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Complementary and Alternative Medicine: Non-Conventional Therapies

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PREFACE

In recent years, the search for alternatives to conventional medical treatments has intensified, particularly in response to the global rise of antimicrobial resistance (AMR) and other complex health challenges. *Complementary and Alternative Medicine: Non-Conventional Therapies* offers a thorough exploration of innovative approaches, focusing on how alternative methods can address a range of medical and veterinary health issues. This volume brings together cutting-edge research on non-traditional therapies, including botanicals, probiotics, and nanotechnology, highlighting their potential to complement or replace conventional treatments for a more sustainable healthcare future. The book opens with an investigation into natural compounds like anthocyanins and their role in combating AMR. As antimicrobial resistance continues to threaten both human and animal health, alternative solutions are crucial. Studies on bacterial resistance, such as those on *Staphylococcus aureus* and *Escherichia coli*, offer promising methods for controlling these pathogens. Additional sections explore innovative approaches in animal health, examining how products like Activo can replace conventional antibiotics in poultry, helping to improve productivity and reduce antibiotic reliance. Throughout, the book underscores the role of precision treatments and advanced therapies in modern medicine. Nanotechnology applications, for instance, show how nanoparticles can enhance the effectiveness of antimicrobials against pathogens such as Salmonella. Furthermore, insights into the immunomodulatory effects of probiotics demonstrate how harnessing natural immune responses can provide effective treatments against infections, potentially reshaping preventive care strategies. Beyond infectious disease, this work examines the broader scope of CAM in chronic disease management. Natural therapies for autoimmune disorders, obesity prediction using artificial intelligence, and alternatives to antipsychotic medications reflect a growing interest in holistic and non-conventional approaches. These sections showcase how CAM can offer solutions for managing both physical and mental health conditions without solely relying on pharmaceuticals. Particularly relevant is the focus on AMR in agriculture, with discussions on the integration of prebiotics and probiotics as defense mechanisms in animal health. Natural compounds and feed additives are explored as means to support immune health in livestock and poultry, presenting a proactive approach to disease prevention and animal welfare. Moreover, topics on environmental influences on health and vaccine development provide a broader context for CAM's role in public health. The closing sections of *Complementary and Alternative Medicine: Non-Conventional Therapies* delve into emerging therapies such as Reiki for animals, herbal medicine's role in managing inflammation and infection, and the potential of CAM in both human and veterinary vaccine advancements. These discussions highlight the ongoing shift towards an integrative health model, blending traditional wisdom with scientific innovation to create more adaptable, resilient healthcare systems. With a focus on both established and emerging therapies, this book serves as an inspiring resource for researchers, practitioners, and students interested in expanding the possibilities of healthcare. By integrating alternative perspectives into mainstream medicine, *Complementary and Alternative Medicine: Non-Conventional Therapies* demonstrates how a holistic, innovative approach can meet some of the most urgent health challenges of our time.

Editor

Contents

Chapter 01

Role of Anthocyanins in Combating Antibiotic Resistance

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ABSTRACT

Antibiotic resistance becoming a global challenge nowadays. The use of antibiotics as growth promoters is a serious health concern for consumers because of antibiotic resistance. So, the world is moving toward alternatives like prebiotics, probiotics, herbal products, and trace minerals. Anthocyanin is a class of phenolic compounds that are extracted from fruits, vegetables, and grains. Due to various antibacterial, anti-inflammatory, antiviral, and antioxidant effects, anthocyanin is being interesting field of research for researchers. It is found that anthocyanins have strong anti-microbial activity but their usage is limited due to low bioavailability and unstable structure. However, this compound improves the growth health by improving the gut microbiota and immunity of the host. The encapsulation, nanoparticle formation, and targeted drug therapy will improve the dark side (bioavailability and stability) of anthocyanins and specific targeting sites improve the host from most food-borne pathogens. The discovery of encapsulation, nanoparticle formation, and targeted drug therapy will be a big through of replacing the anthocyanins in the future. In this chapter the details of anthocyanins, their merits being used as a replacement to antibiotics, and their impact on the animal and food industry.

KEYWORDS
Antibiotic resistance, anthocyanins, growth promotor

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INTRODUCTION

Anthocyanins, the phytogenic bioactive compounds, can be found in a variety of plant species, such as fruits, vegetables, flowers, and cereal crops. Anthocyanins, being water-soluble pigments, possess a diphenyl propane skeleton (C6C3C6) and can display a range of colors, such as red, purple, or blue, depending on the pH level. Cyanidin, delphinidin, and pelargonidin are among the most commonly occurring anthocyanins. In addition, malvidin, peonidin, and petunidin are the methylated derivatives of these three anthocyanins. Humans typically consume around 12.5 milligrams of anthocyanins per day through diet. Because, the food we eat contains a plentiful number of anthocyanins, which are perfectly safe to consume. For individuals, a safe daily dose is typically around 2.5 milligrams per kilogram of body weight (Pojer et al., 2013). Extensive research has provided data to support the idea that anthocyanins, a class of naturally occurring bioactive chemicals, can potentially enhance health in various ways, including relieving metabolic symptoms (Tena et al., 2020). The increasing demand for anthocyanins in the food industry is due to the use of it the reason, as its addition improves the quality of the food items. For instance, the worldwide wine output in the year 2022 amounted to around 25.8 billion liters, resulting in a substantial quantity of grape skins abundant in anthocyanins. However, anthocyanins, which are the primary natural colorants, possess a wide range of by-products that make them a promising candidate for use as animal feed additives and in several other applications.

Numerous scientific studies have provided data supporting the notion that the addition of anthocyanins has a positive impact on gut health. This is accomplished by maintaining the integrity of the intestines, regulating the immune response, and preventing the growth of harmful bacteria in the gut (Morais et al., 2016). In recent research, it has been found that the utilization of anthocyanins has the potential to be a substitute for antibiotics that are being utilized in food and feed. However, the researchers face numerous challenges in presenting anthocyanins as an alternative to antibiotics, including (1) The price of production, and storage are very costly (2) Isomerization and degradation by heat are also technical challenges (3) The mechanism of utilization is still needed to be explored and euthanatized (4) Bioavailability in the intestine is also

questionable and need to explore, that how it controls the harmful microbes. In this context, this book chapter summarizes the recent scientific literature and evidence for the potential of anthocyanins in replacing or reducing the use of antibiotics.

Current Demand of Antibiotics and Emerging Threat

Antibiotics are commonly used in livestock animals from the total antibiotic production, 66% is being used in food animals, to protect from diseases and optimize growth. The meat-producing industry widely utilizes antibiotics as a growth promoter, especially in the USA. However, In the USA as well as in the world, extensive utilization of antibiotics in farm animals is a big challenge due to antibiotic resistance that is harmful and alarming for the human future. The discovery of antibiotics was a great achievement in human history due to their best activity against pathogens (Nelson et al., 2019). They have been used in the food industry for many years, however, increased demand was observed in the 1950s and afterward as enhancing animal production and health improvement (Bouwman et al., 2013). In animals, the feed conversion ratio (FCR) and feed efficiency both improve by supplementing antibiotics in their food, On the other hand, meat quality and health of animals were also improved (Tian et al., 2022). The wide use of antibiotics in food animals with almost 70% has been exceeding the consumption for human health purposes (Khoo et al., 2017). The statistical data indicated that if it is not being controlled, the economic losses will increase by up to $100 trillion and the deaths are predicted to extend up to 10 million per annum by 2050 (Duong et al., 2010).

AMR is emerging due to the high consumption of antibiotics in the dairy, poultry, fattening, and food industry (Nelson et al., 2019). The AMR is reported high in developing countries that utilize antibiotics in animals for their rapid growth and high meat demand like the sulfonamides, tetracycline, and penicillin shows resistance (Nhung et al., 2016). The higher resistance is a big challenge for human health (Speer et al., 2020). AMR was observed by different literature they found it in the bacteria that were isolated from animals and food. More concern is that the common pathogens observed are *Salmonella* spp and *Escherichia coli* (Hur et al., 2012). The presence of these antibiotic-resistant bacteria needs further research and investigation.

Considering the well-known connection between the use of antimicrobials and the development of resistance, several countries have developed plans to reduce or eliminate the use of growth-promoting antimicrobials in food animals. Most of these efforts are focused on agents that are important in the field of human medicine. Several sources have been cited to support this claim (Gerwick and Moore, 2012). Accordingly, several efforts have been made to identify alternative methods that may be used to maintain the health and well-being of animals particularly the use of lactobacillus species in the poultry and livestock sector as probiotics (Corsello et al., 2017). Similarly, other techniques like the use of prebiotics, herbal products, and trace minerals are also being used to optimize the health and growth of animals. However, due to their low efficiency and side effects, these efforts have not been successful. Foodborne infections, which are brought on by a wide range of bacteria, viruses, and parasites, are a major worldwide health problem that causes a wide range of illnesses in both people and animals, according to the Centers for Disease Control and Prevention (CDC).

When viruses penetrate the human body, they may form colonies inside the intestinal lumen, which can result in foodborne illnesses. These diseases may cause major harm, such as significant financial losses or even fatalities (Kemp et al., 2022). Every year that goes by, the stock density of contemporary animal husbandry rises because of the growing demand for meat consumption around the world. Therefore, the animal production industry uses antibiotics a lot to treat bacterial infections and reduce the risk of infectious disease epidemics in animals raised for meat. Newborn animals are more vulnerable to stress-inducing stimuli including illnesses, poisons, and free radicals because of their impaired gastrointestinal systems. Higher death rates and worse growth performance are the outcomes of this greater susceptibility. The present practice of increasing animal growth performance via the management of pathogen-induced diarrhea and other disorders involves the widespread use of antibiotic growth promoters (AGP) in animal feed. According to a recent study by (De Briyne et al., 2020), pigs and hens both consume considerable amounts of antimicrobials annually (148 mg/kg BW and 172 mg/kg BW, respectively) that play an important role in the development of the AMR as shown in Fig. 1. During the next twenty years, it is anticipated that the use of antimicrobials in cattle will increase by a factor of two, with developing countries contributing thirty percent of this expansion. This is mostly due to the development of agricultural activities in middle-income countries. As the worldwide demand for meat consumption increases, there is a potential for the development of antibiotic resistance (Monger et al., 2021). The issue of antibiotic resistance significantly affects both the environmental microbiome and human health, leading to increased concern about the regulation of antibiotic use in animal feed.

Sources of Anthocyanins

Anthocyanins, which are abundant in plants, including fruits, vegetables, and cereals, are a significant class of water-soluble pigments (Martín et al., 2017). They have crucial functions in human nutrition, health, and overall well-being. This chapter elucidates prevalent and uncommon natural origins of anthocyanins, drawing upon research conducted over the course of the last twenty years, including botanical sources. The use of modern analytical methods, such as LC-MSn, to define minor aglycones and complicated substituted or acylated sugar moieties is expected to contribute to the ongoing expansion of structural diversity, as shown by recent investigations. Moreover, the augmentation of study endeavors about the health advantages associated with anthocyanins will concurrently facilitate the commercial advancement of certain naturally prevalent variants in the realms of functional foods, nutraceuticals, cosmetics, and pharmaceuticals (Mohammed and Khan, 2022). Fig. 2 shows the sources from which anthocyanins can be extracted, fostering study endeavors in the screening and profiling of natural anthocyanins derived from understudied plant species, therefore furthering our comprehension of anthocyanins and their inherent occurrence in the foreseeable future.

Fig. 1: The systematic diagram representing the mechanism of antibiotic resistance transmission to human beings

Anthocyanins as an Alternative to Antibiotics

Anthocyanins do not serve as a direct replacement for antibiotics; rather, they possess certain virtues and benefits in specific circumstances, including as the rise of antibiotic-resistant bacteria, which poses a substantial danger to public health, may be attributed to the excessive and improper use of antibiotics (Silva et al., 2021). One possible alternate approach to infection treatment that anthocyanins provide is reducing the selection pressure that leads to antibiotic resistance. However, they are also related to health improvements like hepatic, cardiovascular, and renal health. Due to their health-beneficial effect and human well-being, these anthocyanins reduce the infection and ultimately decrease the burden on antibiotics (Ma et al., 2019). As anthocyanins are present in vegetables and fruits, they are easily available and safe for the environment as well (Khoo et al., 2017). Their preparation is, however, easier than antibiotics. The synergetic effect of antibiotics is near to be explored by the researchers which may open a new window to reduce the load of antibiotics and minimize the risk of antibiotic resistance.

Role of Anthocyanins in Health and Performance

a) Antioxidant

In cellular settings, prooxidants such as nitric oxide (NO), hydroxyl radical (OH), and superoxide anion (O2) are often present, as are reactive oxygen/nitrogen species (ROS/RNS). These species possess the amazing capacity to stop dangerous microbes from invading and to destroy malignant cells. When present in the body's normal quantities, they also have a significant impact on wound healing. An imbalance between prooxidants and antioxidants leads to oxidative stress (Carini et al., 2017). Numerous investigations have shown a link between oxidative stress and a range of illnesses, including diabetes, cancer, heart disease, and inflammation. Research has revealed that cells can be easily damaged by lipid oxidation, DNA cross-linking, and apoptosis (Pisoschi et al., 2021). A bunch of detoxification and antioxidant enzymes, like glutathione S-transferase (GST), heme oxygenase (HO1), superoxide dismutase (SOD), and NADH(P)H-quinone-oxidoreductase 1 (NQO1),

are controlled by the Nrf2/Keap1/ARE pathway. It is widely believed that these enzymes have a significant role in maintaining the balance of redox (Neves et al., 2019). Animals in the livestock industry possess inherent mechanisms to combat oxidative stress. Nevertheless, these stressors can have a significant impact on the well-being of these animals. Factors such as soaring temperatures, air pollution, metal contamination, dietary influences, and overcrowding can all contribute to a weakened immune system, increased susceptibility to diseases, and a decline in the quality of their meat. According to a study conducted by (Lazalde-Cruz et al., 2021), it has been observed that oxidative stress can have an impact on the milk production, reproductive performance, and lifespan of sows. In addition, studies have demonstrated that goats experience a certain degree of oxidative stress during the middle of their lactation period when they are subjected to hot summer weather conditions (Mutwedu, 2022). Furthermore, oxidative stress has been associated with factors such as energy balance, milk production, nitrogen dioxide (NO2), and ozone (O3) in the air (Taysi et al., 2019). The economic implications of oxidative stress in cattle and poultry are quite substantial, as it also presents challenges in terms of disease prevention and management. Ethoxyquin, butylated hydroxyanisole, and butylated hydroxytoluene are commonly employed as synthetic antioxidants. Nevertheless, the evaluation of biological effects and toxicological safety of these substances is not yet fully complete, and using them in high concentrations has been associated with negative outcomes (Smeriglio et al., 2016). As a result, there has been a lot of attention on natural substances that have powerful antioxidant properties.

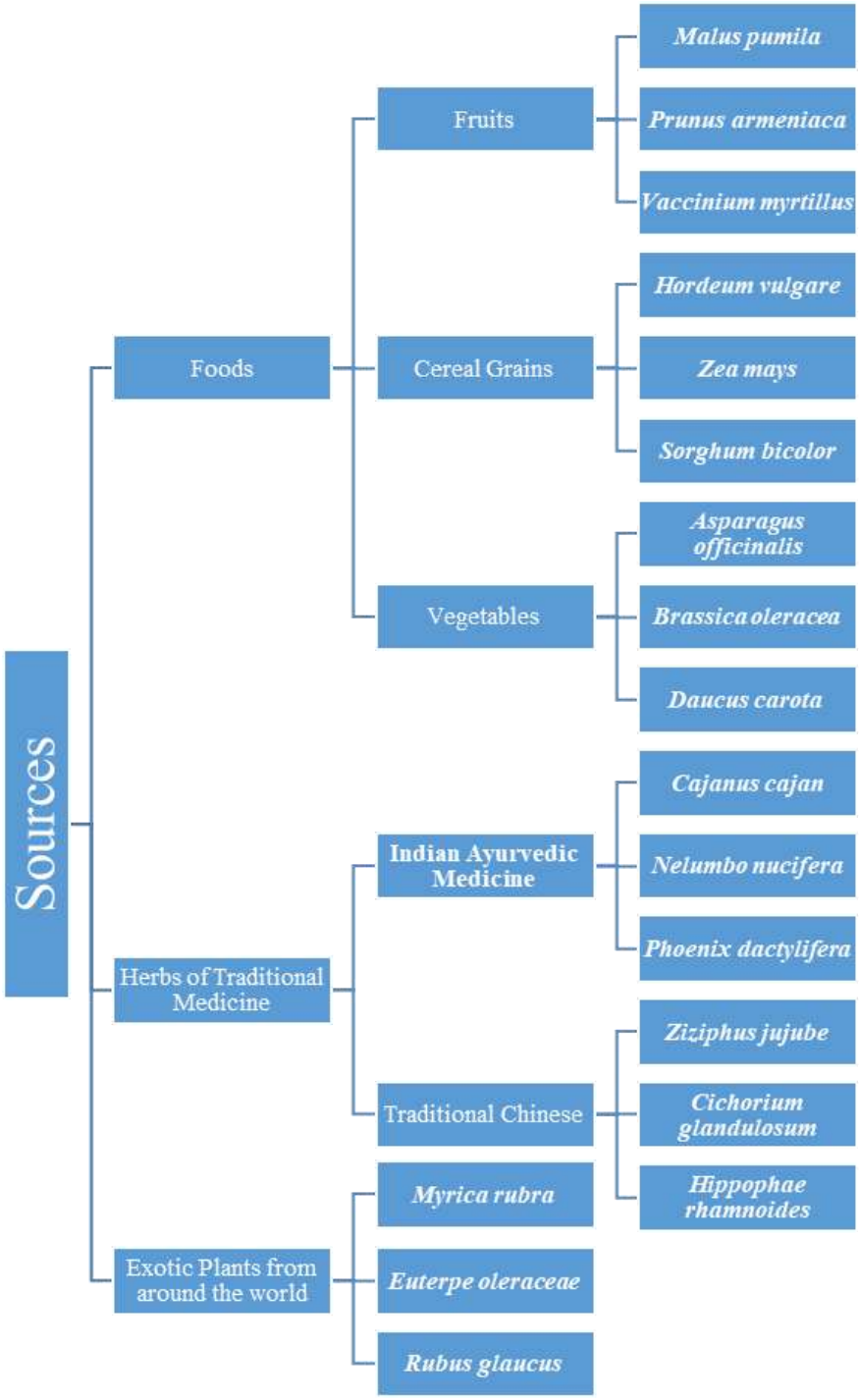

Fig. 2: Exploring Anthocyanin Sources

b) Anti-inflammatory

Several studies, both epidemiological and experimental, have shown that anthocyanins found in food have anti-inflammatory properties. This suggests that these compounds could help reduce inflammation-related conditions such as colitis, Peyronie disease, periodontal disease, laryngopharyngeal reflux, postprandial inflammatory response, and pain behavior. Many intricate mechanisms play a role in inflammatory responses, such as cytokines, enzymes, lipid mediators, and vasoactive mediators (Krga and Milenkovic, 2019). (Moreira et al., 2021) also found that anthocyanin-rich extract of mulberry suppresses edema and peritonitis by downregulation of COX-2 expression and inhibition of PGE2 Production. A study conducted by (Sarikaphuti et al., 2020) revealed that incorporating cyanidin 3-glucoside and wild mulberry extracts into one's diet can effectively reduce inflammation and edema caused by carrageenan. To achieve this objective, the synthesis of PGE2 is inhibited and the expression of COX-2 is reduced. The production of nitric oxide (NO), which plays a role in inflammation, relies heavily on the iNOS enzyme. According to (Rupasinghe and Arumuggam, 2019), some inflammatory disorders tend to progress at a faster pace when there is an excessive production of nitric oxide (NO). In a study conducted by (Mahalanobish et al., 2019), it was found that cyanidin 3-O-β-glucoside exhibited inhibitory effects on the zymosan-induced inflammatory response in rats. The levels of iNOS, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 were found to be decreased in the peritoneal exudate cells. As per a study conducted by (Henriques et al., 2020), it was found that treating BV-2 microglial cells with an extract from acai pulp, which is rich in anthocyanins, had positive effects in preventing the release of nitric oxide (NO), synthesis of indole-3-acetic acid (iNOS), and expression of COX-2 induced by lipopolysaccharide (LPS). A recent study conducted by (Carey et al., 2017) has confirmed the anti-inflammatory properties of a specific component of red raspberries that is abundant in anthocyanins (RR-ARFs). The study examined the impact of RR-ARFs on RAW264.7 cells in a model of acute animal colitis.

c) Improve Gut Health

Anthocyanins are naturally occurring compounds that are commonly found in the human diet. There is a growing body of evidence indicating that the consumption of specific substances is linked to beneficial biological effects. The microbiota has gained recognition as a metabolic organ, with its role in metabolizing phenolic compounds and the effects on bioavailability and biological outcomes being highlighted. The objective of this study was to gather information on the interaction between anthocyanins and the microbiota, focusing on two main aspects: (i) the discovery of their colonic metabolites as possible bioactive compounds, and (ii) their function as prebiotic agents. These viewpoints are crucial aspects in the field of anthocyanin metabolomics. Various metabolites, namely phenolic acids and simple phenols, have been identified as possible health advantages after the ingestion of anthocyanins. Conversely, the alteration of microbiota is strongly linked to several physiological dysfunctions, and its alteration has been seen as a potential route via which phenolic chemicals may exercise their impact. The whole picture is summarized in Fig. 2.

Integrating Anthocyanins into Food and Feed Industries

Food Industries

Anthocyanins exhibit a diverse range of hues in the natural environment, and their plentiful and readily available properties make them a primary choice for use as a natural pigment (Câmara et al., 2022). Concurrently, the favorable antibacterial characteristics shown by these substances have garnered the attention of scholars and are progressively being included in diverse food products. Using fig peels and blackthorn fruit extracts, which are abundant in anthocyanins, we have crafted delectable bakery treats such as Iceing and Beijinhos. This technique not only resulted in the attainment of light pink and dark purple hues but also enhanced the longevity of the products (Becerril et al., 2021). According to a study by (Tamkutė et al., 2019), adding cranberry anthocyanins to the preparation of pork and beef may help decrease the presence of Staphylococcus aureus. This phenomenon can be ascribed to the remarkable capability of anthocyanins to induce a disturbance in the cell membrane, resulting in the release of cytoplasmic contents. Not only can anthocyanin be added directly to food products, but there have also been advancements in producing antibacterial films for food packaging using this natural compound. According to a study conducted by (Staroszczyk et al., 2020), films made using blue-berried honeysuckle extract and fish gelatin have demonstrated remarkable antimicrobial properties against *E. coli*, *P. fluorescens*, *S. aureus*, and *L. innocua*. According to a study conducted by (Yong and Liu, 2020), a movie was created using chitosan/starch and enriched with extracts of blueberry, cranberry, and pomegranate. These extracts are packed with anthocyanins. This film exhibited impressive antibacterial properties against *Aspergillus niger* and *Penicillium notatum*. Anthocyanins possess remarkable antibacterial properties, and the film derived from these compounds serves as a reliable indicator of food spoilage owing to its sensitivity to pH. The film displays a vibrant array of hues that vary depending on the pH conditions, as evidenced by the investigations carried out by a multitude of researchers in recent years.

Feed Industry

In traditional farming like livestock, poultry, and aquatic products, a wide range of broad-spectrum antibiotics are used for the prevention of diseases and growth promotion (Yong and Liu, 2020). Nevertheless, this practice has given rise to antibiotic resistance and posed a significant threat to human health. Consequently, regulatory bodies have taken notice of this issue and have progressively implemented bans on the utilization of antibiotics (Gomiero, 2018). Because of their useful properties, anthocyanins, which are present in many different types of plants, may eventually take the role of antibiotics. These properties include immunological modulation, antibacterial, antioxidant, anti-inflammatory, and gut ecology

maintenance. In a recent study, the feces of lambs fed an additional 90 mg/kg daily of anthocyanin-rich red-orange and lemon extract compared with a normal diet showed significant increases in Firmicutes, Bacteroidetes, Lactobacillus, and Bifidobacterium genera, and significant decreases in Proteobacteria, Actinobacteria, Escherichia coli, and Salmonella species. These findings suggest that adding anthocyanins to a lamb's diet can regulate intestinal flora and inhibit potentially pathogenic bacteria (Tian et al., 2021). Additionally, the neuropeptide Y immunoreactive cells significantly increased in the abomasal epithelium and pancreatic islets (De Felice et al., 2021). Anthocyanins can improve an animal's general health by reducing oxidative stress.

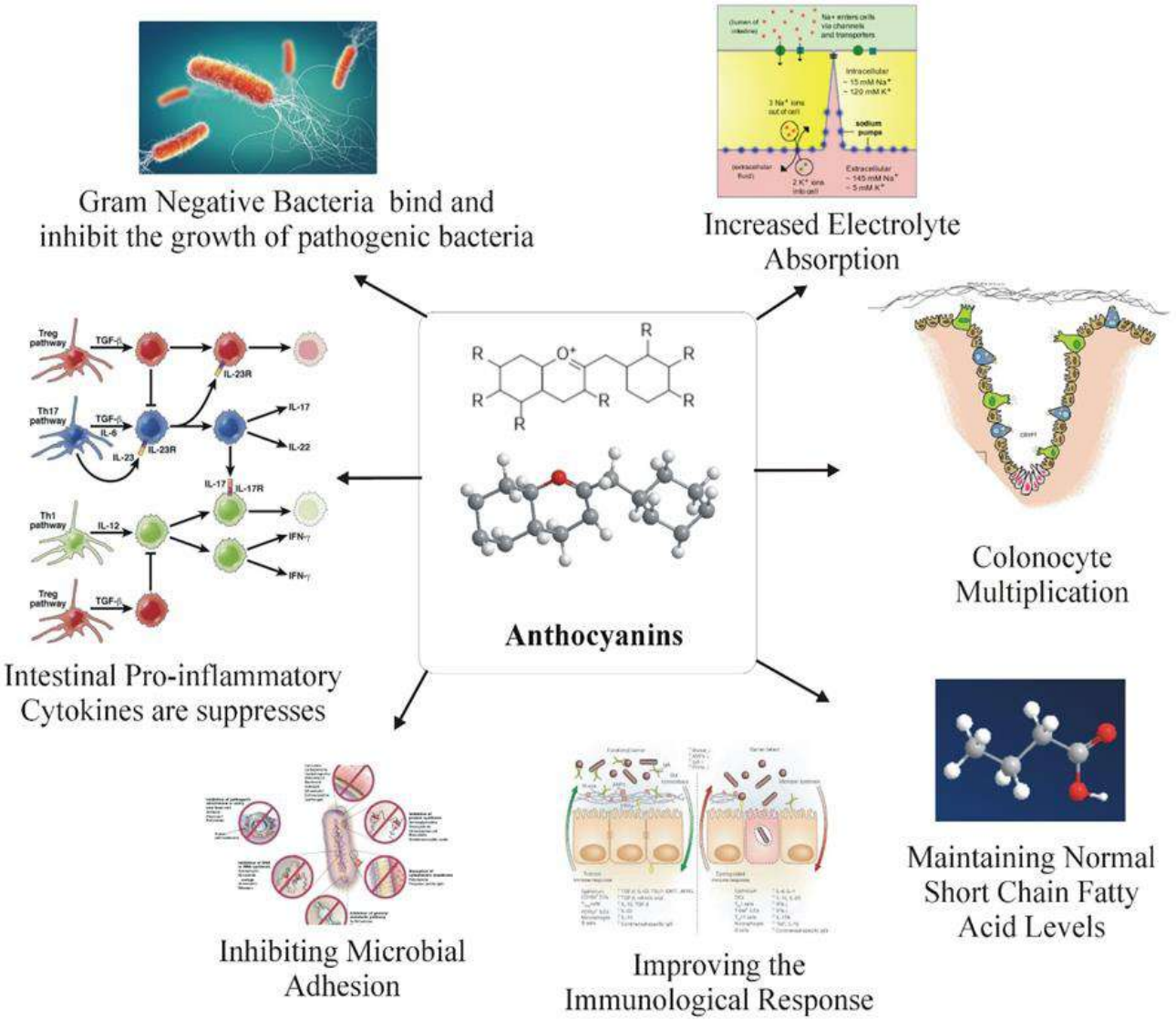

Fig. 2: Representing the beneficial effect of Anthocyanins on the intestine

Conclusion, Future Directions and Technological Innovations

The significance of illness prevention has seen a notable rise in contemporary society. Health is a fundamental component of a well-rounded existence and a primary concern for individuals. Public policy entities are actively promoting preventive health interventions as a strategy to mitigate dependence on costly medical interventions. Both governments and people are using scientific studies to inform their dietary recommendations and choices, aligning with this prevailing trend. It is well acknowledged that certain food sources and naturally occurring chemicals in meals may contribute to the maintenance of an organism's health. Anthocyanins are a category of naturally occurring bioactive compounds that can be visually detected in food and beverages. This characteristic greatly aids in the translation of nutritional and pharmacological research findings into practical recommendations for health-conscious consumers. This has been exemplified by the color diet, as proposed by (Panda et al., 2022). Phytopigments known as anthocyanins, which have health-promoting characteristics, are present in several "functional foods" including red, blue, and purple berries as well as red wine. Extensive studies have been conducted to enhance the production of these compounds in plant-based food, particularly in the context of red fruit-flesh apples (Cappellini et al., 2021). Extracts with high levels of anthocyanins derived from grape skin, blackcurrant, purple maize, or red cabbage (Codex Alimentarius INS 163) have been approved natural colorants in Europe, Australia, and New Zealand. These extracts are widely used in the food industry.

Anthocyanins have gained immense popularity over the past two decades. This article offers a detailed explanation of the numerous health benefits linked to anthocyanins, along with the underlying molecular mechanisms at play. These positive effects are influenced by various signaling pathways such as MAPK, NF-κB, AMPK, and Wnt/β-catenin, as well as important cellular processes like cell cycle, apoptosis, autophagy, and biochemical metabolism. In the future, these pathways hold promise as therapeutic targets and strategies for enhancing the treatment of various diseases. Nevertheless, there remains a considerable amount of information that requires clarification to utilize them effectively for the improvement of human health. Additional research is required to explore the biological impacts of particular anthocyanin metabolites. There is a proposal suggesting that anthocyanins are mainly absorbed and transported in human serum and urine as metabolites. These metabolites might have a significant impact, either completely or partially, on the biological activities of the previously mentioned anthocyanins. In addition, numerous studies conducted on both animals and humans have shown that consuming anthocyanins in the diet can have various health benefits. However, achieving the desired beneficial results is a significant challenge due to the limited bioavailability of anthocyanins (Braga et al., 2018). There is a need to develop novel methodologies aimed at improving the bioavailability of these advantageous compounds inside the human body. Thankfully, emerging technologies like nanotechnology provide potential solutions to address this issue. Furthermore, anthocyanin individuals that have been purified may have distinct biological activity as a result of their unique chemical structures. Future research should prioritize the meticulous and precise characterization of various anthocyanins, to gain a deeper understanding of the molecular mechanisms underlying their health-promoting properties.

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Chapter 02

Prevalence of *Staphylococcus* especially *S. aureus* Resistance and Alternative Methods to Control

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ABSTRACT

Infectious diseases remain a serious problem in the whole world. *Staphylococcus* which is a gram-positive grapelike cocci bacterium causes many infectious diseases. *Staphylococcus* has many species but *S. aureus*, *S. epidemidis,* and *S. saprophyticus* are considered most clinically important. *S. aureus* is the most dangerous among other species because it has developed resistance to multiple drugs quickly. *S. aureus* showed resistance to the first drug penicillin within 12 months. Methicillin-resistant strains (MRSA) were identified within two years after its development. The MRSA showed the highest prevalence rates in China (18.07%), the United Kingdom (18.66%), Spain (15.45%), Italy (16.34), Singapore (22.72%), and France (13.89%). Vancomycin, which is used to treat MRSA, showed resistance in 2002. The prevalence of VRSA was 1% among 456 *S. aureus* isolates in Europe, 5% among 11,074 *S. aureus* isolates in Asia and 4% among the 395 isolates in America. However, the mortality level due to MRSA has reduced but still it is a global problem. Therefore, the interest in the development of alternative treatment strategies for bacterial infections has been increased recently. Nowadays, anti-virulence agents, plant derivatives, nanoparticles, and phage therapies are used as alternative methods to treat multiple drug-resistant *S. aureus*.

KEYWORDS
Staphylococcus aureus, Penicillin-resistance, Methicillin-resistance, Vancomycin-resistance, Anti-virulence agents, Plant derivatives

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INTRODUCTION

The *Staphylococci* are gram-positive cocci arrays in irregular grapelike clusters (Organji, Abulreesh et al. 2018). All *Staphylococci* are catalase positive (catalase deteriorate H_2O_2 into O_2 and H_2O (Hadwan and Abed 2016)). There are three clinically important species of *Staphylococci*: *S. aureus*, *S. epidermidis*, and *S. saprophyticus*. The coagulase produced by *S. aureus* differentiates it from other *Staphylococcus* species (Argemi, Hansmann et al. 2019). The coagulase-negative *Staphylococci* are known as *S. epidermidis* and *S. saprophyticus*. The coagulase-negative *Staphylococci* are separated by novobiocin sensitivity testing. This coagulase-negative causes serious endocarditis in prosthetic valve and urinary tract infection, respectively. Of these three species, *S. aureus* is the most crucial. Because infections due to *S. aureus* are predominantly challenging as a consequence of recurrently happening *S. aureus* resistance to antibiotics (Turner, Sharma-Kuinkel et al. 2019).

The *S. aureus* is a well-known human pathogen and becomes the reason for serious mortal disorders (Balasubramanian, Harper et al. 2017). Among the infectious agents in community and hospital infection, *S. aureus* is one of them (Wurster, Bispo et al. 2018) and causes bacteremia, toxic shock syndrome, osteomyelitis, infective endocarditis due to soft tissue infection, and incurable pneumonia infectious diseases (Guo, Song et al. 2020). It has become the cause of critical inflammation and sepsis that is often caused by multi-drug resistant strains (Schuster and Bertram 2016).

The resistance of essential bacterial pathogens to ordinary antimicrobial treatments and the development of multidrug-resistant microscopic organisms are expanding at a disturbing rate and constitute one of our most prominent challenges within the conflict of bacterial contamination and went with difficulties (Mühlen and Dersch 2016). The antibiotic resistance comes up by various contrasting mechanisms, such as edited drug target area, enzymatic drug demission, enhanced outflow of antimicrobial components, and changed drug availability

(Vestergaard, Frees et al. 2019). By the accomplishment of antibiotic-resistance genes from *S. aureus* strains or even from other genera of bacteria, antibiotic-resistant clones quickly come out chiefly. By the process of conjugation or bacteriophage transduction, various complement of mobile genetic elements transformation occurs (Haaber, Penadés et al. 2017) in strains of *S. aureus*.

The *S. aureus* is contemplated as a supergerm that has expanded its defended operation to many types of antibiotics in the recent years. Cases of the ineffectiveness of penicillin against *S. aureus* were reported within three years after Penicillin came to market from 1942 to 1945 (Saraiva, Sestito et al. 2024) and within 12 months after extraction of its pure form (Khan and Aziz 2016). This circumstance was repeated with many other drugs, such as methicillin. After the development of the first methicillin, methicillin-resistant strains (MRSA) were identified within just two years (Saraiva, Sestito et al. 2024). Methicillin-resistant *S. aureus* (MRSA) strains possess resistance to almost all β-lactam antibiotics through the accomplishment of the staphylococcal cassette chromosome mec (SCCmec), which bears the antibiotic-resistant gene mecA. The MRSA consists of livestock-associated MRSA (LA-MRSA), healthcare-acquired MRSA (HA-MRSA), and community-acquired MRSA (CA-MRSA) strains (Yang, Zhang et al. 2016). In some countries, the healthcare-associated MRSA (HA-MRSA) prevalence is higher than health care related to methicillin-susceptible *S. aureus* (Pathare, Asogan et al. 2016).

In different regions of the world, Methicillin-resistant *S. aureus* (MRSA) has increased that is shown in various studies. More than 80% of *S. aureus* infections contain MRSA shown in publication by WHO in 2014 (Sapkota, Sharma et al. 2019). The prevalence of MRSA in England peaked at 7700 in 2003–2004, and the numbers decreased gradually from 2006 (Duerden, Fry et al. 2015). The percentage of MRSA in Saudi Arabia reached 50% in 2011 that was indicated by a group of clinicians infections in King Fahad Medical City in Riyadh (Alghizzi and Shami 2021). In the late 1980s, Vancomycin developed as an antibiotic of the best choice for the treatment of MRSA infections in hospitals after being accepted for use in humans in 1958 (McGuinness, Malachowa et al. 2017). However, in 2002 the first vancomycin-resistant *S. aureus* (VRSA) was discovered from the foot wound of a diabetic patient who had taken long-term vancomycin (Hu, Peng et al. 2016). The prevalence of VRSA was shown in different countries. Identification is the first step to treat any infection.

Identification of *S. aureus* is mostly done by using selective media having a high concentration of salt and chromogen to produce colour for detection (Perry 2017). Methicillin-resistance *S. aureus* is detected by using CHROM agar, and Baird-Parker RPF agar specific culture media (Beça, Bessa et al. 2015). At present-day the insufficiency of efficient drugs, deficiency of protective measures, and a small number of new antibiotics used in clinical events give chances to improve the treatment and to change the antimicrobial therapies (Mühlen and Dersch 2016). A lot of studies have been done to treat of methicillin-resistant *S. aureus* (MRSA) but it is still a more prominent worldwide concern. MRSA infection treatment is trying to be enhanced by using new agents. These new agents come to market in the last 10 years (Horn, Danziger et al. 2017).

Penicillin is mostly used as a medicine of excellent choice if isolated bacteria is sensitive (MSSA, or methicillin-sensitive *S. aureus* strains) and vancomycin for MRSA strains (Taylor and Unakal 2017). However, antimicrobial therapy requires alternative therapy to be added due to greater resistance cases (Tong, Davis et al. 2015). For instance, the relevant use of a cysteine-capped hydrogel binding with copper method that stop the growth of united state strain of *S. aureus* and minimize the production of inflammatory protein 2-alpha (MIP-2) cytokine and pro-inflammatory macrophage (Yang, Huang et al. 2020). Anti-virulence agents are made available as alternative methods (Dickey, Cheung et al. 2017). Plants also assist as sources for the detection of natural products that are aimed at the bacterial virulence factors (Wu, Liu et al. 2019) and many other therapies such as phage, lysine therapy, and therapy by using nanoparticles.

Staphylococci

The *Staphylococci* are gram-positive cocci arrayed in irregular grapes-like clusters (Organji, Abulreesh et al. 2018). All the *staphylococci* are catalase-positive (catalase deteriorates H_2O_2 into O_2 and H_2O (Hadwan 2018)). The catalase is a dominant virulence factor of *Staphylococci*. The neutrophils cannot be able to kill catalase-positive bacteria by using H_2O_2 (Kobayashi, Malachowa et al. 2018). There are three clinically important species of *Staphylococci*: *S. aureus*, S. *epidermidis*, and *S. saprophyticus*. The coagulase produced by *S. aureus* (Tam and Torres 2019) differentiates it from other *Staphylococcus* species. The coagulase is an enzyme that leads extracellular fluid to coagulate by energizing prothrombin to form thrombin. Then catalyses the stimulation of fibrinogen to form the fibrin clot. Coagulase-negative *Staphylococci* are known as *S. epidermidis* and S. *saprophyticus* and both coagulase-negative *Staphylococci* are differentiated by the novobiocin sensitivity testing. Of these three species, *S. aureus* is the most dangerous. because *S. aureus* can become resistant to clinically available antibiotics (Vestergaard, Frees et al. 2019).

Staphylococcus aureus

The *S. aureus* is both mutuality micro-organism and human infectious agent. The *S. aureus* infected almost 30% of the human population. The *S. aureus* causes many infectious diseases such as, endocarditis due to soft tissue infection, device-related infection, bacteremia, osteoarticular and skin infections (Tong, Davis et al. 2015). It plays altered roles in numerous stages of its replication that are its main virulent factors; for example alteration of surface proteins such as Protein A, fibronectin-binding protein, and clumping factor in the exponential-growth phase (Gajdács 2019).

The history of *S. aureus* started when Alexander Ogston discovered grape-like cluster bacteria from surgical wound infection in 1880 (Høiby 2024) and when the abscesses were produced in animals during the insertion of pus

from human staphylococcal infections by Louis Pasteur with Alexander Ogston. The word *Staphylococcus* (Greek staphyle, "a bunch of grapes; kokkos, "grain or berry) for the genus was stamped out by Ogston in 1882 (Lakhundi and Zhang 2018).

Antibiotic Resistance of *S. aureus*

The *S. aureus* is a dangerous antibiotic-resistant gram-positive bacterium. It is major worldwide problem for antimicrobial resistance.

Antibiotics

Penicillin

Penicillin is the first naturally occurring antibiotic and the second antibiotic after the practice of applying a poultice (Lalchhandama 2021). During the investigation of different patterns of *S. aureus*, Scottish physician Alexander Fleming discovered penicillin accidentally in 1928 during his work at St Mary's Hospital in London (Lalchhandama 2020). Then Howard Walter Florey and Ernst Boris Chain successfully extracted pure penicillin compound from the mould in 1939 (Khan 2017). During the World War II, penicillin was first time clinically used by sprinkling its powder on wounds before they were sealed and penicillin injections were injected to inspire infection rescue and inhibition (Mancini 2021). This drug has been used in the treatment of various bacterial infections and it became famous by the name of magical bullet or miracle drug in a short time. The European pharmaceutical companies produced a large number of various types of penicillin after the end of the second war. The era of 1950 and 1970 is known as the antibiotic era or golden era because various types of antibiotics were produced against different bacterial varieties (Khan 2017). There was 12 years difference between the discovery of penicillin and extraction of its pure form but *S. aureus* developed resistance to it within 12 months. Fleming said during an event in 1945 "But I would like to sound one note of warning. It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body (Khan and Aziz 2016). Penicillin resistance occurs due to penicillinase enzymes that digest the β-lactamase ring in human isolates (Lindsay 2019).

Penicillin-mediated resistance in *S. aureus* was the first wave of antibiotic resistance. It has become a pandemic in 1960. Resistance to penicillin was discovered in 80% of hospital and community-acquired *S. aureus* by late 1960 and by early 2000, more than 90% of cases were reported (Gnanamani, Hariharan et al. 2017).

Methicillin

After resistance to wonder antibiotic penicillin was spread, then methicillin was developed by Beecham, a British Pharmaceutical Company in 1959. It was the first antibiotic that was also known as the first semi-synthetic penicillin. It was used to cure infection initiated by penicillin-resistant *S. aureus*. After the development of methicillin, then it was considered that being of resistance to *Staphylococcus* had been stopped. Even Ernst Chain said that "no more resistance problems, methicillin is the answer". But there was bad news *S. aureus* strains developed resistance to methicillin in 1961 (Khan 2017). MRSA is also classified as a Multiple drug resistance because it showed resistance to oxacillin or cefoxitin and gathers no susceptibility to all classes of β-lactam antimicrobials i.e., all categories of penicillin, cephalosporin, β-lactamase inhibitors, and carbapenems (Kaur and Chate 2015). The *S. aureus* uses expression of the exogenous mecA gene to develop resistance to methicillin and all other beta-lactam representatives. It encodes for a variable penicillin-binding protein PBP2 (PBP2a) with reduced affinity for beta-lactams, thus preventing the suppression by beta-lactams of cell wall synthesis (Team 2015).

The Hospital Acquired-MRSA, Community Acquired-MRSA, and Livestock Acquired-MRSA strain infections are different for their specific risk factors (Lee, De Lencastre et al. 2018). MRSA contains eleven different types of SCCmec (I-XI). SCCmec types I, II, III, VI, and VIII are commonly known as hospital-acquired MRSA or (HA-MRSA). SCCmec Types IV, V, and VII are called community-acquired (CA-MRSA) while types IX, X, and XI are known as livestock-associated MRSA (LA-MRSA) (Mitsan and Oladeinde 2016).

The prevalence of MRSA in England peaked at 7700 in 2003–2004, and the numbers decreased gradually from 2006 (Duerden, Fry et al. 2015), 25–50% in southern Europe (for example, Portugal, Spain, Italy, and Greece) and <5% in northern Europe (for example, the Netherlands, Norway, Sweden, and Denmark) of *S. aureus* reported as invasive infections caused by methicillin-resistant (Lee, De Lencastre et al. 2018). The prevalence of MRSA had been shown at greater rates in Singapore (22.72%), United Kingdom (18.66%), Italy (16.34%), the United States (23.78%), China (18.07%), France (13.89%), Poland (22.18%), Switzerland (13.15%), Israel (14.82%) and Spain (15.45%) (Hasanpour, Sepidarkish et al. 2023). The percentage of MRSA in Saudi Arabia reached 50% in 2011 that was indicated by a group of clinicians infections in King Fahad Medical City in Riyadh (Alghizzi and Shami 2021).

Identification of *S. aureus* is mostly done by using selective media having a high concentration of salt and chromogen to produce colour for detection (Perry 2017). Methicillin-resistance *S. aureus* is detected by using CHROM agar and Baird-Parker RPF agar specific culture media (Beça, Bessa et al. 2015). The modern laboratories consider matrix-assisted laser desorption of flight mass spectrometry (MALDI) as the gold standard for staphylococcal identification and differentiation of MRSA and MSSA (Van Belkum and Rochas 2018).

Vancomycin

In the late 1980s, Vancomycin had become an antibiotic of the greatest choice to treat MRSA infections in hospitals after being accepted for use in humans in 1958 (McGuinness, Malachowa et al. 2017). The strains of *S. aureus* are

categorised as VCM-intermediate resistant *S. aureus* (VISA; MIC = 4–8 µg/ml) or VCM-resistant *S. aureus* (VRSA; MIC ≥16 µg/ml) by the resistance level according to the Clinical and Laboratory Standards Institute (Ishii, Tabuchi et al. 2015). In Japan, the first Vancomycin Intermediate *S. aureus* (VISA) was reported in 1997 with a Minimum Inhibitory Concentration (MIC) of 8 µg/ml (Shariati, Dadashi et al. 2020). So in 2002, the first vancomycin-resistant *S. aureus* (VRSA) was recovered from the foot wound of a diabetic patient who had taken long-term vancomycin (Hu, Peng et al. 2016). The vanA-mediated resistance and resistance due to thickened cell walls are two mechanisms of *S. aureus* to develop resistance to vancomycin (Bitrus, Peter et al. 2018).

The prevalence of VRSA was 3% among 171 *S. aureus* isolates in South America, 16 among 720 isolates in Africa, 4% among 395 isolates in America, 5% among 11,074 isolates in Asia, and 1% among 456 isolates in Europe. The most recurrent VRSA prevalence was 29% in Nigeria, followed by 18% in Saudi Arabia (Wu, Sabokroo et al. 2021).

An Alternative Method to Control Resistance

Although, many other antibiotics have been introduced for the treatment of MRSA and VRSA, but in the development of alternative cure approaches for bacterial infections has been increased because of the greater level of *S. aureus* resistance to multiple antibiotics (Cheung, Bae et al. 2021). The development of new antibiotics is becoming outpacing due to the quick evolution and distribution of antibiotic resistance among bacteria but anti-virulence agents are made available as alternative methods (Dickey, Cheung et al. 2017). The plant derivatives and many other therapies are also used.

Anti-virulence Agent

The anti-virulence agents targeting pore-forming toxins lead to three major toxins: α-hemolysin (Hla, α-toxin), Panton-Valentine leukocidin (PVL), and leukocidin AB (LukAB) (Ford, Hurford et al. 2021). The Alpha-hemolysin is a virulence factor of *S. aureus* which is encoded by the HLA gene and causes damage to a large variety of the host cells (Divyakolu, Chikkala et al. 2019). The Panton-Valentine leukocidin (PVL) and LukAB (sometimes known as LukGH) are encoded on the core genome or phage and oligomerize to form a pore structure (Ahmad-Mansour, Loubet et al. 2021). A human anti-AT IgG1 monoclonal antibody (MAb) and MEDI4893 (suvratoxumab) (Tkaczyk, Semenova et al. 2018) is used as an alternative method to detoxify the alpha-hemolysin toxin. The combination of ASN100 two mAbs is neutralizes six cytolytic toxins of *S. aureus* together containing PVL, LukAB and Hla (Magyarics, Leslie et al. 2019). In addition to Mbs therapy, Sphingomyelin-cholesterol micelles were used *in-vitro* that hindered Hla toxicity (Henry, Neill et al. 2015) and the activity of detoxification of toxins also showed by poly (lactic-co-glycolic acid) (PLGA)-based nanoparticles coated with natural membranes of human RBCs (Chen, Chen et al. 2018).

Anti-virulence therapy for staphyloxantin and staphylococcal protein A, that have virulence of immune evasion, are neutralized by the effect of human squalene synthase inhibition when targeting CrtM, CrtN for inhibiting staphyloxanthin biosynthesis (Gao, Davies et al. 2017) and passive ote-immunization with anti-SpA mAbs neutralizes SpA activity (Thammavongsa, Rauch et al. 2015).

The *S. aureus* quorum senses regulates many virulence factors by agr protein containing phenol-soluble modulins and PFTs (Le and Otto 2015). The agr quorum sensing system does the production and sensing of auto-inducing peptide (AIP) (Khan, Yeh et al. 2015) and for therapy, foambuic acid and sulfonamide B are used to prevent AIP production and sensing (Sully, Malachowa et al. 2014).

Plant Derivatives

Plants and microorganisms are not only important food and medicinal resources but also assist as sources for the detection of natural products that aim for bacterial virulence factors (Wu, Liu et al. 2019). The concentrate of *P. emblica* seeds, which directed the efficiency of methanolic and ethyl-acetate extracts (Anbuselvi and Jha 2015), and the seed extract of *L. shawii* (Aldoweriej, Alharbi et al. 2016) presented high antibacterial activity against *S. aureus* strains. In China, *Z. nitidum* is mostly used in traditional Chinese medicine as a detoxifying, analgesic, and hemostatic herbal medicine and for the treatment of MRSA infection (Zeng, Wang et al. 2022). There is a high number of phytochemicals in extracts of medicinal plants such as *Acacia catechu*, and *Psidium guajava* that are used in anti-MRSA therapy. These medicinal plant categories include phenolics, alkaloids, steroids, terpenes, saponins, etc. (Okwu, Olley et al. 2019).

The alternative therapies of mutual benefits such as essential oil *Austroeupatorium inulaefolium* and essential oil extracted from the leaves of *Leoheo domatiophorus Chaowasku* together applied to treat infections of MRSA (Bua, Usai et al. 2018) and a mixture of *Aloe Vera*, tea tree oil, and propolis and a mixture of *Myrtus communist*, *tretinoin,* and *Origanum vulgare* (MOTC) also used for the treatment of dermal infections affected by MRSA (Mazzarello et al., 2020; Mazzarello et al., 2018).

Others Therapies

By using phage and lysine therapy, the prevalence of antibiotic resistance to *S. aureus* may be reduced. The nanoparticle technology is also used in the field of medicine (Barbero et al., 2017). The nanoparticles that are merely 25 nano-meters in diameter but have the extraordinary power of inhibition and killing effects on *S. aureus* (Li et al., 2017) such as zinc oxide nanoparticles used for isolated MRSA (Umamageswari et al., 2018). By using cysteine-capped hydrogel

incorporating copper, the growth of MRSA has been suppressed. During this method, cysteine-capped hydrogel absorbs and releases copper ions to reduce MRSA production (Yang et al., 2020).

Prevention

Numerous actions are frequently taken to inhibit transmission and infection with MRSA (Lee et al., 2018). Transmission and infection risk of MRSA can be reduced by active surveillance programs because of the enormous asymptomatic resources of carriers and direct interventions (Fätkenheuer et al., 2015). To reduce the spread of MRSA through hand, then hand hygiene with alcohol-based hand rub or soap and water is necessary (Magiorakos et al., 2017). To minimize MRSA transmission related to contamination of hands and clothing, healthcare workers should practice interaction safety measures (use of disposable gowns and gloves) during dealing with patients of MRSA colonization (Lee et al., 2018).

Conclusion

S. aureus is more dangerous and clinically important than other *Staphylococcus* species. *S. aureus* can become resistant to various clinically available antibiotics. Penicillin was discovered by British bacteriologist Fleming in 1840 and was used clinically to control *S. aureus*. However, it showed resistance to penicillin within 12 months after the discovery of its pure form. Many antibiotics were continued after this. But this large-scale investigation also raises hidden concerns about people's lives. The increased use of antibiotics has led to an increase in the number of risky bacteria that started with the development of multidrug-resistant strains of Methicillin resistance *S. aureus*. It developed resistance to multiple drugs within a short time. It showed resistance in short due to high mutation in its genes. The prevalence of antibiotic resistance *S. aureus* had remained at a high rate. It has become the cause of many deaths in Europe, the US, and other different countries. Although methicillin-resistant *S. aureus* has caused less death recently but still it is a severe public health problem in worldwide. Due to *S. aureus* high resistance to multiple antibiotics, scientists have been trying different other therapies. They have discovered anti-virulence therapies such as developing different monoclonal antibodies against its toxins, therapies by using plant derivatives such as use of combine essential oil of *Austroeupatorium inulaefolium* and essential oil extracted by leaves of *Leoheo domatiophorus Chaowasku* and using nanoparticles such as zinc oxide. By using bacteriophage and lysine therapy, the prevalence of MRSA can be reduced. The used of cysteine-capped hydrogel incorporating copper, suppressed the growth of MRSA.

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Chapter 03

Antibiotic Resistance in Escherichia coli: A Global Challenge for Human and Veterinary Health

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ABSTRACT

Antibiotic resistance in *Escherichia coli* (*E. coli*) is a major global health risk in both human and veterinary medicine. This resistance, either inherent or acquired, is caused by a variety of processes, including gene mutations as well as horizontal gene transfer. The ability of *E. coli* strains to acquire resistance genes is remarkable especially those that encode genes that give resistance to polymyxins, carbapenemases, plasmid-mediated quinolone resistance (PMQR) genes, extended-spectrum β-lactamases (ESBLs), and MCR genes. While the development of carbapenemase genes is mostly documented in human health settings, the growth of colistin resistance in *E. coli* can be related to its widespread usage in veterinary medicine globally. Although genetic studies indicate that there are genetic distinctions between ESBL manufacturers that affect both humans and animals, the transmission of resistance genes between the human and animal sectors is still up for debate. Furthermore, *E. Coli* strains originating from animals frequently display resistance to previous antimicrobial treatments, which is enabled by transposons and plasmids, which are mobile genetic elements. The use of antimicrobial medicines in veterinary medicine is associated with the co-selection and persistence of resistance to these vitally important drugs in human medicine. Maintaining the efficacy of antimicrobial drugs in both human and veterinary medicine as well as creating efficient techniques to counteract *E. Coli* antibiotic resistance requires an understanding of these mechanisms.

KEYWORDS
Antibiotics, Resistance, Global Challenge, Solutions

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INTRODUCTION

The problem of bacterial resistance often results from the administration of antimicrobic drugs in medical establishments. An organism that was once vulnerable to antibiotics may develop resistance when exposed to them for an extended period (Serwecińska, 2020). As a result, bacteria and microbes die in the susceptible organism while others are left behind, and this creates resistance. Several variables need to be considered in the development of resistance (Mancuso et al., 2021). These may include how resistance is expressed, an organism's ability to withstand resistance systems, and how initial colonization occurs. For example, resistance develops more rapidly in plasmid-borne resistance in the case of resistance of antibiotic proteins whereas it might develop rather slowly in chromosomal gene-associated resistance (Poole, 2004).

One of the most contributing factors to the emergence of antibiotic resistance is the lack of implementation of infection control measures that contribute to the spread of MRSA may include aerosols, hand-to-nose contact, and lack of handwashing by healthcare workers (Coia et al., 2006). MRSA is commonly found in hospitals and the community and in animals that expose humans to drugs. Consumption of antimicrobials in animal feed allows the growth of antimicrobial resistance in human flora (Palma et al., 2020). According to the European Food Safety Authority, consumption of antimicrobials among common animals contributes to the acquisition of resistance by *Salmonella* and *Escherichia coli*, also known as *E. coli* has a strong ciprofloxacin resistance, which is associated with the use of fluoroquinolones in chicken production (Romero-Barrios et al., 2020).

Notably, MDR which is also known as multiple drug resistance is a result of microorganisms developing resistant mechanisms with time due to extensive use of antibiotics. In all likelihood, these mechanisms evolved from genes discovered in species that make antibiotics on their own (Bakkeren et al., 2020). According to facts, methicillin-resistant against strains of *Staphylococcus aureus*, vancomycin-resistant against strains of *S. aureus*, extended-spectrum β-lactamases, and extremely drug-resistant *M. tuberculosis* have all been proven to produce MDR Hurdles (Mlynarczyk-Bonikowska et al., 2022). These bacteria make up *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *E. coli*, and *Klebsiella pneumoniae*. *Haemophilus influenza*, *Burkholderia*, *Shigella dysenteriae*, *Salmonella enterica* serovar *Typhi murium,* and *stenotrophomonas* are other resistant bacteria (Giedraitienė et al., 2011).

Antibiotic resistance can result from chromosomal changes, but it is more frequently linked to mobile extrachromosomal genetic elements such as integrons, transposons, and plasmids that are obtained from other bacteria (Partridge et al., 2018). One important mechanism of bacterial multidrug resistance is known to be efflux pumps.

Genetic Mechanisms Contributing to Antibiotic Resistance

Bacterial resistance against antibiotics can be classified into two categories: innate and acquired. For instance, the natural resistance of *E. coli* against vancomycin is a demonstration of its innate antibiotic resistance. In the meantime, numerous mechanisms, inclusive of mutations in the genetic material, and horizontal gene transfer can ultimately result in acquired resistance, apart from other causes (Urban-Chmiel et al., 2022). Rare occurrences known as spontaneous mutations frequently result in resistance to substances with similar structures for instance, resistance to quinolone in *E. coli* might be caused by modifications in the amino acids of particular genes. Some bacteria, such as *S. aureus* and *E. coli*, may become hypermutable, with their rate of mutation being significantly increased as a result (Ruiz, 2019). In the cells that are either slowly dividing or have stopped division, adaptive mutagenesis can occur under nonlethal selection pressure, leading to the production of adaptive mutations, which further strengthen the resistance to antibiotics. Transfer of the genes of resistance from one diffused bacterium to another through various processes collectively called horizontal gene transfer, which can include entailing the uptake of free DNA, viral delivery, and plasmid transfer. Resistance genes are borne by small self-replicating circular units of DNA known as plasmids. Plasmids are a greater means of transmitting resistance from one bacterium to another than chromosomal change (Johnston et al., 2023). The genes of resistance can be transferred through transformation, conjugation, and transduction. The so-called "jumping gene systems" (commonly referred to by the name "transposons") can rapidly increase their copy number in bacterial populations and mobilize resistance genes (Algarni et al., 2022). Integrons are gene capture systems that capture and express genes for resistance via a specific type of recombination system (Ghaly et al., 2021). Understanding these genetic routes is key in the struggle against antibiotic resistance and in developing actionable strategies for the preservation of antibiotics.

Antibiotic Inactivation or Modification

Different mechanisms have been developed by bacteria to counter the action of antibiotics. Three major enzymes are involved in antibiotic inactivation: β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferases (Varela et al., 2021).

β-Lactamases

β-lactamases are a group of enzymes that hydrolyze the β-lactam ring of antibiotics and thus inactivate them. There are approximately 300 various types of β-lactamases, but only a few produced by gram-negative bacteria are of clinical significance (Alfei and Schito, 2022). These enzymes are chromosomal. They reside on plasmids and are transferrable among bacteria. Another class of β-lactamase produced by several bacteria, including carbapenem-resistant clinical isolates of *P. aeruginosa* and *K. pneumoniae*, are metallo-βlactamases. The extended-spectrum β-lactamases are a grouping of enzymes that mediate resistance to a wide spectrum of β-lactam antibiotics, including inhibition of the activity of the cephalosporins, but are inhibited by other antibiotics, such as clavulanic acid or tazobactam, and also by specific inhibitors (Ejikeugwu et al., 2021).

Antibiotic Modification by Hydrolysis

E. coli produces erythromycin esterase II encoded by the EREB gene that hydrolyzes the lactone ring of erythromycin A and oleandomycin, thus providing resistance against the said antibiotics. Opening of the ring by epoxidase can result in resistance to Fosfomycin (Nag et al., 2021).

Antibiotic Inactivation by Group Transfer

Aminoglycosides, chloramphenicol, streptogramin, macrolides, and rifampicin can become inactivated. These molecular alterations involve addition of adenylyl, phosphoryl, or acetyl groups to antibiotics and result from enzymes belonging to the class of transferases (Haider et al., 2023). As a result, the affinity of the antibiotic for its respective target is reduced, thus inactivating it. Aminoglycoside modifying enzymes (AMEs) include phosphoryl transferases, nucleotidyl transferases, and acetyltransferases, which neutralize aminoglycosides and provide resistance to them (Revuelta and Bastida, 2023).

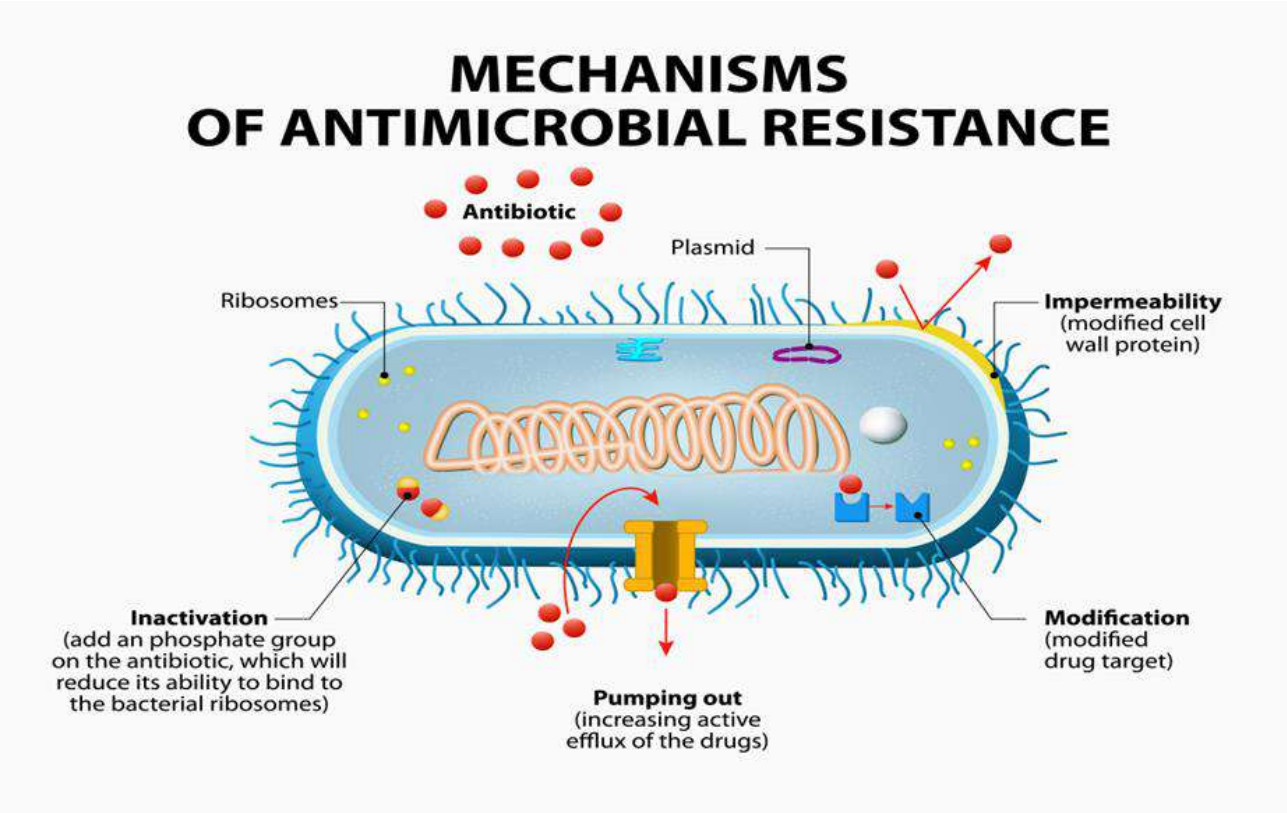

Fig. 1: This pictorial presentation shows the Mechanism of Antimicrobial Resistance

Antibiotic Inactivation by Redox Process

Some bacteria, however, exert resistance to antibiotics through oxidation and reduction reactions. In the case of *Streptomyces virginiae*, for example, it produces virginiamycin M1 and hence saves itself from its activity through the replacement of a ketone group with an alcohol residue to prevent effective binding of the antibiotic with its target (Jaka et al., 2015). Such mechanisms are of central importance to understanding in devising strategies on how to counteract antibiotic resistance to maintain the effectiveness of antibiotics.

Target Modification

Antibiotics bind to specific target molecules in bacteria, and often small changes in the targets affect how the antibiotic binds. Sometimes, when a target is altered, other changes in the cell are needed that will allow the cell to live with the altered target (Wilson et al., 2020).

Peptidoglycan Structure Alteration

Cell wall synthesis inhibition by antibiotics such as β-lactams (e.g., penicillins). However, mutations in penicillin-binding proteins (PBPs) leading to a loss of affinity for these antibiotics can compensate for cephalosporins and glycopeptides, such as vancomycin (Dörr, 2021). Methicillin resistant *Staphylococcus aureus* (MRSA) is due to the acquisition of the "*staphylococcal* cassette chromosome mec" SCCmec, which carries the resistance gene mecA, encoding PBP2a. PBP2a confers high resistance against β-lactams and guarantees cell wall synthesis at lethal β-lactam concentrations (Gajdács, 2019). Other mechanisms of resistance include mutations in membrane proteins, resulting in cross-resistance between β-lactam antibiotics and fluoroquinolones (Kakoullis et al., 2021).

Protein Synthesis Interference

Different antibiotics will bind to different steps of protein synthesis. For example, aminoglycosides (e.g. gentamicin) bind to the 30S ribosomal subunit, while chloramphenicol binds to the 50S ribosomal subunit, thereby inhibiting protein synthesis (Ahmed et al., 2021). The macrolides, lincosamides, and streptogramin B prevent protein synthesis by binding to the 50S ribosomal subunit resulting in an inhibition of RNA methylation. Mechanisms of resistance related to rRNA resistance to these very antibiotics. Oxazolidinones have their mechanism of action in inhibiting protein synthesis, which involves the binding of 23S rRNA of the 50S subunit (Osterman et al., 2020)**.**

DNA Synthesis Interference

Resistance to the quinolone and its derivatives, fluoroquinolones, arises due to the alteration of DNA synthesis enzymes: DNA gyrase and topoisomerase IV, which are involved in this process (Bush et al., 2020). The mutations in genes

gyrA and parC can decrease the affinity of these enzymes to the fluorescence, thereby rendering the antibiotics ineffective and leading to replication failure. These mutations can be in chromosomes or be plasmid-mediated, as reported by Martínez et al. (2019). Knowledge about these resistance mechanisms is very critical. It means developing strategies to overcome antibiotic resistance effectively.

Efflux Pumps and Outer Membrane Permeability

Efflux pumps are transmembrane proteins that export antibiotics from the bacterial cells, keeping their intracellular concentrations at a low level. They are major factors of antibiotic resistance, since removing the antibiotic from the cell, prevents the activity of these antibiotics. Besides, a reduced outer membrane permeability can also be involved in resistance through the reduction of uptake of antibiotics into the cell (Aslam et al., 2022).

Efflux Pumps

One of the most challenging issues in antibiotic resistance is efflux pumps, and of all, the multi-drug efflux pumps. Such pumps might extrude a huge variety of antibiotics, involving the major classes, including macrolides, tetracyclines, and fluoroquinolones, thus providing multi-drug resistance. For example, there are several efflux pumps involved with *Pseudomonas aeruginosa*, such as MexAB-OprM and MexVW-OprM, which provide resistance to different antibiotics (Lorusso et al., 2022).In addition, bacteria can produce more membrane proteins that act as export or efflux pumps, which pump out the toxic compounds from the cell (Henderson et al., 2021).

Outer Membrane Permeability

The outer membrane of gram-negative bacteria has an inner layer of phospholipids and an outer layer of lipid A, thereby making the uptake of drugs and their transfer through the OM less extensive. Small hydrophilic molecules such as β-lactams and quinolones may diffuse through the OM by using the porins(Giordano et al., 2020). Larger molecules, however (like aminoglycosides and colistin), self-promoted uptake by binding to lipopolysaccharides on the outer side of the OM (Ropponen et al., 2021). Acquired resistance in *P. aeruginosa* is often linked to low OM permeability, leading to high resistance against most antibiotics, especially aminoglycosides (Langendonk et al., 2021). The contribution of efflux pumps and outer membrane permeability towards antibiotic resistance should be known to devise strategies against multi-drug-resistant bacteria because of overcoming them with efficacy.

Bypass of Antibiotic Inhibition

Such mechanisms of resistance mean that bacteria will produce another target, usually an enzyme, which is resistant to the antibiotic's inhibitory action. This alternative target acts in place of the native target, which remains sensitive to the antibiotic (Annunziato, 2019). For instance, MRSA produces a different PBP that is resistant to the action of methicillin. With this alternate target, the bacteria can survive in the presence of the antibiotic (Lade and Kim, 2021). The lowering of the sensitivity and affinity of the altered enzymes, such as DHPS and DHFR, results in resistance to trimethoprim and sulphonamides respectively (Sánchez-Osuna et al., 2020). This is another example of how bacteria are resourceful in developing alternate bypasses against the action of the antibiotics.

Antibiotic Resistance in *Escherichia coli* (*E. coli*)

Escherichia coli, better known as *E. coli*, is very famous in microbiology as it represents an organism that can live either as a commensal or cause serious, life-threatening infections in humans and animals (Peng et al., 2024). The general term for a disease caused by the bacterium *E. coli* is "Colibacillosis". The bacterium resides naturally in the intestines' microbiota in warm-blooded mammals. While the majority of strains are beneficial, a few possess virulence characteristics that can cause minor intestinal infections or serious infections outside of the intestines, and some even cause minor to serious infections outside of the intestines. There are two elements that influence *E. Coli's* ability to cause disease: the pathogenic type of the strain and the presence of particular virulence genes (Basavaraju and Gunashree, 2023).

The concern has been rising over the past years that deadly bacteria may become resistant to antibiotics and then find their way into humans from animals through animal product consumption. Possible transmission of this infection, according to Carvalho et al. (2019), is through direct contact, contact with the waste produced from the animals, or ingestion of contaminated food. *E. coli* stores antimicrobial resistance genes. *E. coli* has the capacity for horizontal gene transfer between strains, horizontal gene transfer may also be infecting different species of bacteria. *E. coli* may, therefore, have the potential to spread through bacteria and cause therapeutic implications in veterinary medicine and human medicine (Sarowska et al., 2019). One of the most important characteristics of *E. coli* is that it is capable of multidrug resistance through horizontal gene transfer, developing resistance to many antibiotic families. It is quite a considerable issue with the management of E. coli because it is capable of rendering most of the conventional antimicrobials inefficient (Pérez-Etayo et al., 2020).

In veterinary healthcare, *E. coli* is also a common source of infection in animals. The pathogenic *E. coli*, especially in young animals and particularly in those reared for food, for example, poultry, swine, and cattle, may cause diarrheas that can lead to fatal electrolyte imbalances and dehydration in animals (Balestracci et al., 2023). Moreover, E. In this respect, *E. coli* can cause dairy cattle mastitis, and coli-bacillosis in poultry, and further result in infections of the urinary tract (Fahim et al., 2019).

Table 1: Types, Mechanisms, and Examples of Antibiotic Resistance, an Overview

Type of Resistance	Mechanism of Resistance	Antibiotic Class	Common Examples	Reference
Genetic	Spontaneous Mutations	Quinolones	Changes in specic genes (e.g., gyrA, parC) leading to altered target binding	(Sabet et al., 2020)
	Hypermutators		Increased mutation rate if in bacterial populations, enhancing adaptation to antibiotics	(Mehta et al., 2019)
	Adaptive Mutagenesis		Mutations in non-dividing or slowly dividing cells under selection pressure, contributing to antibiotic resistance	(Windels et al., 2019)
	Horizontal Gene Transfer		Transfer of resistance genes between bacteria via plasmids, transposons, or other mobile genetic elements	(Horne et al., 2023)
Antibiotic Inactivation	β-lactamases	β-lactams	Hydrolysis of β-lactam ring, rendering antibiotics inactive	(Shamsuddin, 2019)
	Aminoglycoside-modifying enzymes	Aminoglycosides	Addition of modifying groups to aminoglycosides, reducing their affinity for bacterial ribosomes	(Sati et al., 2019)
Target Modification	PBPs alteration	β-lactams	Changes in penicillin-binding proteins, reducing antibiotic binding	(Clark et al., 2019)
	Ribosomal mutations	Macrolides	Mutations in ribosomal RNA, reducing binding affinity of antibiotics	(Jednačak et al., 2020)
Efflux Pumps	MexAB-OprM efflux pump	Fluoroquinolones	Pump extrudes antibiotics from cell, reducing intracellular concentration	(Thakur et al., 2021)
	MexXY-OprM efflux pump	Aminoglycosides	Extrudes aminoglycosides from cell, contributing to resistance	(Davin-Regli et al., 2021)
Bypass of Inhibition	Production of alternative targets (e.g., altered enzymes)	Various	Bacteria produce alternative targets that are not affected by antibiotics	(Darby et al., 2023)

The treatment of *E. coli* infections in animals involves the usage of a high share of antimicrobial medications. This can result in the development of multidrug-resistant bacterial strains through overuse or misuse of antibiotics for veterinary treatment, which is becoming more concerning (Puvača and de Llanos Frutos, 2021). Hence, prudent antimicrobial use practices have been recommended in an effort toward prevention of this. One prime consideration should be the use of all other available treatment options and reserving antimicrobials only when necessary. Especially, AmpC and extended-spectrum beta-lactamase genes were identified in *E. coli* isolated from animals, primarily from commensal flora in fecal samples, and this fact underlines selective pressure due to antibiotic use in animal husbandry (Ewers, 2023). The identification of the genes thus calls for management concerning antibiotic resistance with a One Health approach - acknowledging interdependence between environmental, animal, and human health - as indicated by Aslam et al. (2021).

The pathogenic ability and the capacity of *E. coli* to develop antimicrobial resistance makes the bacterium's challenge in both human and veterinary medicine. A multidisciplinary approach encompassing surveillance, judicious use of antimicrobial agents, and cross-dialoguing is required regarding public health and animal welfare on the fact of this bug of importance (Singh et al., 2020).

Mechanisms of Resistance of Various Antibiotics in *Escherichia coli*

Resistance to β-lactams

The β-lactam resistance problem caused by many genes in *Escherichia coli* is regarded as serious in both humans and animals. While some, such as blaTEM-1, are very common in animal *E. coli,* their resistance to β-lactams is poor (Waithiru et al., 2022). More recently, carbapenemase genes have been occasionally found in animal *E. coli*, along with the emergence of genes encoding ESBLs/AmpCs in human as well as animal *E. coli*. Since ESBLs confer resistance to a variety of β-lactams, including third- along with fourth-generation cephalosporins authorized for veterinary use, they are particularly relevant in veterinary medicine (Ramos et al., 2020a). ESBLs are classified as class A in the Ambler classification and as group 2be in the Bush-Jacoby functional grouping (Sawa et al., 2020). Since ESBLs are increasingly being detected in *E. coli* from food-producing along with pets worldwide, including healthy people and wild animals, their presence can result in treatment failures and restrict therapeutic options (Widodo et al., 2020).

TEM- along with SHV-ESBLs were once common, but they have been completely substituted by CTX-M-ESBLs after 2000, which have been found in commensal along with pathogenic *E. coli* of human as well as animal origin worldwide (Rahim et al., 2020). Food-producing animals vary in their prevalence of cefotaxime resistance depending on the nation and species. The most often found ESBL genes are blaCTX-M-1, blaCTX-M-14, blaTEM-52, as well as blaSHV-12, along with a number of additional blaCTX-M, blaTEM, and blaSHV variants (Silva et al., 2019).

Isolates of ESBL-producing *E. coli* from sick food-producing animals were examined in a German study conducted between 2008 and 2014. It was discovered that 69.9% of ESBL producers had blaCTX-M-1, which was followed by blaSHV-12 (1.4%), blaTEM-52 (1.9%), blaCTX-M-15 (13.6%), and blaCTX-M-14 (11.7%). Less commonly, 1.0% as well as 0.5% of isolates, respectively, had blaCTX-M-3 and blaCTX-M-2 detected. ESBL gene distribution differed depending on the animal host and isolation location (Mwendwa, 2021).

In companion animals in Europe, blaCTX-M-1 and blaCTX-M-15 were both often seen. When ESBL-producing *E. coli* from infections of the urinary tract in companion animals was found in the US, blaCTX-M-15 dominated (Ewers, 2023). On the other hand, blaCTX-M-14 was more common in people, companion animals, and poultry in Asia but less common in Europe (Chenouf et al., 2021). Although it has not been widely reported, ESBL-producing E. coli from chickens, dogs, as well as wild birds in both Germany and Spain were shown to carry the ESBL gene blaSHV-12 (Chenouf et al., 2021).

The most common ESBL gene found in *E. Coli* isolates of human origin worldwide is blaCTX-M-15, which is mostly connected to the global epidemic of *E. Coli* clone O25:H4-ST131 (Sewunet et al., 2022). There have been isolated reports of this clone in animals, mostly companion animals. Diverse sequence kinds of animal *E. coli* have been shown to harbor a variety of ESBLs; some of these types are more commonly found in both people and animals, which may aid in the transmission of ESBL genes (Athanasakopoulou, Reinicke, et al., 2021).

The spread of ESBL genes in animal *E. coli* is mostly caused by horizontal gene transfer, which is linked to several genetic components. Plasmids contain the majority of ESBL genes, with some types being more common than others (Ramos et al., 2020). Additional resistance genes may be carried by plasmids containing ESBL genes, which could aid in their persistence (Hagel et al., 2019).

Research into the potential transmission of ESBL-producing *E. coli* from animals to humans has been conducted. One study suggested that ESBL-encoding plasmids of identical genetic origin may be transmitted from humans to chickens via a food-borne route; however, most studies did not show a direct link (Cardozo et al., 2021). In various parts of the world, hens have been shown to be a significant reservoir of ESBLs. ESBL-producing *E. coli* has been associated with infections in broilers and laying hens in several countries and contamination of chicken meat at retail (Lay et al., 2021).

Acquired AmpC Cephalosporinases and their Role in Antibiotic Resistance

Besides the class A ESBL enzymes that contribute to resistance, the acquired AmpC cephalosporinases, also known as AmpC-type enzymes, are another group of enzymes that confer *E. coli* with high-level resistance to broad-spectrum cephalosporins. Plasmid-encoded AmpC enzymes of the CMY-, DHA-, and ACC-types have been prevailing in mammals. Among these types, the CMY type is distributed more widely in the world (Galani et al., 2021). A study in Denmark and Sweden showed the existence of CMY-2-producing *E. coli* in dogs, poultry, and poultry products. The research indicated that the IncI1-γ and IncK plasmids are basic vectors used in the dissemination of blaCMY-2 (Börjesson et al., 2016). In Sweden, the introduction of one-day-old chicks from countries that apply a prophylactic use of broad-spectrum cephalosporins in young birds has been implicated in the first detection of CMY-2-producing *E. coli* in broiler farms. Furthermore, it has been discovered that migrating birds harbor CMY-2-positive *E. coli*, indicating a possible role for them in the long-distance transmission of isolates resistant to several drugs (Athanasakopoulou, Tsilipounidaki, et al., 2021).

Acquired Carbapenemases and their Role in Antibiotic Resistance

There is limited application of carbapenem in veterinary medicine, but still carbapenemases have been isolated from animals all over the world, although being infrequently detected in animal *E. Coli*. VIM-1 was the initial carbapenemase determinant found in animal *E. Coli*; it was found in a German pig. OXA-48, OXA-181, KPC, NDM-1, NDM-5, IMP-4, and other carbapenemases have also been identified in E. coli (Köck et al., 2018). Several carbapenemases have been found in a range of animal species as well as geographical areas, which is consistent with the frequency of human isolates found in those areas. The usage of penicillins, that serve as substrates for carbapenemases, may have an impact even if the selective pressure responsible for choosing carbapenemase producers in animals is still unknown (Hammoudi Halat and Ayoub Moubareck, 2020). In general, colonization in animals by Enterobacteriaceae producing carbapenemase is considered not to be of great public health importance. Until now, the potential for dissemination of these bacteria to humans from animals or humans to animals remains poorly understood.

Resistance to Quinolones and Fluoroquinolones

The main mechanism of quinolones and fluoroquinolones resistance, critical antibiotics in treating various infections in humans and animals, is mutation in the genes for DNA gyrase together with topoisomerase IV genes (Millanao et al., 2021). These alterations reduce the activity of the drugs, mainly at GyrA and ParC. Plasmid-mediated mechanisms of resistance, resulting in quinolone resistance, were also found to be linked with efflux pumps and Qnr proteins (Shariati et al., 2022). The genes are isolated in animals that have a wide distribution, with a high prevalence noted in animals for food in China. According to Luiken et al. (2022), in Europe, these genes have isolated in pigs, cattle, and poultry. This implies that the genes have spread since they can also be isolated from companion animals. There is a need for surveillance on these systems to ensure antibiotics are applied effectively.

The mechanisms of resistance against quinolone and fluoroquinolone in *E. coli* represent a rather complex process, including both plasmid-mediated and chromosomal changes (Phan et al., 2022). The mutations in the genes for DNA gyrase and topoisomerase IV lower the efficiency of the drug, while plasmid-mediated ways of resistance involve efflux pumps and Qnr proteins (Azargun et al., 2020). Resistance genes are harbored by a variety of animal hosts, which again are

very widely distributed. For example, in China, food-producing animals excrete these genes at high rates, as do many animal species in Europe (Liu et al., 2021). Effective management of the use of antimicrobials in agriculture and prevention of the spread of resistant bacteria requires both an understanding of these mechanisms and monitoring of their occurrence.

Resistance against Aminoglycosides

Aminoglycosides represent the crucial antimicrobial agents applied in the combined therapy against a number of diseases both in humans and animals. These pharmacological agents are prepared from the species Streptomyces and Micromonospora (Abdel-Razek et al., 2020). Amikacin is applied only in pets and horses; neomycin, streptomycin, gentamicin, kanamycin, as well as paromomycin, have been commonly applied in the sphere of veterinary medicine (van Duijkeren et al., 2019). These are bactericidal drugs that interfere with bacterial translation and act against various Gram-positive and Gram-negative bacteria (Breijyeh et al., 2020).

Some genetic material mutation encoding ribosomal RNA and ribosomal proteins also result in resistance to aminoglycosides; however, the mechanism is less common in E. coli since it carries a high number of copies of the rRNA gene (Foudraine et al., 2021). Aminoglycosides include tobramycin, amikacin, gentamicin, and netilmicin, among others. High-level resistance to them may be provided by the methylating of some residues at 16S rRNA (Al-Tameemi et al., n.d.). These residues get methylated by the action of enzymes such as ArmA, RmtA/B/C/D/E/F/G/H, and NmpA, which creates an effective barrier to antimicrobial therapy (Davies and Wright, 1997).

The armA gene has been confirmed in isolates of Pseudomonas aeruginosa, Acinetobacter baumannii, and enterobacteria, although it was first described in Klebsiella pneumoniae in 2003 (Jouybari et al., 2021). Moreover, while RmtB was first described in E. coli from Chinese pigs in 2007, it has since been found in a number of other animals (Li et al., 2023). Much less commonly, other methylases have been reported, such as RmtD and RmtE. Many of these enzymes are carried on a mobile genetic element, which assists in their diffusion through the bacterial population (Yang and Hu, 2022).

Development and diffusion of these methylases are ruled by the habits in the use of antimicrobials. Although not as frequent as other mechanisms of resistance, they do represent a serious obstacle to the effective use of aminoglycosides for the treatment of bacterial infections. The other mechanism of resistance is enzymatic inactivation, whereby aminoglycoside-modifying enzymes alter the chemical structure of the drug so that it cannot bind to the target site (El-Khoury et al., 2022). The three main types of these enzymes are phosphotransferases, nucleotidyltransferases, and acetyltransferases; subgroups of each of these enzymes have been isolated in E. coli from both human and animal sources (Abushaheen et al., 2020). Such resistance mechanisms have been described in several animal species, such as pigs, cattle, poultry, and wild rabbits.

Tetracycline Resistance Mechanisms

Since tetracyclines are often used in veterinary treatment, bacteria like *E. coli* frequently develop resistance to them. In *E. coli*, a number of tetracycline resistance genes have been identified, including ribosome protection genes (tet(M) and tet(W)), efflux genes (tet(A), tet(B), tet(C), tet(D), tet(E), tet(G), tet(J), tet(L), also tet(Y)), and an inactivating gene (tet(X)) (Sheykhsaran et al., 2019).

These resistance genes can be found in *E. Coli* from a variety of animal origins in different combinations and frequencies. Tet(A), tet(B), and tet(C) are common genes in cattle, but tet(A), tet(B), tet(C), tet(A) + tet(B), and tet(A) + tet(B) + tet(M) are common genes in pigs (Cheng et al., 2021). Tet gene-carrying plasmids frequently co-occurring with other resistance genes contribute to multidrug resistance in *E. Coli* from various animal species across the globe.

Resistance to Phenicols

In veterinary medicine, antimicrobial drugs are frequently utilized, such as chloramphenicol as well as its derivative florfenicol. However, the EU outlawed the use of chloramphenicol along with its derivatives in animals raised for food in 1994 due to its toxicity and negative effects on humans (Ghimpețeanu et al., 2022). At the moment, florfenicol is permitted for usage in animals that produce food, whereas nonfluorinated phenols are limited to companion animals.

Three main mechanisms are involved in *E. Coli* resistance to phenicols in animals: target site methylation by an rRNA methylase (encoded by the CFR gene), active efflux by major facilitator superfamily proteins (encoded by the cmlA and floR genes), and inactivation of enzymes by chloramphenicol acetyltransferases (encoded by cat genes) (Pokludová, 2020). Research has indicated that *E. coli* from different animal species have high levels of resistance to chloramphenicol (Pokludová, 2020). Commonly, resistant isolates possess resistance genes such as catA1, catA2, cmlA, and floR (Williams et al., 2019). These gene sequences are frequently present on plasmids, which may also carry other resistance genes. When nonphenicol antimicrobial drugs are applied, this could result in co-selection (García et al., 2022).

Sulfonamides and Trimethoprim Resistance Mechanisms

Such antimicrobial drugs as trimethoprim and sulfonamides are applied both in human and veterinary medicine. They act by inhibiting different steps in the pathway of folic acid biosynthesis. In most cases, *E. coli's* resistance to these drugs is due to genetic mutations of the genes encoding the target enzymes or due to the acquisition of genes that render the action of the drug on the target enzymes (Schaenzer and Wright, 2020).

Table 2: This table illustrate the antibiotic classes and their resistance mechanisms with some common examples across different Animals and Regions.

Antibiotic Class	Resistance Type	Resistance Mechanism	Common Examples	Animal Affected	Geographical Region of Resistance	Reference
Clavulanic-Acid Inhibited Class A ESBLs	Beta-lactam resistance	Production of extended-spectrum beta-lactamases (ESBLs) that hydrolyze beta-lactam antibiotics	TEM, SHV, CTX-M	Food-producing animals, companion animals, humans	Global	(WIBISONO et al., 2020)
Acquired AmpC Cephalosporinases	Beta-lactam resistance	Production of plasmid-mediated AmpC enzymes that hydrolyze cephalospor-ins	CMY, DHA, ACC	Poultry, dogs, pigs	Global	(Athanasakopoulou et al., n.d.)
Acquired Carbapenemases	Carbapenem resistance	Production of carbapenem-ase enzymes that hydrolyze carbapenem antibiotics	VIM, NDM, IMP, OXA, KPC	Various animal species	Global	(Bonardi and Pitino, 2019)
Quinolones and Fluoroquinolones	Quinolone resistance	Mutations in genes encoding DNA gyrase and topoisomera-se IV, reduced outer membrane permeability, target protection, increased efflux pump activity	Various mutations	Various animal species	Global	(Chaudhari et al., 2023)
Aminoglycosides	Aminoglycoside resistance	Enzymatic inactivation, target site modificatio-n	AAC, APH, ARM, RMT	Various animal species	Global	(Reynolds et al., 2022)
Fosfomycin	Fosfomycin resistance	Mutations in genes affecting fosfomycin uptake, acquisition of fosfomycin-modifying enzymes	fosA, fosB, fosX, fomA, fomB	Various animal species	Global	(Zheng et al., 2022)
Tetracyclines	Tetracycline resistance	Efflux pumps, ribosomal protection, enzymatic inactivation	tet(A), tet(B), tet(C), tet(M), tet(W), tet(X)	Various animal species	Global	(Roberts, 2019; Hormeño et al., 2020)
Phenicols	Chloramphenicol resistance	Enzymatic inactivation, active efflux, target site modification	cat, cmlA, floR, cfr	Various animal species	Global	(Preena et al., 2020)
Sulfonamides and Trimethoprim	Sulfonamide and Trimethoprim resistance	Enzymatic inactivation, target site modification	sul, dfr	Various animal species	Global	(Kordus and Baughn, 2019)
Colistin	Colistin resistance	Chromosom-al mutations, plasmid-mediated MCR enzymes	PmrA, PmrB, MgrB, mcr-1, mcr-2	Various animal species	Global	(Gogry et al., 2021)

Animal-derived *E. coli* sulfonamide resistance is largely mediated by sul genes, particularly sul1, which often appears in class 1 integrons with other genes related to resistance (Lima et al., 2020). The streptomycin resistance genes, typically located on plasmids, are also often coupled with the disseminated gene sul2. Sul3, initially discovered in *E. coli* of pigs, was plum localized on plasmids in *E. coli* of other animals and is frequently associated with aberrant class 1 integrons along with other resistance genes also (Jiang et al., 2019). The trimethoprim resistance in *E. coli* is due to the dfr genes, mainly dfrA and dfrB, that are regularly borne on gene cassettes inside integrons (Ambrose and Hall, 2021). Isolation of the *E. coli* from several animal species showed the presence of multiple dfr genes. Among them, dfrA1 was found to be the most prevalent one (Sacher-Pirklbauer et al., 2021). The availability of these resistance genes in these genetic components depicts the necessity of regulation and monitoring of the use of antibiotics in veterinary treatment in order to avoid resistance generation.

Mechanisms of Colistin (Polymyxin E) Resistance

In the context of veterinary medicine, colistin (polymyxin E) is an antibiotic drug applied for infection or prophylaxis in the intestines of animals like pigs, poultry, and cattle (Joshi et al., 2024). It works by attacking a substance called lipopolysaccharide, which forms part of the outer membrane of Gram-negative bacteria. China and Europe have imposed a ban on the use of colistin following concerns that colistin resistance may spread from animals to humans. The

chromosome gene mutations in *Escherichia coli* confer resistance to the antibiotic colistin by affecting the expression of the LPS-modifying enzymes PmrA, PmrB, and MgrB (Ahmad et al., 2023).

These changes have been identified in *E. coli* isolates from humans and investigations are ongoing as to whether similar mechanisms form the basis of resistance in animals (Riquelme et al., 2023). It has been identified that *E. coli* isolated from pigs harbor mutations in genes including pmrA, pmrB, mgrB, phoP, or phoQ (Kim et al., 2019). The first report of plasmid-mediated colistin resistance was that of the MCR-1 gene producing the MCR-1 phosphoethanolamine transferase in 2016 (Hussein et al., 2021). This enzyme gives polymyxins resistance through alteration of the lipid A portion of the LPS (Moffatt et al., 2019). The MCR-1 gene has been detected in isolates of both human and animal origins, including *Salmonella*, *E. coli*, *Shigella*, *Klebsiella*, and *Enterobacter*, from Enterobacteriaceae across the globe (Mmatli et al., 2022). The frequent carrying of this gene in plasmids, sometimes together with other co-carried resistance genes, is on plasmids with different sizes and incompatibility groups. Further proof that calls for strict surveillance and measures of control to avoid their dissemination and to maintain colistin for its effectiveness in veterinary and human medicine is given by the isolation of further plasmid-mediated colistin gene mutations, namely, MCR-2 to MCR-7 and their variants, from different animal and human isolates (Anyanwu et al., 2020).

Conclusions

The indiscriminate use of antibiotics has undoubtedly made bacteria, like *Escherichia coli*, resistant to them. Resistance to antibiotics is a serious threat to the health of humans and animals, which is attributed to innate as well as acquired mechanisms. Multi-drug resistance of *E. coli*, apart from complicating the treatment process, acts as a gene pool for resistant genes in other animal species, including humans. Knowledge of transmission routes and resistance mechanisms becomes very vital in formulating effective control measures. Although the impacts of animal-produced carbapenemase-producing *E. coli* on human health appear limited; the presence of plasmid-mediated antibiotic colistin gene resistance in animals currently poses a severe threat to human health. There is, therefore, an immediate need for a One Health approach that brings together veterinary and human activities to end the development and spread of antimicrobial resistance within *E. coli*.

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Chapter 04

Use of Activo in Poultry to Replace Tilmicosin

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ABSTRACT

Since beginning of poultry industry it has become a key player in food industry as a crucial contributor for poverty alleviation. Despite being a good producer of food items poultry industry has its own challenges that often affect the farmers and poultry industry overall with economic downturns and decline in production. One of such problems is finding alternatives to antibiotics since the ban on antimicrobial agents in Europe. Although vaccines have saved a lot of disease curbing effort that used to happen with antibiotics there are still some diseases and problems that require measures beyond common vaccination protocols. For instance, the disease caused by bacterial agents like *Mycoplasma spp.* are usually not properly controllable through vaccines and require additional agents like antibiotics for reducing their impact on poultry production. This realization among researchers lead towards a trend of looking for alternatives besides antibiotic drugs, having antimicrobial characteristics with least toxic effects. An example of such agent is the Activo supplements that supply birds with cinnamaldehyde and carvacrol to boast their immunity and increase their vitality.

KEYWORDS
Poultry, Antibiotics, *Mycoplasma*, Activo, Cinnamaldehyde, Carvacrol

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INTRODUCTION

In present era, after the ban on antibiotics came in effect all to prevent their utilization as growth promoting agents. Since then alternative medicinal agents have been under consideration for use as substances inducing effects of growth promotion and increased immunity. Such potent agents include phytochemicals, probiotics, enzymes and other organically obtained substances (da Silveira Deminicis et al., 2021; Ghasemıan et al., 2022). The purpose of this ban on antimicrobial drugs is to counter the spread of antibiotic resistance among bacteria. This step will ensure the no antibiotics are used so the development of resistance strains among bacterial species can be prevented simultaneously stopping its transfer to other strains of non-resistant bacterial populations (Rahimi et al., 2012; Gholami-Ahangaran et al., 2021a).

Phytochemicals, also referred to as phytogens include a diverse range of plant-based substances of bioactive nature. These useful phytochemicals can be derived from various kinds of plant parts including identified in fruits, vegetables, whole grains, legumes, nuts and herbs. More than 800 types of these phytochemicals have been discovered that chemical belong to the groups of phenols, flavonoids, tannins, saponins, essential oils and many other types. The usefulness of these compounds has earned them the name of phytobiotics (Yadav et al., 2016). These phytobiotic agents can be used as feed or dietry additives to be used in the rations of commercial animals for improving their productivity through improvement in characteristics and quality of feed. The increased feed quality ultimately leads to animal production enhancement while simultaneously improving the quality of food and food other byproducts obtained from such animals (Gholami-Ahangaran et al., 2021b).

The change in trend of medication from traditional drugs to plant based medicine during past few years can be attributed to the number advantages that come with these drugs as compared to the chemical options available. Along with low to no toxicity the plat derived drugs are also available naturally hence cutting down the synthesis costs and environmental stress. Such characteristics make them ideal feed inclusive supplements (Gholami-Ahangaran et al., 2021c). The plat-based products consist of various classes including essential oils that are well known for possessing bioactive components. These ingredients have been reported to show antibacterial activity against bacterial agents, yeast and fungal molds (Bahmani et al., 2014). Out of various types of the groups that are major ingredients in the plant-based drugs some important ones imparting antibacterial characteristics are thymol, eugenol, saponins, flavonoids, carvacrol, terpenes and their precursors that display such properties in their essential oils (Gholami-Ahangaran et al., 2020). There are several types of phytobiotic agents classified chemically into six groups as these compounds can be: phenolic, alkaloids, nitrogen-containing, organosulfur, phytosterols and carotenoids. These groups have been further divided into sub-groups. A number of investigations regarding the biological impact of phenolic compounds and carotenoids have been conducted to determine their qualities and characteristic (Kikusato, 2021).

Composition of organically derived plant products may be inconsistent with regards to its of individual components. For instance, the amount of two major ingredients, thymol and carvacrol in essential oil of thyme, can range between the lowest value of 3% and going as high as 60% (Gholami-Ahangaran et al., 2019). Same kind of inconsistency can be seen in the *Origanum vulgare ssp. hirtum*, oregano essential oils. These oils are ontained through steam-distillation of the plant parts containing a number of biologically active compounds that goes beyond 30. Majority of these compounds are chemically phenolic and display a inconsistency in their activities. Main ingredients of essential oils usually carvacrol and thymol, which make upto 80% of the whole oil. In this composition carvacrol is usually in a higher percentage in the oil as compared to thymol, although it may also occur as a trace component (Skoufos et al., 2020). The amount of bioactive phytochemical compounds and their chemical makeup of these products may result in widely varying composition of the final product on the basis of plant parts utilized (seeds, leaves, etc.), geographical location of the source plant and season of harvesting those plants. Suggestions have been presented by researchers that the variations in composition of final products also means varying beneficial results form the administration of oregano essential oils as its functionality is directly dependent on its composition ratio (Ghasemıan et al., 2022).

During these recent two decades, biologically active phtyochemicals have been reported to display various types of effects. The impact of these phytobiotic includes anti-inflammatory, antibacterial, anti-oxidative and metabolic-regularity effects. Phytobiotic chemicals are utilized for enhancement of growth and improvement in quality of egg and meat products obtained from poultry birds (Kikusato, 2021). The proper mechanism of action for these phytobiotics isn't well known yet. However, they have been reportedly found to impact gut microflora by indicing a decline in population of the pathogenic organisms. It is suspected that the phytobiotics act by producing changes in permeability level of membranes for hydrogen ions (Gholami-Ahangaran et al., 2020).

Phytobiotics contain antioxidant substances of both hydrophilic and lipophilic active nature. These substances are being utilized for countering stress conditions, such as heat stress, due to their antioxidant nature of activity. Their antioxidant characteristics can prove to be helpful in enhancement and the sustenance of processed meats products in terms of quality. It also prevents losses through the muscle drip during thawing of freeze-stored products (Yadav et al., 2016). Additionally, various studies have reported that phytobiotic chemicals can improve activity of the digestive enzymes as well absorption capacity of the gut. Furthermore, the outcomes of several studies have shown that phytogens may also stimulate mucus production in the intestines and it may even lead to the reduction in numbers if pathogenic bacteria by preventing their adherence to the gut mucosa (Mohammadi Gheisar and Kim, 2018).

Tilmicosin

As an antibiotic drug Tilmicosin is classified as a semi-synthetic lactone macrolide consisting of 16-members with strong antibacterial action in vitro against Gram-positive and Gram-negative bacteria, as well as other pathogenic species such as *Mycoplasma spp.* (Xiong et al., 2019). Tilmicosin has been approved for treatment of respiratory disease in cattle and sheep. It is especially effective against respiratory disease causing bacterial species that are *Mannheimia haemolytica* in cattle and sheep, *Actinobacillus pleuropneumoniae* in swine and *Pasteurella multocida* in chickens, similarly it also affects *M. gallisepticum* and *Mycoplasma hyopneumoniae* in swine species (Womble et al., 2006). Tilmicosin has also seen widespread application in treatment against *M. gallisepticum* that accumulates in lungs leading to respiratory disease, with high levels of Tilmicosin being retained in tissues of lungs. This effect is desired by farmers as *M. gallisepticum* is an intracellular pathogen of facultative nature and the amount of lung tissue residual levels should be considered when formulating treatment regimen for poultry (Zeng ZhenLing and Feng QiHui, 1997; Huang et al., 2003). Additionally, Pharmaco-Kinetic and Pharmaco-Dynamic integration models of tilmicosin against *M. gallisepticum* and *M. hyopneumoniae* have been performed only utilizing in vitro methodologies and lacks researches that used mycoplasma-infected chickens (Huang et al., 2019; Huang et al., 2020).

Development of tilmicosin has beenain accordance for its extensive use in the veterinary field administrations. Its application also involves its utilization as a preventive medication besides being a treatment drug for pneumonia of pigs, sheep and cattle caused by *Pasteurella*, *Staphylococcus*, *Streptococcus*, and *Actino-bacillus pleuropneumoniae*; *Mycoplasma* along with mastitis in ruminants (Xie et al., 2011; Wang et al., 2012; Ibrahim and Abdel-Daim, 2015). Additionally, use of tilmicosin is also increasing for the treatment of respiratory tract infections induced by various tilmicosin-susceptible

organisms, in poultry (Yapar et al., 2006; Clark et al., 2008; Han et al., 2009). Tilmicosin performs its antimicrobial activity by the mechanism of forming bonds with the bacterial ribosome subunits. This type of binding blocks the transferase activity to prevent translocation of the mRNA, stopping the extension of the peptide chain which inturn pauses the synthesis microbial proteins and ultimately leads to expression of antibacterial effects of tilmicosin. Clinically utilization of tilmicosin was reported to show various types of useful qualities. These properties included requirement of lower concentration for inducing inhibitory effect, broad range of antibacterial activity, large volume of distribution, longer active life span hence longer half-life before elimination, and accumulating rapidly in the target bacterial cells (Ziv et al., 1995; Ramadan, 1997).

Adverse Effects

Previously conducted researches and observations from clinical practice have reported that despite exhibitkng low toxicity, oral administration of tilmicosin can lead to serious issues such as incresead rate of respiration, vomiting, convulsions, or even death. Similar effects may also occur in animals after administration of tilmicosin through subcutaneous or intravenous routes or through repetitive high-dose usage Potentially tilmicosin toxicity can occur in several organs mainly damaging heart, liver and kidneys (Kart et al., 2007; Oda and Derbalah, 2018). Generally, the undesired effects of tilmicosin on cardiovascular system lead to an increase in heart rate and disruption of functionality of ventricules, this effect is easily evident in younger animals (Jordan et al., 1993; Main et al., 1996). However, these effects are usually dose dependent, other factors like animal species, and route of administration can also lead to variation in severity of these effects (Altunok et al., 2002; Yazar et al., 2002). Despite its cardiotoxicity being well known tilmicosin, is still being studied for other types of metabolic toxic effects it can induce in animals, especially poultry birds. Data available today reveals that changes in levels of creatine kinase (CK), CK-MB, total sialic acid, glutathione, and malondialdehyde (MDA) in blood are the key indicators of cardiotoxicity caused by tilmicosin as observed in lab mice, and humans. Cardio toxicity caused by tilmicosin happens through the mechanism of reactive oxygen species (ROS) generation and disruption of the antioxidant enzyme systems in cardiac tissues (Yapar et al., 2006; Kart et al., 2007; Cetin et al., 2011).

Defense against Diseases

The chronic respiratory disease in poultry happens primarily due to *Mycoplasma gallisepticum* (*M. gallisepticum*) infection. This agent also causes sinusitis in turkeys. The major signs of these respiratory infections include air sacculitis, nasal discharge, and development of keratoconjunctivitis. Furthermore, *M. gallisepticum* are able to infect birds through both horizontally as well as vertically mode of transmission via eggs (Levisohn and Kleven, 2000). Embryonic death, decline in body weight, and reduced egg production level are some of the adverse effects induced by these infections in poultry. Such effects on large scale lead an increased economic stress on poultry farms (Kleven, 1990).

Various types of antibiotics are being used in present era for controlling *M. gallisepticum* infections on poultry farms. Some of such major antibiotics include pleuromutilins, tetracyclines, quinolones, and macrolides (Zhang et al., 2016; Zhang et al., 2017). The Pharmacokinetics (PK) of macrolides are distinguishable as they large distribution volume with qualities of prolonged retention and sustenance in the lung tissues. That is why they are most preferred treatment drugs against bacteria invading respiratory tracts of livestock (Yang et al., 2019). However, there has been a rise in resistance against *M. gallisepticum*. This threat appeared and progressed in a very short time, hence demanding a vigilant approach towards development of effective dosing schedule through utilization of PK/Pharmacodynamics (PD) modeling system to make sure that proper treatments are formulated (Gerchman et al., 2011; Ammar et al., 2016).

Reportedly *M. gallisepticum* has been mostly eliminate from layer type hens although it still found persisting through many generations of egg-laying hens all across the world. This vertical transmission results in loss of egg production percentage, and increase in efficiency regarding feed conversion in birds along with a dramatic increase in mortality rate among birds (Ferguson-Noel et al., 2020). For several years, enough consideration has not been given to the control of *M. synoviae*, until it was recorded causing a high amount economic losses *M. synoviae* affected flocks (Collett et al., 2020). These losses served as the key driving force for a heightened consideration by researchers towards study, comprehension, effects of infection, control and eradication of *M. synoviae* in affected layer hens. As a pathogen *M. synoviae* is a significant player in domestic poultry, as it can lead to severe economic losses due to its pathogenesis in layer hens giving it the second place on the list of most important *Mycoplasma spp.* of avian especially domestic poultry (Michiels et al., 2016). *M. synoviae* is transmitted vertically through eggs and then it can infect horizontally through the respiratory tract. It has a 100% morbidity rate for all hens with in the same house (Michiels et al., 2016; Ferguson-Noel et al., 2020). After infection, the layer hens remain infected with *M. synoviae* persistently becoming lifelong carriers of the pathogen. This persistence creates the logic behind continuous treatment with the antibiotics (Arafat et al., 2018). Such conditions mandate an alternative medication option like the essential (Laptev et al., 2019). Frequent, occurrence of infection can be seen as an asymptomatic infection of upper respiratory tract. This infection can later lead to the lesions in respiratory tract that are in turn become sites of aggravation for other pathogens of respiratory tract. After 2000, the issues in the egg production of layer hens have started falling under lens of consideration more than ever worldwide especially the ones caused by strains of *M. synoviae*. The upper portion of the eggshell is translucent, thinner and more fragile to break, although sometimes the poor eggshell is defined genetically (Nikolova[1] and Kocevski, 2018).

Activo Ingredients

An important role is played by the plant based products and spices in form of standalone compounds or as a mixture of several substances. The role of these products is improving the performance of birds, quality of food products and the health condition of laying hens (Florou-Paneri et al., 2005; Puvača et al., 2013; Puvača et al., 2019).

Cinnamaldehyde

Another perspective adopted for bypassing the bacterial resistance threat is utilization of medicinal extracts sourced from plants or essential oil to be use alone or as a mixture. This also provides protection to the birds against the probable side effects of the antibiotic drugs. Furthermore, it also saves the utilizing entities from the adverse effect of the antibiotic residual tests and rejection that may lead to loss of meat and other industry useful organs of animals (Devi et al., 2010). For a long time, the antibiotic effect of essential oils and their ingredients have been identified. A complex mixture of various volatile substances makes up the essential oils. These essential oils are obtained through distillation of secondary metabolic reaction products that have been conducted upon aromatic and medicinal plants parts of different plants (Devi et al., 2010). Similarly, herbs and spices have found application as antioxidants and flavoring agents for a long time, as well as for their antibiotic activity (Nabavi et al., 2015). These essential oils obtained from aromatic plants l have been mainly utilized as preservative and flavoring agents. Such agents include cinnamon (*Cinnamomum zeylanycum Boiss*), a notable key player belonging to *Lauraceae* family usually found in South Asian regions. It has been observed in previous studies that the oils of cinnamon, thyme, and rosemary are the most efficient agents against foodborne bacteria. It aligns well with reports in other studies that the phenolic group compounds are the most effective micro-organism control agents. (Rezaei et al., 2010).

During the analysis of cinnamon oil main biologically active substance that was most of the times observed was cinnamaldehyde (Prabuseenivasan et al., 2006). One of the previous studies on this matter (Denyer, 1995) concluded that in case of cinnamaldehyde the antimicrobial mechanism of action occurs in various steps consisting of : pool metabolites loss, bloackage of active transport, and DNA, RNA, proteins, lipids, and polysaccharides synthesis disruption, proton motive force stoppage, respiratory chain, electron transfer, and substrate oxidation. Hydrophobic nature is another mechanism of action for volatile oils that assist them in disrupting the cell membrane lipid bilayer. Cell membrane disturbance leads to an increase permeability for protons (Trumpower and Gennis, 1994). The protons exiting from bacterial cells along with the leakage of essential molecules causes death of the bacterial cell (Prabuseenivasan et al., 2006). The level of populace decline was lowest when observed for *M. gallisepticum* and *O. rhinotracheale* among all the bacteria being considered for the test. Although the level of inhibition was not enough to be interpreted as inhibition of phenotypic nature. The dispute in results of phenotypic and molecular test may be caused by failure in RNA. The translation failure can happen due to various reasons resulting in production of a nonsense codon in the mRNA through error in transcription (Brégeon et al., 2003), ribosome being unable to bind with mRNA, frameshifting of ribosome on mRNA resulting in premature elimination of translational activity or endonucleolytic cleavage of mRNA (Brégeon et al., 2001).

Carvacrol

Carvacrol (C10H14O) is a liquid belonging to the phenol group. It is also known as 2-methyl-5- (1-methyl ethyl) phenol. It is mainly found in oregano oil (*O. vulgare*), thyme (*T. vulgris*), pepperwort (*Lepidium flavum*), wild bergamot (*Citrus aurantium var. bergamia Loisel*) and other plants. Commercially synthetic carvacrol is produced through chemical reactions (Yadav and Kamble, 2009). The International Union of Pure and Applied Chemistry has termed carvacrol as chemically to be 5-isopropyl-2-methylphenol. This substance has various biologically active aspects, especially its ability to combat bacteria and fungi (Chavan and Tupe, 2014), viruses (Sánchez et al., 2015), oxidative stress, regulating of immune response (Khazdair et al., 2018), controlling inflammation (Fitsiou et al., 2016) and preventing carcinogenic development (Özkan and Erdoğan, 2011). Having suitable flavor and antibiotic properties are the main reasons why carvacrol has found its major application as a feed additive and a natural preserving agent (Salehi et al., 2018). Carvacrol is one of many ingredients mainly found in several medicinal plants, such as black cumin (*N. sativa*), oregano (*O. compactum*), *M. didyma, O. dictamnus, O. microphyllum, O. onites, O. scabrum, O. vulgare*, *thyme* (*T. glandulosus*), *savoury* (*S. hortensis*) (Figiel et al., 2010; Attia and Al-Harthi, 2015). Additionally, carvacrol has also been synthesized by chemically and biotechnologically with the help of metabolic-engineered microorganisms (More et al., 2007). Substances like carvacrol, 6-gingerol and thymol have various effective antioxidant characteristics hinting towards the fact that they might be useful in future for finding a 'natural' alternative besides the synthetically produced antioxidant food additives (Aeschbach et al., 1994).

Conclusion

With the boom of poultry industry came the rise of issue that affected the industry in terms of growth and economy. A prominent problem out of these issue was the spread of disease among poultry flocks. Various of types of diseases both viral and bacterial affected poultry farms adversely and led to various issues regarding production of the flocks. For instance, the diseases caused by Mycoplasma spp. had a significant impact on economics of poultry and production potential of birds. This disease was then effective controlled and treated with Tilmicosin. The most effective treatment designated against this disease was use of the antibiotics which proved to useful for some time until the rise of antibiotic resistance. Emergence of antibiotic was immediately met with ban on antibiotics in an effort to curb it. This resulted as a gap for disease control efforts that threatened to falter the progress of poultry framing industry. This threat soon forced the researchers to focus on alternative of boosting bird health and immune status to counter diseases. One such alternative was use of Activo as a feed supplement to that contained cinnamaldehyde and carvacrol as active ingredients. Both of these substances have been proved to be effective for their antibiotic, antioxidant, gut health boosting and immunity enhancing effects. Hence, it is expected that with further improvement and consistency in composition these compounds can be effectively used to replace antibiotics like Tilmicosin in near future.

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Chapter 05

Effectiveness Comparison of Amoxicillin versus Activo against diarrhoea in Poultry

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ABSTRACT

Diarrhoea and loose dropping is one the major issues of poultry leading to lowered production in terms of eggs and weight gain. Diarrhoea not only reduces FCR but also nutrient absorption leading to reduced immunity. Reduction in immunity consequently leads to the rise of diseases that can result in large-scale mortalities among poultry flocks which increases the losses of poultry farmers by manifold. Such losses were inevitable previously as even small changes in feed, feeding regime or water contents led to defecation of loose droppings and production problems in poultry. Digestive issues were a nightmare for farmers as little to no effective treatments were available and losses were huge. Later on, some farmers tried using different techniques and substances to reduce the cases of diarrhoea among birds hence limiting the losses. Along with the application of slow feed change regimes and provision of clean drinking water, some farmers also tried giving drugs like Amoxicillin to limit the population of gut microflora that caused diarrhoea. This tactic proved effective, as amoxicillin killed many of the harmful bacteria in the gut, restoring it to its normal function and preventing losses of weight and production. This triumph over the diarrhoea problem was however a short-lived one. Soon, the bacteria started developing resistance against the antibiotic and it became useless. After that, the diarrhoea started persisting even after using amoxicillin. Additionally, the abundant use of antibiotics stressed the liver and reduced its performance. All these signs denoted the need for a new solution that could resolve the diarrhoea issue all while keeping the birds safe from antibiotic resistance, antibiotic residues and loss of liver performance. Such a solution was to be present in phytochemicals like carvacrol and cinnamaldehyde. These compounds were collectively added to several products for commercial use such as in Activo.

KEYWORDS
Poultry, Diarrhoea, Antibiotic resistance, Amoxicillin, Activo

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INTRODUCTION

Before the antibiotics restriction came into effect intestinal health was maintained cost-effectively and industrial-scale chicken farms heavily relied on the administration of antibiotics. The antibiotics helped the chickens to be healthy, produce better and grow rapidly. However, the antibiotic ban in the European Union in 2006 led to severe consequences for the agriculture and poultry farming industry. These challenges included increased risk of disease, reduced production performance, elevated administration of drugs for therapeutic purposes, and rise in cost of farming (Shao et al., 2021). Antibiotics are banned from use in animal feed additives too. This practise practice also encouraged researchers to find alternative feed additives. Antibiotic alternatives like probiotics, organic acids, prebiotics, phytochemicals, and other agents

are now being used for maintaining the health of poultry flock (Hernandez-Patlan et al., 2019).

Amoxicillin

Clostridium perfringens causes necrotic enteritis in chickens which often leads to bloody diarrhea. Coccidiosis is often found in close association with necrotic enteritis in poultry hence demanding an investigation of coccidiosis in case of an outbreak. As a commensal *C. perfringens* is found commonly in the alimentary tract of chicken under normal conditions. However, sometimes these bacteria may extend their population beyond normal amounts, leading to the production of clinical disease, upon exposure to substances of exceedingly high nutritional value in the jejunum and ileum. This overgrowth leads to variation in the environment of the intestine. In case of necrotic enteritis suspicion, the drug of choice for preliminary treatment till confirmation is amoxicillin to be administered for three to five days at a dose rate of 20 mg/kg each day. Dosing can be managed according to the recovery speed and requirement of withdrawal time needed before chicken enters the food chain. This strategy improves efficacy against *C. perfringens*. Although amoxicillin has a short withholding period as it is water-soluble, it can be applied immediately (Kandeel, 2015). Previously, antibacterial agents like amoxicillin and tylosin were effective against the likes of *C. perfringens* strains, in vitro. Although this issue came to the discussion table recently, the isolates from broiler chickens already started showing antibiotic resistance of acquired nature against lincomycin, earlier (Martel et al., 2004). The development of this mechanism meant that fewer antibiotics like amoxicillin were able to effectively control the problem of diarrhoea in poultry flocks before the antibiotic bans came into effect.

Plant-based Solution

Researchers have claimed that plant extracts and their parts can be used to enhance nutritional value and absorption for animal feed. It also inhibits the growth of certain bacteria that are undesirable for gut health. This in turn beneficial for balancing the population of beneficial intestinal bacteria and for keeping the gut healthy (McGaw, 2013). An important source of compounds with antioxidant properties is the plant-based essential oils. They can control and eradicate free radical species, stop the action of oxidases, improve the activity of enzymes that catalyze antioxidant reactions, and produce effects similar to antioxidant substances (Miguel, 2010). Several of the naturally occurring compounds in plants show antibiotic characteristics. These compounds also act as appetite stimulants and enhance the activity of enzymes (Basmacioğlu Malayoğlu et al., 2010; Hou et al., 2022). Being an agent of antibiotic nature, these compounds can also serve as alternatives to antibiotics. Recently, these substances have started receiving attention for use and development. It is important to note that despite having similar general characteristics the essential oils obtained from different plants are comprised of different chemical substances with varying compositions. This in turn leads to the production of differing antibacterial activity (Lee et al., 2004).

Activo the Commercial Product

Activo is a liquid supplement solution, intended for veterinary use by EW Nutrition. Activo is used for the preservation and improvement of gut health in animals. Its ingredients balance nutrients, and intestinal flora and mitigate challenges. It is a part of the Antibiotic Reduction program - EW Nutrition's Gut Health Management. This program is designed to support animal agriculture operations with innovative products and services while reducing antibiotic misuse at the same time. It also includes additional benefits like customer support and consultancy by EW Nutrition experts. Activo is made up of Carvacrol and Cinnamaldehyde. These are beneficial phyto-molecules that improve the production of poultry flocks by resolving issues of the digestive tract like diarrhoea without mandating the administration of an antibiotic (Heinzl and Caballero, 2020).

Ingredients of Activo

Carvacrol and its Impact

In the past, researchers like (Ultee et al., 1998) identified and reported the antibacterial activities of plant extracts like carvacrol on *Bacillus cereus, a pathogenic* agent. Being a hydrophobic compound agent with the ability to impact biological membranes, carvacrol is effective against *B. cereus*. Carvacrol is metabolized and excreted from the body pretty quickly, hence reducing its withdrawal period. The main pathway of metabolism for carvacrol is through the reaction of phenolic groups such as its esterification with sulfuric acid (H_2SO_4) and glucuronic acid ($C_6H_{10}O_7$). However, a substitute metabolism that happens much less frequently is the conversion from primary alcohols to the end methyl groups. It was during an experimental activity of (Austgulen et al., 1987) on male albino rats when it was discovered that rats defecated a large portion of administered carvacrol (1 mmol/kg) through the urinary route. The main sulphate and glucuronide conjugates of carvacrol give the products of 2-phenylpropanol and benzyl alcohol derivatives mainly due to oxidation reactions that occur extensively with the methyl groups. Additionally, they also release respective carboxylic acids. A minute amount of another metabolite has also been reported that forms as an outcome of ring hydroxylation. Furthermore, the carvacrol residual or its derivatives are found in minute quantities in urine for one day only. These discoveries indicate the high-speed metabolism of carvacrol and its expulsion from the body within the first 24 hours.

Improving Nutrient Bioavailability and Growth/Productive Performance

Several trials among poultry and animals have been conducted to determine the extent of the functionality provided by carvacrol and the dependency of the supplement on it as a feed additive. It was revealed through research by (Hashemipour et al., 2013) that augmentation of feed with compounds like a mixture of carvacrol and thymol compounds

with increasing concentrations of 60, 100, and 200 mg per kg of animal feed enhanced growth rate, activity of digestion-related enzymes and antioxidant enzymes. Additionally, this mixture also stops the peroxidation of lipids in the chicks of broiler poultry birds. The extent of immunoglobulin (IgG) presence in serum was improved due to supplementation of the diet with several types of plant extracts including compounds like oregano and thyme in contrast to the groups of mice and pigs with a controlled diet (Namkung et al., 2004). Administration of diet to one-day-old broiler chicks that were supplemented with carvacrol at 5.0 ppm concentrations for seven consecutive days resulted in enhanced body weight gain rate and reduced shedding of oocytes. Furthermore, there were also markedly reduced gut lesions seen in the carcasses and the expression of pro-inflammatory cytokine gene during the invasion of coccidiosis also lowered as the birds faced the challenge of *Eimeria acervulina* infection (Namkung et al., 2004).

Antiviral Activity

Parts of plants with medicinal properties and their extracts or derivative products have been explored for a long time to determine the existence of antiviral properties in them. Even the cold-pressed and extracted essential oils of various well-known herbs used for culinary purposes (Jassim and Naji, 2003; Soković et al., 2010). Carvacrol is the main ingredient from the perspective of antiviral components that effectively work against human rotavirus (RV). Similarly, the extracts and oil from Mexican oregano (*Lippia graveolens*) containing carvacrol components are capable of reducing and inhibiting the diseases caused by viral agents in animals and humans. A specific example of the antiviral action shown by the phenolic compounds of oregano is seen in its action against human respiratory syncytial virus (HRSV) and acyclovir-resistant herpes simplex virus type 1 (ACVRHHV-1). In the same context, the antiviral properties of carvacrol have been documented after observing its effects on RV (Bernstein, 2009; Pilau et al., 2011). Carvacrol is found in essential oils extracted from Oregano. These compounds have been strongly advocated as agents with natural antiviral properties making them effectively active against several diseases of a viral nature such as the widespread pandemic caused by the infection of the H_1N_1 virus (Vimalanathan and Hudson, 2012). On the other hand, (Sökmen et al., 2004) observed that the addition of oils or oregano extracts was unable to affect the anti-influenza virus. It has been reported by (Gilling et al., 2014) that the presence of carvacrol as a food ingredient in nature and being a diet component is extremely useful in reducing human infection of norovirus by lowering its concentration within the first hour of invasion by attacking directly on the viral capsid.

Antimicrobial Activity

Essential oils show antibacterial effects due to the presence of compounds with phenolic. These compounds include curcumin, carvacrol, cinnamaldehyde, thymol, and eugenol. These compounds are found in plant-based oils extracted from oregano, thyme, clove, turmeric and cinnamon, respectively (Tsao and Zhou, 2000; Lambert et al., 2001; Veldhuizen et al., 2006; Alagawany et al., 2015). Several researches have shown that the bacterial growth inhibition impact by phenolic compounds occurs due to the reactions that happen between these compounds and the molecular components of the membrane of target bacteria. This interaction is often connected to the hydrophobic nature of such compounds (Sikkema et al., 1995; Weber and de Bont, 1996; Adam et al., 1998; Ben Arfa et al., 2006; Nostro and Papalia, 2012). 1% carvacrol supplementation has shown a reduction in numbers of campylobacter. Similar effects can be seen in the case of a thymol and carvacrol combination at 0.5% (Arsi et al., 2014). The antibiotic effect of carvacrol has been observed against several bacterial species microbes such as *Fusarium*, *Salmonella*, *Streptococci*, *Listeria*, *Pseudomonas*, *Aspergillus*, and *Bacillus* (Friedman et al., 2002; Soković et al., 2002; Nostro et al., 2004; Can Baser, 2008). It was observed that carvacrol has a supplementary effect as an antimicrobial component on bacteria of harmful nature including *Escherichia coli* and *Salmonella Enteritidis*. The growth of these bacteria in the intestines of chickens affects the health of the digestive tract negatively. So, this effect of inhibiting the bacterial growth against pathogenic agents happens due to carvacrol vapours (Burt et al., 2005; Burt et al., 2007). It has been seen that eugenol and carvacrol reduce ($P \leq 0.05$) growth of *Salmonella Enteritidis* and *C. jejuni* in the caecal portion of chicken's intestine to <1.0 $\log_{10}$ cfu/ml at 50 and 75 mM and 20 and 30 mM, respectively (Johny et al., 2010).

Improving Immunity

Enhancing the immunity of poultry birds is a major goal for preventing infectious diseases. Lowered immunity can occur due to several reasons including attack of immunosuppressive disease, misuse of antimicrobial drugs or vaccination failure. For enhancing the immunity of poultry birds and reducing their susceptibility to the attack of infectious diseases, immune system stimulating agents could be used. It was reported by (Acamovic, 2005) and (de Cássia da Silveira e Sá et al., 2013) that plants enriched with flavonoid compounds including thyme and carvacrol could enhance the immunity function of birds by acting as antioxidant agents hence complementing the activity of vitamin C. It was also expected by (Botsoglou et al., 2002) that an enhancement in the immunity of chicks will be observed because of the surefire antioxidative, antimicrobial and antiviral actions of carvacrol. These actions have been reported to occur by various researchers previously. It was pointed out by (Namkung et al., 2004) that birds fed with diets composed of herbal phytonutrients like carvacrol, thymol, cinnamaldehyde, capsicum and oleoresin notably increased the degree of immune system responsiveness in poultry birds especially chicks hence, lowering the occurrence of infectious diseases in poultry.

Cinnamaldehyde and its Effects

Cinnamaldehyde is an essential oil product derived from plant-based sources. It is also one of the key ingredients that actively bestow cinnamon with its various virtues. Cinnamaldehyde has various beneficial characteristics including its

antagonising impact on harmful fungi and bacteria. Researchers have reported that cinnamaldehyde has a notable antibiotic characteristic that is effective against both Gram-negative and Gram-positive bacteria (Moleyar and Narasimham, 1992; Chang et al., 2001; Goñi et al., 2009). It was reported by (Tiihonen et al., 2010) that the addition of carvacrol and cinnamaldehyde to the feed of broiler chicken feed can enhance the growth rate of beneficial flora in the alimentary tract of the chicks. Researchers have also reported an improvement in the growth rate of animals, efficiency of feed conversion, maintenance and improvement of health, along with the prevention of feed value degradation (Chaves et al., 2008). Monoglyceride cinnamaldehyde and lauric acid are useful in enhancing production capacity, gut health and antimicrobial defence characteristics of broiler chickens in poultry. The interactions between the ingredients of compounds through several pathways and mixtures of various plants may be more impactful than the products from a single plant (McGaw, 2013).

It was observed by (Shirzadegan, 2014) that supplementation of cinnamon plant extract in the feed of broiler chicken's diet with varying amounts for each experiment group led to a notable improvement in the body weight by the end of the experiment. The most significant observation was that the 0.5% concentrated solution level of cinnamon extract provided the optimum results. It was also reported by (Lee et al., 2004) that the addition of cinnamaldehyde to the feed of broiler chickens resulted in enhancement of chicken growth performance. The addition of monoglyceride lauric acid and cinnamaldehyde in the diet has been discovered to be linked with a drop in the progression of virulent bacteria and hinder the reproductive process of parasites that inhabit intestines (Fortuoso et al., 2019). It was also reported, (Castillo et al., 2006) that cinnamaldehyde extract shows antioxidant qualities at the intracellular level that help it serve as a protective agent for intestinal villi from the corrosive action free radical species of toxic nature hence improving the height of intestinal villus. It was discovered by (Tiihonen et al., 2010) that the addition of carvacrol and cinnamaldehyde to the main regular feed of broilers resulted in an improvement of the amount in terms of butyric acid production in the intestines of the birds. Butyric acid is the agent responsible for speeding up the growth and multiplication of epithelial cells lining the small intestinal additionally, it also assists in repairing intestinal mucosa.

Conclusion

The rise of the problem of diarrhoea among poultry has been very concerning for farmers since the beginning. Diarrhoea is considered a serious issue because it leads to several other kinds of problems. Diarrhoea means there is low absorption of nutritional components in the intestine of the bird, which will mean lower FCR. Reduced FCR can ultimately lead to a decline in production both in terms of weight gain and egg laying. Not only production will be reduced but immunity will be also lowered. Being sensitive birds the decline in immunity will mean that the flocks will begin to succumb to different kinds of diseases resulting in huge losses for the farmers. In past, such problems were countered by farmers through the use of antibiotics like amoxicillin. Amoxicillin served as a competent agent that attacked and killed any kind of harmful bacteria found in the intestines of poultry birds. However, this solution was soon abolished as the antibiotics were banned to reduce antibiotic residues and hence prevent the spread of antibiotic resistance. Hence, soon the poultry industry felt the need for a new solution to battle this problematic issue. Such a solution was found in the form of phytochemical compounds extracted from plant-based sources, like carvacrol and cinnamaldehyde found in commercial preparations such as Activo. Activo was hailed as a major game changer for poultry as it effectively eliminated the need for the use of antibiotics and produced much better results. Activo not only controlled bacteria but also produced other beneficial effects such as increased immunity and improved nutrient absorption.

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Chapter 06

Precision Tactics against *Salmonella*: Leveraging Nanoparticle Innovations to Confront Antimicrobial Resistance for Enhanced Treatment Strategies

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ABSTRACT

Salmonella is rod-shaped and belongs to the family Enterobacteriaceae. It is a microscopic threat to the life of humans and animals that has caused severe illness for centuries. These organisms are motile and facultative aerobic with 2500 serotypes identified. The primary mode of transmission is through food and water contaminated by Fecal. They are present in the feces of infected persons only in humans but the serotype causes serious infection in animals like poultry birds. Meat and egg of poultry can also be a vehicle of spread of it. Due to the huge amount of meat and eggs consumed by humans, there is a great chance of salmonellosis. The mechanism of infection is completely different in typhoidal and non-typhoidal serotypes. Other sites of infection and different symptoms appear. But they both enter the body through the gut. When *Salmonella* enters the mouth, it passes through the strong acid of the stomach, detergent activity of bile, and gut microflora. They sense the stress, survive through extreme conditions, and form virulence factors. Antibiotic resistance is a big threat to the world. Overuse of antibiotics makes *Salmonella* resistant to these antibiotics. Nanoparticles are being investigated as a treatment against multi-drug-resistant *Salmonella*. They are tiny particles that can kill bacteria very accurately. They can overcome antimicrobial resistance. Vaccines are also present to treat salmonellosis but no one is present orally. About 90% of infections occur through the mucosal surface but vaccines cannot pass harsh conditions of the gut nanoparticle vaccines can do it and kill *Salmonella* in the intestine.

KEYWORDS
Bacteria, *Salmonella*, Resistance, Alternatives, Nanoparticles

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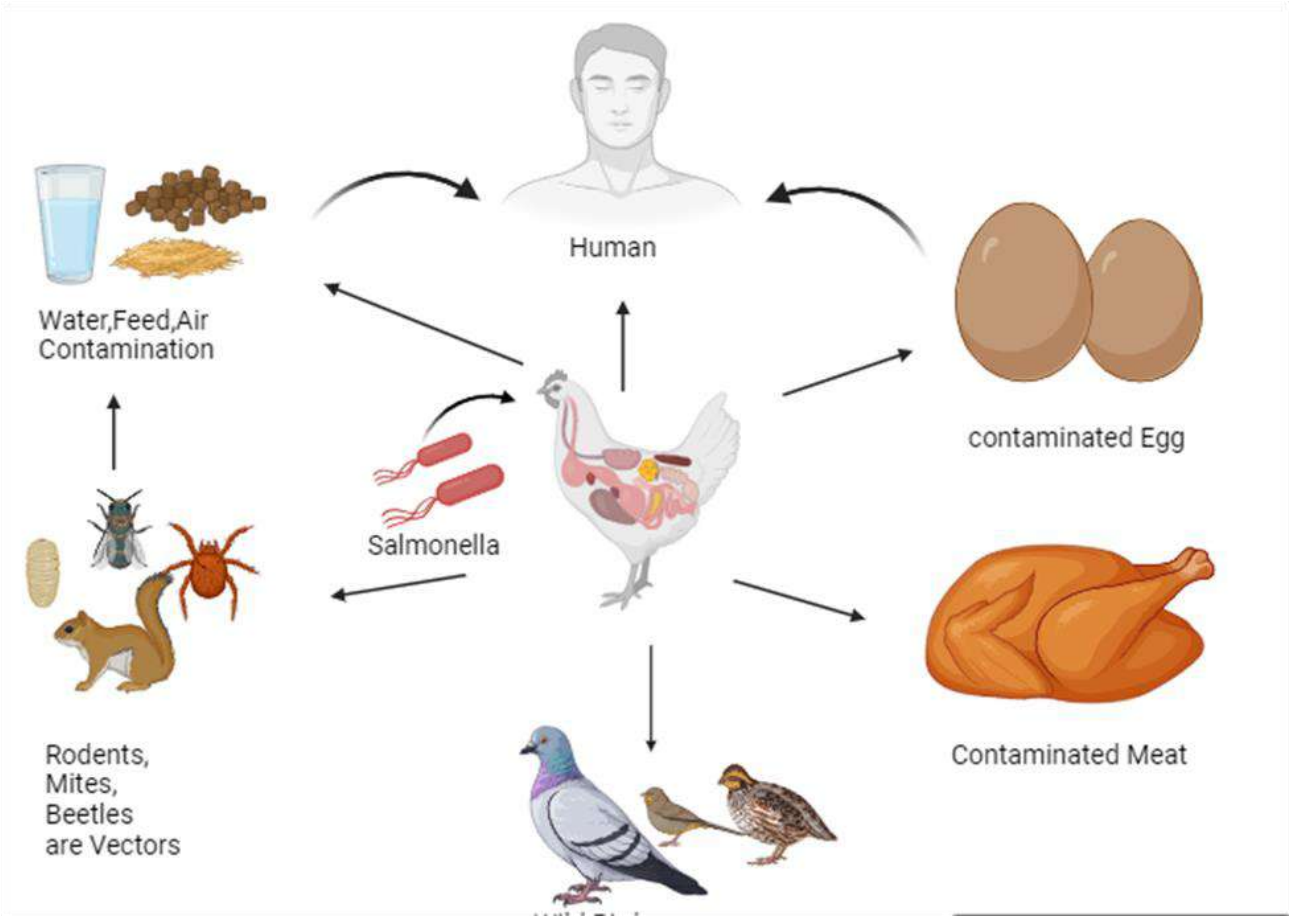
INTRODUCTION

Salmonella is rod-shaped (Pradhan et al., 2023; Sajjo et al., 2019). *Salmonella* belongs to Family Enterobacteriaceae (Makangara, 2023) which is non-spore-forming, motile, gram-negative bacteria (Castanheira and Portillo, 2023), with a cell diameter that varies from 0.7 to 1.5 µm (Hanning et al., 2023). It is a microscopic danger that has tortured the world for centuries.

Salmonella was named after an American veterinary pathologist Daniel Elmer Salmon (Bhat et al., 2022). Karl Eberth observed the pathogen in Peyer's patches and spleen of typhoid patients in 1880 (Al-Khafaji et al., 2021). Theobald Smith discovered *Salmonella enteric* when he was working as a research laboratory assistant in the veterinary division of the United States Department of Agriculture in 1845 (Wilson, 2014) under the supervision of Daniel Elmer Salmon. The name *Salmonella* wasn't used until 1900. In 1900 Joseph Leon Lignieres suggested that this pathogen was discovered by the

Salmon's group so in honor of his name they named it *Salmonella*. In the 1930s an Australian scientist Nancy Atkinson established a *Salmonella* typing laboratory in which he described multiple strains of *Salmonella* and discovered *Salmonella Adelaide* in 1943(McEwin, 2018). It has about 2500 serotypes (Eng et al., 2015). There is an antigen present on the cell surface of bacteria that helps in the differentiation of different serotypes. In *Salmonella* Somatic O antigen and Flageller H antigen based on *Salmonella* can be classified (Guyassa and Dima, 2022). By Knowing the serotypes, it is easy to monitor the outbreaks.

Food and water that have been tainted can spread them. These pathogens are present in the feces and when some person makes contact with it, transmission occurs (Levantesi et al., 2012). Many serotypes of *Salmonella* cause severe infection in other animals like poultry birds. *Salmonella gallinarum* and *Salmonella pullorum* are the most virulent serotypes of *Salmonella* that cause high loss to poultry (Fowl Typhoid). They can spread from a contaminated environment to poultry birds and also from parent flock to offspring (Tariq et al., 2022). These pathogens spread from poultry birds to humans and other animals. Poultry meat and eggs have been considered as a vehicle of *Salmonella* spread. Due to huge amounts of meat and egg consumption by the human population, it is a major issue of salmonellosis. *Salmonella* is very much controlled in Europe by minimizing it in meat and egg but this is not done in third-world countries so Salmonellosis is at its peak (Antunes et al., 2016).



There are two types of *Salmonella*: Typhoidal and Non Typhoidal. *S. typhii* and *S. paratyphii* are two more subtypes of Typhoidal *Salmonella*. This serotype only infects humans; it does not infect animals (Ngolo et al. 2023).
Non-typhoidal serotypes generally cause GIT disease (Fierer, 2022) and infect both animals and humans.

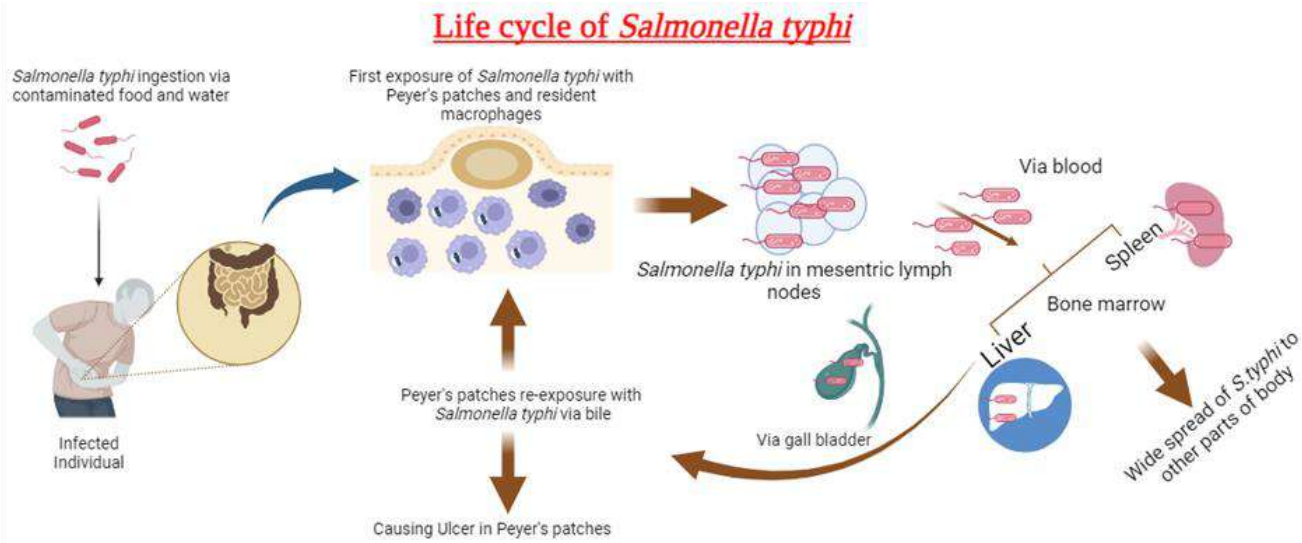
Among the several serotypes of *Salmonella*, Typhoidal *Salmonella* is a serious threat to human health (Chatterjee et al., 2023). They are the source of both typhoid and paratyphoid fever in addition to foodborne illnesses (Khuhro et al., 2019).

Typhoid fever is the most severe embodiment of *Salmonella* infection, a life-threatening disease caused by *Salmonella* enterica typhi that enters the bloodstream, spreads in the whole body, invades vital organs, secretes endotoxin, and causes severe infection. It can result in hypovolemic shock in which severe loss of blood volume occurs and septic shock in which systemic inflammation occurs in response to infection. These are very complicated conditions. These behaviors of this microorganism make it very harmful and capable of causing high mortality in affected persons.

In the systemic form of typhoid fever, *Salmonella* enters very smoothly into the host body. They cross the lymphatic system of the intestine and enter the bloodstream there they start typhoidal form of diseases. From blood, they move to vital organs like the liver, spleen, and kidney. They form secondary foci in these vital organs and cause a septic form of typhoid fever that has severe consequences. The pathogenicity of typhoidal *Salmonella* is distinct by the action of

endotoxin. These toxins cause great damage to the vascular and nervous systems. They increased the vessel's permeability and decreased the tone. Clinical symptoms such as vomiting, diarrhea, and fever result from this. In extreme cases, vomiting and diarrhea produce a significant loss of fluids and electrolytes, and the body's water-salt balance is upset, resulting in hypovolemic shock, a potentially fatal illness. Reduced blood volume, circulation, and arterial pressure are its hallmarks. Septic shock also occurs which increases the severity of infection. Oliguria and azotemia may develop as a result of renal involvement, originating from hypoxia and toxemia.

Non-typhoidal serotypes of *Salmonella* generally cause food poisoning (Chang et al., 2022). When a person ingests food that has a high concentration then bacterial infection occurs. In infants, infection occurs even if they inhale the bacteria in small quantities. They enter through the digestive tract and infect the inside of the GIT. Some microorganisms are killed in the stomach while those that survive enter the intestine where they start multiplication in tissue. Gastric acidity causes the destruction of many bacteria but *Salmonella* has some resistance toward the acidity of the stomach (Alenazy, 2022). After the incubation period is completed the host cell is poisoned by endotoxin produced by *Salmonella* and in response to endotoxin enteritis and gastrointestinal disturbance occur.



In developed countries, Non-typhoidal *Salmonella* causes only GIT problems but in developing countries, they cause severe issues in bloodstream infection. They are most commonly isolated bacteria in patients who have fever in those countries. In Africa, the case fatality rate of bloodstream infection of *Salmonella* is 19% (Uche et al., 2017). Most invasive nontyphoidal infections are caused by *S. typhimurium* and *S. enteritidis* (Uche et al., 2017). The increased prevalence of these infections in Africa is due to a large portion of the African population with some degree of immunosuppression due to the burden of HIV, malaria, and malnutrition. Moreover, there is resistance developed against some antibiotics due to which treatment becomes tougher.

Serotype	Main Host	Disease	Host Specificity	Reference
S. Typhi	Human	Typhoid Fever	Host Specific	Jahan et al., 2022
S. Paratyphi	Human	Enteric Fever	Host Specific	Stepien et al., 2024
S. Abortusovis	Sheep	Abortion Storm in Sheep	Host Specific	Barrow, 2024
S. gallinarum	Poultry	Fowl Typhoid	Host Specific	Lee et al., 2020
S. pullorum	Poultry	Fowl Typhoid	Host Adapted	
S. abortus equi	Horse	Abortion in Horse and Donkey	Host-specific	Liu et al., 2022
S. choleraesuis	Swine	Paratyphoid	Host restricted	Papic et al., 2021
S. Dublin	Cattle	Diarrhea, Respiratory infection, Abortion	Host restricted	Velasquez et al., 2024
S. Typhimurium	Human	Acute Gastroenteritis		Sivasankar et al., 2024
S. enteritidis	Poultry	Gastroenteritis		

Mechanism of Infection

The mechanism of infection of *Salmonella* is different in typhoidal and non-typhoidal serotypes. They have different target sites and show different symptoms. Both groups enter through an intestinal wall but when they pass this barrier, they do different strategies to cause infection.

When *Salmonella* enter GIT they face stomach acid, the detergent-like activity of bile, decreasing oxygen supply encounter with normal gut flora, and also with antimicrobial peptides present on the intestinal cell surface. All these stress *Salmonella* can sense and react against them and then it forms virulence factors.

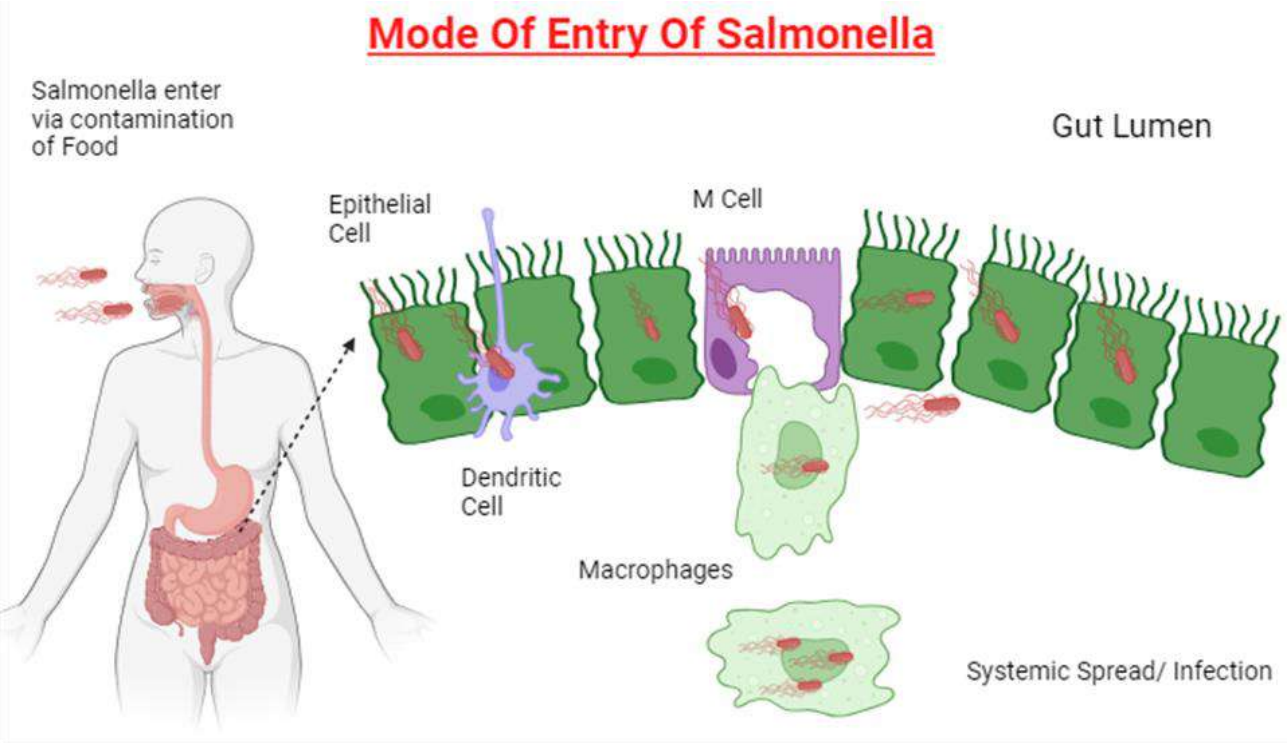
When *Salmonella* is ingested, it travels to the intestine when it reaches the intestine it travels toward the host cell through intestinal peristalsis and movement by flagella then penetrates the mucus barrier. Then it adheres to the host cell

by using bacterial adhesion and a type 3 secretion system (Li et al., 2023). Then they invade the host cell. Inside the host cell, they start replication (Zhou et al., 2023).

After the replication is complete, they enter into blood and start spreading themselves to vital organs (Naushad et al., 2023). Some of them go back to intestinal cells reseed the population and repeat the same procedure.

Non-typhoidal serotype enters M cells on the intestinal wall by bacterial-mediated endocytosis (Chijioke et al., 2023). They can also disrupt the tight junctions between intestinal cells, stop the flow of water ions and immune cells in and out of the intestine and this can induce diarrhea.

Salmonella is also able to invade the intestinal barrier through phagocytosis and by CD18-positive immune cells. It is also able to invade macrophages via macropinocytosis (Dia et al., 2024). *Salmonella* uses the Type 3 secretion system(T3SS) to cause infection. It is a crucial virulence factor that injects bacterial protein directly into host cells allowing *Salmonella* to manipulate the host cell processes (Marshall and Finlay, 2014). These factors stimulate the formation of membrane ruffles which allows the uptake by nonphagocytic cells. *Salmonella* remains within a membrane-bound compartment called *Salmonella* containing vacuoles (SCV), when SCV is acidified it leads to the expression of T3SS-2 (Han et al., 2024). The secretion of the T3SS-2 effector is required for *Salmonella* to survive in the host cell and cause disease. T3SS is also involved in colonization in the intestine, inflammation of cells, and diarrhea (Gul et al., 2024).



Treatment of *Salmonella* with Antibacterial Resistance and Use of Nanoparticle

Salmonella is a dangerous microorganism that can cause severe disease in animals as well as humans. There is a high mortality rate. This genus is very adaptable according to the environment and has much resistance to many factors. These characteristics make it a more dangerous and harmful microorganism. So, it is very important to figure out a specific treatment for it. As they are bacteria, they can be killed by using an antibiotic. However, it becomes resistant to many antibiotics. Antibiotic resistance is a natural phenomenon that has existed for millions of years (Depta and Niedźwiedzka, 2023). But from the 1940s the more use of antibiotics by humans and animals, the rate of antibiotic resistance increased at high speed, which is not good for the ecosystem. Antibiotic resistance is a major health issue and it leads to more mortality, morbidity, and cost of treatment. The most concerning antibiotic resistance occurs in the family Enterobacteriaceae and other zoonotic species that cause food poisoning. Antibiotic resistance is causing treatment failure which can lead to more deaths. Antibiotic resistance is important for two reasons. It can lead to therapy failure in animals and humans. It can lead to the development of antibiotic-resistant zoonotic pathogens which can spread to humans and can be a public threat (Cella et al., 2023).

Resistance characteristics are naturally present in bacteria due to genetics and phenotypic variations (Wiradiputra et al., 2023). A well-planned increase in antibiotic-resistant microorganisms occurs when a selective pressure is present. This pressure comes from the use of antibiotics which promote the survival and multiplication of resistant bacteria. Without pressure the chance of resistance is low. The huge concern is the increase in the pool of antibiotic-resistant genes and the spread of it carried on genetic elements like a plasmid (Ghaly and Gillings, 2022). The main selective pressures on *Salmonella* arise from the overuse of antibiotics for prophylaxis and treatment. The use of antibiotics without diagnosis puts pressure and it leads to resistance.

The history of antibiotic resistance is not simple. It has gone through various stages and trends in the last few years.

The treatment of *Salmonella* by antibiotic started in 1940 while the first report of resistance in *Salmonella* was filed in the 1950s, this resistance is against streptomycin. This report shows the cases with monoresistant strain. There were many examples given by researchers of resistance to isolated *Salmonella* that had resistance to specific antibiotics at that time (Naushad et al, 2023). In the mid-1960s a specific phage type PT29 of *S. typhimurium* became a highlight which was causing infection in both bovine and humans in England. It isolated and became the first pentaresistant phage type which has resistance to streptomycin, sulphonamides, tetracycline, ampicillin, neomycin, kanamycin, and furazolidone.

In the late 1960s, Dr Datta described transmissible drug resistance in *S. Typhimurium* (Viji, 2018). This finding pushes more genetic studies of *Salmonella*.

In the 1970s research started on the localization of resistance genes on plasmid and transposons. PT505 in the Netherlands was found to carry resistance determinants on a plasmid and it allows the transfer. It leads to resistance (Helmuth, 2000). In 1973 the appearance of antimicrobial resistance occurred against chloramphenicol, ampicillin, and co-trimoxazole (Chowdhury et al., 2024). Resistance against ampicillin occurred by Chromosomal and plasmid-borne *blaPSE* and *blaTEM* coding for β-lactamase enzymes which inactive the work of ampicillin (Chowdhury et al., 2024). Between 1975 and 1980s S. Dublin isolates in Germany which have multiple resistance genes. These strains initially carry resistance genes on a plasmid and then integrate them into chromosomes and resistance becomes inherited (Haung and Naushad, 2024).

In the 1980s and 1990s, the spread of multiple resistant *S. Typhimurium* clones occurred. These clones played an important role in increasing the prevalence of antibiotic resistance in *Salmonella* (Davis et al., 2002). In the 1990s the prevalence of multiple resistance in *Salmonella* increased. The current treatment depends on the severity of the infection and the patient's factors. Most people with low infection recover with treatment. While some need intensive care to survive the infection. In *Salmonella* infection, there is diarrhea and vomiting that cause severe dehydration. To tackle this dehydration fluid therapy is given. Antipyretic medicine is given to treat fever. Antibiotics are prescribed only in severe cases. The specific antibiotic that is prescribed depends on the type of bacteria that causes infection. Due to antibiotic resistance, some *Salmonella* bacteria are not susceptible to the antibiotics that were previously used to treat the infection. So, it is important to test three types and prescribe the specific antibiotic. Following are the latest antibiotics that work on *Salmonella*: Ciprofloxacin, azithromycin, ceftriaxone, ertapenem, and meropenem but the threat of AMR is not eradicated so it is very near when *Salmonella* becomes resistant against these antibiotics so there is need of alternative treatments.

As the world progresses, some inventions will be modified in the future and used as a good source of treatment for this type of disease.

Nanoparticles will be used to treat the infection of *Salmonella*. Nanoparticles are matter particles (Griffin et al., 2017), have a ting size of 1-100 nanometers (Paulami et al., 2023; Tripathi et al., 2023), and can't be seen with the naked human eye or even with the compound microscope. (Diaspro et al., 2022; M. Ismail et al., 2019). Nanoparticles are classified into different types based on physicochemical compositions (Khan, 2019; Stone et al., 2010) e.g. Organic based nanoparticles (nanocapsule, nanosphere, liposomes, dendrimers), Inorganic based nanoparticles (silver, gold, magnetic and alloy nanoparticles.), carbon-based Nps, semiconductor-based nanoparticles, etc. Nanoparticles are ting fighters that fight against pathogens (Mobeen et al., 2021). They can treat infections caused by bacteria and viruses (Baptista et al., 2018). They can target the bacteria very accurately and kill them in different ways even if they develop resistance to antibiotics (Natan and Banin, 2017). This seems very dramatic but it's reality now. Nanoparticles play a role in water purification systems by effectively eliminating bacteria and eliminating bacterial contaminants (Joseph et al., 2023) as we know *Salmonella* spreads by contaminated water so it can be helpful. Nanoparticles have various mechanisms of action with bacteria. Some invade bacterial cell walls (Mei et al., 2013), destroy cell wall integrity, and cause cell death. Some release antimicrobial compounds or generate reactive oxygen species (ROS) which inhibit bacterial growth and interact with important cellular processes, making them effective antibacterial agents (Karanwal et al. 2023). The rise of antibiotic-resistant bacteria can be treated with nanoparticle solutions. Nanoparticles can fight against resistance strains through multiple MOA making them effective against multidrug-resistant bacteria. (Cui, Zhao, and Zhang, 2012). Silver nanoparticles are very lethal against *Salmonella enterica* serotype typhimurium. When this serotype is treated with silver nanoparticles, there is no effect on the outer membranes but permeability of the inner membrane changes with the concentration of nanoparticles. This means that silver nanoparticles affect the inner membrane without affecting the outer membrane. Ag nanoparticle-induced the accumulation of ROS and ca^{2+} by using 2′,7′-dichlorodihydrofluorescein and Fura-2 AM respectively. Destruction of the inner membrane plays a vital role in the antibacterial role of Silver Nps (Seong and Lee, 2017).

Vaccines are also a big problem-solving thing in bacterial infection. Most vaccines are given through injections. About 90% of infections occur through the mucosal surface but there is no vaccine available to give by oral route and go to the intestine to kill *Salmonella* due to harsh conditions produced by gut chemicals. Nanoparticles can be used as a covering of these vaccines to pass through gastric juice of the stomach to the intestine and their vaccine kills *Salmonella* but this research is at the very starting phase and a lot of work is required to make it commercial. When it becomes commercial it is a big revolution against food-borne, especially *Salmonella* (Acevedo et al., 2021).

Conclusion

Salmonella, a versatile and adaptable genus of bacteria, has a long history of affecting human health. Its diverse serotypes, with the ability to cause typhoidal and non-typhoidal infections, have been a major concern for public health worldwide. Typhoid fever, caused by *Salmonella Typhi*, is a life-threatening illness with severe consequences, including

hypovolemic and septic shock. On the other hand, non-typhoidal *Salmonella* serotypes primarily cause food poisoning but can lead to bloodstream infections, particularly in regions with high rates of immune suppression. Understanding the mechanism of *Salmonella* infection is crucial, as it helps to identify potential treatment strategies. *Salmonella* uses various strategies to infiltrate the human body, including adhesion, invasion, and manipulation of host cells through the Type III secretion system (T3SS). This knowledge is essential for the development of effective treatments. However, the rise of antibiotic resistance in *Salmonella* is a concerning issue. Antibiotic resistance, driven by the overuse of antibiotics in both humans and animals, poses a significant threat to public health. *Salmonella* has evolved to resist multiple antibiotics, making treatment more challenging and increasing the risk of therapy failure.

To address this challenge, nanoparticles can be used. Nanoparticles like silver Nps kill *Salmonella* by destroying the inner membrane of pathogens. Nanoparticle-based vaccines can also be developed to treat infection in the intestine. In the face of these evolving challenges, it is imperative to continue research and develop strategies that not only treat *Salmonella* infections effectively but also mitigate the growing threat of antibiotic resistance. Public awareness and hygiene practices are equally important in preventing *Salmonella* infections.

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Chapter 07

Lactobacillus casei Stimulates *in vitro* Production of CCL2, Interleukin-12 and Gamma Interferon in Bovine Monocytes

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ABSTRACT

In order to understand more about the direct immunostimulatory effect of lactic acid bacteria *Lactobacillus casei* (Lc) on immune cells, the production of important molecules such as CCL2 chemokine and the cytokines Interleukin-12 (IL -12), and Interferon gamma (IFN-g) in the initiation of the immune response the present study was carried out. Briefly, monocytes were obtained from peripheral blood mononuclear cells of six-month-old bovines (6MO) and adults of 12MO which were subsequently cultured *in vitro* in the presence (Lc) or not (T) of *Lactobacillus casei*. Seventy-two and 96 hours after exposure to Lc, the presence of CCL2, IL-12 and IFN-g in culture supernatants were determined by means of a commercial quantitative immunoenzymatic assay (ELISA). Results indicate that Lc-stimulated CCL2 production was better in monocytes from 6MO bovines as compared to chemokine production in monocytes from 12MO animals. The production of both IL-12 and IFN-g in monocytes from young and adult animals was significantly stimulated by Lc compared to the production of these cytokines observed in cells from untreated bovines. These results indicate that *L. casei* stimulates monocytes to produce cytokines involved in the innate immune response of bovines, which is fundamental for the correct development of an acquired protective immune response and suggests that such a bacterium could be used to induce protective responses against various pathogens in the cattle. These findings are in agreement with the growing knowledge about the notion of trained immunity of the last years.

KEYWORDS
Chemokine, Cytokines, Monocytes, Lactobacillus, Bovine

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INTRODUCTION

This manuscript deals mainly with unpublished research results. The interest for using substances other than chemical parasiticides for controlling parasites in animals of economic importance, which generate resistance in parasites to those substances and contaminate the environment, gave rise to the study of *Lactobacillus casei* as a non-specific immunostimulating agent that promotes natural resistance against parasites in the host. In this regard, the induction of a protective response of this Lactobacillus was demonstrated when it was inoculated intraperitoneally in mice before infection with various parasites such as *Trichinella spiralis*, *Hymenolepis nana* (Bautista and Figueroa 2004) *Babesia microti* (Bautista et al., 2005) and *Trypanosma cruzi* (Bautista et al., 2008) and in poultry against *Eimeria* spp. (Bautista et al. 2003).

Subsequently, the generation of protective responses in cattle was demonstrated when co-administered, intramuscularly, with a bivalent vaccine against bovine babesiosis (Bautista et al., 2008b, 2012). However, the protective mechanism(s) stimulated by lactobacillus in the host are not well known. In previous studies, it was shown that the lactic acid bacteria *L. casei* transforms the morphology of bovine peripheral blood monocytes when these cells are exposed to lactobacillus *in vitro* indicating an activation (Bautista et al., 2015).

In another study, researchers observed that *L. casei* stimulated the *in vitro* production of nitric oxide (NO) in monocyte cultures of young and adult bovines as compared with the production in cells non stimulated with saline solution (Bautista et al., 2016). On the other hand, it is not known whether *L. casei* is also capable of stimulating the production of the chemokine CCL2, important in regulating macrophage recruitment during inflammation and tissue repair (Boring et al., 1997; Hembruff et al., 2010) and the cytokines IL-12 and IFN-gamma in bovine monocytes. IL-12 is a cytokine produced mainly by macrophages, monocytes, dendritic cells, and B-lymphocytes in response to antigens from bacteria and intracellular parasites.

Likewise, this cytokine is responsible for the subsequent secretion of interferon-gamma (IFN-gamma) and tumor necrosis factor alpha (TNF-alpha) (Trinchieri and Gerosa 1996). IL-12-induced IFN-gamma secretion increases phagocytosis, NO production, and respiratory burst, leading to increased destruction of pathogenic microorganisms (Trinchieri and Gerosa, 1996). Based on the above and the lack of information on bovines, aim present study is to evaluate the quantitative production of CCL2, IL-12 and IFN gamma in supernatants of peripheral blood monocyte cultures of young bovines and adults exposed or not to *L. casei in vitro*.

Materials and Methods

Experimental Animals

Nine male Holstein cattle were used as peripheral blood donors to obtain mononuclear cells: four six-month-old calves and five 12-month-old (MO) cattle. All animals were healthy and free of brucellosis, tuberculosis, anaplasmosis and babesiosis. The animals were kept in suitable pens and were provided with a diet consisting of oat straw, alfalfa, commercial concentrate and water *ad libitum*. It is important to note that the animals came from Amecameca (tick free area), State of Mexico and had no history of any type of vaccination.

Experimental Design

With the monocytes obtained from each of the nine cattle, three replicates were established for each treatment (*Lactobacillus casei* and control) and evaluation date (72 and 96 hours) as indicated.

Obtaining Monocytes from Peripheral Blood

From each donor bovine, a sample of 10 to 25 mL of peripheral blood was taken by puncture of the jugular vein in vacutainer tubes with heparin as an anticoagulant, after asepsis with cotton swabs and 70% benzal. The procedure described by Birminham and Jesk (1980) was used and modified by this work team. The blood obtained was centrifuged at 450 x g for 15 minutes in sterile conditions. Then the phlogistic layer containing white blood cells was suspended 1:1 in Hanks Buffered Saline Solution (HBSS) Subsequently, four parts of the white blood cell suspension in the HBSS solution were carefully deposited on three parts of Ficoll-Hypaque (Histopaque 1077®) and centrifuged at 450 X g for 15 minutes.

Cell Culture

Mononuclear cells isolated from peripheral blood had a viability greater than 95% and were incubated for 120 minutes at 37°C in a CO2 atmosphere in sterile polyethylene Petri dishes (6 cm in diameter) (1 x 10^6 mononuclear cells /Petri box). For the immunostimulation test, all plates were washed with Vega and Martínez solution - VYM - (Vega et al., 1985), in order to remove non-adherent cells; so that only adhered monocytes stayed in the plate (Birminham and Jesk, 1980) and in the MF12 medium, in the presence of *L. casei* (1x10^8 unit forming cells (ufc)/plate), which were obtained according to Bautista-Garfias et al., (2001), and the control treatment (physiological saline solution –SSF). Each treatment was carried out in triplicate, maintaining the *in vitro* cultures in an incubator at 37°C with a CO2 atmosphere for 96 hours, performing medium changes every 24 hours.

Determination of Chemokine and Cytokines

The supernatant was collected from each replicate of the cultures at 72 and 96 hours for the quantitative determination of the chemokine CCL2 and the cytokines IFN-gamma and IL-12 using commercial ELISA packages. For CCL2, the Bovine CCL2 package, ELISA Kit (Cat. No. E11-800) from Bethyl Laboratories Inc. (Montgomery, TX, USA) were used. For IL-12, the Interleukin 12A (IL12A) ELISA Kit (Cat. No. MBS2019480) from MyBioSource (San Diego, CA, USA) was used. For the determination of IFN-gamma, the Bovine IFN-gamma package, ELISA Kit (Cat. No. E11-805) from Bethyl Laboratories Inc. (Montgomery, TX, USA) was used. In all cases, the manufacturer's instructions were followed. Readings were carried out in an ELISA reader at 450 nm. On the other hand, after 96 h, a plate from the group treated with *L. casei* and another from the control group (both young and adult bovine monocytes) were fixed with methanol, stained with Giemsa and observed under an optical microscope. with 100 X objective to verify the morphology of the adhered cells.

The data obtained were analyzed (random blocks) with the FAUNL experimental designs package, v.2.5 (Olivares, 1994). Differences were considered significant when P values were < 0.05.

Results

For the chemokine CCL2 in 6 MO cattle, at 72 h, an average (+ SEM) (picograms per mL) of 2.45 + 0.25 for T and 2.8 + 0.1 ($P<0.05$) for Lc was obtained and at 96 h of 2.35 + 0.3 for T and 2.9 + 0.1 ($P<0.01$) for Lc (Fig. 1). At 72 h in 12 MO adults, the values were 1.3 + 0.3 for T and 0.6 + 0.4 for Lc ($P<0.05$), and at 96 h they were 3.0 + 0.2 for T and 3.2 + 0.4 for Lc (Fig. 2).

For IL-12 in 6 MO cattle at 72 h, pg per mL values of: 0.7 + 0.01 for T and 1.8 + 0.02 ($P<0.01$) for Lc were observed and at 96 h the values were: 0.6 + 0.01 for T and 1.9 + 0.02 ($P<0.01$) for Lc (Fig. 3). At 72 h in 12 MO cattle the values were 0.1 + 0.01 for T and 1.1 + 0.01 ($P<0.01$) for Lc; while at 96 h, the values were 0.1 + 0.01 for T and 2.1 + 0.01 ($P<0.01$) for Lc (Fig. 4).

In the case of IFN-gamma (nanograms per mL), at 72 h in 6 MO cattle, values of 0.12 + 0.01 for T and 0.13 + 0.01 for Lc were obtained and at 96 h, the values were 0.08 + 0.01 for T and 0.11 + 0.02 for Lc (Fig. 5). At 72 h in cattle of 12 MO, values of 0.16 + 0.01 for T and 0.30 + 0.01 ($P<0.01$) for Lc were obtained; while at 96 h, the values were 0.16 + 0.02 for T and 0.48 + 0.01 ($P<0.01$) for Lc (Fig. 6).

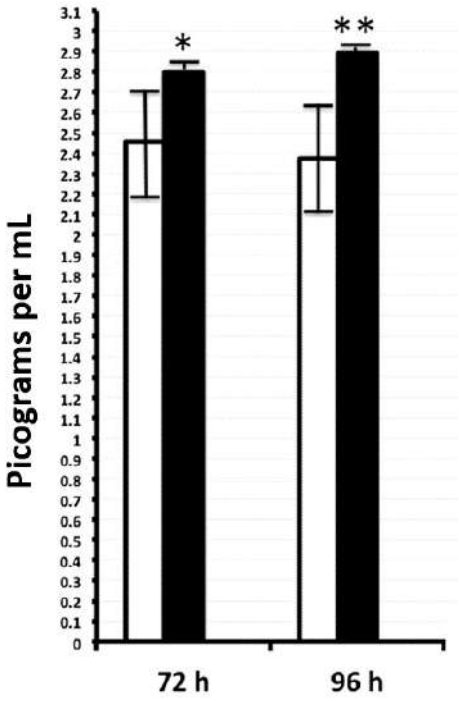

Fig. 1: CCL2 production (picograms/mL) by six-month-old bovine monocytes (MO), 72 and 96 hours after exposition to *Lactobacillus casei in-vitro*. Each point represents the average (+ SEM) of four animals (three repetitions/bovine). White bars, untreated controls; black bars, treated with *L. casei*.* P<0.05; **P<0.01.

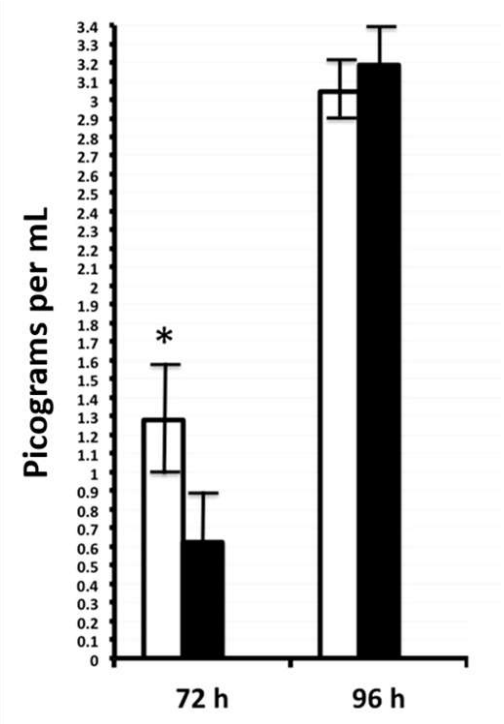

Fig. 2: CCL2 production (picograms/mL) by 12-month-old bovine monocytes (MO) 72 and 96 hours after exposition to *Lactobacillus casei in vitro*. Each point represents the average (+ SEM) of five animals (three repetitions/bovine). White bars, untreated controls; black bars, treated with *L. casei*. *P<0.05.

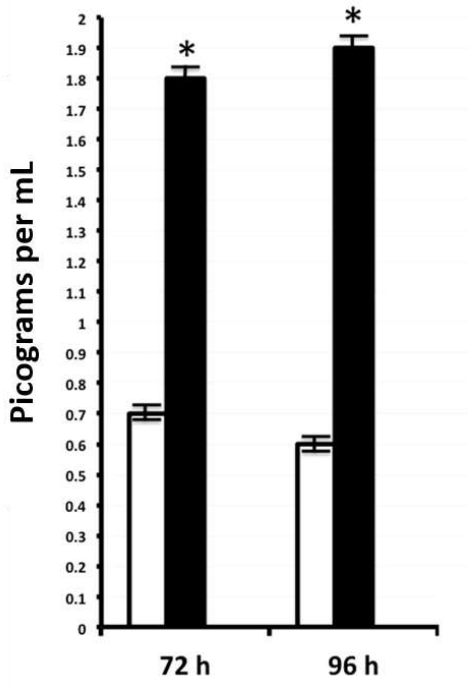

Fig. 3: Production of IL-12 (picograms/mL) by six-month-old bovine monocytes 72 and 96 hours after exposition to *Lactobacillus casei in vitro*. Each point represents the average (+ SEM) of four animals (three repetitions/bovine). White bars, untreated controls; black bars, treated with *L. casei*. *P<0.01.

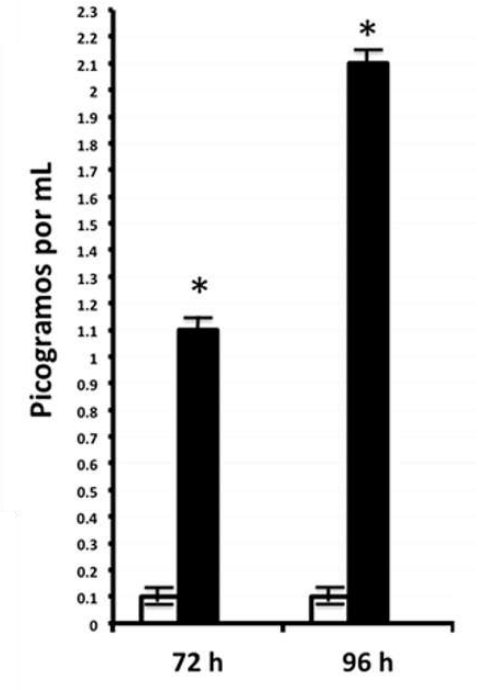

Fig. 4: IL-12 production (picograms/mL) by 12-month-old bovine monocytes 72 and 96 hours after exposure to *Lactobacillus casei in vitro*. Each point represents the average (+ SEM) of five animals (three repetitions/cattle). White bars, untreated controls; black bars, treated with *L. casei*. *P<0.01.

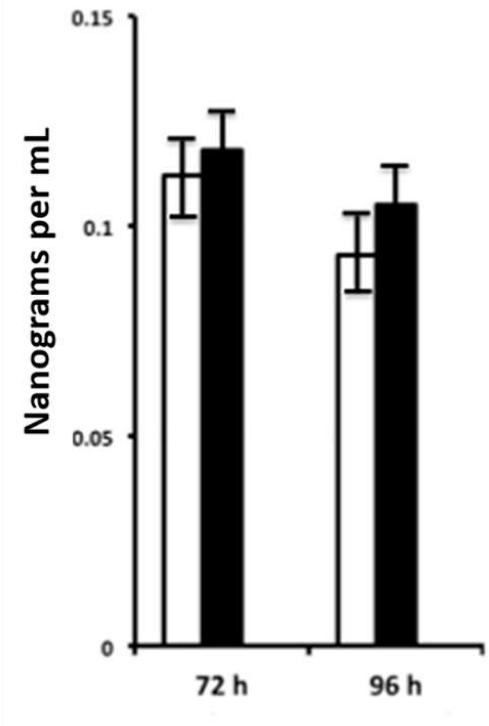

Fig. 5: Production of IFN-gamma (nanograms/mL) by six-month-old bovine monocytes 72 and 96 hours after exposure to *Lactobacillus casei in vitro*. Each point represents the average (+ SEM) of four animals (three repetitions/bovine). White bars, untreated controls; black bars, treated with *L. casei*.

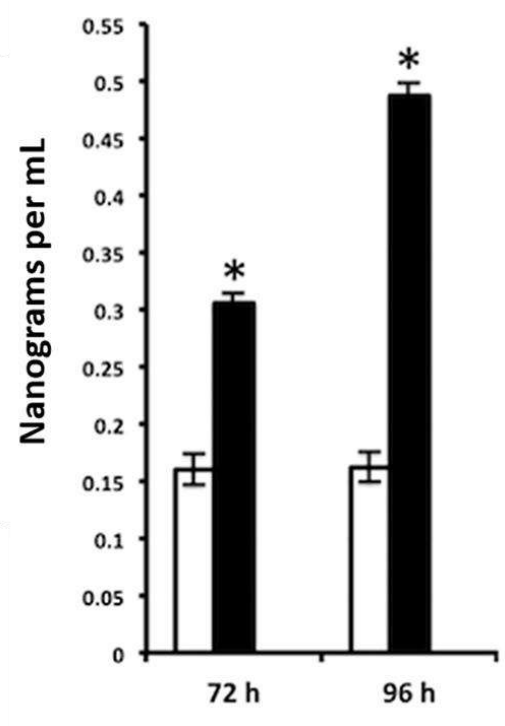

Fig. 6: Production of IFN-gamma (nanograms/mL) by 12-month-old bovine monocytes 72 and 96 hours after exposure to *Lactobacillus casei in vitro*. Each point represents the average (+ SEM) of five animals (three repetitions/bovine). White bars, untreated controls; black bars, treated with *L. casei*.* P<0.01.

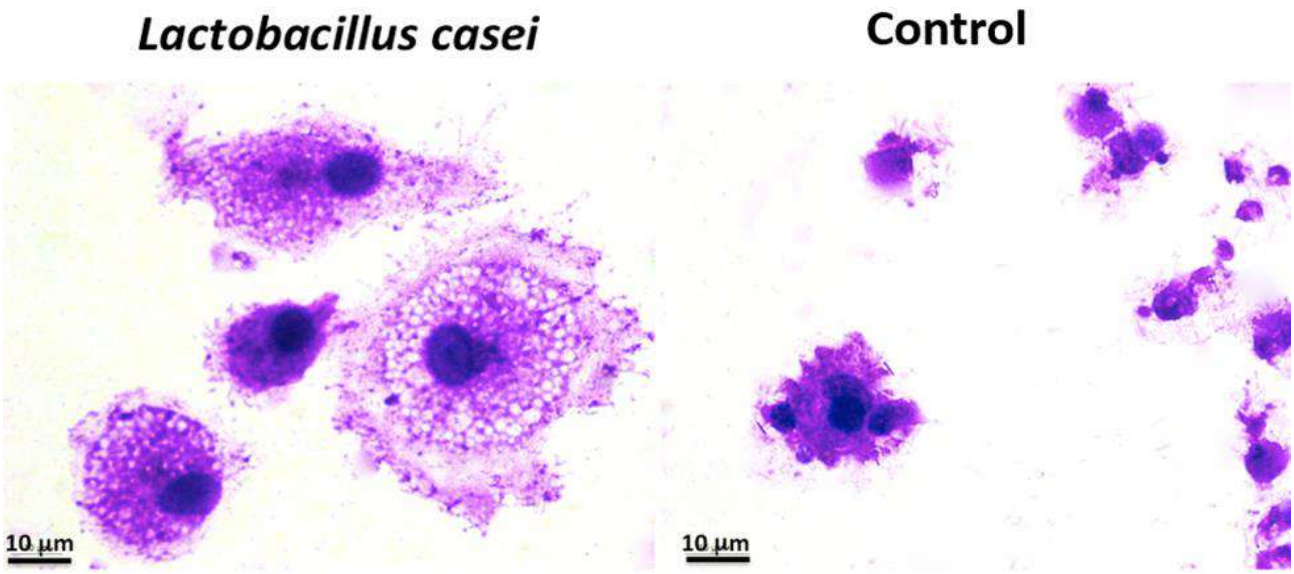

Fig. 7: Morphology of adult bovine peripheral blood monocytes, treated (left) or not (right) *in vitro* with *L. casei* after 96 hours (100X). Cells exposed to lactobacillus have the appearance of activated macrophages and have a large number of lysosomes. The morphology of monocytes from young animals exposed to lactobacillus was similar. The cells were colored with Giemsa stain.

Regarding the morphology of monocytes exposed to *L. casei* determined by optical microscopy, they presented an appearance of macrophages, rather than monocytes, compared to control monocytes not exposed to lactobacillus (Fig. 7).

Discussion

In relation to the technique of isolating monocytes from peripheral blood, it has been pointed out that the method of adhesion to plastic – used in the present study – is appropriate since the cells are less disturbed and it avoids their loss during their collection (Delirezh et al., 2013). In the case of the chemokine CCL2, which was only produced significantly by *L. casei* in monocytes from young animals, unlike in adults (Fig. 1 and 2), it is probably due to particular characteristics of the cells of the calves. In this sense, it has been shown that the modulation of innate immunity, in which there is stimulation of chemokines such as CCL2, results in a better acquired immune response (Morel et al., 2011). Likewise,, the results obtained are consistent with those of other researchers whom demonstrated that lipoteichoic acid (LTA) from Gram positive bacteria - such as *L. casei* - are powerful stimulators of the innate immune response (Seo et al., 2008), and, in particular, they are potent inducers of IL-12 in monocytes/macrophages (Cleveland et al., 1996; Hessle et al., 2000), a cytokine that plays a central role in the immune response against pathogenic microorganisms (Trinchieri and Gerosa 1996).

it has been demonstrated that the activation of Toll-like receptors (TLRs) –present in different cells, including monocytes and macrophages– promote the production of pro-inflammatory cytokines in macrophages (Zhang et al., 2001; Werling et al., 2004), and the fast differentiation of monocytes to macrophages and dendritic cells (Krutzik et al., 2005). This previous information is in agreement with the results obtained when treating bovine peripheral blood monocytes with *L. casei* (Fig. 7).

The results of the present study are also consistent with those of a previous study, in which it was demonstrated that peripheral blood monocytes from bovines of three age groups (4 to 6 months, 8 months and 12 months) exposed in vitro to *L. casei*, produced significantly more NO compared to control monocytes not exposed to lactobacillus (Bautista et al., 2016). It should be noted that NO production is stimulated in monocytes/macrophages by IL-12 (Trinchieri and Gerosa 1996), an immunoregulatory cytokine produced primarily by antigen-presenting cells such as macrophages (Hamza et al., 2010).

In this sense, bovine monocytes/macrophages stimulated by lactobacillus in vitro secreted significantly more IL-12, compared to unstimulated cells, both in young cattle (Fig. 3) and in adult cattle (Fig. 4). On the other hand, it has been shown that human macrophages in vitro are also capable of producing IFN-gamma when stimulated with IL-12 and IL-18 (Darwich et al., 2009), which agrees with the observation that monocytes /bovine macrophages exposed to *L. casei* secrete IFN-gamma significantly by cells from adult bovines (Fig. 6) unlike those from young bovines (Fig. 5) which did not show a significant difference with respect to untreated control cells. This difference between adults and young people is probably due to the fact that the monocytes of calves are less mature than those of adults but could, however, be associated with particular characteristics that are not completely known (for example, the production of NO) that They allow them to resist infections by *Babesia* spp., compared to adults (Zintl et al., 2005).

It should be noted that the information generated in this study and in other vaccination trials against bovine babesiosis using *L. casei* (Bautista et al., 2008b; 2012) is consistent with the notion that substances that stimulate innate immunity are excellent adjuvants for generating solid acquired responses (Coffman et al., 2010) and should be taken into account for the establishment of immune control measures for bovine pathogens.

Regarding the levels of cytokines detected in this study, it has been reported that bovine macrophages, derived from peripheral blood mononuclear cells, exposed to Toll-like receptor ligands such as the Gram-negative pathogenic bacteria *Listeria monocytogenes* and *Salmonella dublin* or LPS induce the production of a greater amount of IL-12 than the CpG oligonucleotide (Werling et al., 2004). It should be noted that CpG oligonucleotides have been used as adjuvants in vaccines against various pathogens and tumors (Shirota et al., 2015). It is likely that the aforementioned bacteria induce excessive levels of cytokines in macrophages that could cause damage to the host, but not the bacteria of the normal flora such as *L. casei* that possibly promote small but adequate amounts of cytokines to stimulate a protective response. In this context, it has been shown that a polysaccharide from the cell wall of *L. casei* acts as an immunomodulator of cytokine production in macrophages, thus preventing exacerbated immune responses caused by the lactobacillus (Yasuda et al., 2008), which could explain the apparent low levels of CCL2 and IL-12 and IFN-gamma observed in the present work. To evaluate the biological significance of stimulation of bovine monocytes/macrophages with *L. casei*, a study is being carried out to compare the effect of cells treated or not treated with lactobacillus on the in vitro growth of the bovine protozoan *Babesia bovis*. These findings are in agreement with the growing knowledge about the notion of trained immunity of the last years (Netea et al, 2020, Divangahi et al, 2021; Hartung and Esser-von Bieren 2022; van Leent et al, 2022; Ochando et al, 2023).

Conclusions

Exposure of peripheral blood monocytes/macrophages from young and adult cattle to *L. casei in vitro* induced significant production of the chemokine CCL2 (in young and adults) and the cytokines IL-12 and IFN-gamma (only in adults). , particularly highlighting the production of IL-12. The information obtained in the present study contributes to the understanding of the immune response generated by *L. casei* in bovine cells. More studies are required for a better understanding of the non-specific immune response induce by lactobacilli; however, the results suggest that these bacteria could be used to induce innate protective responses against various pathogens in cattle.

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Chapter 08

Understanding and Managing Emerging and Re-emerging Viral Diseases of Animals

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ABSTRACT

Animals' viral diseases are a critical factor posing a threat to animal well-being, food security, and human health. This chapter exposes the multifaceted characteristics of viral diseases in depth by confronting different elements triggering their emergence and re-emergence. The process of viral evolution, environmental changes, internationalization, and animal-human interactions are the main factors facilitating the spread of these viral diseases. This chapter explores the features of the viruses, the ways these viruses spread, and how this may affect either human health or animal populations. This states the role of a surveillance system for early detection, preventive measures such as vaccination and biosecurity management, and well-coordinated response checks which often involve different stakeholders. Additionally, here is a thorough discussion of some specific viral diseases like lumpy skin disease, bluetongue virus, canine distemper virus and foot-and-mouth disease which are accompanied by pathogenesis, diagnostic techniques and the current treatment and control strategies that are known. Through a solid analysis and practical lessons, readers will understand why managing new and old viral diseases is important. The key point is that helps to take preventive action in order to safeguard not only human health but also animal health as well minimizing economic losses.

KEYWORDS
Viral diseases, Emergence, Transmission, Control strategies

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INTRODUCTION

The world has been facing several difficult issues due to new and old diseases caused by viruses which are animal associated. Such diseases can be very harmful to animal health, food production and even human health as well (Jones et al., 2008). For the sake of animals, the livestock industry, and our communities, it's best to figure out what these viral diseases are and how to stop them.

This section focuses on the intricacies of new and re-emerging viral diseases of domestic animals that are a threat to public health. It covers such ailments by illustrating their causes which are responsible for their onset and recurrence. The chapter discusses special attributes of the virus and its possible consequences. Additionally, it presents comprehensive strategies for prevention, control, and response to these disease threats, aiming to equip readers with a thorough understanding and ability to manage these evolving challenges.

The Emergence and Re-emergence of Viral Diseases

The viral diseases can pop up or come back because viruses are always changing and evolving. These modifications may result in new virus strains or facilitate the infection's spread to previously uninfected humans and animals (Morse, 2001). Most viruses continually change through genetic mutation. Sometimes, these changes cause them to become stronger or spread faster. Other mutations even allow viruses to infect species that were previously not affected (Holmes, 2009). Another crucial element to consider involves the evolving dynamics among individuals, animals, and their surrounding ecosystem. As human populations expands and encroach on natural habitats, the chances of new animal-to-human (zoonotic) disease transmission increase (Daszak et al., 2000). Furthermore, the rapid cross-border spread of viral infections might be aided by the globalization of trade and travel (Kilpatrick and Randolph, 2012). Environmental changes, such as climate shifts, habitat loss, and changes in the farming methods, can contribute to the spread of viruses. Some changes can make the natural balance of ecosystems unhealthy. This often helps viruses and their carriers (like bugs or rats) to do well and grow more (Lafferty, 2009).

Understanding Viral Diseases

Knowing the key parameters for new and old viral diseases is vital toward their proper management. Viruses, small pathogenic pathogens, can only thrive due to other living organisms like plants or animals. The viruses consist of genetic information in the form of either DNA or RNA which is shielded by a protein layer. If they are in a host, they can rule over the functions of a cell to produce more of their own type. They can destroy the host cells with the release of new virions, able to infect other cells or other persons (Flint et al., 2020). The viruses possess the ability to infect just about any animal and spread in multiple ways. Some viruses spread through direct contact of the individuals, whilst other viruses can only be transmitted with the intermediary of insects or rodents, for instance. To develop effective strategies aimed at preventing and managing viral diseases, it is important to determine exactly what the particular features are which are specific to each virus (Woolhouse and Gowtage-Sequeria, 2005).

The Impact of Viral Diseases

The effect of pandemics such as newly spreading, re-emerging diseases has very negative effect on the feeding mechanisms among the animals, humans and food supply. In the animal husbandry, these diseases can result in the widespread occurrence of diseases and deaths which can be a subject of concerns as to productivity and profitability. It can thus potentially cost enormous money to countries, as external trade may be blocked, global market access might be prevented, and disease control via for example, killing infected animals is rather expensive. Another aspect to consider is that some viral conditions can be a threat to human welfare as well. Many of these are zoonotic, that is, the disease can be transmitted from animals to humans (Woolhouse and Gowtage-Sequeria, 2005). The current COVID-19 crisis, caused by the SARS-CoV-2 virus, shows how devastating it can be when a virus jumps from animals to humans and spreads worldwide (Cucinotta and Vanelli, 2020). The emergence or resurgence of such viral diseases can also have far-reaching effects on society. They can exhaust healthcare systems, disrupt the flow of goods and services, and lead to a lack of food, especially in regions where people are already struggling (Lindahl and Grace, 2015).

Managing Emerging and Re-emerging Viral Diseases

Managing new viral illnesses and reappearing dormant diseases presents a massive test. No single approach works - we require a multipronged strategy monitoring outbreaks, preventive measures, spread control, and coordinated response systems. However, no single group can tackle it alone. We need collaboration across the government, veterinary, medical, scientific, agricultural, and farming sectors. Only by combining expertise and resources we can craft and execute a thorough plan against viral threats (Karesh et al., 2012).

Surveillance and Early Detection

Early identification of viral diseases is crucial for effective control. The enhanced surveillance systems are vital to promptly detect illness or high mortality in domestic and wild animals. This surveillance enables authorities to react swiftly and contain outbreaks to minimize their impact (Rodríguez-Prieto et al., 2015). The viral surveillance involves: Testing animal samples (e.g., blood, tissue) and environmental samples for known and new viruses. Tracking disease reports, lab results, and epidemiological data to identify potential outbreaks or re-emerging viruses. By monitoring these trends, we can detect and respond to viral threats more effectively (Hoinville et al., 2013).

Prevention and Preparedness

To minimize the risk of viral disease outbreaks, proactive prevention and preparedness strategies are vital. These include:

1. Strengthening Biosecurity: Enhanced animal production facility and supply chain biosecurity to reduce pathogen entry and spread (Nöremark et al., 2013).
2. Applying techniques such as vaccination, disease surveillance, and quarantine procedures to promote animal well-being (Ellwanger and Chies, 2021).
3. Collaboration between the Veterinary and Public Health sectors is essential to establish partnerships aimed at improving early warning systems and promoting coordinated preparedness (Coker et al., 2011).
4. Teaching people about animal diseases is very important. Farmers, vets, and others must know signs of sickness. They must report illness right away. They also need to learn how to stop diseases from spreading (Ritter et al., 2017).

Control and Response Strategies

In the case of a viral disease outbreak, some important steps are needed. These include:

1. Travel restrictions and the implementation of quarantine to stop the spread (Cowling et al., 2010).
2. Conducting quick diagnostic tests and tracing the spread of the outbreak (Halasa et al., 2016).
3. Providing treatment and care to animals infected, including antiviral drugs or other support (Chevalier et al., 2014).
4. Communicating clearly with the public, involved parties, and other countries to share information and coordinate efforts (Marty and Jones, 2020).

Here we discuss some emerging and re-emerging viral diseases:

Lumpy Skin Disease

LSD arises from the LSD virus, which infects cattle and buffalo. The lymph nodes of the diseased animals are impacted, resulting in the formation of 2-4 cm in diameter masses on various body parts such as the head, udder, neck, genitalia, limbs, and perinea. These masses are referred to as cutaneous nodules (Datten et al., 2023). Additional red flags and symptoms include a high fever, abrupt reduction in milk yield, ocular and nasal discharge, excessive salivation, depression and appetite loss. As per the Food and Agriculture Organization (FAO), the incubation period is around 28 days, which is the time span between the infection and the development of symptoms (Sprygin et al., 2019). Animals are infected not only by contact with vectors but also through excreting the virus directly from the nose or mouth, sharing of watering troughs and food as well as artificial insemination (Liang et al., 2022).

The disease was restricted to some sub-Saharan African countries between 1929 and 1986. The lumpy skin disease (LSDV) is a contagious and WOAH-marked disease caused by the Capri poxvirus. It affects water buffalo and cattle and it is highly contagious(Manzoor et al., 2023). It spreads non-vectorially through bodily fluids and contaminated food, taking one to four weeks for viremia to develop. The illness can affect animals of any age or gender (Whittle et al., 2023). LSD morbidity and mortality vary significantly based on cattle breed, population immunology, transmission vectors, and virus isolates. Typically, endemic areas have a 10% morbidity and a 1%–3% mortality rate (Mulatu and Feyisa, 2018).

Symptoms

- The sickness takes 1-4 weeks to incubate, and when the virus enters the body, it takes another 4-14 days for fever and depression to appear. LSD can have acute, sub-acute, or in-apparent clinical courses, among others (Gumbe, 2018).
- Elevated temperature of the body (>40.50C) and skin nodules (10–50 mm in diameter) that typically result in necrosis and affect the udder, limbs, perineum, genitalia, head, inner ear, and eyelids, and other areas are typical symptoms of LSD.
- Other clinical manifestations include decreased milk production, increased subscapular and prefemoral lymph nodes, and lachrymation and nasal discharge. Infected animals may also have lameness, emaciation, infertility, abortion, and persistent fever (Hailu et al., 2014).

Transmission

Ways of transmission are shown in Figure 1 below and the details are following:

- **Transmission without a vector**

Sick animals spread illness through touch. This is a kind of non-vectored disease transmission. It happens without living or non-living spreaders. Saliva, sinus, and ocular secretions comprising infectious LSDV are released into public eating and drinking places, so dispersing the illness. In addition, eating milk, gestational transmission, dispersion by contaminated semen during coitus, and immunization via contaminated needles can all spread ailments (Das et al., 2021).

- **Transmission with Vector**

The role that arthropod vectors play in aiding this virus's dissemination. The bloodsucking hard ticks that have been related to the spread of LSDV include *Amblyomma hebraeum, Rhipicephalus decoloratus* (blue tick), *Rhipicephalus appendiculatus* (brown ear tick), and flies *Stomoxys calcitran, Haematobia irritans*, and *Aedes aegypti* mosquitos. LSDV is transferred both transstadially and transovarially in the tick host under frigid conditions. Because animals are free to travel across international boundaries, the virus can spread over greater distances as well as shorter ones, up to a few kilometres (Carn and Kitching, 1995).

Diagnosis

Because the nodule has a larger load of virus particles than blood or viscera, samples taken from skin lesions provide higher positive findings in PCR tests. Animals that are clinically infected may provide entire blood, nasal swabs, or saliva to be used in molecular testing and virus isolation. Serological testing, such as the ELISA, VNT, Serum Neutralization Test, IFAT, and Indirect Immunofluorescence Test, can also be used to diagnose the illness. ELISA exhibits greater sensitivity and specificity when compared to IFTA or VNT. It has been determined that the Immuno-peroxidase Monolayer test, a novel test, useful in the diagnosis of LSD. It's inexpensive. Upon autopsy, tiny nodules resembling pox knobs were seen in the fluid-filled membranes of several viscera and cavities, including the testicles, tongue, esophagus, trachea, lungs, and oro-nasal cavities (Al-Salihi, 2014; Datten et al., 2023).

Treatment

Cattle can be protected against LSD infection with live-attenuated vaccines that are homologous (Neethling LSD virus strain) or heterologous (goat pox viruses or sheep pox). Some of the commercially available strains of the *Capripoxvirus* (CaPV) vaccine are the Gorgan goat, Yugoslavian RM65 sheep pox (SPP), and strains Romanian SPP. A study on the potency of three CaPV strains against LSD found that the Gorgan GTP vaccination can effectively protect cattle against LSDV. The Neethling and KSGP O-180 vaccinations, on the other hand, seemed to be ineffectual, suggesting the need for further molecular diagnosis for those ineffective vaccinations (Babiuk, 2018).

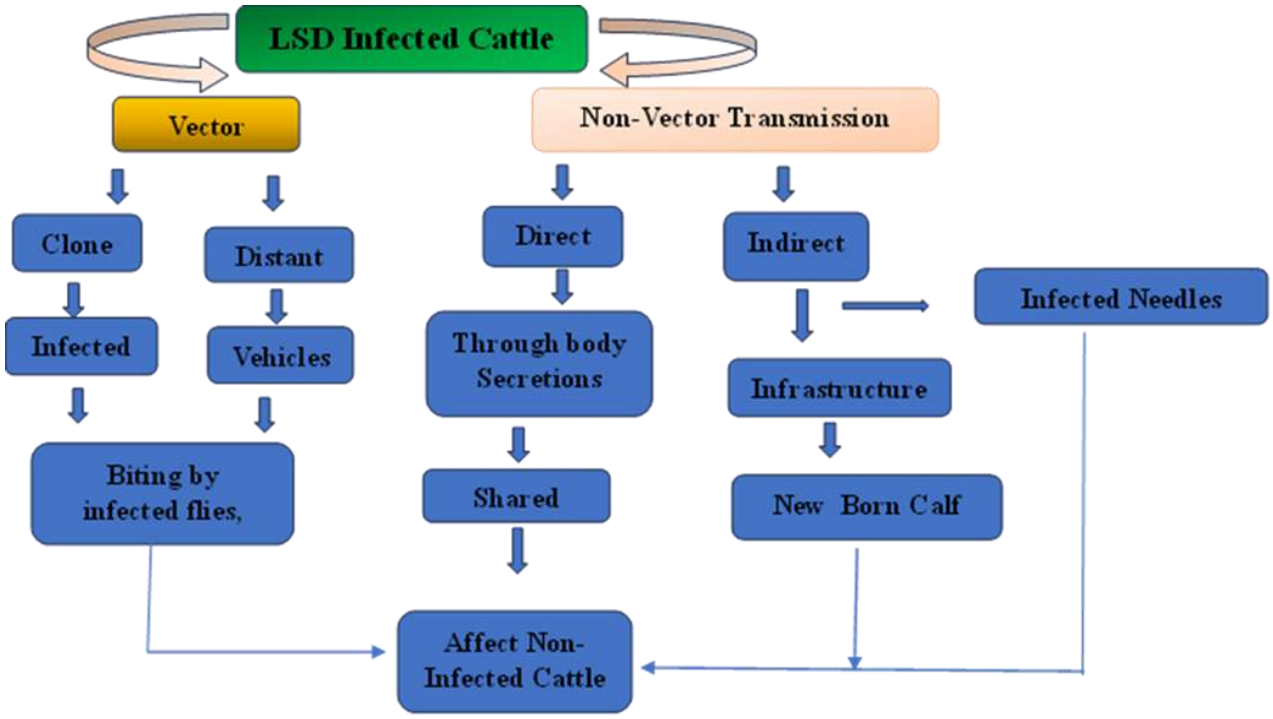

Fig. 1: LSD transmission ways

Bluetongue Virus (BTV)

Bluetongue, also known as BT, is characterized as an infectious viral disease that is non-contagious and transmitted by arthropods, affecting both wild and domestic ruminants. A virus that is a member of the *Reoviridae* family and belongs to the genus *Orbivirus* is the Bluetongue Virus (BTV) – the one that causes the disease. Bluetongue virus is transmitted by culicoides midges and it may bring the illness in sheep, deer, white-tailed deer, bighorn sheep, pronghorn antelope, and those presenting no signs of the disease in goats, cattle, and camelids (Saminathan et al., 2020).

The BTV was first identified in South Africa in 1876 when European immigrants introduced intensive sheep-farming practices to the region. Lastly, the increase in the range of the vectors, animals' movements as a result of trade accelerated the spread of BTV throughout Africa. The very first recorded cases of BTV among sheep in Cyprus took place in the year 1924 (Alkhamis et al., 2020).

BT is distributed across countries where this vector family is typical, which is usually the biting midge species *Culicoides*. These areas include severe and tropical and subtropical zones of the globe, covering continents, such as Asia, Africa, Australia, North America and Europe, as well as a great number of tropical islands. The virus is still in the regions where the environment of chilly nipping midges survives in winter due to climatic conditions. In this way, the propagation of pathogen is confined to the areas where the essential insect vector species of vector exist. Human cases are usually reported at the same times when the vectors are multiplying with higher temperatures and excess rainfall marking the peak of these episodes, while their activity decreases significantly during the first frost or extreme cold conditions (Rojas et al., 2019).

Pathogenesis of Bluetongue Virus

The main route of BTV transmission is through a midge bite, although it is important to be aware of the possible alternative transmission pathways.

Non-Vector Transmission

Scientifically, there are documented examples of vertical transmission from mother to fetus. Thus this form of transplacental infection is the key to many economic problems resulting in abortions, stillbirths, and non-viable offspring. Besides, placenta is another form of contagious route of transmission through oral transmission. Apart from this, needles for subcutaneous injections can also serve as a pathway for the transmission of BTV. Another mechanism for horizontal transmission has been identified among some serotypes through direct contact between animals that are housed in environments without vectors (Rojas et al., 2019; van der Sluijs et al., 2016).

Vector Transmission

After being bitten by an insect vector, the virus travels to the lymph nodes, where it replicates. The replication of the virus occurs in endothelial cells of the vascular system. After that, it is released into the bloodstream, where it interacts with erythrocytes which cause necrosis, vascular thrombosis, and infarction of tissue. In sheep, viremia can last more than 30 days during this period, whereas in cattle, it can last up to 100 days. Platelets and erythrocytes are mostly associated with BTV.

The virus is also spread through the lymph and vascular system to sites of secondary replication like in the lymph nodes, spleen, and lungs. In BT-infected animals, the spleen plays the role of the primary location for secondary viral replication. BTV is released from the spleen and causes viremia, which is a condition in which the virus is closely linked to cells. BT infection induces cell apoptosis and necrosis by activating p38MAP kinase, which prompts vascular permeability and increased production of thromboxane and prostacyclin in the bloodstream, culminating in an exaggerated inflammatory response and replication and transmission is discussed in Figure 2 (Đurić et al., 2018).

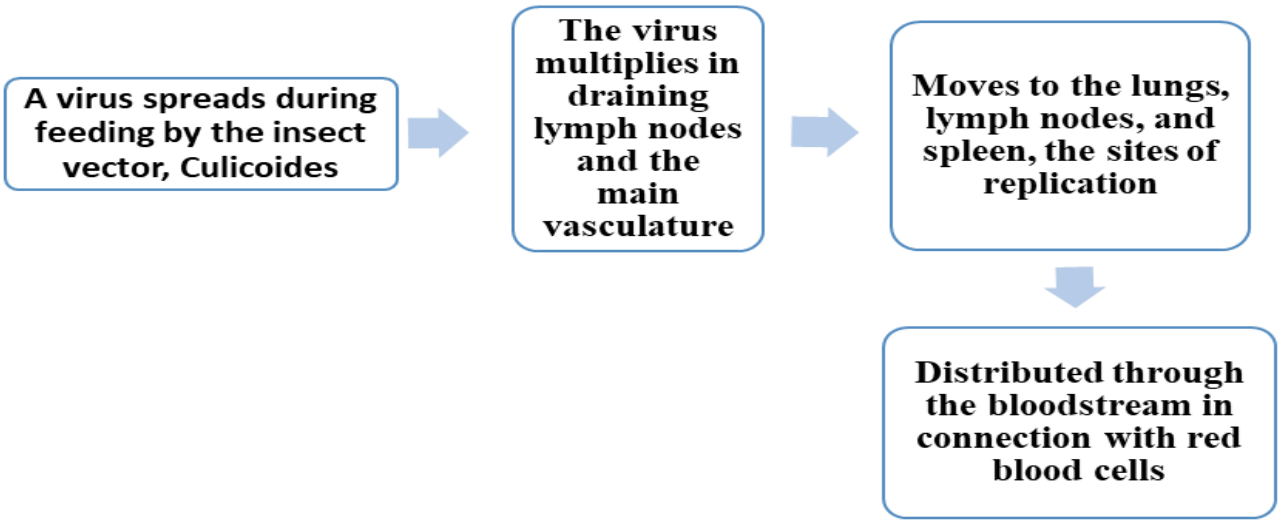

Fig. 2: Bluetongue Virus Replication and Transmission

Symptoms

Clinical manifestations of BT disease arise from the ulceration and erosion observed in the oral cavity and muzzle, in addition to hyperemia, edema, vascular congestion, hemorrhage, and tissue infarction caused by virus-induced vascular damage. In addition, the ulceration and erosion that damage the forestomach, esophagus, and nasal and oral cavities, as well as the inflammation, fluid buildup in the lungs, and internal hemorrhages that result in cyanosis and the characteristic blue look of the tongue, all contribute to death. The clinical presentation may encompass symptoms such as lethargy, altered mental status, respiratory distress, excessive salivation, and loss of appetite.

Standing can be difficult for some animals as a result of coronitis and laminitis, which can cause an arched back, drooping head posture, and sensitive hooves. Distinctive alterations have been documented in two prominent ruminant species most affected by the disease. Sheep often display extensive edema, particularly in the pulmonary region, in cases of severe bluetongue, while white-tailed deer experience "extensive vascular damage leading to disseminated intravascular coagulation and widespread hemorrhaging (Rivera et al., 2021)."

Diagnosis

Various assays have been developed to detect BTV infection, utilizing either antibody or virus detection methods**.**

Clinical Techniques

To diagnose BTV, vets first examine animals for symptoms like fever, runny nose, mouth sores, trouble walking, and inflammation of the mouth and nose. However, these signs can also indicate other diseases, so they can't be used on their own to confirm BTV.

Serological Assays

Serological assays are used to identify the antibodies present in the body after exposure to the BTV virus. Two commonly used techniques include ELISA: A highly sensitive method that measures the presence of antibodies. * SNT (Serum Neutralization Test): A more specific method that measures the effectiveness of antibodies in neutralizing the virus. These tests are crucial for tracking the spread of the virus (surveillance), determining how many people have been exposed (seroprevalence), and evaluating the success of vaccinations.

Molecular Techniques

Detecting the genetic material of the BTV offers quick and accurate diagnosis from patient samples. RT-PCR is widely used for the detection of BTV. RT-PCR amplifies particular RNA regions of the virus which code for either structural or non-structural proteins, making the virus identification and serotype determination possible. Advanced molecular tests can be performed by using the LAMP method, showing rapid and highly specific results to increase diagnostic speed and accuracy (Rao et al., 2017).

Treatment

Vaccinations were being applied as the brilliant way of disease prevention, and they might also interrupt the chain transmission from infected animals and hematophagous vectors. The particular research in BTV vaccines is carried out on approaches that are based on neutralizing antibodies which are specific for one serotype. Mainly the viral serotype identity of BTV which is attributed to the interaction of the Nabs with the capsid proteins of BTV is counted on. Cytotoxic T lymphocytes (CTLs) play their main role in viral defenses of BTV on the active side. The general way through which the cell-mediated immunity works for T lymphocytes is by them targeting the non-structural proteins that are vital for all types of the virus resistance. The choice of vaccine platform used has a major impact on the efficacy of antibody and CTL-based protection. Recently, Live – attenuated vaccines (LAV) based on reverse genetics and viral vector vaccines have been developed to combat BTV(Jiménez-Cabello et al., 2021).

Canine Distemper Virus CDV

Canine distemper virus (CDV), commonly referred to as canine morbillivirus, is a member of the *Paramyxoviridae* family., specifically in the *Morbillivirus* genus. This genus includes the measles morbillivirus, which affects humans and nonhuman primates, the small ruminant morbillivirus, also known as the peste-des-petits-ruminants virus, which affects sheep and goats, and the marine mammal-causing phocine and cetacean morbillivirus. Moreover, the artiodactyl-targeting Rinderpest morbillivirus is extinct. Six structural proteins and two non-structural proteins are encoded by the enclosed, non-segmented, single-stranded, negative-sense RNA virus known as CDV. The etiology of this condition can be attributed to canine morbillivirus or CDV, which belongs to the genus *Morbillivirus* within the family *Paramyxoviridae*. Veterinarians commonly identify canine distemper by integrating clinical manifestations with laboratory examinations. The absence of a definitive treatment and the lack of authorized antiviral medications to counteract the disease underscore the critical significance of vaccination in this context (Jiménez-Cabello et al., 2021).

Clinical Symptoms of CDV

The CDV primarily targets the immune system cells, which results in a compromised immune response in dogs, elevating their susceptibility to additional infections.

Upon propagation to the respiratory and gastrointestinal systems, canines commonly exhibit the subsequent clinical manifestations:

- Ocular and nasal discharges
- Elevated body temperature
- Respiratory distress
- Diminished activity levels
- Decreased appetite
- Emesis (Özkul et al., 2004)

Controlling of CDV

Vaccination is an essential tool for the prevention of CDV. The Puppies should be vaccinated when they are 6-8 weeks old. In order to be protected, adult dogs must be given booster shots. The transmission of CDV can be prevented by keeping away from unvaccinated dogs and diseased animals.

The good hygiene practices are vital in combating CDV. Cleaning the place where dirty dogs are kept is a great way to control the virus. Dogs exhibiting symptoms such as fever and coughing should be taken to the veterinarian as quickly as possible. Currently, there is no known cure for distemper; however, supportive care may help the survival of some dogs. Besides these, following vaccine recommendations and practising biosecurity measures are the ideal ways to protect dogs from this deadly ailment (Portela et al., 2017).

Foot and Mouth Disease (FMD)

The outbreak of other diseases the foot and mouth disease (FMD) is a disastrous disease all over the world. It is caused by foot and mouth disease viruses (FMDV), a retrovirus, a member of the *Picornaviridae* family, mostly caused disease in cloven-hoofed animals such as cows, sheep, pigs and including 70 further species that may be domestic or wild. FMDV is a single-stranded positive-sense RNA virus. FMD is a highly contagious disease spread by a retrovirus that is further divided into seven serotypes distributed in Asia, Africa, and South America. The serotype C was the main cause of two outbreaks in Europe in 1996 and 2004 occur in Kenya and Brazil. The accidental introduction of FMDV in cattle can cause an abrupt outbreak and cause a disastrous economic loss. In Pakistan first originated in 2006, FMDV serotype A lineage ASIA/Iran-05 was the main cause of disease in buffalos and cows in Pakistan. Nowadays the FMDV is one of the most spreading viruses causing disease in buffalos and cattle all over in Asia including Pakistan. More the 100 countries are affected with FMDV. The vaccine is available for FMDV but the vast variations of viruses it is difficult to control (Jamal and Belsham, 2013).

Transmission and Effects of FMDV on Cattle

This virus mainly leads to vesicular illnesses that impact the mouth lining, hoof regions, and udder skin in pigs, cattle, and sheep. Additionally, it's a zoonotic ailment, meaning it can transmit from animals to humans. Researchers have

extensively studied the airborne spread of FMDV in natural settings, particularly emphasizing the development of mathematical models. These models integrate factors like weather conditions and wind movements to better understand the transmission dynamics. Hugh-Jones' findings suggest that under favorable weather conditions like wind and rain, the aerosol transmission of FMDV on land can extend over distances ranging from 60 to 150 kilometers. Additionally, the likelihood of viruses being transported over long distances through plumes is notably high across seaways. This results from the fact that airborne particle concentrations are kept constant for more distance as compared to those regions with more turbulent flow at ground level. The greatest airborne range is believed to be around 300 km adapt at the ocean surface.

In cloven hoofed animals including cows, buffaloes, sheep, pigs and 70 other species the FMDV through aerosol infection, primarily cause infection in pharyngeal epithelium, and then extensively divide into pneumocytes in the lungs. In the beginning, virus starts replicates in the epithelial cells after takeover the cattle body. After couple of days of infection, the virus gets into the bloodstream and spreads to various organs and tissues where it multiplies again. This causes viremia (a clear sign of the virus in the blood). The lining of the throat (pharyngeal epithelium) is closely linked to how long the virus sticks around in cattle. The typical signs of FMD in infected animals are fever and lameness. You can also see blisters in their mouths and on their feet (Paton et al., 2018).

Controlling of FMDV

Current Vaccines

Many countries have set up vaccine banks storing concentrated antigens in liquid nitrogen's gaseous phase. The current FMD vaccine is made from deactivated whole virus and mixed with adjuvant before being used in the field. Antigen stored under these specific conditions maintains stability for an extended duration compared to formulated vaccines. These banks house antigens for various virus serotypes, ensuring member countries have swift access to vaccines when needed.

Live Attenuated Vaccines

Another method for treatment of FMD through live attenuated vaccines. Efforts to create live attenuated FMD vaccines involved traditional methods like serial passage in animals or cell cultures. These methods aimed to weaken the virulence of FMD strains for cattle by passing them through non-susceptible species like mice, rabbits, or embryonated eggs. Field trials with these attenuated viruses were conducted across Africa, the Middle East, and South America. Though some of these attenuated vaccines showed promise by providing a level of protection, there was a significant challenge. Viral strains attenuated for one host species often retained virulence when introduced to other susceptible species. This highlights the complexity of developing effective and safe vaccines for diseases like FMD (Blacksell et al., 2019).

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Chapter 09

Investigating the Potential of Microbiome as Regenerative Medicine for Treating Autoimmune Disorder

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ABSTRACT

Autoimmune disorders (AIs) are linked to gut dysbiosis, an imbalance in the gut microbiome. This disruption is associated with chronic inflammation and potentially contributes to bone and joint degeneration seen in AIs like rheumatoid arthritis. The gut microbiota of an individual plays a crucial role in human functioning due to its close integration with the body's metabolic, mechanical, immunological, and neurological pathways. Although the gut microbiota is always changing, dysbiosis in the gut may have a major impact on both mental and physical health. We delve into the communication between gut microbes and bone/cartilage cells, investigating the potential for a two-way influence between AIs, skeletal tissues, and the gut microbiome. This chapter explores the potential of manipulating the gut microbiome as a form of regenerative medicine for AIs**.** The aim is to elucidate the mechanisms of microbiome communication between cartilage and bone cells and investigate the potential bidirectional influence between AI, skeletal and cartilaginous tissue, and the gut microbiome. This concept of altering gut microbiota is a crucial aspect of regenerative medicine (RM) and must be integrated into the field. This chapter could provide additional evidence for studying the impact of gut microbes on bone health and AI related pathology of muscles and joints.

KEYWORDS
Regenerative Medicine, Autoimmune Disorders, Microbiomes, Rheumatoid Arthritis

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INTRODUCTION

The AI disorder is a category of more than eighty epidemic disorders that have an impact on a large number of people in the United States. The AI is a growing issue in developed nations which disproportionately affects women as compared to men. Several AI disordered exhibit elevated levels of inflammatory markers such as TNF-α, IL-6, and IL-17, contributing to arthropathy (joint inflammation) and associated with increased levels of gut dysbiosis. Even though these disorders possess unique pathologic characteristics, but they are all caused by an adaptive immune system that targets the body's tissues in a self-reactive manner (Behera et al., 2020). Furthermore, the small organisms residing in the human body, referred as microbiome, or microbiota, may influence the management of rheumatic illnesses. However, due to the variety and unpredictable nature of treatment outcomes, this interaction can be challenging to comprehend. Additionally, the AI disorders deteriorate and are irrevocable at the time of clinical diagnosis because the initial sign of the disease are ambiguous and delicate (Ostrov and Amsterdam, 2017).Therefore, more effective early intervention measures must be developed in order to improve illness management. Moreover, certain AI disorders that lead to bone loss are linked to abnormal bone regeneration processes associated with chronic inflammatory conditions. It would be necessary to investigate the possibility of changing and maintaining the gut microbiome as a type of regenerative therapy and as a potential additional treatment for AI disorders that affect the bones and joints, because the human gastrointestinal microbiota is crucial for maintaining bone homeostasis (Behera et al., 2020). The gut microbiome as a form of regenerative therapy has the potential to treat AI disorders that affect the bones and joints, because gut microbiota is essential for preserving strong bones (Grüner et al., 2023).

This chapter discusses the impact of gut microbiota on bone metabolism, degenerative changes in joint, and skeleton related with several AI disorders, such as RA, PsA, and SLE. Our aim is to elucidate the mechanisms of microbiome communication between cartilage and bone cells and investigate the potential bidirectional influence between AI, skeletal and cartilaginous tissue, and the gut microbiome. In this chapter, we will use the terms microbiome and microbiota interchangeably, and also discussed the distinct AI disordered pathophysiology linked to inflammatory bowel disease (IBD), SLE, PsA, and RA.

Concept of Regenerative Medicine (RM)

The RM is an emerging field that integrates different technologies to replicate the natural healing mechanisms of the body for disease treatment. The primary goal of RM is to support the body in self-healing, and advancing the treatment of disorders through an understanding of the body's physiology and surrounding environment (Orlando et al., 2019). The RM is responsible for addressing and rectifying issues with stimulating the body's innate functions by cooperating with its natural systems rather than using drugs to inhibit its healing. The RM is neither a medication or a prosthetic substitute, or even a traditional surgical procedure. Nevertheless, the definition should not be so narrow as to confine its concepts to tissue engineering, stem cells, genetic engineering, autologous or allogenic biomolecules, or tissue transplantation (Lau et al., 2021). As recent as 2022, Preethy and others studied the impact of intestinal bacteria on the efficacy of cell treatments in the RM (Preethy et al., 2022).

According to the evidence, alterations in the bacteria within our bodies due to diet and lifestyle, such as the use of pre- and probiotic supplements, and the transfer of bacteria from one person's stool to another all align with the definition of RM (Murtaza et al., 2024). In addition, these small organisms help to protect us by producing chemicals that kill germs and outcompeting harmful bacteria for food and surfaces to colonize (Vyas and Ranganathan, 2012). There are two types of immune systems in the body: the innate and the adaptive. If the RM is dedicated to supporting the body's natural healing processes and taking into account various disease factors, we think altering gut bacteria could be an effective in the RM treatment. Additionally, these methods of aiding individuals in improving their health may be more convenient to utilize than alternative medical interventions, such as stem cell usage, genetic modification, or tissue regeneration (Lau et al., 2021). The concept of RM is neither a medication treatment, a prosthetic substitute, nor a traditional surgical procedure. Nevertheless, the definition should not be so narrow as to confine its concepts to tissue engineering, stem cells, genetic engineering, autologous or allogenic biomolecules, or tissue transplantation as shown in Fig. 1.

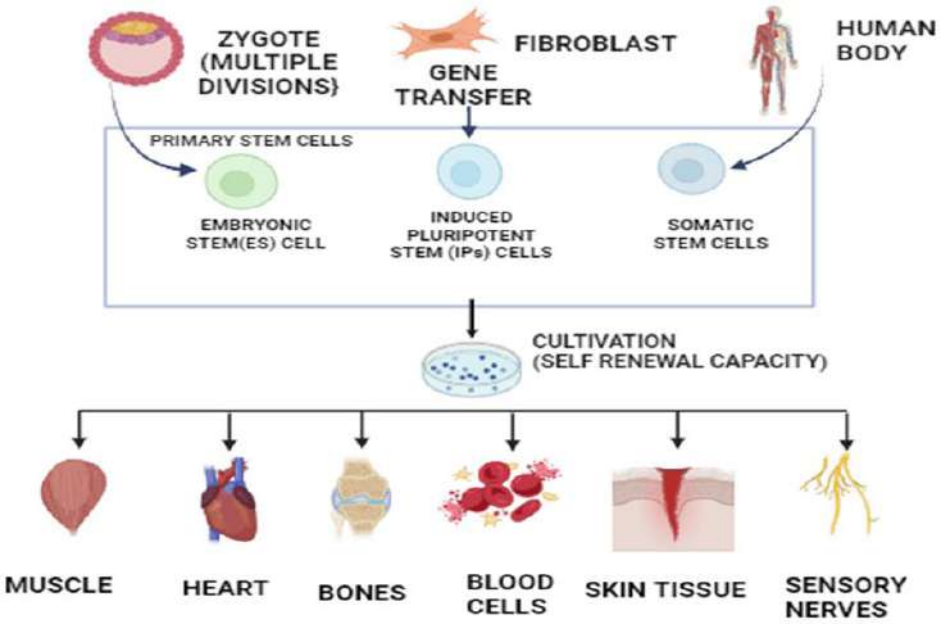

Fig. 1: Concepts of Regenerative Medicines

Role of Microbiome as Regenerative Medicine for Treating Autoimmune Disorder

Many distinct paths have been proposed to explain how microbiome contributes to the development of AI diseases as shown in Fig. 2. These include the microbiome and the probable mechanisms of AI disorders. According to several theories, disturbed microbiome can lead to the development of AI diseases. These include the translocation of pathobionts and their pro-inflammatory products, such as lipopolysaccharides (LPS); molecular mimicry, which refers to the similarity between self-antigens and bacterial antigens in genetically predisposed individuals; and disordered metabolome, which typically aids in the containment of inflammatory pathways in a healthy state. Normally, several microbiota metabolites SCFA in particular can promote the growth of innate lymphoid cells (ILC-3), immunoregulatory T-helper cells (T-Reg), and their immuno-regulatory cytokines (IL-10, 22) while obstructing Th-17-mediated proinflammatory pathways. The gut epithelial barrier is often dysfunctional in patients with AI disorders, and enhanced permeability that makes bacterial translocation easier.

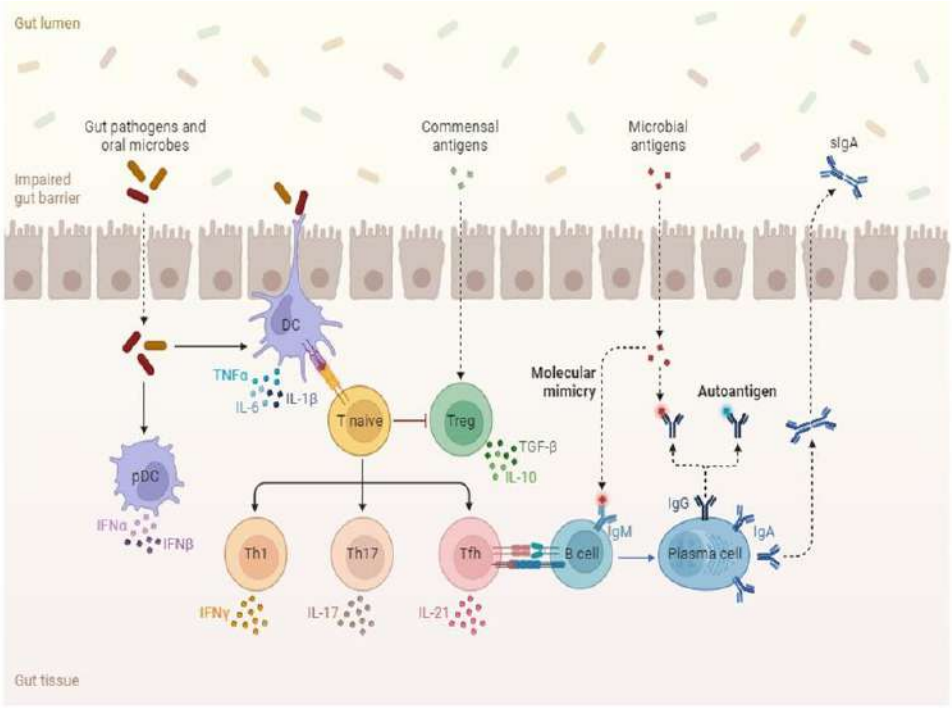

Fig. 2: Role of Microbiome as Regenerative Medicine for Treating Autoimmune Disorder

Autoimmune Disorders

Psoriatic Arthritis (PsA)

The PsA is an inflammatory complex condition where the immune-mediated arthropathy affects the joints and other structures. It's a bit like having a double-edged sword: it can show up in various clinical ways, causing inflammation such as nail disease, uveitis, osteitis, and dactylitis. Recent studies have linked PsA to several other health problems such as diabetes, high blood pressure, and heart disease, showing that it's not just related to joint problems (Van den Bosch and Coates, 2018). The co-occurrence of several health problems, especially in PsA patients, points to a degree of complexity that is not completely understood. According to current theories, PsA develops similarly to plaque psoriasis because certain people are more sensitive to specific stresses that increase inflammation. Swelling and inflammation are caused by the migration of neutrophils, macrophages, mast cells, T cells, and dendritic cells to the sites where tendons link to bones and ligaments as well as inside joints. Additionally, the skin, joints, and surrounding tissue of people with PsA showed higher levels of many cytokines linked to inflammation, including tumor necrosis factor (TNF) and several interleukins (IL)-1, IL-9, IL-17, IL-18, IL-22, and IL-23 (Zhang et al., 2018).

In-depth Imaging techniques, aided researchers in grasping the process of bone formation in people with PsA. It was determined that the IL-23/IL-17 pathway has significant implications for the transformation of bones in PsA. Furthermore, the elevation of these inflammatory proteins has been associated with shifts in the microbiota or microbiomes present in the bodies of PsA patients (Veale and Fearon, 2018). It is essential to discover the illness at an early stage and determine the exact variation of PsA. This can assist in stopping sustained chronic structural damage that may impair the patient's disability and lead to additional complication of PsA (Ogdie et al., 2020) as shown in Fig. 3.

Using the screening tools, carefully examining the patient's medical history, and doing a thorough physical examination can help confirm a PsA diagnosis and pinpoint the exact location of the illness in the patient's body. Relieving symptoms, improving the patient's quality of life, and preventing the disease from getting worse are the goals of PsA treatment. In order to reach therapeutic goals, it is crucial to examine the patient's health and the severity of the illness when contemplating the usage of pharmaceuticals such as disease-modifying antirheumatic drugs (DMARDs) (Paine and Ritchlin, 2018; Veale and Fearon, 2018). There are already medications intended to treat particular features of PsA, although around 40% to 50% of individuals do not respond well to these treatments. Apart from researching the effects of drugs and lifestyle choices on PsA, there is significant interest in the connection between PsA and gut microbiota. In order to support the treatment of PsA, they are especially interested in using medicines that particularly target the restoration of gut microbial equilibrium. For this particular demographic, altering the microbiota may be a therapeutic option (Olejniczak-Staruch et al., 2021).

On the other hand, there is a belief that a decrease in helpful gut bacteria and overall bacterial diversity can lead to a weaker intestinal barrier, which could potentially lead to the movement of bacteria from the gut to other parts of the body. This has been demonstrated to enhance specific aspects of the immune system, such as IL-23, IL-17, and IL-22. So due to the existence of TNF-a can result in inflammation within the body and might factor into conditions like psoriasis, PsA, and other illnesses as shown in Figure 2 (Shi et al., 2021). In 2015, Scher and colleagues had their study published by the American College of Rheumatology. It was discovered that individuals with PsA have lower levels of certain beneficial

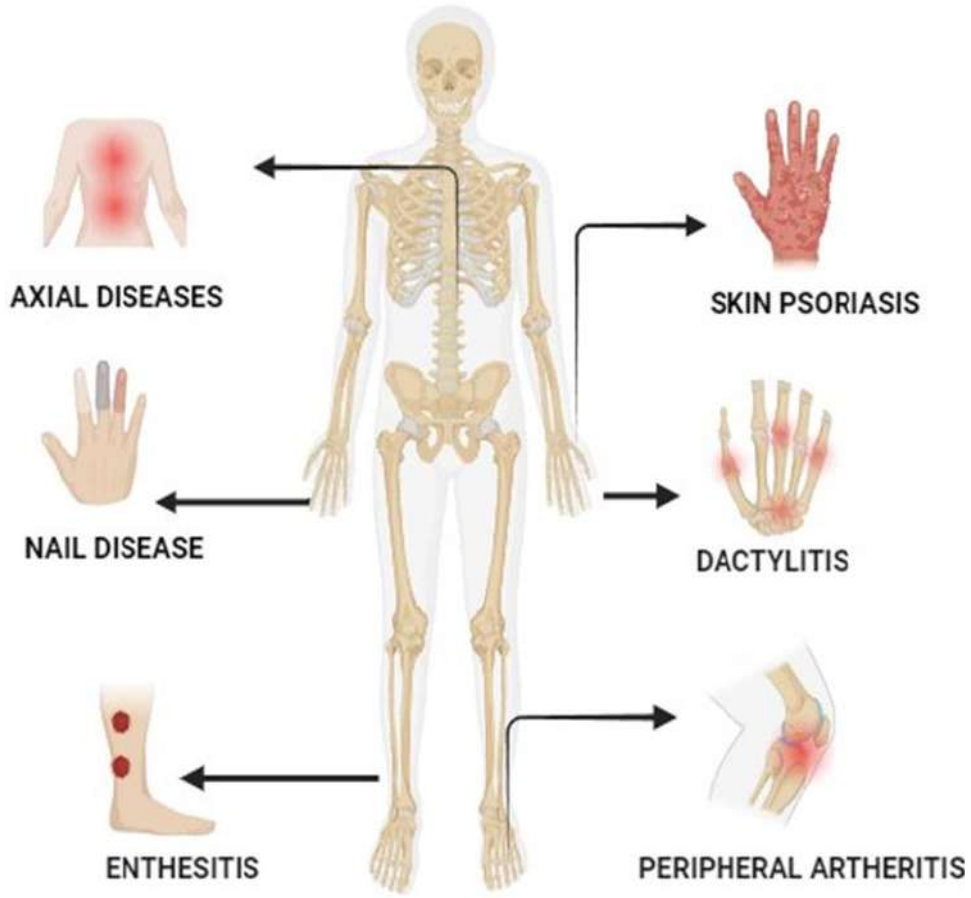

Fig. 3: Additional Complication Related to Psoriatic arthritis

bacteria in their gut compared to those who are healthy such as *Ruminococcus*, *Pseudobutyrivibrio*, and *Akkermansia* (Scher et al., 2015). Some research has concentrated on certain microbiota and attempted to determine the proportion of helpful to harmful taxa, while other research has examined the possibility that the food itself has a role in the pathophysiology of PsA. In January 2021, (Shi et al., 2021) discovered that a diet heavy in sugar and moderate in fat, similar to the western diet, encouraged the infiltration of γδ T cells and IL-23-mediated skin inflammation. Moreover, it increased sensitivity to IL-23-mediated joint inflammation and Th17 cytokine production. Further reinforcing the connection between the western diet and its pro-inflammatory effect, reduced consumption of fat and sugar also led to enhanced microbial diversity, including a drop in *Proteobacteria* and an increase in *Bacteroidetes,* and lower IL-23-mediated inflammation (Shi et al., 2021). The study also showed that reducing fat and sugar intake resulted in a decrease in the IL-23-mediated inflammation as well as an increase in microbial diversity, which included a decrease in *Proteobacteria*, an increase in *Bacteroidetes*, and a subset of *Firmicutes*. These findings serve to further support the link between the western diet and its pro-inflammatory effects. This suggests that the western diet, which similarly raises the body's pro-inflammatory state, may worsen the dysbiosis in PsA patients. This implies that dietary practices may exacerbate inflammation and intestinal dysbiosis in PsA patients. Knowing the links between gut flora, inflammation, and PsA may help develop novel strategies for treating the illness, which might provide relief for patients who don't react well to existing therapies (Olejniczak-Staruch et al., 2021).

Rheumatoid Arthritis (RA)

The RA is characterized by symmetric inflammatory polyarthritis and its etiology remains uncertain. It often results in joint deformities due to tendon and ligament stretching and deteriorating joints through erosive bone and cartilage damage. Inadequate treatment or treatment ineffectiveness can lead to joint inflammation and degradation, reducing physical function and impairing daily activities. Uncontrolled inflammation may also increase the risk of cardiovascular disease and osteoporosis, among other health complications (Aletaha and Smolen, 2018). The body's production of anti-citrullinated peptide antibodies contributes to the development of RA. The activation of T and B cells, as well as the presence of rheumatoid factor, are considered to be highly significant, and it appears that there is a reduced number of Treg cells, a factor that could be significant, and may play a crucial role in disease pathogenesis. Additionally, cytokines such as, like TNFα, IL-1, and IL-6 are crucial in the progression of RA as they trigger inflammation and deteriorate bone and cartilage as shown in Fig. 4. The body experiences immune complications when its multisystem immune dysregulation are not functioning effectively in various systems, Imbalances between pro- and anti-inflammatory cytokines. One of the predominant environmental factors associated with RA is dysbiosis of the gut microbiota and its metabolic products altering T-cell differentiation (Mateen *et al.,* 2016).

In the past ten years, it has been revealed that an unbalance in the gut's microbiome can be a prominent indication of RA. Reduced gut microbial diversity and proliferation of rare microbial lineages are characteristic of RA-associated intestinal microbiota (Chen et al., 2021). RA diagnosis typically involves assessing inflammatory arthritis involving three or more joints, positive rheumatoid factor (RF) and/or anti-citrullinated peptide/protein antibody (ACPA), elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), and ruling out alternative diagnoses. The RA diagnosis can still be made in patients not meeting all criteria (Gupta et al., 2021).The current recommendations for RA management include glucocorticoids, biologic disease-modifying antirheumatic drugs (bDMARDs), targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs), and conventional synthetic disease-modifying antirheumatic drugs (csDMARDs). The treatment selection depends on disease severity, patient preferences, regulatory guidelines, and comorbidities (Fraenkel et al., 2021).

The nonsteroidal anti-inflammatory drugs (NSAIDs) and/or glucocorticoids may be added for initial clinical inflammation control while awaiting DMARD therapy response. Additionally, non-pharmacological interventions and measures to prevent atherosclerotic cardiovascular disease (ASCVD) are associated with improved outcomes in RA patients. Research indicates that potentially anti-inflammatory diets (vegetarian, vegan, ketogenic, and Mediterranean) may reduce pain compared to regular diets (Schönenberger et al., 2021). The gut flora of RA patients is disrupted, suggesting complex changes related to RA. Several theories have been proposed, including alterations in intestinal mucosa permeability, enzymatic citrullination of peptides, modulation of the host immune system, promotion of T helper type 17-mediated mucosal inflammation, and activation of antigen-presenting cells through toll-like receptors (TLRs) or nucleotide-binding oligomerization domain-like receptors (NLRs) (Horta-Baas et al., 2017).

A study by (Bennike et al., 2017) detected twenty-one citrullinated proteins in colonic tissue from RA patients and controls. These peptides were also found in pulmonary tissues and joint fluid from RA individuals. The colon mucosa may serve as a potential site for immunological tolerance disruption to citrullinated epitopes, with citrullinated vimentin, fibrinogen-alpha, and actin being recognized targets for anticitrullinated protein antibodies (ACPAs) (Bennike et al., 2017). To investigate the gut microbiota, fecal samples were obtained from RA patients and healthy individuals, revealing marked differences in the microbial composition. Dysbiosis, characterized by changes in microbial populations, affects T cell groups including Th1, Th2, Th17, and Treg cells, leading to autoimmune and inflammatory disorders (Li and Wang, 2021).

The therapeutic benefits of probiotic bacteria in RA remain debated despite evidence of gut microbiota changes. A recent study suggests potential therapeutic outcomes in RA individuals with varying rheumatoid factor (RF) levels, indicating potential anti-RA effects. Additionally, research analyzing fecal specimens from SLE patients and controls revealed reduced *Firmicutes* to *Bacteroidetes* proportions in SLE patients' feces, linked to an elevated oxidative phosphorylation process in the gut microbiota. Caloric restriction has also been shown to induce changes in intestinal microbes and slow SLE progression, highlighting potential dietary interventions for AI (Jeong et al., 2021).

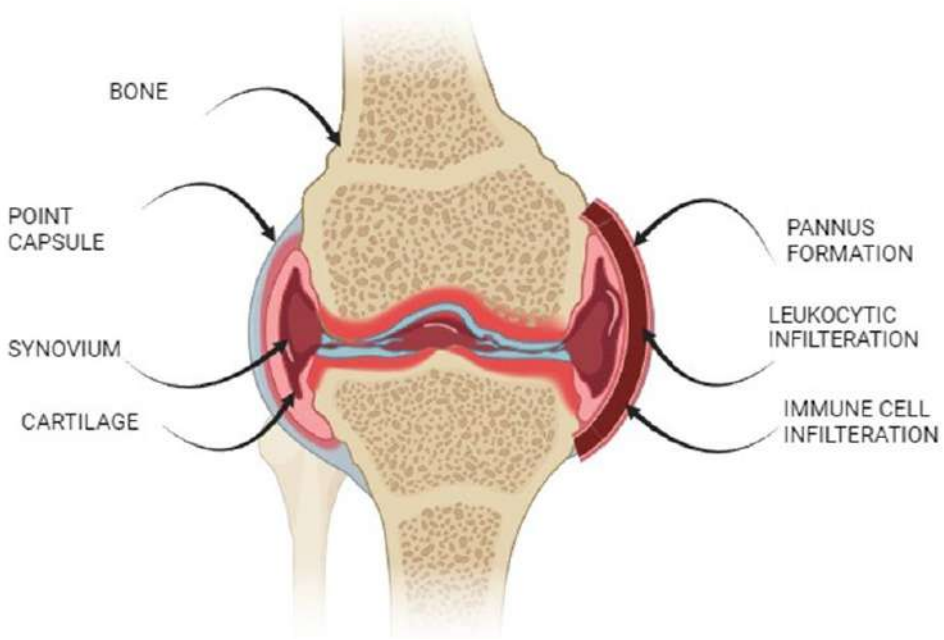

Fig. 4: AI-related Joint Disordered (Cartilage Degradation, and Joint Inflammation) can Result from Interleukin in Rheumatoid Arthritis

Inflammatory Bowel Disease (IBD)

The IBD comprises autoimmune digestive disorders, namely ulcerative colitis (UC) and Crohn's disease (CD), characterized by symptoms such as diarrhea, abdominal discomfort, rectal bleeding, and weight loss. The CD typically presents with watery diarrhea and nebulous symptoms like bloating and lethargy, while UC is more likely to manifest with diarrhea and bleeding. Additionally, around 40% of individuals with IBD experience musculoskeletal symptoms due to extraintestinal consequences (Williams et al., 2023). The pathophysiology of IBD involves three major host compartments: the microbiota, immune cells, and enteropathogens, forming an integrated "supraorganism. The environmental factors,

such as smoking, antibiotics, and enteropathogens, along with genetic susceptibility, disrupt homeostasis, leading to chronic abnormal inflammation or IBD. The genome-wide association studies suggest enhanced T-cell-mediated responses in UC and CD, indicating multi-genic processes (Santana et al., 2022).

The microbial imbalance is implicated in IBD pathophysiology, as drugs affecting the gut microbiota increase the risk of developing IBD. The endoscopy, particularly colonoscopies, is the primary diagnostic method for IBD, with findings such as erosions, ulcers, pseudopolyps, and loss of vascularity in the mucosa. The biopsy results differ between UC and CD, with UC showing crypt abscesses and CD exhibiting granulomas and fibrosis (Nakase et al., 2021). Treating IBD is challenging, with medical treatment aiming to induce and maintain remission, often starting with 5-aminosalicylic acid (5-ASA) for UC. Regarding nutrition and dysbiosis, lifestyle modifications likely play a significant role in managing IBD. Susceptible individuals exposed to gut antigens, such as microbiota alterations, prompt abnormal immune responses. The experimental mouse models devoid of microbes do not develop the disease or show significant improvement, indicating the role of bacteria in IBD-related intestinal damage (Williams et al., 2023).

Distinct subtypes of IBD may correlate with microbiome component changes, with decreased alpha diversity observed in fecal specimens from IBD patients. Increased *Proteobacteria* relative abundances and decreased *Firmicutes* relative abundances are noted in IBD patients, with elevated *Enterobacteriaceae* family levels particularly pronounced in individuals with concomitant arthritis (Nakase et al., 2021). The IgA-coated *Escherichia coli*, a member of the *Enterobacteriaceae* family, are more prevalent in individuals with CD-associated spondylarthritis compared to those with CD alone. The antigen-specific immune responses against intestinal antigens at non-intestinal sites may underlie extraintestinal symptoms of IBD. The microbiome could represent a new target for treating arthritis and other joint issues associated with IBD by modulating specific antigen immune reactions at joints (Williams et al., 2023). Modifying everyday routines can be highly beneficial in managing IBD, especially with the emergence of new treatment options. Investigations are being conducted on the potential connection between nutrition and the dysbiosis in relation to IBD. At present, the prevailing theory suggests that IBD develops when individuals at a greater risk are exposed to substances in their gut that elicit an immune reaction, and this can occur when there is a change in the gut microbiome, causing the immune system to malfunction (Santana et al., 2022).

Furthermore, different types of IBD could be associated with alterations in the gut microbiome found in our bodies. The study, which included 303 individuals with Crohn's disease, 228 with ulcerative colitis, and 161 healthy individuals, revealed that the range of gut microbiome present in the stool of the sick individuals was reduced in comparison to their healthy counterparts. The stool of patients with both Crohn's disease and ulcerative colitis showed a decrease in the variety of certain gut microbiome compared to those without the conditions. When comparing patients with and without symptoms, those with active ulcerative colitis had considerably less diverse gut microbiomes (Clooney et al., 2021). The extraintestinal symptoms of inflammatory bowel disease (IBD) may be caused by the cross-reactivity of antigen-specific immune responses against intestinal antigens that arise in non-intestinal sites. Treating the microbiota may be a novel approach to treating arthritis and other joint problems associated with IBD, if it modifies the immune responses specific to antigens at joints (Viladomiu et al., 2017).

Systemic Lupus Erythematosus (SLE)

The SLE is an autoimmune disorder that affects and regulates the gastrointestinal tract. It manifests as flare-ups of systemic lupus and causes symptoms such as pancreatitis, lupoid hepatitis, lupus enteritis, peritonitis, ascites, intestinal dysmotility, regional vasculitis, and protein-losing enteropathy. A dominance of *Lachnospiraceae* in the gut microbiota of female rats has been linked to more severe lupus symptoms, according to research. High concentrations of *Lachnospiraceae* and *Clostridiaceae* have also been shown to slow disease progression. Additionally, research on probiotic properties of *Lactobacillus rhamnosus* and *Delbrueckii* suggests that increasing *Lactobacilli* levels may treat leaky gut, promote IL-10 production, improve kidney function, and prolong the lifespan of mice (Zhang et al., 2021).

In the realm of RM, the significance of the gastrointestinal microbiome in our well-being is increasingly recognized. The RM aims to improve standard treatments by gaining a deeper understanding of specific diseases. Altering diet and daily routines can support the proliferation of diverse good microbes in gut. Adjusting the gut microbiota to reduce intestinal permeability may offer benefits for individuals with AI. In the future, this may have the potential to be used as a treatment for AI (Williams et al., 2023). Detectable antibodies in SLE include anti-dsDNA, anti-Smith, anti-La, and anti-Ro. Comparisons with other AI like IBD, diabetes mellitus, multiple sclerosis, and RA reveal correlations with dysbiosis, though in-depth research on the similarities between AI and underlying dysbiosis traits is lacking. The diagnosis of SLE relies on clinical signs as shown in Figure 5, blood testing, and histology of affected organs, with skin lesions being the initial symptom in 85% of cases. Constitutional symptoms such as anorexia, fatigue, malaise, fever, and weight loss are often observed first (Thong and Olsen, 2017).

Musculoskeletal involvement is common in the SLE, affecting 80% to 90% of patients at some point during the disease course. This involvement ranges from mild arthralgias to erosive arthritis, with lupus arthritis primarily affecting the wrists, knees, and small joints of the hands. Avascular necrosis, inflammatory myopathy, fibromyalgia, and RA nodules are also frequent manifestations of SLE. The treatment options include corticosteroids, immunosuppressant's antimalarial medications, and biologics (Williams et al., 2023). The non-pharmacological treatments such as increasing physical activity or physiotherapy can also effectively manage inflammation-related symptoms. Increased oral probiotic in take has been

shown to reduce autoimmunity and decrease lupus severity. The patients using these medications should undergo regular monitoring for co-morbidities. Other pharmacological therapies include DMARDs and biologics (Thong and Olsen, 2017).

Both the pathophysiology of the illness and the gastrointestinal system are impacted by SLE. The symptoms include pancreatitis, lupoid hepatitis, lupus enteritis, leptonitis, ascites, esophageal dysmotility (particularly in the upper portion of the esophagus), intestinal vasculitis, and protein-losing enteropathy. An overrepresentation of *Lachnospiraceae* in female mice's intestinal biomes was discovered by L. Zhang et al. in their study, and this overrepresentation was linked to more severe lupus symptoms (Zhang et al., 2021). Moreover, the presence of many *Clostridiaceae* and *Lachnospiraceae* bacteria in the gut can assist in decelerating the progression of the disease. Additionally, *Lactobacillus rhamnosus* and *delbrueckii* have been studied for their probiotic properties due to their ability to obtain nutrients and decrease inflammation. A greater presence of *Lactobacilli* in the body can contribute to the repair of leaky gut, the enhancement of IL-10, and the improvement of kidney function, and extend the life expectancy of mice (Zhang et al., 2014).

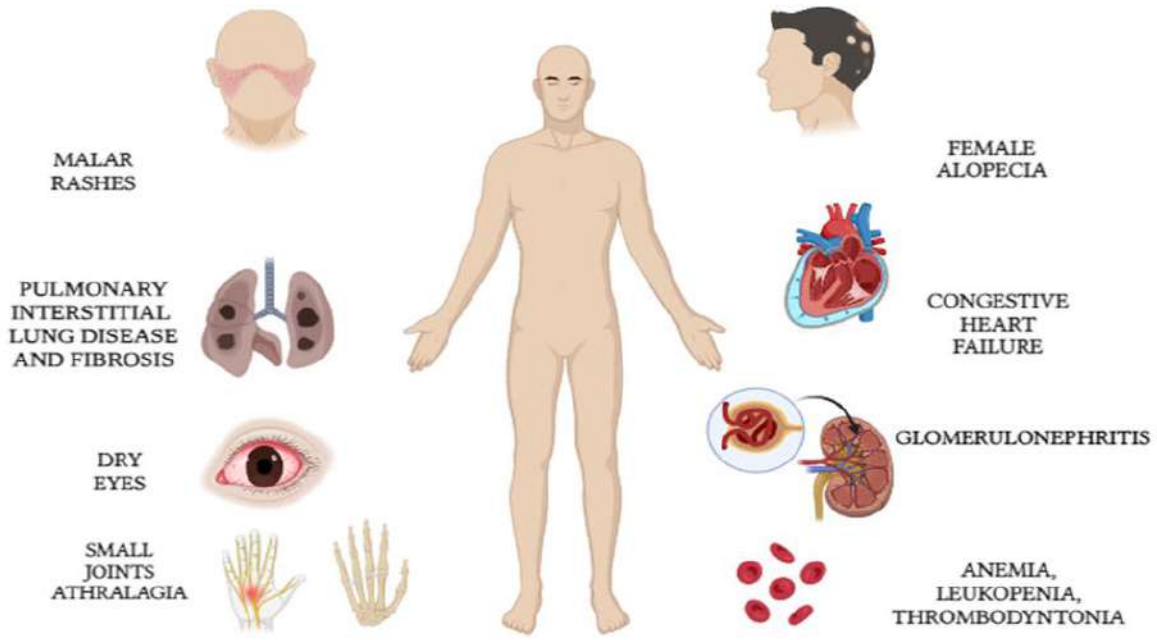

Fig. 5: Additional Complication Related to Systemic Lupus Erythematosus

Optimizing Autoimmune Treatment, Personalized and Regenerative Medicine

The PsA, RA, IBD, and lupus can all cause degenerative damage to the joints. There are numerous medications available to alleviate symptoms of autoimmune conditions, but some can disrupt the balance of bacteria in the gut. Furthermore, these conditions bring about indications that influence the complete body, including continual discomfort, exhaustion, and head pain. It is difficult to effectively treat diseases when the treatments are typically limited to addressing one aspect of the disease, such as a specific symptom or marker (Williams et al., 2023). Alternatively, patients may find themselves having to take numerous medications to manage all their symptoms, resulting in potential negative consequences. The current approach to treating patients may lead to them being overmedicated or not receiving adequate treatment. The significance of personalized treatments that cater to an individual's body and health requirements has been highlighted by AI. The use of RM is beneficial as it enhances the body's natural healing process. RM can also provide relief for various joint issues caused by these diseases and has fewer side effects than the medications currently prescribed (Grüner et al., 2023). The potential of microbiome as RM for treating AI is shown in Table 1.

Table 1: Autoimmune Disorders, their Treatment Options, and Impact of Gut Microbiome

Autoimmune Disorders	Key Features	Treatment Options	Impact of Gut Microbiome	References
Psoriatic Arthritis	Joint inflammation, skin lesions, nail disease	NSAIDs, DMARDs, biologics	Dysbiosis associated with increased inflammation	(Veale and Fearon, 2018)
Rheumatoid Arthritis	Symmetric polyarthritis, joint deformities, systemic complications	DMARDs, biologics, glucocorticoids	Gut dysbiosis triggers inflammatory pathways	(Chen et al., 2021)
Inflammatory Bowel Disease	Diarrhea, abdominal discomfort, weight loss, musculoskeletal symptoms	5-ASA, corticosteroids, immunosuppressant's	Alterations in gut microbiota linked to disease pathophysiology	(Williams et al., 2023)
Systemic Lupus Erythematosus	Skin rash, joint pain, organ damage, fatigue	Corticosteroids, immunosuppressant's, antimalarial	Overrepresentation of certain bacterial populations associated with disease severity	(Zhang et al., 2021)

Microbiome's Impact on Autoimmune Disorders, a Path to Regenerative Medicine

The microbiome is integral to the pathogenesis and progression of AI. PsA and IBD exhibit similar microbiota shifts, with elevated levels of *Salmonella*, *Campylobacter*, and other pathogens, along with suppressed species like *Prausnitzii* and *Bifidobacterium*. The RA involves gut dysbiosis triggering inflammatory pathways, while lupus severity correlates with specific microbial populations. The fecal microbiota transplantation supports the hypothesis that gut microbiota alterations influence autoimmune responses (Williams et al., 2023).

The importance of treating the microbiota of the intestines as a kind of RM is shown by the robust association found between musculoskeletal complaints and gut dysbiosis in AI diseases. The complexities of microbiome modification and its potential as a substitute or adjunctive strategy to currently available therapies for AI diseases require more research (Behera et al., 2020). RM innovations in targeting the gut microbiome for AI Disorders is shown in Table 2.

Table 2: Innovations in Targeting the Gut Microbiome for Autoimmune Disorders

Treatment Target	Mechanism	Examples	Potential Benefits	References
Macronutrient Intake	Altering gut permeability	Decreasing *Firmicutes* and *Proteobacteria* Balancing mucin-degrading bacteria	Reducing gut permeability associated with AIs	(Williams et al., 2023)
Pathogen Enzymes	Targeting unique enzymes	Proteases from *Bacteroides vulgatus*	Improving barrier dysfunction and preventing colitis Potential for new treatment options	(Grüner et al., 2023)
Probiotic Therapy	Modulating immune reactions	*Lacticaseibacillus* spp.	Well tolerated by general population Possible risk of systemic infections	(Preethy et al., 2022)
Pharmacobiomics	Influencing drug response	Gut microbiota altering drug bioavailability Converting drugs to potentially toxic forms	Enhancing drug performance Potential for drug toxicity	(Lau et al., 2021)
Fecal Microbiota Transplantation (FMT)	Restoring microbial balance	Transfer of healthy gut microbiota	Effective treatment for gastrointestinal disorders Potential for treating extra-gastrointestinal conditions	(Fraenkel et al., 2021)
Specific Bacterial Group Targeting	Modifying microbiota composition	Increasing beneficial bacteria (e.g., *Lactobacilli*)	Promoting anti-inflammatory effects Alleviating symptoms associated with AIs	(Behera et al., 2020)
Modifying Lifestyle and Diet	Promoting a healthy and diverse microbiome	Dietary changes promoting beneficial bacteria Lifestyle modifications reducing inflammation	Supporting the body's repair mechanisms Complementing traditional therapies for AIs	(Murtaza et al., 2024)
Targeting Gut Epithelial Cells	Strengthening gut barrier function	Enhancing tight junctions and mucosal barrier Supporting intestinal health and immune function	Preventing bacterial translocation Reducing inflammation and autoimmune responses	(Williams et al., 2023)

Conclusion

This chapter summarized the potential of microbiome as innovative RM for the treatment of AI disordered. Several clinical studies have confirmed that, maintaining diversification of microbiome and dietary adjustments play a significant role to address gut dysbiosis and reduce the systemic inflammation of AI disordered contributing to joint pathology, while promoting natural healing processes of the body. Although the underlying mechanism remains to be further investigated, maintaining, and modification of microbiome could significantly improve AI disordered treatment, and also advanced the therapeutic effects of AI disordered.

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Chapter 10

Alternatives to Antipsychotic Drugs

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ABSTRACT

Schizophrenia and depression are global problems nowadays and they have prevailed all over the world. There are various factors which affect the psychology of the people and the biggest one is the economy of the people. Antipsychotic drugs are used to treat symptoms of episodes of psychosis by inhibiting dopamine D1 or D2 receptors, they also affect GABAergic receptors. Some drugs showed positive effects but some s caused severe adverse effects. To avoid these, people preferred the use of alternatives to pharmaceutical antipsychotics. Several plant sources showed similar positive effects against episodes of psychosis without severe adverse effects. The research findings showed the useful herbs and plants employed by traditional practitioners in Bangladesh that helped in calming the effects of psychosis experienced by schizophrenic or similar disorder-experienced patients. Several species of plants were also recorded that showed promising effects but are yet to be scientifically researched.

KEYWORDS
Antipsychotics, Schizophrenia, Psychosis, GABA, Dopamine, Herbs

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INTRODUCTION

Antipsychotic drugs are used to treat the signs and symptoms of psychosis even in the absence of overt psychotic symptoms. They are mostly administered to treat agitation, marked mood instability, and aggressive behavior. Their effects are mostly related to sedation hence they were considered as major tranquilizers. However, in later years it was discovered that not all antipsychotics produce such adverse effects and were then separated into the class of antipsychotics (Samreen et al., 2020).

The idea of psychosis has a history that spans from its invention in 1845 to the contemporary. When psychosis was first defined, it encompassed mental disability in addition to a few other severe mental illnesses. However, a year later, it was interchangeable with the terms "psychopathy" and "psychoneurosis." Before long, all mental illnesses were grouped under this general title amid a bewildering plethora of terms. To distinguish psychosis from psychopathy and neurosis, it is investigated in detail. In modern psychiatry, the term "psychosis" is fundamental. Both specialists and laypeople regularly use it. Currently, it has the broad definition of "insanity." The word Psychosis is a combination of two Greek words, “psyche” means soul, and in advanced terminology, we apprehend it as "mind." The suffix "osis" means "ailment related to" (Singh et al., 2020).

The word "psychosis" was initially used in German-speaking psychiatry, which provided a rich environment for its invention due to its solid foundation in university philosophy faculties. Using novel ideas in the categorization of mental disorders (J.C. Reil, for example, first used the term "psychiatry" in 1808) (Beer, 1996). A psychological state of disorder in behavior and thought processes is referred to as psychosis. The thought process is chaotic and the mental ability is wildly

warped. This results in an incapacity to understand or associate with others in significant ways what does the term "psychotic" imply, whether it means illusions, hallucinations, disordered speech, or disorganized or catatonic behavior in people with a psychiatric disorder (Richards et al., 2000).

The following conditions are included in the category of psychotic disorders.

- Schizophrenia
- Schizoaffective disorders
- Schizophreniform disorders
- Delusional disorders
- Brief psychotic disorders
- Psychotic disorder because of general medical conditions
- Substance-induced psychotic disorders
- Shared psychotic disorders

Psychosis affects the way the brain perceives the information. It can cause an individual to hallucinate and hear things that may not exist in reality, they start to lose sense of reality. Psychosis is presumed to be a symptom rather than an ailment. It can be prompted by mental infirmity, physical injury, illness, traumatic stress, or abuse of substance. Psychosis disorders can be caused by schizophrenia within the late teen years or early adulthood with minor changes in behavior, these are before the first episode of psychosis appears; known as a prodromal period. It could last from weeks to years. Secondary psychosis can also be a case when an individual is not suffering from primary psychosis (schizophrenia or bipolar disorder) yet they lose touch with reality (Lieberman et al., 2000).

Furthermore, studies show that the symptoms of psychosis are triggered by dysregulation of dopaminergic activity in the brain this led to the discovery of the first antipsychotic agents in the 1950s. Studies worldwide also highlighted a critical window for treating schizophrenia. Early intervention leads to better outcomes. However, these same studies reveal a concerning gap between the onset of psychosis and receiving treatment. Data from over 10 studies across continents show an average delay of 1-2 years before treatment starts (Tost et al., 2010).

Prevalence

Schizophrenia influences about 24 million people or 1 in 300 around the globe. This ratio is 1 in 222 among elders. It's not as widespread as other mental illnesses. Inception is more frequent during the twenties and tends to occur primarily among men than females (Jaeschke et al., 2021). The encounter of psychotic experiences escalated at age 13-24, climaxing during late adolescence. Of 4000 individuals interviewed at 25 age, 314 had explicit psychotic episodes from age 12. Among 109 individuals met the criterion for a psychotic up to age 25, from whom 70% had sought professional assistance. Anticipation of recent psychotic disorder at age 24, by both self-claimed and interviewer-rate of psychotic encounters at age 18 was rectified by integrating information on frequency and distress although sensitivities were declined. World Health Organization (2022 January 10).

The average observation time was 433 days. At the 18th month, 38 subjects had experienced psychosis. The iIR of transitional to psychosis after 6, 9, 12, and 18 months was 7.05%, 11.0%, 14.00%, and 19.01%, respectively as being expressed in figure 1. The average duration to transit from baseline examinations was 497 days. Psychosis prognoses in accordance with *DSM-IV* were brief psychotic disorders (Sullivan et al., 2020).

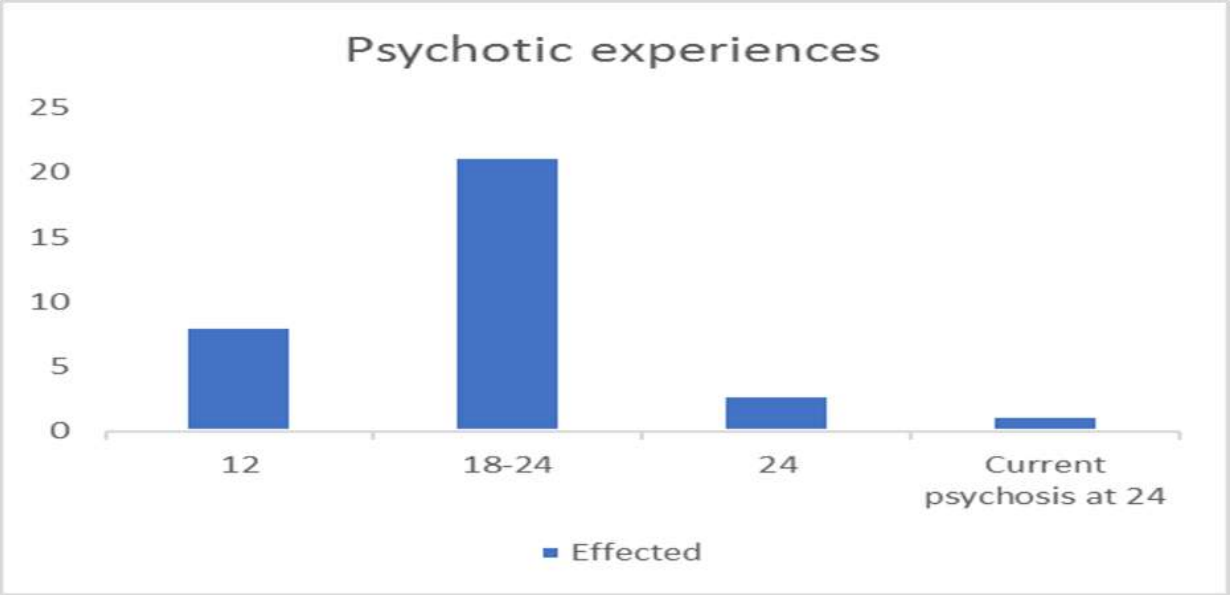


Fig. 1: Percentage prevalence of psychosis in relevance to age

Assessment of Psychosis

Identification of untreated psychosis has caught a great deal of attention from scientists as it is considered a modifiable condition impacting the quality of life of the individual and if treatment starts earlier, it maximizes its effectiveness. There are several ways an individual can be tested for psychosis. First, a self-report questionnaire that reviewed the attenuated symptoms of psychosis followed by a practitioner-administered structured interview of Psychosis Risk Syndromes (SIPS) which are frequently practiced (Kline and Schiffman, 2014). Secondly, scientists have made strides in identifying people who might develop psychosis. Tools like SIPS and CAARMS interviews help assess this risk by looking for subtle signs called "attenuated positive symptoms," which suggest a potential mental state known as an "at-risk mental state" (ARMS). Studies have shown these methods can predict psychosis onset within two years, with success rates ranging from 16% to 52%. However, despite this progress, there's still room for improvement. These approaches based on "attenuated positive symptoms" need further development before they can be safely and effectively used in everyday community settings (Klein et al., 2012). Lastly, in some cases, brain imaging may also be necessary to rule out medical-related psychosis-like symptoms. However, this happens in rare cases such as those related to certain tumors and brain dysfunctionality (Wiles et al., 2006).

Differential Diagnosis

An extensive number of pathologies is included in the differential diagnosis of psychosis some of these are listed below in Fig. 2.

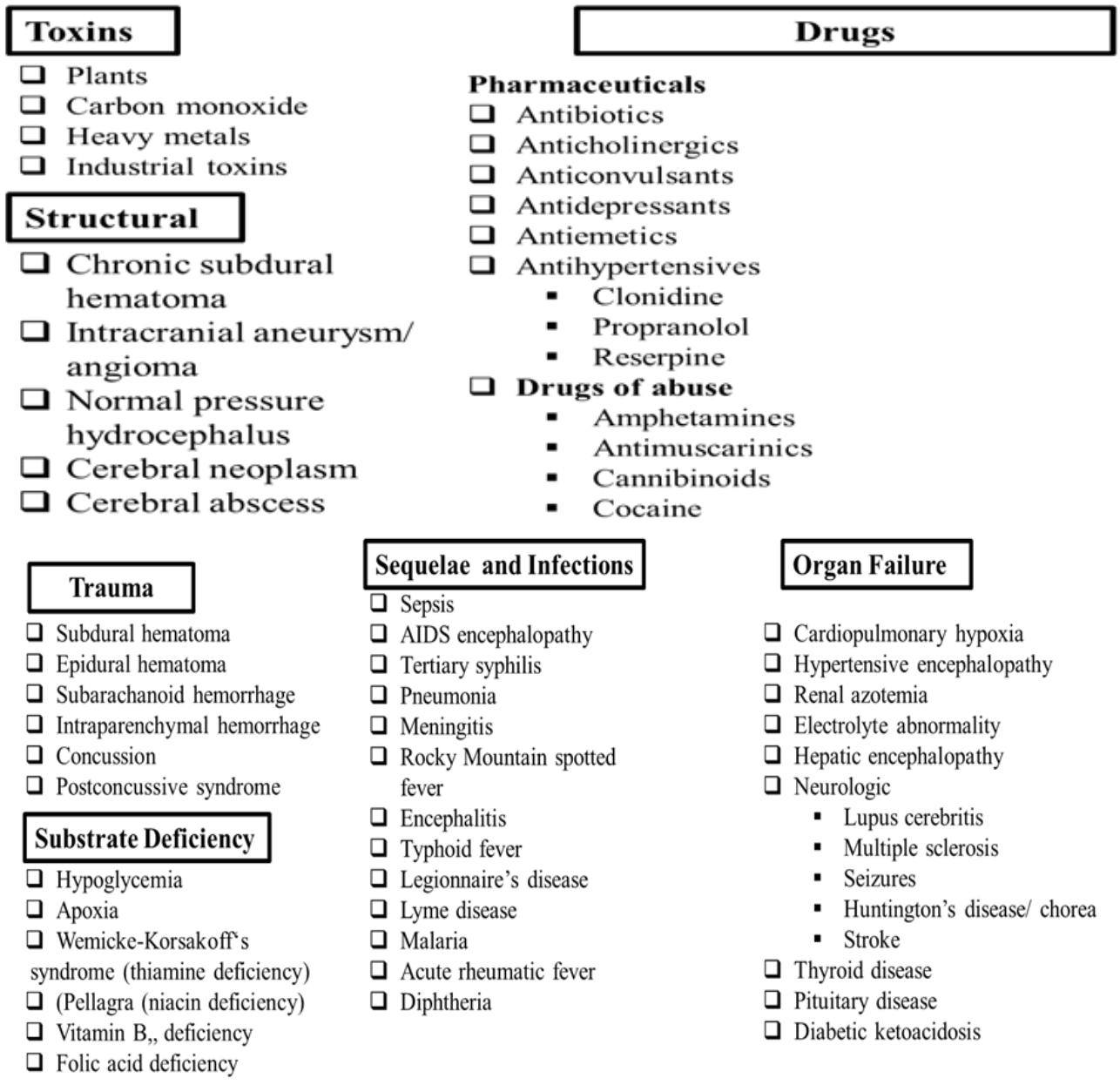


Fig. 2: Possible pathologies associated with differential diagnosis of psychosis

Trauma

Any kind of traumatic brain damage, no matter how severe, has the potential to cause behavioral changes that have psychotic characteristics (Evans, 1992). Older individuals may exhibit subtle, deferred, and unusual complaints, as well as intense and prolonged symptoms (Mazzucchi et al., 1992).

Drugs

Medicinally driven psychosis may be brought on by ingesting toxic substances, as well as by the use of OTC or prescribed drugs. This type of psychosis can be brought on by an excessive dosage, or an idiosyncratic response to a typical dosage (Ceti et al., 2019).

Toxins

Numerous harmful assaults by toxins can have an impact on a child's developing brain. Numerous chemicals work through anticholinergic and sympathomimetic pathways to produce their effects. A thorough list and explanation of cerebral, psychological, and cognitive conditions are outside the purview of this article (Kontzialis and Huisman, 2018).

Infections

Acute psychosis can appear in patients with systemic infections. A shift in personality or mental state, especially in older people, may be the initial indications of an infection (Mufaddel et al., 2014). While infections of the central nervous system are not as prevalent as pneumonia and UTIs in producing delirium, they may nonetheless, manifest subtly and have significantly higher morbidity and fatality rates (Fong et al., 2015). Acyclovir ought to begin empirically if encephalitis is diagnosed. The HIV patient poses a unique diagnostic conundrum. Individuals suffering with AIDS are prone to several different illnesses that can result in psychosis (Degano et al., 2019). Patients with HIV illness also frequently experience primary mental disorders (Larsson et al., 2009).

Psychiatric Illness

Individuals suffering from mental health disorders may arrive at the emergency department (ED) due to an acute episode or a worsening of their long-term sickness. In the emergency department, schizophrenia and mood disorders are frequently seen as psychiatric causes of psychosis. Individuals experiencing a significant depressive episode may also exhibit psychotic traits, especially in older individuals (Kessler et al., 2003). Typically, emergent functional (psychiatric) psychosis takes several days to months to manifest and culminates in the incident that sends the patient to the emergency department. A past medical history of sleeplessness, attention problems, anxiety, agitation, withdrawal from social circumstances, and paranoia may exist. Psychomotor hyperactivity or apathy, auditory hallucinations, emotional instability, and paranoid or grandiose delusions are all common in mental patients. Even if it develops slowly, acute psychiatric psychosis needs to be treated immediately. A healthcare initial evaluation and psychiatric evaluation ought to be completed while the patient's symptoms are under control in a secure setting; these patients shouldn't be released from the emergency department (Saarni et al., 2010).

Treatment

Treatment routes for mental ailments are mostly complicated, often interfering with traditional or religious healing beliefs. Traditional healers are mostly preferred for patients with anxiety or depression in the continents of India and Africa. However, patients with psychosis require additional bio-medical care. Biomedical includes medicinal and non-pharmaceutical cures like antipsychotic medications, recover-orientation care, and psychotherapeutics. Customary treatments are designated to tackle primordial epistemology (Singh and Madhavan, 2015). Customary healing practices do not hold training in medicine but are believed as health care in the local community, they employ animal floral substances and incorporate ideologies relating to psychological wellness and the harms of mental ailments (Lilford et al., 2020). Over the last twenty years, there has been a growing curiosity about how the mind contributes to mental conditions. The evolution of theory revolves around concepts like the theory of mind, mentalizations, reflective functioning, and metacognitions are supported by research. The induction of an array of renewed treatments aimed at addressing metacognitive deficits for patients presenting with psychosis, including schizophrenia spectrum disorders, demonstrates the renewed enthusiasm for addressing deficits associated with various of the most crippling neurological situations simultaneously (Lysaker et al., 2018).

Medically approved treatments include antipsychotic drugs, psychotherapy, and sometimes support therapies (mostly for people recovering from drug abuse).

Pathophysiology

There has been extensive research in the identification and understanding psychotic symptoms and related ailments. Parkinson's patients present with many symptoms sometimes intrinsic, extrinsic, or recurrence of both simultaneously. The most primeval factors linked with psychotic symptoms include dopaminergic medications, neurochemical and structural deformities, insomnia, visual processing defects, and brain stimulation disturbances (Zahodne et al., 2008). Figure 3 demonstrates the linkage of pathophysiology of psychosis in Parkinson's disease (PD) and involves the interaction of inherent and external variables.

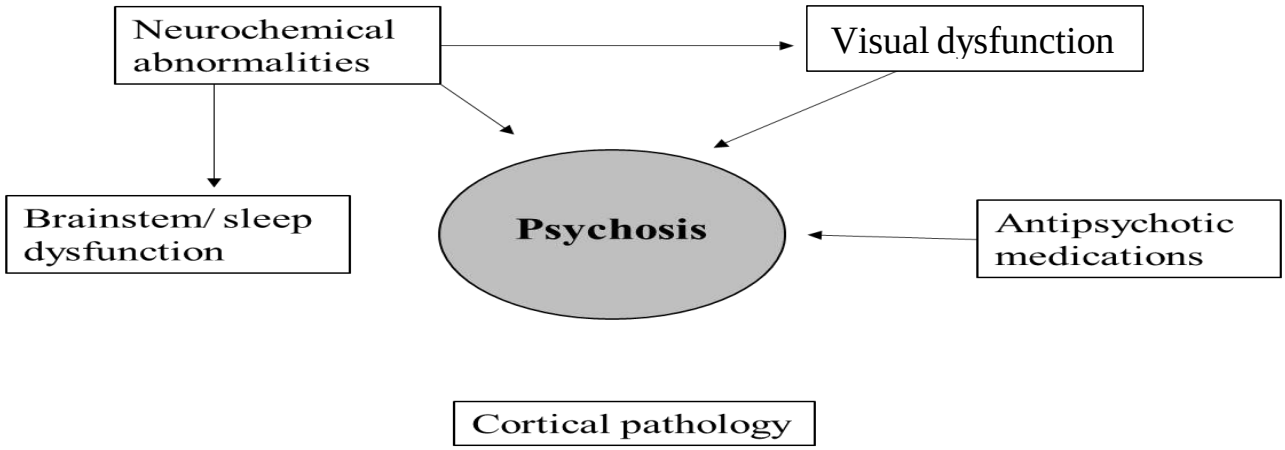


Fig. 3: The pathophysiology of psychosis in Parkinson's disease (PD) involves the interaction of inherent and external variables

Dopaminergic Medications

Dopamine's significance in the pathophysiology of psychotic symptoms has long been investigated. Conventional theories on the biology of psychosis in Parkinson's disease (PD) focused on the role of dopaminergic drugs as the causative agent (Friedman, 1991). Cocaine and amphetamine are examples of dopaminergic receptor agonists that can cause psychotic symptoms (Factor et al., 1995). On the other hand, dopamine is assumed to act in the biological process of action of certain antipsychotic medications that are used to lessen the prevalence of similar symptoms occupancy of D2 receptors (Goetz et al., 1982).

Psychotic traits have been linked to all PD drugs, not just levodopa, and they frequently go away when drug therapy is cut back on or stopped. The idea that psychosis arises as a side effect of antiparkinsonian medication is supported by clinical experience. The initial factor linked to the onset of psychosis in people with Parkinson's disease (PD) was the use of dopaminergic drugs, and it seems that patients are more vulnerable to dopamine receptor agonists in particular (Kapur et al., 2000).

Antipsychotic Agents

"Atypical", antipsychotics cause fewer, milder extrapyramidal side effects and lower serum prolactin levels as compared to first-generation or traditional antipsychotics (Keltner and Johnson, 2002). Elevations, maybe a result of antagonistic dual serotonin-dopamine receptors. Because of this, second-generation antipsychotics are usually the recommended course of treatment for PD-related psychosis (Noel, 2007). Nonetheless, the US FDA has required that all producers include a boxed warning on product labels noting that the use of these drugs in elderly dementia patients has been linked to an increased risk of death (Schneider et al., 2005).

Antipsychotic drugs affect the neurotransmitter system of the body. Their main interaction is with dopamine receptor subclasses D_2, D_3, and D_4. Subclasses D_2 and D_3 are of greater importance. However, it is found that antipsychotics that block 80% of dopamine receptors produce extrapyramidal side effects. The antipsychotic drugs act as agonists of D_2 and D_3 receptors. Before the 1950s there only used to be supportive or institutional care for the patients. Then in 1952, chlorpromazine was discovered and the first class of antipsychotics was introduced. They were first, however, developed as synthetic antimalarial drugs.

There are six classes of First-Generation Antipsychotics (FGAs) based on their structures. There are the phenothiazines, which are further subdivided into diphenylbutylpiperidine (pimozide), aliphatic (chlorpromazine), butyrophenones (haloperidol); piperazine (trifluoperazine), and piperidine (thioridazine); thioxanthines (thiothixene); dibenzoxazepines (loxapine); and dihydroindolones (molindone) (Strange, 2001).

The mechanism of action of all these FGAs is similar that is blocking the dopamine receptors resulting in reduction of forebrain activity and signaling. It may also cause the effects of dizziness. Additionally, reduction of dopamine activity can also result in Parkinson's like symptoms experienced by patients. These symptoms were not experienced by patients who took second generation antipsychotics that are: asenapine, clozapine, iloperidone, lumateperone, lurasidone, olanzapine, pilaperidone, quetiapine, risperione and ziprasidone (Hudepohl et al., 2012).

Clozapine

A 2007 meta-analysis found that the only 2nd generation antipsychotic entirely approved for the cure of psychosis in PD is clozapine, a derivative of dibenzodiazepines. Many exposed-label and double-blind placebo-controlled studies have shown that clozapine is both effective and tolerable in people with Parkinson's disease (Frieling et al., 2007).

Clozapine is noted to produce agranulocytosis in 0.38% of patients, despite its proven efficacy (as determined in a sample of over 99 000 US patients with schizophrenia). That's why, its frequent use is avoided (Honigfeld et al., 1998).

Because of this, when using clozapine, monitoring white blood cell counts needs to be done every week for the first six months of treatment and every two weeks after that. Side effects from clozapine that are more commonly seen include sialorrhea, orthostatic hypotension, and drowsiness. In addition, the use of second-generation anti-psychotics in individuals with schizophrenia has been linked to a condition known as the "metabolic syndrome," which is characterized by aberrant glucose metabolism, insulin resistance, weight gain, and dyslipidemia (Holt et al*.,* 2004).

In conclusion, the evidence is consistent with the use of low-dose clozapine to treat PD patients' psychosis. But because clozapine treatment necessitates close observation, efforts are being made to find a more workable medication to treat PD-related psychosis (Pollak et al., 2004).

Aripiprazole

Aripiprazole, sometimes referred as the precursor of third wave of antipsychotics, is partially an agonist that acts on both D2 and 5-HT1 receptors, in contrast to first- and second-generation antipsychotics, which are D2 antagonists antipsychotic agents. Its high 5-HT2/D2 affinity ratio is also thought to confer a comparatively reduced likelihood of extra-pyramidal side effects (Stahl, 2001). Aripiprazole's effectiveness and stability in people with Parkinson's disease appear to vary, according to the studies that are currently accessible. Based on the existing data, it appears that aripiprazole has a significant risk of side effects and has variable efficacy for some individuals with PD psychosis (McGavin and Goa 2002).

Risperidone

Risperidone, a drug that is structurally distinct from clozapine and was initially approved as an "atypical" antipsychotic in the United States, has since been proven to have more characteristics of a "typical" antipsychotic, such as a dependent on dosage prevalence of extrapyramidal adverse reactions and prolactin elevation (Grant and Fitton 1994). Risperidone investigations for people with Parkinsonism are almost exclusively open-label. As a whole, the studies show that psychosis has significantly improved (Ford et al., 1994). Findings on negative effects on motor function, however, differ significantly between research (McKeith et al., 1995*).* According to certain investigations, every patient receiving risperidone experiences substantial motor deterioration (Rosebush and Mazurek, 1999).

The majority of findings on risperidone are open-label, which is partly responsible for the variation in findings across research. Some of the possible supplementary treatments involved against psychosis are enlisted below in figure 4. Varied trials used varied titration regimens and observation lengths, and doctors may have differed in their abilities to diagnose Parkinsonism. Physicians are cautious about using risperidone for managing PD psychosis due to the drug's "typical" antipsychotic behaviour and multiple instances of motor deterioration in those with Parkinson's receiving the treatment (Rustembegovic et al., 2006).

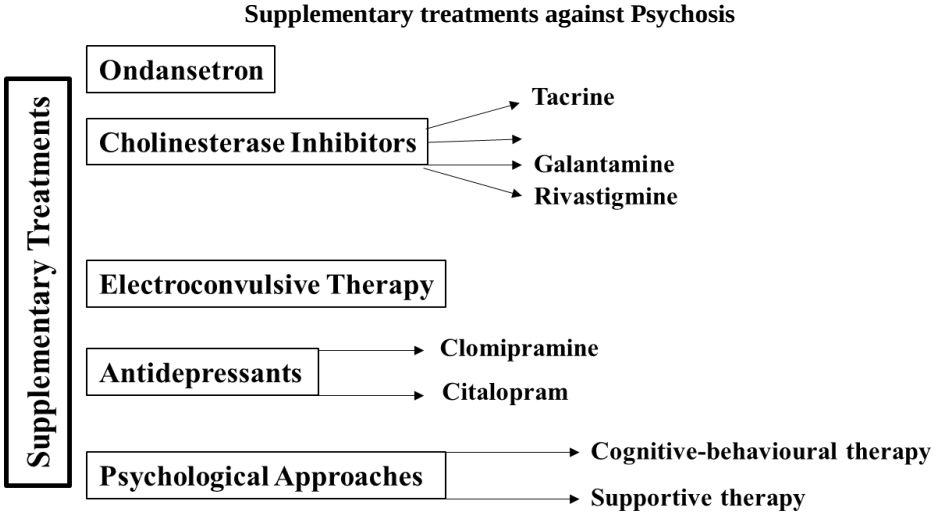


Fig. 4: Supplementary treatments against psychosis

Extrapyramidal Intricacy Associated with Antipsychotic Drugs

As mentioned before, FGAs were recorded to cause neurological disorders due to dopamine reduction such as Parkinson-like symptoms. 'Haloperidol' is reported to have movement disorders due to its property of binding tightly to dopaminergic neuroreceptors. Weaker-binding first-generation antipsychotics, and even the second-generation antipsychotic clozapine, are particularly prone to causing anticholinergic effects such as constipation, dry mouth, blurry effect, tooth decay, urinary retention and sometimes cognitive impairment. 2nd generation antipsychotics mainly clozapine and olanzapine create more metabolic disorders such as obesity and type 2 diabetes. Some of the cognitive impairments interlinked with antipsychotic drugs are portrayed in figure 5. However, all the antipsychotics are likely to show effects of sedation, cardiac arrhythmia, sexual dysfunctionality, postural hypotension, and sudden cardiac death (Muench et al., 2010).

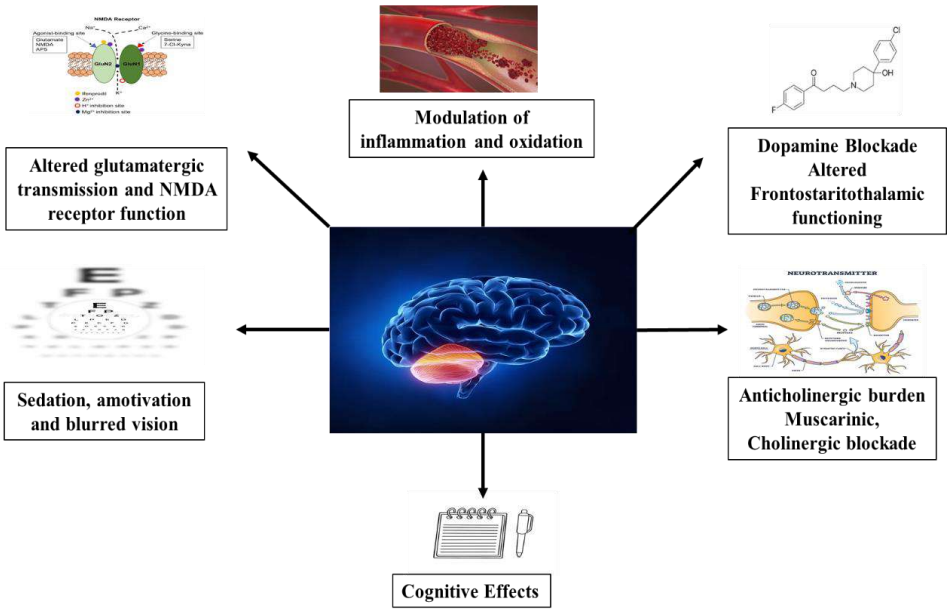


Fig. 5: Antipsychotic-associated cognitive impairment

Antipsychotic Drugs in Weight Gain Induction

Instead of increasing muscle mass, the body weight gain brought about by these medications is most likely the result of the increased accumulation of fat. According to a prompt study, people on antipsychotic medications had higher waist-to-hip ratios, which is an indicator of the formation of abdominal fat. According to an investigation, subcutaneous as well as troublesome intra-abdominal fat was found to be significantly increased in formerly drug-naive patients consuming antipsychotic medicines (Zhang et al., 2004) that used magnetic resonance imaging measures. Patients on antipsychotics exhibit a considerable increase in leptin. This is not surprising because patients taking antipsychotic medications and exhibiting weight gain typically respond to fat deposition by increasing their release of leptin (Jin et al., 2008).

Antipsychotic Drugs as Inducers of Diabetes

A documented result of obesity and metabolic syndrome is an elevated chance of developing diabetes; there is no reason to believe that psychiatric patients would not have a similar association taking antipsychotic medications in the same way as the general public. Clozapine and olanzapine are linked to an increased risk of diabetes, according to a recent meta-analysis involving over 270,500 subjects (Newcomer, 2007). However, treatment with quetiapine or risperidone does not appear to be associated with an increased risk of diabetes compared to patients receiving conventional care or no treatment at all. Though antipsychotic medication-induced excess weight and metabolic syndrome can lead to serious complications such as diabetes, there have also been cases of diabetes and rapid-onset glucose intolerance in individuals receiving antipsychotic medication who do not have obesity. An immediate metabolic affect unrelated to weight gain is not common, although it can result in a potentially catastrophic ketoacidosis. Notably, olanzapine and clozapine, the two antipsychotic medications that induce the most weight gain, are also specifically linked to this fast-onset diabetes. Thus, certain cases of diabetes are unrelated to the use of antipsychotic drugs, even though these conditions will inevitably raise the risk of glucose intolerance and type II diabetes due to weight gain and obesity (Best et al., 2005). The two antipsychotic medications that most strongly impact weight gain are also most associated with impaired glucose regulation, which may indicate a shared pharmaceutical mechanism. However, the pathophysiological mechanisms underlying both detrimental effects which are mutually exclusive must be distinct. The antipsychotics' sedative properties, which are partly caused by antagonistic actions at histamine H1 and α1 adrenergic receptors, may also lead to a decrease in activity and a spike in body fat. According to certain lab research, these medications may also affect the activity of pancreatic beta cells (Reynolds et al., 2010).

Antipsychotic Drugs Effect on Autonomic System

The autonomic nervous system, which is comprised of the sympathetic and parasympathetic nervous systems, regulates the functioning of the cardiovascular systems (Galanter and Lowenstein, 2008). Antipsychotic medications may affect the cardiovascular system and circulatory system directly by blocking adrenergic and cholinergic nerve receptors (Agelink et al., 2001). They may also have a secondary impact on these organs via central autonomic control and baroreceptor impulses (Mackin, 2008). In a particular investigation, methoxamine, an α1-adrenoceptor agonist, markedly elevated blood pressure in the rats administered with chronic thioridazine when compared to controls after a four-day washout time frame. This suggests that α1-adrenoceptors are either overexpressed or more sensitive, potentially as a result of antipsychotic-induced receptor blockade (Elman et al., 2004). it is incredibly intriguing to note that sympathetic hyperactivity is linked to the possibility of cardiac arrhythmias and the likelihood of QT lengthening in certain antipsychotic therapies (Leung et al., 2012).

Herbs against Psychosis

Despite the progress in medication over the years the pharmacological treatment of psychotic disorders is often unsatisfactory. Psychotic symptoms are often partially resolved especially cognitive and negative symptoms. Other than clozapine, SGAs are as effective as FGAs for positive symptoms. Many patients continue to experience persistent symptoms due to inconsistency in their medication schedule or due to adverse effects of these medications.

Many people prefer the use of nonconventional medication to decrease the symptoms without the adverse effects either by changing their lifestyle or by using alternative medicines. Alternative medicines comprise of diagnostics, treatments and prevention strategies. This is different from biomedicine. People prefer plant or herbal source medicine hoping for minimal adverse effects however, this is not always as depending on the source natural can also be toxic to human or can cause undesired effect combined with prescription medicine (Hoenders et al., 2018).

Plants

Plants contain several phytochemicals that can play a role in enhancing organ functions to overcome occurrences of many diseases. Many plants have been researched for their medicinal value present in their roots, leaves or stems. Many modern medicines used today were also discovered from observing traditional methods of indigenous people. In a research in Bangladesh it was found that the natives used 14 formulations from 15 plants to cure psychosis episodes in schizophrenic patients. The Lamiaceae and Rubiaceae and families supplying 2 plants while other contributed single plants. The numerous floral parts were employed like leaflets, bark, trunk, blooms, seedlings, and ripen ovary and roots. The investigations demonstrated that various parts or sole plant species are employed in drugs in Bangladesh. Leaflets contributes most portion, forming 47.64% of whole application. Barks and seedlings each contributed 14.31% of total application. The other plant parts mentioned constituted, respectively, 9.50, 4.75, 4.761, and 4.760% of total use as figured in diagram 6. In Bangladesh, Traditional Medicinal Practitioners (TMPs) have employed various plants for generations to manage symptoms. Their knowledge came from centuries of practice and most of the practitioners had their own list of medicinal plants for particular disease or disorders. Most of the medicines were seeds to be ingested with water. Paste of herbs to be eaten with rice or mostly squeezed out juice. Other methods included inhaling plants for calming effects. TMPs also believed that using single kind of herb to cure a disease is not enough so, they used mixed herbs into a medicine.

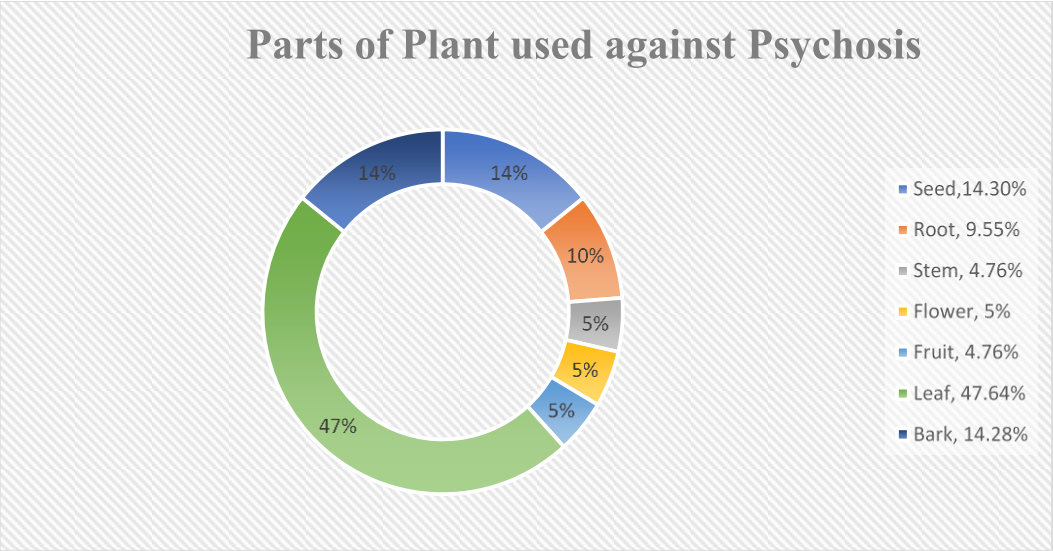


Fig. 6: Parts of medicinal plants used to treat schizophrenia-like psychosis

Since the dawn of civilization, people have used plants as medicine all throughout the world. The safe, affordable, and increasing popularity of using plants for therapeutic purposes is used for its efficient application. Several plants that have previously been studied by various investigators for their antipsychotic potential have been mentioned in this study. Behavioral research on plants has produced a rare chance to discover novel psychotic drug therapies. Clinical trials should be conducted to better assess the herbal extracts and ingredients that have shown psychotherapeutic effects in animal models. Certain nutritional supplements, like omega-3 oil from fish and antioxidant vitamins, can also assist with alleviating psychosis episodes (Yadav et al., 2015).

Euphorbia neriifolia (Crested Indian Spurge tree)

A species of spurge called *Euphorbia neriifolia* was first identified by Carl Linnaeus in 1753. In animal models, a study found that the hydroalcoholic extract of *Euphorbia neriifolia* leaves has antipsychotic, antianxiety, and anticonvulsant properties (Bigoniya and Rana, 2005). A different study's finding revealed that the ethanolic extract of *Randia dumetorum* Lam fruits does not have antidopaminergic or antiserotonergic properties, although exhibiting an anticataleptic effect. Catatonic schizophrenia is typically linked to cataleptic seizures (Ghaisas et al., 2008).

***Aegle marmelos* (Bael)**

In albino mice, a methanol extract from *Aegle marmelos* leaves showed possible anxiolytic and antidepressant effects. It may possibly be a useful source for natural psychotherapy agents (Kothari et al., 2010). *Aegle marmelos* is a well-known ayurvedic medicinal tree in India that is used to treat nerve disorders and as a brain tonic (Maheshwari and Singh, 1984).

***Ocimum americanum* (Lime basil)**

Ocimum americanum had calming properties (Boskabady et al., 2006). It is thought to be advantageous for people with mental illnesses. The Tonchongya tribe of Bangladesh used a combination of *Brassica juncea* seeds, *Ocimum americanum* renders juice and and *Acorus calamus L.* (Acoraceae) leaves to treat people with mental illnesses and those who were possessed by evil spirits. While it is controversial whether or not such entities are present any kind of possession by "evil spirits" can be regarded a form of mental disorder with someone in an episode of delirium (Rashid et al., 2012). *A. calamus* has also been used to treat schizophrenia and is a component of the Ayurvedic medicine system brahmyadiyoga. The financial burden of therapy is a significant cause for worry because Ayurvedic remedies are more affordable than chlorpromazine and, consequently, more readily available to the impoverished than newer unusual antipsychotics. Some of these are enlisted below in table 1. (Agarwal et al., 2010).

While undergoing this traditional knowledge we can get an extraordinary glimpse into historical practices, it's important to acknowledge the limitations. Scientific research is lacking on the effecacy and safety of these plants in the treatment of psychosis. In fact, some plants, like *Thevetia peruviana*, can prove to be toxic and pose serious health risks.

While many of these plants have not yet been scientifically researched but they hold the potential to be effective against psychosis such as:

- *Aegle marmelos* (leaves) exhibit potential anxiolytic (anti-anxiety) and antidepressant effects in mice.
- *Vitex negundo* (roots) may possess antidopaminergic properties, suggesting a possible role in reducing movement problems caused by other medications used for psychosis.
- *Cannabis sativa* (specifically the CBD compound) shows promise for antipsychotic effects, but further research is necessary to confirm its safety and efficacy in humans.

(Ahmad et al., 2014).

Table 1: Medicinal plants used by practitioners to treat psychosis

1	Leaves of *Piper retrofractum* (Piperaceae) are enmeshed then taken up orally until fully cured.	Leaf	*Piper retrofractum* Vahl/Choi
2	Extracted juice from softened roots and leaves of *Ficus hirta* (Moraceae) is taken per os. until fully cured.	Leaf, root	*Ficus hirta* Vahl./Pakur
3	A paste of combined bark and seed of *Thevetia peruviana* (Apocynaceae) is taken until fully cured.	Bark, seed	*Thevetia peruviana* (Pers.) K. Schum./Holde Korobi
4	*Datura metel L.* (Solanaceae) leaves can be crushed and ingested as a paste, which is or the flattened leaves can be applied to the nose	Leaf, seed	*Datura metel* L./Dhutura
5	Juice squeezed from *Euphorbia neriifolia* (Euphorbiaceae) leaflets is taken per os.	Leaf	*Euphorbia neriifolia* L./Monshaseez
6	The nectar that comes from the macerated bark of the *Rubiaceae* plant, *Randia dumetorum*, is obtained, evaporated, and ground into a powder that is combined with glucose. The combination is prepared into five gram tablets, which are consumed until they cure.	Bark	*Randia dumetorum* (Retz.) Lam/Monkata
7	Smashed leaves and roots of *Aegle marmelos (Rutaceae)* are used to make tola-sized tablets. For three days, the pills are taken twice a day.	Leaf, root	*Aegle marmelos* (L.) Corr./Bel
8	*Litsea polyantha* (Lauraceae) outer part is decocted with half gram of sweet to extract its pulp, which is then taken by mouth.	Bark	*Litsea polyantha* Juss./Uruijja, Menda
9	Leaves of *Coccinia grandis* (Cucurbitaceae) are cooked in hot waters and then decoction obtained is drunk orally.	Leaf	*Coccinia grandis* (L.) J. Voigt/Telakuchi
10	Seeds of *Brassica juncea* (Cruciferae) are combined with leaflet juice of *Ocimum americamum* (Lamiaceae) and taken orally until fully cured.	Leaf, seed	*Ocimum americanum* L./Radha tulshi *Brassica juncea* (L.) Czern./Shorisha
11	Aroma of flower of *Abroma augusta* (Malvaceae) is sniffed through nostrils or crushed stems are eaten with *Aloe vera* and glucose two times weekly till cured fully.	Flower, steam	*Abroma augusta* L.f./Ulot Kombol
12	Leaves of *Vitex negundo* (Lamiaceae) are taken with body. Another solution is to take tablets early in the day made from the leaflets.	Leaf	*Vitex negundo* L./Nishinda
13	Leaves from *Cannabis Sativa* (Cannabaceae) are used as oil then massage over scalp until recovered. If a patient is in severe condition then the leaves were used to make steam and the steam is inhaled in by nostrils.	Leaf	*Cannabis Sativa* L./Bhang, Siddhi
14	Ripe fruit of *Morinda citrifolia* (Rubiaceae) are taken as raw and mashed leaves are taken as vegetable.	Fruit, leaf	*Morinda citrifolia* Linn./Holdi Kachu, Noni

Plants and their benefits

Kingdom	Order	Family	Genus	Specie
Plantae	*Caryophyllales*	*Amaranthacae*	*Spinacia*	*S. oleracea*

Spinacia oleracea also known as spinach.

Rich in antioxidants, neuroprotective, anti-epileptic, anti-Alzheimer and anti-inflammatory effects.

Attenuated induced stereo typed behavioral symptoms in mice.

Restored total brain proteins, reduced dopamine level, acetylcholinesterase activity and TNF - alpha, increased levels of GABA and reduced glutathione.

Fig. 7: Classification and Medicinal properties of *Spinacia oleracea*

Kingdom	Order	Family	Genus	Specie
Plantae	*Myrtales*	*Combretaceae*	*Terminalia*	*T. macroptera*

Terminalia macroptera Guill and perr. (Combretaceae) is a commonly used African medical plant.

7 consecutive treatments of ethyl acetate from *T. macroptera* significantly attenuate stereotyped behavioral symptoms from mice induced with ketamine and apomorphine for psychosis effect. It showed protective effects in psychosis.

Fig. 8: Classification and Medicinal properties of *Terminalia macroptera*

Kingdom	Order	Family	Genus	Specie
Plantae	*Asterales*	*Asteraceae*	*Crassosphalu*	*C. bauchiense*

Crassocephalum bauchiense (Hutch) has many medical uses. Used to treat epilepsy, cerebral malaria, cerebral deficit, anxiety and behavioral disturbances. Likewise, it is used to treat insomnia, psychosis and other CNS disorders

An apomorphine induced psychosis animal was observed with its leaves extract. The results observed showed that it possibly mediated via blocking Dopamine D2 receptors and GABAergic activation.

Fig. 9: Classification and Medicinal properties of *Crassocephalum bauchiense*

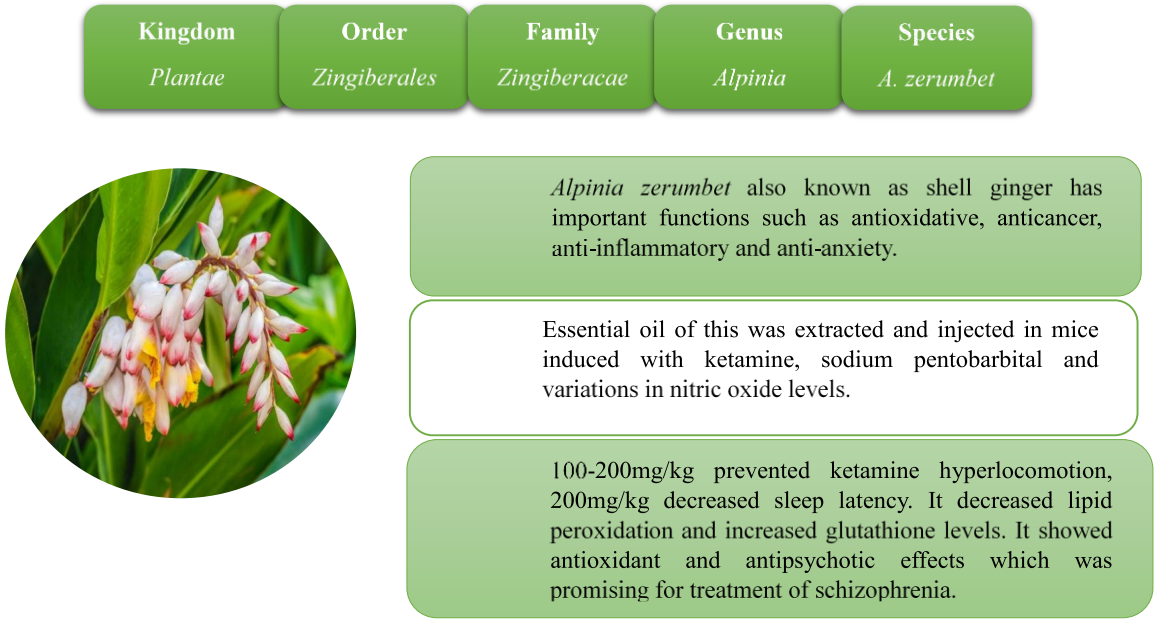
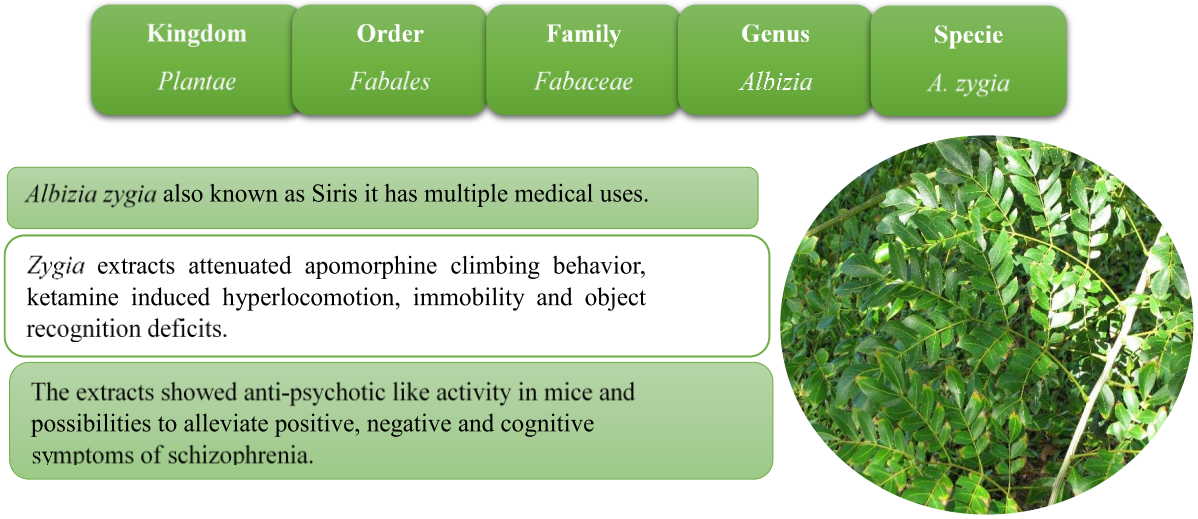


Fig. 10: Classification and Medicinal Properties of *Alpinia zerumbet*



(Otimenyin and Ior, 2021).

Fig. 11: Classification and Medicinal Properties of *Albizia zygia*

Conclusion

Use of antipsychotic drugs over the years has proven effective for the treatment of psychosis however, has also shown great adverse effects due to which patients prefer the use of herbal medication or changes in their lifestyles. Scientific literature also includes information on some of the benefits of herbal medicine that showed promising effects against psychosis. Moreover, research conducted in Bangladesh had also resulted in a list of plants that proved effective for the symptoms of psychosis and other mental disorders but still need to be medically or scientifically researched.

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Chapter 11

Genomic Insights: Predicting Obesity through AI and Machine Learning

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ABSTRACT

Obesity is a complex chronic disease characterized by an excessive accumulation of body fat that leads to adverse health outcomes and is considered a global epidemic by the World Health Organization (WHO). The imbalance between energy intake and expenditure results in the storage of excess fat in adipose tissues, causing hyperplasia and hypertrophy, ultimately disrupting normal body mass regulation. Obesity is intricately linked to the development of various diseases such as diabetes, coronary heart diseases, respiratory complications, cancers, and osteoarthritis, exacerbating healthcare systems and economies globally. Sedentary lifestyles, unhealthy dietary habits, and socio-economic disparities play a significant role in the prevalence of obesity. This emphasizes the need for urgent and coordinated efforts to promote healthy lifestyles and create supportive environments for weight management and overall well-being. Addressing obesity-associated complications through sustainable changes in diet, physical activity, and sleep, along with engaging families, is crucial for effective obesity management and prevention of related social, behavioral, and psychological issues.

KEYWORDS
Obesity, Artificial Intelligence, Alternatives, Genes

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INTRODUCTION

Obesity is a chronic disease and due to its high prevalence, it is considered a global epidemic. The imbalance between the food needed for the normal and healthy functioning of the body and the food ingested and utilized through the different body activities of a person leads to the development of obesity. The energy from the extravagant food is stored in the fat cells, causing an increase in their number and size, i.e., hyperplasia and hypertrophy, respectively. The superfluous fat cells in turn agitate the normal body mass (Bray, 2004).

According to the World Health Organization (WHO), obesity is defined as "abnormal or excessive fat accumulation that presents a health risk" (WHO, n.d.-a). The body weight is governed by an intricate interplay of different complex regulatory mechanisms, homeostasis, and emotional and cognitive processes. All these mechanisms alter greatly when the body weight exceeds in uncontrolled manners. All these mechanisms undergo significant alterations when the body weight exceeds beyond control (Kansra et al., 2020). Obesity aggravates many maladies, both independently and in liaison with other diseases. Conspicuously it is involved in the development of diabetes mellitus, coronary heart diseases (CHD), gallbladder disease, respiratory complications, different types of cancers, and osteoarthritis (Kopelman, 2000). The contributing factors include sedentary lifestyles, unhealthy dietary habits, environmental influences, and socio-economic disparities. Obesity is intricately linked to an increased risk of chronic diseases such as type 2 diabetes, cardiovascular disease, certain cancers, and mental health disorders, placing a significant burden on healthcare systems and economies. Urgent and coordinated efforts are essential to address the multifaceted determinants of obesity, promote healthy lifestyles, and create supportive environments conducive to weight management and overall well-being.

These medical problems also entail many social, behavioral, and psychological problems. Obesity could be treated by addressing the obesity-associated complications, engaging families, and cultivating sustainable changes in diet, physical activities, and sleep (O'Connor et al., 2017).

Global Prevalence and Impact on Public Health

Obesity is a persistent condition described by an overabundance gathering of muscle-to-fat ratio, prompting adverse consequences for well-being. It is normally characterized by utilizing the weight record (BMI), determined as a singular load in kilograms partitioned by the square of their level in meters. As indicated by the What obesity's identity is, named a BMI of 30 or higher, with extreme obesity delegated a BMI of 35 or higher (Ng et al., 2014).

The predominance of heftiness has been consistently expanding around the world, with critical ramifications for general wellbeing. In 2016, more than 1.9 billion grown-ups were overweight, and of these, north of 650 million were corpulent. The commonness of obesity differs by district and financial status, with higher rates seen in created nations and among populaces with lower financial status. Obesity is a significant gambling factor for a few ongoing sicknesses, including cardiovascular illnesses, type 2 diabetes, outer muscle problems, and specific sorts of disease. Tending to the commonness of heftiness is in this way a basic general wellbeing needs to diminish the weight of related illnesses and further develop by and large wellbeing results. Weight has formed into an overall pandemic, which has grave repercussions for the soundness of everybody. One meaning of stoutness presented by the World Wellbeing Association (WHO) depicts it as an unusual or exorbitant development of fat that represents a danger to one's wellbeing (*WHO*, n.d.-b). The World Wellbeing Association distinguishes it as a complicated problem that is influenced by different factors, including hereditary, natural, and social variables. Because of the way that nourishment has a major impact in both the turn of events and control of obesity, one of the main parts of stoutness the board is acquiring information on the dietary propensities for individuals (Ng et al., 2014).

Need for Innovative Approaches to Tackle Obesity

The growing obesity crisis demands novel strategies that go beyond traditional techniques. When it comes to tackling the intricate interactions between genetic, environmental, and socioeconomic factors that contribute to obesity, traditional techniques frequently fall short. To tackle this intricate problem, innovative methods must use technology advancements, employ big data analytics to offer customized interventions and advocate for legislative modifications that promote hygienic environments. To co-design and execute sustainable solutions, a range of stakeholders must be involved, including legislators, healthcare providers, and impacted communities. By adopting innovative approaches, we can transform the way obesity is managed and prevented, leading to significant improvements in public health outcomes (Sumro et al., 2021).

Role of Genetics in Obesity

It is becoming more widely acknowledged that genetic factors play a major role in the development of obesity, affecting a person's vulnerability to weight gain as well as how they respond to dietary and lifestyle changes (Satia, 2009). These inherited tendencies combine with environmental factors to determine a person's susceptibility to obesity and the related health risks.

Numerous genes linked to obesity have been found through genetic investigations, illuminating the complex interactions between metabolism, genetics, and control of adiposity. The FTO (fat mass and obesity-associated) gene, which influences energy metabolism and the formation of adipose tissue, is one of the most well-known genes linked to obesity. Variations in the FTO gene have been linked to a higher body mass index (BMI) and an increased risk of obesity in several populations. Additionally, two genes involved in controlling appetite - leptin and ghrelin - play a crucial role in controlling signals of hunger and fullness, which in turn influences food consumption and the balance of energy. Individuals who possess genetic variations in specific appetite-regulating genes may be more vulnerable to overindulging and gaining weight (Bandura, 2004). Furthermore, genes associated with energy expenditure - such as uncoupling proteins - have an impact on thermogenesis and metabolic rate. These factors also have an impact on overall energy balance and weight control.

Adipogenesis-related genes that affect the distribution and storage of fat, such as adiponectin and peroxisome proliferator-activated receptor gamma (PPARγ), control adipocyte formation and lipid metabolism. Despite significant progress in understanding the genetic basis of obesity, it is important to remember that genetic factors alone do not determine an individual's risk of obesity. Environmental factors such as diet, exercise, financial status, and cultural influences can affect a person's weight trajectory (*Articleobesity.Pdf*, n.d.). Gene-environment interactions, which modify the way genes associated with obesity, are expressed in response to environmental cues, further muddy the genetic-obesity relationship. Lastly, genetic factors exacerbate the complexity of the obesity etiology, highlighting the necessity for comprehensive approaches that integrate behavioral, environmental, and genetic factors into obesity prevention and treatment programs. Understanding the underlying genetic causes of obesity may eventually improve obesity treatment and preventative outcomes by enabling the development of tailored therapies and medications based on each individual's genetic profile (Sahu et al., 2022).

Understanding the Heritability of Obesity

Understanding the heritability of obesity requires figuring out how much genetic variation contributes to individual differences in body weight and fat accumulation. Heritability estimates provide insight into the genetic component of obesity risk in a community by displaying the proportion of phenotypic variance attributable to genetic variation. Research on twins and families has consistently demonstrated a significant genetic impact on obesity susceptibility, with heritability estimates ranging from 40% to 70%. These findings demonstrate the

important role that genetic factors play in influencing a person's propensity to become obese. That being said, since environmental factors also play a significant role in the development of obesity, heritability does not imply determinism. The further modification of obesity risk by gene-environment demonstrates the complex link between genetic predispositions and environmental exposures (Chawla and Davis, 2013).

Convergence of Genomics Technology

The advances in genomics and artificial intelligence (AI) have brought about dramatic improvements in the fields of biotechnology, agriculture, and healthcare. Combining genomics and AI enables a paradigm shift in biomedical research and tailored treatment by revealing hitherto undiscovered details about the complex relationships between genes, environment, and disease. The ability to examine enormous genomic datasets at a speed, precision, and depth never before feasible is made possible by this convergence, which facilitates the discovery of novel genetic variants, biomarkers, and therapeutic targets (Singh et al., 2022). By applying AI techniques like machine learning and deep learning, researchers may identify patterns and prediction models from genetic data. This procedure promotes innovation in diagnostics, pharmaceutical discovery, and precision healthcare (*Articleobesity.Pdf*, n.d.).

Significance of Leveraging Technology for Personalized Healthcare

In the fast-paced world of modern technology, the delivery of healthcare has changed with the integration of cutting-edge tools and platforms, particularly in personalized medicine. Using technology, healthcare providers can tailor interventions and treatments to the specific needs of each patient, improving patient care and optimizing results. The current paradigm shift towards individualized healthcare presents numerous opportunities to effectively serve the complicated needs of diverse patient populations and improve overall health outcomes (Guni et al., 2021).

Precision Diagnostics

Wearable technology, imaging modalities, and genomic sequencing are a few examples of technology-enabled diagnostic technologies that provide medical professionals with accurate information on a patient's physiological parameters, genetic makeup, and disease risk factors.

In particular, genome sequencing has made genetic variants linked to illnesses more visible, which has made it possible to customize treatment regimens and diagnose patients with greater precision (Chawla and Davis, 2013).

Targeted Therapeutics

Technological advancements have enabled the development of targeted medications that are tailored to an individual patient's genetic profile, illness subtype, and response to therapy. Pharmacogenomics is a useful tool for designing personalized treatment plans since it enables medical professionals to identify genetic variations that affect drug efficaciousness and metabolism. The choice of medication and dosage can then be optimized using this information (Dias and Torkamani, 2019).

The Interplay between Genetics and the Environment

Epidemiological studies have highlighted several modifiable risk factors for obesity, including consumption of sugar-sweetened beverages, fried foods, poor diet quality, sedentary lifestyle, and physical inactivity. There is growing evidence that these environmental risk factors can influence the genetic risk of obesity and related diseases. Recent Genome-Wide Association Studies (GWAS) have emphasized the importance of considering interactions between genes and the environment in the discovery of new genetic variants linked to obesity (Kamal and Razia, 2018).

Integrating Nutrigenomics into Obesity Management

Understanding the role of nutrigenomics, diet, and exercise is essential to address obesity and its associated health issues. Nutrition plays an eminent role in health management and preventing obesity-related diseases. Nutrition has a positive correlation with the health and well-being of the individual as it is the factor that could prevent the onset and even cure the ailments. The study of the different genetic responses in retaliation to certain nutrients, and their association with disease, is termed "nutrigenomics". This protocol could be used to provide personalized nutrition recommendations based on the genetic makeup of the individual. This approach presents a novel blueprint to improve health and manage obesity by regulating the dietary factors only (Salma et al., 2019).

Personalized nutrition emerged as a promising approach to address obesity and its related medical conditions. It tailors the nutritional suggestions depending upon individual genetic, metabolic makeup, and lifestyle factors (Barrea, Annunziata, Bordoni, Muscogiuri, Colao, Savastano, and Obesity Programs of nutrition, Education, Research and Assessment (OPERA) Group, 2020) (Barrea, Annunziata, Bordoni, Muscogiuri, Colao, Savastano, and on behalf of Obesity Programs of nutrition, Education, Research and Assessment (OPERA) Group, 2020). Personalized nutrition aims to offer more targeted and effective solutions by taking the complex interactions between diet, genes, and health into account, rather than the traditional obesity management strategies.

Different specific genes have been identified that play crucial roles in the weight regulation and metabolic processes. These genes affect various factors like physical activity levels, energy exertion, appetite control, insulin signaling, and fat cell differentiation. Due to Single nucleotide polymorphisms (SNPs), different variants of these genes are constructed that

produce different responses to weight loss interventions. This advocates that the genetic susceptibility of an individual plays a crucial role in obesity management.

Categorization of genes based on their involvement in the metabolic processes and genetic variations, targeted interventions to control obesity can be developed. Such as targeting the genes that are involved in energy intake and expenditure, lipid metabolism, and thermogenesis. Through the incorporation of genetic information into obesity management system, leads to more precise and personalized treatments thus, improving the health status of individuals struggling with obesity and its associated health issues (Joffe and Houghton, 2016).

Role of Artificial Intelligence in Healthcare

Artificial Intelligence (AI) is revolutionizing healthcare by enhancing clinical decision-making, improving patient well-being, in drug discovery (Gallego et al., 2021) and development process (Larabi-Marie-Sainte et al., 2019), and remotely treating patients (Fröhlich et al., 2018). Through machine learning algorithms and deep learning techniques, AI enables healthcare providers to analyze vast amounts of data, identify patterns, and make predictions accurately (D. Lee and Yoon, 2021).

AI has modernized the healthcare, particularly in diagnostic imaging. AI algorithms could analyze medical images like X-rays, MRIs, and CT scans to detect abnormalities and diagnose diseases at their early stage. The advanced AI algorithms could not only identify and quantify microorganisms but also aid in diagnosing and predicting various medical anomalies. Through the use of AI, there is an increase in diagnostic accuracy, a decrease in human error, and a decrease in the cost and time needed to interpret images. Hence, it enables rapid diagnostic decisions and ultimately improves patient care (Choi et al., 2018).

AI has also transformed personalized medicine by analyzing the genetic data, medical history, and lifestyle factors of the patient to generate customized treatment interventions. This tailored treatment protocol improves treatment efficacy and minimizes side effects, leading to better patient healthcare.

Furthermore, AI plays a significant part in diagnostic analysis preventive care, and treatment protocols. By utilizing the health data from electronic and wearable devices, AI can predict the patients at risk of developing or experiencing certain adverse conditions. This allows the healthcare providers to intervene early thus, preventing the progression of disease and improving the overall population health. The integration of AI in healthcare not only improves patient health but also increases access to quality care for the whole population (Quazi, 2022).

Advancements in healthcare through artificial intelligence

The implementation of AI in healthcare system causes the inception of a new era of medical innovation. The ability of AI to analyze complex data and learn continuously, make it an essential element of clinical decision-making. Advanced AI based algorithms for enhanced risk assessment, diagnostic accuracy, and workflow efficiency, make AI a crucial component of modern medicine.

AI-enabled tools have the extraordinary ability to unleash complex patterns within raw data, making them invaluable across various medical fields. AI is becoming an integral element of the healthcare landscape, ranging from drug to treatment development, patient care, and even management strategies. Its growing role in medicine highlights the significance of this technology, in tackling challenges that exceed human cognitive capabilities within limited time frames.

The collaboration between AI and medical professionals highlights that AI enhances decision-making capabilities, allowing for more precise interventions and treatment strategies. This is particularly evident in diagnostics, where AI-driven algorithms have modernized and automated disease diagnosis, making it more reliable and cost-effective (Choi et al., 2018).

It is anticipated that AI in healthcare will advance to get more industrious, through the integration of diverse data sources to provide comprehensive assessments of diseases and their progression. The healthcare sector is also actively utilizing AI to improve care quality, from drug development to patient monitoring (Weerakoon et al., 2019). Specifically, AI is widely used in visualization tasks, such as analyzing CT and MRI images, giving deeper insights into patients' conditions and improving the decision-making. Moreover, it offers predictive insights and manages vast medical datasets effectively (Cudney et al., 2019).

AI-driven solutions are also transforming operational challenges within healthcare institutions, such as enhancing efficiency, improving patient experiences, and motivating staff. The utilization of these protocols contributes to better organizational effectiveness and cost management (Kumar et al., 2018).

Machine Learning in Genomic Analysis

Machine learning involves the development of computer algorithms and their application to improve experience. These ML algorithms predict and interpret data and make decisions based on analysis data.

Basics of Machine Learning and its Application in Genomic Analysis

In genomics, it's a revolutionary approach to improving knowledge about genetic information. Genomics deals with the understanding of the complete DNA, or genome of organisms. ML algorithms are suitable for analyzing complex genomics and patterns (Monaco et al., 2021). For example, ML in genomics can help you find the location of transcription start sites in a genome sequence. Firstly, machine learning will help researchers learn through an algorithm. Secondly, the algorithm will provide a large set of transcription start sites (TSS). A list of sequences that are not exactly known to be TSS is also provided. TSS and Not TSS are labeled and processed by algorithms and then stored in the form of a model. The sequences that were not known to be TSS are again given for labeling. If the learning remains successful, then the predicted labels are considered to be correct. For validation testing the TSS predictions given by machine learning are performed in the lab.

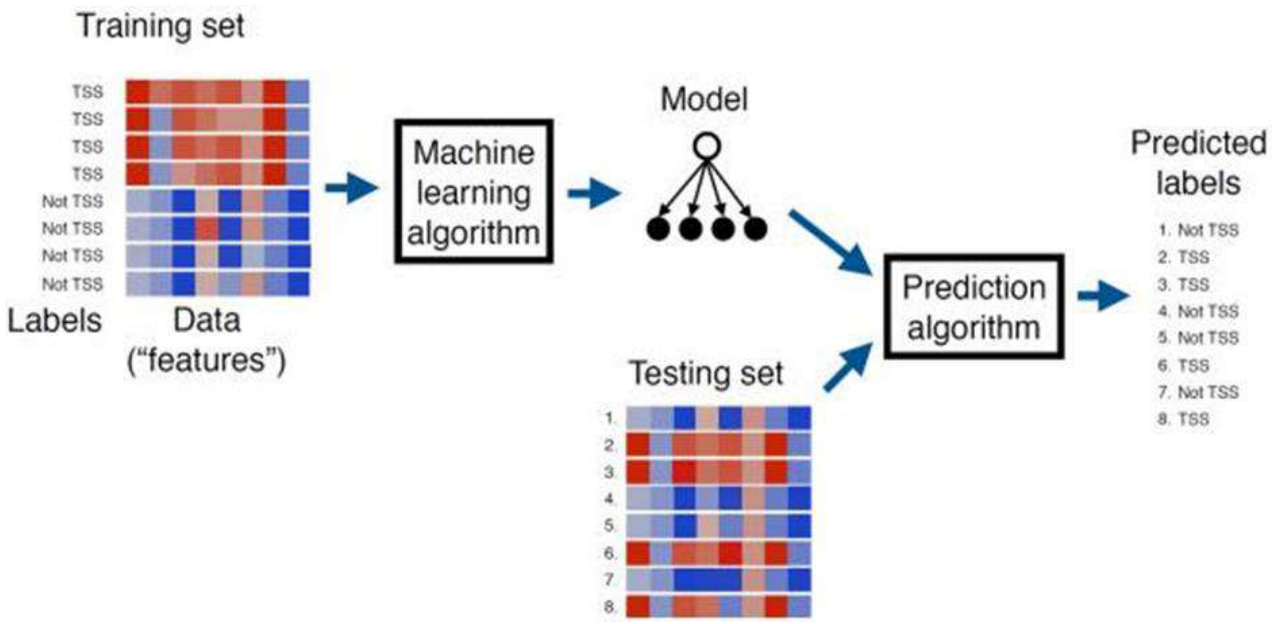

Fig. 1: The stages of the machine learning process.

This is just an example to dictate supervised learning i.e. a subtype of machine learning. When machines are allowed to learn in an unsupervised manner this is deep learning. Deep learning is modern and unsupervised learning that does not include any assistance from humans to make predictions about the real world. A question can arise as to why there is a need for machine learning to study genomics. Since the completion of the draft human genome sequence, the project has led to the generation of highly exceptional genomic data. It is estimated that research will produce almost 2 and 40 exabytes of genomic data within a decade (Larrañaga et al., 2006).

Using machine learning to uncover patterns related to obesity risk

Obesity brings multiple risk factors contributing to increased morbidity and mortality rates. Some factors associated with health risks and causes of obesity must be understood in a better way. Machine learning provides valuable insight into the application stage of data analyzing on obesity. By using publicly available dataset obesity is analyzed by Classification and regression trees, and Naive Bayes. It is essential to control the mortality and morbidity due to obesity by providing precise data to facilitate finding risk factors. ML models play role in understanding general health, any disease contribution, detection, and identification of risk factors. For this purpose, some models are used to predict the obesity risk based on the intake of diet and adherence to diet recommendations (Thamrin et al., 2021a).

Researchers can also use ML algorithms to analyze genetic disorders that can play a role in obesity. Genetic profiles of people with and without obesity are compared and genetic markers are identified that make individuals susceptible to the condition. These models also develop personalized interventions to mitigate obesity.

Predictive Modeling for Obesity

Building obesity prediction models represents overlapping of healthcare, data science, and public health (Thamrin et al., 2021b) (Haghi Kashani et al., 2021). This struggle takes an integrated approach to understand and recognizing the variables affecting prevalence and progression. Researchers collect and preprocess a variety of data sets to find the best possible predictor including clinical records, lifestyle, genetics, and environmental factors. Then they select and engineer features from the data sets. Predictive models are trained, assessed, and refined to accurately predict obesity risk and outcomes by using machine learning algorithms ranging from ensemble to regression and decision (Alsharef et al., 2022). Interpretability and explainability are critical so that healthcare professionals and policymakers can understand model predictions and devise targeted interventions. Building predictive models for obesity offers promising avenues for personalized interventions, population health management, and policy formulation. Embracing advancements in data analytics and healthcare informatics helps to combat this global epidemic.

Methodologies for Developing Predictive Models for Obesity

The development of predictive models for obesity involves a combination of methods that integrates data science techniques, medical knowledge, and epidemiological insights. The selection and compilation of datasets that capture many aspects of obesity, including as genetic predispositions, lifestyle behaviors, and environmental factors, is a crucial step in this process. Foundational sources include clinical data from electronic health records (EHRs), demographic surveys, and longitudinal research. These data offer important insights into people's health condition and medical history (Huguet et al., 2020). In addition to clinical data, lifestyle and behavioural data gathered by wearable technology, mobile health applications, and questionnaires provide detailed information on stress management, physical activity levels, food habits, and sleep quality (Nagpal et al., 2021).

Feature selection and engineering play an important role in identifying relevant predictors from the large amount of available data. Techniques such as correlation analysis (Aisike et al., 2023), feature importance ranking (Shi et al., 2021), and domain expertise are employed to identify key determinants of obesity risk and progression (Kohavi and John, 1997). In order to improve forecast accuracy, feature engineering procedures may also involve the development of composite measures, such as waist-to-hip ratio or body mass index (BMI). There are many different machine learning methods available to academics, the choice of model depends on the goals of the study and the type of data.

The frequently used techniques for obesity prediction include Linear regression, decision trees, support vector machines, and neural networks. Ensemble methods, such as random forests and gradient boosting machines, are also preferred for their ability to improve prediction accuracy and robustness (Kaur et al., 2022).

Detailed validation processes are needed to evaluate prediction models to judge their generalizability and performance. Cross-validation methods, such bootstrapping and k-fold validation, are used to reduce overfitting and guarantee accurate model results (Tan et al., 2022) (Ivanescu et al., 2016). Quantitative measures of the efficacy of the model are provided by performance metrics, such as accuracy, precision, recall, F1-score, and area under the receiver operating characteristic curve (AUC-ROC) (Naidu et al., 2023). The methods for building obesity prediction models include feature engineering, machine learning algorithms, validation processes, and a methodical integration of various data sources. By using these approaches, researchers hope to improve population health outcomes, inform targeted therapies, and deepen our understanding of the origin of obesity.

Data Sources and Features used in Model Development

Data sources and features used in model development for obesity prediction are diverse and cover various characteristics of individuals' health, behavior, genetics, and environment. Clinical data, which includes electronic health records (EHRs), include key information such prescription history, diagnosis, and medical history (Gupta et al., 2022). Population surveys and longitudinal studies provide important insights into the course of the disease and the demographic characteristics linked to obesity (Huguet et al., 2020). Lifestyle and behavioral data provide complete details on eating habits, levels of physical activity, sleep patterns, and stress management. This data is obtained through questionnaires, wearable technology, and mobile health applications (Ferdowsy et al., 2021). Genetic data, obtained from genome-wide association studies (GWAS) and genetic sequencing, enable the exploration of genetic predispositions and susceptibility to obesity (Y.-C. Lee et al., 2022).

Understanding the broader factors that impact the prevalence of obesity requires an awareness of environmental and socioeconomic data. Variations in obesity risk and access to health resources can be understood through socioeconomic markers such as income, education level, and neighbourhood feature (A. Lee et al., 2000). Environmental information provides insight into the obesogenic environments people live in, including food availability, built environment characteristics, and accessibility to recreational opportunities (Kahan and Mehrzad, 2020). Geographic information system (GIS) data, satellite imagery, and other geospatial data provide spatial insights into neighbourhood features, urbanization trends, and environmental factors influencing obesity (Slotman et al., 2022).

Predictive models for obesity can reflect the complex connection of genetic, environmental, social, and behavioural factors that contribute to obesity risk and progression by integrating these various data sources and features. By taking a comprehensive approach, prediction models become more accurate and applicable. This helps guide targeted interventions and policy decisions that address obesity on both an individual and a population level.

Evaluating Model Accuracy and Reliability

In order to guarantee the efficacy and dependability of predictive models in a variety of domains, it is critical to assess model correctness and reliability. Thorough validation processes are used to evaluate the model's performance against unknown data and determine how well it can be applied to real-world situations. Quantitative measures of the model's performance are provided by metrics like accuracy, precision, recall, F1-score, and area under the receiver operating characteristic curve (AUC-ROC). These measures indicate the model's capacity to accurately categorize instances and reduce errors. Additionally, cross-validation methods like bootstrapping and k-fold validation reduce over-fitting and offer reliable estimates of model performance. Additionally important are interpretability and clarity, which enable stakeholders to comprehend the model's decision-making process and have trust in its forecasts. Researchers and practitioners can guarantee that predictive models consistently inform decision-making processes and effectively contribute to the solutions of real-world problems by maintaining strict evaluation demands (Chicco and Jurman, 2023).

Challenges and Ethical Considerations

The principal technical challenge while carrying out genomic predictions is guaranteeing the quality as well as the adequacy of the data being used for analysis. Genomic data of different populations may vary considerably in terms of quality because of various factors i.e. methods of sample collection, techniques of sequencing, as well as pipelines of data processing. Severe steps of quality control as well as protocols for standardization are necessary to address these issues to reduce partialities and imprecisions in the data.

Furthermore, one of the other challenges associated with genomic predictions is the quantity of data (genomic) accessible for study. Although developments in sequencing technologies have led to an increase in data generation exponentially, still there is a need for more general datasets, specifically from lessened populations, to expand the precision and generalizability of already prognostic models.

Handling large genomic datasets during the analysis poses challenging computational challenges. Genomic data is intrinsically sophisticated and high-dimensional, ultimately requiring sophisticated algorithms as well as computational means for analysis. Additionally, the absolute volume of generated data from studies like genome sequencing requires accessible as well as effective computational structures.

Researchers are discovering innovative methods such as distributed computing, parallel processing, and cloud-based solutions to counter these challenges. Furthermore, developments in machine learning algorithms modified for genomic data, like deep learning architectures, are undertaken to enhance the competence as well as scalability of genomic predictions.

Emerging Technologies and Research Directions and Genomics and AI

Compared to the traditional statistical models, machine learning models mainly neural networks provide more specific predictions for obesity. They also find out the variables that have an impact on the onset and risk of obesity. The model's findings enable users to implement personalized goals and strategies using real-time data analysis systems. Furthermore, healthcare professionals can utilize these tools to monitor progress, predict obesity risk, and guide interventions (D. Lee and Yoon, 2021).

Recent studies have analyzed different AI and ML tools and models to manage obesity and related diseases. These include a range of systems such as a Decision Support System customized for bariatric surgery patients, an app named MOPET designed to encourage physical activity, and a Neuro-Fuzzy Model developed for the refinement of body mass index results. Along with these different techniques of Parameter Decreasing Methods and Artificial Neural Networks are developed to find out the correlations between obesity and cardiovascular disease. An artificial neural network, for the prediction of resting energy expenditure, is developed. Furthermore, image processing model, Support Vector Machine, is developed to monitor the food intake of a person. These investigations shows that these AI systems offer more specific results, hinting at their potential as effective tools for managing obesity and its associated diseases (Marmett et al., 2018).

The combination of AI, personalized medicine, and genomics shows a paradigm shift in the management system for obesity. By combining genetic insights and personalized treatment methods with modern techniques of machine learning and artificial intelligence, healthcare providers can provide more effective treatment strategies personalized for individual needs. This holistic method empowers early detection and intervention, helping individuals to make informed decisions about their health. AI-based intervention should be investigated in future studies to optimize their efficacy in handling obesity focusing on personalized medications.

Conclusion

The broader implications of AI for healthcare systems and public health are multifaceted and transformative, moving from the boundaries of traditional medical practice to impact various fields of healthcare delivery and population health management. At the systemic level, AI has the potential to reform healthcare systems by improving patient outcomes, enhancing operational efficiency, and reducing costs. By streamlining administrative processes, optimizing resource allocation, and facilitating remote patient monitoring, AI-driven solutions enable healthcare providers to deliver high-quality care more effectively and efficiently. Furthermore, AI enables patients to take charge of their health by providing access to specific health information, remote monitoring tools, and telehealth services, resulting in increased patient involvement and happiness. Additionally, AI accelerates medical research and innovation by facilitating data-driven discovery, predictive analytics, and precision medicine. By analyzing large-scale healthcare datasets, AI algorithms uncover patterns, correlations, and insights that drive scientific discovery and inform clinical practice.

Furthermore, AI holds promise for addressing broader public health challenges, such as disease surveillance, outbreak prediction, and targeted interventions. Predictive analytics models powered by AI can outbreaks of illness identify those at greatest risk, and campaigns to prevent infectious disease transmission. However, realizing the full potential of AI in healthcare and public health requires addressing ethical, regulatory, and societal considerations to ensure equitable access, safeguard against bias, and uphold patient rights and privacy. To summarize, AI has far-reaching implications for healthcare systems and public health, providing unprecedented opportunities to improve outcomes for patients, improve operational efficiency, and address societal challenges, all while requiring careful consideration of ethical, regulatory, and societal implications that guarantee fair implementation.

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Chapter 12

Pharmacologic Advances in Animals Reproduction

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ABSTRACT

This chapter underscores the crucial role reproduction plays in the survival of species and animal breeding endeavors, providing a detailed overview of the anatomical aspects involved in the male and female reproduction. The intricate processes of fertilization, including sperm-egg fusion and influential factors, are thoroughly elucidated. The pharmacological substances are recognized as potent instruments, facilitating the synchronization of estrous cycles, aiding in conception, and inducing ovulation through various drug categories such as GnRH analogs, prostaglandins, estrogens, and progestogens. The chapter deliberates on the notable advancements facilitated by Assisted Reproductive Technologies (ARTs), with a specific emphasis on the transformative impacts of techniques like *in-vitro* fertilization (IVF) and embryo transfer methods in managing infertility concerns. It chronicles the historical progression of IVF, explores its application in the animal models, and acknowledges the associated ethical considerations.

KEYWORDS
Reproduction, Estrous cycle, ART, IVF, Embryo transfer

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INTRODUCTION

Over the ages, humans have honed their observational abilities, studying both humans and animals with growing expertise. They've utilized progressively powerful and advanced tools and methodologies, gathering crucial insights essential for their survival, education, and health (Kinter et al., 2021). For wildlife populations to be viable, both reproduction and survival are essential fitness components. At times, the importance of reproduction for sustaining gradually growing animal populations is overlooked when evaluating wildlife management options (Manlik, 2019). Over the past ten years, significant advancements have been made in the treatment choices for gynecologic emergencies and companion animal fertility. There are now more effective and less side effecting pharmacological treatments accessible. There exist novel medications for inducing estrus, terminating pregnancy, and managing pyometra (Wiebe and Howard, 2009).

Reproductive Physiology in Animals

A complex process which is necessary for species to survive is reproduction. To develop into a healthy and productive member of the species, certain physiological and metabolic requirements must be satisfied (Evans and Ganjam, 2017). Developing successful, long-term breeding processes requires a strong understanding of reproductive processes.

Puberty

Puberty marks the start of an animal's reproductive life. For males, the first sperm production indicates the onset of puberty. The puberty is marked in female non-primate and some primate species by the first ovulation and, in menstrual primates, by the first menstruation. The male and female adolescents experience a significant rise in hypothalamic secretion of GnRH during puberty. This, in turn, triggers the anterior pituitary gonadotropins' stimulation of LH and FSH hormone secretion. The hormone profiles undergo significant changes which lead to the development and activation of germ cells, gonads, and secondary sex traits that are frequently species-specific. Male testis and accessory sex organ weight, such as seminal vesicles and the prostate, are positively connected with increases in LH, FSH, prolactin (PRI) from the anterior pituitary and androgens from testicular leydig cells. Many of the processes are comparable in females, where an increase in the ovarian weight is accompanied by increases in the pituitary LH, FSH, and PRL levels as well as ovarian progesterone and estradiol (Kleiman et al., 2010).

Reproductive Anatomy: Male and Female Systems

Male Reproductive Anatomy

The male reproductive system comprises the testes, epididymis, vas deferens, and accessory sex glands such as the prostate and seminal vesicles. Additionally, it includes organs like the ejaculatory ducts, bulbourethral gland, and efferent ducts (Picut and Remick, 2016). The mature testis is divided into two sections: the endocrine portion secretes testosterone and the gamete portion generates sperms. Inside the seminiferous tubules of the gamete region, spermatogenesis takes place. Here, Sertoli cells are responsible for the production and maintenance of spermatozoa. Meanwhile, leydig cells, situated in the interstitium between the seminiferous tubules, produce androgen, including testosterone. The process of spermatogenesis also depends on this androgen synthesis. Nevertheless, the only androgen that stimulates spermatogenesis is the local intratesticular one. The exogenous androgen inhibits spermatogenesis by suppressing the pituitary (Silber and Silber, 2018). The spermatogenesis entails the intricate process through which a relatively undifferentiated diploid cell, known as spermatogonium, undergoes gradual transformation into a finely specialized haploid cell, known as spermatozoon (Sharma and Agarwal, 2011). During spermatogenesis, meiotic cell division gives rise to haploid spermatids from telosploid initial spermatocytes. Additionally, spermatogonial stem cells undergo mitotic cell division to differentiate into spermatocytes. The spermiogenesis marks the concluding phase of spermatogenesis, wherein spermatids mature into spermatozoa (Nishimura and L'Hernault, 2017). The spermatogenesis in humans begins during the onset of puberty and continues throughout life. Optimal functioning of the gonadotroph axis, along with functional androgen receptors (ARs) in testicular sertoli cells, and intratesticular testosterone (T) synthesis by leydig cells are essential. This process is regulated by the FSH and testicular androgens, primarily testosterone (Christin-Maitre and Young, 2022).

Female Reproductive Anatomy

The female reproductive system comprises the ovaries, oviducts, uterus, cervix, uteri, vagina, and external genitalia. The broad ligament provides support to the internal genital organs, which include the first four components mentioned. This ligament consists of the mesometrium, providing support to the uterus, the mesosalpinx supporting the oviduct, and the mesovarium supporting the ovary. Unlike certain species (such as rats and mice), where the ovaries are housed in a closed sac, farm mammals have their ovary housed within ovarian bursa. The uterus is comprising a body, a cervic (neck), and two uterine horns (comua). The uterus of cattle, sheep, and horses is of the bipartite type (uterus bipartitus), in contrast to the bicomuate type (uterus bicornis) found in pigs (Hafez and Hafez, 2000). The ovary is a special organ that performs a wide range of physiological tasks. These tasks include the regulation of oocyte production, steroid hormone production, and support of early pregnancy. The support of growth, behavior, and improved immune function against several cues that are both endogenous or environmental (Orsi et al., 2024), are among the very less known functions of the ovary. The ovary is a dynamic organ that has multiple functions. The ovary does two main things. It generates fertile oocytes that can both develop and are fully competent for the completion of an entire life cycle and releases a set of sex steroid hormones to prepare the reproductive tracts to be ready for steps towards conception and subsequent development of pregnancy. Follicles are the functional units of ovary containing one oocyte surrounded by one or more layers of somatic cells, called the granulosa cells. The oocyte and its surrounding granulosa cells are segregated from the interstitial (stromal) tissue by a membrane known as the basal lamina (Oktem and Oktay, 2008). The follicle development initiates during fetal life and progresses through a dynamic process known as folliculogenesis during various postnatal phases (Das et al., 2023). The oogenesis is a prolonged procedure which includes birth, development, and maturation of a cell that is special in its capacity to reproduce organisms into new generations. The ultimate aim of folliculogenesis and oogenesis is the ovulation of a healthy egg that is prepared for fertilization, which is ensured by a coordinated communication network among the oocyte and the somatic cells (Rodrigues et al., 2008).

Fertilization

Almost all animal species undergo fertilization, which is the joining of male and female gametes, or sperm and eggs, to create a zygote or one-cell embryo. To produce new offspring that display every trait of the species, the procedure is extremely necessary (Wassarman, 1988). The spermatozoa are ejaculated from the male and deposited in the female (internal fertilization) or into the oocyte's surroundings (external fertilization), the process of fertilization starts and it concludes when the zygote is created (O'Rand, 1986). The spermatozoa are responsible for fertilizing eggs. Nevertheless, during ejaculation, mammalian spermatozoa are unable to complete this duty. They must first go through a morphological change in the female reproductive tract known as the acrosome response, which releases several enzymes and proteins from the acrosome, followed by physiological changes known as capacitation. The acrosome is a subcellular organelle which is present at the tip of the head of sperm (Okabe, 2013). The processes of sperm chemotaxis and capacitation are crucial to mammalian fertilization. The former speaks about sperm maturational alterations in the female reproductive system, whereas the latter describes sperm moving up a chemoattractant gradient in the ampulla of the oviduct toward the ovulated egg (Wassarman, 1999).

Chronology of Fertilization

In case of mouse, the process by which eggs are fertilized involves a number of processes that must happen in a certain order. First, the sperms loosely bind with eggs that are ovulated at the surface of the zona pellucida, forming an attachment. This attachment progresses to a more tenacious binding, facilitated by mutual orientation of gametes. The

sperm attach to the zona pellucida through the specific sperm receptors and egg-binding proteins. Subsequently, the sperms that are attached undergo the acrosome reaction, readying themselves to penetrate the zona pellucida and fuse with the egg's plasma membrane. This penetration is facilitated by acrosin, a trypsin-like proteinase. Once the sperm reach the perivitelline space, binding with the egg's plasma membrane takes place, resulting in fertilization. When a single sperm merges with the egg it prevents polyspermy, ensuring normal embryo development (Wassarman, 1987).

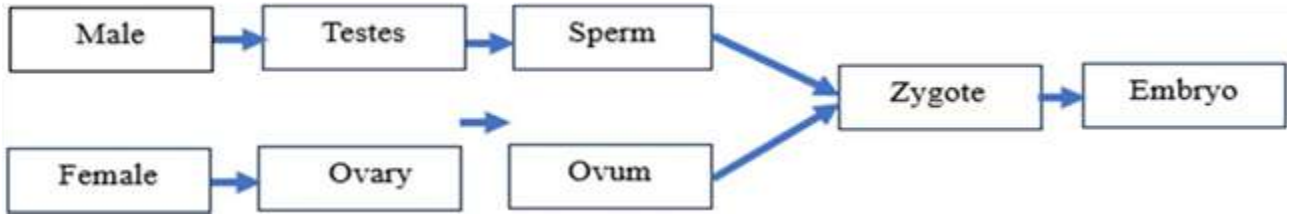

Fig. 1: This figure illustrates the basic reproductive process, showing the flow from male to female reproductive organs, culminating in the formation of a zygote and subsequent embryo.

Pharmacological agents used in Animal Reproduction

In the field of pharmaceuticals, a plethora of hormones exist naturally, comprising a diverse group of compounds that impact complex physiological processes. Broadly speaking, these hormones can be classified into three main categories: proteins, compounds derived from amino acids and steroids that are formulated to modify specific bodily functions.

GnRH Analogs

The utilization of GnRH-I and its synthetic agonists was primarily aimed at inducing decreased fertility or potential infertility in females by leveraging their LH releasing and ovulation-inducing characteristics. The evolution of pro-fertility interventions has yielded various therapies such as ovulation induction, management of cystic ovarian disease, and mitigation of embryonic mortality. Extensive research has been conducted utilizing GnRH-I and agonists across diverse environments, farming systems, reproductive statuses, etc., occasionally in conjunction with other hormonal therapies. Originally designed for addressing infertility, GnRH-I agonists have demonstrated a paradoxical suppression of reproduction when administered in high dosages or continuously, a phenomenon leveraged in anti-fertility treatment modalities (Schneider et al., 2006).

Prostaglandins

Several compounds of prostaglandin F_2alpha (PGF) have been accessible for fifty years as veterinary medications for the treatment of food animals and horses. Their unique properties of luteolysis and uterotonic effects have proven to be advantageous not only in large animals but also in small animals. Surprisingly, no pharmaceutical company has yet shown interest in developing a prostaglandin product specifically for dogs and cats. In recent times, the utilization of human prostaglandin E (PGE) products has been gradually adopted by small animal veterinarians (Romagnoli, 2017).

Estrogens

The estrogens predominantly act by engaging with isoforms of the estrogen receptors. Subsequently, these receptors bind to the hormones within the cytoplasm of cells, facilitating the transportation of this complex to the cell nucleus. Consequently, the transcriptional process begins with the activation of response elements in gene promoters. These receptors are classified into nuclear estrogen receptors (nERs) and membrane estrogen receptors. In addition to conventional estrogenic actions, alternative mechanisms have been elucidated. The influence occurs through cell signal transduction linked with mERs instead of the genomic activity process (Wojnarowski et al., 2021).

Progesterone

The progesterone plays a vital role as a hormone in the reproductive system of animals, particularly in ruminants. Its synthesis and secretion are mainly carried out by the corpus luteum, with fluctuations in levels occurring throughout the sexual cycle and pregnancy. The effects of progesterone involve inducing changes in the uterine environment, which in turn support the survival of embryos and the elongation of the conceptus. Various strategies exist to enhance the peripheral concentrations of P_4 following artificial insemination, although the available data on the outcomes often present conflicting or inconclusive results (Genazzani et al., 1990).

Prolactin Inhibitors

The prolactin is a hormone produced by pituitary gland that has a notable influence on various physiological processes in animals, such as lactation, growth, metabolism, osmoregulation, behavior, and reproduction. In the case of females, prolactin serves as a trigger for the initiation and maintenance of lactation, and elevated levels of serum prolactin during lactation contribute to a decrease in fertility, thereby offering protection against untimely pregnancies. Additionally, prolactin assists in maintaining pregnancy by supporting the corpus luteum in the ovary, which acts as the main source of

the female sex hormone progesterone (Wiebe and Howard, 2009).

The antibiotic agents are employed within the animal industry for three primary objectives:

- Firstly, for therapeutic reasons, in order to manage existing medical conditions.
- Secondly, as a proactive measure, using dosages lower than those required for therapeutic effects.
- And lastly, sub-therapeutically, with the aim of enhancing productivity through increased growth rates and more efficient feed utilization (Council, 1999).

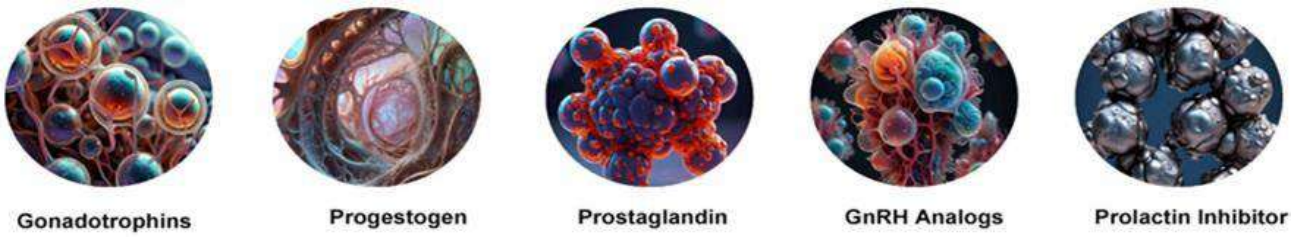

Fig. 2: This image depicts five key hormones involved in reproductive physiology

Advancements in Associated Reproductive Technologies

In July 1978, the world witnessed the birth of Louise Brown, heralding the first successful pregnancy through *in-vitro* fertilization (IVF), with assistance from Dr. Robert Edwards and Patrick Steptoe. Since then, the field of assisted reproductive technologies (ART) has seen rapid growth and development. In fact, what was once thought to be a contentious medical curiosity has drastically altered the prognosis for infertile couples and is causing increasing numbers of births worldwide (Brezina et al., 2012). For any livestock production system to be sustainable, adequate reproductive performance is essential. This goal is believed to be somewhat achievable with traditional reproductive methods. The advancement and utilization of modern reproductive technology have unlocked vast potential for investigating, treating, and manipulating the reproductive process both *in-vitro* and *in-vivo*. The overarching aim is to enhance the reproductive capacities of domestic livestock animals (Choudhary et al., 2016).

ART Technique Effects in Animal Models

Employing animal models eliminates fertility as a potential confounding factor, allowing for detailed analyses of ART technique effects without the variability linked to infertility. Furthermore, interpreting data is further complicated by the probability that infertility in humans could be a secondary symptom of another medical condition, such as endometriosis, which might have a genetic or epigenetic origin.

Various results have been reported by mouse models studying the effects of fertilization which is *in-vitro*. Overall, when the *in-vitro* conditions deviate more from the natural reproductive tract, the resulting changes in postnatal phenotype tend to become more pronounced. The epigenetic modifications are thought to mediate reprogramming and developmental plasticity, and there is copious evidence that altering the epigenetic landscape in embryos occur *in-vitro*. Evaluating that preimplantation development involves significant chromatin restructuring.

Evidence from Epidemiological data

There are several reviews that detail the obstetric and pediatric IVF outcomes, along with the epidemiological evidence supporting DOHaD principles. The preterm birth and multiple gestations are notable risks associated with antiretroviral therapy. The low birth weight has been associated with elevated blood pressure, fasting insulin levels, insulin resistance, and incidence of type 2 diabetes, coronary heart disease, hypertension, and hyperlipidemia. It is a well-established (though not very reliable) indicator of intrauterine stress. In comparison to children conceived naturally by sub-fertile parents (defined as conception after one year of infertility), adolescents conceived through IVF display slight yet noteworthy variances in growth patterns, fat accumulation, blood pressure, and glucose levels. Subfertility is the most moral method of accounting effects of various fertility issues and makes up a valuable control group (Feuer and Rinaudo, 2016).

Ovulation Induction Therapies

The therapies for anovulatory infertility are ovulation induction. In order to treat hypogonadotrophic hypogonadism, patients must receive injections of HCG to cause follicle rupture in addition to FSH and LH. The pulsatile GnRH has the benefit of a low multiple pregnancy rates along with the same effectiveness as gonadotrophins. The initial treatment option for polycystic ovarian syndrome (PCOS) is clomiphene citrate. When this medication is used appropriately in chosen patients, pregnancy rate comes close to healthy women. The second line of treatment, low-dose FSH protocols, is effective in inducing monofollicular development (Messinis, 2005). The management and prevention of ovarian hyperstimulation syndrome have received attention. This study looks at the use of controlled ovarian hyperstimulation (COH) and induction of ovulation as adjuvants to intrauterine insemination (IUI). It has been decided, quite firmly, that women with polycystic

ovary syndrome or hypogonadotrophic hypogonadism should be provided with methods of ovulation induction at this juncture, taking into account treatment costs, complications, and efficiency.

Reproductive Physiology and Hormonal Therapies

Reproduction is a multifaceted phenomenon encompassing various components, some of which serve as indicators of reproductive efficacy. However, many of these attributes are not easily quantifiable directly within the animal's physiology, except through the outcome of conception or pregnancy (Moreno Klemming, 2019).

Hormonal Therapies

While the anti-Müllerian hormone (AMH) concentration and antral follicle count (AFC) are dependable indicators of ovarian reserve, there is ongoing debate about their suitability as fertility markers (Schwarzmann et al., 2023).

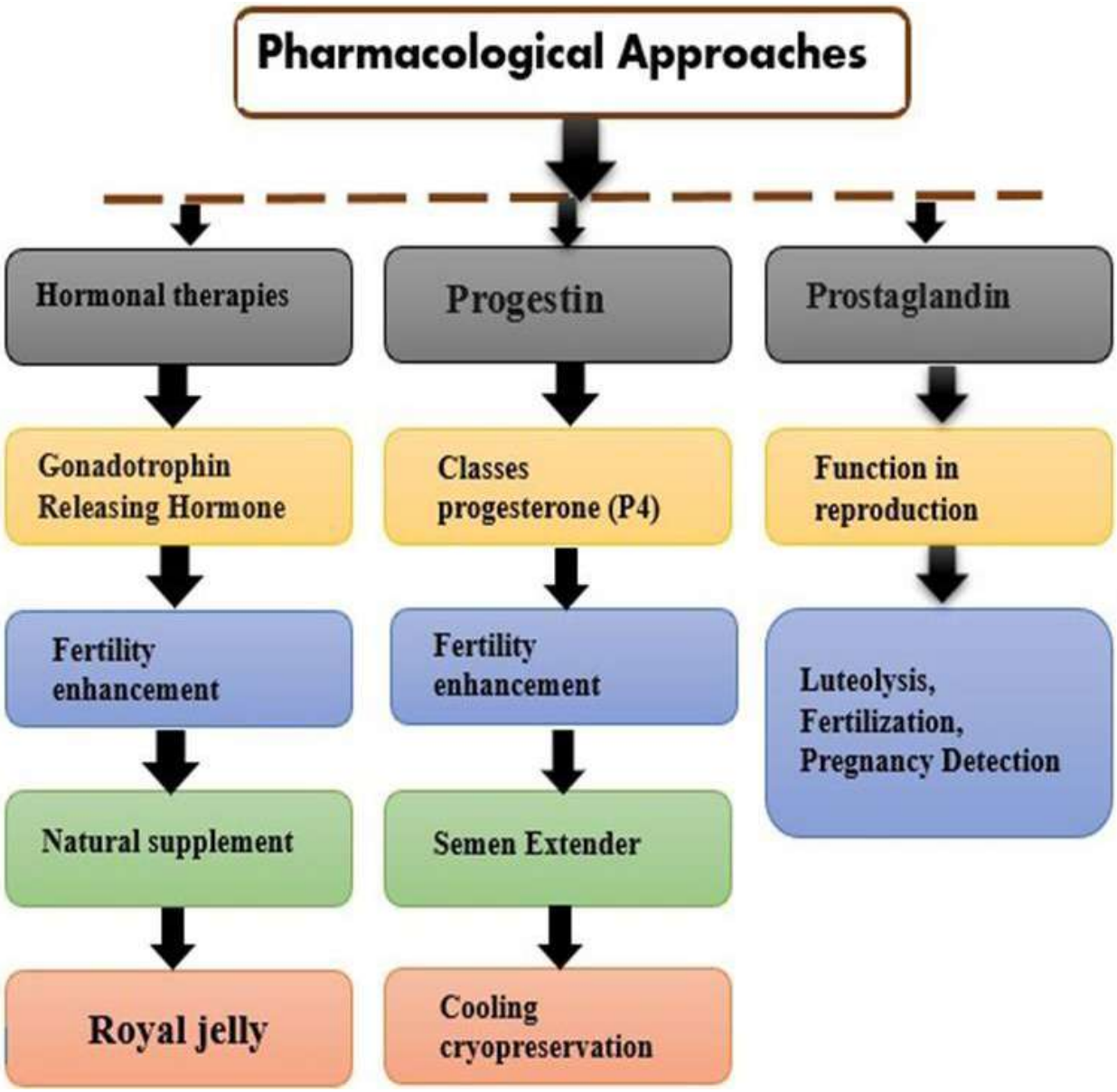

Fig. 3: The image illustrates pharmacological approaches to reproductive physiology, such as cooling cryopreservation, progestin and prostaglandin, their respective functions, and hormonal therapy.

Gonadotrophin Releasing Hormone

The pig is the only mammal raised for livestock that can produce a functional protein for both the receptor (GnRHR-II) and the second type of mammalian GnRH-II. Although mature GnRHR-II KD males showed similar levels of LH, their circulating testosterone amount were lower compared to those of boars from the control litter.

Progestins

The hormonal contraceptives (HC), with a global user base exceeding 150 million, have been authorized for over 60

years. The primary components of HC include synthetic progestin (progestin-only) or a combination of synthetic progestin and estrogen (Dasgupta et al., 2020).

Fertility Enhancer

Many different treatment modalities have been employed to improve the likelihood of conception because male infertility can stem from a variety of causes. Feed additive efficiency is crucial for preserving the general health and physiological well-being of animals (Elnesr et al., 2019). Natural supplements are used by most people to enhance their health. (Alagawany et al., 2019). The German Federal Board of Health officially categorizes royal jelly, commonly abbreviated as RJ, as a medicinal product. Consequently, in China, RJ has been utilized both as a dietary supplement and a form of medicine (Brindza et al., 2010). Moreover, being exposed to various environmental stressors, such as heat stress, smoking, pollution from light, air, water, and food, contamination from heavy metals, and the consequences of industrialization, can have adverse effects on the health, fertility, and overall well-being of both humans and animals. RJ is a byproduct of the secretions made by honeybees and is fed to their larvae. Additionally, it has a variety of uses for enhancing human health and is generated from specific glands in the heads of young laborers (hypopharyngeal and mandibular glands) (Abdelnour et al., 2019; Han et al., 2011).

Semen Extender

Semen extenders function as a medium for the preservation of sperm, aiding in conception, while also regulating the pH of the medium pre-thaw and post-thaw, preventing bacterial contamination, minimizing cryogenic damage, and preserving the metabolic functions of sperm (Malik et al., 2018; Raheja et al., 2018). Essential attributes of semen extenders encompass maintaining a pH level between 6.8 and 7.2, supplying energy antioxidants to mitigate oxidative stress, antibiotics for contamination prevention (Schulze et al., 2020), and anti-freezing shock (Amirat-Briand et al., 2010; Tariq et al., 2020). These characteristics enable the transportation, storage, and utilization of sperm for various purposes such as research, intracytoplasmic sperm injection, and IVF involving AI.

Animal Communication and Distress Signals

Certain species of animals, produce ultrasonic vocal expressions as a means of communication when faced with stressful situations like aggression, fear, and pain. Although inaudible to the human ear, these vocalizations are indeed detected by fellow rodents. When these animals are in close proximity to a location where another animal is in distress or undergoing a painful procedure, they might feel discomfort because of the distress signals emitted by their suffering counterpart. Additionally, the presence of blood odor serves as another aversive signal, as it is linked to harm, peril, and mortality. Thus, precautions must be implemented to prevent such scenarios and minimize unnecessary distress among group members.

The Three Rs Principal

In the modern world, Reduce, Replace and Refine are the three principles on which the utilization of animals in research are based on globally. To maintain the welfare of animals that are used in experimentation, law makers formed laws (Dias et al., 2021). When the 3Rs concept was introduced and the emphasis shifted to humankind towards animals that are used in experiments, model characterization got more attention. The limitations of animal studies were acknowledged. The data that was generated from the animal models were concluded to be useful only in one context (Van Meer et al., 2015).

Replacement

The primary rationale for using animals in scientific research is to serve as substitutes for humans. It involves using different models in place of animals, such as cell cultures, microbes or other invertebrates, organs, or even cellular fractions. The best alternative would be a procedure carried out without using any animals (Andersen and Winter, 2017). Strategies for replacement include:

- Culturing of tissues
- Circulated organs
- Slice of tissue
- Fractions of cells
- Components of the subcellular (Badyal and Desai, 2014).

Reduction

Methods that minimize the quantity of animals utilized in research while enabling the collection of comparable volumes of data from fewer animals or increasing the amount of data obtained from the same number of animals (Badyal and Desai, 2014). When substitution of animals is not feasible, reduction should be prioritized. Reduction involves minimizing the number of animals utilized in research procedures (Dias et al., 2021).

Refinement

Refinement comes into play once all efforts have been exhausted to reduce the number of animals used in

experiments, and there are no other viable alternatives. Refinement involves reducing the frequency and/or intensity of inhumane procedures applied to animals during experimental protocols to minimize pain and distress and improve overall welfare (Dias et al., 2021).

Non-invasive methods and use of suitable anesthetic and analgesic regimens for pain management are two examples (Badyal and Desai, 2014). Russell and Burch cited a study on a medication designed to lessen fear as an example. Rather than making the pigeon, the study animal, experience something horrifying, the medication could teach it to avoid the platform by giving it a very mildly upsetting experience—finding food that is inaccessible, for example. The medicine could then be given to the pigeon and observed by the researcher to determine if it now visits the forbidden platform on its own accord. This approach demonstrated the drug's efficacy and spared the animal needless pain (Dias et al., 2021).

Conclusion

Pharmacological agents emerged as a powerful tool in animal reproduction, enabling interventions for estrous cycle synchronization, conception facilitation, and ovulation induction. Various classes of drugs, including GnRH analogs, prostaglandins, estrogens, and progestogens, were explored for their specific functionalities. The advancements in ARTs, highlights the revolutionary impact of /IVF and ETTs on overcoming infertility challenges. The application of IVF in animal models, and the related ethical considerations encompasses the historical milestones. Cryopreservation and germplasm conservation methods were introduced as strategies for long-term preservation of genetic diversity and enhancing reproductive performance in the future. The chapter conclude by emphasizing the importance of ethical considerations in animal experimentation within the domain of reproductive pharmacology.

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Chapter 13

Methicillin-Resistant *S. aureus*: An Overlooked Threat to Livestock Production

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ABSTRACT

The use of antimicrobial agents is frequently used for the prevention, management, and controlling of animal diseases. Antimicrobial agents promote the growth and health of livestock. Methicillin-resistant *Staphylococcus aureus* (MRSA) is commonly linked with livestock, is referred to as livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA). Several livestock-associated methicillin-resistant strains of S. aureus infected pigs in the Netherlands in 2005. The disease has been perceived in pigs all over the world since then. There is a possibility that humans can be infected with methicillin-resistant strains of S aureus from pigs (zoonoses), as well as humans can be infected with pigs (zoonoses). There are both asymptomatic carriers of LA-MRSA and diseased individuals infected with the pathogen which pose a significant risk to public health. There is an extensive range of antimicrobial resistance in the strains as well as a pathogen is associated with a broad range of antimicrobial resistance. The purpose of this paper is to conclude the research that has been conducted on MRSA prevalence and distribution, infection dynamics, health implications, antibiotic usage, and resistance, as well as antimicrobial resistance control in livestock.

KEYWORDS
MRSA, Livestock, Prevalence, Transmission Dynamics, Health Implications, Antibiotic Usage, Antibiotic Resistance, Antimicrobial Resistance, Control Strategies.

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INTRODUCTION

In the genus *Staphylococcus*, 81 species are known or believed to exist, along with various subspecies. A majority of the species in the genus are opportunistic pathogens or commensals that live in mammals. Many species are important not just from the veterinary perspective, but also from a medical standpoint. In terms of pathogenicity, *Staphylococcus aureus (S. aureus)* has become one of the most important and prodigious staphylococci species (Haag et al., 2019). According to the definitions under the microscope, Staphylococcus is referred to as a "bunch of grapes" by two Greek words, "staphyle" and "kokkos.". Hence the phrase "golden staph" which translates to "golden cluster seed." for this bacterium (Ogston, 1881). Gram-positive *Staphylococcus aureus* is a non-motile, non-spore-forming, bacterial species with a wide range of biochemical profiles including catalase, nucleases, lipases, coagulases, catalases, proteases, collagenases, and -lactamases. A staphylococcus is categorized as either CoNS or CoPS depending on whether it produces coagulase. Staphylococcus species are the ones that cause yellow/orange colonies, while Staphylococcus species cause white colonies, according to Friedrich Rosenbach (Furuya and Lowy 2006; Rossi et al., 2020; França et al., 2021). As a persistent and widespread pathogen in the environment, Staphylococci tolerate temperatures ranging from 7 to 40° and can survive

dryness and dehydration. The CoPS group is most often dominated by *Staphylococcus aureus*, while the CoNS group is dominated by Staphylococcus epidermidis (França et al., 2021).

MRSA infections are now a serious problem for food animals and the food industry as well, even though they were initially thought to spread more slowly in companion and food animals. Mastitis caused by LA-MRSA results in a decrease or no milk production in cows and buffaloes (Javed et al., 2021). Besides infections in poultry such as comb necrosis, chondronecrosis, and septic conditions (Fluit, 2012), LA-MRSA also causes infections in humans (Walraven et al., 2012). There is a high risk of LA-MRSA transmission to humans from companion animals such as dogs, cats, and horses. It has also been reported that LA-MRSA colonizes foxes, roes, rabbits, wild boars, and wild species (such as pigeons, ducks, buzzards, gulls, and rocks). The present study seeks to consolidate findings on the prevalence and distribution, dynamics of transmission, health consequences, patterns of antibiotic use and resistance, challenges regarding antimicrobial resistance, and strategies for controlling MRSA in livestock.

Prevalence and Distribution of MRSA in Livestock

In epidemiological terms, MRSA emergence and transmission worldwide are of particular importance. Many countries have reported the spread of MRSA. MRSA is known to spread in two ways: either through the transmission of existing clones from one host to another, from either human to human, from one animal to another, or through horizontal gene transfer (Lee et al., 2018). Nosocomial pathogens tend to be more endemic in hospital settings, and MRSA is among the most prevalent. According to the Centers for Disease Control and Prevention (CDC), MRSA is a leading threat to public health because it is prevalent in hospitals, community centers, and animals, it is transmitted from one to another, and its rates of infection, resistance, and therapeutic challenges are high (Ferri et al., 2017).

The European Food Safety Authority (EFSA) conducted a baseline study on swine farming in 2008 to assess the burden of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) (European Food Safety Authority, 2009). MRSA infections were observed in 14.0% of breeding and production holdings, respectively, across 26 countries with more than 5,000 participants. The prevalence estimates in most countries were significantly lower than in those in severely affected countries such as Germany, Spain, and Italy. At that time, 40 breeding farms were surveyed, but only 3/141 of them were positive. Genetic testing in most countries identified clonal complex 398 as the dominant genetic lineage. Several countries with a previously low prevalence have conducted similar investigations in the following decade. The results of the study indicate the rapid spread of the pathogen within a short time and increased genetic variability (Mroczkowska et al., 2017).

LA-MRSA is more likely to be transmitted by pig farmers and veterinarians on farms (Lewis et al 2008). The prevalence of MRSA among people working in MRSA-positive farms has been estimated to be 77–86% (Cuny et al., 2009). There is evidence that colonization is transient in most cases (Angen et al., 2019), but there is also evidence that it persists over the long term (Köck et al., 2012). Accordingly, it is not surprising that LA-MRSA CC398 has also become an occupational hazard for patients who work in animal husbandry in some European countries (Lewis et al., 2008). Recently discovered readaptation of LA-MRSA to humans, however, suggests that the bacteria are spreading among people without livestock contact (Sieber et al., 2019). Besides its zoonotic potential, MRSA infections are difficult to treat because they are resistant to many antibiotics.

Transmission Dynamics of LA-MRSA in Livestock

Human health is at risk due to pig-to-human transmission of LA-MRSA, especially in countries with large pig populations. In studies that mostly involved the swine industry (Chen and Wu, 2021), LA-MRSA was found to be associated with an increased risk of infection and colonization. However, this rate is also rising in the general public (Oliveira et al., 2022).

Livestock-to-Human

Among the most common sources of LA-MRSA transmission, pigs are responsible for most of it (Crombe et al., 2013). Due to pigs' important role as LA-MRSA reservoirs, it has been investigated how LA-MRSA is transmitted within pig farms and herds. Among the methods of transmission of LA-MRSA, direct contact between pigs appears to be the most common (Elstrøm et al., 2019; Karlsen et al., 2021) speculate that LA-MRSA-positive pigs could spread the infection to LA-MRSA-negative pigs. The LA-MRSA infection is spread by pigs that carry the infection to other pigs, both on pig farms and outside of them. Studies have also demonstrated LA-MRSA transmission between pigs in abattoirs because of the high density of livestock in cages (Karlsen et al., 2021). If exposed to LA-MRSA through indirect contact while being transported to the abattoir and while being stunned, LA-MRSA-negative pigs could quickly become LA-MRSA-positive.

Furthermore, sows have been reported to transmit livestock-associated-methicillin-resistant S aureus to their piglets (Köck et al., 2013). During an experimental study conducted by Roth et al. 2020, LA-MRSA was found to be transmissible from sows to piglet babies. The study also found that LA-MRSA is more likely to be passed along to piglets from sows with positive LA-MRSA. Piglets from LA-MRSA-negative sows may have been infected with LA-MRSA due to other factors. In contrast, farms with high LA-MRSA colonizations have lower colonization rates of LA-MRSA, whereas farms with low LA-MRSA colonizations have higher colonies of LA-MRSA. This study found that LA-MRSA colonization in pigs is affected by environmental factors, including pollution, on each pig farm. A piglet may become a carrier of LA-MRSA or re-colonize

with it in the future. LA-MRSA colonization status in sows needs to be considered when implementing control measures. Nonetheless, standardized farm hygiene practices are difficult to establish due to the wide variation in LA-MRSA prevalence across farms, requiring farms to use well-tested control methods.

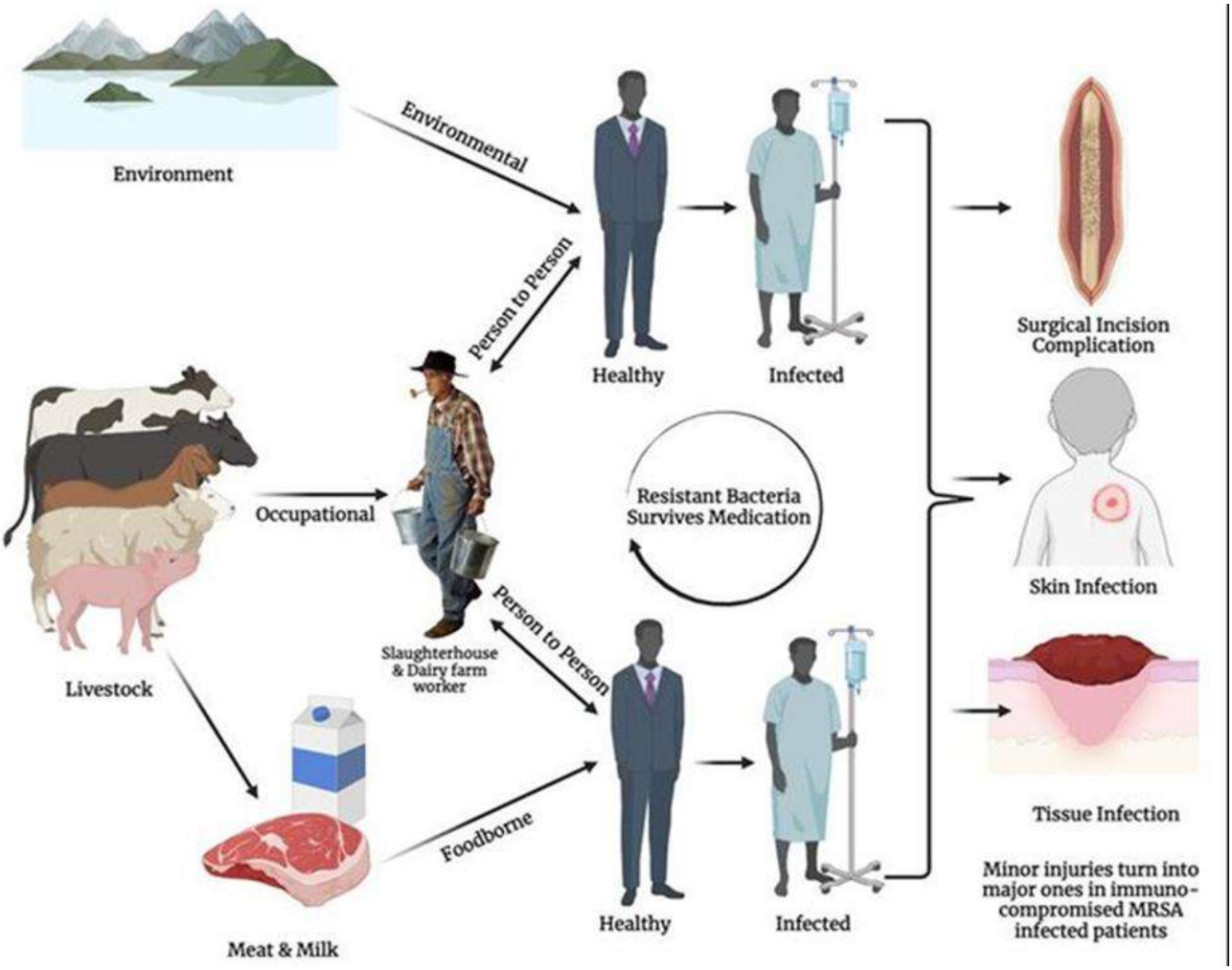

Fig. 1: An outbreak of methicillin-resistant *Staphylococcus aureus* caused by zoonotic transmission (Akhtar et al., 2023).

Human-to-Livestock

A farmer's family, abattoir staff, and veterinarians working on pig farms are at high risk for contracting LA-MRSA from pigs (Köck et al., 2013). Verhegghe et al (2016) found that 37.8% of people living or working on 25 out of 49 pig farms investigated in Belgium were infected with LA-MRSA CC389. According to another study, 7.5% of the veterinarians in Belgium who participated in this study are infected with LA-MRSA CC398, which is highly contagious. As reported by Normanno et al. (2017), the prevalence rate of LA-MRSA in the Netherlands was significantly higher (0.1%) than the LA-MRSA prevalence rate reported by the Netherlands (5.7%). Although the exact route of transmission has not been determined, humans and pigs are likely to share the same route. S aureus associated with livestock can be spread directly or indirectly through direct or indirect contact with contaminated air or the environment. LA-MRSA is more likely to be transmitted to humans through pigs and viapigs that have a high prevalence of LA-MRSA (Kinross et al., 2017). According to Krukowski et al. (2020), LA-MRSA prevalence has decreased from 26% to 11% among young cattle farmers and their families without direct contact with cattle, with only 7% carrying LA-MRSA CC398, a variant. Due to its persistent nature, LA-MRSA can cause serious health problems in humans. In addition, Mascaro et al. (2018) found that 17% of farmers and 94% of breeders initially detected LA-MRSA in pigs that were infected with the bacteria. In addition, LA-MRSA-positive individuals had no negative tests for LA-MRSA, while LA-MRSA-positive breeders did not have any negative tests for LA-MRSA. In Zomer et al. (2017) study, 59% of farmers who were previously LA-MRSA-positive did not become LA-MRSA-negative during the summer vacation. This suggests that pig farmers did not colonize LA-MRSA during their summer vacation, regardless of having direct contact with pigs. Further research is required to determine whether LA-MRSA CC398 can reinfect humans.

Environmental to LA-MRSA

LA-MRSA, or livestock-associated methicillin-resistant *Staphylococcus aureus* , has raised concerns primarily within communities with close contact with pigs, where the bacteria originate. Recent studies have delved into its transmission

dynamics, particularly within hospital settings. Würtz et al. (2017) suggested that LA-MRSA CC398, a common genotype, poses a lower risk of spreading within hospitals compared to other genotypes. Similarly, Lekkerkerk et al. (2012) found CC398 to be significantly less infectious than other strains of LA-MRSA, shedding light on its transmission patterns. Interestingly, investigations into its genome have revealed insights into its journey from humans to livestock. Bünter et al. (2017) observed a decrease in MRSA's transmission ability and virulence during the human-to-pig transmission of the CC398 lineage. These findings underscore the intricate dynamics of bacterial transmission across species boundaries.Despite its prevalence among human populations, in-hospital infections remain relatively uncommon (Boswihi and Udo, 2018). Boswihi et al. (2018) highlighted the challenges in identifying LA-MRSA strains using pulsed-field gel electrophoresis (PFGE), with a notable difference in detection rates between PFGE-positive and PFGE-negative cases.Moreover, the diverse virulence factors of LA-MRSA CC398, as identified by Stone et al. (2022), suggest a multifaceted nature to its pathogenicity. Continuous monitoring of its determinants and epidemiology, including key virulence factors like staphylococcal enterotoxins and bicomponent Panton-Valentine leucocidin, remains crucial.

The regional variations in MRSA infection rates, particularly in Northern Europe as highlighted by Goerge et al. (2017), emphasize the need for a nuanced understanding of LA-MRSA's epidemiology and its implications for public health beyond hospital settings. Further research endeavors hold promise in unraveling the complexities surrounding the spread of LA-MRSA within healthy communities.

Health Implications of MRSA in Livestock

The spread of LA-MRSA CC398 within pig populations is concerning due to its high transmissibility and adaptability in intensive farming conditions (Hoefer et al., 2022). Pigs serve as reservoirs for this pathogen, contributing to its widespread presence in pig farms. However, eliminating LA-MRSA CC398 from such environments poses significant challenges, especially given the costs and feasibility concerns associated with implementing national control and eradication programs. Despite its relatively low impact on pig health, there's a looming worry about its potential transmission from pigs to humans, particularly for individuals in frequent contact with these animals (Höjgård et al., 2015; Li et al., 2019).

The comparison between LA-MRSA CC398 and other MRSA strains, particularly those associated with hospital settings, sheds light on the different public health risks they pose. While LA-MRSA CC398 currently presents a lesser threat, there's a crucial caveat: the potential for it to acquire resistance and virulence genes, leading to the emergence of more dangerous strains (Würtz et al., 2017; Laustsen and Omland, 2019). The presence of genes like CFR, conferring resistance to multiple antibiotic classes, including those commonly used in treating MRSA infections in humans, raises alarming possibilities. Additionally, the transfer of genes like VGA further complicates the landscape, potentially leading to treatment failures, a worrisome scenario for public health (Xiao et al., 2022).

Antibiotic Usage and Resistance in Livestock MRSA

Infections are commonly treated and prevented with antibiotics since they inhibit or eliminate microorganisms' growth. They can either be bactericidal or bacteriostatic. A bacteriostatic drug inhibits bacterial growth and multiplication, while a bactericidal drug directly kills the microorganism (Crofts et al., 2017). Antibiotics with bactericidal effects include aminoglycosides, beta-lactams, fluoroquinolones, glycopeptides, and oxazolidinones, while bacteriostatic drugs include lincosamides and macrolides. In addition to blocking cell wall synthesis, inhibiting membrane synthesis or damage, inhibiting protein synthesis in ribosomes, altering nucleic acid synthesis, and altering metabolic processes, bacteria can become resistant to antimicrobials through several mechanisms (Tenover, 2006). A bacterium can develop two types of antibiotic resistance: intrinsic resistance resulting from its unique biology, or extrinsic resistance resulting from horizontal gene transfer when new genes are acquired through plasmids, transposons, integrons, or bacteriophages. Genetic changes caused by these mechanisms can enable bacteria to perform certain biochemical functions (Kapoor et al., 2017), including inactivation of enzymes, efflux pumps, or altered targets that can be specific for different antibiotic classes.

The Issue of Antimicrobial Resistance in Livestock

Farms that raise animals for human consumption have a primary goal of raising healthy and nutritious animals. Veterinary supervision, good nutrition, clean and dry conditions, and good ventilation are essential in these places (Kasimanickam et al., 2021). The same antibiotics are prescribed for humans as well as animals for bacterial infections. In some cases, antibiotic treatment is the only option, and the only way to prevent the illness from spreading, promoting animal welfare, and promoting food safety is to treat sick animals with antibiotics. The use of antibiotics for non-medical purposes has been around for a long time, however. Using these medications at subtherapeutic levels, industries can achieve highly efficient production, reducing mortality and morbidity and improving reproductive performance in livestock (Kasimanickam et al., 2021). In addition to all the benefits antibiotics may offer animal health and welfare, continued use at low levels poses health hazards to humans due to the emergence of microbes that are resistant to these drugs. Animal production is frequently prevented using antimicrobials in low-middle-income countries. Antimicrobial consumption in animal production has almost doubled human consumption due to their easy availability and non-restricted use (Chowdhury et al., 2021). A significant part of this problem is posed by unqualified animal healthcare providers in developing countries (Chowdhury et al., 2021).

A global rise in AR is often associated with antibiotic use in food animals for disease prevention, treatment, and growth promotion. Approximately 63,151 trillion antibiotics were consumed globally in 2010 (Ma et al., 2021), and in 2030

it is estimated to increase by 67%. There are national and international guidelines to ensure therapeutic efficacy and mitigate antimicrobial resistance through the responsible use of antibiotics. It is important to note, however, that compliance with such guidelines varies greatly from country to country. There is a large amount of literature discussing the risks of bacteria resistance directly affecting human health when it is transferred from production animals to humans. There are, however, far more serious consequences associated with antibiotic resistance originating from animals. Behind all the suffering caused by bacterial diseases, production animals can suffer immense economic losses. As many species are susceptible to respiratory and enteric diseases, others, such as cows, goats, and sheep, suffer from mastitis (Bengtsson and Greko, 2014). Because these infections are contagious, they become more prevalent when animals are housed in large groups. Aquaculture is also affected by bacterial diseases since fish and shrimp are raised in large numbers and are in close contact (Bengtsson and Greko, 2014). In 1951, turkeys were observed to be resistant to streptomycin after being fed streptomycin (Starr and Reynolds, 1951). There have been increasing cases of antimicrobial resistance to antibiotics, such as tetracyclines, sulfonamides, lactams, and penicillin (Ma et al., 2021). There are two main routes through which antibiotic-resistant bacteria enter the human body in terms of public health. Indirect acquisition occurs through exposure to niches with high antimicrobial pollution or through direct contact with food-producing infected animals (Vidovic and Vidovic, 2020). According to several studies, farm workers and veterinarians who work closely with food-producing animals have a high prevalence of antimicrobial resistance (Jackson and Villarroel, 2012). The use of antibiotics causes environmental pollution in animal-producing sectors although very few antibiotics are administered to animals (Tian et al., 2021). A study conducted by Cheng et al found that 30-90% of antibiotics are excreted from animals as main metabolites in their urine or feces. There is a high concentration of these metabolites that can enter soils and waters in a variety of ways, causing ecological problems (Cheng et al., 2019). As a result, soil or water bacteria living there will become resistant to antibiotics due to selective pressure. There are many similarities between the use of antibiotics in agriculture and that in human medicine. If they are transmitted horizontally, such as when they are on mobile genetic elements, like plasmids, they may pose a considerable threat to public health (Vidovic and Vidovic, 2020). Due to these factors, antibiotics can cause antibiotic-resistant bacteria to emerge when used in animal settings, especially when they are used heavily to promote growth. Indirectly or directly, humans interact with animals, which will affect their well-being and the public's health. A global economic burden is also associated with antimicrobial resistance.

Control of MRSA in Livestock

In addition to performing LA-MRSA screening tests on pigs periodically, pig farms can prevent the spread of LA-MRSA by avoiding some antibiotics (Verhegghe et al., 2015). It is the government's responsibility to punish or warn farmers when they exceed the reasonable threshold for antibiotics used on pigs (McKernan et al., 2021). In Denmark, farmers who used unnecessary antibiotics for their pigs were issued yellow cards several years ago (Kirchhelle et al., 2020). Among the updates that were made to the "yellow card" policy in 2016, a different class of antibiotics was considered for reducing the overuse of antibiotics that are vital for human health, and that also the source of antibiotic resistance (Kirchhelle et al., 2020). During 2017, fluoroquinolones were weighted 50, quinolones 50, cephalosporins 50, and tetracyclines 50%. A ban on third and fourth-generation cephalosporins has also been imposed by the swine industry because they have been found to be associated with a higher rate of LA-MRSA transmission (Bartsch et al., 2021). In addition, several European countries have implemented national antimicrobial resistance targets between 2010 and 2013 (Bartsch et al., 2021).

Farmers working in pig pens are advised to wash their hands before leaving the pen and to change their clothes regularly to prevent the spread of LA-MRSA. Veterinary professionals and farmers should work together to prevent LA-MRSA infection. Pig herds should be stopped receiving antibiotics routinely to reduce the risk of LA-MRSA infecting humans and entering human herds via pigs. Veterinary examinations should be the only time antibiotic therapy is administered to pigs. Furthermore, pig farmers and veterinary staff must have access to consultation services and antibiotics must be administered appropriately. Furthermore, increasing the use of vaccines would be beneficial (Verhegghe et al., 2016). Maintaining the cleanliness of pig pens is essential to prevent indirect LA-MRSA contamination. LA-MRSA should be checked regularly on pork farms. Furthermore, international efforts are required to reduce antibiotic resistance and to analyze LA-MRSA transmission routes (Tattevin et al., 2020). Several European countries in 2018 required adherence to hygiene protocols when handling pigs (Raasch et al., 2020).

Future Approach to Prevent LA- MRSA Infections

The use of antimicrobials, hand hygiene, controlling interactions with natural reservoirs of S aureus, preventing transmission from infected patients, decolonization, isolation, disinfecting the hospital environment, and active surveillance are among the interventions currently being used to prevent and control LA-MRSA (Lee et al., 2018). Animal and human cadres can be effectively controlled by vaccination against MRSA. The concern over antibiotic resistance, including the formidable challenge of MRSA, is well-documented by authoritative bodies like the Centers for Disease Control and Prevention (CDC) and the World Health Organization. As antibiotic resistance escalates, the urgency to develop novel vaccines intensifies. Unlike antibiotics, MRSA is not inherently resistant to antibodies, as highlighted by Anderson et al. (2012), prompting significant efforts in MRSA vaccine research by various companies (Adhikari et al., 2012). Creating an effective MRSA vaccine demands a comprehensive approach, necessitating multiple antigens to confer immunity against diverse strains (Aqib et al., 2018a). To enhance vaccine efficacy and absorption, adjuvants are incorporated, as advocated by Adamczyk-Poplawska et al. (2011). Key antigenic factors, including clustered factor A (ClfA), alpha-enolase (Eno1), and

iron-regulated surface determinant protein B (IsdB), are pivotal in developing robust staphylococcal multiepitope component vaccines. The presence of Eno1, a polypeptide found in well-preserved lineages of S. aureus, holds promise for vaccine development, given its crucial role in adhesion and microbial spread (Ghasemi et al., 2016). Similarly, ClfA, a surface protein utilized by the pathogen for host adhesion, is deemed instrumental in staphylococcal infections (Garcia-Lara and Foster, 2009). Thus, including ClfA in vaccines is imperative to stimulate an effective immune response (Brouillette et al., 2002). Moreover, IsdB emerges as another significant epitope marker involved in cell adhesion, as elucidated by Zapotoczna et al. (2013), underscoring its potential in vaccine design against MRSA. This long-lasting infection-prevention agent reduces antibiotic usage by preventing infection before it begins and disrupting the colonization of infection-causing organisms in host cells (Pozzi et al., 2017). In combination with antibiotics, antibodies developed by MedImmune, a company based in the United States, may provide protective immunity against x-hemolysin factors of S aureus. An American company also prepared and tested two monovalent vaccines, but they were not successful at producing protective levels. StaphVax and V710 vaccines produced by Nabi Pharmaceuticals use CP5 plus CP8 and IsdB as capsular polysaccharides. Shinefield et al., 2002 found that the vaccines were ineffective in phase III trials, despite being immune in animal models. USA 300 is a strain of S aureus that has no capsaicin, and vaccines are prepared without adjuvants. Several virulence factors that allow S aureus to evade immunity, including the IgG binding protein A, can compromise antibodies' function, leading to an ineffective immune response (Fowler and Proctor, 2014). Although trials are underway involving antigens such as ClfA, CP5, CP8, QT-toxin, ESAT-6, LUKS-PV, and MntC, there are no vaccines available right now. According to Lehar et al. (2015), rifampicin antibiotics and WTA-targeted antibodies were included in the monoclonal vaccine tested in preclinical trials. Researchers are working on a better vaccine, and hopefully, it will be available by the end of next year for multiple strains of S aureus, including MRSA.

Conclusion

MRSA was detected in moderate amounts (29%) in quails slaughtered for human consumption. Antimicrobial resistance and resistant genes were widely distributed among all strains, and they were all multidrug-resistant. Pigs are commonly infected with livestock-associated methicillin-resistant S aureus CC388, a pathogenic strain. Methicillin-resistant *S.aureus* associated with livestock is spread via direct and indirect contact. The LA-MRSA infection is particularly common among farmers, abattoir workers, and veterinarians. Even though it may benefit the quail industry to indiscriminately use antimicrobials, especially those considered essential for human health, this would probably lead to the spread as well as an increase of antimicrobial-resistant pathogens. In light of this, all sectors of the poultry industry should implement more restrictive legislation. Furthermore, frequently monitoring MRSA strains from poultry and other livestock will allow you to understand how the strains spread and how the genetic repertoire changes, as well as their potential as zoonotic agents.

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Chapter 14

Cytotoxic Effects of Chemotherapeutic and Immunotherapeutic Drugs in Cancer Treatment

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ABSTRACT

The phenomenon of uncontrolled cell growth and aggressive malignancies, cancer, has been the cause of millions of deaths annually. Commonly used methods for cancer treatment are immunotherapy and chemotherapy. Chemotherapy has been the most commonly used and effective treatment for various cancers but it hasn't been very successful in some cancers. The exact cause of this malfunction has not yet been determined. Chemotherapeutic medications aim to destroy rapidly proliferating cells, like cancer cells, as well as some normal cells, like intestinal epithelium. On the other hand, immunotherapy is a kind of cancer treatment that efficiently uses the body's defenses against cancer cells. This method accounts for the fact that the surface of cancer cells expresses a variety of antigens. The immune system is capable of identifying these tumor antigens. On the other hand, cancer cells typically become immune system resistant to their defense systems. Although there are several obstacles in the way of cancer immunotherapy, given its remarkable clinical efficacy, it is regarded as a promising and successful therapeutic option for cancer of two distinct kinds. Body cells can be modified to effectively identify and attach to tumor antigens and develop natural immunity. The term "active immunotherapy" is used for this kind of treatment. Another kind of therapy is Passive immunotherapy which can also modify the natural defense of the body against cancer.

KEYWORDS
Cytotoxic, Chemotherapeutic, Immunotherapeutic, Drugs, Cancer

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INTRODUCTION

Cancer is an incurable disease that has a long-lasting impact on wellness and the standard of life, and it is more common in the elderly. Globally, cancer is another most common cause of death (Siegel et al., 2023). Overall, the incidence of malignancies has grown; by 2014, there were over 1,665,540 cancer patients in the nation's capital alone, and 585,720 among them had passed away from the illness (Arowolo and M. A., 2022). Consequently, cancer is a major issue that effects human beings worldwide. Unfortunately, tissue level variability is exhibited by the disease and it poses abundant challenges for both specific diagnosis and successful therapy (Li et al., 2020). Men are more likely to develop prostate, intestinal, respiratory, and urinary bladder cancers (Siegel et al., 2024). The chest, diaphragm and airways, colon and rectum, vaginal corpus, and thyroid are the areas in women where cancer risk is highest (Tolou-Ghamari and Z., 2020). According to this data, the majority of malignancies in men and women are prostate and breast tumors, respectively. Cancers of the nervous system and lymphatic system, as well as blood cancer, account for the greatest proportion of cancer cases in children (Miller et al., 2020).

Cancer develops by a succession of sequential genetic changes that alter cell performance (Novikov et al., 2021). Chemical substances may lead to gene mutations and cancer. Furthermore, smoking contains various oncogenic chemical components that contribute to lung cancer (Hecht et al., 2022). Notably, ambient chemical compounds with carcinogenic tendencies effect either completely or partially the cell's cytoplasm and cell's nucleus, resulting in genetic abnormalities and gene alterations (Choudhuri et al., 2021). Pathogens such as bacteria, viruses, and radiation beams cause approximately 7% of malignancies (Das et al., 2020). Cancer causes disturbances in cellular interactions and cause the genes to malfunction. This results in disruption of the cell cycle and causes uncontrolled growth. Under normal conditions,

oncogene prototype is liable for cellular division and proliferation, but following genetic changes they can become oncogenic and can be detrimental to the cell survival (Stasevich et al., 2021). In addition, the absence of genes that regulate tumor suppression leads to unregulated proliferation of cells (Matthews et al., 2022). Repair genes produce mending proteins and enzymes, with over 30 kinds identified (Koliadenko et al., 2020). Removing uracil from DNA prevents UV light-induced DNA damages, which are needed for salvaging genes. The study of cell destiny and epigenetic alterations is termed as epigenetics, including DNA methylation, nucleosome location, and histone modifications, that play a crucial role in cancer formation (Kciuk et al., 2020). Pharmaceuticals used for curing cancer are called as anticancer drugs and they can be categorized by their method of execution including DNA-interactive substances, antitubulin substances, antimetabolites, molecular targeted agents, monoclonal antibodies, hormones, and other biological substances (Singh et al., 2023). This review discusses the most frequently applied anticancer medications (i.e., conventional cytotoxic agents).

Antimetabolites, an ancient class of anticancer medicines, work by interfering with biosynthesis routes (Thurston et al., 2021). Pyrimidine or purine structural analogues are introduced into components of cells to inhibit nucleic acid synthesis (Zenchenko et al., 2021). The typical pyrimidine and purine counterparts are 5-fluorouracil and mercaptopurine, respectively. Other antimetabolites, like a drug called methotrexate disrupt critical metabolic enzymatic activities (Marin et al., 2022).

DNA interactive agents are a major class of anticancer drugs that use many methods (Sharma et al., 2021). Alkylating chemicals cause DNA bases to be alkylated in smaller or larger channels (Karati et al., 2022). Such as: dacarbazine, procarbazine, and temozolomide.

Cross-linking agents that can cross-link DNA work by attaching themselves to the strand, either intra- or inter-strand (Sosic et al., 2021). The two primary subgroups of this anticancer medication subfamily are nitrogen mustards (such as cyclophosphamide and ifosfamide) and platinum complexes (such as cisplatin, carboplatin, and oxaliplatin) (Cetin et al., 2021). Cross-linking agents also include thiotepa, busulfan, and nitrosurea compounds. Intercalating agents work through base pair binding. Actinomycin-D, mitoxantrone, and anthracyclines (such as doxorubicin and epirubicin) are members of this group of drugs (Das et al., 2023).

Irinotecan and etoposide chemicals are examples of topoisomerase inhibitors. These medications block the enzymes that cause DNA to cleave, anneal, and change topologically (Yakkala et al., 2023). The site of attachment of DNA is the location of strand dissection caused by DNA-cleaning agents like bleomycin (Gendron et al., 2023).

Antitubulin drugs cause cell death by intervening with microtubule dynamics, such as building spindles or dismantling, and by preventing nucleus division. The principal constituents of this group consist of vinca alkaloids and taxanes (Čermák et al., 2020).

Treatment Therapies of Cancer

There are several cancer therapies available. Options for therapy will vary depending on a number of variables, including preferences, overall health, kind, and stage of the cancer. Options for treating cancer include:

1. Chemotherapy
2. Immunotherapy

Chemotherapy

Chemotherapy is a medication treatment that employs strong chemicals to destroy your body's rapidly proliferating cells. German scientist Paul Ehrlich developed chemotherapy techniques for the treatment of infectious disorders in the early 1900s. Concurrently, after doing several tests on animal models, he discovered that chemotherapy medications may also be used to treat cancer. The development of standard chemotherapy techniques to treat various tumors was facilitated by these groundbreaking investigations (Behranvand et al., 2022). Chemotherapy medications can be injected intravenously, intramuscularly, or orally to achieve systemic levels. Inevitably, they lack a specific antitumor activity. As a result, they not only prevent cancerous cells from proliferating unchecked, but also suppress healthy cells with high rates of emergence, such as bone marrow stem cells, gastrointestinal epithelial cells, and hair follicles, which can have grave adverse reactions and damage normal cells and tissues. As a result, it is not advised to administer them frequently, and it is crucial to weigh the risks against the benefits. Based on information on treatment resistance, drug toxicity, and the quantity of targeted tumor cells, the right dosage of chemotherapeutic drugs may be chosen. Ultimately, the number of tumor cells that remain and the reported adverse effects are used to calculate the effectiveness of chemotherapy medications (Al Shoyaib et al., 2020).

The drugs used in chemotherapy to kill tumor cells through a variety of mechanisms, such as: (1) provoking cell cycle arrest by activating the p53 gene in response to damaging DNA and suppressing kinases; (2) inducing both inner and outer apoptosis pathways through boosting the expression of Fas ligand (FasL) and subsequently cytochrome C release; (3) stimulating the autophagy pathway via the action of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinases/mammalian target of rapamycin (PI3K/mTOR); (4) DNA alkylation resulting in a single- or double-strand break that ultimately results in cell death; (5) inhibiting the synthesis of DNA or RNA because of structural similarities with antimetabolites, which are nucleotides with greater activity during the S phase of the cell cycle; (6) disrupting microtubules, which stops cell development; (7) producing reactive oxygen forms (ROS), which damages DNA directly or indirectly; and (8) inhibiting topoisomerases, which halts cell growth. The majority of these pathways cause oxidative stress and trigger

the Rad3-related (ATM/ATR) and ataxia-telangiectasia mutated (ATM) repair response. This response, which uses p53 and its subordinate p21 protein, may cause a stop in the cell cycle or accelerated fading away (Behranvand et al., 2022).

Certain chemotherapeutic drugs may potentially exacerbate the inflammatory response. Research has demonstrated that cyclophosphamide at low dosages can trigger the synthesis of many inflammatory mediators, including interferon gamma (IFN-γ), GM-CSF, IL-1β, IL-5, IL-10, as well as tumor necrosis factor alpha (TNF-α). Moreover, TME's intake of MDSCs was boosted and it inhibits the reaction of the immune system to the tumor cells. Furthermore, a significant amount of RNS (reactive nitrogen species) and ROS are produced by these cells in response to cyclophosphamide (Hwang et al., 2022). A byproduct of cyclophosphamide, Acrolein, also causes ROS generation triggering the transcription factor NF-kB, and that is essential for inflammatory reactions. It causes the activation of cyclooxygenase-2 (cox-2) and the production of cytokines that are inflammatory such TNF-α and IL-6 (Iqubal et al., 2019). Other chemotherapeutic agents, including taxanes, have the ability to stimulate the production of TNF-α, an essential inflammatory cytokine, in tumor cells found in the breast and ovary (Mercogliano et al., 2020). The natural taxane paclitaxel is used to treat breast cancer, but sadly, it may additionally stimulate a receptor called toll-like receptor 4 (TLR4), that is expressed extensively in some tumor cells. In addition to causing receptor signaling and localized inflammation in the TME, this activation also causes systemic inflammation, which may promote blood vessel development, lymphogenesis, and metastasis (Lupi et al., 2020). Additionally, paclitaxel can activate NF-kB, which results in the production of IL-1, IL-6, and IL-8, among other cytokines (Fakhri et al., 2022).

Immunotherapy

Immunotherapy, which is often referred to as biological treatment, targets cancer by boosting your body's immune system. Because cancer is not perceived as an invader by your immune system, it may thrive unchecked in your body. Your immune system can detect and combat cancer with the aid of immunotherapy. The use of immunotherapy has been acknowledged from the start of 1883. William Cole first proposed the idea of immunotherapy, which is predicated on the several defense mechanisms that our immune system exhibits to shield our body from unwanted chemicals by identifying and eventually getting rid of them. Elie Metchnikoff received the Nobel Prize in recognition of his contributions to the immunotherapy sector. In 2018, Allison and Honjo get recognition for the finding of proteins that function as restraints on T cells that combat tumors (Kaur et al., 2020). Using immunosuppressives or immunomodulators, immunotherapy either suppresses or stimulates an immune response implicated in the onset and spread of the illness. It is regarded as one of the foremost cutting-edge and effective discoveries for the treatment of many cancer kinds (Sangeetha Vijayan et al., 2023).

Cancer immunotherapy, aims to eliminate the cancerous cells by utilizing the body's natural defense systems. During this process the cancer cells are specifically targeted and the normal cells in their close proximity are prevented from any kind of harm. This process considers the fact that the tumor antigens (specific chemicals) are expressed on the surface of the cancer cells which are recognized by the immune cells. Many cancer vaccination approaches and other forms of targeted cancer immunotherapy are based on tumor antigens. On the outermost layer of tumor cells, a number of significant histocompatibility type I or II molecules present. There are situations when they are solely expressed by malignant cells rather than by healthy body cells. In this instance, they are known as tumor-specific antigens, or TSAs, and they are typically the product of a tumor-specific mutation. Tumor-associated antigens, on the other hand, are antigens that are expressed by both tumor cells and healthy body cells (TAAs). Because deleterious T lymphocytes detect these antigens, the tumor cells may be destroyed before they have a chance to proliferate. Normally, the body's immune cells can recognize these tumor antigens with ease. However, because of genetic instability, tumor cells multiply continuously, which makes them resistant to the immune system's destruction. As a result, the tumor's surroundings become immune system-suppressed, which presents a number of difficulties for the successful treatment of cancer (Babalola et al., 2021). Genetic engineering can be used to overcome this barrier in the realm of cancer immunotherapy (Chuntova et al., 2019). Immune system fortification is mostly dependent on cancer treatment. It has been demonstrated that the genetic modification of immune cells is an effective cancer therapy method. The patient's immune cells are removed from their body and genetically altered to enable them to recognize tumor antigens with greater efficiency. These altered immune cells find tumor antigens, attach to them, and eventually kill the tumor cells when they are re-administered into the patient's body (Kaur et al., 2020).

Types of Immunotherapy

Many kinds of cancer immunotherapy methods can be used, each having their own unique characteristics and mode of actions. These medication-assisted cancer treatments seek to either enhance the body's innate immune response or engage immune system components that have been altered. Cancer immunotherapy may be roughly divided into two categories:

i. Active Immunotherapy
ii. Passive Immunotherapy

Active Immunotherapy

Active immunotherapy is designed to boost the immune system of cancer patients by administering specific TAAs (tumor associated antigens) that trigger an immune response that kills tumor cells. It includes the application of

vaccination techniques, oncolytic viruses, and immune checkpoint inhibitors. Immune checkpoint inhibitors function by primarily targeting PD-1, PD-L1, or CTLA-4 to stop T cells from deactivating (Elshahidi and M. H., 2018). They are regarded as the ground-breaking turning point in the development of cancer treatments. A small percentage of the cancer patients can be effectively treated by immune checkpoint inhibitors, still some of them may experience severe immune-related side effects. The body's natural defenses against autoimmunity are strengthened on suppression of the immunological checkpoints, leading to a number of pathological events. This can have adverse repercussions on the body. Therefore, in order to distinguish between responders and non-responders and avoid any negative consequences, the development of biomarkers with predictive value is essential. Along with increased release of several cytokines and large amounts of T effector cells, the responders often have a very high neoantigen burden (Kaur et al., 2020). It has been demonstrated that the abundance of MIP1β+ NK cells and CD69+ may function as a predictive biomarker for the anti-PD-1 therapy, and the presence of CD4+ and CD8+ memory T cells can be regarded as a predictive indicator for the antiCTLA-4 therapy. Naturally existing or genetically altered viruses that specifically reproduce within cancer cells and destroy them without harming healthy body cells are known as oncolytic viruses (Rahman et al., 2021). Thus, compared to other traditional anti-cancer medications, immunotherapy against cancer can be regarded as an efficient therapeutic method with little adverse effects.

Passive Immunotherapy

An array of immune system components are employed in passive immunotherapy. These immunological components are known as monoclonal antibodies because they are often manufactured or altered in a lab. These antibodies, such as Avastin, Campath, Herceptin, and Cetuximab are widely utilized in clinical settings. They function by adjusting the immune system's reaction to cancer cells, so stopping tumor cells from evading the immune system. Herceptin's efficacy makes it a highly useful medication for treating metastatic breast cancer (Kaur et al., 2020). When it interacts with Human Epidermal Stimulating Factor Receptor 2 (HER2), monocytes and natural killer cells cause cell-mediated cytotoxicity (Collins et al., 2021). However, there is a drawback to using monoclonal antibodies; the immune system of the body could view them as alien substances and, as a result, mount an attack to eliminate them. Utilization of humanized antibodies, which are antibodies produced from non-human animals, can readily get beyond this barrier. Another component of passive immunotherapy is cytokines. These consist of interferons, IL-2, and IL-12. IL-2 has an indirect effect on cancer cells. It enhances the production of antibodies and starts a few crucial immune system processes. It has been discovered that IL-2 treatment works well for metastatic carcinoma of renal cells and melanoma. In a different research, patients with metastatic melanoma who received high dosage interleukin-2 therapy had an overall response rate of 18.1% (Clark et al., 2021). It became apparent that patients with sarcomatoid-like metastatic kidney cell carcinoma who received large doses of interleukin-2 had a better efficacy and a greater chance of surviving (Rathmell et al., 2022).

Conclusion

Millions of people die from cancer each year, making it the second leading cause of death. Regretfully, this elevated death rate would suggest that several treatment regimens are futile. Worldwide, chemotherapy is currently the primary treatment option for a variety of malignancies. Research suggests that the reason why some cancer therapies like chemotherapy are ineffective might be because they cause persistent inflammation. Actually, a number of chemotherapy medications have the potential to worsen TME inflammation, which creates an environment that is more conducive to the development, multiplication, and even recurrence of tumor cells even in cases of total remission. Unlike previous anti-cancer medicines, immunotherapy for cancer does not severely damage healthy body cells that are in close proximity to cancerous cells. Compared to other therapies, it has fewer adverse effects and yields longer-term therapeutic results. Despite being thought to be less harmful than chemotherapy, immunotherapy nevertheless has many drawbacks and adverse effects. The kind, location, and patient of the cancer all influence the wide range and dramatic nature of the treatments. The science of cancer immunotherapy has advanced, and as a result, the focus is now more on treating tumor biologic characteristics. Cancer immunotherapy in particular has to find ways to built-up resistance and almost eliminate resistant reactions in tumors in order to be effective. As a result, we should closely monitor and manage patients receiving immunotherapy.

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Chapter 15

Assessing Long-Term Non-Genetic Risk Factors for Breast Cancer

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ABSTRACT

As the most common cancer diagnosed worldwide, breast cancer affects one in eight women. The long-term risk factors for breast cancer are examined in this chapter, with a focus on psychological stress, lifestyle, nutrition, and obesity. Due to hormonal and immunological reactions, psychosocial stress, which arises from unfavorable life events like divorce and ongoing stressors, has been connected to an increased risk of breast cancer. Prolonged stress can interfere with the SAM system and the HPA axis. This can lead to elevated cortisol levels and compromised immunological function, which can further encourage the growth and spread of cancer. Inactivity and a bad diet are two major lifestyle variables that increase the risk of breast cancer. A diet high in whole grains and low in alcohol can lower the risk, whereas high consumption of red and processed meats, saturated fats, and alcohol are linked to higher risk. Exercise has been demonstrated to reduce the incidence of breast cancer by enhancing insulin sensitivity, regulating hormones, and reducing inflammation. Obesity, especially central obesity, raises the risk of breast cancer by interfering with hormone balance, causing chronic inflammation, and altering metabolism. Increased risk of breast cancer is associated with higher body mass index (BMI), particularly in postmenopausal women. Several mechanisms, including changed adipokine levels, insulin resistance, and increased estrogen production, influence this risk. As it explores these non-genetic elements, this chapter emphasizes how crucial it is to handle lifestyle changes, stress reduction, and weight control to lower the risk of breast cancer and improve outcomes.

KEYWORDS
Breast cancer, Diet, Obesity, Lifestyle, Psychosocial stress

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INTRODUCTION

As the most frequent cancer diagnosed worldwide, breast cancer has surpassed lung cancer to account for one out of every eight, diagnosed cancers and 2.3 million new diagnoses in women and men combined. It was by far the most often diagnosed cancer in women in 2020, accounting for a quarter of all cases of cancer in females. The burden of this disease has been increasing globally, especially in transitioning nations. In 2020, approximately 685,000 women lost their lives to breast cancer, making up 16% of all female cancer deaths (Arnold et al., 2022). Breast cancer is divided into 4 different types, based on the hormone receptor (HR) on cancerous breast cells HR^+ types are classified into Luminal breast cancer based on estrogen and progesterone receptors divided into Luminal type A and Luminal type B, $HER2^+$, and HR-breast cancer type includes TNBC. TNBC is the most aggressive type as compared to other types of breast cancer, which is further divided into 7 subtypes based on immunohistochemistry. This classification includes Immunomodulatory, mesenchymal, mesenchymal stem-like, basal-like (1 and 2), unstable, and luminal androgen receptors (Dai et al., 2017).

Risk Factors of Breast Cancer

A risk factor is anything that influences a person's likelihood of contracting an illness as in the case of breast cancer. There are certain significant risk factors for breast cancer that a person cannot control. For instance, since breast cancer strikes women 100 times more frequently than it does males, being a female is the primary risk factor for the disease. Risk factors are of two types: genetic risk factors in the case of breast cancer, and family history is the main risk factor. This type of cancer involves the BRCA1, BRCA2, EGFR, c-Myc, and Ras gene families. Environmental factors are anything other than genetic causes, like in the context of breast cancer, estrogen intake, aging, gender, poor lifestyle, unhealthy diet, etc.(Sun et al., 2017). According to a study, 1 in 8 women is at the risk of developing this cancer globally (Rojas et al., 2016).

Genetic predisposition and many non-genetic factors increase the chance of breast cancer to occur (Ciszewski and Jopacka-Szatan, 2015). These included late menarche, delayed first pregnancy age as above 27 to 30 years is an important risk factor, fewer pregnancies usually considered as less than 4 pregnancies may be considered a risk factor, breastfeeding duration as short or no breastfeeding, later menopause, increased obesity, poor diet and consumption of alcohol, stress, inactivity, and hormone replacement therapy (HRT). Hereditary breast cancer has also become more prevalent. For instance, the prevalence of the BRCA2 founder mutation is thought to have quadrupled in Iceland during the last century, and the total frequency of intermittent, by the age of 70, breast cancer cases also quadrupled from 2.5 percent (Howell et al., 2014). The frequent genetic causes of breast cancer include mutation in BRCA1 and BRCA2 (Petrucelli et al., 2010).

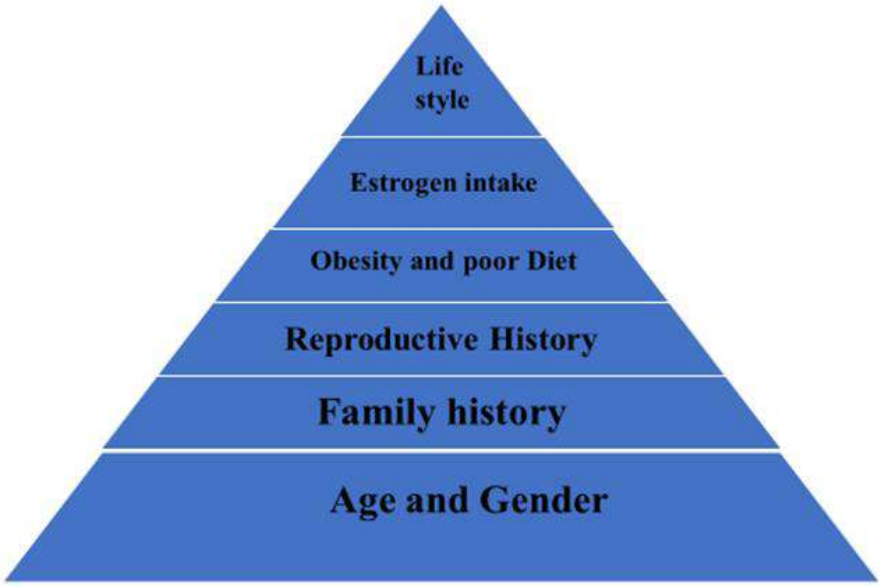

Fig. 1: Trends of non-genetic risk factors of Breast Cancer

In this chapter, the long-term risk factors in developing BC are discussed in detail. These factors are non-genetic and cause high chances of occurrence of breast cancer. The cumulative increase in the incidence of BC is all mainly due to poor lifestyle, psychosocial stress, obesity, and intake of unhealthy diet.

Psychosocial Stress Related to Breast Cancer

An inherent and everyday part of life, "stress" may be both a motivator and a burden for different people. The majority of definitions of stress refer to an external or internal stimulus, disturbance, or challenge; they can also refer to how one feels about a challenge or the body's physiological reaction to it. According to a comprehensive definition, stress is a series of events that begin with a stimulus, or stressor, that causes the brain to react, which then triggers the body's natural fight-or-flight reactions. The brain reaction is known as stress perception while the body response regarding that is known as stress response. Every person is different from others to perceive and respond to a stressful event. Short-term stress is sometimes protective and helpful since it helps the organism face difficulties, whereas long-term stress is usually damaging (Dhabhar, 2014).

Stressful events in life, death of a spouse or friend, divorce, health issues, financial burden, and environmental changes are common examples of adverse life changes. Many epidemiological studies have been conducted to determine whether unfavorable experiences in life increase the risk of developing breast cancer. For example, the risk of breast cancer following a broken marriage considerably triples due to changes in marital relationships such as divorce or the death of a spouse. The study discovered that a significant rise in the incidence rate of breast cancer was linked to intense strain and a lack of social support (Chen et al., 2023).

An important feature of stress is how long its biological effects last. Stress can be classified as short-term or chronic, depending on whether it lasts for minutes or hours. Examples of short-term stress include job interviews, any assigned work, public speaking, exercise, and sports activities, while chronic stress includes relationship problems, the death of a close one, career failure, social comparisons, self-imposed demands, caregiving, negative thoughts, worries, and prolonged financial hardship. When long-term stress starts to have negative effects, instability of the circadian cortisol cycle and reduced levels of GR receptors frequently occur at the same time (Sephton et al., 2003). Chronic stress is related to many disorders, as compared to acute stress, and this is mainly caused by environmental factors. Research indicates that differences in a wide range of socio-economic status (SES) factors, including education, job, and income, significantly explain variations in medical morbidities and fatalities. This leads to depression and post-traumatic disorders (Adler and Stewart, 2010).

The Mechanism Linking Psychosocial Stress to Breast Cancer

The relationship between cancer and stress is complicated. There may be a connection between the risk of cancer mortality and prolonged stress reaction, which may make patients more susceptible to depression. Depression and anxiety

are common outcomes of prolonged stress that lead to poorer quality of life and compromise health in different ways as hormonal changes, stress-induced changes in genetic expressions, and immune responses related to chronic stress. These all lead to many metabolic disorders and increase the chances of development and metastasis of cancer (Smith, 2015).

Hormonal Pathways

A stress response is mediated by a complex interplay of neurological, hormonal, and immunological processes, activating the immune system, the sympathetic adreno-medullar axis, and the hypothalamic-pituitary-adrenal axis. There are three hormones involved in the stress mechanism cortisol, epinephrine, and norepinephrine mainly (Mifsud and Reul, 2018).

An essential bodily system is the HPA axis which involves the hypothalamus, and two glands, the pituitary, and adrenal glands. Stress stimulates the HPA axis, which in turn increases the adrenal glands' release of glucocorticoids. Emotional and physiological conditions of fear or worry are referred to as stress. Immediately upon receiving the message, the HPA axis activates. The hypothalamus then releases a corticotropin-releasing hormone. This triggers the sympathetic nervous system, a portion of the nervous system that responds by, among other things, raising heart rate and perspiration. The pituitary gland is also impacted by corticotropin-releasing hormone in addition to those bodily alterations. Adrenocorticotropic hormone is released by the pituitary gland, released into the blood. It moves to adrenal glands through blood circulation which is the signal to begin manufacturing cortisol and other chemicals are then sent to the adrenal glands. The prolonged high cortisol level is harmful as it affects memory, sleep, weight, heart, brain, bones, kidneys etc.(Liyanarachchi et al., 2017).

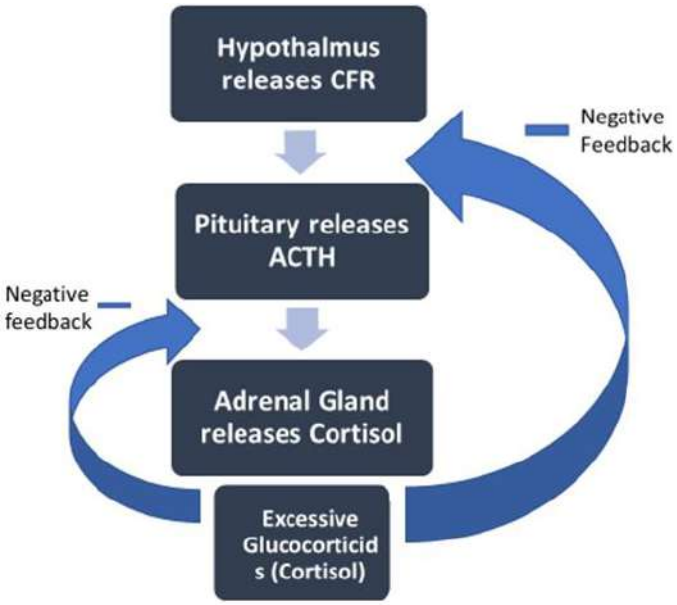

Fig. 2: Normal Mechanism of HPA axis

Another hormonal mechanism is involved in this. The stress that results from SAM activation elevates norepinephrine levels in the brain by causing the adrenal medulla to produce more of it and epinephrine into the bloodstream, as well as more norepinephrine from the sympathetic nerves. The body's smooth muscles, various organs, and the α- and β-adrenergic receptors are all impacted by the released adrenaline and norepinephrine. The release of norepinephrine and adrenaline triggers the cAMP signaling pathway that swiftly triggers cellular responses by binding to particular G-protein receptors on membranes. These receptors cause vasoconstriction, increased BP, high heart rate, high cardiac output, increased blood supply to skeletal muscle, bronchiolar dilatation, increased retention of sodium, glycogenolysis, gluconeogenesis, lipolysis, increased oxygen requirement, focused attention, analgesia, thermogenesis, alertness (Szczepek and Mazurek, 2021).

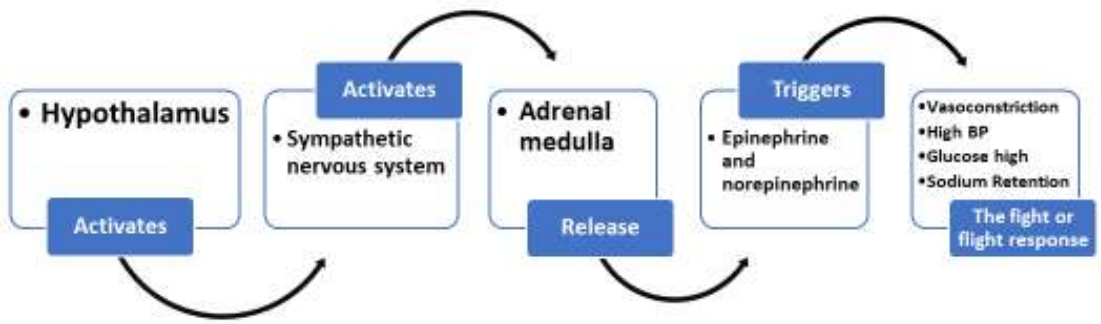

Fig. 1: Normal Mechanism of SAM Axis

Numerous studies have investigated the relationship between adverse childhood experiences and the adult risk of breast cancer. These events have been linked to a range of stressors that fall into different categories, such as psychological, physical, sexual, and parental separation, as well as domestic drug and alcohol abuse. Additionally, these events have been connected to other medical conditions that are linked with breast cancer, for example, increased levels of cortisol, alcoholism, and tobacco use (Holman et al., 2016). From a biological point of view, four biomarkers, C-peptide, adiponectin, high sensitivity C-reactive protein, and insulin-like growth factor-1, were studied about both estrogen receptor-negative disease and breast cancer overall. These results suggest that there is a connection between these biomarkers, ACEs, and a higher risk of getting estrogen-receptor breast cancer. These modifications may have revealed an explanation for the ACE/stress effect, as well as a general explanation for the overall stress and unfavorable disease outcomes (Warner et al., 2016).

Immune Response Related to Stress

The production of catecholamines and glucocorticoids is chronically triggered when stress responses are activated. It has been shown that many immune cells have glucocorticoid receptors, which take cortisol and interfere with the activity of NF-κB, an enzyme that regulates immune cells' cytokine output. Adrenergic receptor binding of norepinephrine and adrenaline triggers the activation of the cAMP response element binding protein and transcription of genes encoding several cytokines. Changes in gene expression mediated by catecholamines and glucocorticoid hormones can dysregulate immune function (Padgett and Glaser, 2003). β-adrenergic receptor stimulation typically enhances tumor cell metastasis and suppresses the immune response, NK cell cytotoxicity, and dendritic cell antigen extraction capacity. Research has indicated that elevated NE secretion and pressure-activated neuroendocrine system can enhance CD11b+F4/80+ cell infiltration and differentiation into M2 macrophages, thereby accelerating breast cancer cell metastasis to lung and lymph nodes without impairing the growth of the primary tumor (Antoni and Dhabhar, 2019).

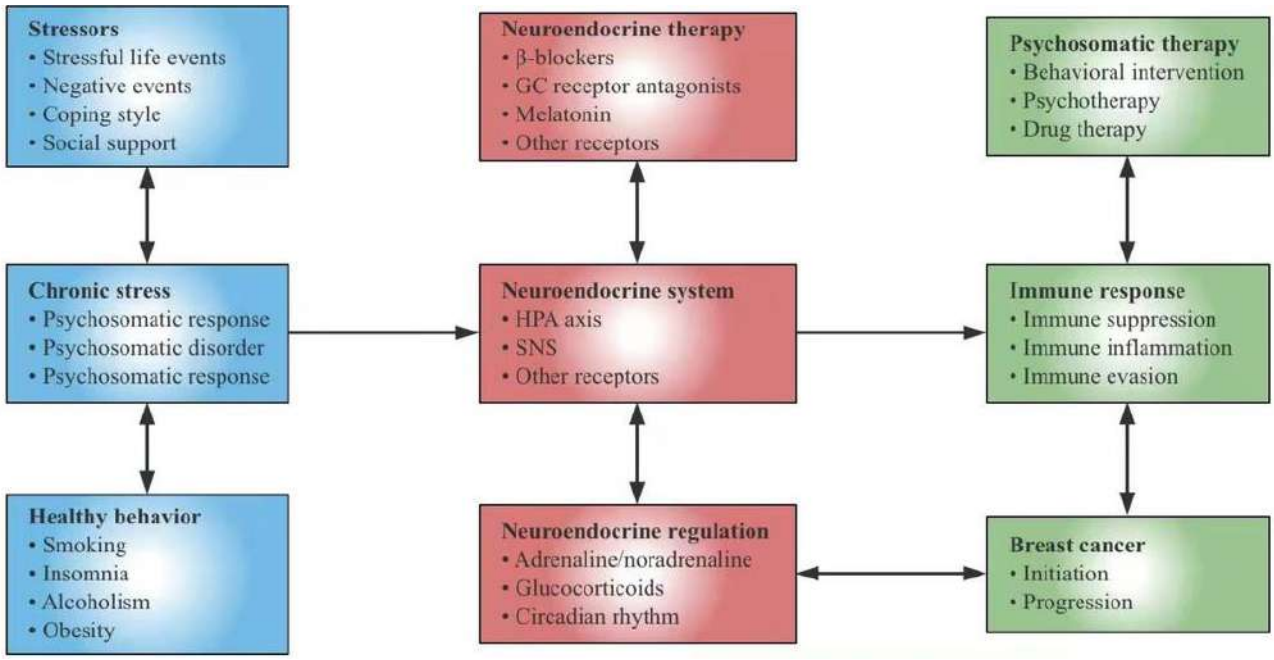

Fig. 2: Chronic stress leading to breast cancer (Chen et al., 2023)

Poor Lifestyle Risk Factor of BC

Randomized controlled trials are the only way to accurately define the genuine anticancer effects of how one lives without distractions, but they are exceedingly costly and time-consuming, which is why they are rarely done. However, a few small lifestyle adjustments lower the chance of getting breast cancer, according to several Cohort research. Rather than particular dietary variations, five trials involving postmenopausal women revealed 16% to 60% risk reductions, primarily associated with reduced body fat percentage and alcohol intake (Hastert et al., 2013; McKenzie et al., 2015). Nevertheless, compared to other lifestyle factors, investigations of a cohort of premenopausal and postmenopausal women revealed a 31% lower incidence of breast cancer in women who followed particular dietary recommendations of increasing wholegrain items and reducing meat and alcohol (Catsburg et al., 2014).

Being overweight is primarily linked with a high risk of both ER+ and ER- breast cancer beyond menopause, and it seems to have a role in both family history and individual women so try to be as lean as possible without becoming underweight. Irrespective the estrogen hormone is positive or negative, women, are more likely to develop it after drinking alcohol. Research indicates that women having a family history, defined as a first-degree relative, who is affected by alcohol are either more or equally affected by alcohol as compared to women without a family history (Scoccianti et al.,

2014). The reason behind this is the downregulation of the BRCA gene, DNA mutation, chromosomal aberrations induced by acetaldehyde, or increased estrogen receptors (Suzuki et al., 2008).

To lower all-cause mortality and increase quality of life, the American College of Sports Medicine suggests engaging in low-impact cardiorespiratory exercise for almost 150 minutes a week (Garber et al., 2011) With an estimated 3% risk reduction for every 180 minutes of additional moderate-intense exercise per week, the ideal requirement of physical activity for reducing the risk of breast cancer appears to be more than current recommendations (Wu et al., 2013). Physical activity may benefit anticancers by lowering insulin resistance, endogenous female hormone concentrations, and inflammation. Furthermore, newer routes that may be significant include those involving telomere length and oxidative stress, global DNA hypomethylation, and immunological function (Neilson et al., 2014). Smoking is also another risk factor for developing breast cancer, especially among post-menopausal women so try to avoid direct or passive smoking (Glantz et al., 2014).

Diet-related to Breast Cancer

Foods containing nutrients for body growth and the maintenance of essential activities, as well as nutritional components that function as antigens. Food has nutrients for physical development and the maintenance of essential functions, but it also contains substances that function as antigens (Soldati et al., 2018). The consumption of red meat or processed meat is significantly linked with an increased incidence of breast cancer. A study was conducted to check the link between cancer development and meat consumption and evidence was present as 6 percent more chances of developing breast cancer by consumption of processed meat. Reducing the amount of red and processed meat consumed may be a major modifiable lifestyle factor in lowering the kinds of cancer (Farvid et al., 2021).

Consumption of animal fat or unhealthy fat intake is considered as one of the risk factors in Breast Cancer. Unsaturated fat is usually safer than consuming saturated fat (Sun et al., 2017). The mechanism involved in the increase in Leptin is the over- and long-term intake of saturated fat. Leptin is a hormone that plays a role in the development of tumors and their growth and proliferation. Leptin interacts with other signaling factors like growth factors, inflammatory factors, cytokines, and estrogen receptors. Thus, by triggering it also undergoes the transition of epithelial cells into mesenchymal cells. This transition leads to metastasis of breast cancer and invasion of tumors also (Atoum et al., 2020). An unhealthy diet is related to weight gain and obesity which is also related to breast cancer.

Obesity and BMI Related to Breast Cancer

The World Health Organization defines obesity and overweight as excessive or abnormal fat buildup that poses a risk to one's health. In 1997, the World Health Organization (WHO) proclaimed obesity a global epidemic and a serious public health issue (Haththotuwa et al., 2020). In 2017–2018, the age-adjusted prevalence of obesity was defined as a body mass index (BMI) that uses weight and height to estimate overweight and obesity. A BMI of 25 kg/m^2 or greater or 30 kg/m^2 or greater is considered obese (Hales et al., 2020; Haththotuwa et al., 2020). Previous studies found that obesity and a family history of cancer significantly increase breast cancer risk in women. Higher BMI levels correlated with greater risk, particularly in those with a family history. Premenopausal and postmenopausal women are additionally at risk for breast cancer due to an increase in BMI. The combined presence of these factors amplified the risk beyond their individual effects (Chlebowski et al., 2019).

BMI Categories

Body Mass Index (BMI) categories are frequently used to divide people into various weight status groups according to their BMI estimates. The usual categories are as follows:

Table 1: BMI INDEX according to categories including normal to obese body

Category	BMI (kg/m^2)
Underweight (extreme slimness)	<16.0
Underweight (moderate slimness)	16.0-16.9
Underweight (mild slimness)	17.0-18.4
Normal Range	18.5-24.9
Overweight	25.0-29.9
Obese (Class I)	30.0-34.9
Obese (Class II)	35-39.9
Obese (Class III)	≥40.0

Limitations of BMI

Although widely used, BMI has several limitations. BMI does not provide information about the distribution of body fat. It cannot distinguish between abdominal or central obesity, which is linked to increased health risks, solely based on BMI measurements. BMI thresholds for overweight and obesity may not be universally appropriate across all age groups, sexes, and ethnicities. For instance, certain Asian populations may face higher health risks at lower BMI levels compared to other ethnic groups (Orgel et al., 2018).

Changes in Body Composition Over Time

BMI does not account for changes in body composition that occur with aging, such as loss of muscle mass and increase in fat mass. This limitation can result in misclassification of an individual's weight status as they age (Orgel et al., 2018). Studies have shown that 45% of individuals classified as overweight or obese are metabolically healthy, whereas 30% of those classified as normal weight are cardio metabolically unhealthy. This suggests that BMI alone does not reliably predict health risks (Buss, 2014).

Waist-to-Hip Ratio

An indicator of central obesity, the waist-to-hip ratio (WHR), has been associated with higher odds of breast cancer survival. With the rise in 5-year survival rates of breast cancer patients across the globe, particularly in China, there is growing interest in the impact of obesity on long-term consequences. Patients who have received adjuvant endocrine therapy for estrogen receptor (ER)-positive breast cancer, in particular, may still be at significant risk of recurrence in the years that follow (George et al., 2014). The study found that the relationship between BMI and late all-cause mortality did not significantly vary by ER status, TNM stage, or menopausal status. However, more pronounced U-shaped associations were observed in ER-negative and postmenopausal patients, and lower BMI was linked to more mortality risk in stage I breast cancer patients. The ER-positive status itself was linked with a 36% higher risk of late all-cause mortality (Zhang et al., 2017).

Central Obesity with Breast Cancer

Obesity-related visceral fat raises breast cancer risk by disrupting hormonal and inflammatory balances, activating pathways such as NFκB, JAK, STAT3, and AKT. It also intensifies oxidative stress and affects miRNA levels involved in cancer development. Additional research is required to explore these mechanisms and the epidemiological effects of central obesity on breast cancer (Zimta et al., 2019)

Body fat Percent Measurement

To find the body fat percentage the information about breast cancer patients with different stages of cancer is taken. Data on demographics, exercise habits, treatment, and body measurements were gathered, and outcomes such as metastasis and mortality were evaluated over several years. The following methods can be used.

Anthropometry

Participants' height, BMI, weight, and body fat were measured using a digital scale and Bioelectrical Impedance Analyzer (BIA). The BIA assessment was performed with specific positioning and preparation to ensure accurate body composition results.

Data on disease progression were gathered from medical records and phone interviews, tracking the time from cancer diagnosis to events such as metastasis and mortality.

Disease Progression

To understand the disease progression data on disease progression, such as metastasis and mortality, were gathered through medical charts and phone interviews. The interval between cancer diagnosis and each event was measured in months (Khan et al., 2023).

Demographic

Demographic and disease information is gathered in this type, focusing on disease stage, ER/PR status, age, and exercise habits. The treatment modality is excluded due to its strong association with the disease stage (Kumar et al., 2015).

Mechanisms Involving in Obesity and Cancer Association

Hormones

Breast cancer risk is increased by obesity via several hormonal pathways. Body obesity increases the synthesis of estrogen, which leads to hormone receptor-positive breast tumors. Obesity-related insulin resistance increases insulin and IGF-1 levels, which promotes the development of cancer cells. Chronic inflammation and abnormalities in adipokines, including adiponectin and leptin, also have a role in the development of breast cancer (Emons, 2022; Zattarin et al., 2020)

Chronic and Acute Infections

Breast cancer can be triggered by chronic inflammation, often associated with obesity. This inflammation creates tumor-friendly conditions through elevated levels of pro-inflammatory cytokines, angiogenesis, and cell proliferation. The immune response to this inflammation can alter the local breast tissue environment, facilitating cancer development. Additionally, changes in the breast microbiome may increase this risk. In contrast, short-term inflammation caused by acute infections typically has less long-term carcinogenic potential than chronic inflammation (Ma et al., 2023).

Metabolic Syndromes

Metabolic syndrome (Abrahamson et al.) is a collection of metabolic disturbances characterized by insulin resistance, low HDL cholesterol, high triglycerides, obesity, and hypertension, which links obesity to liver damage and breast cancer. Excess fat from obesity accelerates tumor development and progression by fostering insulin resistance and chronic inflammation. Elevated estrogen levels due to obesity increase breast cancer risk, while insulin resistance promotes cancer cell growth. Those with metabolic syndrome also face additional health complications such as liver damage, diabetes, hypertension, and dyslipidemia. Moreover, the mechanisms linking obesity to breast cancer onset are complex, involving factors such as increased estrogen levels, insulin resistance, inflammation in mammary fat, heightened aromatase expression, and elevated leptin levels(Coleman, 2016; Grossmann et al., 2010; Xu et al., 2020).

Managing obesity and its related health risks, such as metabolic syndrome and breast cancer, necessitates a range of approaches, including exercise, intermittent fasting, medication, bariatric surgery, and calorie restriction. Effectively managing obesity and its associated health risks, like metabolic syndrome and breast cancer, requires a multifaceted approach. Caloric restriction aids weight loss by reducing daily intake while maintaining nutritional and metabolic health. Pharmacotherapy, using medications such as liraglutide and orlistat, should be supervised by a healthcare provider due to potential side effects. Bariatric surgery, including sleeve gastrectomy and gastric bypass, provides significant weight loss and improves obesity-related conditions for those with severe obesity. Regular exercise, combining resistance and aerobic training, enhances cardiovascular health and insulin sensitivity. Intermittent fasting, involving cycles of eating and fasting, can lead to weight loss and improved metabolic health. Combining these methods often yields the best results, and personalized plans developed with medical professionals are essential for optimal outcomes(Chomiuk et al., 2024; Golbidi et al., 2012).

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Chapter 16

Toxicity Induced by Commonly used Antimalarial agents

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ABSTRACT

Various species of plasmodium cause malaria and it remains one of the most infectious diseases present globally. Antimalarial drugs have been used over the years to control the disease to control it. The number of antimalarial drugs is not high but they are used in excess because of their low prices and are easily accessible. Aryl Aminoalcohol Compounds, Anitfolate compounds (antifols), 8-aminoquinoline, and Artemisinin compounds have been used to reduce the intensity of the disease. These groups include Quinine, chloroquine, Mefloquine, sulfadoxin, primaquine, and amodiaquine. These drugs are used as a single dose or combined when the disease frequency is very high. These groups include the over and prolonged time use of the drugs will lead to toxicity of various organs. The toxicity of various drugs includes cardiotoxicity, nephrotoxicity, hepatotoxicity, Gastroenterotoxicity, ocular toxicity, hematotoxicity, ototoxicity, cutaneous toxicity, and myopathy. On the other hand, drug toxicity is little acceptable because it produces fewer harmful effects than the disease itself. For a pregnant woman, its prescription is little advisable because of the health risks of the mother and fetus.

KEYWORDS
Malaria, Plasmodium, Antimalarial drugs, Toxicity

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INTRODUCTION

The term malaria is deduced from the Italian word *Malus aer* meaning bad air. Malaria is a febrile illness caused by the small intracellular protozoan pathogen, plasmodium parasites transmitted by the bite of female *Anopheles* mosquito among vulnerable populations belonging to tropical areas of the world especially in South East Asia and Africa. The genus *Plasmodium* consists of amoeboid intracellular parasitic organisms found to harbor an insoluble hemoglobin metabolite as a malarial pigment. Four out of 120 plasmodial species are involved in causing infections in their human host. These include *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malaraie,* and the one primate-originated *Plasmodium knowlesi*. Among these, *Plasmodium falciparum* is associated with 60-70% fatalities. (Daily, Minuti, and Khan, 2022; Khan et al., 2023; Talapko, Škrlec, Alebić, Jukić, and Včev, 2019; Varo, Chaccour, and Bassat, 2020). Plasmodium falciparum carries the highest burden of case fatalities in South Africa (95%) and South East Asia (63%) (Rathod et al., 2022). All age groups are equally susceptible to these species but pregnant ladies, infants, and young children under age five are at higher risk of getting disease due to partial immunity. Other vulnerable groups include refugees, and travelers. Malarial disease is characterized by the onset of high fever with chills, perspiration, abdominal discomfort, nausea, anorexia, fatigue severe headache, and muscles and joint aches triggered by the injection of schizonts from the gut of the female Anopheles mosquito into the blood of the intermediate host, the human where these passes through some stages to complete its asexual cycle (Talapko et al., 2019; Tuteja, 2007). The sporozoites invade hepatocytes to develop multinucleated liver-stage schizonts which upon segmentation develop into merozoites. The intracellular multiplication of merozoites bursts the erythrocytes resulting in exponential expansion of merozoites population. After this, the plasmodial parasite enters its gametocyte stage in its definite host, the female *Anopheles* mosquito where it enters after sucking a blood meal and completes its life cycle, thereby again developing into sporozoites as shown in the malarial life cycle in Figure 1. (Guttery, Zeeshan, Ferguson, Holder, and Tewari, 2022; Milner, 2018; Talapko et al., 2019; Tan and Blackman, 2021; Tuteja, 2007; Warren, 2016).

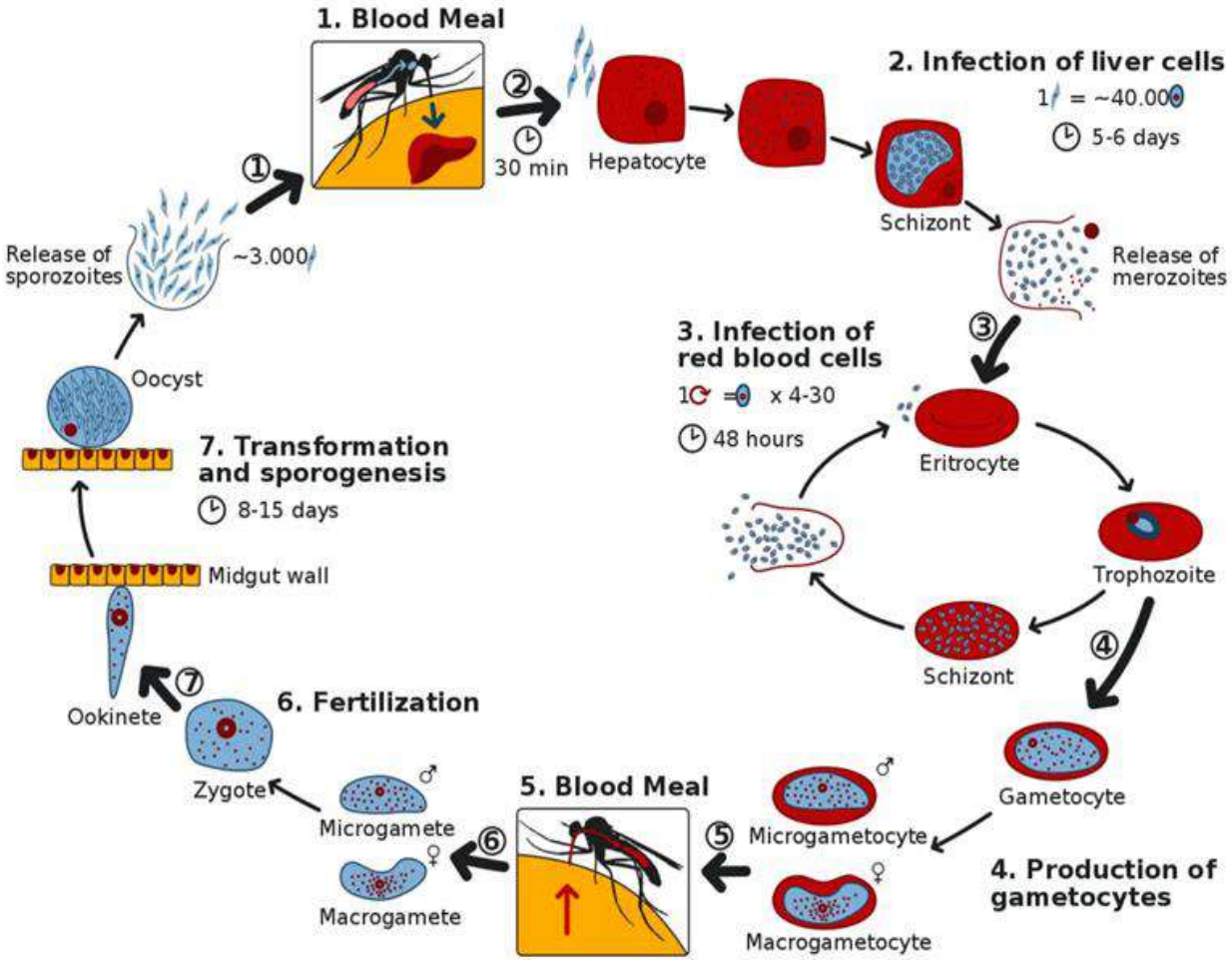

Fig. 1: The different stages of the life cycle of the Plasmodium parasite within the Human host (intermediate host) and the mosquito vector (definite host).

If left untreated, malaria causes severe complications like anemia, and hypoxia, induces hepatomegaly, splenomegaly, and kidney disease, and can even cause coma and eventually death until it reaches its severity (Balaji, Deshmukh, and Trivedi, 2020). It is associated with high mortality and morbidity in the areas of the world where it is endemic. Africa and some regions of some tropical regions of Asia are the most endemic regions of the world where it is prevalent. According to an estimation conducted in 202, there appeared to be 241 million cases of malaria worldwide with 627000 fatalities, and most of those belonged to the African Sub-Saharan desert. These stats elevated to 247 million by 2021 and caused 619000 deaths per world Malaria Report, 2022 (González-Sanz, Berzosa, and Norman, 2023; Siqueira-Neto et al., 2023). There appears approximately 2000 cases of malaria in the United States, each year (Daily et al., 2022; Fatima et al., 2023). A study conducted by Islam, Dhar, and Rahman (2023) reported approx. 3.2 million suspected cases of malaria in Pakistan between January to August 2022. 17000 of those were confirmed in laboratory testing. *Plasmodium falciparum* (23%) and *Plasmodium vivax* (77%) were reported to be the most prevalent species in Pakistan. A cross-sectional study conducted in Sindh, Jamshooro following flood indicated Plasmodium vivax as the as the highest prevalent species of malarial parasite (Humaira et al., 2023).

The prevalence of malaria is having a great impact on the economic crisis of countries like Sub-Saharan Africa as it caused a US $19 billion annual loss to the economics of the said country with the highest level of morbidities (95%) and mortalities (96%) as per stats published by Siqueira-Neto et al. (2023).

Various antimalarial agents have been used as prophylaxis and the treatment of malarial disease but at the same time, they exert some toxic effects on human host leading to organ damage. Antimalarial agents are broadly categorized as follows based on their chemistry and mechanism of action:

I. Aryl Aminoalcohol Compounds
II. Anitfolate compounds (antifols)
III. 8-aminoquinoline
IV. Artemisinin compounds
V. Others (Antibacterial compounds and atovaquone (Na-Bangchang and Karbwang, 2019; Taylor and White, 2004).

Mechanism of Action of Antimicrobial Drugs

Antimalarial drugs work uniquely by killing parasites at various phases of their life cycle given as in Fig. 1.

1) Blood Schizonticidal drugs work by targeting the erythrocytic stage of the plasmodial life cycle thereby preventing the invasion of erythrocytes. Primaquine and proguanil are the primary tissue schizontocides. Two types of antimalarial agents are used as follows:
Fast acting drugs: chloroquine, quinine, mefloquine, atovaquone and artemisinin.
Slow-acting drugs: pyrimethamine, sulfonamides, sulphone, proguanil, and tetracyclines.
2) Tissue Schizonticidal drugs work by interfering with the dormant stage of the life cycle of the plasmodial parasite, the hypnozoite formation stage.
3) Gametocytocidal drugs affect the sexual erythrocytic phase of the *Plasmodium* in blood when infections pass to mosquitoes. These drugs prevent the transmission of infection to the sexual gametocyte stage of the plasmodial life cycle by interfering with its gametocytocidal stage. Examples of such drugs are chloroquine, primaquine, and artemisinin. E.g primaquine and Artemisinin protect against all Plasmodium species, chloroquine, and quinine act on *P. vivax*.
4) Sporontocides work by stopping the proliferation of oocytes to its sporozoites form so that infection may not start again. Examples include primaquine, proguanil, and pyrimethamine.
5) The prophylactic antimalarial agents block the erythrocytic stage of the life cycle by preventing the invasion of erythrocytes and killing parasites in hepatocytes. Examples include primaquine, proguanil, and pyrimethamine. A study carried out stated that these drugs work by preventing the initial developmental stage of malarial parasite in the liver cells by blocking the initial asexual phase of the malarial life cycle, thereby preventing the relapse of *Plasmodium ovale* and *Plasmodium vivax* induced by hypnozoites of the liver (Aderibigbe and Mukaya, 2016; Alam et al., 2009; Fontinha, Moules, and Prudêncio, 2020; Grace, 2023; Shibeshi, Kifle, and Atnafie, 2020).
The mechanism of action of antimalarial drugs according to their target site as per study by Siqueira-Neto et al. (2023) is shown in Fig. 2.

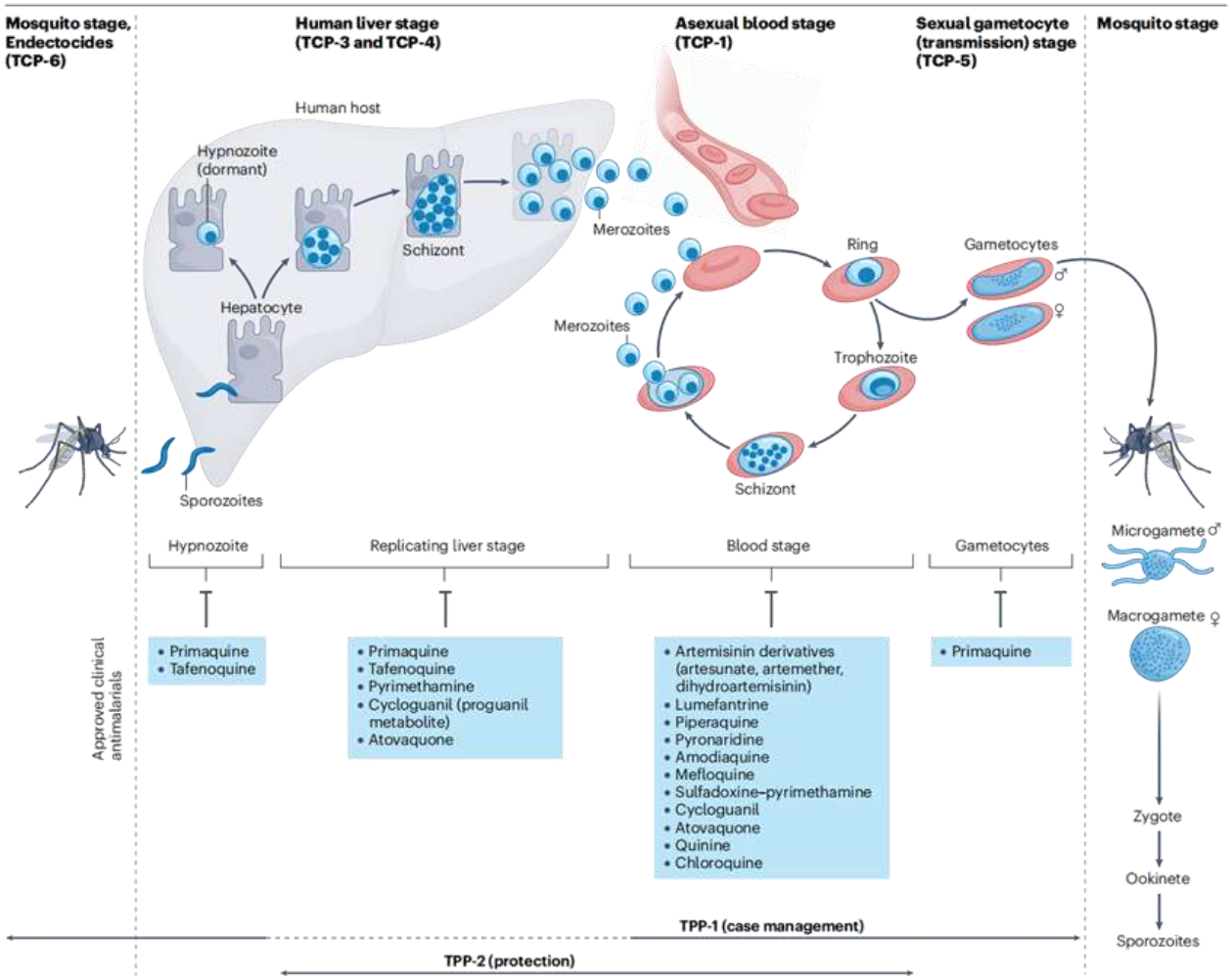

Fig. 2: The target sites of Antimalarial Drugs in the context of *Plasmodium* Life cycle

Toxicity Induced by Antimalarial Drugs

Apart from killing and interfering with various developmental stages of the life cycle of *Plasmodium*, antimalarial agents are found to induce some adverse effects on the health of the patients known as toxicity. The detailed discussion on the toxicity induced by the following classes of antimalarial drugs is as follows:

1. Aryl Aminoalcohol Compound Quinolines affect the polymerization of hemozoin.
2. Antifolate Compounds (works by blocking dihydrofolate reductase and dihydropteroate synthetase enzyme
3. 8-aminoquinoline
4. Artemisinin Compounds
5. Other Drugs

Neurotoxicity Induced by Antimalarial Drugs

Chloroquine has been used as prophylaxis along with proguanil since long but it induced several neuropsychiatric effects by hindering the transporter protein p-glycoprotein with the induction of neurotransmitters, anticholinergic effects and interfering with the cellular metabolism of antimalarial drug as per reports by Grabias and Kumar (2016).

Quinine being employed against *Plasmodium falciparum* was tested using serial audiometry test performed on 10 patients which reported tinnitus in 7 patients with resolved high tone issue with reduced plasma level of 5mg/ml. The ingestion of a high dose of 6.5-7.8g of quinine sulfate resulted in deafness and mutism in a 14years child as reported by Nagarajan and Lam (2000).

Another study by Nagarajan and Lam (2000) explained that overdosage of tetracycline may lead to benign intracranial hypertension.

Mefloquine is used to treat the blood stage of the parasite and its neurotoxic reactions were first reported in 1971. Grabias and Kumar (2016); Nevin (2012) reports the neurotoxicity associated with this drug appeared in the form of sleep disturbance, anxiety, depression, nausea, and psychoses. Further in vivo studies observed the appearance of similar types of adverse reactions with the introduction of relevant Pamaquine and Plasmocid.

Mefloquine causes neurotoxicity by interfering with the degradation of acetylcholine, acetylcholinesterase, and butyl cholinesterase, thereby activating the post synaptic neurons which further interfered by the drug to restore cell resting potential with the inhibition of calcium pump and ATP-sensitive potassium pump as reported by Dow, Hudson, Vahey, and Koenig (2003); Gribble, Davis, Higham, Clark, and Ashcroft (2000); McArdle et al. (2005).

Oxidative stress-related neurotoxicity induced by mefloquine causes oxidative damage to cells and its constituents like DNA, proteins lipids etc. The lipid contents of the brain need more oxygen to function efficiently thereby undergoing oxidative stress eventually causing neurological disorders by disrupting CNS function. Oxidative stress is modulated by Reactive oxygen species (ROS) that is unable to get balanced by antioxidants present in cell such as superoxide dismutase, glutathione, and glutathione peroxidase as per studies conducted by Martins et al. (2021); (2009); Valko et al. (2007).

In a study conducted by Hounkpatin, Kreidenweiss, and Held (2019) reported that the severe psychiatric issues associated with the higher therapeutic dose of tafenoquine appear in the form of sleeplessness, mood swings, anxiety, and abnormal dreams in patients with a previous history of psychological disorders. The prolonged Cmax and half-life of the drug might have delayed the onset of the associated signs and symptoms. The study reported the first observed mood disorders in 52% of the patients aged 60years old (Brodaty et al., 2001; Thakkar et al., 2018).

Cardiotoxicity Induced by Antimalarial Drugs

The release of nitric oxide and histamine may mediate cardiovascular response leading to postural hypotension. Chloroquine induces cardiotoxicity by stabilizing membrane, direct negative inotropic effects, and arterial vasodilation (Badhe, 2019; Mubagwa, 2020).

Cardiotoxicity is associated with the overdosage and long-term dosage of the antimalarial agents as the high dose of chloroquine and hydroxychloroquine induces cardiomyopathy in patients by disturbing the conductance of heart and inducing congestive heart failure as per case report published by Costedoat-Chalumeau et al. (2007). They further reported that a 59years lady was presented with cardiotoxicity induced owing to the long-term high dose administration of antimalarial agent for 13years against discoid lupus erythematosus which induced congestion disturbances and congestive heart failure ultimately leading to the transplantation of the heart. Severe cardiotoxic effects were reported when patients were introduced to chloroquine and hydroxychloroquine for the long term for the study of conduction disturbances (45 patients) and heart failure (25 patients), respectively.

According to study reports by Chattopadhyay, Mahajan, and Kumar (2007), it is been reported that chloroquine has a very low margin of safety. Chloroquine beyond recommended doses leads to cardiotoxicity and eventually death. The dosage of 20mg/Kg is the recommended safe dose of chloroquine. The ingestion of higher dosage of even 30mg/kg proved to be fatal leading to cardiac arrest and respiratory distress, eventually causing death.

Desmarais et al. (2021); Doyno, Sobieraj, and Baker (2021); Tselios, Deeb, Gladman, Harvey, and Urowitz (2018) reported the prolongation of QTc interval and cardiac arrhythmia with prolonged use of chloroquine and hydroxychloroquine, thereby inducing cardiac toxicity. Similar kinds of adverse reactions were observed with the additional use of Plaquenil associated with the above drugs as ventricular arrhythmia and torsade's de point (tdp) like severe toxic effects were observed with overdosage along with other combinations.

Bouchaud et al. (2009); Haeusler, Chan, Guérin, and White (2018) reviewed Halofantrine as the sole drug associated with the unacceptable risk of arrhythmogenic toxicities when employed as an antimalarial agent. This drug has the indications of inducing serious cardiovascular effects in term of conduction delay and extreme QT prolongation. A GlaxoSmithkline Global Safety Database reported 26 out of 35 cardiotoxic related mortalities associated with the use of halofantrine in his review published in 2009 between 1995-2005. This was associated with five pediatric deaths along with

70% of the female death reports. This drug was employed against the malarial infection associated with the highly lethal *Plasmodium falciparum* as per reports by WHO (2017) .

Ototoxicity Induced by Antimalarial Drugs

Ototoxicity induced by quinine has induces negative impacts on the central peripheral auditory system in terms of impairment of disturbance of cochlea in the form of impairment of outer hair cells (OHCs), microangiopathy and reduced blood flow owing to induced thrombocytopenia and disseminated coagulation in blood vessels. High dose induced several morphological and physiological changes in cochlea i.e. elongation and contraction of OHCs and forms central microtubule cores inside it (Alvan et al., 2017; Martins et al., 2021).

Ding et al. (2013); Ding et al. (2020); Jozefowicz-Korczynska, Pajor, and Lucas Grzelczyk (2021) stated Ototoxicity induced by the use of increased therapeutic concentration of mefloquine in term of damage induced to cochlear base hair cells and supporting cells present before hair cells. In addition to this it caused the oxidative stress in the hair cells and SGNs, and induce apoptosis activated by caspase-3.

Nephrotoxicity Induced by Antimalarial Drugs

Nephrotoxicity related to antimalarial drugs is correlated with the long term and high dose use of drugs. Chloroquine induced nephrotoxicity is infiltered by the prolonged usage of high dose of the drug. This drug induces serious consequences related to kidney in patients with renal impairments (Wiwanitkit, 2016). Thorogood et al. (2007) addressed serious consequences of renal toxicity correlated with high dosage of the drug leading to kidney failure with megaloblastic anemia, exfoliative dermatitis and pancytopenia.

Mahmoudi, Sadigh-Eteghad, Salehi-Pourmehr, Gharekhani, and Ziaee (2020) reported kidney failure in among old age Covid-19 patients when treated with chloroquine and hydroxychloroquine.

Mahmoudi et al. (2020) has reviewed Quinine as the safe drug but allergy to this drug my induce acute renal failure, neurological impairments, neutropenia, anemia, thrombocytopenia, nephrotoxicity, disseminated intravascular coagulation, and liver toxicity.

Wiwanitkit (2016) reported Artesunate as the effective and safe antimalarial drug against *Plasmodium* species but when employed for a prolonged duration it shows serious kidney injuries by lowering the function of the glomerulus, increasing kidney blood flow, and urinary excretion of ions of sodium, potassium, and nitrogen. When employed in the normal amount it excretes the normal amount of blood urea nitrogen (Azlan et al.) but the prolonged treatment with Artesunate may lead to serious renal injuries due to increased concentration of BUN with time as analyzed by Nwankpa, Ogbonna, Udekwu, and Nwokafor (2020).

Another study by Plewes et al. (2015) reported kidney failure associated with artesunate use against *Plasmodium falciparum-infected* malarial patients.

Hepatotoxicity Induced by Antimalarial Drugs

The adverse reactions caused by antimalarials to the liver are categorized as antimalarial drug-induced hepatotoxicity. McGready et al. (2000) reported hepatotoxicity of the amodiaquine causing liver damage induces the release of amodiaquine-quinone imine, an electrophilic metabolite involved to initiate hypersensitive reactions by binding to cellular macromolecules. Repeated exposure to the antigen triggered by this metabolite induces organ damage such as hepatitis and agranulocytosis and disrupts its hepatocytic function.

Another study by Ihegboro and Ononamadu (2022); Manov, Motanis, Frumin, and Iancu (2006); Shimizu et al. (2009) also added that the hepatotoxic effects achieved by the oxidation of amodiaquine by liver microsomes and peroxidases stimulate the discharge of iminoquinone metabolite by causing reversible binding to protein which in turn disrupts hepatocytes thereby inducing direct liver toxicity.

Artemether and lumefantrine also induce oxidative stress to the cell by exerting ROS species which induces apoptotic cell death therefore interfering with the liver proliferation and G_0/G_1 phases of the cell cycle via the apoptotic mechanism as mentioned by Audu, Patel, and Idowu (2023); Yin et al. (2020).

A meta-analysis was performed by Xiong and Huang (2021) on thousands of patients to study the hepatotoxic effects of artemisinin therapy employed on malarial patients. 1.3%, 1.1%, and 0.9% of all the reported cases were presented with neutropenia, elevated QT interval, and increased liver enzymes, respectively. Another study by Deeken et al. (2018) added that the patients presented with advanced solid tumors were able to tolerate even 18mg/kg of the therapeutic concentration of ART when injected intravenously but surprisingly, they showed severe manifestation of infusion reaction even at the lowest therapeutic concentration of 8mg/kg, intravenously.

Myopathy induced by Antimalarial Drugs

The risk of myopathy induced by antimalarial drugs is noticed very rarely. This results from concomitant diseases like hyperthyroidism, renal disease, hepatic disease, and diabetes characterized with muscle weakness owing to the use of high doses of antimalarial drugs (Taylor and White, 2004).

A software-based Comprehensive meta-analysis of chloroquine and hydroxychloroquine was carried out by Biguetti, Junior, Fiedler, Marrelli, and Brotto (2021) at a confidence interval of 0.05 for qualitative analysis of 23 studies including 17 case reports and quantitative analysis of 5 case reports, respectively. 21 patients from Case reports were presented with

muscle weakness and a biopsy confirmed the induction of myopathy with the use of chloroquine and hydroxychloroquine. 37 of 13367 patients chosen for observational studies from five studies appeared with muscle weakness while 252 were found to exhibit an elevated level of enzymes such as phosphokinase, creatinine, adelase, and lactate dehydrogenase, thereby inducing myopathy related to drug.

Ocular Toxicity Induced by Antimalarial Drugs

Ocular toxicity is defined as the severe adverse reactions associated with the overdose or prolonged use of antimalarial agents on the eye. Ocular toxicity was first introduced in literature in1957 (Badhe, 2019; Badhe, Aher, and Saudagar, 2019). Lewis, Gregorian, Portillo, and Goad (2020b) reported the adverse photosensitive reaction of prolonged usage of chloroquine is related to keratopathy and retinopathy.

Marmor, Kellner, Lai, Melles, and Mieler (2016) reports that the ratio of renal toxicity increases to 1% in patients up to 5 years, 2% up to 10 years, and 20% upto 20years of patients with the use of chloroquine for five years as per baseline and annual screening criteria set for retinal toxicity by the American Academy of Ophthalmology.

Another study by Marmor et al. (2016) reports the increased risk of adverse effects of tamoxifen rise in the form of retinotoxicity when taken by patients with impaired renal function.

AlKadi (2007); Neubauer et al. (2003) reviewed the prevalence ratio of retinopathy among unmonitored patients that ought to be 10% in case of chloroquine administered patients while 3-4% for hydroxychloroquine administered patients. These drugs cause visual impairment in affected patients taking high dose. The elevated plasma level of quinine may result in blurred vision leading to complete blindness within hours. Blurred vision may proceed to erythema which can be observed in fundoscopy as blurred vision causes the dilation of pupils which in turn become photosensitive resulting in narrowing of arteriole of the retina of the eye.

Taylor and White (2004) reported the prevalence rate of severe ocular toxicity induced by quinine in patients affected with severe and uncomplicated malaria is less than o.1%. It causes severe intermittent and permanent blindness in patients taking high therapeutic doses of quinine.

Badhe (2019) addressed a 34-year-old man being administered with 1250g of amodiaquine hydrochloride for up to a year was presented with diffused changes induced in conjunctiva and cornea thereby inducing retinal function impairments and vision impairments.

Gastroenterotoxicity Induced by Antimalarial Drugs

Doyno et al. (2021); Mulenga-Cilundika, Ekofo, Bagalwa, Kabuya, and Chenge (2020) reported gastrointestinal toxicity even with the short-term administration of hydroxychloroquine and chloroquine characterized by nausea, vomiting, and diarrhea-like symptoms. In addition to this, hydroxychloroquine was found to induce glucose level abnormalities by altering the glucose concentration in the blood but rarely at standard therapeutic doses.

Mulenga-Cilundika et al. (2020) reported the adverse reactions associated with primaquine in the form of gastrointestinal discomfort, abdominal pain, nausea, lack of appetite, hypo-gastric pain, nasogastric pain, choluria, myalgia, arthralgia, breath shortness, dizziness, and bronchospasm, etc. Another study by Grace (2023); World Health (2015) revealed similar kind of adverse effects induced by dihydroartemisinin-piperazine when employed against *P. malaraie, P. ovale,* and *P. knowelsi*.

Another antimalarial induces mouth ulceration and further gastrointestinal tract disorders characterized by abdominal pain, nausea, vomiting, and diarrhea in reports presented by World Health (2015).

Studies suggested ACTS exerts a high index of gastric ulcers on a patient's gastric mucosa and mucosal layer, thereby raising therapeutic challenges. This therapy induces oxidative stress which causes mucosal ulceration as per reports mentioned by Kalange et al. (2020).

Cutaneous Toxicity Induced by Antimalarial Drugs

Cutaneous toxicity involves the adverse reactions of antimalarial drugs to the skin. As reported by Lewis et al. (2020b) the prolonged overuse of chloroquine is involved with the spread of pruritus in dark-skinned people.

Sulphadoxine/pyrimethamine when used as prophylaxis caused severe adverse reactions in the form of Steven Johnson syndrome, multiform erythema, and toxic epidermal necrolysis with a prevalence ratio of 1/8000 found to cause severe adverse reactions among American travelers and 1/1100 to 1-25000 fatal outcomes as per stats reported by Taylor and White (2004). The stats related to the risk associated with cutaneous toxicity in UK and Sweden showed 1/4, 900, and 1-10, 000 cases while these stats elevated case fatalities to 1/11, 100, and 1/35,000., respectively.

Another severe cutaneous reaction was observed as erythroderma with the presumptive use of Sulphadoxine/Pyrimethamine and chloroquine as prophylaxis as per a study review by Taylor and White (2004).

Hematoxicity and Immuno Toxicity Induced by Antimalarial Drugs

Primaquine is active against all phases of the *Plasmodium* life cycle. It induces hemolytic toxicity in patients characterized by the deficiency of Glucose 6 Phosphate Dehydrogenase (G6PD) even after 2-3 days of use. The severity of hemolytic toxicity induced by primaquine depends on its therapeutic dose, length of therapy, and degree of G6PD deficiency as mentioned by Lewis et al. (2020b).

Maokola et al. (2011); Mutabingwa et al. (2009) Co-trimoxazole induces various life-threatening cutaneous disorders like toxic epidermal necrolysis, Stevens-Johnson syndrome erythema multiforme, and some forms of allergies.

Further studies by Lewis et al. (2020b) showed associated hemolytic toxicity in the form of hemolytic anemia, leukopenia, granulocytopenia, and methemoglobinemia owing to the deficiency of G6PD. Another study by Chattopadhyay et al. (2007) addresses that pyrimethamine blocks the folic acid synthesis enzyme, dehydrogenase reductase enzyme which in turn causes folic acid deficiency and malnourished pregnant ladies and children due to overdosage of the concerned drug. Furthermore, this drug induces severe hematological reactions in the form of megaloblastic anemia, thrombocytopenia, leukopenia, agranulocytosis, and purpura.

Another study by Lewis, Gregorian, Portillo, and Goad (2020a) reported the elevation of methoglobin with the use of a high dosage of tafenoquine antimalarial agent.

Future Directions and Conclusions

Antimalarial drug-related toxicities are posing serious health concerns and economic burdens on poor and developing countries of the malarial endemic regions of the world. There is a dire need to develop safe, economical, and efficient drugs to ensure safe and effective treatment of malarial patients with minimal and effective therapeutic concentration causing minimal adverse effects, therefore drug repurposing is the need of the time to overcome these challenges. Preventive measures like vaccination should be adopted at a mass level to lower disease burden, worldwide. High and prolonged dosages of antimalarial drugs should be avoided. The new combination of chemotherapeutic agents for the minimization of malaria should be developed using new strategies of drug development. New analogs of the existing drugs should be developed. Coordinated strategies for the effective control of vectors, their eradication, safe and efficient drug development, and effective vaccines are essential for malarial control. There is an immediate need for the development of novel strategies to eradicate malarial from the world is essential to avoid the overburden of drug toxicities induced by antimalarial agents. Desired amendments are essential for the guidelines in the use of antimalarial drugs particularly when prescribed in the case of pregnant ladies, immuno-compromised patients, and children up to five years of age.

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Chapter 17

Strengthening the Inner Shield – Prebiotics, Probiotics and Antimicrobial Resistance

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ABSTRACT

Antibiotic resistance is a rapidly increasing global issue. Microbes are increasingly developing resistance against drugs, possessing a threat to global health. It's not just bacteria but fungi, viruses, and other parasites are also becoming resistant to the medicines we use against them. The factors that contribute to this drug resistance include misuse of antibiotics, antivirals, antifungals, or other drugs specific for parasites along with inadequate diagnosis. Additionally, gut microflora plays an important role in not just maintaining body's defenses but also keeping the overall body healthy. A well balanced gut microbiome significantly plays a role in not just preventing diseases but also supporting the immune functions. This highlights the potential of probiotics and prebiotics in the battle against antimicrobial resistance. Since the overuse of antibiotics is leading to resistance and becoming a global issue, exploring natural and alternative options have become necessary to avoid this resistance, the concept of using probiotics seems a promising natural approach to fight off the antimicrobial resistance.

KEYWORDS
Antimicrobial Resistance, Gut Microbiome, Prebiotics, Probiotics, Gut Health

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INTRODUCTION

Antibiotic resistance is a huge worldwide problem. With each passing day, Microbes are getting better at defending themselves against drugs, possessing a threat for global health (Ventola, 2015). It's not just bacteria; fungi, viruses, and other parasites can also become resistant to the medicines we use against them. This resistance occurs when these microbes build a shield by developing certain genes that help them to survive the effects of antibiotics, antivirals, antifungals, or other drugs specific for parasites. When they develop the strength against multiple drugs, they're called multi drug resistant (MDR) or superbugs, which are really hard to treat. This resistance usually occurs as a natural process when these drugs are not used as prescribed or used too making them ineffective against the germs (Mukuna et al., 2023). When the genes are shared horizontally by the bacteria, even the harmless ones can also develop the resistance traits around their communities. The harmless bacteria, duet to development of resistance, could also into ones that cause diseases. This resistance also acts as the survival of the fittest through natural selection, especially when antibiotics are used excessively or not in the right way. This leads to more bacteria and other microbes becoming resistant to drugs.

Effects on Public Health

With every passing day, drug resistance is becoming a huge challenge which impacts everyone and everything, humans, animals, plants, and the environment. It's like a huge web where everything is connected. To win this battle, One Health approach is the key where everyone would work together in this battle. Since human and animal health is connected with the environment and everything is a part of the same picture, understanding this link is important. If we fail

to understand this resistance against antimicrobial drugs, this could have adverse impacts leading to disruption in many aspects of modern medicine, like treating cancer or doing organ transplants etc. (Salam et al., 2023)

The seriousness of the problem is actually alarming as in 2019 alone, a huge number of 1.27 million death were reported just from antimicrobial resistance. There were 1.27 million deaths just from the bacterial resistance only. Even in the United States, antimicrobial resistance is also considered as a big threat to public health. That is why the hospitals and the labs have special surveillance programs in public health to keep a close eye on microbes that are resistant to antibiotics. Even though, there are a lot of efforts to fight antimicrobial resistance, there still are gaps in progress that are creating a hindrance from finding complete solutions to this huge threat to public health. Dealing with antimicrobial resistance means that everyone needs to work together, the doctors, pharmacists, farmers, finance people, trade experts, teachers, and groups that aren't part of the government, not just in the country but around the world as well. It is important to control the impact this resistance has on society.

Causes and Contributing Factors

Resistance against antimicrobial drugs has become an issue of concern across the world. There are many factors that cause tis resistance. One of the major reasons for antimicrobial resistance is that either the body's own microflora learns to fight off the antibiotics or one catches the resistant bacteria from other people and the surrounding (Burroughs et al., 2003). This development of resistance against antimicrobial agents is driven by blend of various factors like selective pressure, mutations, where bacteria undergo changes in their genetic makeup leading to resistance, inappropriate use of antimicrobials which includes using the medicines when not needed and using the wrong type of drugs, and inadequate diagnostics where the physician is unable to identify the infection type and might prescribe wring treatment. All of these factors could either be natural or even man-made (National Research Council, 1999). The excessive use of antibiotic drugs in fish farming, livestock and other different sectors, substandard cleaning in healthcare, poor hygiene and improper diagnostics etc. adds up to the increase in AMR. Furthermore, it's not just the overuse of antibiotics that causes exposure and resistance but may also be because of the transmission of resistant germs within community and across the environment (CDC, 2022). Even though, the clinicians are trying to cut back on antibiotics, there still is a need to study about its spread to actually control it (Collignon ET AL., 2018). Moreover, the problem of antimicrobial resistance not just localized to certain sectors, it can also travel from food animals to the food chain, travel, trade and waste ultimately leading to resistance against, drugs in humans (Goryluk-Salmonowicz et al., 2022).

Gut Microbiome – Body's Inner Shield

The gut microbiome is a complex community made up of trillions of bacteria that are important and play a huge role in maintaining the overall health and wellness. This complex system interacts with different organs and systems in the body including brain, lungs, liver, bones and heart among others (Feng et al., 2018). The bacteria residing in our gut have many functions. They help ferment the food, keep the harmful germs and pathogens away, strengthens the immune system and even produce the vitamins that are essential for the body to function properly (Hou et al., 2022). Additionally, the gut microbiota helps and plays an important role to keep the gut lining and barrier strong and healthy, while also acting as a shield to keep all the invaders out, whose strength depends upon the microbial composition (Kelly et al., 2015). This balance is important because any disturbance in the gut microbiome could have a huge impact on health and overall immunity as well. The gut associated lymphoid tissue (GALT) works along with the mucosal associated lymphoid tissue (MALT), and forms a team to protect the body from possible invaders which highlights the significant role of gut microbiome in defending the body against harmful intraluminal threats. Furthermore, the gut microbiome is not just important for the digestion and absorption of nutrients, it is also important for keeping the immune system strong while also maintaining the overall health. (Dupont et al., 2020).

What is the Gut Microbiome?

The gut microbiome is composed of bacteria that reside in the form of large number of colonies in the digestive tract and play a main role in various body functions, not only related to the gut but also affecting other organs and systems outside the gut. Change in the composition of gut microbiome can effect greatly on the overall health which may lead to health problems. For example, when there is an imbalance in the gut microbiota, it can disturb the bacterial composition affecting the host's immunity and triggering a long term inflammation. This may add up to the development cardiovascular diseases, cancers, respiratory diseases and other such problems (Hou et al., 2022). Additionally, the communication among the gut bacteria and the host is done using the nerve connections, immune cells etc. The gut microbiota, as the main member, play a significant role in keeping the balance, regulating the immune system and affect many other processes throughout the body. Various methodologies like microbiota modulations and fecal microbiota transplantation etc. are being investigated by the researchers as possible treatments to adjust the gut microbiome and enhance the overall health (Hou et al., 2022).

Role of Balanced Microbiome in

A well balanced gut microbiome plays a huge role in keeping the body healthy. It basically is a small ecosystem made up of good and bad bacteria both, mainly consisting of the good ones through which the host and microbiota both

benefits (Wischmeyer et al., 2016). These good microbes communicate with the gut cells, helps the intestinal in smooth digestion and prevent the harmful bacteria to take over, adding up to the better gut health and over all wellbeing as well. When the gut microflora is healthy, they stay stable, strong and work well in keeping the body healthy (Hou et al., 2022). Moreover. Probiotics like live bacteria and yeasts, when consumed in sufficient amounts, help create a balanced environment in the gut while inflicting positive effects on the health (Valdes et al., 2018). The gut microbiome also have a very important role in breaking the food down and turning it into the useful nutrients that are required by the body for not just good physical but mental health as well. Furthermore, what we eat impacts greatly therefore consuming a healthy diet that includes fruits, vegetables and fiber improves the quality and composition of the microbiota in the gut and help them thrive better which again is important for the overall health mora et al., 2019).

Prebiotics

Prebiotics play an important role in keeping the gut healthy. They are different from probiotics in a way that like probiotics, prebiotics are not live microorganisms but they act like food ingredients that are mainly non-digestible. They provide nourishment to the good bacteria that is already present in the gut. Prebiotics, mainly plant fibers like fructogluco saccharides (FOS), insulin, arabinogalactan etc. act as fertilizers or fuel for the growth and nourishment for the good bacteria in the gastrointestinal microbiota. (Salvin et al., 2013 and Ansari et al., 2023). While probiotics are live microbes that brings health benefits when ingested, prebiotics act as their trusted substrates which boosts up the efficiency and effectiveness of the probiotics. Moreover, the production of short chained fatty acids in the colon is enhanced by consumption of foods like whole grains, bananas, onions etc. that are rich in prebiotics. These short chain fatty acids are important to keep the gut microbiome healthy as they provide energy to the colon cells, enhance the mucus production, reduce inflammation and strengthen the immune system. Since, the digestive system cannot digest the prebiotics, they remain unbroken and once they reach the colon they play an important role in maintaining the overall health maintain the right balance of gut microorganisms which is necessary for the overall gut health.(Ansari et al., 2023).

Prebiotics and Growth of Healthy Gut Bacteria

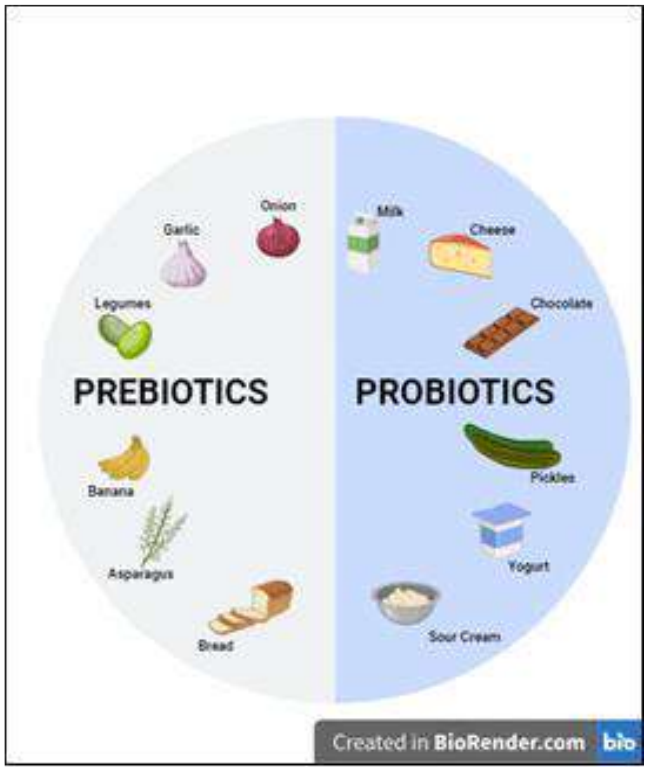
Prebiotics act as a fuel for the growth of good bacteria in our gut hence, they are of significant importance to maintain the health of our digestive system and the overall health (You et al., 2022). These as special compounds that cannot be broken down or digested by our digestive enzymes making their way to the intestines while remaining intact (You et al., 2022) where they serve as a source of energy for the probiotics, promoting growth and proliferation of good bacteria like *Lactobacilli* and *Bifidobacili* in the gut (Carlson et al., 2018 and You et al., 2022), ultimately increasing the growth and diversity of gut microflora (Zhou et al., 2024). Moreover, prebiotics also help to calm down any inflammation in the intestines, making the gut environment even more favorable for the gut microbiota to flourish (Zhou et al., 2024). Adding foods that are a rich source of prebiotics in diet can be a great way to boost and balance the beneficial gut bacteria which not only results in strengthening the gut defenses but also keep whole digestive system healthy (Fernández et al., 2015). That is why it is important for researchers to further explore deeply about the selective promotion of probiotics by prebiotics, their required concentration and other factors that may impact the efficiency of probiotics (You et al., 2022).

Prebiotics and Reduction of AMR Risk

Prebiotics are special type of plant fibers that keeps our gut healthy by supporting the growth and function of good bacteria in our digestive system. These prebiotics could be found in foods like whole grains, bananas, leafy greens, onions, and garlic etc. which when consumed, cause significant changes in the types of microbes residing in our gut, also impacting the mucosal immune system (Vieira et al., 2013). These prebiotics act as nutritional source for the gut microflora providing favorable environment and helping them grow and thrive adding up to the overall gut health (You et al., 2022). Moreover, prebiotics have been found to strengthen the gut barrier, decrease mild intestinal inflammation and reduce metabolic disturbances resulting in potential benefits for managing weight and improvement in overall health conditions (Fernández et al., 2015), furthermore acting as a key source to strengthen the gut lining and the microbes to fight off the armful invaders like *E. coli*, Salmonella spp., Campylobacter and other pathogens (Carlson et al., 2018). Prebiotics produce highly beneficial compounds once they reach the gut and get broken down there. This really helps in strengthening the growth and function of the healthy gut bacteria (Ansari et al., 2023). Moreover, this also helps to support the relationship even more among the prebiotics and gut health which is mutually beneficial. Hence, basically, prebiotics mainly work by feeding the wide range of gut microflora that are important to keep our gut healthy. This highlights the importance of retaining a stable gut microbial community which would potentially decrease the chances of microbial resistance (Ansari et al., 2023).

Probiotics

The World Health Organization (WHO) describes probiotics as “Live Microorganisms” that, when ingested in sufficient quantities, provide a lot of health benefits to the host. Commonly used probiotics from bacterial genera include *Lactobacillus, Bifidobacterium*, *Escherichia, Enterococcus*, *Bacillis*, and *Streptococcus* etc. Some of the fungal strains like *S. boulardii* from *Saccharomyces* genus have also been utilized as probiotics. (Gupta and Garg, 2009).



FOOD SOURCES
PREBIOTICS & PROBIOTICS

Probiotics and Their Contribution to Healthy Gut Microbiota

Probiotics play a very important role in supporting the healthy gut microbiota through different methods. These microorganisms are very helpful and important to keep the intestinal microbiota balanced by producing important vitamins and contributing in processes like the digestion of bile salts, enzyme activation and neutralization of toxins (Maftei et al., 2024). Moreover, probiotics not just plays a part in producing vitamins, they also play a role in keeping the harmful microorganisms from growing in the gut while keeping the environment friendly favorable for the good bacteria, which is highly important for keeping the gut microbiota healthy. It is done by making more bioactive substances like short chain fatty acids which helps to lower the acidity in the colon, making the gut a friendly and conducive place for good bacteria to flourish, supporting the overall gut health (Maftei et al., 2024). Furthermore, Probiotics have proved to have a positive and fruitful impact on changes in the microbial community of the gut, which highlights their importance in maintaining a healthy gut microflora (Hemarajata and Versalovic, 2013). Moreover, while maintaining the balance of intestinal flora, probiotics also help them to boost and strengthen the gut immunity, prevent the adherence of pathogens to the intestinal lining and overall strengthening the gut barrier, all of which are highly important to maintain a healthy gut microbiota (Wang et al., 2021).

Specific Strains of Probiotics and Their Efficacy in Combating Antimicrobial Resistance

The ongoing researches focus on the effectiveness of certain strains of probiotics in fighting of antimicrobial resistance. Probiotics have shown the ability to change the composition of the gut bacterial community which helps greatly in preventing harmful pathogens from establishing colonies in the gastrointestinal tract (Wang et al., 2021). There are certain strains of probiotics that may influence the microbial communities in the intestines and boost the production of immune factors like β-defensin and IgA by the host. This mainly helps to suppress the growth of pathogens and create a healthier environment in the gut (Hemarajata and Versalovic, 2013). Furthermore, multi-strain probiotics have proven to show potential in strengthening the stress related cognitive performance, also indicates a possible connection between gut health and cognitive function (Bloemendaal et al., 2021). Since probiotics are live microorganisms, they can adjust the gut microbiota by strengthening the activity of beneficial bacteria leading to improvement in overall health (Wang et al., 2022 and Maftei et al., 2024). Furthermore, the impact of probiotics on the health extends beyond gut microflora as it directly affects the host's immune response. This highlights their role in maintaining the overall health (Valdes et al., 2018). Similar to the body's natural microbes, probiotics travel through the digestive system, brining positive and beneficial effects to the microbiota along with improving the overall health (Tuohy et al., 2003). Studying about how probiotics might affect the gut-brain axis and their connection to mood disorders show how closely gut health is related to the overall body's physical function. This suggests that probiotics offer a wide range of benefits that go beyond just fighting the antimicrobial resistance (Ng et al., 2023).

Role of Probiotics in Strengthening Immunity

Probiotics play an important role in strengthening the immune system by serving as body's natural defense against invaders and the harmful pathogens. A balanced diet that includes healthy fats, probiotics help to protect against the diseases while boosting the body's strength and ability to fight off the infections (Appanna, 2018). Adding the probiotics to the diet can additionally strengthen this immune shield even more by encouraging the growth of the beneficial bacteria in the gut, which further boosts up the body's immune functions. Studies have demonstrated that probiotics cannot just help in regulating the body's immune response but it also supports the intestinal barrier and decrease the inflammation; all of which play an important role in maintaining the overall immune health and resilience (Appanna, 2018). Individuals can develop a strong internal shield in the form of immune system by nurturing a healthy gut microbiome through probiotic supplements, which also helps maintain a balanced overall wellbeing. This allows the body to recover better and bounce back quickly when it faces any sudden immune challenges. This strength of body's defenses with probiotics is like building up the inner strength and providing the protection to improve the overall health and energy (Appanna, 2018 and Li, 2023)

Incorporation of Prebiotics and Probiotics and Fight against Antimicrobial Resistance – Challenges

There are some challenges that should always be kept in mind while adding prebiotics and probiotic supplementation to fight-off the antimicrobial resistance, and always be considered before adding them to the treatment protocol. One of the major challenge is the need of further research to study how exactly probiotics and prebiotics are effective in fighting antimicrobial resistance (Joseph et al., 2023). Another issue that we face is understanding the optimal amount and duration that these prebiotics and probiotics could take, to make a difference against antimicrobial resistance. Furthermore, it must also be considered that how these supplements would interact with other treatments or medications (Joseph et al., 2023). While antibiotic resistance is becoming a concern for the clinicians, probiotics are being considered as alternative treatments (Rabetafika et al., 2023). Although the concept of using probiotics seems a promising natural approach to fight antibiotic resistance, there is still a need for research because of mixed results of studies about rebalancing of the gut microbiome (Li et al., 2022). Another problem is finding and selecting the right probiotics to reduce and fight the infections. Since the overuse of antibiotics is leading to resistance and becoming a global issue, exploring natural and alternative options have become necessary to avoid this resistance (Li et al., 2022). Moreover, antimicrobial and antibiotic resistance has also been labelled as a worldwide hazard by the World Health Organization (WHO), which also highlights the need of alternative treatment protocols by using prebiotics and probiotics in the best possible way (Rabetafika et al., 2023).

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Chapter 18

Perspectives of Maggot Therapy in Veterinary Medicine

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ABSTRACT

Wound infection is the root cause of delayed healing in open acute/chronic wounds via secondary infection. If this infection gets neglected then it leads from contamination to colonization and local infection and processed towards systemic infection including sepsis, and MODS (Multiple organ dysfunction Syndrome) leading to life threatening conditions. An infection could be processed so long as the presence of Biofilms, which means bacteria have a self-preserving mechanism in nature and have a parasitic relationship which would produce further evolving factors including tachycardia, tachypnea and increased white blood cell count. Maggot therapy can be an alternative approach to treat chronic wounds. Most regularly used specie for maggot therapy are the Greenbottle fly (*L. seritica*) and other one which have are minimal or lesser extent are the sheep blowfly *(L. cuprina*). Maggots secrete proteolytic enzymes that result into debridement of wound. Maggot had caused a rapid effectiveness in wound healing. This capability incorporates the maggot to do faster healing of wound by promoting angiogenesis, improving oxygen perfusion and enhanced migration of fibroblast to wound site. Maggot therapy has shown potential in treating various veterinary diseases, including Actinomycosis, panniculitis, and coffined bone osteomyelitis. However, it has not been widely used due to its lack of comparison with conventional treatments and lack of supportive information. The potential adverse effects of maggot debridement therapy include throbbing pain, alkalosis and ammonia toxicity. Few of the animals reported by getting treated with large number of maggots and in result toxemia as well as alkalosis which would target to attack on the kidneys as well as the heart so death occurred indirectly by the myiasis.

KEYWORDS
Maggots, Wounds, Healing, Toxemia, Therapy

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INTRODUCTION

Biofilms are present in almost 70% of chronic as well as the acute infections which causes delayed healing of wounds and this type is the complication of major joint replacement. Furthermore late infections are mainly due to the coagulase negative staphylococci along with multi-resistant (Sanchez et al. (2013).

In addition, biofilms are made up of complex protective Glycocalyx that are basically produced from bacterial communities and this protective layer provides major role at host defense mechanism and in antimicrobial therapy either by the topical (antiseptic) or systemic antibiotics (Leaper et al., 2015). However as in the biofilm's bacteria are able to communicate via 'Quorum sensing' and these bacteria will persistent in their slimy matrix and through the channels exchange of nutrients, gas and chemical agent molecules. It is an autoinducers phenomenon and bacteria will kept metabolically inert and protected. As soon as the bacteria get favorable conditions they will change their phenotypic type and ultimately form new proteins, become plankton and free of biofilm (Sauer et al., 2002).

Biofilms cannot be seen with the naked eye as they are persistently present in chronic wounds especially when there is a prolonged or delayed healing occurs. A diagnostic guidelines are given below to investigate presence of biofilms in Fig. 1 (Hall et al., 2014).

As biofilms factors could be there so there is no diagnostic tool to detect or find the evidence of biofilms unless by observing the following signs as mentioned in Fig. 2 given below: Maggot therapy can be an alternative approach to treat chronic wounds.

History of Maggot Therapy

Back in the centuries when wound infections got improved by the maggot infestations and it get evident via military record .so the first person who documented the benefits of maggots in wound state was the Dr William Baer and the results of very unintentional maggot infestations could be clearly seen over the Soldiers of first world war (Manring and Calhoun, 2011).

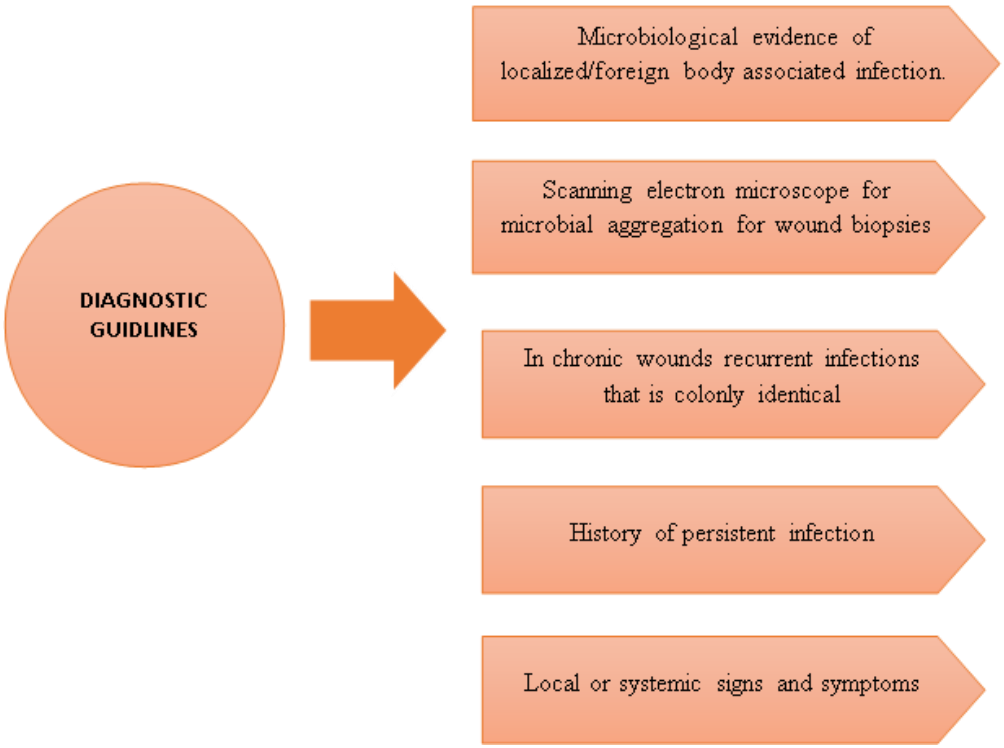

Fig. 1: Diagnostic guidelines to investigate presence of biofilms

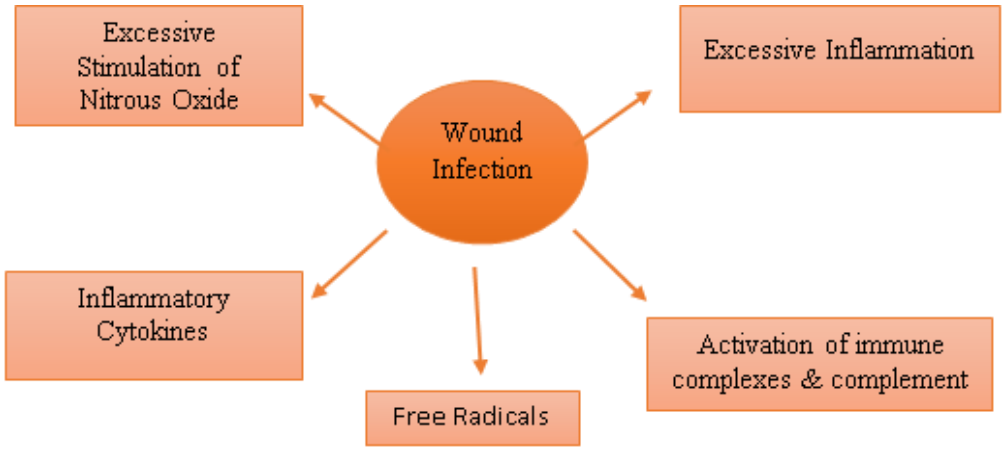

Fig. 2: Wound Infections signs indicating biofilm formation

After the war, he worked as a professor of orthopedic surgery at the John Hopkins University in USA and after this he was the pioneered who used the sterile maggots for the treatment of wound and that technique was accepted. In the early 1930s and 1940s his outstanding and the revolutionary study had led to the widespread application of maggot therapy (Leaper et al., 2015) . As when the industrial era of antibiotics had emerged then the use of maggots come to an end due to the progressively use of antibiotics (Leaper et al., 2015). Afterwards the most of the strains of the bacteria become resistant to almost of the antibiotics treatments and this issue started to evolve with the passage of time. Now this issue is a main global health concern having patients of antibiotic wound resistant infections.(ALfadli et al., 2018).

So, currently the scenario of the world is that most of the laboratories that are properly licensed/approved provide aseptically medicinal maggots or clinically graded maggots. These can be directly ordered via the monitoring bodies and then directly send towards hospitals and other collection centers for the treatment of conditions including leg ulcers, pressure ulcers, diabetic and necrotic tissue ulcers, infected surgical wounds, burns and traumas.

Fly Species

There are almost many of the fly species in the world wide that are used for the treatment of wound healing. But most importantly fly specie belongs to the family Calliphoridae that are having 1500 species have been known. Some of the species

that can be used in maggot therapy are given in Table 1.
However, most regularly used specie for maggot therapy are the Greenbottle fly *L.seritica* (Andersen et al., 2010)and other one which have are minimal or lesser extent are the sheep blowfly *L.cuprina* (Nair et al., 2021).

Table 1: List of specific species that are used in maggot therapy

SPECIES
• *Calliphora vicina*
• *Lucilia caeser*
• *Lucilia illutris*
• *L.seritica*
• *L.cuprina*
• *Phormia regina*
• *Protophormia terraenovae*
• *Chrysomya megacephala*

Feeding Behaviour of Fly Species

Maggots mainly forms the aggregations that are specifically known as feeding masses and use adduction by the cephalopharyngeal skeleton with the help of mouth hooks just to grab, scratch and rubbing of the food source. The specific fly larvae have two salivary organs that produces stomach related proteins and one powerful siphon like pharynx that help them to suck liquefactive food and then drains into the alimentary canal.so these larvae specie saliva contains collagenase, chymotrypsin and trypsin like compounds that will ultimately breakdown the necrotic tissue in to that particular form that will be suitable for the ingestion (Chambers et al., 2003).

Wound debridement could further help by the larvae specie that will circulate around the wound and then further dislodge the necrotic tissue from the wound side. The maggots have three larval stages: A larval stage having high metabolic capacity could result in rapid growth rate, Feeding stage of larvae just complete in three days on the living host and undisturbed or the uncontained larvae would exit out of the wound and pupate in the environment (Jones and Wall, 2008).

It can also be seen that the feeding larvae are the result of the Auto disinfection. However it's the particular process of that saliva produces antimicrobial substances that will ultimately destroys the bacteria in the larval alimentary canal (Robinson and Norwood, 1934).The process of Auto disinfection has played an important role in the larvae ,because before enter in pupation it needs to get detach itself from any of the bacteria in their alimentary canal. Otherwise, bacteria have a key role in multiplication of larvae eggs, infect and then ultimately kill pupa. The secretory products of the maggots have a significant role in anti-microbial activity against Staphylococcus aureus, Streptococcus A and B as well as some activity against Pseudomonas species and a clinical isolate of multiple resistant S. aureus (Kerridge et al., 2005).

The larvae have a significant role for creating unfavorable condition for the bacterial growth by disruption of wound environment. The larvae secrete allantoin ammonium bicarbonate as well as urea that will alkalinize the wound bed. In addition to ,this action or larvae maggots and in their response from the host tissue has led to the production of large amount of fluid that help to detachment of bacteria from the wound side. Meanwhile the granular tissues of the maggots have led towards the fast and abrupt healing of the wounds. At the initial phase it was assumed that its basically the antimicrobial secretory products and the step forwardness of the maggots itself cause stimulatory effects in wound healing but with the passage of time different researchers have worked over it. Furthermore in 1997 Prete work on the growth promoters had published in which the particular effects of growth promotors on the maggot secretions by exposing them into the human fibroblast and culture them either in the presence or the absence of Epidermal growth factor/EGF and IL6 /Interleukin. According to her research she observed that by the significant addition of the secretions made by the maggots towards EGF as well as the interleukin /IL6 → undergoes stimulation of fibroblast →growth rate has gone to be increased which have deviation from the EGF when used alone.

The secretory and excretory products of maggot have a proteolytic activity so due to this activity it caused fluctuations in the adhesion of fibroblast and in accordance with the maintenance of cell viability (Prete, 1997).The biodegradation of the fibronectin into potentially small fragments which will increase the Fibroblast extracellular matrix.

Types of Fly Larvae

The two species preferably used in maggot therapy for the wound treatment include *Lucilia Seritica* which is commonly known as Greenbottle fly and *Lucilia cuprina* which is commonly known as Blowfly. These both belong to the Family Calliphoridae (Diptera: Calliphoridae)

Life Cycle of Fly Larvae

L. sericata eggs hatch in about 21 hours at 21°C and in about 18 hours at 27°C after being deposited. At 20°C, larval development takes about four days, while at 27°C it takes three days. The larvae go through three instars of development (Wall et al., 1992). But a lot of other things affect development as well, like humidity and the availability of food. When completely grown, the larvae of the third instar depart from their host or carrion and burrow into the surrounding soil or substrate. The adult fly emerges after about 10 days of pupal development at 21°C and 7 days at 27°C (Pruna et al., 2019).

Adult females deposit up to 200 eggs in clusters on the host or carcass after mating. In clinical use sterilized eggs are hatched under sterile conditions. Hungry first-stage (L1) larvae are fed once on a high-protein cereal-based diet after emerging and right before being packaged for delivery, allowing them to survive for up to 24 hours during transit (Nigam, 2021). The larvae can feed and develop to their final third stage (L3) once they are applied to a wound; they typically stay there for four days before being removed. Used larvae are considered infectious clinical waste. The life cycle of medicinal maggot is summarized in Fig. 3.

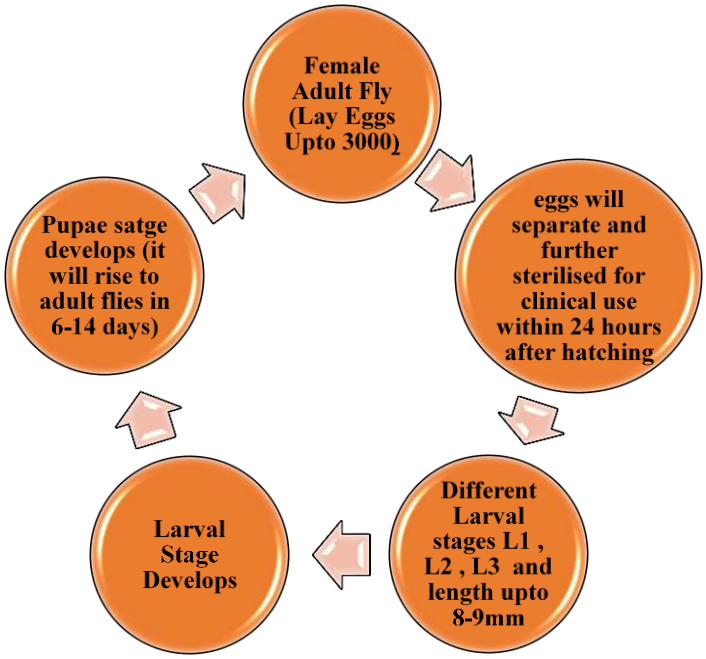

Fig. 3: Life cycle of medicinal maggot

Maggot Debridement Therapy

Chronic wounds are not well treated with the normal healing process and they are specifically characterized by: delayed/prolonged inflammation, inhibition of cell proliferation, incomplete remodeling of ECM, and failure of epithelialization.

Therefore, the inefficient debridement of extracellular matrix components. Likewise, fibronectin and fibrin it will cause failure of chronic wounds to heal. The extensively most of the methods of debridement of chronic wounds includes: surgical and sharp debridement with the help of scissors and scalpel to remove the necrotic tissues, mechanical debridement like wet to dry dressings, wound irrigation, whirlpool techniques, and application of exogenous enzymes follows enzymatic debridement and use of hydrogels and hydrocolloids follows for autolytic debridement. But the major disadvantage is that, all these techniques will cause prolonged treatment time and mechanical damage as well as pain to the specified underlying tissues (Nigam et al., 2006). Hence it can be truly said by seeing all the disadvantages that for the better, quick and effectively treatment of chronic wounds as well as the other infected wounds which could not damage the viable tissue, so maggot debridement therapy can be used**.**

Maggots are photophobic in nature and will normally move with the help of locomotory mechanism of itself into the deep portions where the wound debris is present which probably said that its far beyond from surgeon's scalpel approach. In most of the Reports that have been published well explained the outstanding results of maggot debridement therapy in all aspects of wounds like: abscess, burns, gangrenous wounds, arterial and venous ulcers, osteomyelitis, diabetic foot ulcers, and pressure sore.

Furthermore, to cross check the results of maggot debridement therapy Vs conventional debridement therapy just in comparison the pressure sore treatment. Hence it had clearly indicated that 80% of the maggot treated wound were completely debrided and in comparison, with other only 48% conventionally treated wounds were completely debrided. The ratio had been the 80:48 (Sherman, 2002). By utilizing MDT technique surface area has decreased while with the conventional methodology surface area of wound increased. The report known to that ending point that Maggot debridement therapy was much more effective and efficiently work on the wounds as compared to the conventional therapy for wound healing.

Maggot Debridement therapy is also termed as larval treatment is one of the best choices for the treatment of the contaminated and necrotic wounds. The medically clinical graded aseptic maggot's larvae of the common green bottle fly (*L. sericata*). For the clinical use eggs that are being disinfected hatch under normal sterile conditions. When hatched eggs before going to packed for transportation, Hungry first larval stage L1, so these egg stage could be fed high protein diet only once so they can easily survive up to 24 hours.

Whenever larvae L1 gets placed on the contaminated site where they get favorable conditions and immediately L1 stage converts into L3 stage and they stay up to four days here before being eliminated from that field of contamination and the

utilized larvae treated as irreversible infections clinical waste. When the wound gets favorable with the selected maggot for the treatment then the treatment goes towards advanced clinical supervision. Any chronic wound that is suitable for maggot therapy has moist slough or the necrotic tissue on it debris area.(Naik and Harding, 2017).

The utilization of appropriate placement of larvae over the wound requires clinical expertise and that maggot larvae could be in one of the any form either free range maggots or bagged maggots. In addition to with the passing of several days, by doing this technique of larval placement at the wound it's not only slough the necrotic tissue but also eliminate the infection. Afterward the removal of larvae from the wound, the site could available for further therapy. By using maggot debridement therapy, it could facilitate the wound disinfection and wound healing.

Wound Debridement

The main objective of the maggot therapy is to basically done the whole process of wound debridement that is to get rid of the dead tissues. The capability of the *Lucilia Sericata* to accomplish very effective and quick getting rid of the dead tissue just because of their necrophagous nature that is basically having the capability of killing or ingest the dead tissues very quickly and effectively.

Mechanism of Action

The helpful impacts of maggot therapy on the infected wound are includes: debridement, disinfection and enhances healing. So, in accordance with the wound debridement which is the removal of cellular debris as well as non-viable necrotic tissue from the infected wound. The very initial and important step that can commence before healing. As there had been the removal of necrotic tissues it will destroys the associated bacteria and also lessen up the wound odor. And the other beneficial effect of removal of necrotic tissue and it has acts as a microbial substrate which may reduce the risk of infection. In addition to when the inflammatory stage of wound healing happens then the host leucocytes has played an integral role in wound debridement, degradation of damaged extracellular matrix components via release of protease enzyme. Protease are released from the neutrophils, macrophages, fibroblasts, epithelial and endothelial cells. Afterwards when the healing proceeds ECM components like collagen , Elastin and proteoglycans can be produced or formed in a new way and the damaged extracellular matrix could be removed by the protease.(Granick et al., 2006). Researches have been shown the mechanism of the maggots that is secrets a rich quantity of digestive enzymes during feeding. These enzymes are Carboxypeptidases A andB, Lucine aminopeptidases, Collagenases and serine proteases /Trypsin like and chymotrypsin like enzymes. (Nigam et al., 2006)

Furthermore , in recent studies workers in Nottingham ,UK, has well demonstrated the in-vitro series of enzymes that has secreted from the larvae i.e., *P. sericata* (Chambers et al., 2003) are four proteolytic enzymes, two serine proteases, one metalloproteinase and aspartyl proteinase.

These particular enzymes were detected having molecular weight that's range from 20 to 40kDa, having a wide pH range activity. A chymotrypsin like serine protease showed brilliant degradation of the Extracellular matrix components includes laminin, fibronectin and collagen type I andIII.

These are significantly played an integral role in the wound management and clearing of debridement. The basic movements or the mechanical activity of the various wriggling/freely moveable maggots help in the necrotic filled wound that will going to help the wound debridement. Maggots have apparently couple of mandibles which helps to maintains a connection as well as locomotory mechanism between the tissue and the body. These locomotory body activities via mouth hooks and attachment of wound tissues with the maggot's larvae helps or speed up the activity of debridement. Therefore, also the key role of the hooks during feeding to enters into the membranes and also facilitates the penetration of secretory enzymes (Barnard, 1977).

Maggot Therapy and Wound Healing

From the very longer period of time the episodic reports come at the outcomes that the wounds treated with maggot therapy have effective results and are healed very quickly and faster as compared to those who are not treated with maggots. These results can be clinically as well as scientifically investigated.

To check the clinical effectiveness of larval therapy that are one basically being dressed with debridement treatment mainly hydrogel and to get the one randomized trial sample on the venous or the arterial ulcers. The result has shown as 96.9% ulcers debrided in the larval arm and 34.4% from the hydrogel arm (Mudge et al., 2014).

Medicinal maggots have a combination mix of enzymes like proteolytic as well as digestive enzymes. The locomotory movements of maggots on the tissue surface help to get entered into the necrotic tissue and then cause breakdown and nutrient rich product that will help in ingestion. These complex combination of enzymes having the capability to bear the endogenous inhibitors and then kill the other harmful enzymes in the necrotic tissues (Telford et al., 2011)**.**

Maggot had caused a rapid effectiveness in wound healing. This capability incorporates the maggot to do faster healing of wound by promoting angiogenesis, improving oxygen perfusion and enhanced migration of fibroblast to wound site (Nigam and Morgan, 2016).

Clinical use of Maggot Debridement Therapy

Maggot therapy has been started at time when other conventional techniques of wound healing had failed or the non-

surgical debridement treatment being considered. According to the National institute of health and care recommends that if debridement is needed then maggot therapy in chronic pressure ulcers but it will be contraindicated in sharp debridement. So, Maggots have been mentioned in that particular institute prescription since 2004. Maggot could be considered as medical devices in USA, and it was the first live organism that can be used for medical purpose. The most of the moist wounds having characteristics of revitalizations are the most appropriate for the maggot therapy. When maggot debridement therapy have much clear clinically approval reports over it and includes clinical hematomas as well.(Borst et al., 2014).

Application and Management of Maggot Therapy

The most importantly two applicable techniques are used in the larval maggot therapy are free ranged maggots and bagged maggots.

Free ranged maggots are directly or straightforwardly applied over the surface of the wounds. These maggots could apply using sterile saline with the facilitation of small pods in the sterile coated tube-like structure. So, in accordance with the breathable dressings wound will be well sealed. Particularly larvae are freely allowed to locomote here and there in the surroundings of wound and then inculcate into the deep tissues. The utilization of free larvae overwhelmed clinical use for the quite long period of time just before the modification in the maggot bag.

The bagged larvae are just enclosed in the two close layers of very thin polyvinyl netting, as by the bag corners undergoes the treatment of heating and small cube shaped spacious material could be inserted just to make safety from the bag collapse. Hence bagged larvae are prepared for the transportation. Furthermore, Biobag could be placed as it is on the wound as well as some sticky adhesions could also be places on the wound edges. As the maggots Larvae having necrophagous nature and these larvae release digestive enzymes via netting and ultimately transform into dead as well as devitalized the tissues into 'soup' like for so they can ingest easily.

Free ranged maggots as well as the Biobag could stay upon three to seven days on the wound site as per technique or application procedure. Maggot debridement application could be applied according to the dense contamination of the bacterial debris in it but for procedure protocols therapy could be applied over upon three treatments.

Maggot Therapy in Veterinary Medicine

The effective utilization of maggot therapy in veterinary as well as the human medication are particularly staying non-significant. The utmost major trouble in the maggot therapy is that researchers could not mentioned in comparison with the conventional therapy. Furthermore, maggot therapy is depicted however with no supportive information.

The most outbreaking utilization in single Guernsey bull having issue of Actinomycosis was treated with maggot therapy and the maggot therapy treatment in the aged donkey having an issue of panniculitis. When there was the very deep wound abscesses infection prevails then ultimately disinfected maggot therapy could be used.(Thiemann, 2003).

In the horses the main infection of laminitis having necrotic tissues contamination so this bacterial outflow of the debridement causes the improper balance and facing difficulty in walking due to the weaken angle of the foot. The utilization of maggot debridement therapy in the coffined bone osteomyelitis with the prevailing pain of laminitis along with septic navicular bursa as well as persistent distal interphalangeal joint sepsis, canker, acute caudal coffined bone rotation along with necrosis of collateral cartilage and non-healing foot ulcers. Coffined bone Osteomyelitis has significantly an out breaking effect by using Maggot debridement therapy. However, in addition to the other disease like quitter which is collateral cartilage has no effect by using maggot debridement therapy because of the major digital instability problem arises. As soon as the stability of digits of foot has achieved then treatment with the help of larvae could also show remarkable changes and healing. Furthermore, it had been concluded that maggot debridement has extensively an intrinsic approach to heal the highly contaminated wounds by creating no particular changes in the normal angle of the foot of the horse.

A clinical survey by the US practitioners had treated the horses by using Maggot debridement therapy and thirteen horses could be used as a control to check the effectiveness of healing. Half of the horses had euthanized and half were lame. There had never been adverse effects regarding larval therapy.

The rabbits having an issue of chronic bed sore as well as ulcers and these rabbits undergoes maggot debridement therapy. They utilized the Phormia Regina larvae at the width as well as thickness of 80-12cm.These larvae Manipulated the effectiveness of one single procedure for almost of the 6 days and it was observed that the maggot debridement was rapid as well as selective and very well treated and favorable by this treatment.

Maggot debridement therapy could also be used in the small animals by the practitioners which includes two canines four felines and one rabbit more than the age of 6 years. By the treatment of all these small animals one goes towards the sepsis after treated by the Maggot debridement therapy. Researchers have demonstrated that the results could be more reliable and more authentic when it get treated in initially early animals rather than those who are already septic. Predominantly the significant disadvantages by the medicinal clinical graded larvae and many troubleshoots has to face during applying of dressing. However in concluding remarks that being the fear of being necrotizing of tissue abrasion and cost of therapy might prevent the Veterinary specialists and other clients respectively (Sherman et al., 2007).

Some of the Researchers could also claimed that the treatments which was earliest unsuccessful by the conventional methods of treatment but the bearing capabilities of the animals having healthy layer of wounds could be placed over it.

Furthermore, the advanced studies revealed that larval treatment results are as rapid and selective. Maggot debridement therapy could also show excellent results in the un-approachable place at the foot rot where keeping of dressing could be a quite challenging task. while keeping the sheep in field conditions are significantly more challenging task (Kočišová et al., 2006).

Adverse Effects

Although most of the task that had faced doing Maggot debridement therapy and it has also disadvantaged as well includes bleeding and pain. These two troubleshoots of the larval therapy the bleeding could be in the surroundings of uncovered blood vessels, body cavities and wound debridement in the body tissues. In addition, maggots have capability to produce anticoagulant which will ultimately encounter the bleeding mechanism.

When there is any wound occurred at the tissue necrotic site the surrounding area skin is loose, soft, and liquefactive adhesive secretions have been made through the wound along with some secretory enzymes activity that will might be help in healing process. Hence by using prophylactic treatment any ointment so that this wound debrided area has been away from the healthy skin tissues.

After the maggot debridement therapy done then rightly after 24 hours larval sensational movements become more prominent as the larval size had increased. Some of the patients had claimed baseline pain right before the larval treatment but some claimed significantly prominent pain receptors has been activated at wound site. Majority of the pain has done with some analgesics and few would suggest to give analgesic medications just prior to treatment just to get rid the pain effect and the sensation of pain at its deep tissue level (Jordan et al., 2018). Cost of the larval Maggot treatment could also be the considerable factor for the choosing whether going to adopt larval therapy or not. Hence, it's very cost-effective treatment as it can be estimated by the smallest maggot bad which cost at round figure of 250 dollars.

Potential Hazards

The following are the particular specific troubleshoots that has arisen after getting treated with the maggot debridement therapy. In human patients, just rightly after the larval therapy it will cause severe discomfort level and too much body pain happens. Somewhere when the larva gets entry into the wound surface sharp throbbing knife like pain could be severely felt. Basically, pain due to the fluctuations in the pH level because of the large number of secretory as well as excretory products and also the locomotion behavior of maggots when get initially treatment started. When there is talk of human Vs animals so to differentiate and to get relief from pain in humans is quite easier and more detectable as compared than the animals, because animals' owner as well as the patient could not communicate to reach at its pain level. In these ways, animals are continuously encountering the pain. By all the way it become quite mandatory to mainly observe and clearly note down the fluctuations in any physical and behavioral response when they get in pain. Other main reason for the drawback of larval therapy in animals because maggots cannot get stayed on the wound for longer period of time just due to animal get detached them by their own movements of forelimbs as well as hindlimbs. This behavior can signal towards clear indication as animal is in trouble. As maggots have secretory as well as excretory nature, and absorption of ammonia from the bloodstream results in the iatrogenic ammonia toxicity which can be very poisonous for the body physiology. By having loss of weight and anorexia as well as off feed in sheep and getting inculcate with the larvae *Lucilia cuprina* results in the pyrexia as well as Tachypnea. Few of the animals reported by getting treated with large number of maggots and in result toxemia as well as alkalosis which would target to attack on the kidneys as well as the heart so death occurred indirectly by the myiasis. So, to avoid ammonia toxicity after getting animal size as well as wound size could relatively important factor as small animal has large surface area wound so it could be treated more focused and maggot's ratio on the per centimeter square area would also less than the large animal. By the maggot debridement therapy, it could also be the result of bleeding which could mainly due to the small blood vessels which surround the wound. Larval therapy could efficiently significantly cost for the treatment so it could also keep mind that the placement of maggots could not too distant or too close to the wound. The main trouble in the veterinary medicine is the cost that is too high and quite very challenging task to be accomplished over it.

Conclusion

Maggot debridement therapy has significantly an outstanding feature that has really helpful in wound healing specially by their enzymatic activities and anticoagulant as well, but still some problems specially in veterinary medicine still unknown. As in the early recent studies claimed that larval therapy could arise multiple resistant bacteria that why antibiotics could not be much effective and recent world should move towards alternative therapies. Treatment regimens should be monitored individually very closely to get to the extent of the diagnosis. Maggots that could retained in the necrotic wound should have a proper supply of oxygen, the necrotic wounds that are very exudatory in nature and deep close texture. So, its dire need to recommend a penrose drain in the necrotic wound so that it creates a persistent drainage or the entry of aeration and sufficiently aerated. Financial cost should also be scrutinized more specifically towards the veterinary medicine side.

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Chapter 19

Control Strategies of Crimean Congo Hemorrhagic Fever (CCHF) in Pakistan: An Overview

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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) is a primary vector-borne disease known to be transmitted through tick bites from *Hyalomma* species. CCHF is endemic in Africa, Balkans, Middle East and Asia, including countries south of the 50th parallel north. CCHF outbreaks have a case fatality rate up to 40%. Contact with infected tissues, blood, patients, livestock, tick bites, and manual tick removal are risk factors for CCHF. Clinically, the illness is characterized by fever, muscle soreness, and progressive hemorrhages. Prolonged clotting durations in pro-thrombin tests, elevated concentrations of Alanine transaminase (ALT), creatinine phosphokinase (CPK) and Aspartate aminotransferase (AST) are some common serum biochemical findings. The pathogenesis of the disease is linked to endothelial activation and functional loss because of cytotoxic molecule secretion and epithelial damage during the viral replication process. Medication involves supportive therapy to patients through blood or plasma infusion, while an antiviral drug named Ribavirin has shown excellent results in preventing the disease from spreading further. The disease poses a serious threat to the health and safety of healthcare workers during the onset of the hemorrhagic phase. This chapter focuses comprehensively and covers the transboundary effect of CCHF in Pakistan, with the implementation of detail control strategies to prevent and control the spread of the disease. It also discusses the policies and legislation regarding public education campaigns, research, and development for disease prevention and control at the global level.

KEYWORDS
Hyalomma Tick, Crimean-Congo hemorrhagic fever (CCHF), Endemic, Zoonotic

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INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a medically significant and deadly viral infection. It is found throughout the world and is the most common of the tick-borne viral infections. CCHF is an infection caused by a tick-borne virus (genus: *Nairovirus*, family: *Bunyaviridae*) (Patil et al., 2020). The most important vector for CCHF is the ixodid tick specie named *Hyalomma*. Along with tick bites, direct contact with the virus-affected animal is responsible for its spread. The virus is kept alive in tick species by horizontal and vertical transmission, and it spreads to domestic animals, which then spreads the sickness ultimately to humans. It is classified as a zoonotic illness (Akıncı et al., 2013). CCHFV first appeared 3,100–3,500 years ago (Papa et al., 2014), the infection was originally documented in Crimea in 1944. In 1969 it was recognized that the pathogen causing Crimean hemorrhagic fever was the same as that responsible for an illness identified in 1956 in the Congo Basin. The infection is currently widespread throughout the world, including Africa, Asia, Central and Southern Europe, Eastern Europe, particularly in the former Soviet Union, the Mediterranean, northwestern China, the Middle East, and the

Indian Subcontinent (Burt and Goedhals, 2014). Pakistan witnessed its first case of CCHF in 1976 and has been a major victim of CCHF for years, since 2002, the virus has also cropped up in a number of Balkan nations, raising concerns that CCHF is spreading beyond its present geographic range (Alam et al., 2013). Africa-1, Africa-2, Africa-3, Europe-1, Europe-2, Asia-1, and Asia-2 are the seven genotypes of the virus. The genetic diversity in short segments of RNA is used to classify these seven genotypes (Mild et al., 2010). The CCHF is severe since it causes substantial medical complications and, if left untreated, can lead to death in humans. The presence of blood in sputum, gums, rectum, and urine is the most common symptom of the condition (Temur et al., 2021). Another cause for concern is that CCHFV is highly pathogenic, easily transmissible to humans, and has a 10–40% case fatality rate. Because of its pathogenic nature, the culture of CCHFV is only allowed in biosafety level four (BSL-4) and maximum-security laboratories; there is a possibility that this virus could be exploited as a bioterrorism or biological warfare agent (Whitehouse, 2004; Aslam et al., 2016). The virus must be recognized as a worldwide health issue because of its extensive geographic dispersion.

Because of high mortality rate, CCHF is currently endemic in Pakistan. Attributable to the fluctuating temperatures, there is a biennial spike in CCHF infections from March to May and from July to September. CCHF affecting nearly all four provinces of Pakistan, but the number of cases has been recorded more in Balochistan and Khyber Pakhtunkhwa, fewer cases in Sindh and Punjab. The major concerned of Balochistan and Khyber Pakhtunkhwa provinces is due to sharing their borders combined with Afghanistan nearly about 2,640 Kilometers, where annually millions of sheep and goats are imported, especially on the occasion of Eid-Ul-Adha every year. About 2.4 million Kuchis, nomads typically, travel from eastern, central and southern Afghanistan as shown in (Figure 1) to Balochistan and Khyber Pakhtunkhwa in winter to graze their sheep, goats, cows, camels, horses and donkeys before entering to southern Punjab. Every summer, a number of nomadic pastoralist groups would pass via Zhob, Peshawar, and Kohat, etc. On their way back to Afghan territory after spending the winter on the trans-Indus plains, along with their animals. This increases the chances of the spread of CCHF in the local permanent urban and rural communities (Kronenfeld, 2008).

Fig. 1: Afghan nomad's sheep herd and donkeys drove with their open pavilions southern Afghanistan (Radio free Europe .com)

The widespread CCHF outbreaks in Pakistan are currently beyond the capabilities of the country's healthcare system. Appropriate surveillance systems are lacking in order to discover cases early, and there are insufficient diagnostic tools available for CCHF. More preventive measures must be implemented and needed immediately in order to remove the CCHF reservoirs from the country, since Crimean-Congo hemorrhagic fever virus (CCHFV) could pose a severe threat to the country (Palomar et al., 2013).

Life Cycle

Trans-stadial transmission occurs when the CCHF virus replicates in the host tick during the larval to adult stages. Transovarial transmission is the process by which the virus spreads from one generation to the next. As a result, through vertical transmission, the tick as shown in (Figure 2), can serve as both a reservoir and a vector for the virus (Bernard et al., 2022), while most *Hyalomma* spp. ticks are multi host and employ bigger vertebrates as the host for the adult stage of their life cycle, small rodents, lagomorphs, and birds have all been implicated as sites of infection of the tick's immature stages (Serretiello et al., 2020).

Fig. 2: *Hyalomma* tick

Transmission and Zoonotic Impact

CCHF is transmitted to humans by a bite from an infected tick or direct contact with body fluids, blood, and the viscera of confirmed cases in animal husbandry practices as shown in (Figure 3) i.e., slaughter or in human communities. These ticks have small vertebrates (sciurid and birds) as hosts for the immature stages, with livestock or other large terrestrial wildlife serving as the preferred host species of adult Hyalommas. Humans get infections through direct contact with infected animal blood, tissues, or tick-biting (Gürbüz et al., 2009). Secondary transmission through tissues, blood, and other bodily fluids poses a significant threat to healthcare worker's life (Tsergouli et al., 2020).

CCHF poses a significant zoonotic threat, primarily affecting people involved in livestock farming, animal husbandry, and slaughtering (Chiuya et al., 2021). These individuals are at higher risk because of frequent contact with potentially infected animals and ticks (Spengler et al., 2016). Seroprevalence studies have shown higher anti-CCHF IgG antibody titers in people with close contact with livestock, indicating a higher exposure risk. Humans are considered accidental hosts, as they are not part of the virus's natural transmission cycle. However, the disease's severe clinical manifestations and high mortality rate in humans highlight the importance of controlling CCHF in animal populations to prevent congestion in human communities (Gilbride et al., 2021).

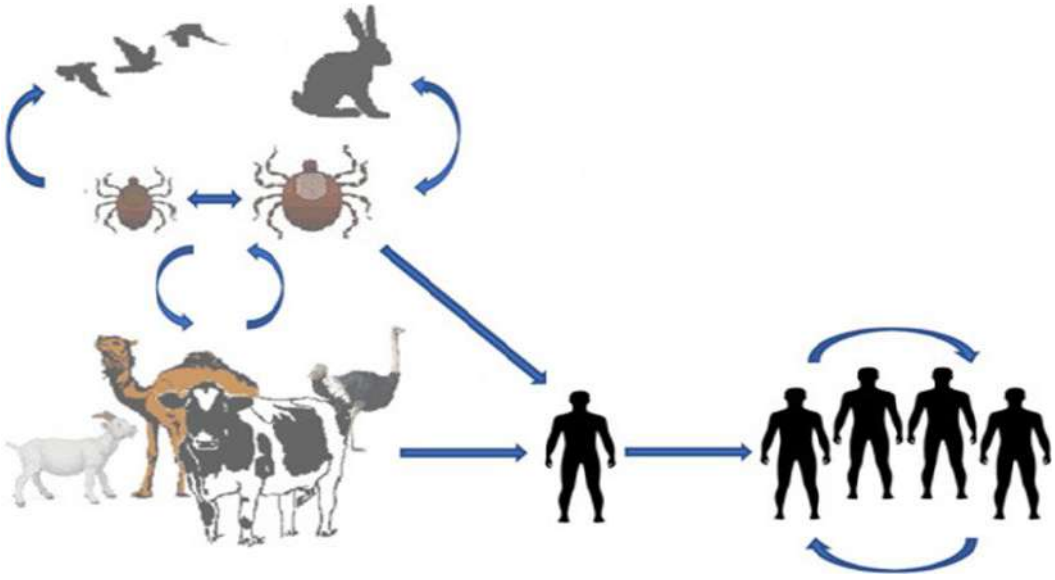

Fig. 3: Transmission of CCHF (Fillâtre et al., 2019)

Pathophysiology of CCHF

Viral Entry and Replication

After viral entry into the body, it directly attacks endothelial cells, hepatocytes, and macrophages. An infected tick bite or contact with contaminated blood or tissue causes the entry of the virus into the body. The virus causes endothelial activation and dysfunction once it has entered the host by targeting the endothelial cells that line the blood arteries. Chemokines and pro-inflammatory cytokines are released during this process, exposing the immune cells to the site of infection.

Dissemination and Immune Response

Virus disseminates through the bloodstream, reaching various organs, including the liver, spleen, and lungs. Infected macrophages spread the virus throughout the body and amplify the immune response. The immune system attempts to control the infection resulting in the production of cytotoxic molecules, which can damage endothelial cells and disrupt the blood-brain barrier.

Hemorrhagic Manifestations

The hemorrhagic phase, marked by extensive endothelial damage and coagulation abnormalities, stands as the primary hallmark of CCHF. Endothelial dysfunction results in increased vascular permeability, due to which fluid moves out of blood vessels into tissue and causes edema. The release of pro-coagulant factors and consumption of clotting factors contribute to disseminated intravascular coagulation (DIC), a serious illness marked by irregular blood clotting and bleeding.

Organ Dysfunction and Failure

Multiple organ failure and dysfunction may result from significant endothelial injury and coagulation problems. Renal failure and hepatic necrosis are common complications that can affect the liver and kidneys. Hypotension and shock may arise from a compromised cardiovascular system.

Immunopathogenesis

The immune response is pivotal in CCHF pathogenesis and infection control. However, an excessive or dysregulated response can significantly worsen the disease. Elevated levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), are linked to severe conditions and poor prognosis.

Clinical Findings and Diagnosis

CCHF has been divided into four distinct phases: incubation phase, pre-hemorrhagic phase, hemorrhagic phase, and the convalescent phase graphically as shown in (Figure 4).

Incubation Phase

This phase begins immediately after an infected tick bite, typically lasting 3-7 days, depending on the viral load and exposure route. It can take around 5 days to develop blood and tissue infection in an infected animal, while it takes 5-7 days for human-human transmission.

Pre-hemorrhagic Phase

This phase is characterized by high fever (39-41°C), severe headache, dizziness, and muscle pain, and may last about 3 days. Additional symptoms may include diarrhea, vomiting, and nausea. The face and neck may become hyperemic, and conjunctivitis is common.

Hemorrhagic Phase

The hemorrhagic phase is marked by various types of hemorrhages, including petechiae and large hematomas. Clotting time increases, leading to bleeding from natural orifices such as the nose, gums, and vagina, and in urine and feces. Internal hemorrhages can mimic appendicitis. Hepatomegaly and splenomegaly are occasionally observed. These signs appeared between 3rd-5th days post-infection.

Convalescent Phase

For survivors, this phase begins 10-20 days after infection. Symptoms include dyspnea, polyneuritis, xerostomia, partial or complete hair loss, tachycardia, faint pulse, memory loss, and visual issues. Some people may have low blood pressure and bradycardia.

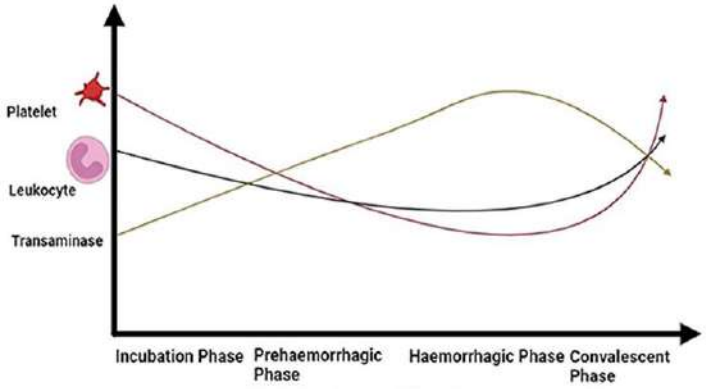

Fig. 4: Dynamics in Hematology of different phases (Aslam et al., 2023).

Laboratory Findings

Laboratory tests play an important role in diagnosing CCHF. Common findings include:

1. Liver damage is indicated by elevated levels of ALT, and AST.
2. Tissue injury is indicated by higher levels of LDH (lactate dehydrogenase) and CPK.
3. Prolonged clotting times, including prolonged PT (prothrombin time) and activated partial thromboplastin time (aPTT), indicate coagulation abnormalities.
4. Low platelet count (Thrombocytopenia) and low WBC count (leukopenia) are common, reflecting bone marrow suppression and increased consumption of platelets.
5. Hematocrit may decrease due to bleeding and hemoconcentration.

Diagnostic Methods

RT-PCR (Reverse Transcription Polymerase Chain Reaction)

The gold standard for detecting CCHF is RT-PCR. It detects viral RNA in blood samples and provides a definitive diagnosis within hours. This method is highly sensitive and specific.

Serology

Detection of anti-CCHF IgM and anti-CCHF IgG antibodies using ELISA (Enzyme-linked immunosorbent assay) can confirm recent or past infections. IgG antibodies point to prior exposure, whereas IgM antibodies show a recent infection.

Viral Isolation

Although less commonly used due to biosafety concerns, viral isolation involves culturing the virus from blood or tissue samples. It requires specialized BSL-4 facilities and is time-consuming.

Immunohistochemistry

With the use of certain antibodies, this technique finds viral antigens in tissue samples. It is helpful for research and post-mortem diagnosis.

Treatment and Management

Supportive Therapy

The mainstay of CCHF management is supportive care, which emphasizes preservation of essential functions and symptom relief.

Fluid and Electrolyte Management

Rehydration and electrolyte balance are crucial, especially in patients with severe hemorrhagic manifestations and fluid loss.

Blood Products

Red blood cells (RBCs), platelets, and fresh frozen plasma transfusions can all be used to treat coagulation issues and control excessive bleeding.

Oxygen Therapy

Providing supplemental oxygen supports patients with respiratory distress and hypoxia.

Pain Management

Analgesics and antipyretics help control pain and fever, improving patient comfort.

Antibiotics

In individuals with impaired immune systems, prophylactic antibiotics may be used to avoid subsequent bacterial infections.

Antiviral Therapy

Ribavirin

The most used antiviral medication for CCHF is ribavirin. It is an analog of a nucleoside that prevents viral replication by obstructing the creation of viral RNA. Early administration of ribavirin, preferably within the first few days of illness, has been shown to reduce mortality and improve outcomes. The standard dosage involves an initial loading dose followed by a maintenance dose for 10-14 days.

Other Antivirals

Research is ongoing to identify additional antiviral agents effective against CCHF. Favipiravir and interferon-alpha have shown promise in preclinical studies but require further investigation.

Experimental Therapies

Convalescent Plasma

The use of convalescent plasma from recovered CCHF patients is an experimental therapy being explored. It involves transfusing plasma containing antibodies against the virus to critically ill patients, providing passive immunity and potentially improving outcomes.

Monoclonal Antibodies

Research is underway to develop monoclonal antibodies targeting specific viral proteins. These antibodies could neutralize the virus and prevent its replication, offering a potential therapeutic option.

Prevention and Control

Vector Control

Controlling the tick population is essential for reducing the risk of CCHF transmission. Effective vector control measures include:

Tick Management

Implementing tick control measures on livestock farms and in rural areas where tick populations are high. This can involve acaricide treatment of animals, tick habitat modification, and environmental management.

Personal Protective Measures

Encouraging individuals at risk, such as farmers and livestock handlers, to wear protective clothing, use insect repellents, and regularly check for ticks on themselves and their animals.

Tick Removal

Educating the farmers on the proper techniques for tick removal to minimize the risk of infection. Ticks should be removed promptly and carefully using fine-tipped tweezers, ensuring the entire tick is extracted.

Public Awareness and Education

Raising public awareness campaigns about CCHF and its transmission routes is crucial for preventing the disease.

Information Campaigns

Conducting information campaigns through media, posters, and educational materials to inform the public about CCHF, its symptoms, and preventive measures.

Community Engagement

Engaging with local communities, especially in high-risk areas, to promote awareness and encourage participation in preventive measures.

Training Programs

Providing training programs for healthcare workers, veterinarians, and livestock handlers on the proper handling of animals, tick control, and infection prevention practices.

Occupational Safety

Healthcare workers are at high risk of CCHF due to their exposure to infected patients. Implementing strict infection control measures in healthcare settings is essential. Key measures include:

Personal Protective Equipment (PPE)

Ensuring that healthcare workers wear appropriate PPE, including gloves, gowns, masks, and eye protection, when handling suspected or confirmed CCHF cases.

Isolation Protocols

Isolating CCHF patients to prevent nosocomial transmission. This includes dedicated isolation rooms and strict adherence to infection control protocols.

Safe Handling of Blood and Body Fluids

Implementing safe handling and disposal procedures for blood and body fluids to prevent accidental exposure.

Vaccination and Immunization

Developing and distributing vaccines for at-risk populations and healthcare workers. While no licensed, CCHF vaccine exists yet, research is ongoing to develop effective vaccines.

Surveillance and Reporting

Early detection and reporting of CCHF cases are crucial for preventing outbreaks and controlling the spread of the disease.

Surveillance Systems

Establishing powerful surveillance systems to monitor CCHF cases and track virus spread. This includes reporting systems for healthcare facilities and collaboration with veterinary and agricultural sectors.

Rapid Diagnostic Testing

Ensuring the availability of rapid diagnostic tests, such as RT-PCR and serology, in healthcare facilities to facilitate early diagnosis and timely intervention.

International Collaboration

Collaborating with international organizations, such as the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), to share data, resources, and expertise in managing CCHF outbreaks.

Legislation and Policies

Effective legislation and policies play a critical role in preventing and controlling CCHF.

Infection Control Guidelines

Developing and enforcing infection control guidelines for healthcare facilities to prevent nosocomial transmission of CCHF.

Animal Health Regulations

Implementing regulations for livestock management, tick control, and the safe handling of animals to reduce the risk of zoonotic transmission.

Public Health Campaigns

Financing and endorsing public health initiatives aimed at promoting preventative care and increasing awareness of CCHF.

Research and Development

Funding for Research

Providing funds for CCHF research, which includes creating vaccinations, antiviral treatments, and diagnostic instruments.

Collaborative Research

Promoting cooperation between international organizations, governments, and research institutes in order to improve knowledge and management of CCHF.

Data Sharing

Encouraging the exchange of information in order to better coordinate international efforts to control CCHF outbreaks.

Emergency Preparedness

Emergency Response Plans

Creating and carrying out emergency response plans for CCHF epidemics, which should include quick deployment of medical personnel, supplies, and diagnostic equipment.

Training and Drills

Conducting training and simulation drills for healthcare workers and emergency responders to ensure preparedness for CCHF outbreaks.

Resource Allocation

To guarantee that emergency responders and healthcare personnel are ready for CCHF epidemics, training and simulation exercises are conducted.

Future Directions and Research

Vaccine Development

The creation of a successful CCHF vaccination is still a top objective. The main goals of research are to find appropriate viral targets and create safe, immunogenic vaccine candidates. Numerous strategies are being investigated, such as

recombinant vaccines, inactivated vaccines, and live-attenuated vaccinations. The CCHF vaccination must pass clinical trials and receive regulatory approval before it can be made available to at-risk groups.

Antiviral Drug Development

The predominant antiviral therapy for CCHF is still ribavirin; nevertheless, new antiviral medications with better safety and effectiveness characteristics are required. Novel antiviral drugs and treatment approaches, such as combination treatments and immunomodulators, are the subject of continuing research. To assess the safety and effectiveness of these possible medicines, preclinical research and clinical trials are required.

Epidemiological Studies

Continued epidemiological studies are essential in monitoring the spread of CCHF, identify risk factors, and assess the impact of control measures. These studies can provide valuable data on the effectiveness of preventive strategies and inform public health policies.

Public Health Interventions

Research is needed to evaluate the effectiveness of public health interventions, including vector control measures, public awareness campaigns, and occupational safety protocols. Implementing evidence-based interventions can help reduce the incidence of CCHF and improve outcomes for affected individuals.

Conclusion

In Pakistan, CCHF is widespread. To enhance CCHF prevention tactics, antiviral treatments, and diagnostic instruments, more research and development is required. International cooperation and effective laws and regulations are essential for halting the disease's spread and lessening its effects on public health. Individuals who are in close proximity to animals in laboratory settings, as evidenced by PCR and ELISA-IgM results, are very susceptible to contracting CCHFV infection due to the significant role animals play in the virus's transmission to humans. The worldwide growth and reappearance of CCHF underscores the necessity of additional efforts to contain the disease, which poses significant risks to the health of humans and animals. The creation of therapeutic alternatives, countermeasures, and immunocompetent CCHF disease models must be the main areas of concentration for future study. Apart from the dearth of research, the data also indicated a significant knowledge gap about the implementation of personal protective measures. It is necessary to develop a comprehensive national strategy on CCHF that spans all pertinent sectors and places a strong emphasis on heightened surveillance, quick action to save precious human lives, forming partnerships, and research to inform public policy. Thus, now is the perfect time to launch instructional initiatives, especially for those involved in high-risk professions like veterinary care, agriculture, herding, and slaughterhouse labor.

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Chapter 20

Antibiotic Alternatives: Recent Therapeutic Approach to Tackle AMR

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ABSTRACT

Antimicrobial resistance is a primary one-health concern, posing a significant threat to humans, animals, and the environment. Unnecessary and uncontrolled usage of antibiotics led to this alarming situation that is causing the death of almost 0.7 million people every year worldwide and is estimated to increase up to 0.01 billion by the end of the next few decades if left unchecked. The treatment of infectious diseases has further become challenging amid the appearance of multidrug-resistant bacteria, especially in patients with low immunity. The most commonly affecting pathogens, like Pseudomonas *aeruginosa,* E. *coli,* Streptococcus *pneumoniae, and* Klebsiella *pneumoniae,* are responsible for causing fatalities between 50,000 and 100,000 annually. Different studies are being conducted worldwide to explore the possible alternatives/methods of antibiotics to tackle this issue; however, either few or none are entirely implemented in the field. Examples of alternative methods are a) Strategies targeting antimicrobial-resistant enzymes b) Strategies targeting antimicrobial-resistant bacteria, and c) Physiochemical, b) Strategies targeting antimicrobial-resistant bacteria, and c) Physicochemical methods.

KEYWORDS
Antibiotics, Bacteria, Antimicrobial Resistance, Multidrug-Resistant (MDR) Pathogens, Antibiotic Alternatives.

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INTRODUCTION

Pathogenic microbes have continued to be one of the most potent enemies of humanity in the past. Back in 1900, the majority of American people had an estimated life span of 40-50 years as they died as a result of cholera, plague, tuberculosis, and other infectious diseases (Streicher, 2021). However, a sharp decline in mortality and morbidity was seen in the middle of the 20th century due to the discovery of antibiotics (Ventola, 2017). Along with this effective outcome of antibiotic discovery, the discovery and use of alternative medicines almost stopped completely (Summers, 2012). However, antibiotic-resistant bacteria have emerged just as fast as the new antibiotics are introduced. One reason behind this emergence may be that antibiotics mainly originated from nature and have been used for ages. The purpose of producing antibiotics by microorganisms is to protect against their rivals. Still, just as these rivals have developed resistance, the antibiotics no longer remain effective in providing defence. This resistance is developed even against not-yet-discovered antibiotics (Spellberg et al., 2013).

Antimicrobial resistance is a natural process in bacteria through gene mutation or gene transfer, which can even occur with zero human interference (Melnyk et al., 2015). However, the excessive and uncontrolled use of antibiotics for many years has played a significant role in its occurrence (Merker et al., 2020). Antibiotics work by either inhibiting the growth of microorganisms or killing them; each antibiotic group has a different working mechanism, like inhibiting DNA synthesis, cell wall synthesis, protein synthesis and so on (Kapoor, 2017). These working mechanisms of antibiotics have introduced so much pressure that it resulted in the development of defense mechanisms or resistance in microbes in different ways, like minimizing the permeability of their membranes so that antibiotics cannot gain access into the cell, similarly producing specific enzymes that can degrade the antibiotics. Along with this, antibiotics, particularly broad-spectrum, also disturb normal flora, resulting in an increased probability for opportunistic bacteria to cause infection (Streicher, 2021).

AMR is a global health concern causing the death of 7,00,000 people patients every year and may rise to 10 million in the next 2-3 decades (Ghosh et al., 2019). The treatment of infectious diseases and medical procedures, such as surgeries, has become challenging due to the ineffectiveness of many antibiotics (Dadgostar, 2019). The upsurge of superbugs that are resistant to multiple drugs, termed multi-drug resistant (MDR) pathogens is another serious concern for the whole world (Matar et al., 2020). Treatment of diseases caused by MDR pathogens using commonly available drugs has become a great challenge for the world. It is predicted that MDR pathogens will be responsible for the death of 10 million patients per year by the end of the next 2-3 decades, which is much higher than the deaths caused by cancer today, which is 8.2 million per year (Andersson and Hughes, 2010). Unluckily, there are far too few antibiotic medicines in trial stages to keep up with the increasing tide of AMR. Not many new antibiotics are being developed, even though much effort has been put into the field (Appelbaum, 2012).

Antibiotic resistance is a growing problem that requires audacious and creative solutions. It resulted from a complicated process of evolution, as different organisms have adopted various strategies for this purpose. For example, Pseudomonas *aeruginosa* develops a biofilm and layer of persister cells that are drug resistant. Another example is the adopted ability of Mycobacterium *tuberculosis* strains to complete their life cycle within granuloma (a tiny cluster of immune cells) and to become dormant in undesirable conditions, which ultimately provides them protection from both the host-related and environment-related stress (Merker et al., 2020). Various studies conducted in the US poultry industry reveal the possibility of propagation of resistance genes via poultry waste into the soil (Yang et al., 2019). A comprehensive knowledge of all these mechanisms may be fruitful in improving the treatment strategies using already available medications. Even though AMR genes can persist in an environment for years, even in an environment deprived of antimicrobials, decreased usage of antimicrobials is the first and most crucial move to tackle AMR. The significance of a "One Health" strategy in addressing the issue of antimicrobial resistance (AMR) cannot be overstated, considering the intricacy of the challenge it poses to both human and animal health and the environmental implications. Thus, a multifaceted strategy is needed to coordinate efforts to address and resolve this concerning issue.

Understanding bacterial resistance mechanisms, applying multimodality therapeutic techniques for possible clinical alternatives, and developing novel antimicrobial drugs and/or targets are the three main objectives that highlight the key features of this mission (Matar et al., 2020). Immediate and fruitful action is needed in an hour amid the looming challenges of AMR. The ongoing activities have mainly focused on discovering new antibiotics and improving the efficacy of existing ones, like enhancing the shelf life of antibiotics, limiting or stopping their use in agriculture, and improving the practices to control infections. However, these strategies are essential but are inadequate to tackle the situation when all the antibiotics are useless due to their ineffectiveness. Various alternative antibiotic strategies are being discussed, and research is being conducted. A few of the crucial strategies will be addressed in this chapter.

Strategies Targeting Antimicrobial-Resistant Enzymes

Medicinal Plants

Medicinal plants are an attractive therapeutic approach to control antimicrobial resistance due to their capability to target the mechanisms of antimicrobial resistance. The significant mechanisms targeted by medicinal plants are biofilms, efflux pumps, and cell membranes (Tiwari et al., 2023). Plants have phytochemical mechanisms to shield themselves from microbes. Phytochemicals are secondary metabolites in leaves, roots, stems, fruits, and flowers (AlSheikh et al., 2020).

Plant-derived substances (PDSs) of significant medicinal value with the highest antibacterial action are terpenes, alkaloids, organosulfur, phenolic compounds, and coumarin. (Gupta and Birdi 2017; Khameneh et al., 2019)Plant-derived substances have in vitro antibacterial activity against an extensive range of bacteria, with mechanisms including inhibition of bacterial cell wall synthesis, inhibition of biofilms, impediment of bacterial physiology, lessening of bacterial virulence, and inhibition of efflux pumps. (Khameneh et al., 2019). For example, alkaloids and phenolic compounds resist *E. coli, Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus* (MRSA) due to their inhibitory effect on efflux pumps. (Dwivedi et al. 2019; Maurya et al., 2013). Plant steroidal Alkaloid like Tomatidine has bactericidal activity against *Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), and colony variants of *Staphylococcus aureus* through inhibition of ATP synthase. (Guay et al., 2018). Berberine causes the death of *Streptococcus agalactia* by destroying the cell wall due to the inhibition of protein and DNA synthesis. (Peng et al., 2015). Flavonoids belong to phenolic compounds that restrict the beta ketoacyl acyl carrier protein synthase (KAS) III, targeting novel antibiotics. (Jeong et al., 2009). Organosulphur compounds also have antibacterial potential to inhibit DNA and protein synthesis, ATP Synthase, restricting sulphydryl dependent enzymes and destruction of cell membranes of *Streptococcus agalactia, E.coli, Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Campylobacter jejuni* (Reiter et al. 2017; Stermitz et al. 2001; Wu et al., 2012). Coumarin is a potent bioactive heterocyclic compound possessing antimicrobial activity. (Al-Majedy et al., 2017). Coumarin restricts the DNA gyrase activity *of Helicobacter pylori, P. aeuroginosa, Staphylococcus aureus,* and MRSA. (Murugaiyan et al., 2022). Combined therapies of antibiotics with phytochemicals have synergistic effects that lessen the usage of antibiotics (Rajamanickam et al., 2020). Furthermore, in livestock and poultry farming, the use of phytochemical compounds with stated antibacterial qualities may be a feasible substitute for the use of growth promoters based on antibiotics as feed additives (Jouany and Morgavi, 2007).

Enzyme Inhibitors

Low subatomic weight synthetic particles known as enzyme inhibitors can partially or suppress the catalytic activity of enzymes, either irreversibly or reversibly. The inhibitors of cholinesterase (ChE) and monoamine oxidases (MAO) serve as examples of this when applied to a variety of pharmaceutical objectives. Enzymes tend to be prime candidates for therapeutic medicines since altering an enzyme's chemical function has improved the disease course. Even with the rise in pharmaceuticals for receptors to modify signals from outside the cell, 47% of all currently prescribed drugs block enzyme targets (Ramsay and Tipton, 2017). Many antibiotics block certain enzymes, and a large number of bacterial enzymes are crucial in the development of antibiotic resistance. Most antibiotics are designed to target specific enzymes, and resistance develops when those target enzymes undergo structural alterations or when the components that the antibiotics impact undergo enzymatic modifications. Many examples of enzymes serve as targets for antibiotics that have enzymes involved in nucleic acid replication and cell wall synthesis. PBPs are primary targets for the majority of antibiotics in use today. These antibiotics impede bacterial cell wall formation and operate as competitive inhibitors of PBPs (Egorov et al., 2018). Clavulanic acid is commonly used with beta-lactam antibiotics, a prime example of a beta-lactamase inhibitor. It inactivates the beta-lactamases involved in the destruction of beta-lactam antibiotics. In the fight against clinically relevant antibiotic-resistant microorganisms, several other compounds with inhibitory activity against enzymes that give antimicrobial-resistant activity have been assessed as promising natural or recombinant weapons more recently (Bush and Bradford, 2016; Tooke et al., 2019).

Essential Oils

"Essential oils" (EOs) are combinations of volatile chemicals developed during secondary metabolism from various plant parts. Paracelsus von Hohenheim, a medieval Swiss physician, used the term “Essential oils” for the first time. Terpenoids, aldehydes, terpenes, and phenolic compounds are included in Essential oils and depict their antimicrobial activities (Swamy et al. 2016; Valdivieso-Ugarte et al., 2019). The primary mechanisms by which EOs help break bacterial cell membranes and inhibit efflux pumps that are liable to several types of AMR in Gram-negative bacteria (Ciocarlan et al. 2021; Iseppi et al., 2021). EOs control the AMR in Gram-positive bacteria by inhibiting the peptidoglycan layer synthesis of bacterial cell walls by binding to PBPs (Kuok et al., 2017). Advancements in proteomics and genomics have increased the understanding of the mechanisms of action of essential oils (Yang et al., 2021). Various research articles demonstrate the antibacterial activity of lavender against *Klebsiella pneumonia* with MIC of 10% (v/v), *Salmonella enterica,* and *Pseudomonas aeruginosa* with MIC of 0.5 to 2% (v/v). Cinnamon bark has antibacterial activity against *E.coli* with MIC of 0.009% to 0.078% (v/v). Cinnamon bark essential oil also showed antibacterial activity against *Staphylococcus epidermis, S. aureus, Streptococcus pyogenes, E. coli,* and *P. aerugin*osa, with MIC values of 0.5 to 5% of MIC. Studies showed that peppermint oil was quite effective against *L. monocytogenes* and *S. aureus* with MIC of 0.5% (v/v). Understanding the interactions between the constituents of crude essential oils, the identification of new molecules, and the clinical endorsement of EOs as antibacterial agents have become progressively significant. It is important to emphasize that the bacteria have been shown to acquire tolerance and resistance to EOs; however, cross-resistance to antibiotics has not been documented (Tetard et al., 2019).

RNA Silencing

RNA silencing is a strategy to produce novel antibiotics. It is naturally found in bacteria and involved in the regulation of genes. Cis and trans sequences complement regulatory regions found on single m-RNA and block the translation reversibly upon binding. Cis antisense sequence is also present near regulatory regions on single RNA. Trans sequences comprise most naturally occurring antisense sequences and are transcribed from distant genetic loci. It may be possible to create synthetic antisense sequences that inhibit the translation of enzymes that give bacteria resistance to drugs. RNA silencing is used to identify novel antimicrobial drugs, assess the targets' stringency, create susceptible antimicrobial screens, and determine the mode of action. RNA silencing is also beneficial in antibacterial screening. It helps the genes to knock down the target of interest. The method of RNA silencing has been applied in detecting the mechanism of action of novel antibiotics; for example, it was used to discover novel enoyl-acyl carrier protein reductase inhibitors. (Good and Stach, 2011).

CRISPR-Cas System

Clustered, regularly interspersed short palindromic repeats- CRISPR-associated proteins are a bacterial adaptive immune system that helps control antibiotic-resistant strains. To counter the invasion of foreign genetic material and mobile genetic elements in bacteria, the CRISPR-Cas system uses RNA-mediated, DNA-encoded, or DNA-targeting processes (Briner and Barrangou 2016; Duan et al. 2021; Gholizadeh et al., 2020). By targeting bacterial genomes statistically, specifically, and selectively, these gene-editing techniques can decrease or eradicate antibiotic resistance and open up novel therapies for multidrug-resistant illnesses. In addition to distinguishing between pathogenic and commensal bacteria, CRISPR-Cas systems may also be able to remove specific AMR genes from bacterial populations and virulence factors or make bacteria more sensitive to antibiotics by eradicating plasmids that carry antibiotic-resistance genes. Further studies are needed regarding the limitations in this field.

Strategies Targeting Antimicrobial-Resistant Bacteria

Lantibiotics and Bacteriocins

The term *Lantibiotics* refers to gene-encoded peptides having rare amino acids, containing thioether amino acids and methyllanthionine amino acids, which are made by post-translational amendment and subsequently acquaint with intramolecular cyclic structures in the peptide needed for the specific exporters and for posttranslational modification (Bierbaum and Sahl, 2009). Over a while, many lantibiotics have been discovered, and their different prescriptions have been documented. Mainly, all of these compounds are produced explicitly from gram-positive bacteria and show their responses against this group (M Daly et al., 2012). Many lantibiotics possess antibacterial activity. They are divided into two groups: type A and B. Type A peptide works by disturbing the membrane integrity of targeting bacteria. Type B peptides perform by disrupting the functions of enzymes, for example, by inhibiting the biosynthesis of cell walls (McAuliffe et al., 2001).

The proteinaceous or peptide toxins produced by bacteria, a type of type-A lantibiotic, are called bacteriocins. They perform by killing closely related bacterial strains but will not destroy the original bacteria (Mullur et al., 2014). Through intensive pharmaceutical drug discovery efforts, bacteriocins were discovered and have become a valuable asset to the present toolkit of medications that may be utilized to combat lethal bacterial infections (Cotter et al., 2013). Due to their antimicrobial properties, bacteriocins have gained a lot more attraction in the field. They are specialist against parasites, bacteria, and viruses and notably against complex bacterial biofilms (Mathur et al., 2018). They are helpful in inhibiting specifically closely related ones. There are still many more bacteriotoxins to be discovered besides the numerous bacteriocins that have already been found. Additionally, bacteriocins are effective against microorganisms that produce anti-toxin systems (Simons et al., 2020)The variety of bacteriotoxins makes a wide range of biotechnological and pharmaceutical uses possible. The primary industry affected by the use of bacteriocin is the agro-food industry.

Antimicrobial Peptides (AMP)–Including AMP + Antibiotics Combination

Antimicrobial peptides have been used for a long time, either alone or in combination with traditional antibiotics, to develop novel or effective antibiotics (Zharkova et al., 2019). AMPs have better antimicrobial activity than conventional antibiotics due to the possession of cationic charge and are amphipathic (Blondelle and Houghten 1992; Sheard et al., 2019). Under normal circumstances, AMPs originating from bacteria are a component of the bacterial cell defense mechanism, helping in defense against hazardous invasion (e.g. exogenous molecules, bacteriophages). This process affects inflammation and boosts the killing of pathogens (Kumar et al., 2018). AMPs exert antimicrobial activity against bacteria by targeting nucleic acids, protein biosynthesis, membrane production, and disrupting the cell membrane (Nguyen et al., 2011). Unfortunately, the drawback of clinical usage of native AMPs in both human and veterinary fields is that they are easily degraded by proteolytic enzymes in the gastric acidic environment. Different routes of administration have been favored, but they are also not efficient in providing the results. So, to overcome the issues, a synergistic combination of AMPs with traditional antibiotics has been discovered and given promising results against severely resistant bacteria. For example, the synergistic effect of AMP with vancomycin showed promising results against *Enterococci*. In situ drug delivery of AMPs with antibiotics with the goal of in situ drug delivery increased drug specificity and reduced toxicity (Riber et al., 2015). Since AMPs cannot enter the microbial cell when the antibiotic's resistance mechanism involves membrane alteration, they work better with antibiotics than when administered alone. By overcoming this obstacle, AMPs might be able to make antibiotics that had previously lost their effectiveness active again (Sierra et al., 2017).

Drug delivery methods based on nanotechnology (nanocarriers) are attractive and powerful techniques for the effective delivery of AMPs. Nanocarriers are utilized to protect the peptides from extracellular degradation by proteases. Applying nanocarriers could also help enhance drug pharmacokinetic profiles and target selectivity. In recent studies, different drug delivery systems have been identified, such as dendrimers, liposomes, micelles, and polymeric nanoparticles(Biswaro et al. 2018; Ritsema et al., 2018)

Nonetheless, significant questions remain today regarding the safety and effectiveness of using AMPs as dietary supplements or medications in veterinary medicine. Efforts to enhance their safety and effectiveness shouldn't be given up to overcome these challenges.

Insect Derived Enzymes and AMPs

Numerous insects generate diverse and intricate groups of enzymes for their protection and survival. The variety of insects is enormous, and there is mounting evidence that the natural system of insects is mainly dynamic. Insect-produced AMPs have cationic groups and are smaller in size. Based on the structure and amino acid composition of AMPs, their four groups are found in insects. These groups have proline-rich peptides, cysteine-rich peptides, alpha-helical peptides, and glycine-rich proteins (Otvos, 2000). Cysteine-rich peptides inhibit biofilm formation and are the main component of the innate immune system of insects. Overall, the interaction of peptide and membrane directly stimulates antibiofilm/antibacterial activity. It is now widely understood that AMPs are the primary immunological effector molecules found in insects. In addition, bioactive compounds and signaling pathways found in the natural systems of insects bear a striking resemblance to vertebrates, including humans, as a result of conserved biological evolution. Researchers from across the world are looking for new bioactive compounds that have unique antibacterial properties. Furthermore, AMPs

and traditional antibiotics may work synergistically to fight various infections. Many characteristics of AMPs, including their sequence, charge, helicity, amphipathicity, and general hydrophobicity, are essential for evaluating them as potent antimicrobial agents (Jenssen et al. 2006; Sahoo et al., 2021).

Nanoparticle-Based Strategies

Nanoparticles range from 1 to 100 nm (Zamborini et al., 2012). Nanoparticles (NPs) are increasingly used as bacterial growth inhibitors in applications like antibiotic delivery and coatings for medicinal materials and implantable devices. They could also be administered straight as antimicrobial agents (Wang et al., 2017). The precise mechanism of action by which NPs carry out their antimicrobial activity is not entirely understood; it has been suggested that three processes take place concurrently. These are non-oxidative processes, metal ion release, and oxidative stress (Seil and Webster, 2012). Nanoparticles (NP) exhibit antibacterial activity depending on their size and inhibit bacterial growth by disrupting bacterial membranes and biofilm formation. Antibiofilm activity is also influenced by shape; rod-shaped particles suppress biofilms more effectively than spherical-shaped NPs. Owing to their physiochemical properties, NPs can be administered intravenously, orally, through skin contact, or by inhalation for antibacterial purposes. The toxicity of NPs is one of the primary disadvantages of employing them as new antimicrobials. The long-term impact of NPs in eukaryotic cells and tissues has been evaluated through both in vitro and in vivo research. In general, NPs harm eukaryotic cells at concentrations that suppress bacterial development. Interestingly, toxicity could be overcome by targeted delivery of NPs to the infection site, although this approach affects NPs potential (Herman et al. 2014; Seil and Webster, 2012).

Coinfection Strategies and Probiotic Bacteria against Pathogens

Various complementary approaches are being used to prevent the spread of AMR infections in veterinary and human medicine. These include administering probiotic-effecting microbial species or using coinfection methods (Lee et al., 2013). Probiotics are live bacteria that have a beneficial impact on health. Probiotics' effectiveness in combating antimicrobial resistance (AMR) depends on their ability to impede the direct selection pressure drugs impose on pathogenic microbes. Probiotics combat infection through a range of ecological mechanisms, such as AMP synthesis with specific bactericidal activity, colonization resistance, and competition for nutrients and ecological space (Palma et al., 2020).

Physicochemical Methods

Non Thermal Atmospheric Pressure Plasma (NTAPP)

It is a breakthrough technology that has various applications in the field of medicine. Its antimicrobial property is one of its vital abilities, and it has been demonstrated in different in vitro studies conducted worldwide, where it effectively inactivates various microbes under study (Mai-Prochnow et al., 2014). Its exact mechanism of action is still being investigated. Still, the most probable way is through the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), and ultraviolet (UV) radiations in the gas phase of plasma. All these collectively disrupt microbial cells in various ways, ultimately leading to cell death (Čtvrtečková et al., 2019). It has also been observed in the research that NTAPP is more effective against gram-negative bacteria than gram-positive. Cold plasma effectively killed various bacteria of clinical importance, like Pseudomonas aeruginosa, when studies were conducted targeting bacterium in both the suspension and biofilm form. Most importantly, it doesn't cause any damage to the host cells while targeting microbe (Heinlin et al., 2013).

Photodynamic Antimicrobial Chemotherapy (PACT)

PACT, also termed photoinactivation, has been proven to be another promising alternative to antibiotics. It causes bacterial cell death by the production of reactive oxygen species. It uses visible light, light-sensitive chromophores, and molecular oxygen. It is effective against various bacteria, including both gram-positive and gram-negative bacteria. Different studies are being conducted across the world to understand and evaluate this promising alternative strategy to tackle AMR whereby various improvements are also being made, the usage of porphyrin and nanoparticles along with it that ultimately facilitate the attachment of photosensitisers to the microbial cell wall and cause its death (Oyim et al., 2021). One of the limitations of this technology is that it cannot penetrate deep into the tissue, which might be due to the presence of pigments like melanin that compete for light absorbance. It may be more problematic in the case of local wounds where skin colour changes due to inflammation or bruises or in individuals with dark skin tones (O'Riordan et al., 2005).

Sonodynamic Antimicrobial Chemotherapy (SACT)

This technology involves the usage of ultrasound and sonosensitizer (a chemical compound) to cause the death of microbes through inaudible, low-frequency sound waves. A study conducted on E. coli effectively killed the bacteria when conjugated with common antibiotics from the quinolone group, like levofloxacin and ciprofloxacin. One of the most important advantages of this strategy is its ability to penetrate deep tissue unlike PACT (Ventola, 2015).

Conclusion

Antimicrobial resistance is one of the most alarming issues of the present day worldwide that is continuously

increasing and even worsening with every passing day, having no boundaries. If this issue remains unaddressed, it will ultimately cause all the infectious diseases of humans and animals to be untreatable shortly. Various antibiotic alternatives are available worldwide and are under investigation. However, very few are implemented in the field practice as further studies still need to be conducted on them to understand their efficacy with no or minimal side effects. Multidisciplinary cooperation and collaborative efforts at the national and international levels need an hour to tackle this burning issue. Unnecessary usage of antibiotics should be controlled immediately and extensive efforts should be made to provide awareness regarding this issue, along with its possible alternatives, to the general public.

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Chapter 21

Probiotics: A Strategy for Combating Antimicrobial Resistance (AMR) in Poultry

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ABSTRACT

The extensive use of antibiotics as growth promoters in poultry has led to concerns regarding antibiotic resistance and the presence of antibiotic residues in poultry products. Probiotics present a promising alternative, exerting beneficial effects on gut health and immunity in chickens. The chicken gut microbiota, consisting of a diverse array of bacterial species, plays pivotal roles in metabolic functions and immune regulation. Probiotics, live microorganisms administered to confer health benefits, act through mechanisms such as enhancement of healthy microbiota, enhancing gut barrier integrity, competing with pathogens, modulating immune responses, and producing antimicrobial substances. Various probiotics, including *Lactobacillus*, *Bacillus*, and yeast, have demonstrated effectiveness in chicken health, enhanced production, improved growth performance, and reduced pathogen colonization resulting in lower diseases. Understanding the mechanisms underlying probiotic action and their impact on gut microbiota composition is crucial for developing efficient alternatives to antibiotics in poultry farming, addressing both health and productivity concerns while mitigating the risks associated with antibiotic resistance.

KEYWORDS
Antibiotic alternatives, Probiotics, Poultry gut microbiota, Antibiotic Resistance, Poultry gut health

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INTRODUCTION

Antibiotics have been widely used since the 1940s as antibiotic growth promoters (AGPs) in the poultry industry i.e., sub-therapeutic levels of antibiotics in poultry feeds to build the immunocompetence (i.e. ability of the body to produce a normal immune response following exposure to an antigen) in chickens against infectious diseases and improve growth performance by increasing feed efficiency (Gadde, 2018). The use of AGP's in poultry feed was considered to improve growth performance about 4 to 8% and increase feed utilization by 2 to 5% mainly using two mechanisms of actions i.e. protection of nutrients against bacterial destruction and thinning of the small intestinal barrier which leads to improved nutrient absorption (Gaskins, 2002). Moreover, antibiotic use also resulted in decreased production of toxins by intestinal bacteria resulting in reduced subclinical intestinal infections.

This excessive, irrational non-therapeutic use of antibiotics in food animals such as poultry results in the emergence of antibiotic resistance in humans. The World Health Organization (WHO, 1997) and the European Union (1998) (Economic and Social Committee of the European Union, 1998) declared that the use of antibiotics in animal feed is a public health concern. However, the European Union banned AGPs in 2006 while the Food and Drug Administration (FDA), USA banned them in 2009 (Castanon, 2007).

Along with antibiotic resistance, prolonged sub-therapeutic use of antibiotics in poultry feed results in the accumulation of antibiotic residues in poultry ultimately leading to reduced growth performance and reduced immunity. Keeping in view the looming antibiotic resistance threat, there is a dire need for safe and effective antibiotic alternatives such as probiotics which claim to enhance growth performance and strengthen enteric immunity (Al-Khalaifah, 2018; Gernat et al., 2021).

Probiotics influence chicken gut health indirectly by increasing beneficial microbes, optimizing gut function or regulation of metabolism, and improving intestinal epithelial barrier integrity. Gut microbiota is considered a very important metabolic organ whose potential is underrated so far as it has a profound impact on host metabolism, physiology, nutrition, and immune function. Gut microbiota determines host health depending upon dynamic and mutually beneficial host-microbiota relationship if imbalanced results in intestinal dysbiosis leading to various diseases. Therefore, regulation of intestinal microbiota may avoid diseases and lead to enhanced growth and production in birds (Shehata, 2022).

Several probiotics have been developed, tested, and evaluated to enhance production and reduce disease burden in poultry so far. However, it is very important to focus on research trials for the discovery of potential antibiotic alternatives to cope with antibiotic resistance (Al-Khalaifah, 2018; Gernat et al., 2021).

Role of Gut Microbiota in Poultry

Gastrointestinal tract (GIT) of chicken is colonized by over 600 different bacterial species from more than 100 bacterial genera, this diverse and plentiful microbial population is called "microbiota". These are mainly beneficial microorganisms, densely populated in GIT, which have a profound impact on chicken health, growth, and metabolic functions (López-García et al., 2017).

Chicken Gut or GIT exposure to microbes starts from embryogenesis and hatching and continues until the formation of a diverse and complex microbial society and is influenced by a variety of factors throughout the lifecycle of a chicken including species, breed, age, nutrition, environment, rearing forms, stocking density, stress, and medicine. However, intestinal microflora cannot be cultured outside their ecological niche like most complex ecosystems (Lee et al., 2019).

It is important to study chicken microbiota, microbe-microbe, and microbe-host relationships to better understand the physiological mechanisms of probiotic action. Avian microbiota benefits chicken health by the development of lymphoid tissue in GIT, immune system education, maintenance of intestinal mucosal barrier integrity, regulation of angiogenesis, modulation of enteric nervous system activity and extraction, processing and metabolism of the nutrients consumed by chicken by gut microflora (Shehata, 2022).

Chicken gut microbiota performs various metabolic functions to maintain host health, it can metabolize proteins and their byproducts, sulfur-containing substances, and glycoproteins. They ferment prebiotics into short chain fatty acids (SFCA), mainly acetate, propionate, and butyrate. SCFAs lowers the luminal pH, provides energy sources for epithelial cells, and has profound effects on inflammation modulators and metabolic regulations. Beneficial gut bacteria feed on fermentation by-products including H2, lactate, succinate, formate, and ethanol, convert them to end products, and secret them in the gut (Rabetafika et al., 2023; van der Wielen et al., 2000).

Clostridium species found predominantly in cecum produce significant butyrate which contributes to growth, inhibits inflammatory responses by acting on proinflammatory cytokines, and also serves as an important energy source for intestinal epithelial cells (Eeckhaut et al., 2011).

Composition of Chicken Gut Microbiota

Chicken intestinal microbiota is mainly constituted by phylum Firmicutes followed by phyla, *Proteobacteria* and *Bacteroidetes* additionally minor phyla inhabiting the chicken intestine include members of phyla *Actinobacteria*, *Tenericutes, Cyanobacteria* and *Fusobacteria*. However, different parts of the GIT vary in composition of microbial population considerably depending on the stage of the lifecycle. Crop, gizzard, and duodenum share a similar microbiome consisting of approximately 99% *Lactobacillu*s genera e.g. *L. salivarius* and *L. aviarius* in jejunum. Microbiota in in ileum are more diverse and less stable than the duodenum and the jejunum, dominated by *Lactobacillus, Candidatus Arthromitus, Enterococcus, Escherichia coli/Shigella* and *Clostridium* XI (Gong et al., 2007; Mohd Asrore et al., 2015; Pourabedin and Zhao, 2015).

The cecum is the main site for bacterial fermentation of non-digestible carbohydrates and pathogen colonization. Both paired caeca, the most studied part, possess similar microbiota. As compared to upper GIT, the cecum harbors the most diverse bacterial population and it is the most densely colonized part of chicken gut as well (Stanley et al., 2015). Cecum is dominated by the *Clostridia* genus followed by the genera *Lactobacillus* and *Ruminococcus*. The *Clostridia* genus found in cecum primarily contains three main families*, Clostridiaceae, Lachnospiraceae,* and *Ruminococcaceae*. Intestinal microbiota include *Enterococcaceae, Enterobacteriaceae,* and *Bacteroidaceae* (Yin et al., 2009). It also possesses unclassified bacteria. At the species level, more than 40% of the cecal microbiota consists of *Bacteroides fragilis, L. crispatus, L. johnsonii, L. salivarius and L. reuteri* (Stanley et al., 2015).

The intestinal microbiota of chickens change over time, some bacteria disappear while others remain stable throughout life. *Firmicutes spp* is dominant in younger chicks while *Bacteroidetes* are most common in old birds. In layer birds of day 01 to 60 weeks of age, four different profiles of cecal microbiota have been identified (Pourabedin and Zhao, 2015).

Probiotics

Over the decades, it was assumed that the probiotics effect through direct interaction with commensal microbial population i.e. by contributing to host intestinal microbial balance or by improving the properties of the indigenous

microflora. However, currently, probiotics are not only considered microbiota mediated as different mechanisms of probiotics are well known (Sanders et al., 2019).

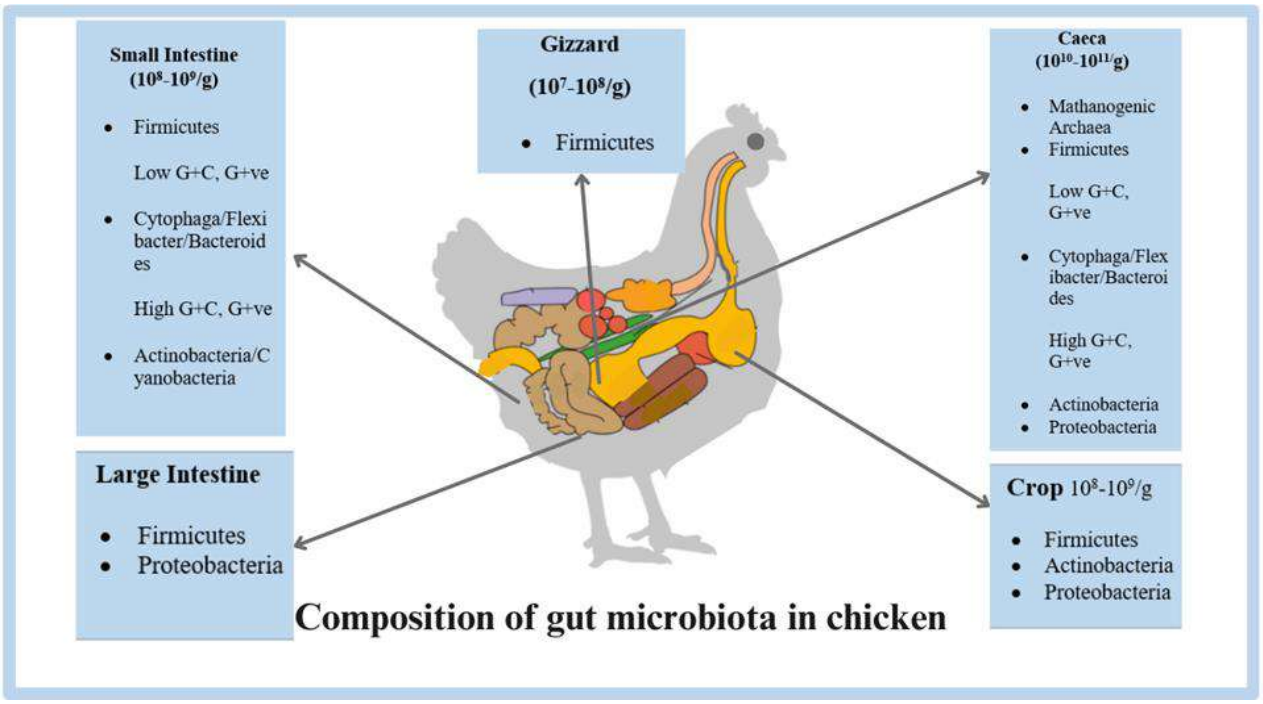

Fig. 1: Composition of gut microbiota in chicken, summarized by (Shang et al., 2018).

In 2001, the Food and Agricultural Organization of the United Nations and the World Health Organization established the term "probiotic" through an Expert Consultation. Probiotics are defined as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host." (FAO/WHO, 2001). Additionally, they should be live or viable microorganisms, non-toxic and non-pathogenic, capable of multiplying and metabolizing in the intestinal microbiota, and remain viable and stable in storage and field conditions (Fuller, 1989).

Strain Designation and Dosage

Probiotics are distinguished by their genus, species, and strain, with each strain potentially offering different health benefits for example strain designation of *Lactobacillus rhamnosus* is "GG" and the strain designation of *Lactobacillus casei* is "DN-114 001". However, the effectiveness of a probiotic isn't solely determined by its strain but also by the dosage consumed (Hill et al., 2014). Consuming a probiotic at a higher dosage doesn't necessarily result in greater health benefits compared to a lower dosage. The dosage needs to match the level proven effective in clinical studies to confer a benefit (Schrezenmeir and de Vrese, 2001).

Probiotic Mechanism of Action in Chicken Gut Health

Probiotics improve healthy gut microbiota, develop disease resistance, and strengthen gut immunity if used properly. Probiotic strains can interact with the gut microbiota through

1. Competitive exclusion i.e., Competition for nutrients and attachment sites
2. Antagonism i.e., Production of antimicrobial substances and organic acids
3. Support of microbiota stability
4. Cross-feeding

Probiotic strains can interact with the host through

1. Enhancement of the epithelial barrier integrity through tight junctions (enterocytes connections)
2. Modulation of the immune system
3. Production of organic acids
4. Production of Enzymes and small molecules (Sanders et al., 2019).

Improvement in Intestinal Barrier Integrity

The epithelial lining of the gut which is densely colonized by microflora acts physical or biological barrier and forms tight junctions that control the paracellular transit of different substances across the gut lining e.g. nutrients, ions, solutes, and water, and also functions as a barrier of extracellular bacteria, antigens, and xenobiotics (Shehata, 2022). Intestinal permeability is controlled by gut microbiota, digestive secretions, physical barriers (mucin, intestinal epithelial cells lining and tight junctions), and chemicals such as cytokines (Bischoff et al., 2014).

Impaired function of an intestinal barrier as a result of any disruption of intestinal epithelium also called “leaky gut” is a condition that results in the infiltration of gut contents including bacteria and bacteria-derived endotoxins to cross epithelial lining and raising their levels in blood leading to cell damage and inflammation of the intestine. This inflammatory condition utilized an ample quantity of nutrients hence, negatively impacting metabolic processes i.e., absorption of nutrients, immunometabolic and endocrine responses, and significantly reduced growth performance of chicken (Abuajamieh et al., 2016).

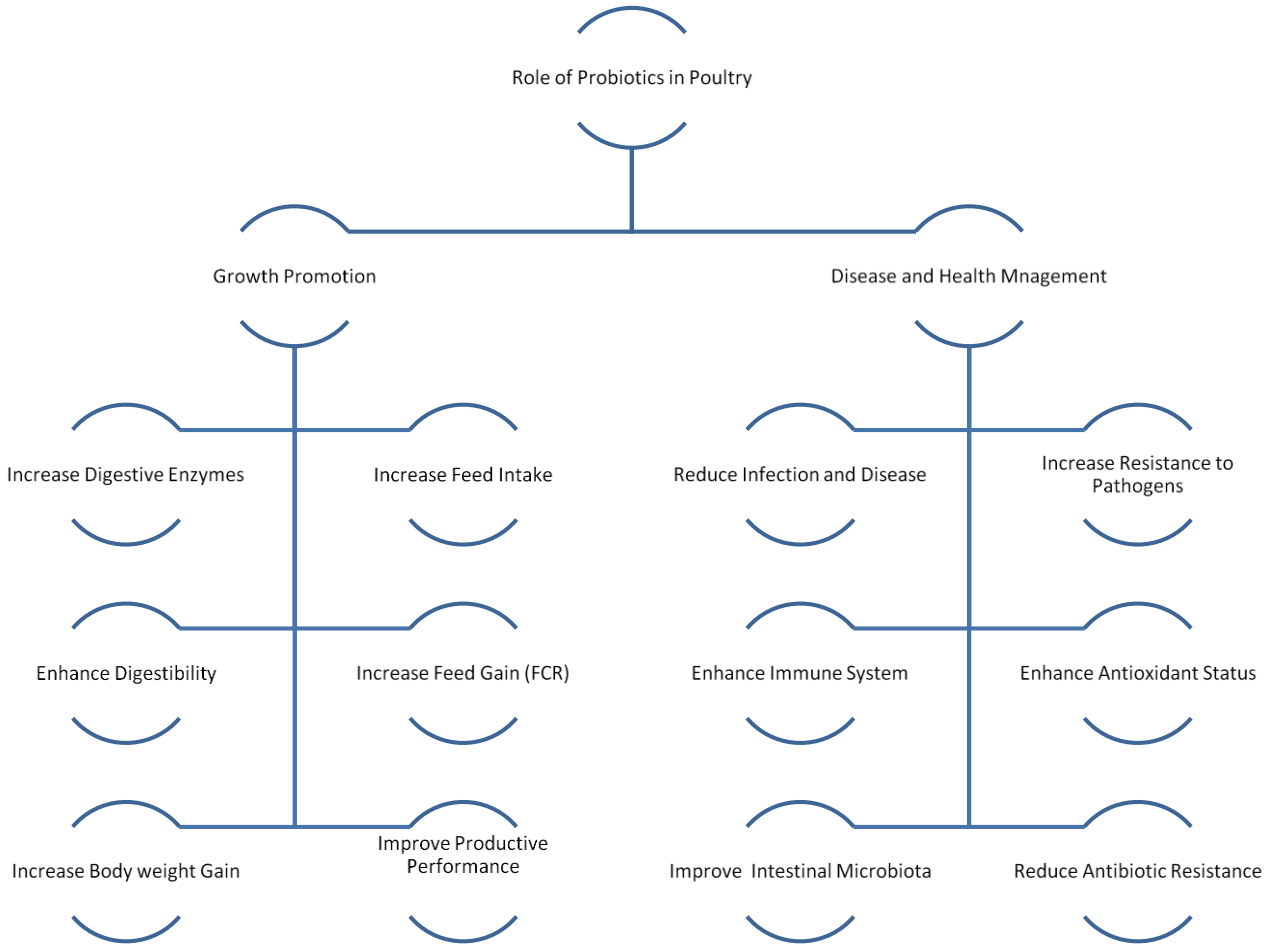

Fig. 2: Impacts of probiotics as antibiotic alternatives in poultry

Several factors contribute to gut disruption i.e., antinutritional factors in feed, heavy metals, toxic substances, bacterial toxins, herbicides, and antibiotics leading to localized inflammation or enteric infections (Krüger et al., 2014). Leaky gut syndromes develop as a defense mechanism response against pathogenic bacteria, feed deprivation, and any environmental stress. Bacterial factors disrupt the intestinal barrier and increase intestinal permeability by following the steps

1. Lipopolysaccharide (LPS) in bacteria activate epithelial cells and macrophages
2. Epithelial cells and macrophages secrete proinflammatory cytokines such as IL-1ß.
3. IL-1ß activates intracellular signaling, such as p38 MAP kinase which activates MLCK (Faralli et al., 2019; J. Wang et al., 2017).

Probiotics interact with host tissues by cell surface macromolecules, including proteins (surface layer associated proteins, mucin-binding proteins, pili, and LPxTG-binding proteins) and non-protein components (lipoteichoic acid, peptidoglycan, exopolysaccharides). They affect binding to epithelial cells, mucin, and immune or dendritic cells, resulting in increased transit times and improved barrier integrity.

There are few enhance other factors that epithelial barrier function for example few probiotics enhance gut integrity through upregulating expression of mucus secretion genes which reduces pathogen binding to intestinal cells. Furthermore, downregulating inflammation is also a factor that improves intestinal barrier function (Rabetafika et al., 2023; Sanders et al., 2019).

Few probiotics improve gut health by enhancing intestinal lining integrity because of their antioxidant capabilities (Prado-Rebolledo et al., 2017).

Competitive Exclusion (CE) of Pathogens

Beneficial gut bacteria and probiotics prevent diseases by a phenomenon called "competitive exclusion" in which beneficial bacteria compete for attachment sites and nutrients and prevent colonization of potential pathogenic bacteria. This phenomenon, used by probiotics as well, is also called colonization resistance in which commensal microbiota occupy the epithelial lining to exclude resident or invading pathogens (Yang et al., 2005).

The gut microbiota plays a critical role in defense mechanisms against enteric pathogens. If the balance between beneficial microbiota is disrupted, it leads to enteric disorders. Few studies reported changes in cecal microbiota in chickens infected with *Clostridium perfringens* (Skraban et al., 2013) *Eimeria spp* (Wu et al., 2014) and *Salmonella Enteritidis* (Nordentoft et al., 2011; Videnska et al., 2014).

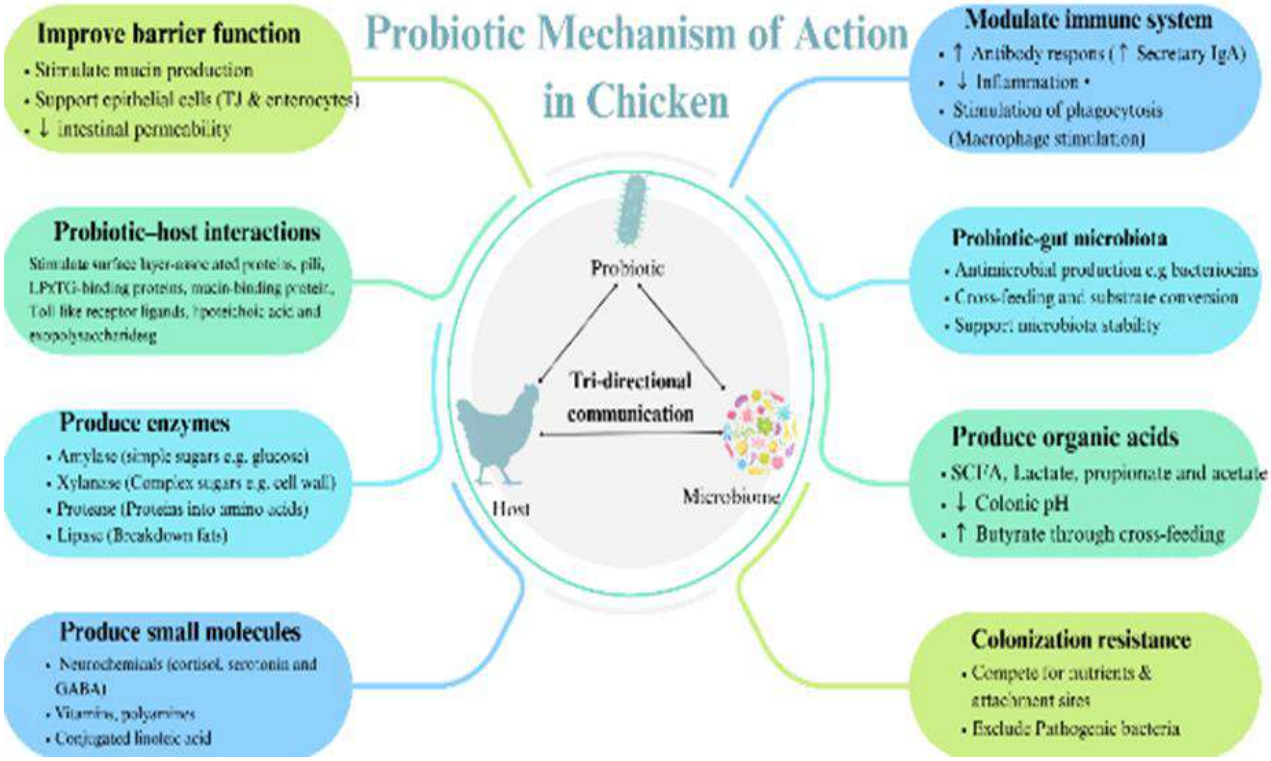

Fig. 3: Probiotic mechanisms of action to improve chicken gut health

Previously it was experimentally proved that Salmonella colonization in day-old chick gut can be avoided by providing it with a suspension of gut contents prepared from healthy adult chickens (NURMI and RANTALA, 1973). Therefore, probiotics benefit chicken health by physically blocking opportunistic pathogen colonization and alteration of environmental niches in the gut (intestinal villus and crypts).

Modulation of Immune System

Probiotics modulate the immune system depending upon the strains of bacteria in probiotics, method of preparation, routes of administration, and housing environment of birds.

i. Modulation of Humoral Immune Response

Probiotics improve innate immune response, the first line of defense, composed of

- Chemical and physical barriers (skin and mucous membranes)
- Immune cells (dendritic cells, macrophages, monocytes, neutrophils, and natural killers) and
- Immunomodulatory agent cytokines

Probiotics enhance the cytotoxicity of natural killer (NK) cells and the phagocytosis of macrophages and directly interact with dendritic cells (Rabetafika et al., 2023; Sanders et al., 2019; Shehata, 2022).

ii. Stimulation of Phagocytic Activity

Probiotics activate phagocytosis by stimulating macrophage activity to initiate pathogen destruction. Activated macrophages

- Increase the production of cytotoxic molecules e.g. nitric oxide (NO) and
- Secrete immunoregulatory cytokines e.g. tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-10, and interferon-γ (IFN-γ)

- Express receptors for a variety of cytokines such as IFN-γ, IL-4, IL-10, and TNF-α, which bind to surface structures of probiotics e.g. LPS

(Rabetafika et al., 2023; Sanders et al., 2019; Shehata, 2022)

Rabetafika et al., (2023). Also reported that Lactobacillus treatment enhanced phagocytic activity against Escherichia. coli and Salmonella spp.

iii. Cell-mediated Immune Response

Probiotics modulate specific immunity by targeting

- Lymphocytes (B and T cells)
- Antibody responses
- Immunoglobulin production and
- Cell-mediated immune response

Probiotics modulate and interact with enterocytes, dendritic cells, and regulatory T cells 12) (Molinaro et al., 2012). Probiotics enhance both natural and specific antibodies, interferon or cytokines as well as activation or suppression of T-cells i.e., by signaling dendritic cells that eventually leads lead to the cytokine expression (Mwangi et al., 2010).

Some probiotics are capable of upregulating defense mechanisms against pathogens and augmenting vaccine responses which helps in the maturation of the gut immune system (Magdalena et al., 2011).

Cell surface structures fimbriae, capsule, and LPS expressed by certain probiotics serve as mechanistic drivers for several activities that trigger and strengthen gut immunity (Sanders et al., 2019). A recent study reported enhanced growth performance and improved immunity i.e. higher antibody titers against IBD in broilers fed probiotics and prebiotics supplemented antibiotic-free diets (Rehman et al., 2020).

iv. Balancing of Pro- and Anti-Inflammatory Cytokines

Immune cells such as macrophages, T cells, B cells, and natural killers produce cytokines to regulate immune response i.e., cell activation, growth, and inflammation. The inflammatory response depends on the balance between pro-inflammatory and anti-inflammatory cytokines.

Pro-inflammatory cytokines include Interleukin-1 (IL-1), IL-2, IL-6, IL-12, IL-18, gamma interferon (IFN-γ), and tumor necrosis factor-alpha (TNF-α) while IL-10, transforming growth factor-β (TGF-β) produced by monocytes, T cells, B cells, macrophages, natural killer cells, and dendritic cells are anti-inflammatory cytokines which inhibit pro-inflammatory cytokines, chemokines, and chemokine receptors.

Several probiotic strains increase levels of pro- and anti-inflammatory cytokines i.e., TNF which prevents colitis. They balance pro- and anti-inflammatory cytokines affecting the enteric immune system (Rabetafika et al., 2023; R et al., 2005; Vanderpool et al., 2008).

v. **Enhancing Immunoglobulin A (IgA) Production**

In digestive infections, IgA produced by plasma cells, is the first-line defense as it protects against pathogen adhesion and gut mucosal penetration. Secretory IgA protects against pathogens as they trap pathogens by using mechanisms of agglutination, disruption of adhesive complex substances, and by setting adhesive proteins on bacterial surfaces. Probiotics improve host defense by increasing overall IgA levels at mucosal sites (Rabetafika et al., 2023; R et al., 2005; Sanders et al., 2019; Shehata, 2022).

Secretion of Antimicrobial Peptides (AMPs)

Probiotics show antimicrobial effects against pathogens as a front line of defense against pathogens. Many strains of probiotics antagonize other microorganisms particularly pathogens by saccharolytic metabolism, which produces organic acids (diacetyl, hydrogen peroxide, and peptides), and by the production of bacteriocins, a heterogeneous group of ribosomally synthesized peptides that directly kill or inhibit the growth of pathogens in the gut by cell wall synthesis inhibition, pore formation, enzyme activity modulation, and quorum sensing (Rabetafika et al., 2023).

Lactobacillus and *Bifidobacterium* genera metabolize carbohydrates into lactic and acetic acids as primary end products, which lower gut pH and inhibit the growth of pathogens (Higgins et al., 2010). They don't produce butyrate and other SCFAs (which affect many physiological and metabolic aspects) but indirectly increase them through cross-feeding other commensal bacteria e.g., *Faecalibacterium*. Several probiotics show antimicrobial effects by gut production of antimicrobial compounds like i.e., SCFAs and bacteriocins or colicins from metabolic reactions. SCFAs reduce cecal pH which inhibits the growth of pathogenic bacteria, enhances mineral absorption, and serves as an energy source for chickens. However, there is stronger evidence that probiotics reduce the adverse effects of antibiotics used for treatments (Rabetafika et al., 2023; Sanders et al., 2019; Shehata, 2022).

Production of enzymes and small molecules

Probiotics produce microbial enzymes like β-galactosidase64 and bile salt hydrolase which help in digestion and blood lipid profiles e.g., *Streptococcus thermophiles* in yogurt, which facilitates lactose digestion in humans. Similarly, certain probiotic strains produce neurochemicals i.e., oxytocin, gamma-aminobutyric acid, serotonin, tryptamine,

noradrenaline, dopamine, and acetylcholine affecting the nervous system (Sanders et al., 2019). However, there is a lack of research data regarding the production of enzymes and other molecules by probiotics in poultry.

Role of Probiotics Supplementation in Growth Performance, Immune Modulation and Pathogen Reduction

Commonly used probiotics in animals include

- *Lactobacillus (L. bulgaricus, L. plantarum, L. acidophilus, L. helveticus, L. lactis, L. salivarius, L. casei, Bacillus subtilis),*
- *Bifidobacterium spp (B. bifidum, B. animalis* and *B.lactis)*
- *Enterococcus (E. faecalis, E. faecium),*
- *Streptococcus (Streptococcus thermophiles),*
- *yeast (Aspergillus oryzae, Saccharomyces cerevisiae)*
- *Lactococcus, E. coli* and fungi (Q. Huang et al., 2015; Yang et al., 2005).

Lactic acid bacteria **(**LAB) and *Bifidobacterium spp* have been used in humans extensively well while *Bacillus, Enterococcus*, and *Saccharomyces* yeast have been commonly used in livestock including poultry (F e r r e i r a C.L., 2011).

However, mixed cultures including multiple strains show better results as compared to single strains because they target multiple sites and act synergistically due to different modes of action adapted by different strains (Klose, 2006; Timmerman et al., 2004).

Table 1: Application of antimicrobial probiotics in poultry

Poultry	Probiotic	Route of Administration	Health benefits	Reference
Broilers	*L. casei, L. acidophilus,* and *Bifidobacterium*	Supplementing 1% of probiotics in water	Increasing growth performance, carcass traits, immune function, gut microbial population, and antioxidant capacity	(Zhang et al., 2021)
Laying hens	*Bifidobacterium spp*. and *L. casei*	Feeding	Improving the growth performance, increase of egg weight, and feed efficiency	(Lokapirnasari et al., 2019)
Newly hatched chicks	*L. plantarum* LTC-113	Oral vaccination	Protection from *Salmonella* colonization by regulating the expression of tight junction genes and inflammatory mediators	(L. Wang et al., 2018)
Chickens	*L. paracasei ssp. paracasei* and *L. rhamnosus*	Feeding	Improving growth performance	(Fesseha et al., 2021)
Broiler	*L. johnsonii* BS15	Feeding	Preventing subclinical necrotic enteritis	(H. Wang et al., 2018)
Chicken	*Bacillus licheniformis*	Feeding	Alleviating intestinal damage caused by SNE challenge, modulating intestinal microflora structure and barrier function, and regulating intestinal mucosal immune responses	(Kan et al., 2021)
Chicken	*C. butyricum*	Feeding	Promoting anti-inflammatory expression and tight junction protein genes Inhibiting pro-inflammatory genes in *C. perfringens*-challenged chickens	(T. Huang et al., 2019)

Lactic Acid Bacteria (LAB)

LAB is a group of fermentative bacteria that are associated with the production of lactic acid from carbohydrates, making them useful for food fermentation. LAB culture probiotics show higher resistance to *Salmonella spp*. infections in chickens and turkeys in the laboratory and field (Higgins et al., 2010; Prado-Rebolledo et al., 2017; Tellez et al., 2021). LAB probiotics reduce mortality due to necrotic enteritis (Hofacre et al., 2003) and reduced gut lesions score due to *Eimeria* and *Salmonella* infection in broiler (Vicente-Salvador et al., 2008).They also produce natural antibodies like intestinal IgA, serum IgG, and IgM (R et al., 2005).

Lactobacillus acidophilus secretes lactic acid that lowers the pH of the intestinal content and helps to inhibit the development of invasive pathogens e.g., *Salmonella spp*. and strains of *Escherichia coli*. Additionally increases antibody responses and seroconversion rates. It also lowers serum cholesterol levels.

Lactobacillus spp helped in the development of normal microflora of chicken, lowered the incidence of *Salmonella spp* in the gut, and reduced idiopathic diarrhea in commercial turkey brooding houses. Another study reported improvement in innate and adaptive immune response against *Eimeria* infection and reduced fecal oocyst shedding as well (Dalloul et al., 2005; Higgins et al., 2010).

Along with antimicrobial compounds, some strains of LAB directly reduce the invasiveness of *Shigella* and *Yersinia* by inhibiting their virulence factor expression (Carey et al., 2008; Lavermicocca et al., 2008).

***Bacillus* Culture Probiotics**

Bacillus supplementation in feed improved gut morphology and gut immunity against *Eimeria* disease. *Bacillus subtilis*

culture supplemented in *Clostridium* and *Eimeria* challenged broiler reduced FCR and intestinal lesions (Jayaraman et al., 2013) and also improved growth performance and enhanced intestinal barrier integrity. Improved carcass and meat quality was noted in broilers when supplemented with *Bacillus subtilis*, *Bacillus licheniformis*, and *Saccharomyces cerevisiae* (Pelicano et al., 2003). *Bacillus licheniformis* supplemented alone also enhanced growth and meat quality (Liu et al., 2012). *Bacillus subtilis* spore was found effective in reducing *Salmonella spp* (Shivaramaiah et al., 2011; Wolfenden et al., 2010).

Direct-fed *Bacillus subtilis* showed a variety of nutritional benefits and prevention of gastrointestinal disorders in commercial poultry along with humans (Wolfenden et al., 2010) and approximately 90 % of spores germinate within 60 min of intake along with feed in different parts of the gut. Inclusion of Bacillus-DFM candidates in different poultry diets i.e. rye, wheat, barley, and oat based-diets resulted in the reduction of *Clostridium perfringens* proliferation and digesta viscosity (Latorre et al., 2015).

Yeast and Other Probiotics

Chicken gut microbiota performs various metabolic functions to maintain host health e.g. *Lactobacillus, Ruminococcus,* and *Clostridium* are reported to enhance performance in chickens. Stimulation of the enteric T cell immune system was observed when fed with *Lactobacillus fermentum* and *Saccharomyces cerevisiae* (Bai et al., 2013). *Saccharomyces cerevisiae* prevents diarrhea and the development of colitis and enterocolitis of pathogenic origin. Yeast culture, *S.cerevisiae*, and supplementation enhanced intestinal mucosal morphology, immune function, and growth performance and reduced the risk and duration of antibiotic-associated diarrhea (Gao et al., 2008). Reduced number of *Campylobacter jejuni* was observed by supplementation of mixed culture i.e. *Klebsiella pneumoniae, Citrobacter diversus*, and *E. coli* (Stern et al., 2001).

Conclusion

In conclusion, the excessive use of antibiotics in poultry farming poses a significant threat to public health due to the emergence of antibiotic resistance and the accumulation of antibiotic residues in poultry products. Alternatives such as probiotics offer promising solutions by improving growth performance, enhancing gut health, and modulating the immune system without the risks associated with antibiotic use.

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Chapter 22

Prebiotics: Enhancing Poultry Health and Combating Antimicrobial Resistance (AMR)

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ABSTRACT

Prebiotics, first conceptualized in 1995, represent a pivotal strategy in dietary modulation to boost beneficial gut microbiota and improve host health. They are substrates selectively utilized by host microorganisms, prebiotics influence gut microbiota composition and metabolic activity, improving chicken growth performance. Prebiotics reach the lower gut, surpassing the upper gastrointestinal tract (GIT), stimulating the growth of beneficial bacteria in the intestine influencing microbiota composition, and modulating the immune system. Fermentation of prebiotics produces metabolites such as short-chain fatty acids (SCFAs), which play crucial roles in enhancing intestinal health, nutrient absorption, and metabolic regulation. Various types of prebiotics, including fructooligosaccharide (FOS), inulin, mannan oligosaccharides (MOS), and others, have demonstrated promising effects on gut health and performance in poultry. These effects include increased populations of beneficial bacteria, improved intestinal morphology, enhanced immune responses, and reduced pathogen colonization. Additionally, prebiotics such as chitosan and yeast cell walls exhibit antimicrobial and antioxidant properties, contributing to overall gut health. This article underscores the significance of prebiotics in avian nutrition and health, and their role in enhancement of production performance in poultry emphasizing further research to optimize their utilization for maximum benefits.

KEYWORDS
Prebiotics, Gut microbiota, Host health, SCFAs, Poultry nutrition, Immune modulation

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INTRODUCTION

The prebiotic concept is a relatively new concept introduced by (Gibson and Roberfroid, 1995) in their publication discussing dietary modulation of the human microbiota. Afterward, researchers focused on the identification of substances that promote beneficial bacteria in the gut which result in better health of the host, for example, bifidobacteria and lactobacilli.

In 2016, a panel of experts in microbiology, nutrition, and clinical research convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) developed the current scientific definition of prebiotics "substrate that is selectively utilized by host microorganisms, resulting in health benefit in host". These selectively fermented ingredients influence the composition of gut microbiota, and their metabolic activities result in particular changes in the composition and metabolic activity of gut microbiota and affect the health of host (Gibson et al., 2017).

Prebiotics mainly use a fermentation process by which a microorganism transforms dietary ingredients into by-products i.e. glucose, lactic acid, ethanol, and other metabolic end products.

Prebiotics characteristically must be utilized by gut microbiota selectively, reach lower GIT undigested, resist acidic pH, stimulate the growth of beneficial bacteria, and modulate the host defense system (Kim et al., 2011; Patterson and Burkholder, 2003).

Prebiotics are dietary supplements that indirectly affect gut health by increasing beneficial microbes, production of metabolites, and increase absorption of nutrients, regulation of metabolism, and enhancement of intestinal epithelial cell health. Gut microbiota regulates nutrient digestion and absorption, metabolism, pathogen protection, and gut immunity. Gut microbiota determines host health depending upon the dynamic and mutually beneficial relationship between the host and colonic microbiota. Intestinal dysbiosis or leaky gut results in indigestion of dietary nutrients leading to various diseases or health issues. Therefore, regulation of intestinal microbiota by prebiotics may avoid diseases, enhance nutrient absorption, and lead to enhanced growth and production in birds (Shehata, 2022).

Commonly prebiotics are confused with the term dietary fibers, however, it's important to note that only a subset of dietary fibers qualify as prebiotics, and they may originate from non-fiber substances like polyphenols (Guarner, 2012). Currently, there is no dietary recommendation for daily allowance or adequate intake of prebiotics. Most prebiotics require a daily oral dose to get health benefits around 5 grams is the target for FOS and GOS in the daily diet. However, the recommended daily amount of fiber is 28 g/day, based on a 2000 kcal/day diet (Gibson et al., 2017).

Role of Gut Microbiota

Gut microbiota is a complex ecosystem, performing multiple functions simultaneously e.g. commensal bacteria in the gut convert dietary carbohydrates, proteins, and fats into metabolites that can be absorbed easily. These metabolites may affect host health positively or negatively (Verbeke et al., 2015). Prebiotics alter the composition of intestinal microbiota by enhancing the number of bacteria utilizing them and may impact host gut metabolism. Healthy gut microbiota may increase absorption, protein metabolism, energy metabolism, fiber digestion, and gut maturation.

Generally, prebiotics are carbohydrate-based but polyphenols and polyunsaturated fatty acids may exert a prebiotic effect on gut health, substantially increasing bifidobacteria, lactobacilli, and butyrate production (Fogliano et al., 2011).

Prebiotics Mechanism of Action

Prebiotics affect chicken health by using these mechanisms

- Increase beneficial bacteria in the gut
- Reduce pathogenic bacteria in the gut
- Improve intestinal barrier health
- Modulation of the immune system
- Improve defense system
- Modulation of metabolic function
- Increased mineral absorption
- Improved bowel function.
- Enhance production performance

Gut microbiota possesses diverse metabolic capabilities and there is delicate metabolic interplay and balanced substrate usages and competition among microbiota (Weiss et al., 2021).

Increase Beneficial Bacteria

Prebiotics proliferate lactic acid bacteria (LAB) and Bifidobacteria in the gut which improves gut health. There are many other bacteria, other than *Lactobacillus* and *Bifidobacteria*, which may utilize prebiotics selectively and may enhance gut health. *Lactobacillus* and *Bifidobacteria*, efficiently metabolize low-molecular-weight carbohydrates as they possess enzymes like glycosidase (cell-associated and extracellular) and specific transport systems (Audrey et al., 2018; Gwen et al., 2009). However, a lot of research is required to explore more options for prebiotics targeting other groups of bacteria in the gut. Such as the Bacteroides genus, breakdown high molecular weight sugars i.e. polysaccharides (Flint et al., 2012) while some bacteria break down particular substrates e.g. *Ruminococcus spp*. Facilitate degradation of resistant starch (Ze et al., 2012).

Increase Healthy Bacteria

Prebiotics pass the upper GIT and reach the lower intestinal tract for the fermentation process, here they increase bacterial growth and enhance the functionality of specific genera or species by selective utilization. Prebiotics in broiler feed have been demonstrated to boost lactobacilli levels. Some investigations on the microbial effects of prebiotic supplementation found increased *Bifidobacteria spp* and decreased *Clostridia spp* (van den Broek et al., 2008). Another study reported reduced *Salmonella* and coliforms population significantly in chicken gut microbiota (Dhama et al., 2008; Janssens et al., 2004) For example, reduction in *Salmonella* colonization was observed by the synergistic effect of beta-glucan, MOS, chitosan, and FOS found in *Aspergillus oryzae* mycelium (Yalçin et al., 2014).

Improve Intestinal Health

Prebiotics increased intestinal villus height in broiler diets, according to intestinal morphology. Detoxification and elimination processes are enhanced by a healthy population of these helpful bacteria in the digestive tract (Teitelbaum and Walker, 2002). SCFAs also improve barrier function and intestinal in the gut. Intestinal dysbiosis allows inflammatory

mediators such as bacterial lipopolysaccharide (LPS) to enter systemic circulation called "metabolic endotoxemia" which activates the immune system and strengthens intestinal barrier functioning (Shehata, 2022).

Immune Modulation

Prebiotic regulate the immune system by increasing bacterial population and cell wall components of the bacteria which stimulate immune responses For example, prebiotics may directly interact with gut immune cells or indirectly interact with immune cells via preferred colonization of beneficial bacteria and microbial metabolites (Collins and Gibson, 1999; Pandey et al., 2015). For example, prebiotic beta-glucan is a unique substance which increases intestinal villi and it is also a potent immunity booster and helps the body fight viral and bacterial invaders (Hooge et al., 2003; Jonker et al., 2010).

Although the exact mechanism of action is not yet clearly understood however, there is evidence that prebiotic supplementation reduces type 2 T helper responses and hence affects allergic responses in infants reducing in incidence of atopic dermatitis, wheezing, and urticaria to less than 50% (Sanders et al., 2019).

Improve Defense System

Prebiotics strengthen host defense and reduce pathogen-induced mortality in birds (Ducatelle et al., 2015). They use the following mechanisms

- Production of organic acids by gut microbiota or probiotics utilizing prebiotics reduces gut pH, inhibiting the growth of potential pathogens in chicken gut.
- Competitive exclusion of pathogens due to the high number of beneficial bacteria that colonize the gut and compete for nutrients and attachment sites (Sanders et al., 2019).
- An increase in the population of *Lactobacillus* spp in the gastrointestinal tract of birds by prebiotics may aid in the competitive exclusion of pathogens.
- Prebiotics also boost the immunological response in chickens, resulting in faster pathogen or disease clearance (Shehata, 2022).
- Gut Bacteria utilizing prebiotics can influence the microbiota composition through the production of antimicrobial agents (for example, peptides) and competitive interactions, possibly reducing infections and
- Bacteria containing LPS cell wall, stimulate immune responses.

Metabolic Function Modulation

Prebiotics reach lower GIT undigested where they are metabolized selectively by the gut microbiota through the fermentation process i.e. the pathway of conversion from a polysaccharide to a SCFA, a complex network of metabolism.

Gut microbes utilize metabolites produced by each other and exist in a mutually beneficial relationship leading to higher microbial population and host health. For example, acetate and lactate, the main metabolic end products of bifidobacteria and lactic acid bacteria are utilized by other microorganisms to produce propionate and butyrate this phenomenon is called "cross-feeding" (Rivière et al., 2016).

Metabolic products influence epithelial integrity, and hormonal regulation, lower pH, reduce pathogenic bacteria, and enhance mineral absorption. They also improve chicken health and production performance by affecting glucose homeostasis, inflammatory response, and blood lipid profile of chickens positively (Adhikari, 2017).

Increased Mineral Absorption

Prebiotic fermentation produces SCFA which reduce intestinal pH, the main site for mineral absorption. Low intestinal pH increases calcium solubility resulting in higher passive uptake through epithelial lining and calcium mineralized into bones which improves production performance in birds (Díaz et al., 2015). They improve eggshell and bone quality, improve mineral utilization, and improve performance in egg-laying hens (Yalçin et al., 2014).

Improved Bowel Function

Due to increased microbial growth fecal bulking and improved bowel functioning take place. Animal studies have shown that SCFAs regulate gut hormones that in turn modulate the local motor responses of GIT (Kanauchi et al., 2013). Prebiotics due to their water-binding capacity, soften feces and make passage easier avoiding gut damage (Lamsal, 2012)

Role of SCFAs

Fermentation is a complex process in the lower gut of chickens where intestinal microbiota breakdown complex carbohydrates (i.e. polysaccharides, oligosaccharides, and disaccharides to simple sugars), proteins, and fatty acids into simpler units which are easily absorbed by the epithelial lining of chicken gut. This process releases short-chain fatty acids, gases, and other metabolites which aid in digestion and intestinal health. Fermentation also creates an acidic environment that inhibits the growth of harmful bacteria while promoting the growth of beneficial colonized microbiota. It plays a crucial role in maintaining the gut health and digestive efficiency of chickens.

Fermentation of complex carbohydrates is the principal process in humans and chickens which produces the end product i.e. SCFA from simple sugars. They change gut physiology. SCFA concentration increases sharply in the ceca of day-old chicks to the greatest concentration at day fifteen (Yang et al., 2011). SCFA's

- Have central role of SCFAs in the metabolism of gut bacteria (Weiss et al., 2021).

- Act as major luminal anions, as they increase the pH of the gut decrease to a value of approximately 4.8.
- Provide50% of the daily energy requirements to intestinal cells (Dimitrov et al., 2016).
- Alter the composition of colonized microbiota drastically.
- Play multiple roles in host health, metabolism, and microbial physiology (Tellez et al., 2006).

Weiss et al., (2021) observed in a study that closely related bacteria produce and consume similar SCFAs e.g., both Bacteroides strains produced acetic acid, succinic acid, and branched-chain SCFAs whereas *Clostridium innocuum* formed butyric acid, valeric acid, and caproic acid.

SCFAs and Muscular Activity

SCFAs such as acetate, propionate, or butyrate increase intestinal blood flow without prostaglandins or adrenoreceptors (Meißner geb. Plöger et al., 2012) by influencing local neuronal networks, chemoreceptors, and direct impacts on smooth muscle cells. (van der Wielen et al., 2000). Colon SCFAs changes upper GIT musculature by entering blood stream therefore the whole GIT depends on the fermentation process other than the colon. An increase in blood flow improves tissue oxygenation and nutrient delivery (Volpe and Braniste, 2014).

SCFAs and Enterocyte Proliferation

SCFA enhances the growth of colorectal and ileal mucosal cells while primary SCFAs (particularly butyrate) showed a reduction in the risk of colon cancer (Shen et al., 2013). As SCFA's reduce pH, colonocytes (cell lining of colon) are unable to take up insoluble bile acids at pH 6 therefore, Inhibition of bacterial conversion of primary to secondary bile acids reduces their carcinogenic potential (Hofmann, 1999).

SCFAs and Mucin Production

SCFA production by gut microbiota enhances mucus formation and its secretion locally, probiotics also increase mucin synthesis (Meißner geb. Plöger et al., 2012) which limits the ability of pathogenic organisms to attach and intestinal epithelial cells. (Montagne et al., 2004).

Few studies showed that gut microbiota and probiotics boost mucin production, which resulted in decreased rotavirus replication, symptoms, and shedding. Another study reported a change in crypt depth and the number of mucus-producing intestinal cells by increasing the concentration of butyrate (Immerseel et al., 2002; Schippa and Conte, 2014).

Types of Prebiotics

Prebiotics may be natural or synthetic, focusing on isolated substances for strict control of substance and dosage as compared to whole plant foods. Most prebiotics are dietary fibers, but not all dietary fibers are prebiotics. Fibers are non-digestible plant-derived carbohydrates comprising at least 3 units of individual sugars. Most fibers are components of plants. Depending on regulations where you live, if fiber is isolated from whole plants or synthesized from sugars a demonstration of physiological benefits is needed to be able to call them ' fiber'. Among the most commonly studied prebiotics are soluble fibers Fructooligosaccharides (FOS) and inulins, Galactooligosaccharides (GOS), and Oligofructose (OF). However, most used prebiotics in poultry include FOS, Inulin, MOS, GOS, Soya-oligosaccharides (SOS), Xylo-oligosaccharides (XOS), Pyrodextrins, Isomalto-oligosaccharides (IMO), and lactulose.

FOS and Inulins

FOS, natural linear polymers, up to 10 monomeric, of β-(2-1)-linked fructosyl units, terminated by one glucose residue) while inulins are longer chain versions of FOS. They remain undigested in the upper GIT and reach the colon to allow fermentation by gut microbiota in chickens (Roberfroid et al., 2010) sourced from plants like onion, chicory, garlic, asparagus, banana, and artichoke. (Flickinger et al., 2003; Ricke, 2015)

The study reported that FOS increases the number of beneficial bacteria e.g. *Lactobacillus spp* whereas restricts the growth of *C. perfringens* and *E. coli* in broilers (Kim et al., 2011). FOS inulin also influences the ability of the chicken macrophage-like HD11 cell line to phagocytose and kill *S. Enteritidis*.

However, fewer studies suggest that FOS can be metabolized by pathogenic *E. coli* strains via a gene cluster called fos locus in the genome of avian extraintestinal *E. coli* (ExPEC) that encodes proteins involved in FOS metabolism (Pourabedin and Zhao, 2015a). FOS have been demonstrated to improve intestinal calcium and magnesium absorption, in addition to bone mineral concentrations (Scholz-Ahrens et al., 2007).

MOS

MOS, mannose-based oligomers linked by β-1,4 glycosidic bonds, are found naturally in some plants i.e., beans or cell walls of yeast *Saccharomyces cerevisiae*. MOS reaches the lower gut because birds don't have enzymes to break down the mannan backbone but *Bacteroides, Bacillus,* and *Clostridium* cleave β-1,4 mannopyranoside in mannan by-products of mannanases enzymes. (Dhawan and Kaur 2007). MOS modify microbiota composition in the gut e.g., they enhance *Lactobacillus spp* and *Bifidobacteria spp* reduce *C. perfringens* and *E. coli* (Corrigan et al., 2011; Kim et al., 2011) promote *Lactobacillus spp* and *Bifidobacteria spp* in cecal microbiota.

MOS enhances disease resistance by increasing the number and oxidative burst activity of heterophils. (Huff et al., 2010) Carbohydrates in mannose bind with lectins in pathogens, avoiding attachment with the intestinal mucosa and pass through the gut without colonization, propagate an inflammatory response by initiating a cascade of cytokine expression,

increase phagocytic and Salmonella-killing activities of chicken macrophage cell line (MQ-NCSU) resulting in increased production of hydrogen peroxide and nitric oxide, upregulate genes involved in innate immunity (Ibuki et al., 2010) and maintain intestinal epithelial integrity. MOS binds toxin-active sites and defends the GIT against invasion (Shehata, 2022).

XOS and other Oligosaccharides

XOS, (oligomers consisting of xylose units linked through β-(1-4) linkages) (Aachary and Prapulla, 2008), produced by partial hydrolytic degradation of arabinoxylans, lignocellulosic materials, found in cereal grains. XOS reaches the lower gut because the glycoside link between xylose monomers cannot be degraded by birds.

Additionally, GOS reported higher *Bifidobacterium spp* when fed in birds (Jung et al., 2008), SMO reported increased *Lactobacillus spp* and also changed volatile fatty acid concentrations after in vitro fermentation by cecal microbiota of broilers (Lan et al., 2007), lactose (Hajati and Rezaei, 2010) and resistant starch have been considered potential prebiotics. Other compounds, such as *Saccharomyces cerevisiae* fermentation products or yeast culture, have shown prebiotic-like effects as well (Pourabedin and Zhao, 2015b). However, more studies are required to confirm the prebiotic properties of GOS and SMO in chickens.

Aspergillus Meal (AM)

Aspergillus meal (AM), a frequently used prebiotic, concerns *Aspergillus oryze*, and includes 16% protein and 44% fiber. AM is reported to enhance production performance in commercial poultry diets with low protein levels (HARMS and MILES, 1988; Torres-Rodriguez et al., 2005). Also, increase weight and FCR (Amirdahri et al., 2012). It also indicated greater digestion and absorption of those nutrients (Reginatto et al., 2011).

AM also showed an increase in

- Number of acid mucin cells
- Neutral mucin cells
- Sulphomucin cells in the duodenum and ileum
- Increase villi height and surface area in the duodenum and ileum of chicks (Tellez et al., 2010).

Londero et al., (2011) studied reduced overall colonization levels of horizontal *Salmonella enterica* serovar Enteritidis transmission and *Salmonella enterica* serovar *Typhimurium* transmission in broiler chickens.

Chitosan

Chitosan is a natural biopolymer created by deacetylating chitin which is the major component of fungal cell walls and arthropod exoskeletons. Chitosan, non-digestible carbohydrates, easily fermented by gut flora found in mycelium or *A. oryzae* also contains beta-glucans, FOS, chitosan, and MOS (Hernandez-Patlan et al., 2018; Uchima et al., 2010). It promotes growth, most likely by enhancing feed ingredient absorption and digestibility (Uchima et al., 2010). It also has antimicrobial and antioxidant properties. (Filipkowska et al., 2014) Moreover, many studies have used chitosan as a mucosal adjuvant, increasing IgA levels (Ravi Kumar, 2000).

Yeast Cell

The yeast cell wall is an effective prebiotic feed additive in broilers as it increases growth performance, humoral immune response, and the reduction in abdominal fat. However, the production of low-cholesterol eggs and improvement in humoral immunity response in laying hens are also reported by (Yalçin et al., 2014).

Role of prebiotics supplementation on growth performance, immune modulation, and pathogen reduction

Reference	Type of prebiotics	Major outcomes
(Fernandez et al., 2002)	MOS	Reduced *Salmonella* infection
(Baurhoo et al., 2007)	MOS and lignin	Increased *Lactobacillus* and *Bifidobacteria*, decreased *E. coli*, low intestinal pH, increased villi height
(Baurhoo et al., 2009)	MOS	Increased intestinal microbes' community and development of intestinal morphology
(Xu et al., 2003)	FOS	Improved body weight gain, feed conversion, and carcass weight increased *Lactobacillus* bacteria.
(Sims et al., 2004)	MOS	Improved body weight gain
(Macfarlane et al., 2008)	GOS	Increased growth of *Lactobacillus* and *Bifidobacteria*, and/ or their fermentation products
(Zhao et al., 2013)	Fructan, FOS	Increased cecal *Lactobacillus* and *Bifidobacteria*, decreased *Escherichia. coli* and *Clostridium. perfringens*
(Janardhana et al., 2009)	FOS, MOS	Increased immunity in GALT, increased IgG, and IgM
(Huang et al., 2015)	Inulin	Increased mucin mRNA expression of jejunum, increased cecum IgA level, increased intestinal immune function at d 21 but did not affect at d 42
(G.-B. Kim et al., 2011)	FOS and MOS	Increased *Lactobacillus* and *Bifidobacteria*
(Geier et al., 2009)	FOS, MOS and inulin	Increased *Lactobacillus* and *Bifidobacteria*

(Hanning et al., 2012)	FOS	Improved villi height and crypt depth
(Cao et al., 2005)	FOS + tea polyphenols	Reduced mortality in 28–42 d old broilers, FOS selectively promoted favorable microbes and inhibited microflora metabolites except for volatile fatty acids in the cecum

Conclusion

Prebiotics play a significant role in enhancing poultry health and production performance by modulating gut microbiota, boosting beneficial bacteria, and improving nutrient absorption and immune response. Their fermentation produces short-chain fatty acids, which contribute to better intestinal health and metabolic regulation. Continued research on optimizing prebiotic use in poultry diets could lead to improved efficiency and overall poultry welfare.

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Chapter 23

Reiki in Companion Animals

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ABSTRACT

The word "Reiki" is derived from two Japanese words "Rei" and "kei" meaning spiritually guided life energy. Reiki helps an individual to feel from disease, grow emotionally, spiritually and mentally. In case of animal, Reiki helps to build trust between pets and owner, promotes healing decrease psychological issues and keep an animal healthy. The major energies Reiki attunement include earth energy, heavenly energy and heart energy. Furthermore, the three degrees of chakra i.e., the heart chakra, the throat chakra and third eye chakra, allow an individual to love unconditionally, open path to consciousness and build trust, respectively. Some practitioner in Reiki train for years to understand the energy and how to navigate delicate and subtle energy which shifts within themselves and their participants, where instead of realigning your bones and muscles tension. The process of Reiki is something anyone can learn and something you can learn fairly swiftly, especially for animals. Reiki allows us to perform at a level where our positive energy flows freely. Reiki should not be an alternative to veterinarian medical care, but seen instead as an aid in the diagnosis to recovery.

KEYWORDS
Healing, Attunement, Chakra, Non-invasive Practice, Energy

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INTRODUCTION

The word “Reiki” is derived from two Japanese words “Rei” and “Kei”, meaning spiritually guided life energy, also known as Universal Life Energy. It is believed that the words “Rei” and “Kei” are originated from Japanese words “Raku-Kei”. Raku described the vertical flow of energy, whereas Kei describes the horizontal energy flow through the body. Raku-kei is the science and art of self-improvement used by ancient Tibetan Lamas dating back thousands of year (Fulton & Prasad, 2006). This energy livens us all and can be found all around us. Reiki is a form of mystical healing using "universal life energy" from the practitioner to the recipient. Reiki helps to harmonize body, mind and spirit for yourself or anyone you want to help. Hands-on healing has been around for centuries and is known in many cultures. Reiki was "rediscovered" in Japan by Dr Mikao Usui during the beginning of the 20th century. In the field of Reiki, 3 persons are prominent in the history of Reiki: Dr Mikao Usui, Dr Hayashi, and Mrs. Hawayo Takata.

Use of Reiki in the Well-being and Promoting Health

Through positive energy Reiki frees us from disease; it prevents and cures illness. Reiki helps an individual to grow emotionally, spiritually, and mentally, providing an opportunity to grow in a wider sense. The preventive and curative qualities of Reiki can be reduced to two simple principles: the cleansing of meridians and the balancing of the chakras to achieve a harmonious energy flow. Following are the Reiki Symbols used by the Reiki healers and practitioners (Honervogt, 1998).

Benefits of Reiki Healing in Animals

Reiki healing is so difficult to explain or simplify. If you are not a practitioner or have done it or witnessed it at least once, you can never truly understand Reiki's full potential. Your animals are there to support, comfort, and ease your pain, while also reducing significant amounts of stress. With Reiki, it allows you to do the same for them through the transfer of your energy, closeness, and love. The benefits that come from a single Reiki session, whether long-distance or close to home, are endless. Reiki relies entirely on the transfer of energy. However, Reiki has been shown to work on many animals as well as humans. It balances the physical, emotional, mental, and spiritual aspects of the patient by releasing any blockages. Any type of energy block can create an overload of emotional baggage, or overwhelming mental illness, and the exhausting effects of physical pain. Through Reiki perusing, you can figure out the root cause of your pet’s ailment which is making it

distressed or otherwise acting atypically (Prasad, 2019). The imperturbable thing about Reiki is that your animal gets to decide how much energy they want to take or can take from you or a Reiki healer and for exactly how long. This way, you don't have to guess or wear yourself down with the transfer.

Source: https://www.centreofexcellence.com/reiki-symbols-meanings/

Following are some proven benefits showing how the practice of Reiki works:

a. Increases bonding and trust between owner and pet
b. Promotes healing after a severe surgery or illness
c. Provides pain relief when medication needs to be taken or before having to go to the vet
d. Balances the energy of not just the practitioner, but the animal too, thus releasing energy blockages
e. Increases relaxation, much like a sedative, which reduces stress and tension in the muscles
f. Generally, reduces feelings of distress and anxiety
g. Helps with separation anxiety or change, such as a big move or plane ride
h. Strengthens the immune system
i. Provides comfort to upset animals
j. Decreases behavioral issues
k. Increases the bond shared between pet and owner beyond the initial foundational bond that comes with ownership
k. Enhances overall well-being

It's safe to say that with these known benefits in mind, it is worth giving Reiki a try on your pet, no matter which stage of sickness they are in. For example, they could be completely healthy, but you merely would like to strengthen your bond together. Reiki can help bring you closer, especially after time apart. Since Reiki is such a gentle and non-invasive practice, the animal will feel special, loved, and well cared for, which initially creates a respectful and well-mannered pet. With this close bond, a sick or dying pet may want to stick around longer than it can because of the shared energy created by the relationship resulting in more pain and emotional distress (Miles, 2008).

Due to Reiki's ability to strengthen the immune system, pets have developed a kind of cancer. They may need chemotherapy or radiation, and Reiki healing will feel more relaxed when they need to get this type of therapy. The stress of these treatments for cancer can be so debilitating already. With Reiki used as a calming technique, the animal will not feel much pain or sickness due to the effects and benefits Reiki offers.

Reiki Attunement

Reiki is considered the most powerful spiritual experience but unfortunately it is not taught as other healing techniques are tough. The ability to attune Reiki frequency is thought to be passed from Master to student. Once Reiki is transferred to students, it allow him to amplify Reiki for himself and others throughout his life. There is no need to alter the thinking process in order to 'turn on' the flow of Reiki. Simply placing your hands on yourself or others opens the heart of love, and you automatically bring through the energy by your intention (Honervogt, 1998).

a. Earth Energy

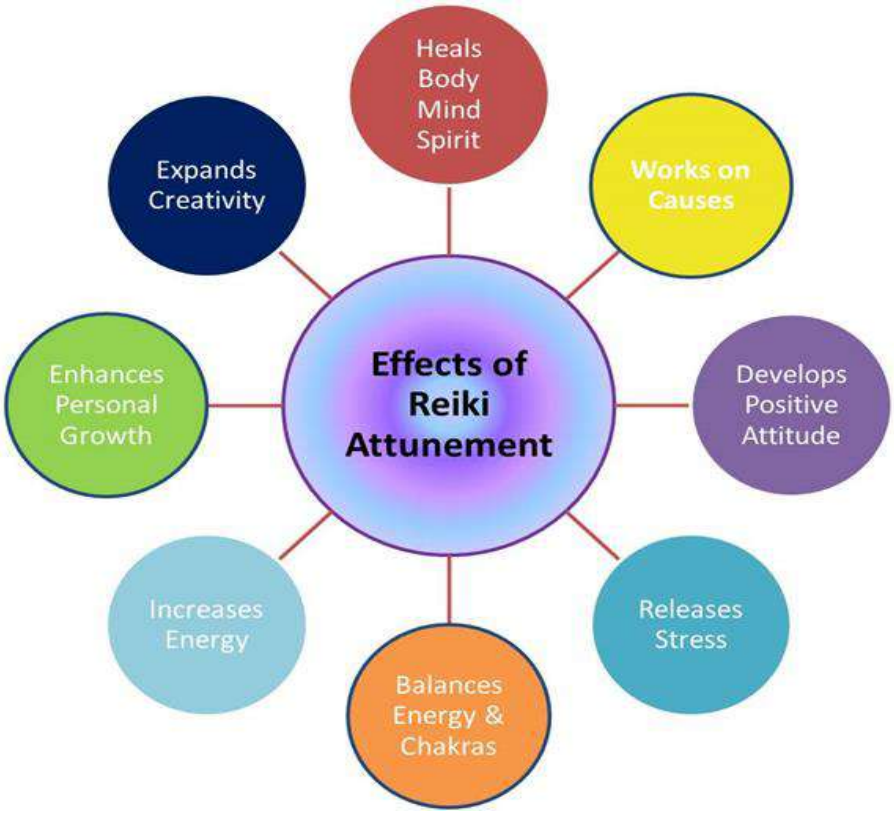
Earth energy (the hara) in Reiki healing means that we use our universe and our environments as tools for healing and grounding. Earth energy is the energetic basis of the triangle of the Reiki system, it is safe to say that this part of the system must always be balanced and healed.

b. Heavenly Energy

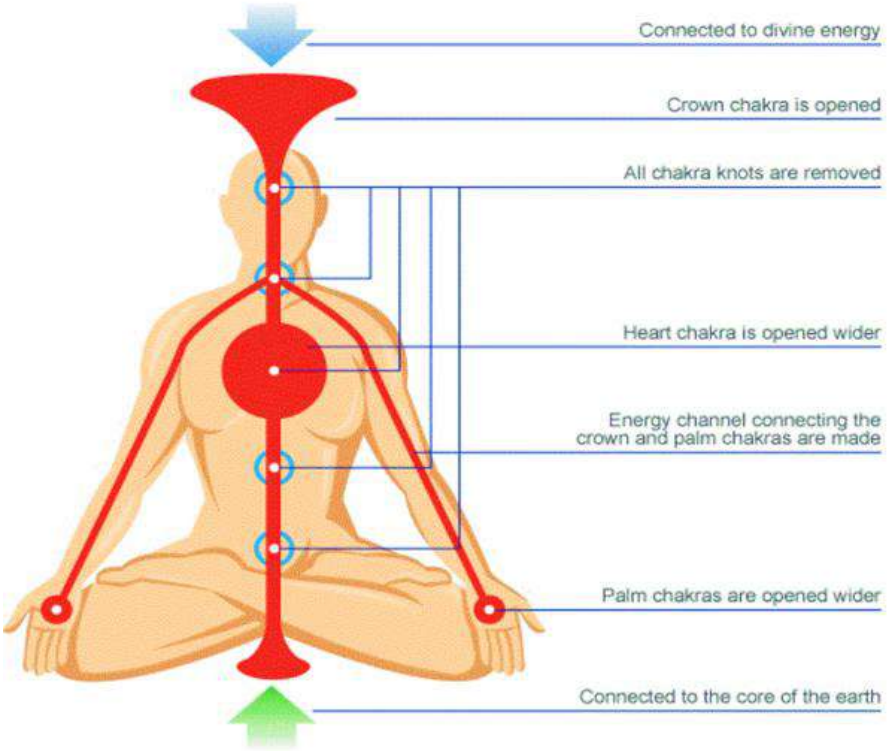
Heavenly energy or spirit energy connects you to your intuition or psychic abilities. It is through these abilities that you may see colours and auras. This type of energy is essential for you to keep balanced so that you will be able to see anything beyond just the immediate. The immediate is this physical world. Maintaining balanced heavenly energy means that you will be able to listen to your intuition.

c. Heart Energy

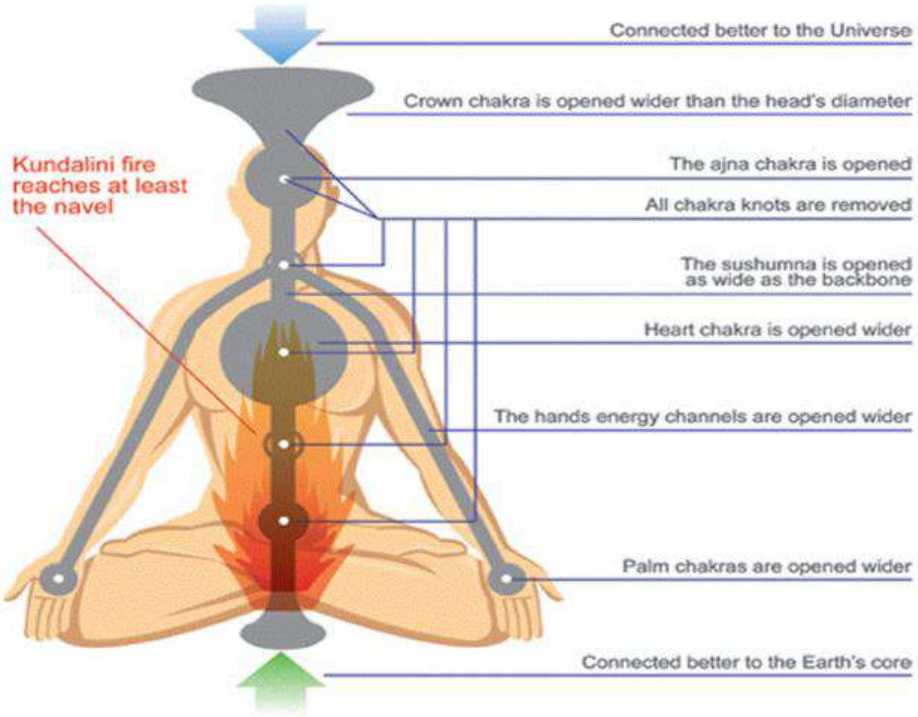
The emotions you experience, both empathetically and personally, are what connect you to your heart's energy. In this center, you learn your life's purpose and what you were created or put here to do and become. In other words, the heart energy is everything that you have experienced from childhood to adulthood and back again.



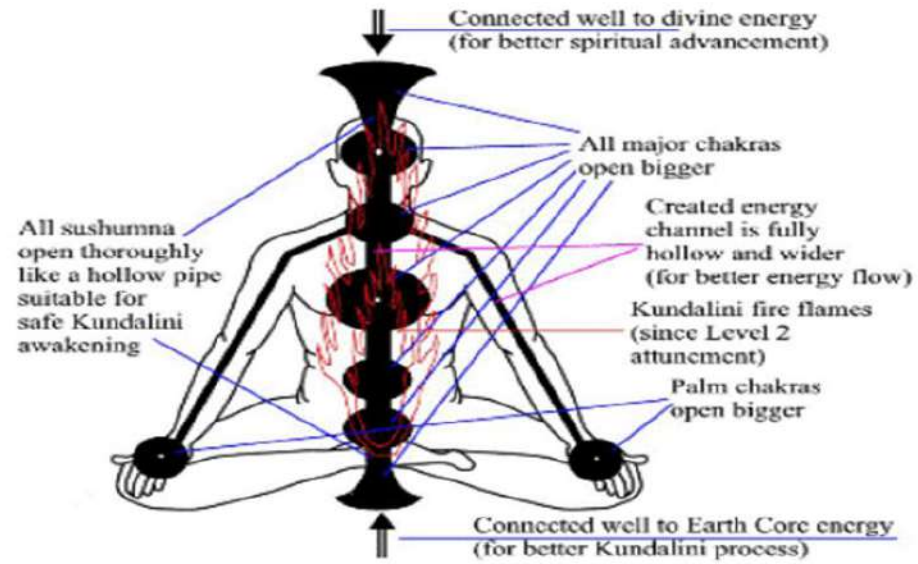
Source: https://enlightenedconcepts.com/reiki-attunements/



Reiki Attunement - Level 1
Source: https://www.willowmoondsm.com/reiki-attunements



Reiki Attunement - Level 2
Source: https://www.willowmoondsm.com/reiki-attunements



Reiki Master Attunement - Level 3
Source: https://www.willowmoondsm.com/reiki-attunements

Degrees and Initiations

Usually, Reiki has 3 degrees. In the first degree, four of the energy centers are reawakened and attenuated. Energy centers are spiritual centers of an individual and the heart act as a bridge between physical and spiritual.

a) **The heart chakras:** Activation of this chakra allows to love unconditionally.

b) **The throat Chakra:** Once activated this chakra opens the path to your higher consciousness and helps an individual to build or established trust.

c) **The third eye chakra:** It is responsible for opening of your intuition.

d) **The Crown Chakra:** Once activated, the crown chakra allows an individual to receive unconditional vibration of the Reiki energy.

Center Finger Method

The founder of Reiki, Mikao Usui, taught this method to improve concentration during all Reiki meditations or healing practices.

a. **Before You Start.** Sit, lie down, or stand anywhere you would like. The place you go to do this concentration method is up to where you feel most comfortable, but it is pre-retired that it happens in an area with no other distractions. When you are ready, place your hands together with each part touching the opposing hand's parts (fingers to fingers, palms to palms).

b. **Preparation.** Close your eyes and find where your energy is placed or resting within your body. As you get better at Reiki healing, your energy should always spread throughout your body. Bring your focus to this energy and then imagine the energy coming all at once to your middle fingers. Keep this energy focused on the middle fingers during the whole process.

Giving Reiki Treatments

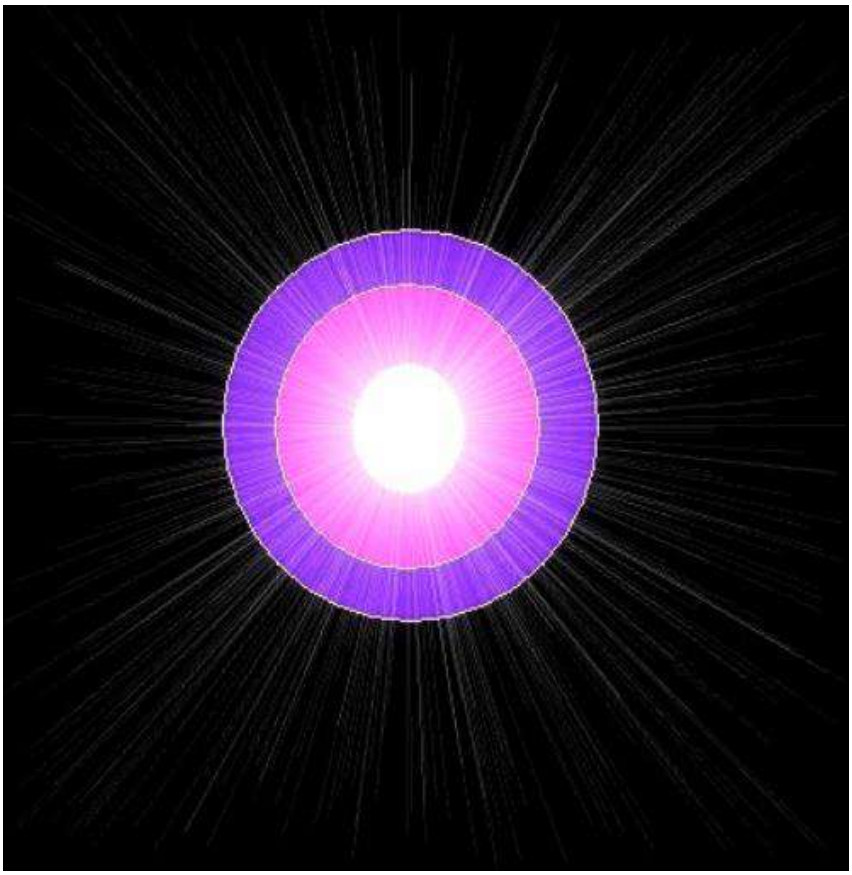
Reiki practitioner's place their hands on the animal and they all are agreed that healing energy flow by itself by doing this. After attunement, as much as Reiki as possible be given in the initial weeks. This process continues and refinement occurs with the sessions. As Reiki is an automatic process, therefore, healing energy will flow to the necessary. The attempts to force the process results in lessening the available power. The reason explained for why treatment does not drain the Reiki practitioners because the giver is a channel for energy to be given and not the source of healing. The Reiki treatment for one session lasts for about an hour during which the energy is communicated to the higher self of the recipient (Miles, 2008). The practitioner should ideally attempt to allow the flow of energy for about 3-5 minutes in each of the hand positions. The best results are achieved when the practitioner is calm and relaxed, becoming one with the energy.

Animal Reiki and the Practitioner

The term Animal Reiki protection describes the protection of animals, pets and endanger3ed species. Animal Reiki protection helps an individual to build a connection with universal energies of the creative source for the healing and protection of animals. This attunement strengthens the abilities of animal to heal faster, allowing an individual's abilities to provide powerful protection to any animal to keep it safe and healthy. The symbol given in the Reiki attunement help people to connect with these energies quickly, so that they can be used easily and, in a hurry, when required.

a. Communicating With Your Pet

Everything is made up of energy. If you don't believe me, think about the invisible sensations or vibrations you get when your gut tells you to run. Upon meeting someone, you might know instantly whether they are easy to talk to or if you want to be closed off to them. People who struggle with mental illnesses such as anxiety, borderline personality disorder, PTSD, etc., might experience energy blockages that create mental illness. For example, when you think negatively, you will view the world as negative. When you think positively, the world and the Universe will reward you. Maybe you and your friend were talking about taking a road trip, and then later by yourself, you thought perhaps it's not the right time right now. Every animal-human relationship is different and unique in its way. So, is it possible to be able to understand and communicate with your pets? The answer is "Yes" the medium of telepathy and the pheromones which we shed while interacting with or pets are the main signals which animals pick.



Animal Protection Reiki Symbol
Source: Channeled and copyrighted by Linda Colibert Sept. 2010- (www.spiritlightreiki.com)

Pet or animal practitioner play a crucial role while using Reiki to heal an animal. The practitioner sets an intention to facilitate the healing process of the animal and also helps in the energy flow. Reiki creates a deep sense of healing and peace, providing an ideal condition for the self-healing of the animal under treatment. Shortly, Reiki practitioners don't play a role in direct healing of the animal, instead simply improve the self-healing abilities of the animal.

a. For healthy animal, Reiki maintains their general health, increases relaxation, and emotional sense of peace and contentment.

b. In ill animal, Reiki is considered a wonderful healing option as well as an alternate to Western Medicine, Chinse Medicine, and different other forms of healing in animal.

c. Reiki is powerful but gentle option to provide comfort and relief from pain, fear, and anxiety, making the dying process easier. Reiki is highly effective in companion animals as it doesn't require physical contact

Reiki is not meant as a substitute for veterinary care, so a*lways consult your veterinarian about the best course of medical treatment for your animals*

Animal Chakras and Auras

The Charkas are the same as Humans and with about the same meanings. Like windmills spinning, these chakras spin within our bodies sending energy to every organ, cell, and drop of blood in our frame; they even fuel our thoughts and our emotions.

1st Chakra - Root/Base chakra found at the base of the spine is the Chakra of survival, our physical needs.

2nd Chakra - The sacral chakra located in the pelvis area, just below the belly button is the Chakra of Pleasure.

3rd Chakra - Solar Plexus chakra at the stomach slightly above the naval is the Social Chakra and that inner knowing or gut feeling.

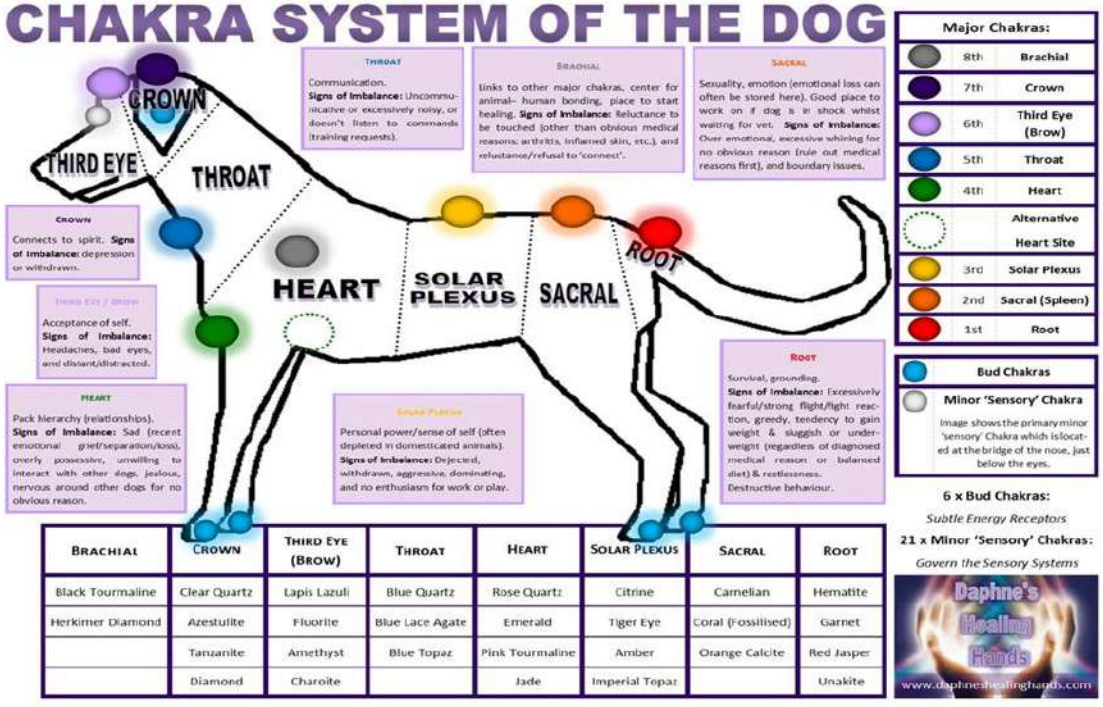
4th Chakra - The Heart chakra is the center of our entire Chakra system connecting the lower three physical and emotional centers with the three higher mental and spiritual centers, the Chakra of unconditional love.

5th Chakra - The Throat chakra is all about communicating and expressing ourselves.

6th Chakra - The Third eye chakra is located in the forehead between the eyebrows just above the bridge of the nose. It is the seat of our higher thoughts, reasoning, understanding, and decisions.

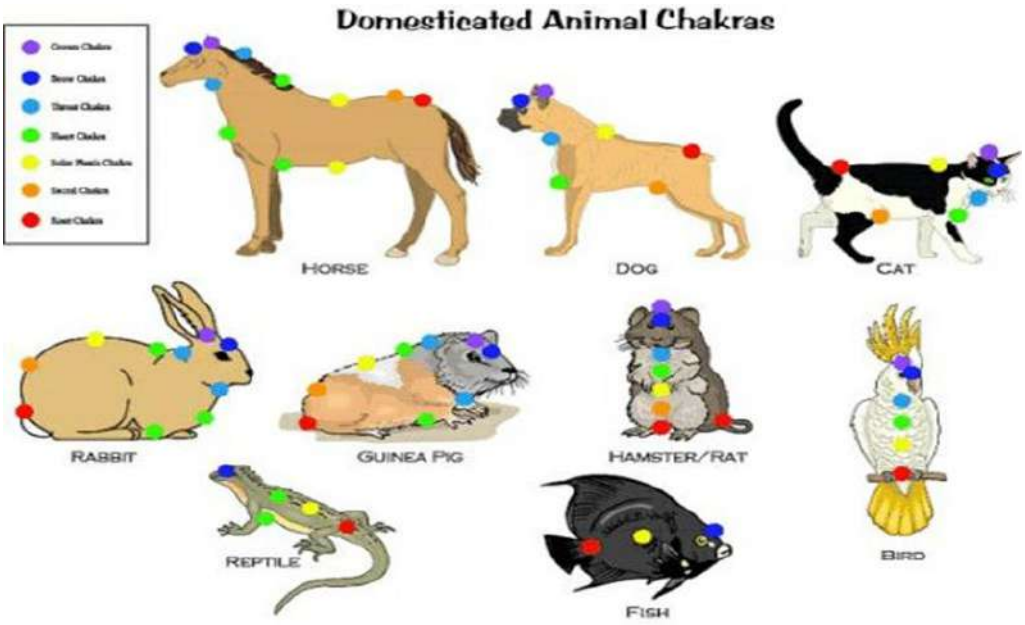
7th Chakra is known as the Crown chakra at the top of the head and is also called the seat of the soul and connection with our Higher Self and higher dimensions, realms, and spirit.

Each Chakra or energy center connects to certain organs and physical functions, when all are aligned and balanced, we realize health and wellness. Blocked and imbalanced Chakras disrupt the flow of energy resulting in illness, aches, and pains. The energy centers not only flow through the body but also radiate outside of the body as well. We see this radiation as layers of various colours, the Auric fields; seven Chakras, and seven layers in the Aura. The colours of these layers, their brilliance and intensity, reflect the conditions of our physical and mental self - each colour represents a different message.

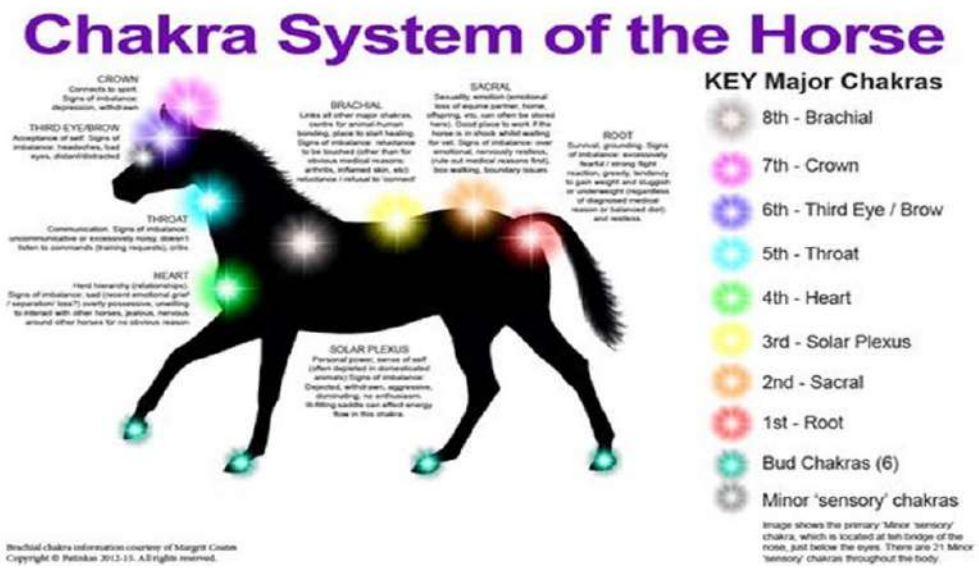


Chakras System of the Dog

Source: https://caninesofmind.com/services/animal-reiki/



Source: https://www.quora.com/Do-animals-have-auras



Source: http://www.reikibycathy.co.uk/equine-chakra-chart

The Difference between Reiki and Animal Reiki

The benefits of using Reiki Healing are not restricted to humans. The Reiki that is given to humans is precisely the same Reiki that is provided to creatures. The Universe has all types of animals that can go through sickness, anxiety, and grief. While we decide to apply the energy given by the Universe, animals are not very fortuitous. Humans can support other humans with Reiki healing by sending levels of energy to another if they are willingly accepting. Their systems are constructed to obtain communications of the mind that deal with emotions and discomfort. Animals only experience that they hurt and that they are doomed to experience. Meditation is the primary distinction in human and animal Reiki healing. You have to discover that relationship that makes an animal feel loved and cared for. Animals are exceedingly perceptive to energy. They can feel an array of emotions like stress, grief, worry, and suffering. Through Reiki healing, you can move inside a pet or animal and build an understanding of compassion and kindness. They will perceive this as a link. Once this individuality has been created, you can settle and soothe whatever ails a pet or animal through a metaphysical bond and trust. Connecting with pets can be a sympathetic means of displaying to them how much more satisfying their ways of life can be. These animals may not see the interpretation behind the movement, but it brings positive outcomes just the same.

Approaching Animals with Reiki

The approach of the practitioner is key when working with dogs and Reiki. Dogs appreciate being given control of the treatment: in other words, being allowed to say "yes" or "no" to the treatment as well as determining the way the treatment will unfold. This means the practitioner needs to follow a few basic rules to be successful in the treatment:

a) Always take permission from the animal before approaching it with Reiki. Or at least set your intention to facilitate the healing process for the animal with an adequate amount of energy.

b) At the beginning of the treatment, never start with the hand-on contact. Always allow the animal to contact you first, as some dogs are too sensitive to receive the energy provided by the human presence, finding the physical contact too uncomfortable.
c) Always allow the animal to move freely in the treatment area. Pay a detailed attention to the signs or things that an animal wants to tell you. Licking of hands or coming forward for a pet are few important signs that show a willingness of animal to connect with the energy.
d) Always approach animal with "passive" or "open" manner. "Offer energy in a non-incentive manner. For this purpose, your body language should match with this passive intention. For instance, always maintain a distance that an animal wants to keep and ensure a non-threatening pose.
e) After you finish the treatment, always thank the dog for participating in the treatment.

Reiki for Sick Animals

As mentioned previously, Reiki healing is beneficial to all life forces on Earth. These life forces consist of plants, animals, humans, and any other living thing created on the planet. The reason Reiki is so beneficial is that it can be done on almost any animal. Ranging from farm animals such as horses and sheep or chickens to smaller animals such as mice, ferrets, and guinea pigs. Every animal in between like dogs, cats, tigers, reptiles, and monkeys can also benefit because it can be done from far away or up close and personal. Almost every animal on this planet operates on an energy wavelength that is sometimes difficult for people to wrap their minds around. Reiki will never harm your animal. It is the most natural aid in healing diseases like cancer and arthritis to solving mental issues such as behavioral problems and depression. It's the act in which you transfer energy from your- self drawing from the universe into your subject and through that process, Reiki will do most of the work. Reiki finds the problem, takes your energy and the energy surrounding you, and gives strength and wellness to the animal you are working on. The animal senses these invisible vibrations and can also choose for themselves how much for how long they want to take from you. Open the mind to the belief in this energy force. Hold the goal of compassion and healing behind the practice. If your pet or an animal you know or don't know is sick, injured, or a senior, Reiki is there to ease the transition toward what happens next. Essentially, Reiki brings peace, comfort, relaxation, emotional, mental healing, and reduced physical pain.

a. Reiki for Senior Animals

Senior animals struggle with walking around. That puppy that you first brought home is no longer bouncing with ex-excitement, or that kitten that you adopted thirteen years ago is no longer playful. You reminisce on so many memories and good times together but feel pain in your heart as you watch them become slower every day. They are on medication to help with their joints, and maybe you have had to buy stairs so they can lay with you on your furniture. The time is here; your pet is a senior. In this stage of their lives, it is essential to support them through the difficult time they are experiencing. Your mouse or ferret may not be able to speak verbally to you. Despite that, within everything you have learned thus far from Reiki, you already know what they are thinking and feeling. Reiki therapy is so helpful for these aging horses and elderly sheep. Whatever pet you have, it is now the best time to welcome Reiki into your lives. Here is why:

b. Pain Relief

When we are children, we experience growing pains or restless leg syndrome. When we become teenagers, we may suffer from headaches or back pain. As we age into adults, we can experience neck-aches, joint problems, arthritis, etc. Finally, as an elder, it is more than likely we need to take pills like Tylenol or muscle relaxers to escape our body pain. Something which radiates through us all the time. Our animals go through these same stages of emotions throughout their lives. The most significant difference is that they cannot tell us what is wrong unless we tap into the communication between humans and animals. Reiki helps injuries from aching bones heal faster. Just as cortisol hormones rise in us when we feel stressed, it does the same for our senior animals. Animals can become stressed when they cannot perform for us as they once did. Reiki has proven to change these stress-induced hormones for an amount of time to relax and bring peace of mind to our senior pets. To help our senior animals, we need to Reiki transfer healing energy to them daily. *Again, whatever treatments the vet has prescribed should never be replaced by Reiki.* Reiki aids in helping them feel comfortable. With Reiki, they can feel more relaxed.

c. Emotional Well-Being

The older we become, the more at risk we become for Alzheimer's and dementia. We may become very forgetful or even confused. Our emotions become higher, and our bodies become slower. So, if this happens to us, can you imagine what your senior animal is going through? Have you noticed your dog bumps into things due to blindness? Maybe you have seen your cat walk into the kitchen but stand there dazed because of forgetfulness. Reiki helps with emotional well-being by giving your pet a sense of deep peace and relaxation, which promotes focus and concentration. To know whether or not Reiki is working, there are a few clear signs. These signs might include your pet lying down in front of you to gain your healing energy. Or, they may breathe out a sigh of relief while in the session.

d. Fear Reduction

Fear comes with age due to the ageing brain. Things you may not have feared before may become a intense emotion for you now. For example, when you are an adolescent, you may enjoy going to an amusement park and being in a large group of crowds for the excitement and thrill of everything you are ex- experiencing. Pets may fear car rides but loved them before. They may fear meeting other animals, but they had the patience for it earlier. They may jump at loud noises, whereas before it never seemed to bother them. Being fearful of these things can lead to heightened levels of anxiety, Reiki helps reverse these types of blockages and leaves your senior pet feeling relaxed. Just in case they do need to meet another animal or go for a car ride to the Vet.

e. Dying

Almost all humans fear the unknown. We fear what we cannot control and part of that fear, as we become elderly, is death. Is it time for your feline friend canine companion, or rodent playmate to pass on? You may know it's time for them to go, but they may be holding on a little longer for your sake. Death brings sadness and grief. However, death also brings new beginnings and learning experiences. Reiki helps your animal feel better about passing away, mainly when you perform the Reiki. Reiki can help them feel at peace with the idea of moving on. Reiki offers grounding and serenity to their mind and body so that it is easier for them to let go. Not only has Reiki proven to be helpful in the passing away process for your animals, but it has also shown to give you some closure as well. Yes, you may feel sad and upset that your best friend is gone. But with using Reiki healing for yourself and through the process, you can come to terms with it leaving you with a sense of enlightenment.

f. Reiki for Injured Animals

When it comes to spiritual healing or any type of healing, Reiki can be looked at as a form of acupuncture, acupressure, and chiropractic services. Reiki was developed out of what's called 'Qigong,' a parent of Tai Chi, and has become a wellness technique for both animal and human health and growth. This system uses the body's energetic system revolving around the Chakras. This revolving area is also called the meridian system. Instead of using needles or hot rocks on the body, Reiki stimulates the flow of energy (Ki) in the body through different hand gestures using the flow of one's energy to transfer into their subject. Anyone who experiences Reiki has said or shown that it is incredibly relaxing, bringing the patient (animal and human) to another level of meditation through the guidance and support of the practitioner. The patients benefit from Reiki because it doesn't matter whether they are ill, weak, sore, mentally ill, or emotionally unwell. Reiki is a non-harmful exercise in developing growth wellness in an individual. Through the gentle gestures of the Reiki practice, the body is guided to recover whatever energy imbalance it may be suffering from. It helps the animal absorb their vitamins through food while increasing their appetite if it is lost. It also helps them feel more comfortable before, during, and after a procedure like surgery or chemotherapy for cancer. If we take Reiki out of the equation and just rely on facts around science and research, people who are calmer and more relaxed can get through conflict in relationships, develop a more profound sense of inner peace, and make it through past trauma easier. Animals are just as equal to humans in the sense that they experience and go through changes and challenges as we do. The practice of Reiki, which essentially is a relaxation aid, opens the minds of your subjects to cure and help with the internal growth of injury or other imbalances through the energetic system.

Conclusion

The system of Reiki consists of the heart, heavenly divine, and earth energy. Since the Reiki is a safe, non-invasive practice, it is so quickly developed and learned by anyone open to the idea of it. Reiki helps in all aspects of your life. It can improve the lives of your patients or pets too. The process of Reiki is something anyone can learn and something you can learn fairly swiftly, especially for animals. Reiki allows us to perform at a level where our positive energy flows freely. Many people who have been through a Reiki healing session, describe the experience as tranquil, calming, and enlightening. Some say that their experience felt more like an emotional realignment. The same effects happen to animals through an animal Reiki healing session. Some practitioners in Reiki train for years to understand the energy and how to navigate delicate and subtle energy which shifts within themselves and their participants, where instead of realigning your bones and muscle's tension, you are realigning your emotive energy to create a balance so that you can live happily and make other living beings, including animals. With that said, remember that Reiki should not be an alternative to veterinarian medical care, but seen instead as an aid in the diagnosis to recovery.

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Chapter 24

Environmental Influences on Health

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ABSTRACT

The relationship between human health and environmental factors is an important field of research, especially as pollution and climate change become more widespread worldwide. This study examines the complex relationships between radiation exposure, climate change, air, water, and noise pollution affecting human health. Cardiovascular and respiratory conditions like asthma, chronic bronchitis, and heart attacks become worse by air pollution, which is mostly caused by vehicle exhaust and industrial emissions. Water pollution causes a variety of health problems, from neurological disorders and cancer to gastrointestinal infections. It is primarily caused by agricultural chemicals, industrial runoff, and improper waste disposal. Significant risks are posed by contaminants such as heavy metals and pathogens, especially in areas that lack adequate sanitation infrastructure.

Noise pollution impacts mental health and cognitive functions, causing stress, sleep disturbances, and an elevated risk of hypertension. Genetic damage and an increase in cancer rates are among the additional risks posed by radiation from nuclear power plants, medical procedures, and natural sources. These health issues are made worse by the effects of climate change, which include increasing temperatures, harsher weather, and altered disease patterns. In order to lessen their effects and to protect human health, these environmental stressors collectively highlight the urgent need for integrated public health strategies and policies.

KEYWORDS
Pollution, Climate change, Health impacts, Environmental Stressors, Radiation

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INTRODUCTION

The environment is a layer of the Earth formed over a long time by natural processes in the biosphere, influenced by the solar energy. It includes the lowest layers of the atmosphere, like the troposphere and lower stratosphere (up to 15-20 km high and 4.5 km respectively below the Earth's surface), the deepest depressions on Earth (up to 11 km deep), and the lithosphere (up to 4.5 km below the surface). Humans are also part of the biosphere. Every year, at least 6.5 million people worldwide die from illnesses caused by environmental pollution. The global population is increasing every day. More and more people are being born each day. According to the United Nations, there were 7.8 billion people on Earth at the beginning of 2022. Every year, the world's population is increasing by 82 million individuals. It is estimated that the population on the planet will be 9.7 billion by the end of 2050. This information was shared during the 52nd session of the Commission on Population and Development at the UN headquarters in New York. They predict that by the end of this century, there could be as many as 11 billion people on Earth (Rasulov, 2023).

The environment is everything around us that has an impact on our lives, whether directly or indirectly. It's divided into two categories: biotic (like plants and animals) and abiotic (like air and water). The environment includes various aspects like how people behave, the natural world, culture, and even the air we breathe. As living things grow, they interact with their surroundings and cause changes in the environment. Plants, for example, not only take in carbon dioxide and sunlight but also absorb water and minerals from the soil. Inside the plant, they perform a process called photosynthesis, creating organic materials. Because plants contain organic matter, they release oxygen into the air, which is good for humans and animals, and they also provide nutrients. The relationships between nature, living things, and processes like geology and biology are quite complex and develop over time as living organisms evolve (Uralovich et al., 2023).

Environmental Impacts on Human Health

Our health can be greatly influenced by our surroundings. Pollution from things like the air, water, chemicals, and metals is increasing worldwide because of more industries, burning fossil fuels, and advanced farming. More people are getting sick and even dying because of exposure to higher levels of these pollutants. Pollution is the main reason for early deaths globally, causing one out of every six deaths. The second largest cause of non-communicable diseases in the world is pollution while the first one is smoking. It plays a big role in the increase of diseases like cancer, heart problems, breathing issues, and diseases that affect the brain as shown in the Figure 1. These health problems highlight the importance of understanding how the environment affects human health and figuring out how being exposed to environmental things can harm our health (Pruss-Ustun et al., 2019).

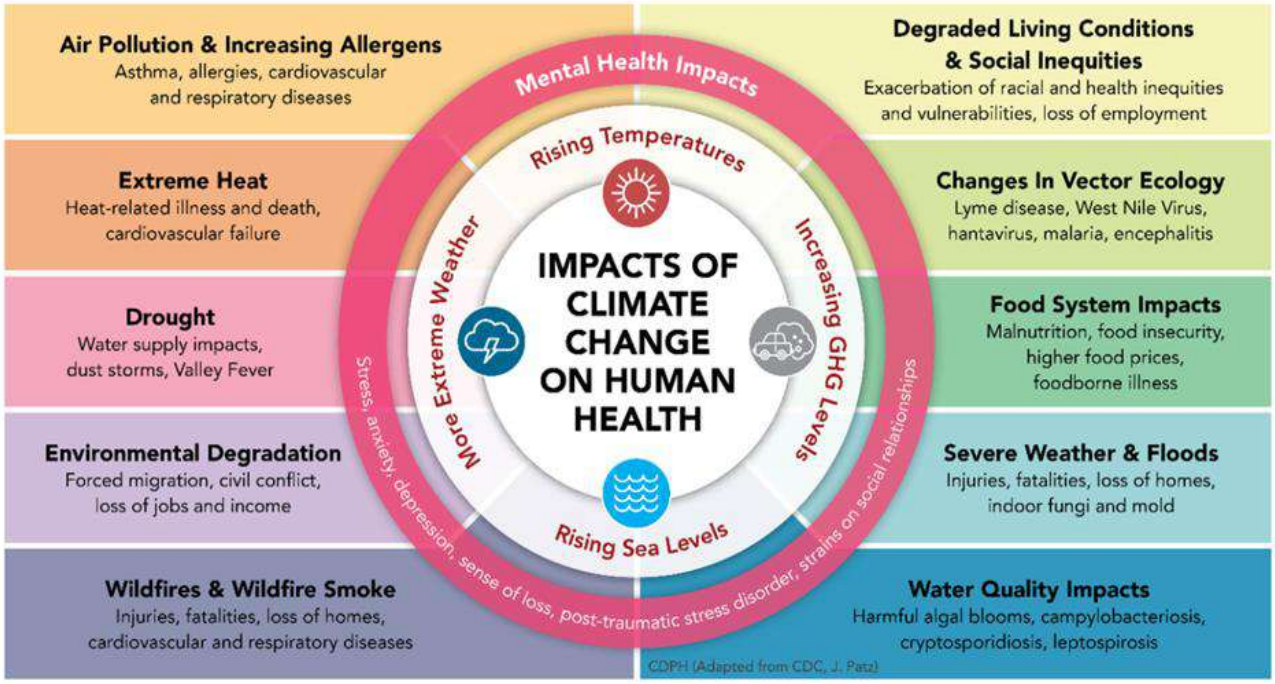

Fig. 1: Impacts of different environmental Change on Human Health. (Impact of climate change)

Air Pollution and Respiratory Diseases

However, even various rules developed by governments, international air pollution is still on the rise. Yearly, seven million people die early due to increased air pollution levels; the latter also has consequential effects on health. Among the leading causes of these deaths it is possible to distinguish the growth in the number of respiratory infections. Even with measures that have been taken by bodies such as the United States Environmental Protection Agency, EPA and the European Environment Agency, EEA, the level of pollutants is rising. The WHO report revealed that more than three billion people breathe air that contains more particulate matter than the legal limit set by their countries' laws. Such a high level of pollution affects the global public health so drastically and has numerous and grave health implications, and the detailed information is provided below. Besides premature mortality, contaminated air may lead to heart diseases and increase tender heartedness to respiratory diseases. Thus, it is vital to investigate and comprehend as to why contaminated air is detrimental to health in order to enhance the ways of preserving the health of the population and the quality of the environment, in which they live (Monoson et al., 2023).

As population investigations have shown, inhaling polluted air triggers the cells in the lungs and causes the formation of tiny extracellular vesicles in the bloodstream. These particles have been reported to bring about endothelial dysfunction, thrombogenesis, as well as inflammation that interferes with the normal functioning of organs. Researchers also found that these particles contribute to increasing the speed of vascular thrombosis, initiate adverse messages in the human brain, and increase muscle stiffness around the lungs (Wu et al., 2023).

Water Contamination

With the increasing world population and the effects of climate change the difficulty of accessing clean and safe water for drinking is increasing as well. Liu and Bridget (2020) established that over 785 million people in the world are still using unimproved water sources. These issues include lack of clean water; issues such as poor quality of water water, hygiene and inadequate supply of sanitation, are some of the causes of water shortage in the world. Unfortunately, due to of such water approximately eight hundred children die every day for diseases such as diarrhoea. Pakistan is perhaps one of the most complex areas as far as information literacy is concerned. It has termed as "the water crisis" where country is suffering

from severe water pollution and scarcity. Pakistan is on the list of countries which are most in danger of deadly issues such as water shortage, contaminated water, air pollution, climate change and global warming as per new Environment Performance Index (EPI). These issues pose serious threats to life of millions of people over the country. The primary causes of the environmental problems include deforestation, carbon emissions, increased urbanization, and industrialization as shown in Fig. 2 (Nsabimana et al., 2021).

Fig. 2: Sources of Water Contamination. (Water pollution)

In recent years, Pakistan has experienced a significant increase in its demand for water due to population growth. Additionally, activities related to the expansion of the agricultural and industrial sectors contribute to the deterioration of water quality. The consequence of these factors is the existence of unsafe drinking water, contaminated by various pollutants like industrial wastes, chemicals, microorganisms, and hazardous materials. (Ebrahim, 2020).

Reports from community health surveys in Pakistan highlight that 40% of fatalities and 50% of water-borne diseases are linked to the consumption of poor-quality drinking water. Alarmingly, high levels of arsenic in drinking water could potentially impact around 60 million people in Pakistan. Over the past four years, the combination of water-borne illnesses and drought has resulted in the tragic loss of approximately 1832 children. Currently, only 20% of population has access to clean drinking water. Most people must still consume contaminated water, mostly by sewage, fertilizers, industrial waste, or pesticides (Ilyas et al., 2019). . Many of the researches conducted researches on the quality of drinking water in different regions of Pakistan have shown that pollutant concentrations are higher than limiting concentrations recommended by the WHO (Kalair et al., 2019).

A fifth and a major problem health wise in citizens is the presence of pesticide in drinking water. Research conducted in the nation shows that effects of exposure to pesticides include headache, vomiting, dizziness, muscle weakness burn sensation in the urethra, breathlessness and skin erythema (Fida et al., 2023).

Noise Pollution and Mental Health

Air and water pollution by such things as factories and technology are on the increase globally and they are Health hazards. This is because pollution, although we know it harms our physical health, is still a relatively new and little-understood culprit for unhealthy states of mind. A number of studies reveal that quite a lot of factors, such as polluted air, metals, radiation, pesticides, as well as factors as light pollution or noise can negatively affect our mental health. There are some of the pollutants that can penetrate our brains or make us stressed in one way or the other so as to develop conditions such as anxiety or depression as shown in figure 3(Ventriglio et al., 2021a).

The UK government have conducted a study among others recently conducted by the World Health Organization on how noise impacts our well-being. Some of the works they identified showed that the quality of evidence on noise and a range of factors, including mental health and well-being, and even birth outcomes and thinking abilities is generally poor.

According to some works, noise such as traffic, and planes could lead to the consumption of medications, anxiety, depression, and might cause cancer as well(Clark et al., 2020).

Several studies have established that exposure to road traffic noise has negative effects on mental health but little is known about the effects of other traffic noises. In order to determine how noise from cars, trains, and airplanes may affect the probability or adherence of grown-ups to suffer from depressions, anxieties, dements, or have impaired thinking, we reviewed many investigations (Hegewald et al., 2020).

Fig. 3: Different sources of Noise Pollution. (Noise Pollution)

Radiation Exposure and its Health Effects

Nanomaterials (NMs) are made and disperse into the environment at a rate of 1000 tonnes per day. Those are mainly natural, but synthetic are used in chemical, engineering, electronics, medical and other industrial processes and can be hazardous to the environment. Because more of these NMs get into the environment, understanding how these NMs behave in nature how they impact on the health of individuals and how they interact with forms of life is very crucial. It is a review of the current body of literature on NMs and their effects on the environment and the health of the people. It tells us how these small materials are transported in the environment, how they penetrate our body, and what effects they have on our health system. It also emphasizes that more work needs to be done to advance the knowledge of them (Malakar et al., 2021).

Issues such as oxidative stress, inflammation, changes in DNA and cellular ageing and working can be attributed to radiation. To enhance the protection of people from radiation in medical treatments, since the population nowadays is aging, we have to know how it influences the body responses (Tong et al., 2020).Ionizing radiation impacts on our immune system in as much as the immune system is complicated in this way: It has to maintain equilibrium of the various components, cells and factors and has a major role in tissue protection and repair especially when it has been damaged by radiation. Low doses of radiation may not show immediate effects but can lead to immune aging and irreversible changes, increasing the risk of diseases like cancer and age-related conditions. However, in some cases, low-dose radiation can actually help with chronic inflammation and pain(Lumniczky et al., 2021).

Urbanization and Public Health

The conflict between protecting the environment and growing the economy has caused health problems due to industrialization and city growth. While we know that carbon emissions affect health because of climate change, there haven't been many studies looking at this in detail. This research used regression analysis to look at how carbon emissions affect people's health in different parts of China from 2002 to 2017. The results show that: (1) more carbon emissions lead to more people needing medical care; (2) higher temperatures also harm health; (3) places with more industry and cities have more health risks from carbon emissions; (4) To improve health, China needs to upgrade industries, improve cities, and balance industrial and city growth based on local conditions. One-size-fits-all policies won't work, and China needs to consider how industries and cities develop together (Dong et al., 2021).

More than half of the world's population lives in cities, which offer many benefits but also change the microbes people are exposed to. These changes, especially in the airborne microbes, might contribute to more allergies and asthma. It's important to standardize research methods and share data to make comparisons easier. We need to study how these microbes affect our immune systems to create healthier cities (Flies et al., 2020).

Cities tend to have higher rates of mental illnesses. Issues like inequality, instability, pollution, and lack of access to nature can make mental health worse. To help city planners and policymakers improve mental health care in cities, we need more research and specific recommendations. We should also use methods from around the world to prevent and reduce mental illness in urban areas (Ventriglio et al., 2021b).

The way cities are expanding, like spreading out into suburbs and peri-urban areas, might make people more vulnerable to infectious diseases. Thus, the future research of health and illness should address quart in what ways the varying patterns of urbanization can potentially cause outbreaks and how it could be prevented (Connolly et al., 2021).

Impact of Climate Change on Mental Health

Climate change exerts a significant impact on a diverse population, manifesting various threats to public health across different geographical areas. Despite this, there has been a notable lag in studying the mental health consequences of climate change, possibly attributed to the issue's complexity and novelty. Research indicates that climate change affects mental health with varying timelines, resulting in a wide range of mental disorders, some common and others specific to atypical climatic conditions. Furthermore, different population groups face disparate impacts based on their direct exposure, vulnerability in geographical conditions, and limited access to resources, information, and protection. Notably, recent literature introduces new terms like ecoanxiety, ecoguilt, ecopsychology, ecological grief, solastalgia, and biospheric concern to describe the connection between climatic events and mental disorders (Cianconi et al., 2020).

Pest Control and Agricultural Health Hazards

Several environmental problems, especially toxicants pollution, have evoked the interest of scientists for as they affect human health and agricultural yields. This review mainly discusses the toxic impacts of heavy metals and pesticides including cadmium, lead, copper, and zinc, insecticides, herbicides, and fungicides on the centre agricultural ecosystems – plants and soils –and on human health. It delves into the accumulation of heavy metals and pesticide residues in soils and plants, discussing characteristics of contaminated soil and plant physiological parameters. The review also explores human diseases resulting from exposure to these environmental toxicants, emphasizing bioaccumulation, mechanisms of action, and transmission pathways.

Furthermore, the bioavailability of contaminants in soil, plant uptake, and the synergistic or antagonistic interactions between heavy metals and pesticides are highlighted. The combined toxic effects of these pollutants are discussed, drawing on previous relevant studies to comprehensively cover various aspects. The information presented in this review offers deep insights into the understanding of environmental toxicants and their hazardous effects (Ahmed et al., 2021).

The Effectiveness and Adverse Effects of Pesticides on Fertility

The social age has recorded a progressive decrease in fertility more alarmingly, adverse reproductive health has increased in the last few decades due to occupational and environmental toxicity of different chemicals. Pesticides, especially insecticides, have been especially blamed for increasing risk of chronic diseases like diabetes, cancer, and neurological disorders, and aggravated birth defects and low fertility (Fig. 5). Occupational and environmental exposures are again important since, for example, a large number of pesticides are known endocrine disruptors which may cause biological effects at very low concentrations. This review seeks to engage in a synthesis and interpretation of knowledge on pesticide exposure and infertility, abnormal sexual development and pregnancy status resulting from occupational, environmental and trans–placental effects that occurred between the year 2000 and 2020. Additionally, it provides insights into current pesticide production, usage legislation, and recommendations for medical surveillance, emphasizing biomonitoring for reduced fertility in occupationally exposed populations. The inclusion of legislative aspects aids in understanding exposure profile variations between countries, offering valuable information for preventive measures (Aleksandra et al., 2021).

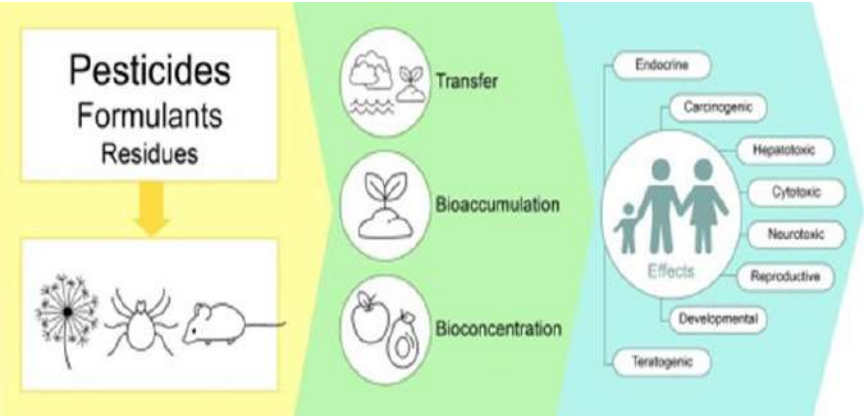

Fig. 5: Distribution patterns of pesticides and their effects on human health. (Pesticides Residues)

Pesticide Risks: Farmers' Perspectives in Punjab, Pakistan

This study addresses the extensive use of pesticides in agriculture, emphasizing the resulting health risks, costs, productivity loss, and environmental degradation. It focuses on farmers' risk perceptions of pesticide residues, employing an ordered probit model with data from 209 vegetable cultivators in Punjab, Pakistan. Key determinants of farmers' perceptions include educational level, family labour, self-consumed food ratio, health effects awareness, integrated pest management (IPM) training, knowledge, and overall awareness. Notably, farmers' awareness and participation in IPM

training emerge as primary indicators of risk perception. The study emphasizes the crucial role of policymakers in addressing farmers' perceptions, as these can worsen pesticide overuse if influenced by internalized social norms, which are mitigated by awareness and knowledge. These revelations indicate the importance of supplementing farmer knowledge, particularly those with restricted information, and increased IPM training programs for human well-being and sustainable agriculture ecosystems (Yasir et al., 2020).

Environmental Pollution and Work Related Ailments

In this context, emphasis is placed on effect of the two types of pollution, that is, ambient and occupational on lung and respiratory diseases. Particular attention is given to particulate matter (PM2.5) as a significant ambient pollutant, generated primarily through combustion. Short-term consequences of PM2.5 exposure include asthma attacks and exacerbations of chronic obstructive pulmonary disease, while long-term effects encompass an elevated risk of lung cancer, mortality, and impaired lung function development in children. Notably, recent findings suggest a potential link between air pollution, especially PM2.5, and a heightened risk of severe COVID-19. Traditional occupational pollutants, namely asbestos and crystalline silica, are associated with diseases such as asbestosis, lung cancer, malignant mesothelioma, and silicosis (Nishida and Yatera, 2022).

This study focuses on assessing and comparing respiratory symptoms and pulmonary functions among individuals exposed to occupational hazards in certain professions (welding, painting, and vehicle repairing) and those who are not exposed. This research establishes that people working in environments that cause them to be frequently exposed to dust, exhausts, fuels, and fumes very often report cases of respiratory symptoms for example chest tightness. Also, the exposed group presents a reduced value of pulmonary functions compared with the control group. Employers and other exposed workers are said to be adopting preventive measures including the wearing of masks in the low levels (Ahmad and Balkhyour, 2020).

Conclusion

In conclusion, the combined effects of air, water, and noise pollution, along with radiation and climate change, pose significant risks to human health. These environmental stressors contribute to a range of health issues, from respiratory and cardiovascular diseases to mental health problems and increased cancer risks. Climate change further complicates these issues by introducing new health threats and exacerbating existing conditions. Addressing these challenges requires a coordinated approach involving policy changes, public awareness, and protective measures to reduce pollution and mitigate climate impacts. By prioritizing environmental health and implementing effective strategies, we can improve overall well-being and build a healthier future for communities worldwide.

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Chapter 25

Innovation and Intersection in Human and Veterinary Vaccine Development

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ABSTRACT

This chapter mainly focuses on the significance of immunization not only in the public health but also in animal health. Also, it reveals striking similarities in human and animal vaccine development. It starts with emphasizing the historical contribution of vaccines to the control of diseases to help dwell on the recent scientific progress in the said field. These include the advent of platforms based on mRNA vaccines and a shift in perspectives from traditional to modern ones being data-driven through science and nanotechnology, which in turn shape how vaccines are designed and distributed. An integral part of the present essay consists of analyzing the intricate symbiotic linkage that is observed between human and veterinary vaccine research where the "One Health" concept depicts itself as an instruction. It is the way to underline and consider the interconnectivity of human, animal, and environmental health and how the improvements in one can be quite positive in ones-side. Although, by now the chapter covers some important achievements, at the same time he puts the challenges that still exist in the line of fire, like the rise of an original pathogen spreading, vaccine resistance, and the gap of access in a global circle. It ends with a prospective direction sheerly emphasizing the fact that the progress should not stop and all fields of science should be running it together seeking solutions for the next challenges in vaccine research and development. This chapter has a lot of relevant information about vaccination in the dynamically changing world that represents a momentary sensation of how interdependent vaccine development is for global health.

KEYWORDS
Animal health, immunization, mRNA vaccine, One Health, vaccine development

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INTRODUCTION

Immunization is essential for the maintenance of good health in both people and animals. Immunology in zoology is fascinating because differences and similarities in immune systems among animals from various orders and species continue to be observed. The immune system of an animal is very complex and individual immune systems of animals very distinct, but the basic principles of pathogen identification and memory upon subsequent exposure to that pathogen are the same for all species (Janeway et al., 2001). The adaptive immune system of humans includes antibodies, B cells, and T cells. From this example, humans and animals use the same elements to defend against infection; however, animals adapt the elements based on what they have encountered in the past with different organisms. In veterinary vaccines, the antigenic determinants, which are focused on intracellular infections for the purposes (Murphy et al., 2012).

This medical breakthrough was the first step for humans to further develop vaccinations to eradicate smallpox (Fenner et al., 1988). A fascinating history of human medicine began in the nineteen centuries with Louis Pasteur, who produced the first vaccine to immunize cattle and saved France's cattle industry from collapse from anthrax (Brock, 1999). The paper introduces the notion of health interconnectedness between humans and animals as discussed by (Geison *et al*., 1978). According to the World Health Organization (WHO) in 2020 the One Health concept (OHC) was established because of the inter connectedness of humans, animals, and the environment. It recognizes that the health of people is connected to the health of animals and the environment. Understanding the importance of vaccination for both humans and animals is crucial. The ongoing research on vaccinations continues to shed light on the role of immunology in disease prevention. This allows us to be ready for emerging diseases that can affect both animals and humans.

Comparative Immunology: Similarities and Differences between Human and Animal Immune Systems

Comparative immunology approaches the understanding of the changes governed by genetics, which are closely linked to the different nature and severity of diseases. Humans have a highly sophisticated immune system that is often classified into two parts: The Innate immunity and Adaptive immune systems. The adaptive system, ironically, is the by-product of vaccination. It shows itself to be a strong innate response system (Janeway et al., 2001). Much as there is an overwhelming difference, an immune system (found majorly in mammals and animals) is simply present. The bursa of Fabricius, which helps B lymphocytes to develop is a specific organ in birds only. Human bodies, meanwhile, have bone marrow, not Fabricius's bursa for this process (Ratcliffe, 2006). Conversely, fish are less adaptive and depend more on their bodies' defences (Uribe et al., 2011).

Despite their diversity, one facet of immunity is shared by all vertebrates when it comes to the interplay between health and illness. Vaccines that are effective in various immune systems may be designed with these similarities and variances in mind (Zhang et al., 2014). Insight into these parallels strengthens our strategy for illness prevention, paving the way for the creation and administration of vaccines that consider the differences between the immune responses of animals and humans.

Historical Milestones in Human and Veterinary Vaccines

The development of vaccinations and their subsequent influence on animal and public health are remarkable examples of human ingenuity. An early kind of smallpox vaccination in Asia before the 17th century, smallpox injection may have been how immunization took place (Behbehani, 1983). Modern vaccines began with Edward Jenner's smallpox vaccine in 1796 (Fig. 1), which introduced the use of the cowpox virus to provide human immunity against smallpox (Riedel, 2005). This not only eliminated smallpox but also laid the foundation for the idea of vaccination. A breakthrough in veterinary research was Louis Pasteur's development of the anthrax vaccine in 1881. His subsequent invention of the rabies vaccine in 1885 marked a significant moment in the treatment of humans and animals (Pasteur, 1885). These findings confirm that control of animal diseases, especially zoonotic diseases, can benefit human health.

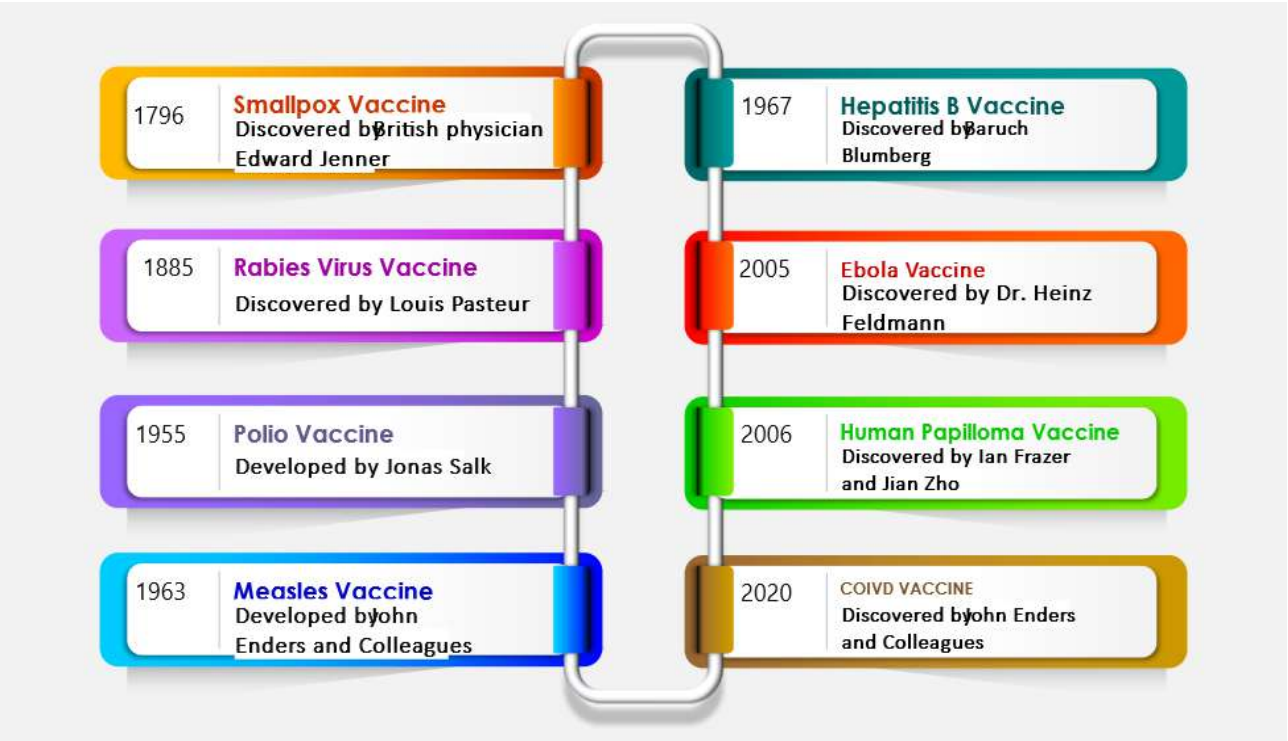

Fig. 1: History of major human vaccine development (Kumari et al., 2022).

Other important developments include the introduction of inactivated vaccines, such as the polio vaccine developed by Jonas Salk in 1955, and later live vaccines, such as Albert Sabin's oral polio vaccine (Offit, 2005). The 20th century saw the development of safe and effective vaccines against various infectious diseases in humans and animals become widespread. The advent of genetic engineering and recombinant DNA technology in the 21st century has revolutionized vaccine development and led to the rapid development of complex vaccines, such as human papillomavirus (HPV) blocking vaccines and the mRNA COVID-19 vaccine (Zimmer et al., 2020). Cooperation between people and veterinarians, promoting the One Health Strategy and joint efforts against infectious diseases.

Types of Vaccines in Human and Veterinary Medicine

Vaccines are biological preparations that provide active acquired immunity to a particular infectious disease. They contain agents resembling a disease-causing microorganism and are often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins.

Live-Attenuated Vaccines: These vaccines use a weakened form of the germ that causes a disease. They prompt a robust immune response and often confer lifelong immunity with just one or two doses. In human medicine, examples include the MMR (measles, mumps, and rubella) vaccine (Plotkin et al., 2012). Similarly, in veterinary medicine, live-attenuated vaccines are used, such as the canine parvovirus vaccine (Roura et al., 2021).

Inactivated Vaccines: Inactivated vaccines use the killed version of the germ that causes a disease. They are safer than live vaccines and are used when a live vaccine is deemed too risky. Human examples include the polio vaccine (IPV) (Orenstein et al., 2017), and veterinary examples include the rabies vaccine for pets (Brunette Gary and Nemhauser Jeffrey, 2020).

Subunit, Recombinant, Polysaccharide, and Conjugate Vaccines: These vaccines use pieces of the germ — like its protein, sugar, or capsid (a casing around the germ). In humans, the HPV vaccine is a notable example (Schiller and Lowy, 1996). For animals, the leptospirosis vaccine is one such vaccine that protects against the bacteria without using a whole-cell preparation (Ellis, 2015).

Toxoid Vaccines: Toxoid vaccines are used against bacteria that produce toxins in the body. They are inactivated toxins (toxoids). The diphtheria and tetanus vaccines are examples in human medicine (Plotkin et al., 2012). For animals, a toxoid vaccine is used for tetanus in horses (Mealey, 2014).

DNA Vaccines: These are the latest type of vaccines that are currently being researched and have been used in veterinary medicine for diseases such as West Nile virus in horses (Kutzler and Weiner, 2008). They involve the direct injection of genetic material into the host, which cells then use to produce an antigen.

mRNA Vaccines: The mRNA vaccines, which include some COVID-19 vaccines, are a new type of vaccine to protect against infectious diseases. They teach our cells how to make a protein—or even just a piece of a protein—that triggers an immune response inside our bodies (Pardi et al., 2018).

Species-Specific Vaccine Development and Use Cases

Strain-specific vaccines play an important role in the prevention of various diseases affecting livestock that pose a threat to human health. For example, vaccines like the feline leukaemia virus vaccine for cats and the equine influenza virus vaccine for horses target specific diseases that are common in these animals (Dodds, 2021). In addition, vaccines like the rabies vaccine work though prevent zoonotic diseases, of animal origin. They can be transmitted to humans, and not only to domestic animals but also to wildlife and livestock (Abidin and Budi, 2021).

Vaccine Development and Regulatory Considerations

The iteration of these drugs needs to follow meticulous knowledge acquisition on the specific immune responses of that species, including the nature of the co-infection, the interactions between the hosts, pathogens, and environment between which (Wakhusama et al., 2019). For the sake of safe and precise performance of such a complex task as it is, such actors as research, public health authorities, and veterinarians need to engage in collaboration.

In a case of regurgitation, the process of regulating vaccine creations which is under few regulatory organizations such as FDA or EMA seems to be very complicated. Drug companies test clinical trials by vaccination to verify for safety and effectiveness before vaccines are authorized for manufacturing and distribution. We should also keep in mind a good strategy through the process of post-marketing surveillance inspections (Cole et al., 2022). We are coming to a new realization that fighting the spread of viral infections needs to abandon the unified plot that considers the health of humans, animals, and the environment (Erkyihun and Alemayehu, 2022). The working together of disciplines is of great historical importance in the reduction of disease and the recognition of coexistence and transfer of different vectors. Human and animal immunization regimens are customized to address unique requirements and dangers. Human vaccination schedules, recommended by health authorities such as the CDC or WHO, are based on factors such as age, risk of infection, and immune competence (CDC, 2020) and animal vaccination schedules also varies in species, genus and disease prevalence, assessing local risk (Yadav et al., 2019). Herd immunity, which is important for the control of infectious diseases, is tracked through vaccination in human and animal populations (Fine et al., 2011). Achieving herd immunity helps protect vulnerable individuals and reduce overall disease incidence, contributing to public health and economic stability.

Despite the benefits of vaccination, challenges remain in both human and veterinary contexts. These include addressing viral infections, particularly vaccine reluctance due to socio-cultural and economic influences, and structural barriers to

vaccine availability in remote populations (Azzopardi, et al., 2009; Dubé et al., 2013). The development and use of species-specific vaccines is critical to protecting animals and populations from infectious diseases. Through scientific advances, regulatory controls, and strategies through the implementation of collective initiatives such as the Single Health System, we can effectively address the challenges posed by infectious diseases and enhance global health security.

Challenges in Human and Veterinary Vaccinology

Zoonotic illnesses pose a tremendous challenge in vaccinology. The transmission of pathogens like the rabies virus or avian influenza from animals to humans requires incorporated vaccination strategies. The improvement and distribution of vaccines must consider potential flora and fauna reservoirs, farm animal's management practices, and the interface between wild and domestic animals (Karesh et al., 2012). Vaccine hesitancy, prompted by socio-cultural, political, and personal beliefs, is a major barrier to reaching vast immunity (Dubé et al., 2013). In veterinary medicinal drug, elements which includes the perceived risk-gain ratio and economic issues can affect compliance (Knight-Jones et al., 2014). Remote human populations often face demanding situations in having access to vaccination due to logistical, infrastructural, and useful resource obstacles (Wiysonge et al., 2013). Similarly, in veterinary medication, delivering vaccines too far off cattle or flora and fauna populations poses substantial logistical hurdles (Emily et al., 2022).

Advances in Vaccine Technologies

Introduction to Adjuvants and Delivery Methods

Adjuvants in veterinary science are important components added to vaccines to enhance the immune response of an animal to a given antigen. These factors play an important role in ensuring that the immune system recognizes and responds to the vaccine effectively (Wilson-Welder et al., 2009). Adjuvants work by promoting inflammation at the site of vaccine injection, attracting immune cells to the site, and improving antigen presentation to these immune cells (Reed et al., 2013). This results in a normal immune response strong, specific, prolonged against target infection or disease.

Significance of Adjuvants in Veterinary Vaccines

Immunogenicity: The primary role of adjuvants in veterinary vaccines is to activate the immune system. Many diseases have antigens that do not provoke a strong enough immune response on their own. Adjuvants help overcome these limitations by enhancing the immune response to the vaccine, thereby providing better protection against the disease (Wilson-Welder et al., 2009).

Increases long-term immunity: Promotes long-lasting immunity. They help produce memory cells, which "remember" the infection and can trigger a rapid and effective immune response should the animal become infected again in the future This is especially important for high-risk diseases worm of reinfection (cited by Reed et al., 2013).

Types of Adjuvants Commonly Used in Veterinary Vaccines

Aluminium salts (Alum): Chemical excipients containing aluminium such as aluminium hydroxide and aluminium phosphate are among the most common ingredients used in veterinary vaccines, they work by forming reservoirs in situ drops it, slows the release of antigen and increases the amount of antibody contact This long-term exposure increases the immune response (Wilson-Welder et al., 2009).

Oil-based excipients: Oil-based excipients, also known as emulsions, are water-based solutions of oil or oil. These adjuvants increase antigen retention at the injection site, resulting in prolonged immune activation. Examples include Freund's helper and Montanide (Reed et al., 2013).

New adjuvants: In recent years, researchers have developed adjuvants with specific ingredients. For example, CpG oligodeoxynucleotides activate the innate immune system by interacting with Toll-like receptors. Put simply, the immune system is more robust in this manner (Reed et al., 2013).

Mechanisms of Action of Adjuvants

Adjuvants promote inflammation because they stimulate prudent inflammation at the site of the eyes. As an explanation for that phenomenon, Wilson-Welder et al. (2009) state that under inflammatory conditions immune cells, specifically dendritic cells and macrophages, come to that locality creating an antigen tile environment. Dendritic cells and other antigen-presenting cells get this antigen by intake and processing and then delivering it more efficiently due to the increased antigen presentation. The immune system is therefore more efficient at comprehending the antigens as a threat and mounting a strong immune response after the ADC system has enhanced its antigen delivering capabilities (Reed et al., 2013).

Immune activation: Adjuvants can stimulate immune cells to serve as a source, and can increase the release of cytokines and chemokine's, thus augmenting the level of immunological activity. Hence, through this, immune cells will reach the area more, thus, the immune response is coordinated well (Wilson-Welder et al., 2009).

A feasible way of looking at this is that adjuvants are a real part of any veterinary vaccine. They contribute to enhancement of the immune system caused by inflammation and as a result the release of immune cells and stimulant of the antigen thus. The adjuvants in veterinary vaccines may have different mechanisms of action; the selected adjuvant depends on parameters of the pathogen, the species, and the desirable time for which the response the immune system will last.

Reverse Vaccinology

This approach stands out in the rapidly evolving genomics technology landscape by utilizing digitalized information on the viral world to analyse genetic material and identify potential vaccine candidates, rather than relying on traditional methods of virus cultivation and study.

Diseases

Vaccines for bovine infections caused by multicide Teams of immunologists are now primarily responsible for developing methods to study Pasteurella's and Hemlitica's viruses. In genomics, scientists have precisely identified the surface proteins on ants and antigens crucial for vaccine development. Antigens are utilized in the creation of vaccines to protect animals from viral infections. *Streptococcus equi* subsp. *equi* is a significant infectious agent that poses a threat to horses. Research on a new vaccine for the disease has primarily utilized reverse vaccinology (Rappuoli et al., 2015). Virulence factors and surface proteins targeted by the immune system have been identified through viral genome analysis. Horses acquired and conscripted during the war played a significant role in advancing horse vaccines for infection and tetanus.

How Genomic Analysis Led to Their Discovery

Identifying specific regions on pathogen proteins or antigens that could initiate an immune response is a crucial step in genomics analysis. These antigens primarily contribute to virulence and are crucial for eliciting the host immune response. Vaccines could be developed based on gene candidates identified through genetic research. Research on vaccines entails introducing test antigens into the immune system of animals capable of producing protective immunity to develop the vaccine. As a result of these vaccinations, certain antigens are introduced to us through biotechnology and vaccinology. Producing the reporting chemical can be achieved through sub-unit vaccines, recombinant proteins, or other existing proteins, depending on the pathogen and the specific immune response required. Simply put, it resides on the surface of bacteria as if it were a bacterium but does not actively infect them. This is the rationale behind the statement (Rappuoli et al., 2015). Participants were instructed to review a persuasive argument advocating for the health advantages of protein-rich foods and offer their assessment of its persuasiveness. Enhancing immunity through adaptive vaccinology involves targeting a region closely aligned with the pathogen's genetic information, resulting in more effective and safer immunity. In addition, this method will render incoming vaccine batches ineffective as they would not target zoonotic diseases.

Animal research has proven to be a valuable method for testing various categories, such as reverse vaccinology, which involves the development of vaccines driven by immune genes (Rappuoli et al., 2015). Vaccinating cattle against respiratory disease and treating horses with crusted disease have significantly contributed to enhancing animal welfare. Identifying the essential proteins or genes that constitute specific vaccines is currently conducted through genomics-based methods. Despite its ancient origins, animal vaccination remains a crucial aspect of medicine.

mRNA-Based Vaccine Models

Their ability to achieve this in human beings forms the basis for a promising future regarding how mRNA-based vaccines might be of importance in veterinary medicine (Zhang et al., 2021). These influence host cells' antigens by changing the genetic code that is fixed in messenger RNA (mRNA) strands. Thereby, these offer immunogenicity and hence help high specificity because they make antibodies against the target pathogen.

Vaccines for Animals Based on Messenger RNA

mRNA-based vaccines are developing as potential game-changers and excellent armoury against a certain class of viral infections such as feline Leukaemia (Zhang et al., 2021). One of the examples: it may be designed to encode antigenic determinants of the feline Leukaemia virus and mimic a natural pathogen that will not cause disease. This stimulates the animal's immune system to produce a strong and specific anti-FeLV antibody that protects against the virus.

Prevention of viral diseases in animals (e.g., porcine reproductive respiratory syndrome - PRRS): mRNA-based vaccines hold promise for livestock, especially in the prevention of PRRS and other viral diseases in mice (Zhang et al., 2021). PRRS and mRNA vaccines can be designed to encode the major antigens of the PRRS virus. When fed to mice, the mRNA is translated into viral antigens in mouse cells. The administration of this preparation with a series of injections incites an immune response to the development of anti-PRRS antibodies. mRNA vaccines are developing very quickly but on high standards that are necessary to be coped with in combating new pathogens in animals.

mRNA-Based Immunotherapy Procedures

mRNA Delivery: This vaccine is usually administered by intramuscular injection. Messenger RNA (mRNA) is inoculated in the musculature; the host cell migrates and locates itself to a certain niche position near the area where it was injected. The vaccine serves as a blueprint to produce synthetic mRNA that carries antigenic information from the target pathogen.

Processing and translation of target antigen: The host machinery processes the mRNA and translates it into target antigens for presentation on the surface of infected host cells. Normally, these antigens could be viral proteins like capsids, or spike proteins in the case of viral infections.

The ones that are newly synthesized appearing on the surface of the host cell show that immune response is kick-started; more simply put, they function as flags that an invasion has taken place. In reaction, the immune system goes into overdrive, making antibodies and training T cells to destroy them.

Memory of Cells: Vaccinations based on messenger RNA also influence Memory of Cells. Immunity is maintained over time because these cells "remember" antigens. An animal's immune system may swiftly develop targeted defences in the event of future infection.

Benefits of Veterinary Vaccines Derived from mRNA

A particular disease may be swiftly and effectively targeted using mRNA vaccines. For the sake of veterinary disease prevention, these alterations are crucial (Zhang et al., 2021). With mRNA vaccines, antigens may be selected with great precision, directing the immune response to target specific portions of the pathogen. There is less chance of infection with mRNA vaccinations since they do not employ live viruses. When applied to animals, they do not pose any health risks. Veterinary applications and companion animals: mRNA-based vaccinations may be used to protect farm animals (such as pigs and cattle) and companion animals (such as cats and dogs) against a variety of viral infections.

Rapid development, accurate antigen selection, and increased safety are three ways in which mRNA-based vaccines might change veterinary medicine forever. It offers an efficient method of reducing infectious disease outbreaks in livestock populations and may be used to reduce viral illnesses in both companion animals and cattle. With the ongoing research in this field, mRNA-based vaccinations are expected to take centre stage in the fight against and control animal illnesses.

Animal Models in Vaccine Development

Vaccine research and development greatly benefit from the use of animal models of human immunity. In most cases, the immune systems of the animals are altered genetically to resemble that of a human being. It is mainly used to test possible vaccine candidates before heading to clinical trials in animals targeted for vaccination. Preclinical animal testing modelling human immune responses may help predict safety and efficiency for animals such as pets, cattle, and the wild (Melkus et al., 2006).

An animal model of human immunity allows a more scientific analysis of diseases but under regulated conditions; this makes them obtain information regarding the disease processes and pathogen interaction as well as immune responses which are the basic components that will help in guiding the design of effective vaccines. Animal models rank as the most important component for assessing a number of possible candidates for vaccination. These are subjected to a rigorous testing process to ascertain safety and effectiveness before vaccination is carried out amongst animal populations. An animal model is one important way of checking the efficacy of the vaccine candidate on protection and induction of the appropriate immune response against the target infection. On the other hand, animals use these models to decide whether a given potential vaccine may be safe or not. It is a part of general vaccination safety, potential side effects, and adverse event screening (Shultz et al., 2007).

Applications of Animal Models in Veterinary Vaccine Development

These vaccines are therefore screened on companion animals, among them horses, cats, and dogs. For example, vaccines can be used in assessing the success of canine parvovirus vaccinations in prevention and guarding against the virus.

The agriculture sector majorly relies on livestock. For instance, some vaccinations can be tested with their use as models of pigs, chickens, and cattle. A model used in the testing of economically significant illnesses such as foot and mouth and respiratory disease in pigs, it might be the model itself (Dwivedi et al., 2012; Zhang et al., 2013). Use animal models to benefit wildlife conservation efforts. Vaccines that have been developed for populations of endangered animals or for infectious diseases to which certain animals are particularly susceptible, like distemper, may also be tested on experimental animals for safety (Daszak et al., 2000, Wolcott et al., 2000).

What a Humanized Immune System Animal Models Operate

Animal models of human immunity are developed using genetic alteration. It is an attempt at mimicking the effectiveness of an immune system through the insertion, deletion, or mutation of some genes that are related to these immunities. Mice can be developed to yield those antibodies and their components which are normal products in human beings. Sometimes animals are adapted as a model of humans after having implanted in them human tissues or cells. To imitate the immunological reaction of people, humanized mice are administered human bone marrow or thymus tissue (Rongvaux et al., 2014). After the construction, these animal models are immunized with the respective vaccine and then deliberately faced by the target pathogen to prove the efficiency of the vaccine. Terms of resistance or immunity to the disease, immunogenicity measures, as well as security, are also evaluated.

Most of the vaccines developed for animals have been developed along with or based on animal models of human immunity. Most likely, they may first research animal diseases to determine potential candidates for vaccines among domestic animals, cattle, and wildlife, besides finding out its safety and efficiency before introducing a vaccine for specific populations. By adoption of this model in the protection and knowledge of animal health, it will be possible to do this job in a very perfect way.

CRISPR Technology in Vaccine Design

Application of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system is highly promising in precise genetic modification, appearing now also as an effective tool in the preparation of animal vaccines. Thus, enhancement

of the immunogenicity of antigens or preparing more effective or less dangerous vaccines based on live attenuated strains of viruses becomes feasible with the help of CRISPR. Using CRISPR, antigens in a vaccine can have their genetic composition remodelled in ways that greatly enhance the potency of stimulating a much stronger and much more targeted immune response. This could include upregulating levels of epitope presentation or even altering programming for improved antigen-antibody recognition or even designing specific epitopes which enhance responses to Abs. Further, weakened vaccines can be delivered that are equipped to elicit disease responses. Where safety concerns are paramount, the use of the method becomes valuable if after where safety concerns are paramount, CRISPR could contribute to drug development and therapeutics if it is used to improve antigen presentation to effectively vaccinate against specific viral diseases in veterinary. Researchers can rapidly target vaccines to respond to potentially harmful new viruses, which is especially important for viral diseases affecting animals and humans Although CRISPR offers precision in genetic analysis in 2010, safety assessment, ethical considerations, and regulatory oversight are essential elements to ensure the safety and efficacy of these new vaccines.

One Health and Global Immunization Efforts

The one health approach recognizes the interplay between human, animal, and environmental health, with an emphasis on communicating strategies for the prevention and control of diseases affecting humans and animals. This approach recognizes that diseases in different populations and the need for concerted efforts to effectively prevent disease, Ebola and many others Infectious diseases are animal vectors of disease, emphasizing the importance of human and animal emphasize health togetherness. One Health emphasizes early detection and surveillance of diseases at the human-animal-environment interface, monitoring human and animal diseases to identify potential outbreaks and prevent their spread. Vaccination is recognized as a key component of preventing viral diseases in One's Health strategies, including to reduce and protect public health, vaccination programs targeting human and animal populations have been developed (Mackenzie et al., 2013).

Case Studies of Successful One Health Vaccination Campaigns

Successful One Health vaccination campaigns have been critical in the prevention of various viral diseases. For example, in areas where rabies is endemic, One Health vaccination campaigns target dogs and humans to significantly reduce human rabies cases through herd immunity in dogs and responsible pet ownership promoting use as well as monitoring, and early detection through One Health vaccination campaigns in poultry farms to prevent avian influenza outbreaks, reducing the risk of human infection and health about vaccinating personnel during One Health's procedures for Ebola virus outbreaks, human and animal populations monitored to prevent the spread of the virus These are examples of One Health vaccination campaigns publication of coordinated veterinary disease prevention and human animal protection health efforts (World Health Organization, 2020).

Future Directions

Predictive Vaccinology and Disease Eradication Goals

Predictive Vaccinology

Predictive vaccinology is a revolutionary approach to vaccine development that uses advances in genomics, computational biology, and immunology to predict and develop vaccines with incredible accuracy. This approach has made a difference in vaccine design and greatly accelerated vaccine development. Here’s a look at how predictive vaccine science is changing the landscape of vaccine development:

Leveraging Genomics

Whole viral genomes are now open for new avenues of prediction with the vaccination candidates in hand. Important antigen determinants would allow the narrowing down of genomic data elements from the virus that are most worthy of being used in vaccines.

Biological Computation

This process will have much to do with computational methods and the application of technology in the forecasting of probable vaccines. Bioinformatics is used for the better understanding of the immune system, for examinations of the bacterial genome, and findings of possible antigenic epitopes, thus avoiding many experimental needs at the vaccine development time.

Customizing Immunizations

Individuals’ unique vaccination requirements may be satisfied with the help of predictive vaccinology. To maximize the effectiveness of vaccinations, it is necessary to comprehend the genetic diversity among the host population.

Progress Acceleration

The time it takes to produce vaccinations is reduced via predictive vaccinology. Researchers may speed up the implementation of vaccinations by accelerating preclinical and clinical studies once they swiftly identify the most promising candidates.

Disease Eradication Goals

On the forefront of global health agendas have been ambitious targets for disease prevention. These objectives seek to eradicate certain diseases by implementing extensive immunization programs. Key elements of preventative aims include the following:

Campaigns to Get Rid of Polio

The Global Polio Eradication Initiative is one of the best examples of an attempt to stop diseases before they happen. The initiative has made significant strides in reducing polio worldwide through widespread vaccination campaigns. But challenges remain, including reaching vulnerable and conflict-affected populations.

Eliminating Guinea Worm Disease

Guinea worm disease caused by *Dracunculus medinensis* is on the verge of extinction. Extensive efforts to provide safe water supply, monitoring and prevention have led to a dramatic reduction in incidents.

Measles Elimination

Goals of gonorrhoea prevention goals are to reduce the incidence of gonorrhoea disease to a point where it no longer circulates within a region. Vaccination campaigns, routine vaccination and surveillance are important components of smallpox prevention strategies.

Interdisciplinary Collaboration Opportunities

Cross-Species Collaboration

Since diseases have a human-animal relationship, cooperation in human-veterinary vaccination is essential. The main features of interracial harmony are:

Zoonotic Diseases

Many diseases, including avian influenza, Ebola, and smallpox, can be transmitted between animals and humans. Collaboration can also lead to shared strategies and research for the prevention and control of viral diseases.

Shared Knowledge

The exchange of knowledge and insights between humans and veterinarians with vaccines can lead to more effective vaccines and better disease prevention strategies.

Include examples of how people have worked together successfully to stop viral diseases, like the "One Health" approach to the flu virus or the group effort to fight new pandemics.

Global Health Partnerships

To overcome obstacles to immunization and reach global safety objectives, global health collaborations are essential. These relationships mostly consist of:

Various Global Groups

Assuring access to vaccinations and supporting research, organizations like WHO, UNICEF, and the GAVI Alliance play a crucial role in global immunization efforts.

Public-Sector Partnership

Immunization programs, border disease monitoring, and vaccine delivery all rely on intergovernmental collaboration.

Not-for-profit organizations and research centres

Global health relationships benefit from the information, funding, and new ideas that NGOs and study groups bring to the table.

Research on New Diseases

To study and deal with new viral diseases, people must work together, and foreign teamwork is very important.

Interdisciplinary Research Teams

To solve complicated health problems and enhance vaccination technology, multidisciplinary research teams consisting of specialists in immunology, genetics, epidemiology, animal science, and public health are necessary.

Extensive Knowledge

Emerging diseases that are transmissible, vaccinations, and other complex health concerns are the focus of these organizations' combined knowledge and experience.

Innovation

Interdisciplinary collaboration supports the development of new products and approaches in vaccine research, such as predictive vaccinology.

Addressing Complex Challenges

Teams are set to tackle the complex challenges at the intersection of human and animal health, including viral diseases and One Health programs.

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