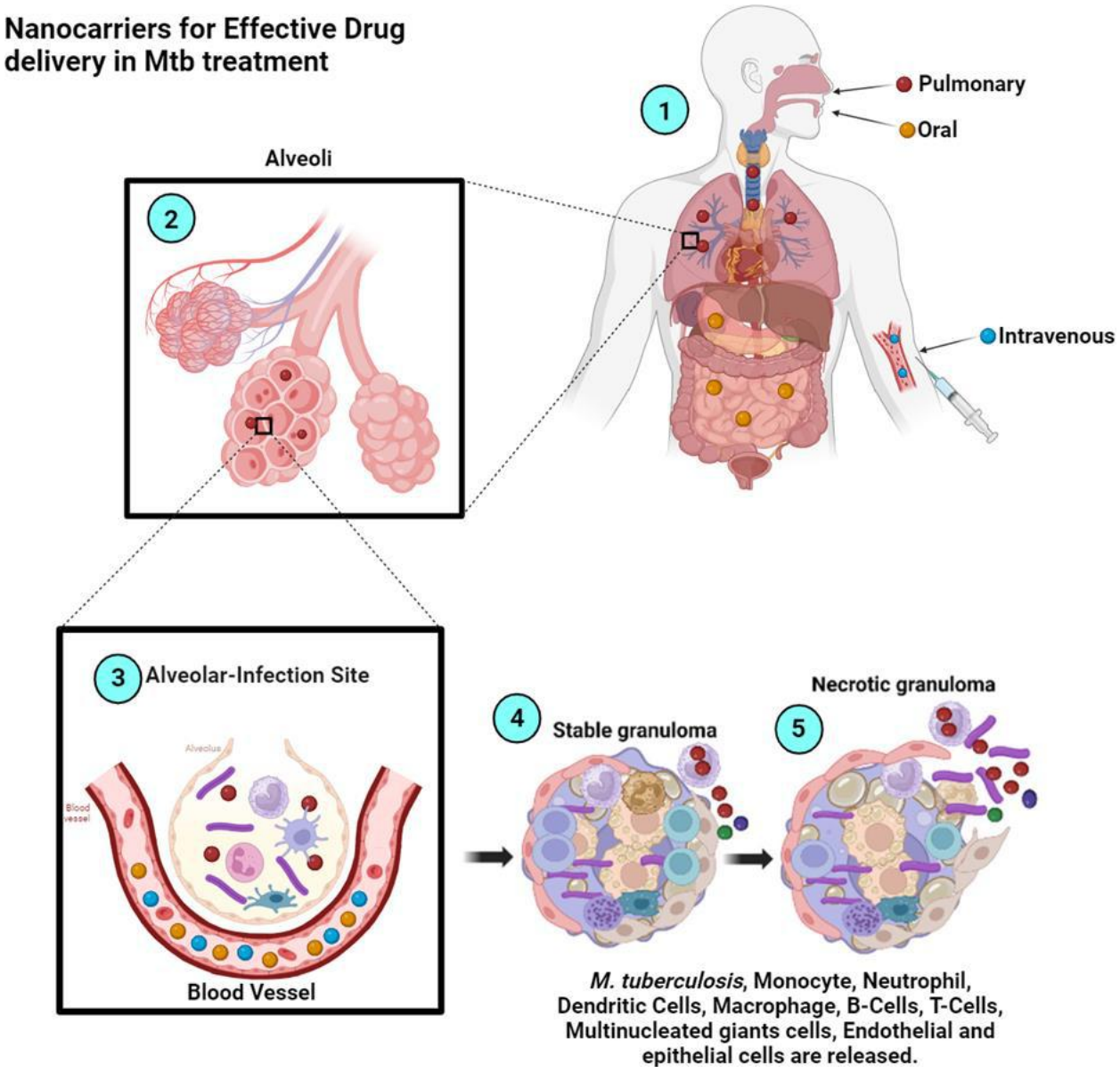

Fig. 2: Nanocarriers for drug delivery in Mtb via different routes of administration.

efficacy against tuberculosis inhabiting macrophages. A variety of techniques have been proposed in the literature for the preparation of polymer nanoparticles (PNs). These can be broadly categorized into methods based on the polymerization of dispersed monomers (alkyl cyanoacrylate) in situ (Gallardo et al. 1993; Chouinard et al. 1994; Lenaerts et al. 1995; Sakuma et al. 1997; Lambert et al. 2000) or the precipitation of preformed polymers (Guterres et al. 1995a; Espuelas et al. 1997; Quintanar-Guerrero et al. 1998; Marchais et al. 1998; Quintanar-Guerrero et al. 1998; Santos-Magalhães et al. 2000). These include poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(-caprolactone) (PCL), methacrylic acid copolymers, and acrylic or methacrylic ester. Irrespective of the method used to prepare, the products are formed as colloidal suspensions in aqueous phase. Nevertheless, the storage time can cause the aggregation of the nanoparticles in the middle of the

suspension, bringing about the formation of precipitates (Molpeceres et al. 1997; Schaffazick et al. 2002) In addition, problems with the chemical stability of polymer and raw materials including drugs, are likely to occur (Guterres et al. 1995b; de Chasteigner et al. 1996; Saez et al. 2000). The mentioned problems can be reduced by employing drying processes, such as freeze drying or sublimation, that allow dehydration while averting particle degradation (Franks 1998; Schmidt and Bodmeier 1999). based on colloidal nature, technical difficulties are dealt with in the physicochemical characterization of NPs. The characterization of the suspensions involves a morphological evaluation, a determination of particle size, an analysis of the molar mass distribution of the polymer, the determination of zeta potential, a measurement of the pH, the determination of the amount of drug affiliated with nanostructures, examination of the drug release kinetics and measurement of stability over some time (Schaffazick et al. 2003).

8. NANO-STRUCTURED LIPID CARRIERS AND SOLID LIPID NANOPARTICLES

Solid Lipid Nanoparticles or SLNs are manufactured from lipids that are stabilized by surfactants and become solid at room temperature as well as body temperature. The waxes, triglycerides, or a mixture of glycerides are the types of lipids utilized for the manufacturing of SLNs. The utilization of the very same types of lipids produces perfect crystals with high organization, which decreases the encapsulation efficiency, which ranges from 25–50% for these systems. Moreover, during storage, the drug may be expelled from the particle after the polymorphic transition from the form to the form, which is more stable. Therefore, to enhance the efficiency of the encapsulation and reduce the expulsion of drugs during its storage in the nanoparticles. NLCs of three different types have arisen from the basic structures. The first model of NLCs is known as “imperfect NLCs”. It comprises a combination of various glycerides made up of variable types of fatty acids. The combination methodology increases the distance among the fatty acid glyceride chains producing imperfections in the crystals. These imperfections produce more open space among the chains to fit in more drugs. These increased spaces in turn increase the encapsulation efficiency of the NLCs. The other model called for NLC formulation is “amorphous NLCs”. It is a combination of lipid liquids with lipid solids. An example of such lipids is Miglyol (triglyceride, caprylic/capric); when large amounts of liquid lipids are mixed with solid lipids it leads to the production of particles in an amorphously solid state. This prevents the expulsion of the drug during storage since the process of lipid crystallization does not occur under these conditions. The third model is called “multiple NLCs;” this is a dispersive immersion of liquid lipids in solid lipids along with water. In this type of NLC, the liquid lipid molecules mix the lipid solid with exceeding solubility producing a separation of phases and the appearance of liquid lipid nanocages into the matrix of solid lipids where the drug is encapsulated (Souto et al. 2007). A major advantage of SLN is its superb chemical and physical inertness, making it a suitable partner for the protection of the labile drugs from falling victim to degradation. It also grants drugs with the ability to be introduced into the body through various routes, such as oral, parenteral and cutaneous due to their minute size and enhanced biocompatibility (Souto et al. 2011). Another advantage of these systems is the controlled drug and the ability of the SLNs to make water-insoluble drugs soluble in this solvent, enhancing drug absorption (Mehnert and Mäder 2012; Taveira et al. 2012; Potta et al. 2010). SLNs and NLCs may be produced by melt emulsification or using ultrasound, mechanical mixing, high-pressure homogenization, emulsification-evaporation of the solvent, or microemulsions. The emulsification solvent evaporation yields particles with smaller sizes due to the lower viscosity of the internal phase since the lipid is dissolved in an organic solvent rather than being melted. This method has the advantage of avoiding exposure to the active compound at elevated temperatures but shows the disadvantage associated with the use of organic solvents (Souto et al. 2011).

9. FUTURE PERSPECTIVES OF NANOTECHNOLOGY

Nanotechnology is a powerful weapon in the battle against drug-resistant TB. Additionally, the combination of nanocarrier-based systems of drug delivery with the pulmonary administration methodology provides physicians with one of the most encouraging approaches to treating TB thoroughly. The use of pulmonary delivery in medication administration systems creates a non-invasive way to consume drugs. This application, especially with systemic action medication intake, has a promising future (Rani et al. 2018). When combined with the pulmonary administration technique, a significant component of the development of a nanomedicine-based strategy with long-term potential can be utilized as a critical component for tuberculosis control, particularly in developing nations. These are the areas without adequate healthcare systems for the majority of its residents (Salamanca-Buentello et al. 2005). The patient itself can use the nanoparticle medications administered with an inhaler device. This increases its use and accessibility for TB patients as a tool for anti-TB treatment. This minimizes the bulk of the expenses associated with treating tuberculosis by lowering the requirement for specialized medical equipment and staff, despite the increase in the number of research reports being published to present the advantages of drug administration through the respiratory route. However, the lack of uniform and effective techniques for administering drugs in pre-clinical trials generally leads to the production of poor results and ultimately translates the methodology as a low-success project. Passive inhalation of the chemical agent is required for the fundamental usage of inhalation devices for delivery through the respiratory route. Variations in lung capacity and inhalation lead to differences in the amount of medication administered. On the other hand, a precise assessment of the amount going into the lungs is required. This is a critical aspect since ineffective medication delivery can lower its effectiveness due to drug loss in the storage container, aerosol drug-generating device tubes, delivery device attachments, and the animal's nasopharyngeal region. These losses are often mistaken for the ineffectiveness of the medications being used to treat tuberculosis. Due to inadequate dosage, it can be a risk factor for the emergence of drug resistance. Due to such issues, researchers prefer invasive techniques to deliver drugs to the respiratory system. These techniques include intratracheal intubation to achieve better and more accurate delivery of the dose of the drugs in the lungs. This is why most preclinical models are markedly different from inhalation methodologies used in humans making the results from experiments and trials a bit unsure for the field application (Kunda et al. 2018).

10. CONCLUSION

Rapid transmission of TB in the human population was already a threat to global health that was amplified by the emergence of antibiotic resistance among *Mycobacteria*. This is a matter of grave concern as it means that all previous medications will fail to battle TB and assist in its treatment. This dire threat This situation called for the development of an alternative method to overcome bacterial resistance and increase the effectiveness of anti-TB drugs. Such an alternative was identified to be nanotechnology. Nanoparticles can be used to bypass antibiotic resistance mechanisms of the bacteria increasing the bioavailability of the drug. On the other hand, the nanopolymers and other nanotechnology-based carrier systems can be used to adjust the dosing regimen of antibiotics to make the treatment less exhaustive for the patient while lowering its toxic effects at the same time. Researchers have been working tirelessly to formulate more effective and less toxic nanoparticles that can be manufactured economically and safely for mass production. This will enable the coating of all drugs in nanomaterials leading to an increased efficiency of the medicine as an overall effect.

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Zoonotic Aspect of Vancomycin Resistant Staphylococcus Aureus

54

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ABSTRACT

Staphylococcus aureus is one of the most ubiquitous organisms found all across the globe. They usually colonize the nasal cavity or outer surface of the human body, behaving as a commensal or pathogen according to the conditions. In the past, they were easily fended off by using regularly available antibiotics. This misuse of antibiotics against *S. aureus* soon led to the development of antibiotic resistance in the bacteria. At first, it became resistant to the methicillin group of antibiotics. An alarm was raised among physicians due to the lost effect of methicillin antibiotics and they soon switched to vancomycin. Vancomycin proved effective even in the case of methicillin-resistant bacteria. However, this remedy soon met its end as vancomycin-resistant isolates of *S. aureus* were discovered later on. Although most of the isolates were from animal sources, it still threatened global public health due to the phenomenon of zoonosis leading to the transfer of vancomycin-resistant *Staphylococcus aureus* (VRSA) to the human population. This is a matter of grave concern for the health security of the human population as zoonosis can lead to further aggregation of already rising antibiotic resistance reducing the overall effect of antibiotics. This will in turn produce diseases that will be incurable with the current antibiotics we have available. A forecast of such incurable maladies suggests that humanity will be once again facing the same era of health problems as it faced in medieval times. Commonly curable diseases will become fatal for mankind and life expectancy will begin to reduce considerably. This will halt the progress of humanity pushing us back hundreds of years. Such issues require the implementation of strict rules regarding the prescription and use of antibiotics. Furthermore, stringent regulations should be implemented to prevent the spread of infectious diseases. Reduction in disease prevalence will ultimately lead to less use of antibiotics and hence lower chances of resistance development in bacteria.

Keyword: *Staphylococcus aureus*, VRSA, Vancomycin, Antibiotic, Zoonosis.

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1. INTRODUCTION

Being an opportunistic commensal, *S. aureus* is becoming a significant human pathogen. Life-threatening infections such as endocarditis, osteomyelitis, bloodstream infections, lung abscesses, and sepsis can be brought on by invasive *S. aureus* which is multidrug-resistant. Septic shock can also be produced by *S. aureus*. In contrast to the previously mentioned structural elements, these superantigens can cause a sepsis-like condition by starting a "cytokine storm." According to Ladhani et al. (1999), certain strains also generate exfoliative toxins called epidermolysins, which can result in bullous impetigo or scalded skin conditions. An opportunistic pathogen that may infect both humans and animals, *S. aureus* can cause food poisoning and a wide range of illnesses, from infections of soft and skin tissue to dangerous conditions like pneumonia, endocarditis, osteomyelitis, septicemia, and toxic shock disorders (Chen and Huang 2014). The abuse of antibiotics (e.g., using antibiotics without a prescription, taking antibiotics in excess, and applying medications needlessly) has contributed to a progressive growth in drug resistance in *S. aureus* in recent decades, which has caused bacterial progression (Gharieb et al. 2020; Guo et al. 2020).

2. VANCOMYCIN-RESISTANT *S. AUREUS*

Resistance to vancomycin One of the most frequent germs to colonize human and animal nasal cavities and exterior body surfaces is *Staphylococcus aureus*. *S. aureus* is a type of bacteria that can cause a variety of infectious disorders and can exist as both pathogenic and commensal bacteria (Weese and van Duijkeren 2010). Vancomycin (VAN) is the preferred antibiotic for the treatment of several infections because methicillin-resistant *S. aureus* (MRSA) strains have drawn attention to their public health relevance since they were first identified in 1961 from human patients (Lowy 1998). But when the USA's Centers for Disease Control and Prevention (CDC) reported the first *S. aureus* strain resistant to both methicillin and VAN in July 2002, things took a turn for the worst (CDC 2002). The dromedary camel, also known as the one-humped camel or Camelsus dromedaries, is a significant type of cattle in the Middle East that is adapted to hot, arid climates. In Egypt, camels are regularly killed and their meat is eaten all year round by people. It was previously believed that camels were immune to the majority of diseases that commonly affect cattle. Nevertheless, new research has shown that camels are susceptible to a wide range of pathogens, and as a result, it is now thought that camels serve as a reservoir or carrier for the spread of various zoonoses and transboundary animal diseases (Graveland et al. 2011). For a very long time, vancomycin has been seen as the last option for treating infections of MRSA (Holmes et al. 2015). Overuse of the drug led to the emergence of vancomycin-resistant *S. aureus* (VRSA), vancomycin-intermediate *S. aureus* (VISA), and heterogeneous vancomycin-intermediate *S. aureus* (hVISA) strains (Amberpet et al. 2019). MRSA strains can generate biofilm as a growth and survivability mechanism in addition to their resistance to antibiotics. This capacity is facilitated by the strains' strong compliance, increased drug resistance, and decreased sanitiser efficiency (Brady et al. 2007; Craft et al. 2019). Coagulase negativity With a few rare exceptions, *S. aureus* (CoNS) and *S. aureus* were both susceptible to glycopeptides. These included vancomycin-resistant CoNS, vancomycin-intermediate *S. aureus* (VRSA), and vancomycin-resistant *S. aureus* (VRSA) (Srinivasan et al. 2002; Tenover 2008). The reason for the emergence of vancomycin resistance is the frequent use of antibiotics for infections other than MRSA. The majority of current antibiotics cannot treat VRSA, hence treatment options are limited. The medical

community is concerned about the introduction of VRSA since *S. aureus* can cause potentially fatal infections in both hospitalized and out-of-hospitalized people (Denys and Relich 2014; Limbago et al. 2014). The cell walls of VRSA strains have been seen to be thicker than those of sensitive strains (Daum et al. 1992). Vancomycin is typically sequestered by the bacterium and trapped in the outer layers, which causes resistance (Billot-Klein et al. 1996; Cui et al. 2000).

3. HISTORY OF VRSA

In 1950, Vancomycin, a glycopeptide isolated from *Streptomyces orientalis*, was discovered. In *S. aureus,* there has been an elevated trend in vancomycin resistance. MRSA containing vancomycin resistance and unrestricted use of antibiotic drugs increased the resistance, calling for the need for additional epidemiological studies. Shockingly, the source and spread of VRSA infections are being substantially noticed across the world encompassing the Indian subcontinent. In 2002, the first Vancomycin-resistant variant of *S. aureus* was estimated in the US (CDC 2002). Lately, VRSA strains have also been reported from Brazil (Palazzo et al. 2005) and Jordan (Bataineh 2006). The incidence of MRSA was reported to be 38.7% which was nearly equivalent to the occurrence in different parts of India (Joshi et al. 2013). While VRSA has a low incidence (4.8%), it is threatening that combined MRSA and VRSA will present a greater risk in the treatment of staphylococcal infections (Mendem et al. 2016). Recently, a study (Thati et al. 2011) indicated an increased prevalence of VRSA variants in MRSA that is of greatest concern (Mendem et al. 2016).

4. VRSA GENES TRANSMISSION

It is commonly known that Enterococci and Staphylococci exchange genetic material and that this results in VRSA (Clewell et al. 2002). According to certain theories, people who are susceptible to VRSA get infected or co-colonized with MRSA and vancomycin-resistant Enterococci (VRE), which allows the vanA gene to transfer from VRE to MRSA in a biofilm environment and produce a VRSA strain (Finks et al. 2009). However, an inadequate and unsuitable dosage of vancomycin also plays a role in the development of VRSA (de Vriese and Vandecasteele 2014). While vanY is involved in the transcription of D, Dcarboxypeptidase, which results in greater glycopeptide resistance, the vanH, vanA, and vanX proteins are important in the development of vancomycin-reduced susceptibility. Teicoplanin-like antibiotic resistance is also mediated by another protein, vanZ, albeit the exact mechanism underlying this resistance is yet unknown (Arthur and Quintiliani 2001; Lee et al. 2004; Depardieu et al. 2007). The vanA type revealed decreased susceptibility to vancomycin, which is neutralized by an alternate mechanism that results in the production of a cell wall antecedent terminus in D-Alanyl-D-lactate. This indicates a decline in the attachment of glycopeptides and downshifting of cell wall production via housekeeping enzymes (Arthur and Quintiliani 2001; Lee et al. 2004; Depardieu et al. 2007).

5. ZOONOTIC TRANSMISSION

RTE food is becoming increasingly popular these days, and it can be seen in countless restaurants and on the streets everywhere, most notably in Egypt. Fast food benefits notwithstanding, there is a risk of bacterial disease and a challenge as these RTE products aren't heated anymore *S. aureus*-contaminated raw meat is a major global source of food poisoning (de Boer et al. 2009; Wang et al. 2014; Raji et al. 2016). When the contaminated meat is undercooked or when this bacterium is cross-contaminated with RTE food, the risk of infection increases (Wang et al. 2017). The majority of epidemiological research on

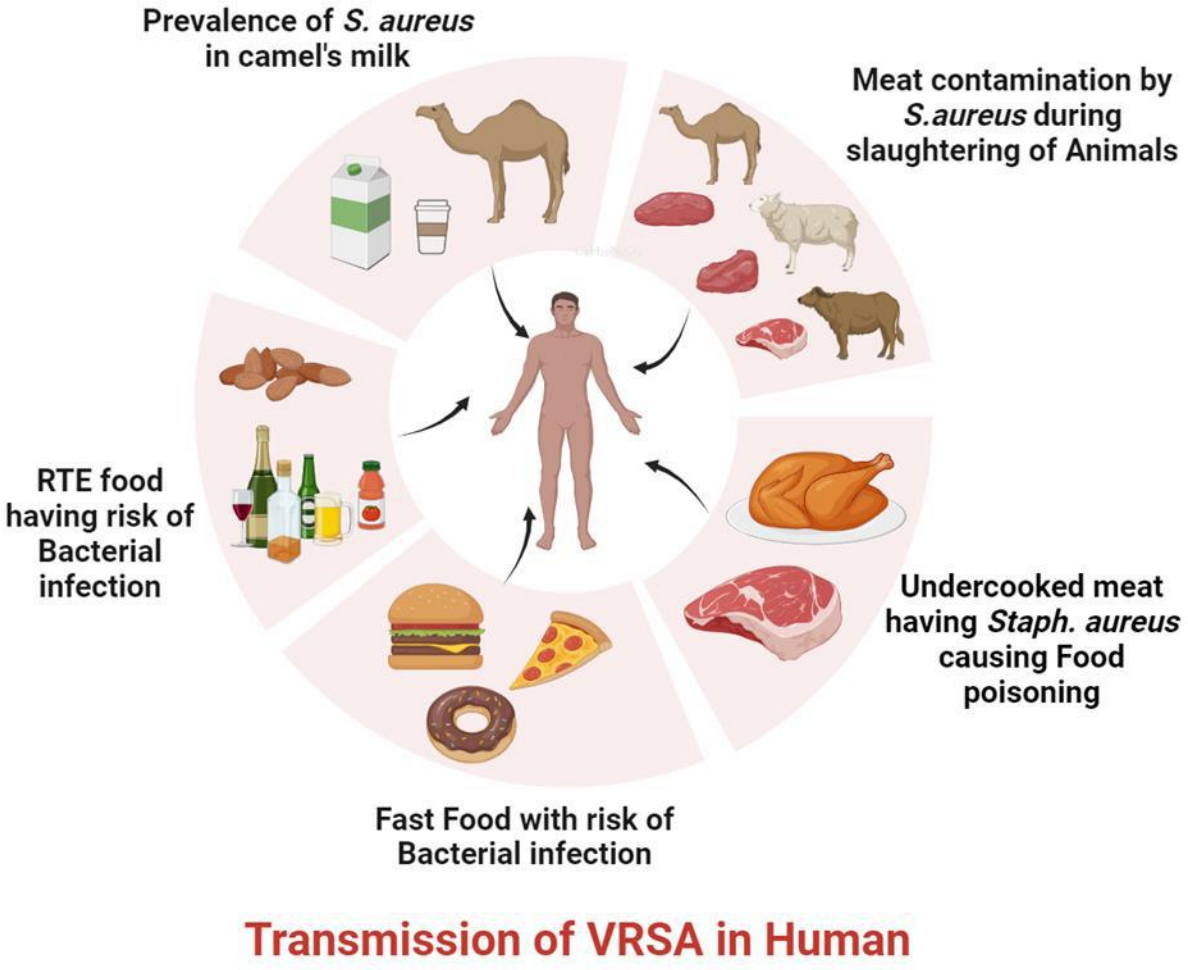

Fig. 2: Zoonotic transmission of Vancomycin-resistant *Staphylococcus aureus*.

resistant *S. aureus* in camels concentrates on the frequency of bacteria in milk (Quddoumi et al. 2006; El Harrak et al. 2011; Mohammad 2011). Few researchers have examined the differences between zoo anthroponotic and anthroponotic transmission resulting from personnel of slaughterhouses or camel breeders getting into contact with camels (Fig. 1). Regarding the distribution, colonization, and transmission of resistant *S. aureus* in camels and their human interactions, there is no data available in Egypt. The purpose of this study was to ascertain whether dromedary camels and abattoir personnel were exposed to VRSA and to investigate the potential zoonotic risk. Generally speaking, VRSA can infect cattle through the ingestion of meat contaminated by viscera during slaughter or by the hands of abattoir workers. Colonization may indicate a potential zoonotic disease risk (Lee 2003; Juhász-Kaszanyitzky et al. 2007). This type of contamination is typically more significant in Asia and Africa than it is in the United States, Canada, or Europe (Pexara et al. 2013).

6. EMERGENCE OF VRSA

When treating multidrug-resistant *S. aureus* and MRSA in healthcare settings, the emergence of vancomycin tolerance has become a major concern (Tiwari and Sen 2006). Assuming the in vitro

exchange of the vanA gene from Enterococcus spp. to *S. aureus*, we estimate the possibility of vancomycin tolerance vanA gene transfer from vancomycin-resistant Enterococci spp. to Staphylococci spp (Whitener et al. 2004). As previous research has shown, the expression of the VanA phenotype depends on the vanA, vanR, vanS, vanH, and vanX genes (Woodford et al. 1998). It has also been found that the cell walls of VRSA isolates are thicker than those of sensitive isolates (Daum et al. 1992). Hetero vancomycin-intermediate *S. aureus* additionally exhibits a thicker cell wall resistance mechanism with a high murein concentration in the cell wall. It has been shown that vancomycin molecules are sequestered by the bacteria and trapped in the outer layers, leading to resistance (Billot-Klein et al. 1996). Transfer of genetic material from one species of bacteria to another is another resistance mechanism for VRSA that has been proposed. According to a theory, people at high risk for VRSA co-colonize and co-infect VRE and VRSA, which facilitates the transfer of the vanA gene from vancomycin-resistant Enterococci to MRSA in a biofilm environment, where it develops into VRSA (Finks et al. 2009). There are only a handful of cases of VRSA found in healthcare environments worldwide, and some of these strains are linked to the population (Whitener et al. 2004). The vanA gene of VRSA was amplified in the current investigation, yet no amplification of the vanA gene suggested that the isolates lacked the gene. It validates the existence of an additional resistance mechanism not dependent on the vanA gene, which requires more research. It is crucial to consider the probability of development and the incidence of VRSA across all populations. Antimicrobial-tolerant microbes like VRSA can be prevented from growing and spreading by using effective contamination control strategies, adequate antimicrobial management, sanitary environments, and increased public knowledge. The reduced susceptibility example of the multi-drug resistant *S. aureus* should be regularly examined. Controlling VRSA infection is crucial because if it isn't, it can cause havoc in both hospital settings and the general community (Riaz et al. 2021).

7. EPIDEMIOLOGY

Recently, human and veterinary medicine have been more interested in the epidemiological distribution of *S. aureus* and its newly discovered strains, mostly due to their potential for zoonosis. Even though the staphylococcal-resistant forms have been known to spread from seemingly healthy pets (Cain 2013) and pigs (Armand-Lefevre et al. 2005), there are no hard data on how common it is in healthy camels or how important a role they play in carrying it. In this investigation, out of 200 dromedary camel meat samples, *S. aureus* was isolated from 14% (29/200); a High isolation rate of 55% (11/20) was also seen in samples obtained from 20 slaughterhouse workers who were directly involved in operations in the investigated facility. Swabs from the carcasses in slaughterhouses in Addis Ababa, Ethiopia yielded comparable rates of isolation of 11.7% (Beyene et al. 2017). However, the incidence of *S. aureus* in the present research was primarily lower than that found in camel nasal samples from Nigeria (20.7%) and higher than that seen in human nasal samples (11.5%) from the same study (Mai-siyama et al. 2014). The issue of antimicrobial resistance has acquired special interest in the African continent over the past decade (Lam et al. 2004). Nonetheless, there's very little information about the actual magnitude of it, as its routine scrutiny is currently being done only in a few countries (WHO 2014).

8. PATTERNS OF MDR IN *S. AUREUS*

In this research, all the isolates of *S. aureus* exhibited distinctive patterns of multi-drug resistance against nine antimicrobial agents. The most dominant resistance patterns were CHL-FOX-OXA-CLI-SXT-ERY-NV for camels and ERY-FOX-OXA-VAN-OFX-SXT for human isolates. The rise of such resistant forms plays a crucial role in therapeutic failure in human and animal diseases. This problem is aggravated by

uncontrolled antibiotic usage, poor diagnostic practices, and improper prescriptions by incompetent physicians (Kimang'a 2012) which creates an enormous hurdle in the prevention and control of the disease agent. Identical resistance patterns were observed by disc diffusion assay in MRSA isolated from emergency care units in Southern India, Hyderabad (Mai-siyama et al. 2014). Furthermore, recently VRSA was reported to be found in a percentage of 16.7 of MRSA forms isolated from skin and nasal samples of buffalo in India using the same method (Kumar et al. 2017). The use of VAN in treating MRSA infections is restricted in both humans and animals due to the issue of antimicrobial resistance, which makes it a last resort (Kumar et al. 2017; Wijesekara et al. 2017). Because of the development of alternative compounds recently, VAN is not the last choice drug anymore; however, it is still the most used antibiotic for the treatment of staphylococcal-related illnesses (David and Daum 2017) In this research, VAN-resistant isolates were also FOX and OXA resistant. MecA gene amplification was performed on all isolates that exhibited resistance to VAN, OXA, and FOX. Consequently, there's a chance that *S. aureus* strains that are more resistant to VAN will arise. Nonetheless, VRSA strains were believed to be rare until recently (Shekarabi et al. 2017).

9. CURRENT TRENDS OF VRSA

The current study on the presence of VRSA in Egypt exposed elevated rates of isolates of VRSA. The prevalence of VRSA was confirmed to be 27.6% (8/29) in dromedary camels and 54.5% (6/11) in human *S. aureus* isolates. Similarly, MRSA was recovered from camel flesh in one study (Quddoumi et al. 2006) and from mastitis female camels in another (Mohammad 2011). Additionally, livestock-associated forms (LAMRSA) have been identified from siblings of the farmers with animal contact (Benito et al. 2014), indicating a possible zoonotic transmission risk to those in contact with animals (Juhász-Kaszanyitzky et al. 2007). Furthermore, previous research in Hong Kong showed that handling meat can result in the acquisition of LA-MRSA (Boost et al. 2013; Ho et al. 2014). It is difficult to compare the findings of our study with previous data from Egypt because, to the best of our knowledge, the incidence of VRSA has never been investigated in camels in the Egyptian region. Five variants of VRSA were identified in our investigation, three of which were human isolates and the other two of which were from camel meat, and all of them demonstrated high-level resistance for VAN (MIC 64 µg/ml). Unsettling evidence of these resistant forms and high VRSA variant prevalence points to a significant public health concern. One mechanism of VAN resistance in *S. aureus* is the conjugation-mediated transfer of a plasmid containing Tn1546 and hence vanA gene cluster from VAN-resistant Enterococcus spp. to *S. aureus* (Saadat et al. 2014). Moreover, vanB has not yet been detected in staphylococci. This study examined the presence of the genes vanA and vanB in *S. aureus* isolates that tested positive for vancomycin (VAN) and discovered that all of the isolates that tested positive for VAN had both genes. According to analyses of VanA gene sequences from camel meat and human samples, the bacteria may have horizontal gene transfer or be of zoonotic relevance (Lee 2003; Juhász-Kaszanyitzky et al. 2007; Boost et al. 2013; Pexara et al. 2013; Ho et al. 2014). VRSA isolates from infected or colonized individuals have been found in Turkey and Asiatic nations (Saha et al. 2008; Cesur et al. 2012; Pahadi et al. 2014). While 4.5% of clinical cases (patients who presented with an evident cutaneous bacterial infection) in Egypt tested positive for VRSA variations, no asymptomatic individuals had these variants detected (ElSayed et al. 2018). Clinical infections may be a major contributor to community-acquired VRSA in Egypt. Even though the front of the nose is commonplace to isolate *S. aureus*, 90% of people also carry the infection on their hands (Wertheim et al. 2005). The lack of nasal swabs from the camels and the workers was a glaring flaw in this investigation; human swabs would have been essential for understanding the colonization and dissemination of VRSA. The requirement to characterize VRSA strains obtained from people and animals

was another deficiency. Additionally, the study hinged on whole genome sequencing, and then core-genome multilocus sequence typing (cg/MLST) was organized with an international laboratory to clarify/assess the zoonotic spread of *S. aureus* in camel butcheries.

10. CONCLUSION

S. aureus is a common bacteria found in commensal or pathogenic relations with its hosts. Recently there has been an emergence of antibiotic resistance among *S. aureus* all across the world. At first *S. aureus* was identified to be resistant to methicillin group antibiotics. At that time Vancomycin was used as the drug of choice against methicillin-resistant *Staphylococcus aureus*. Soon this weapon also became ineffective as *S. aureus* developed resistance against it. These vancomycin-resistant *S. aureus* (VRSA) rendered the antibiotic useless leading to a reduction in the overall effectiveness of antibiotics. This situation was further aggravated as animals became reservoirs of VRSA and its zoonotic transmission prevailed threatening the public health. VRSA was often found colonizing the nasal regions and outer skin surfaces of people who had continuous contact with animals, like pig farmers, veterinarians, etc. These bacteria were also isolated from the family members of such people conclusively proving its secondary prevalence beyond zoonosis.

All of these situations have mainly developed due to the misuse of antibiotics and hence require the implementation of strict policies. Policies should be developed for the use of antibiotics and control of infectious diseases. This will prevent the spread of diseases and simultaneously reduce the usage of antibiotics in turn leading to less misuse of antibiotics. Less misuse of antibiotics will ultimately lower antibiotic resistance among bacteria all over the world hence mitigating the threat of VRSA prevalence among human populations through zoonosis. Such strict control measures have been already implemented in several countries in Europe. In those countries application of control measures was soon followed by a reducing trend of infectious disease prevalence. This trend ultimately led to a pause in the rise of antibiotic resistance among bacteria in those countries. Some of these countries even saw a declining trend of antimicrobial resistance in the bacteria through strict control measures. This proves the importance of control and prevention for disease control which comes before the administration of any kind of antibiotics. It means that if properly implemented, diseases can also be controlled and eradicated through control measures.

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The Threat of Transboundary Zoonosis

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ABSTRACT

Global health security, economic stability, and biodiversity conservation are seriously threatened by transboundary zoonotic diseases, which are transmissible diseases that can transfer from animals to humans over international borders. Transboundary zoonoses have become more prevalent in recent years as a result of the intricate interactions between urbanization, the destruction of animal habitats, intensive agriculture, international commerce, and climate change. These diseases have the potential for fast worldwide spread and devastating effects, as shown by well-known examples like avian influenza, Ebola, and COVID-19. An interdisciplinary approach that integrates epidemiology, ecology, veterinary medicine, public health, and socio-political sciences is required to comprehend the dynamics of transboundary zoonoses. In order to prevent and contain epidemics, it is crucial to build early detection and surveillance systems as well as efficient response systems. Additionally, risk communication and community involvement are essential for promoting collaboration between authorities, medical specialists, academics, and the general public. A key area for the spread of zoonotic diseases is the intersection of wild and domestic animals with humans. The management of transboundary zoonoses is intimately related to efforts to conserve biodiversity, highlighting the need of preserving intact ecosystems and reducing human activities that result in habitat degradation and animal trafficking. Given that transboundary zoonoses have the capacity to cross borders and damage many countries at once, international cooperation is essential in combating them. In order to do this, it is necessary to coordinate response activities, share data, resources, and knowledge, as well as facilitate technology transfer and capacity development in underdeveloped areas. This chapter examines the idea of transboundary zoonosis, highlighting its effects on the environment, global collaboration, and the health of people and animals. This chapter attempts to offer insights into managing the complex danger of transboundary zoonoses by looking at case studies and talking about prevention and control techniques.

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1. INTRODUCTION

At the nexus of human, animal, and environmental health, transboundary zoonotic diseases pose a complex and diverse danger. Global health crises and socioeconomic upheavals are brought on by these diseases, which cross geographic borders. Most of the world's population depends on animals for food and other essential resources like transportation and skins. Animal diseases may thus have serious effects, particularly those with high rates of morbidity or, worse, death. A list of transboundary animal diseases (TADs) is kept up to date by the World Organisation for Animal Health (OIE; previously the Office International des Epizooties) and the Food and Agriculture Organization of the United Nations (FAO) (FAO 2021; WHO 2021). To better regulate and reduce detrimental implications, it is necessary to better understand the transmission, dissemination, and pathophysiology of these diseases. The creation of more accurately described in vitro and animal models will be required. Additionally, more research is required to increase the affordability and effectiveness of diagnostics and immunizations. Rapid diagnosis and/or efficient immunization techniques are essential for the management and prevention of these diseases (Torres-Velez et al. 2019). The idea of one health acknowledges how intertwined the health of people, animals, and the environment are. The few TADs that are zoonotic in origin and so contagious for humans are the subject of most of the One Health literature dealing with animal health. Highly virulent avian influenza, anthrax, Rift Valley fever, Ebola, rabies, and Crimean Congo Hemorrhagic Fever are a few examples of these zoonotic infections. Agricultural animals (livestock and poultry) are employed as draft power to transport goods and cultivate crops, which is an overlooked aspect of TADs and One Health. These animals are an essential component of nourishment and the global economy. To more efficiently coordinate preparedness and response across sectors, zoonotic diseases have been prioritized in several low- and middle-income countries worldwide as a result of the recent growing interest in and advocacy for the "One Health Approach" among non-profit organizations, governments, industries and academia (Salyer et al. 2017). One Health Zoonotic Disease Prioritization (OHZDP) tool developed by CDC (Rist et al. 2014) was used in seven countries throughout the world from 2014 to 2016 during the pilot phase of the tool development and testing (Salyer et al. 2017) to analyze themes from one health zoonotic diseases workshops. This chapter explores the idea of transboundary zoonosis, including its causes, effects, and the need for coordinated remedies.

2. DEFINITION AND SCOPE

Transboundary zoonoses are infectious diseases caused by pathogens that can move across borders through the movement of animals, humans, and their products. The term “Zoonoses” is derived from the Greek word “Zoon”, which means animal, and “nosos”, which means disease. According to the World Health Organization (WHO), any disease or infection that is naturally transmissible from vertebrate animals to humans or from humans to animals is classified as a zoonosis (WHO 2020). Among the human pathogens, about 61% are zoonotic in nature (Taylor et al. 2001). Zoonoses is a great public health concern and a direct human health hazard that may even lead to death. Across the globe, the 13 most common zoonoses were most impactful on poor livestock workers in low- and middle-income countries and have caused an estimated 2.4 billion cases of illness and 2.7 million deaths in humans per year in addition to

their negative effect on human health (Grace et al. 2012). Most of these diseases affect animal health and decrease livestock production (Grace et al. 2012). The review discusses the significance of these diseases in terms of their potential for rapid spread and the challenges they pose to effective control due to their dynamic nature.

2.1. CLASSIFICATION OF ZOONOSES

Zoonotic diseases are caused by a wide range of pathogens. Based on etiology, zoonoses are classified into bacterial zoonoses (such as anthrax, salmonellosis, tuberculosis, Lyme disease, brucellosis, and plague), viral zoonoses (such as rabies, acquired immune deficiency syndrome- AIDS, Ebola, and avian influenza), parasitic zoonoses (such as trichinosis, toxoplasmosis, trematodiasis, giardiasis, malaria, and echinococcosis), fungal zoonoses (such as ringworm), rickettsial zoonoses (Q-fever), chlamydial zoonoses (psittacosis), mycoplasma zoonoses (Mycoplasma pneumoniae infection), protozoal zoonoses, and diseases caused by acellular non-viral pathogenic agents (such as transmissible spongiform encephalopathies and mad cow disease) (Chomel 2009).

The older classification of zoonoses includes the terms anthropozoonoses, zooanthroponoses, amphixenoses, and euzoonoses (Hubálek et al. 2003). Anthropozoonoses are animal diseases that can be transmitted to humans, such as rabies. Zooanthroponoses refers to those diseases that are transmitted to animals from humans such as tuberculosis in cat and monkey. Amphizoonoses are those diseases that can be transmitted in any direction (from human to animal and from animal to human) such as staphylococcal infection. For some parasitic diseases, humans act as the obligatory host. These parasitic diseases are known as Euzoonoses such as Taenia solium and Taenia saginata infections.

Many zoonotic pathogens can replicate in and survive on dead organic materials like saprophytes and the diseases caused by these agents are known as sapronoses. Examples of sapronoses include fungal diseases (such as coccidioidomycosis, histoplasmosis, and aspergillosis) and bacterial diseases (such as legionellosis) (Somov et al. 1988). The term “saprozoonoses,” is defined by the WHO expert committee on zoonoses as pathogens that have a vertebrate host as well as a non-animal reservoir or developmental site (soil, plants, and organic matter) (Schwabe et al. 1964). In many cases, disease transmission may require more than one vertebrate host such as with human taeniasis. These types of zoonoses are known as cyclozoooses. Zoonoses in which both vertebrate and invertebrate hosts are involved are known as metazoonoses such as with arbovirus infection.

Most zoonotic diseases are transmitted to humans from animals. Some reports suggested that animals can also get infected from humans. Such diseases are known as reverse zoonoses. Examples of such pathogens include methicillin-resistant Staphylococcus aureus (MRSA), Campylobacter spp., Salmonella enterica Serovar Typhimurium, influenza A virus, Cryptosporidium parvum, Ascaris lumbricoides, and Giardia duodenalis. In addition, zoonotic diseases caused by pathogens that are occasionally transmitted to animals from humans and then back from animals to humans are referred to as reverse zoonoses.

2.2. ZOONOSES OF DOMESTIC ANIMALS

Domestic animals play a significant role in the transmission of various diseases to humans and in many cases, they work as amplifiers of pathogens emerging from wild animals (Morand et al. 2014). The positive association between domestic animals and humans in influencing pathogen diversity was first hypothesized a long time ago (McNeill 1976). About 60% of human infectious diseases come from vertebrate animals (Taylor et al. 200; Klous et al. 2016). Direct human contact with animals has expanded with the introduction of domestication of different vertebrate animals (Pearce-Duvet et al. 2006). The

possible transmission patterns of zoonotic bacteria, viruses, parasites, or fungi are via direct contact, ingestion, inhalation, through the conjunctiva, or biting (Klous et al. 2016).

Cattle, sheep, goats, dogs, cats, horses, pigs, and other domestic animals act as reservoirs of pathogens of domestic zoonoses and can transmit diseases to humans (Samad et al. 2011). Pathogens can be transmitted through direct contact or animal-origin foods. Examples of zoonotic diseases that can be transmitted to humans from domestic animals include anthrax, rabies, tuberculosis, brucellosis, campylobacteriosis, leptospirosis, toxoplasmosis, balantidiasis, ancylostomiasis, toxocariasis, listeriosis, bovine pustular stomatitis, rotavirus infection, and Q fever (Ghasemzadeh et al. 2015; Samad et al. 2011; Bae et al. 2011).

Of these zoonotic diseases transmitted by domestic animals, anthrax caused by Bacillus anthracis poses a significant public health importance. B. anthracis is soil borne bacteria with the capability to produce spores; thus, allowing them to survive in the environment for a very long time. Anthrax can be transmitted to humans through close contact with infected animals (such as cattle and goats) or their products (such as meat, skin, hides, or even bones). (Goel 2015). Human to human transmission exists, but it is very rare. Every year, about 2,000–20,000 humans are affected by anthrax cases globally (Goel 2015). People from India, Bangladesh, Pakistan, the United States, Zimbabwe, Iran, Iraq, South Africa, and Turkey are occasionally affected (Goel 2015). In humans, it can develop malignant pustule, gastroenteritis, and pneumonitis; conversely, sudden death with some systemic lesions can occur in animals. Mortality can be 25–65% in intestinal anthrax; however, it may rise to 100% in pulmonary anthrax (Kamal et al. 2011). Developing countries whose economy usually depends on agriculture are still facing hazardous effects due to anthrax.

Among the bovine zoonoses having serious public health significance, tuberculosis is the most important zoonotic disease. The disease has been a cause of severe economic loss in animal production. It is caused by Mycobacterium bovis, M. tuberculosis, or rarely M. caprae (Torgerson et al. 2010; Bayraktar et al 2011). Mycobacterium is acid-fast soil saprophytes characterized by the presence of mycolic acid in their cell wall. They are also facultative intracellular pathogens. Though bovine tuberculosis has been greatly eliminated from developed countries, other parts of the globe are still facing serious zoonotic effects. Human tuberculosis is the second most common cause of death after AIDS. About 5–10% of all human tuberculosis has been caused by M. bovis (25% of the patients were children). About 53% of all cases showed that the favorable site of tuberculosis is the extra-pulmonary tract (Samad et al. 2011). Most humans are affected with tuberculosis by handling or milking unpasteurized contaminated milk or via aerosols from coughing of infected animals (da et al. 1996). Importantly, M. bovis infection can also happen in the urogenital system of humans and can impact animals through the respiratory secretions from humans acting as reverse zoonoses (Ocepek et al. 2005). However, direct contact of infected animals with humans such as farm workers, veterinarians, abattoir workers, or village people can pose a significant risk.

Brucellosis is one of the most common bacterial zoonotic diseases causing over 500,000 human cases throughout the world every year (Hull et al. 2018). The disease is classified as a forgotten neglected zoonosis as per the WHO (WHO 2015). Among the twelve species of the genus Brucella, Brucella melitensis, B. abortus, B. suis, and B. canis are zoonotic. The common transmission pattern of brucellosis to humans occurs through the consumption of unpasteurized milk or milk products, though the human–human transmission is rare.

Rabies is one of the deadliest zoonotic diseases caused by the rabies virus, which belongs to Rhabdoviridae. Every year about 30,000–70,000 human deaths occur throughout the globe (Krebs et al. 2004). Though dogs are the main carriers of rabies virus, other wild animals including cats and jackals also act as carriers for the transmission of rabies virus. In developing countries, humans are affected by rabies

through biting because of the stray dog problem (Tang et al. 2005). In developed countries, bats, foxes, and other wild animals are responsible for the transmission of rabies (Tang et al. 2005).

2.3. ZOONOSES OF PETS, COMPANION ANIMALS, AND BIRDS

About 14–62% of pet owners allow their pets to their bedrooms, which could enhance the emergence of zoonoses (Chomel et al. 2011). Companion and pet animals have increased over the past several decades, but they are also a comprehensive source of disease-producing agents. The increased popularity of pets and companion animals has put human health at risk due to the possible spread of infections. In many houses nowadays, pets of exotic species are kept along with common pets. Therefore, huge people are at risk of acquiring new zoonotic diseases from pets, companion animals, and exotic birds and animals. A variety of infectious diseases (viral, bacterial, parasitic, and fungal) are associated with pets and companion animals (Halsby et al. 2014). The zoonotic diseases frequently associated with pets and companion animal include brucellosis, campylobacteriosis, chlamydiosis, catch scratch fever (Bartonella henselae), ehrlichiosis, giardiasis, hantavirus, hookworms, influenza, rabies, Lyme disease, rocky mountain spotted fever, leptospirosis, monkeypox, pasteurellosis, Q fever, plague, roundworms, salmonellosis, staphylococcosis (MRSA), streptococcosis, toxoplasmosis, and tularemia (Halsby et al. 2014: Jacob et al. 2015).

Transmission of pathogens from these animals occurs through direct or indirect contact. The transmission can take place at home, outside, pet shops, hospitals, or other places. In many cases, transmission also takes place when these animals and birds are brought to shows and competitions (Belchior et al. 2011; Vanrompay et al. 2007). Usually, animal bites or scratches are routes through which humans get the infection such as pasteurellosis and cat scratch disease (Chomel et al. 2014).

2.4. ZOONOSES OF FISH AND AQUATIC ENVIRONMENTS

Many microorganisms with zoonotic significance have been isolated from fish (Boylan et al. 2011). Fish-associated zoonotic pathogens are mainly bacteria. Often, fish unsusceptible to these infections are capable to cause serious sickness in humans. However, these opportunistic fish-borne bacterial infections are limited. Fish can get these pathogens from the aquatic environment where they remain as an indigenous part. In addition, aquatic environments may get contamination from agricultural activities, human and animal excreta, garbage from households, and wild animals. These zoonotic infections may be transmitted to humans through the non-hygienic handling of aquatic animals and/or their products. Consumption of raw or improperly cooked aquatic products may also transmit foodborne infections to humans. Among the zoonotic pathogens isolated from fish, Aeromonas hydrophila, E. coli, Yersinia spp, Brucella spp, Shigella spp, Salmonella spp, Streptococcus iniae, Clostridium botulinum, Klebsiella spp, and Edwardsiella tarda are important (Alworth et al. 2007; Haenan et al. 2013).

Several Vibrio species, at least 12, are often known to be potential for fish-associated zoonoses (Abbot et al. 2007). Among them Vibrio(V.) cholerae, V. parahaemolyticus, V. vulnificus, V. damsela are mostly involved in human illness (Austin et al. 2010; Zhang et al. 2016). Eating contaminated raw or undercooked seafood is the major way through which humans get these Vibrio infections, which can cause serious symptoms such as diarrhea, vomiting, and dehydration (Zereen et al. 2019).

In humans, Mycobacterium tuberculosis causes TB. However, fish are susceptible to non-tuberculous mycobacterial infections. The infections are commonly associated with display aquaria and occasionally with commercial aquaculture systems. They can also be transmitted to humans during aquaculture practice on farms and handling of ornamental fish in aquarium and equipment (Kušar et al. 2017). M.

chelonae, M. marinum, and M. fortuitum are main concerns in aquaculture and fish-related businesses. Among these, M. marinum is a well-known zoonotic pathogen.

Erysipelothrix (E.) rhusiopathiae is a fish-borne pathogen that causes systemic skin diseases in marine mammals (Reidarson et al. 2003). It is a Gram-positive pathogen but no reported disease in fish is caused by this bacterium (Dunn et al. 1990). Similar to other fish-borne pathogens, human and non-human animals are exposed to this bacterium through direct contact with cutaneous wounds on fish (Boylan et al. 2011). E. rhusiopathiae can cause diseases in humans (known as “erysipeloids”) and animals (known as “erysipelas”) (Gauthier et al. 2015). Fisheries workers are directly vulnerable to the transmission of E. rhusiopathiae during the handling and processing of live and dead fish, which is the reason that the disease is also referred as fish-handler’s disease (Reboli and Farrar 1989). The disease is also referred to as “fish rose” due to its symptoms, which include purple or red discoloration of the skin (Reboli and Farrar 1989: Wang et al. 2010).

Lactococcus garvieae is an important fish-borne pathogen affecting a wide range of wild fish species (both marine and freshwater fish), giant prawns from freshwater, and wild marine mammals (Gibello et al. 2016). This bacterium causes severe hyperacute hemorrhagic septicemia (known as lactococcosis) in cultured warm-water fish with high mortality rates and an ultimate ominous impact on the aquaculture industry (Gauthier et al. 2015; Meyburgh et al. 2017; Vendrell et al. 2006).

2.5. ZOONOSES ASSOCIATED WITH FOOD-BORNE PATHOGENS

Food plays a significant role in the transmission of infections, particularly food-borne pathogens, which often cause symptoms of diarrhea. Zoonotic infections are the main cause of many food-borne diseases. Both adult and juvenile populations are susceptible to serious diseases and fatalities from food-borne diseases. Millions of people are affected by mortality, which is often linked to digestive disorders brought on by tainted food and water (Newell et al. 2010). 600 million people, or one in ten people worldwide, are thought to eat tainted food and water each year. 420,000 of those impacted, including 125,000 children, pass away (WHO 2015).

Salmonella spp. (Salmonella enterica serovar Enteritidis), Campylobacter spp, Shiga toxin-producing Escherichia coli (STEC), and hepatitis E virus is typical food-borne zoonotic diseases. Salmonella species and Campylobacter species are responsible for more than 90% of bacteria-related food-borne infections (Thorns et al. 2000).

Verodoxin (Verocytotoxin)-producing E. coli is another name for STEC. Direct contact with infected foods may result in their transmission to people (Treacy et al. 2019). In the 1980s and 1990s, Escherichia coli O157:H7 serotype of STEC was identified as a significant contributor to food-borne zoonotic disease. Humans may suffer from renal failure, bloody diarrhea, and other serious diseases as a result of exposure to STEC strains' toxins (Yara et al. 2020; Mir et al. 2019).

Additionally, many hepatitis viruses, mostly present in animal intestines, including Brucella spp, Listeria spp, Clostridium spp, BSE, Norovirus, Calicivirus, and other Hepatitis viruses, may be spread via contaminated food products. There are several risk factors that contribute to the development of food-borne zoonotic diseases, including the globalization of farm animals and the meat market, eating raw or undercooked wildlife food, the rising prevalence of immunocompromised patients, and inadequate awareness of good hygiene and sanitation.

2.6. EMERGING AND RE-EMERGING ZOONOSES

A newly identified, recently evolving, or previously observed zoonosis that exhibits a rise in incidence or an extension in geographical, host, or vector range is referred to as an emerging zoonosis (WHO

2020). Over the last 70 years, at least 250 zoonoses have been reported as developing and re-emerging zoonotic diseases. With a growing frequency and geographic distribution, numerous diseases have spread quickly over the globe (Grace et al. 2012). Close interaction with animals that serve as reservoirs for newly developing and reemerging zoonotic diseases affects humans (Woolhouse et al. 2005). Among the factors that lead to the emergence of zoonotic diseases are changes in human and animal behavior, habitat, ecology, vector biology, pathogen adaptability, change in farming practices, livestock production systems, food safety, urbanization, deforestation, and climate change (Lindahl et al. 2015). Wildlife may serve as a source or reservoir for viruses that cause newly developing and reemerging zoonotic diseases (Kruse et al. 2004).

Diseases that are developing or re-developing have major effects, not only on global socioeconomic challenges as well as public health (Cutler et al. 2010; Liu et al. 2014). 132 of the 175 novel conditions that have been documented are thought to be emerging zoonotic diseases. According to another study, zoonoses account for around 60.3% of all new diseases. They came from animals in 71.8% of the cases (Jones et al. 2008).

2.7. WILD ANIMALS AND RE-EMERGING ZOONOSES

A variety of infectious diseases are spread and maintained by wild animals because of their complicated relationships with people, domesticated animals, and environmental factors (Thompson et al. 2014). The ecological relationships among the one-health components are being disrupted by factors like as globalization, habitat degradation, climate change, and the extinction of species and biodiversity (Thompson et al. 2013) (Akhter et al. 2020). As a result, zoonotic infections develop and the patterns of their transmission shift. Wild animals may carry diseases that affect both human and animal health, lower agricultural output, and disrupt wildlife (Bengis et al. 2004). Animals in the wild, including mammals, reptiles, birds, fish, and amphibians, serve as a reservoir for zoonotic infections that may spread to people or other animal hosts. It is concerning that wild animals are involved in the epidemiology and spread of zoonotic diseases as shown in the Fig. 1.

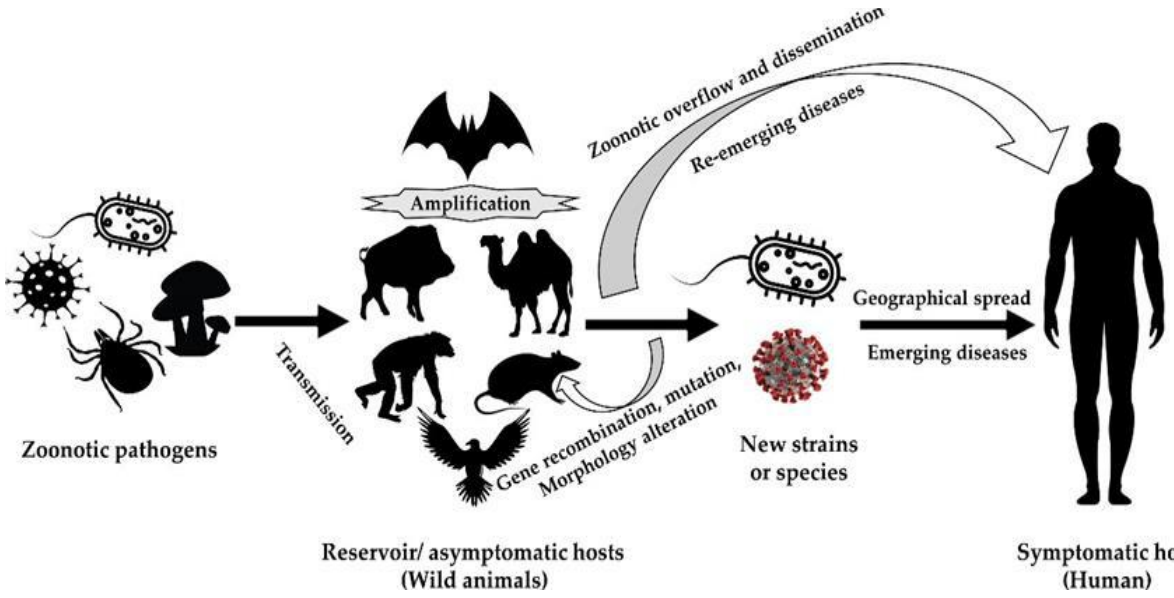

Fig. 1: The involvement of wild animals in the transmission and amplification of etiologicalagents of emerging and re-emerging zoonoses (modified with permission from (Cupertino et al. 2020).

2.8. NEGLECTED ZOONOTIC DISEASES AND IMPLICATIONS

In the developing world, several zoonotic diseases are widespread, which harms the health and standard of living of the underprivileged. Due to their endemic character, neglected zoonoses are more likely to be unreported and to get less financing from funding organizations than emerging and re-emerging zoonoses (Maudlin et al. 2009). The majority of wealthy nations have had success containing and eradicating neglected zoonotic diseases (WHO 2011). Fig. 2 illustrates the key characteristics of neglected zoonoses. Generally speaking, neglected diseases are more prevalent in tropical regions, which is why they are frequently referred to as neglected tropical diseases. Since neglected zoonotic diseases are given less importance in many nations' health systems, they have subtly increased morbidity among rural residents. At the "World Health Assembly" in May 2013, representatives from 32 WHO member nations took several significant decisions to manage 17 neglected zoonotic diseases. Additionally, they put into practice a WHO roadmap for the evaluation of preventative and control measures for those neglected tropical diseases (WHO 2020). Rabies, anthrax, cysticercosis, brucellosis, foodborne trematode infections, leishmaniasis, echinococcosis, and zoonotic sleeping sickness are significant zoonotic diseases. Rabies in Africa and Asia, echinococcosis, and taeniasis (*Taenia solium*) in Asia, Africa, and Latin America, leishmaniasis in Asia and Africa, cysticercosis, and foodborne trematodiasis in Africa are zoonotic diseases that have been neglected (neglected zoonoses) (WHO 2020).

3. CASE STUDIES

To illustrate the diversity and severity of transboundary zoonotic threats, this section examines selected case studies

Avian Influenza (H5N1 and H7N9): The spread of *avian influenza* viruses highlights the impact of migratory birds on the transboundary transmission of zoonoses and underscores the need for surveillance and early detection.

Ebola Virus Disease: The cross-species transmission of the Ebola virus from wildlife to humans exemplifies the complex interplay between ecosystems, human behavior, and disease emergence.

Middle East Respiratory Syndrome Coronavirus (MERS-CoV): This case study emphasizes the role of camels as potential reservoirs and intermediary hosts for zoonotic diseases.

4. PREVENTION AND CONTROL

Effective management of transboundary zoonotic threats requires a multi-pronged approach:

Surveillance and Early Warning: Early detection systems that monitor animal and human health, environmental changes, and cross-border movements are essential for timely responses.

Cross-Sectoral Collaboration: Strengthening partnerships between health, agriculture, environment, and wildlife sectors fosters a comprehensive approach to disease control.

Capacity Building: Enhancing local and national capacities in disease surveillance, diagnosis, and outbreak response is crucial for preventing and mitigating transboundary zoonoses.

4.1. INTERNATIONAL COOPERATION

International institutions must enable cooperative efforts between nations to address the common character of transboundary zoonotic dangers. Every disease management strategy needs a substantial financial investment, which is often not accessible to underdeveloped nations. For successful zoonoses management, the developed nations and international donors must assist the poor nations. One option

for funding is to approach donor organizations like the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), the US Agency for International Development (USAID), the US Department of Agriculture (USDA), the European Union (EU), the Department for International Development (DFID), the Biotechnology and Biological Sciences Research Council (BBSRC), and the Danish International Development Agency (DANIDA). Similar to public funding organizations, private funding organizations may also be contacted for funds to develop zoonoses control projects (Gibbs et al. 2014).

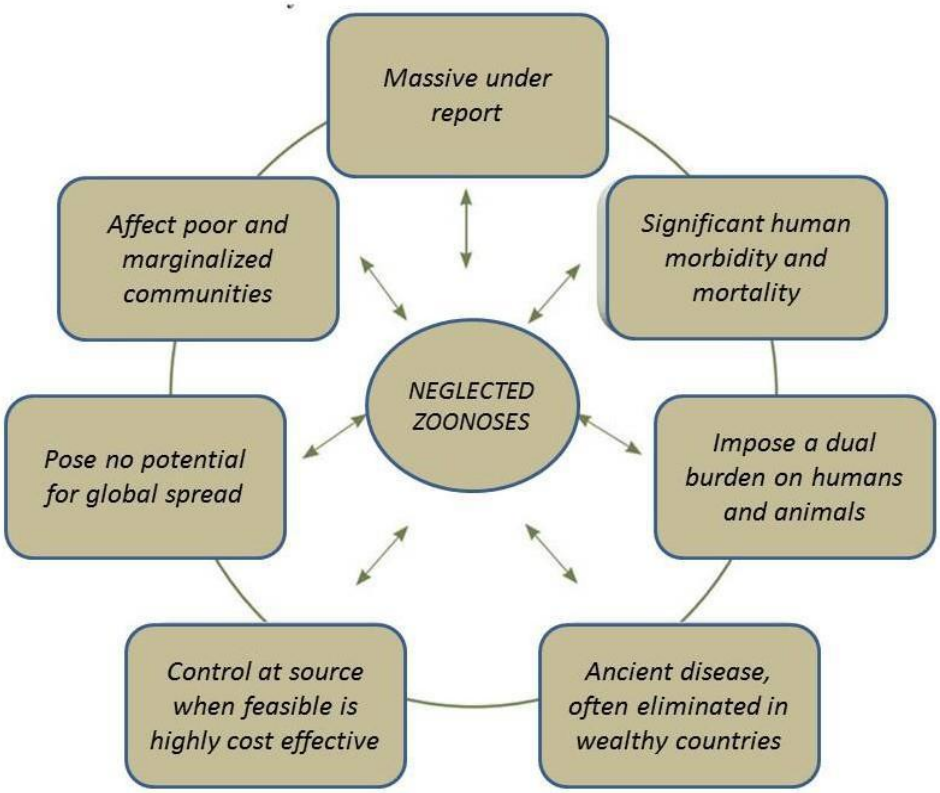

Fig. 2: Basic features of neglected zoonotic diseases (reproduced with permission from (WHO 2011).

The global community faces a major danger to its health from zoonoses. Up to 75% of human diseases are zoonotic, or animal-transmitted, and between 58 and 61 percent of human diseases are communicable (Al-Tayib et al. 2019; Ng et al. 2013). Effective control methods for zoonosis must thus take into account interactions between people, animals, and the environment (Aenishaenslin et al. 2013). For zoonotic diseases to be prevented and controlled, surveillance is essential. It may be used to identify early infection, afflicted people and animals, reservoirs, vectors, and endemic regions including the "hotspots". It assists with the correct management of disease, the adaption of control measures against newly developing and reemerging diseases, and the reduction of human and animal morbidity and death. In order to effectively manage zoonoses, coordinated monitoring strategies at the local, regional, national, and worldwide levels are crucial. Zoonoses (like SARS and HPAI) may quickly travel throughout the world and threaten global societies. All possible zoonoses sources, including rodents, aquatic animals, wild animals, exotic animals and birds, pet and companion animals, need to be monitored. There are several surveillance methods that must be used (Van der Giessen et al. 2010). Effective and functioning surveillance calls for a well-equipped

lab, sufficient diagnostic resources, qualified personnel, and funds. The four methods of surveillance listed below may be used to combat zoonoses:

• Monitoring of pathogens to find and classify them.

• Serological surveillance to identify infections in the blood of people or other animals by tracking immune reactions.

• Syndrome surveillance, which uses data analysis based on symptoms to assess the likelihood of certain diseases. The presence of pathogens cannot be determined using this analysis-based monitoring.

• Risk monitoring to find risk variables linked to disease transmission. The prevalence of various diseases and their clinical characteristics cannot be determined using this control technique.

Zoonoses can also be managed using general principles of disease control like treating sick people, immunizing healthy people and animals, limiting animal movement, managing animal populations, and test and cull (anthrax, glanders, and Rift Valley fever). Infected items must be decontaminated in order to lower the risk of contracting new diseases. For instance, brucellosis may be less common if aborted fetuses are properly disposed of. It is important to practice maintaining personal hygiene and using personal protection equipment such gloves, masks, lab coats, helmets, and goggles. To help stop the spread of brucellosis, salmonellosis, and TB, it is necessary to thoroughly disinfect infected objects and spaces when appropriate.

Even though many zoonoses pose a serious risk to public health, particularly in impoverished nations, they are often ignored and go unchecked. Programs to manage zoonoses must take into consideration both human and animal-related issues. When multiple bordering nations are impacted, coordinated zoonoses management strategies must be used. To effectively manage zoonoses, strategies based on one health policy principles must be established, including veterinarians, medical professionals, occupational health physicians, public health operators, conservation officers, and environmental officers (Murphy et al. 2019). One health-based idea was reinforced among academics and professionals from 21 European and African nations via a research initiative called Integrated Control of Neglected Zoonoses for the control of neglected zoonotic diseases in Africa (Pal et al. 2014).

It is necessary to provide customers with a plentiful supply of safe food in order to manage food-borne zoonoses. Implementing the two major strategies of risk assessment and risk management of food items might help accomplish this. Risk management can be practiced by passing legislation and establishing goals to lower the risk. Risk assessment may be done by gathering and evaluating data, and by offering suggestions based on importance. Foods of animal origin including meat, milk, and eggs must come from healthy animals free of zoonotic viruses. To guarantee the safety of food derived from animals, proper ante- and post-mortem assessment of the animals is essential. For the manufacture of safe food, it is important to provide sanitary conditions at every stage of food processing, including staff members' personal cleanliness. The creation of laws and regulations governing isolation and quarantine, the establishment of robust and efficient disease reporting (notification) systems, farm biosecurity, mass vaccination, testing and slaughter or culling, public awareness campaigns, and health education are additional zoonoses control measures. To better educate the public about zoonoses, mass media, electronic information systems, social networks, text messaging, and other communication channels may all be very helpful.

4.2. ONE HEALTH AND ZOONOSES

International organizations and researchers have characterized the link between humans, animals, and surroundings and embraced a concept known as "One Health Concept" or "One Health Approach" for the prevention and management of infectious diseases such as zoonotic diseases. To effectively address issues

with global health, this paradigm was embraced (Bidaisee et al. 2014). To assure good health for animals, people, and our environment, the one health concept promotes cooperation among wildlife biologists, veterinarians, doctors, agriculturists, ecologists, microbiologists, epidemiologists, and biomedical engineers (Aenishaenslin et al. 2013; Dahal et al. 2014; One health. 2020).

One health is directly linked to the prevention and control of zoonoses. According to (Pieracci et al. 2016), the recommendations provided by one health approach to preventing and control zoonoses are Creating a "Zoonotic Disease Unit" to benefit human and animal health organizations; developing a national strategy for the "Zoonotic Disease Unit"; enlisting the leadership of multi-sectoral researchers and pertinent personnel to prioritize zoonotic disease research; adopting veterinary public health policies with collaborators from other nations; and reviewing the zoonotic diseases regularly (2–5 years) to address the emerging and re-emerging diseases.

Infectious disease onset and dissemination in the One Health domains are being driven by global trends. Recommendations Zoonotic diseases pose a significant risk to the public's health. Even though many zoonoses are already under control, there are still many diseases about which we know little, particularly in terms of their distribution, etiology, pathogen, host, vector biology, dynamics, cycle of transmission, predisposing factors, and risk factors. The balance between the host, agent, and environment may be upset at any time as a result of a variety of anthropogenic activities, such as population growth and natural processes that cause the emission of zoonoses. We are unable to anticipate with any degree of accuracy when or how the next zoonoses epidemic will affect the world. To assure or improve our readiness to combat such a pandemic, the following actions need to be ensured or reinforced.

• Active and extensive zoonoses surveillance and monitoring using cutting-edge methods including molecular epidemiology tools and satellite-based remote sensing systems.
• Giving importance to the creation of action teams and zoonoses.
• The availability of diagnostic resources and qualified personnel.
• International, subnational, national, and regional collaboration.
• One health-based strategy that includes veterinary and medical physicians as well as environmental specialists and other experts. • Providing sufficient ongoing and urgent financing.
• Widespread public education campaigns on zoonoses.
• More investigation into the risk factors, pathogen pathogenicity, host biology, and vector biology for disease.
• Monitoring and safeguarding wildlife

Ensure the safe manufacturing of animal-derived food.

Maintain infectious labs' safety to prevent the unintentional spread of zoonotic diseases and bioterrorism.
• Environment protection.
• Public education campaigns at the national and international levels on zoonoses and cleanliness

5. CONCLUSION

Borders and academic fields are irrelevant when it comes to transboundary zoonotic diseases, which necessitates an all-encompassing strategy. The majority of infectious illnesses that affect people are animal-borne. These pathogens not only infect animals with diseases but also pose a major risk to human health. Because of the growing interaction between people and wild animals, it is often the case that changing eating habits, climate change, and ecologically unfavorable human activities impact the establishment and reemergence of many zoonotic diseases. The present COVID-19 epidemic makes clear how catastrophic zoonosis is for the human population. Research concentrating on the one health approach has to be prioritized to uncover crucial intervention stages in the transmission of infections

because of the close ties between animals, people, and the environment. Societies may better protect themselves against transboundary zoonotic dangers by comprehending the dynamics of disease formation, the consequences for health, economy, and ecosystems, as well as the techniques for prevention and management. Taking on these difficulties benefits the larger framework of sustainable development and the ideals of One Health in addition to improving the security of global health.

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Biosecurity Measures to Control Zoonotic Diseases

56

Gahin A Tayib

ABSTRACT

Zoonoses, diseases transmitted between animals and humans, constitute a significant public health concern globally. Originating from the Greek words "Zoon" and "noses," meaning animals and sickness, the World Health Organization defines zoonosis as infections transferred between people and animals. Approximately 61% of human infectious diseases stem from zoonotic sources. Animals, serving diverse roles in human life, may harbor harmful germs capable of causing zoonotic illnesses, even when asymptomatic. Wild animals, including mammals, amphibians, fish, birds, and reptiles, act as reservoirs for zoonotic pathogens, influencing transmission patterns affected by climatic parameters. This chapter explores major bacterial, parasitic, viral, and fungal zoonotic diseases, focusing on Anthrax, Brucellosis, Salmonellosis, Toxoplasmosis, Trypanosomiasis, Rabies, Crimean-Congo Hemorrhagic Fever (CCHF), Dermatomycoses (Ringworm), and Histoplasmosis. Each disease's transmission, symptoms, prevention, and control measures are discussed in detail. Prevention strategies include biosecurity measures, vaccination, vector control, and public awareness. Additionally, the abstract emphasizes the importance of a One Health approach, recognizing the interconnectedness of human, animal, and environmental health. Understanding these zoonotic diseases, their origins, drivers, and prevalence is crucial for managing emerging infectious diseases and preventing epidemics and pandemics. By exploring various disease types and their implications in different communities, efforts can be directed towards effective prevention and control strategies, ultimately safeguarding both human and animal populations.

Keywords: Zoonosis, One Health, Anthrax, Brucellosis, Rabies

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1. INTRODUCTION

Zoonosis is a word that originated from the Greek word “Zoon”, which suggests animals, and “noses”, refers to sickness. World Health Organization (WHO), suggests that every infection or disease that's normally spread from people to animals or from animal to human will be recognized as zoonosis (WHO 2020). Almost 61% of infectious diseases in human are from zoonotic sources (Taylor et al. 2001). There are over 200 known sorts of zoonoses. Zoonoses include an expansive rate of unused and existing maladies in people. A few zoonoses, such as rabies, are 100% preventable through immunization and other strategies (Acha and Szyfres 2005).

Numerous people in their everyday life deal with animals, both far from home and at home. Worldwide, animals give livelihoods, travel, sport, nourishment, fiber, companionship, and education to people (Steffens and Wilson 2012).

Occasionally, animals carry harmful germs that can infect humans. Germs responsible for causing Zoonotic illnesses have the capacity to produce many different sorts of infections in animals and people, considering infections from slight to serious illnesses and even passing. Sometimes asymptomatic animals even when they have germs can produce sickness in people, relying on the zoonotic illnesses (Acha and Szyfres 2005).

Unpredictably, wild animals are associated with people, domesticated creatures, and subsequently straightly contribute to the transmission and support of diverse irresistible infections, (Kruse et al. 2004). Wild creatures such as warm-blooded animals, amphibians, fish, birds, and reptiles act as a reservoir of zoonotic pathogens with the potential to transfer to people or other hosts. Climatic parameters such as raindrops, humidity, temperature, and by the nature of germs can affect the transmission designs of natural life zoonoses (Thompson and Polley 2014).

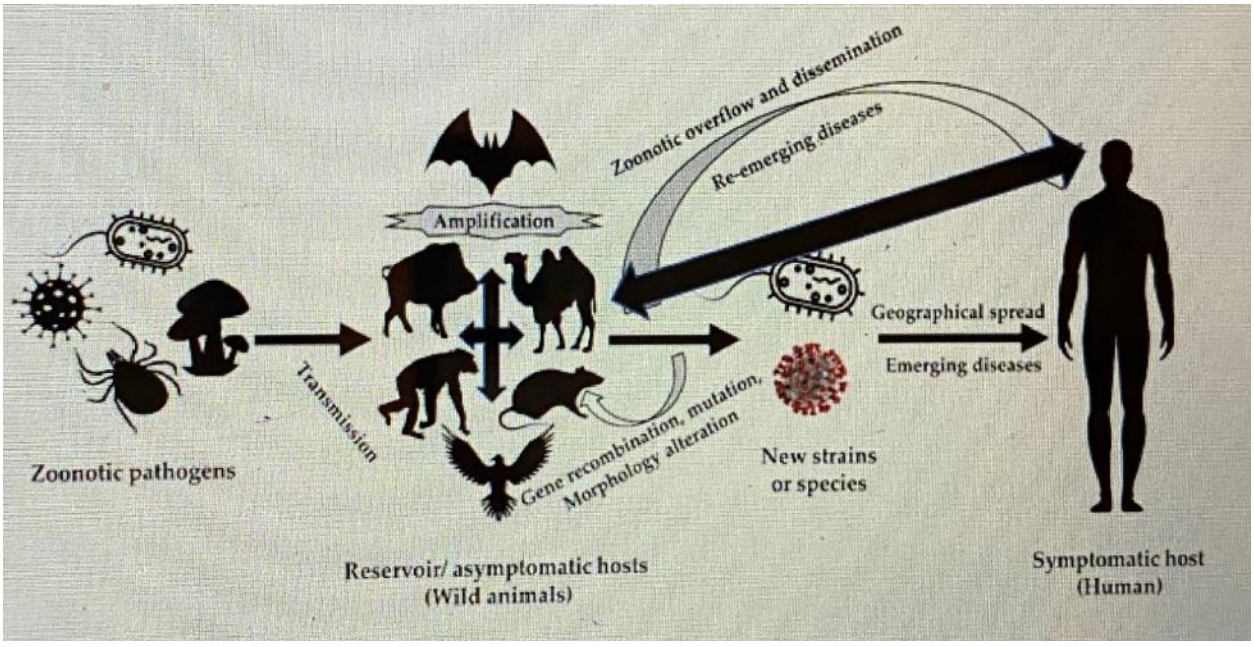

Fig 1: Illustrates the inclusion of wild creatures within the transmission and enhancement of causative operators of the rising and re-emerging of Zoonoses (Kruse et al. 2004; Cupertino et al. 2020). (Rahman et al. 2020).

Globally, Zoonotic diseases are very common. Researchers evaluate that more than 6 maladies out of every 10 known infectious in individuals can be transferred from animal, and in human 3 diseases out of every

4 new or emerging infectious diseases come from animal. Center for Disease Control and Protection has been working 24/7 to keep people from zoonotic diseases (Steffens and Wilson 2012).
In the emergence of managing emerging infectious diseases (EID) including zoonoses, and eliminating the risk of them which have been epidemics and pandemics, we need to search for their various types, their origins, their drivers, and their importance in different communities (United Nations Environment Programme, 2020). Unsurprisingly, livestock, domesticated wildlife, and pets are the large proportion of animal involved in the history of zoonotic diseases or current zoonosis are domestic, with high contact rates. Extremely the emergence of the latest wildlife Zoonosis is rare but can be a very significant (United Nations Environment Programme, 2020).

1.1. MAJOR BACTERIAL ZOONOTIC DISEASES

vectors play a critical part in the transmission of different infections to people and in numerous cases, they facilitate the spread of pathogens originating from wild creatures. The positive affiliation between vectors and people in impacting pathogen differences was, to begin with, hypothesized a long time prior (Robert and Brown 2004). Approximately 60% of human irresistible infections originate from vertebrate creatures (Klous et al. 2016). Coordinate human contact with has extended with the presentation of taming of distinctive animals (Pearce-Duvet 2006).
Sheep, goats, cattle, cats, steeds, pigs, pooches, and other performance as supplies of pathogens of residential zoonoses and can spread the maladies to people (Rahman 2021). Cases of zoonotic diseases that can be transmitted to people from residential creatures include balantidiasis, ancylostomiasis, toxocariasis, campylobacteriosis, leptospirosis, bacillus anthracis, Q fever tuberculosis, brucellosis,toxoplasmosis, listeriosis, bovine pustular stomatitis, rotavirus contamination, and rabies (Rahman 2021).
Worldwide, there are major different bacterial zoonotic illnesses, but few of them with a pretty introduction regarding their cardinal symptoms, transmission, and most important the methods of their prevention and control (monitoring) would be discussed.

1.1.1. ANTHRAX

Bacillus anthracis is abacterial contamination caused by the high-impact, spore-forming, Gram-positive organism, found all through the world. It occurs in residential (such as sheep, goats, and cattle) (Zhang et al. 2022). *Bacillus anthracis* isn't transported from individual to individual. Transmission occurs in one of four ways, and signs and indications be able to change based on how *Bacillus anthracis* inserts the body: Through breaks within the skin. Cutaneous *bacillus anthracis* produces swelling around the sore, an effortless skin sore (ulcer) with a dark center, and causes rankles or bumps on the skin. The sore is ordinarily on the confront, neck, arms, or hands (Steffens and Wilson 2012). In animals, the infection advances very quickly, and commonly no clinical signs are watched some time recently passing. Pastures get sullied with bacillus anthracis spores and Animals get to be contaminated by nourishing on contaminated pastures (Zhang et al. 2022). The transmission of Anthrax from animals to human is described in Fig. 2 (Steele 2016).

2. BIOSECURITY, PREVENTION, AND CONTROL FOR INDIVIDUALS; THE VETERINARY AUTHORITIES ORDERED CONTROL MEASURES

1. Avoid examination of (suspected) infected carcasses.
2. A boycott on the butchering of debilitated creatures and disallowance of the butchering of dead and debilitated creatures.

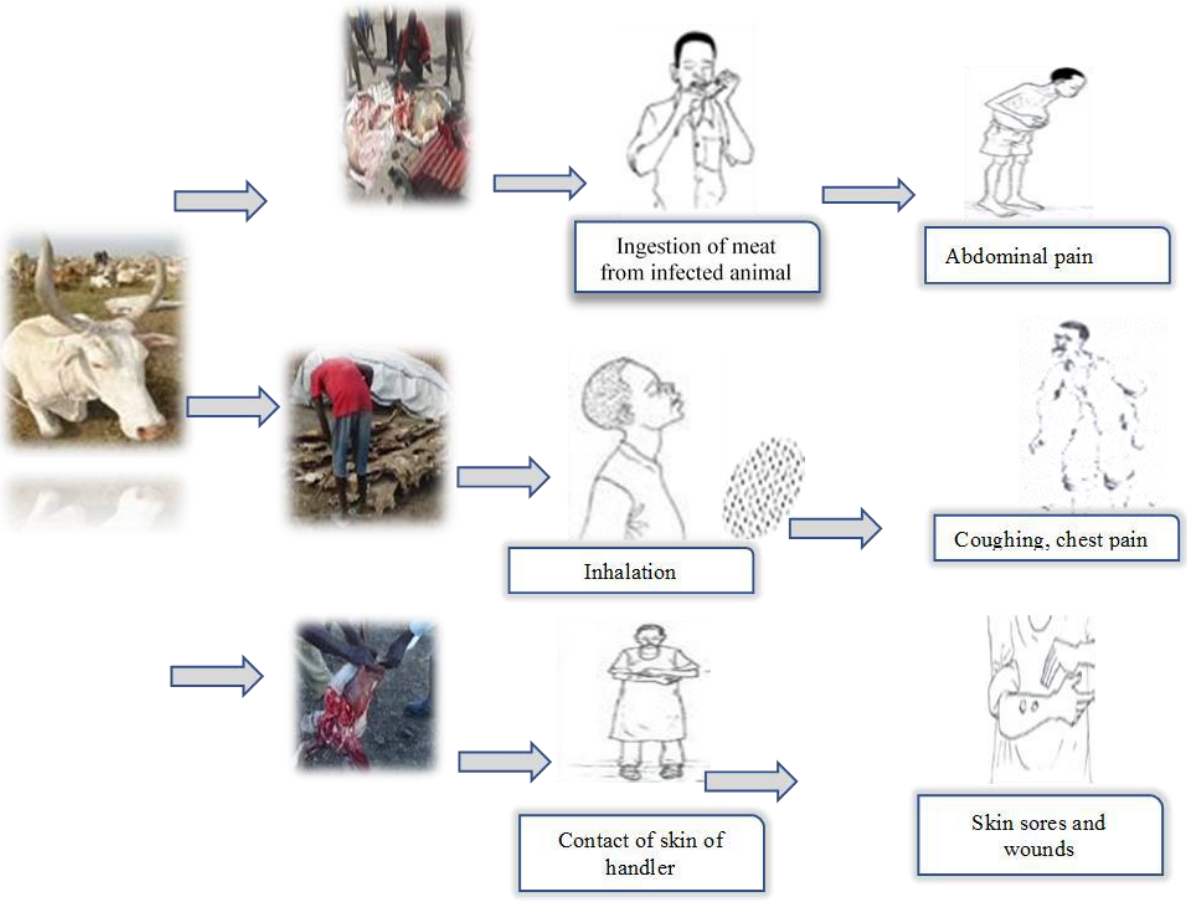

Fig. 2: Description of the methods of Anthrax Transmission to people.

3. Disallowance of the utilization of drain and dairy items, or meat, skin, and other items, as well as with clinical signs of ailment or suspected of contamination,
4. A concise guide for emerging infectious diseases and zoonoses
5. Avoid movement of livestock from affected premises during an outbreak.
6. Dominance or control dust in industry handling wool or hides.
7. Disinfect and wash wool/hair from endemic areas (e.g., with 10% formalin).
8. livestock (cattle, sheep, goats, and Equidae) in endemic areas must be vaccinated.
9. Creatures suspected to have died bacillus anthracis ought to be buried 6 feet profound or burned.
10. Dead creatures ought to not be cleared out to break down within the open touching regions
11. Suspected cases of bacillus anthracis ought to be detailed expeditiously to the veterinary administrations.

Overall, prohibit the use of meat and bone meal as ruminant feed, as these are potential sources of anthrax bacteria.

2.1. BRUCELLOSIS

Brucellosis is an endemic zoonotic disease in Asian and African countries and has a significant impact on both animal and human health. It still remains as one of the major public health concerns throughout

developing countries, accounting for an annual occurrence of over 500,000 cases (Pappas 2002). The malady influences sheep, goats, cattle, pigs, and a few further creatures. It can be able to be passed to individuals by means of coordinated contact with animals or through drinking unpasteurized drains from a tainted animal. Classically, tall fever spikes happen each evening, subsequently the title "undulant" fever. In animals, the microscopic organisms basically influence regenerative organs and are found in expansive concentrations within the uterus of contaminated females. In human, the malady presents as a generalized condition (Steffens and Wilson 2012).

3 BIOSECURITY, PREVENTION, AND CONTROL OF BRUCELLOSIS(UNITED NATIONS ENVIRONMENT PROGRAMME 2020)

1. Animals ought to be appropriately assessed before recently butchering to guarantee no signs of Brucellosis.
2. Non-assessed meat ought to not be taken care of at home.
3. Pasteurized or bubbled drain and dairy items from dairy animals, sheep, and goats only could be utilized.
4. Guarantee meat is completely cooked.
5. Dairy items ought to be arranged as they were from appropriately heated/pasteurized milk.
6. Never suckle milk specifically from the nipples of a goat, sheep, or dairy animal.
7. Prematurely ended material or after-birth ought to never be taken care of without defensive arm sleeves.
8. Wash hands and arms altogether after taking care of births, premature births, and meat.
9. Suspect contaminated fabric ought to be burnt.
10. Suspect cases ought to be detailed instantly
11. In districts where the predominance of brucellosis is tall, guarantee agriculturists and slaughterhouse specialists are mindful of the dangers of taking care of creature tissue, and give enlightening in infection-control hones to play down the hazard of introduction.
12. Bury disposed of the creature's remains.
13. Put in place uncommon safeguards for research facility specialists.
14. Animal brucellosis is best avoided by cautious group administration and cleanliness. Immunization is valuable for the avoidance and monitoring of disease. Destruction can be only accomplished by test-and-slaughter joined with successful avoidance measurements and control of creature development.

3.1. SALMONELLOSIS

Salmonellosis is a zoonotic disease caused by bacteria called salmonella. Worldwide, Salmonella is the most prevalent in impoverished areas that are overcrowded with poor access to sanitation. To date, the highest incidences of typhoidal Salmonella infection in the world occurred in southcentral Asia, southeast Asia, and southern Africa. Salmonella infection is one of the foremost common and broadly conveyed food-borne illnesses. It regards as a major public well-being burden and speaks to a critical cost in numerous nations. Microbes of the Salmonella species include *S. enteritidis, S. typhimurium*, and more than 2500 known types or serotypes worldwide (Vos et al. 2017). Salmonella spp. are highly adapting pathogens to humans and animals. Yearly assessed that tens of millions of human cases happen around the world and the illness comes about in more than 100 000 deaths.83

In spite of the fact that episodes of salmonellosis have been detailed for decades, it is considered a developing malady since it has as of late expanded in rate in numerous landmasses. From the beginning of 1990s, strains of salmonella that are safe to the extent of antimicrobials have risen and undermined to gotten to be a genuine open well-being issue. (Robert and Brown 2004; Taib and Abdulrahman 2022).

Salmonellosis is basically transmitted through the defilement of nourishments, nourishment utensils, nourishment devices, and nourishment hardware by fecal fabric. Destitute administration of human and creature waste is essential to calculate the transmission of these infections (Steele 2016). The symptoms of disease include vomiting, abdominal pain, headache, fever and diarrhea (Taib and Abdulrahman 2022).

4. BIOSECURITY OF THE CONTROLLING OF SALMONELLA ILLNESS(STEELE 2016; CUPERTINO ET AL. 2020)

1. Cook poultry and eggs completely.
2. Don't consume in part baked eggs with runny yolks and don't expend meat that's pink or ruddy after cooking unless persuaded of the new and secure source of the items.
3. Secure food-handling procedures and exercise great individual hygiene and
4. Clean water and cleanser should be used sometime recently while eating or cooking, and after utilizing the latrine.
5. Avoid wild feathered creatures from blending with poultry on ranches and Control rodents.
6. In poultry breeding farms Salmonella testing ought to be conducted.
7. The milk ought to be pasteurized or bubbled sometime recently utilization.
8. Food handlers and the public ought to be taught the dangers of salmonellosis contamination.
9. Always wash your hands after handling animals or animal products.

4.1. MAJOR PARASITIC ZOONOTIC DISEASES

As human pathogens, there are a vast number of parasitic zoonoses such as cryptosporidiosis, toxoplasmosis, and leishmaniasis, trypanosomiasis that have picked up in significance, and this is due to their capacity to cause infection in patients with safe concealment due to HIV. The larger part of the classic parasitic maladies due to helminths, trematodes, cestodes, pentastomids, and protozoa are zoonotic (Weiss 2008). Ignored parasitic zoonoses (NPZ) are a bunch of maladies including trichinellosis, echinococcosis, cysticercosis, and foodborne trematode diseases that proceed to put critical burdens on a few populaces around the world. Control and anticipation of these illnesses require intersectoral collaboration among the open well-being, creature well-being, nourishment security, and WASH divisions (Weiss 2008; Mertz 2016).

4.2. TOXOPLASMOSIS

Toxoplasma infection or Toxoplasmosis is an infestation caused by a parasitic protozoon *Toxoplasma gondii*, ordinarily spread from creatures to people. The condition influence cats, human, sheep, goats, pigs, cattle, rodents, fowls that bolster on the ground, and reptiles. Cats ended up tainted by eating these little birds and warm-blooded animals and after that passing oocyst in their defecation, which are virulent to human. It can have extreme results in pregnant ladies and people with a compromised resistant framework. In both cats and human, invasion happens without clinical signs in typical people. Signs create in people with discouraged insusceptibility (Steele 2016).

Ingestion of undercooked, sullied meat could be the pathway of disease transmission as well as, eating without washing hands completely, after incidentally or unconsciously dealing with sullied nourishment. Also eating nourishment sullied by blades, cutting sheets, utensils, and nourishment that has had contact with crude, sullied meat. Furthermore, drinking water or drain is sullied by *Toxoplasma gondii* (Robert and Brown 2004).

5. BIOSECURITY, PREVENTION, AND CONTROL OF TOXOPLASMOSIS (UNITED NATIONS ENVIRONMENT PROGRAMME 2020)

1. Don't eat crude or improperly cooked meat.
2. Don't drinsssk unpasteurized drain.
3. Don't eat unwashed natural products and vegetables.
4. Wash hands and nourishment planning surfaces with warm foamy water after dealing with crude meat and Wash hands sometime recently eating (particularly for children).
5. Don't drink water from the environment unless it is boiled.
6. Don't nourish crude meat or undercooked meat to cats. Moreover, don't provide them unpasteurized drain.
7. Don't permit cats to chase or meander.
8. Don't permit cats to utilize a plant or children's play region as their location.
9. Pregnant ladies, and people with smothered resistant frameworks, ought to not clean the litter destinations of cats.
10. Control rat populaces and other potential middle hosts.

5.1. TRYPANOSOMIASIS

Trypanosomiasis (Sleeping sickness) are maladies caused by blood parasites. Zoonotic Trypanosomiasis in Eastern Africa, caused by *Trypanosoma rhodesiense*. In West Africa, another shape of zoonotic Trypanosomiasis happens and is caused by *Trypanosoma gambiense*. In animals, zoonotic Trypanosomiasis in Eastern Africa happens in Cattle, elands, and other wild ruminants with cloven hooves. Other species of trypanosomes contaminate and cause illness in cattle, sheep, goats, pooches, cats, steeds, camels, and other wild creatures but are not zoonotic. The cardinal sign of this infestation is sleeping without treatment and the sick person eventually dies (Franco et al. 2022).

6. BIOSECURITY, CONTROL, AND PREVENTION OF TRYPANOSOMIASIS (FRANCO ET AL. 2022)

1. Look for restorative consideration instantly when debilitated
2. Maintain a strategic distance from bites of the tsetse fly
3. In animals utilize of insect repellents such as pyrethrins and counting pour-ons offer assistance keep flies away.
4. A critical epidemiological challenge at the end is the part that supplies such as asymptomatic human carriers and non-human creatures might play in keeping up or rekindling transmission.

6.1. MAJOR VIRAL ZOONOTIC DISEASES

Warm-blooded animals, reptiles, fowls, and likely amphibians are stores or increasing hosts for viral zoonotic diseases. Regularly, these infections cause small or no obvious illness in their non-human vertebrate hosts. A few viral zoonoses have exceptionally constrained host ranges; others may contaminate a wide run of vertebrates. In man, the disease may change from unapparent to lethal

malady. Both modern and ancient viral zoonoses are particularly critical in rising and reemerging infection illnesses. The spreading of zoonotic infections may happen in a multiplicity of courses (Reed 2018). They incorporate direct (e.g., rabies infection) or indirect (e.g., hantavirus) contact; "nosocomial" (e.g., Ebola infection); "aerosol transmission" (SARS coronavirus); "vertical" (in utero) (Zika infection); and "vector- or arthropod-borne" (e.g., yellow fever infection and West Nile infection). Zoonotic viral illnesses happen on each landmass but, maybe Antarctica. Around the world, a few are around, in a variety of environmental settings. Others are found as it were in exceptionally restricted ecologic and geographic foci. In spite of the fact that hundreds of infections are zoonotic, the significance of numerous of these infections has not however been set up. A few of the vital viral zoonotic illnesses will be talked about briefly (Reed 2018).

6.2. RABIES

It causes anxious framework infection that closes in passing. Animals can get to be contaminated without apprehensive framework infection, develop antibodies, and survive, but play no part in transmission. All around the world classical rabies is found but more infection in Britain, the Hawaiian Islands, Australia, Antarctica, and Modern Zealand. Spreading happens by the nibble of a tainted creature. Vaporized (droplet) transmission is uncommon. The saliva of infected animals is highly infectious if it comes into contact with the wound. In tropical developing nations Dog is the most supplied where >99% of all human cases happened. transmitted through bites of infected dogs, cats, bats, wild foxes, squirrels, horses, cattle, monkeys, and other animals (Reed 2018).

7. BIOSECURITY, PREVENTION, AND CONTROL OF RABIES (STEELE 2016; REED 2018)

1. Dogs that appear signs of rabies ought to be slaughtered promptly. Report suspected out-of-control pooches to specialists / veterinary administrations instantly.
2. In regions where rabies is endemic, the dog populace ought to be vaccinated routinely and labeled for ease of recognizable proof.
3. Never throw sticks, stones, or other objects at unusual dogs.
4. Wash dog bite wounds altogether with water and cleanser or cleanser, at that point apply disinfectant such as iodine.
5. Carcasses should be disposed of safely and away from the reach of dogs, cats, and other scavengers.
6. Animal well-being laborers ought to guarantee legitimate control of dogs and cats during taking care of them.
7. Creature well-being specialists ought to ideally get scheduled inoculation against rabies.
8. Education and Public Awareness on rabies prevention and control.
9. Dogs without a vaccination certificate can also be restricted within a country as a control measure.

7.1. CRIMEAN-CONGO HEMORRHAGIC FEVER (CCHF)

Nariovirus or CCHF is a zoonotic tick-borne viral infection, which moves through the normal world in an enzootic cycle that includes vertebrates, ticks, and other ticks. Ticks of the class Hyalomma serve as both vectors and stores of the virus, and the spread of the disease happens through human-human contact or contact with the blood of the asymptomatic animals (Fig. 3). The major complications of CCHF incorporate hepatitis, fast kidney weakening, and aspiratory disappointment or sudden liver disappointment, which may lead to passing (Messina et al. 2015; Mohammed et al. 2022).

8. BIOSECURITY, PREVENTION, AND CONTROL OF CCHF; (MESSINA ET AL. 2015; SORVILLO ET AL. 2020)

1. The foremost effective repellent for avoiding tick nibbles is an Insect repellent that contains N, N-diethyl-m-toluamide, commonly known as DEET.
2. Gloves and other defensive clothing are prescribed for animal specialists.
3. People ought to maintain a strategic distance from contact with the patients or with the blood and body liquids of wiped-out animals.
4. In healthcare settings, Strict adherence to infection-control safety measures is exceptionally critical. Healthcare laborers who have had contact with tissue or blood from patients with suspected or affirmed CCHF ought to be taken up with everyday temperature and indication observing for at slightest 14 days after presentation.
5. Open instruction almost the dangers of tick bites and individual assurance is fundamental.
6. In the interim, there's no secure and successful antibody accessible against this infection for human and creatures to utilize. Subsequently, as it were the method to control the rise of infections is through outside parasitic medications against mindfulness, ticks, and secure butchering methods at authorized abattoirs.

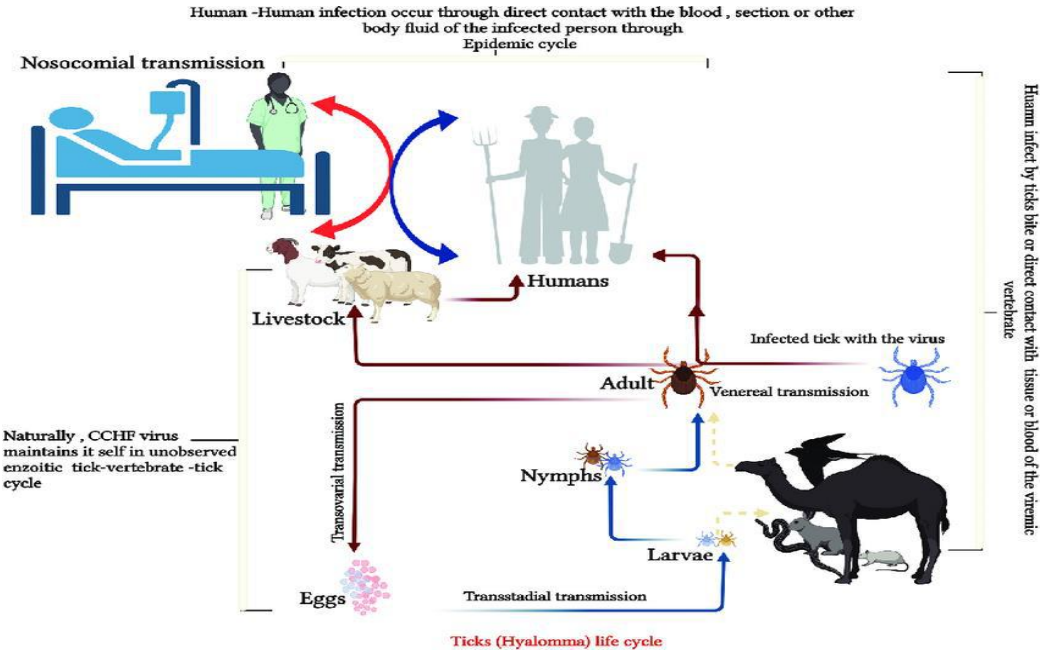

Fig. 3: Ticks (Hyalomma) life cycle (Mohammed et al. 2022).

8.1. MAJOR FUNGAL ZOONOTIC DISEASES

Globally, fungal diseases related to zoonotic transmission are a critical public well-being issue. An amount of these diseases are among the bunch of foremost common fungal illnesses, such as histoplasmosis, dermatophytosis, and sporotrichosis (Sorvillo et al. 2020). Zoonotic fungi can be actually spread between animal and people, and in a few cases cause critical public well-being issues. It is, in any case, notable that a few contagious illnesses with zoonotic potential have needed satisfactory consideration in universal open well-being efforts, driving to inadequate consideration of their preventive methodologies (Sorvillo et al. 2020).

8.2. DERMATOMYCOSES (RINGWORM)

Dermatomycosis (ringworm) could be a major fungal zoonotic illness conveyed around the world and caused by fungi of three genera (Trichophyton, Microsporum, and Epidermophyton), referred as dermatophytes. Ringworm is an infection of the hair, skin, and nails, which is caused in most cases by dermatophytes, and in rarer cases by yeasts and molds. Globally, fungal diseases of the skin are the foremost habitually happening infectious illnesses (Mukrimaa et al. 2016).

9. BIOSECURITY, PREVENTION, AND CONTROL OF THE RINGWORM

The stages for satisfactory Veterinary Public Health (VPH) activity, as concocted by WHO specialists (Mainzer et al. 2002; Mukrimaa et al. 2016) and tried by particular down-to-earth measures in several circumstances, have been distinguished. The taking after operational arrangements has been recognized as appropriate, successful strategies of zoonoses control:

- Observation (Dermatomycoses must always be treated).
- Control of animals
- Successful therapy for fungal diseases is a rapid diagnosis and pathogen typing.
- Control of infective media

Fig. 4: illustrate the importance of one health regarding safe ecosystem.

9.1. HISTOPLASMOSES

It is a zoonotic fungal disease that is associated with bat guano (stool) and could be a sort of infection of the lung. Breathing in *Histoplasma capsulatum* contagious spores is the causative factor of the fungal disease. The spore can be found within the droppings of bats, in soil, and in feathered creatures. Individuals with weaker safe frameworks may involvement serious issues. The infection may advance and spread to other regions of the body (Seyedmousavi et al. 2015). 10 to 15 percent of cases of histoplasmosis have been detailed as skin injuries that have spread all through the body (Mazi et al. 2022). Fig. 5 demonstrate the pathway of histoplasmosis transmission.

10. BIOSECURITY, PREVENTION, AND CONTROL OF THE HISTOPLASMOSIS

It’s difficult to maintain a strategic distance from breathing in H. capsulatum organism in case you live in a range where it’s common. Whereas not completely preventable, there are a few steps you'll be able to take to diminish your chance of histoplasmosis:

1. Maintain a strategic distance from zones where you’ll be uncovered to dirt or dust, particularly in zones where bats or feathered creatures live.

In the event that your work or side interests uncover you to soil that’s likely to have *H. capsulatum*, use an N95 respirator veil to assist channel the air you breathe.

11. CONCLUSION

Wild animals, acting as reservoirs for zoonotic pathogens, contribute to the transmission of infectious diseases. Climatic factors further influence transmission patterns. Major bacterial zoonotic diseases, transmitted through vectors, involve animals such as sheep, goats, cattle, cats, horses, pigs, dogs, and more. Specific diseases include anthrax, brucellosis, and salmonellosis. Prevention measures range from proper handling of carcasses to vaccination. Parasitic zoonotic diseases, caused by organisms like

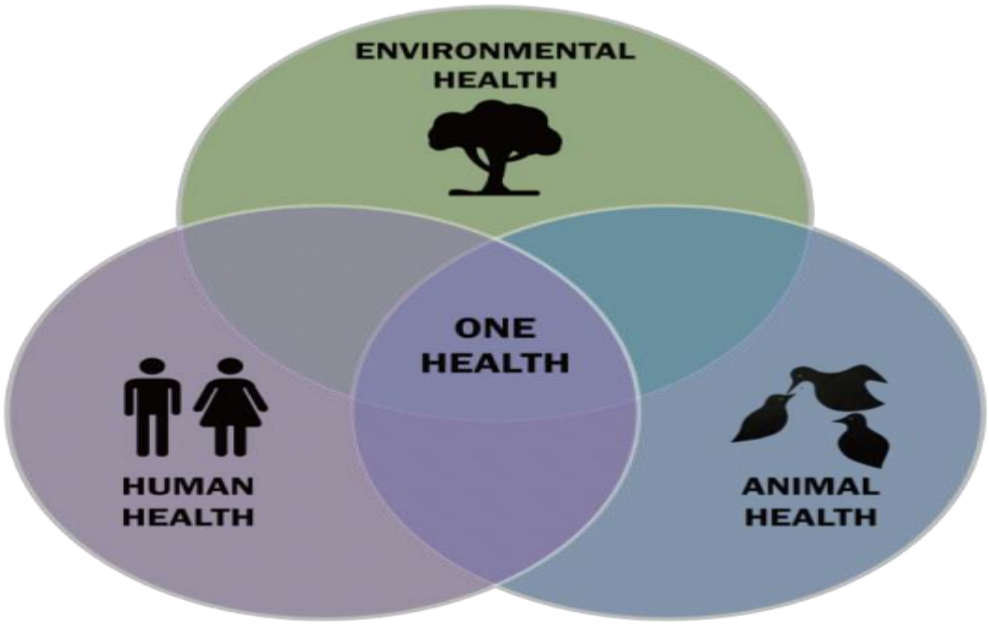

Fig. 5: One health (animal and human health) is interdependent and bound to the health of the ecosystems in which they exist (Rahman 2021).

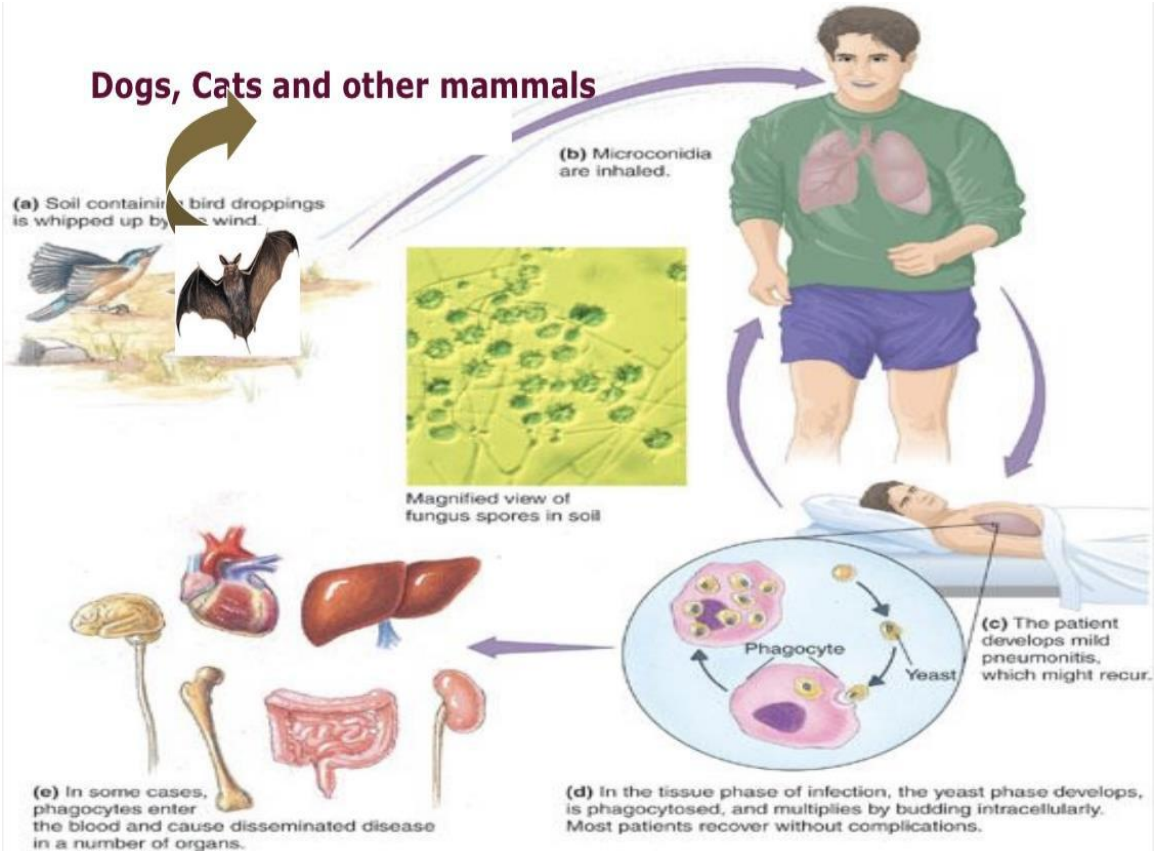

Fig. 5: The pathway of transmission of Histoplasmosis.

Toxoplasma gondii and Trypanosoma species, highlight the importance of avoiding undercooked meat, practicing good hygiene, and controlling vectors. Viral zoonotic diseases, exemplified by rabies and Crimean-Congo hemorrhagic fever, emphasize the need for measures such as vaccination, vector control, and public awareness. Fungal zoonotic diseases, including ringworm and histoplasmosis, underscore the significance of veterinary public health actions, monitoring, and control of infective media.

The complexity of zoonoses necessitates a One Health approach, recognizing the interconnectedness of human, animal, and environmental health. Control strategies involve vigilant surveillance, prompt diagnosis, biosecurity measures, and public education to mitigate the impact of these diseases. In the context of emerging infectious diseases, understanding various zoonotic types, their origins, and drivers becomes crucial for effective prevention and control. As the Center for Disease Control and Prevention works tirelessly, a comprehensive and collaborative approach remains essential in managing and preventing zoonotic diseases globally.

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