Chapter 42

Bacteriophage Applications in Poultry Production and Health Management

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ABSTRACT

The poultry market, particularly chicken, contributes the most protein and macronutrients to the global diet without any religious or cultural taboos associated with it. Infectious agents such as *Salmonella, Campylobacter, Listeria, Staphylococcus spp. and Escherichia coli* are a threat to the poultry industry. There are 18 to 90% of poultry flocks in European countries that are infected with Campylobacter. A severe risk to health of human is posed by antibiotic use and misuse in the livestock and poultry industries that had led to the development of multi-drug-resistant pathogens in animals and transmission of antibiotic resistance genes (ARGs) from animals to humans by the ingestion of animal products. Phage therapy is successful when used at the right time, in the right amount, with the right delivery system, and in combination with other therapies. Bacteriophages are being used in poultry production for the first time, but it will take time to gain a deeper understanding. This book chapter discusses Bacterial Challenges, Bacteriophages' roles in control, food security and safety, molecular applications, antibiotic resistance, and the future of poultry production.

KEYWORDS
Poultry production, Bacteriophages, Antibiotic Resistance, Bacteria

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INTRODUCTION

Bacteriophages, often identified as phages or BPs, are viruses which particularly target and infect arachea or prokaryotes. Frederick Twort as well as Felix d'Herelle made discoveries of bacteriophages in 1915 as well as 1917, individually (Koskella et al., 2022). Through an approximate total no. of 1031 phage molecules in the biosphere 10X more than the estimated number of bacterial cells on Earth—they are incredibly commonplace worldwide (Gómez-Gómez et al., 2019). Bacteriophages are generally considered safe for humans, but their safety is not universally accepted without reservation. Since then, phages have been applied in clinical settings. With the exception of many Eastern Bloc nations, phage treatment was completely replaced in the Western world with the discovery of penicillin, which signaled the start of the antibiotic era. Lately, there has been a renewed focus on antibiotic-resistant bacterial species due to their rising prevalence (Chopra, Hodgson et al., 1997; Sulakvelidze, 2004).

The long-term efficacy of traditional antibiotics as well as human health are seriously threatened by germs that are resistant to many drugs (Cars et al., 2008) Thousands of people die from illnesses brought on by bacteria resistant to antibiotics per year in the European Union alone. Gram-negative bacteria, for example *Acinetobacter baumannii, Pseudomonas aeruginosa,* as well as *Enterobacteriaceae,* that includes *Klebsiella pneumonia* as well as *Klebsiella pneumonia*, are responsible for two-thirds of these deaths.

The speed at which bacteria are evolving and becoming resistant to antibiotics has led to a concerning state of affairs worldwide. But as a result, there is now less interest in the study and creation of new antibiotic chemicals for the pharmaceutical industry. Let's say, the Food and Drug Administration in the United States approved sixteen novel antibiotics between 1983 and 1987; between 2010 and 2016, this number dropped to only six (Luepke et al., 2017). Due to the negative health effects of the carbapenem class of antibiotics, only two antibiotics have been approved for commercialization by FDA and European Medicine Agency (EMA) in the last 20 years, and there is a global need for new antimicrobials. Consequently, the majority of big pharmaceutical corporations no longer want to invest in the creation of novel antibiotics.

Antibiotic resistance was deemed "the greatest and most urgent global risk" in a conference called by the UN General Assembly on September 1, 2016, given the gravity of the problem (Mattar et al., 2020).

Viruses known as bacteriophages exclusively infect bacteria. In contrast to filamentous and temperate phages, lytic phages proliferate inside the bacterial cell and lyse it at the conclusion of their life cycle to release freshly generated phage particles. After attaching itself to the surface of a vulnerable host cell, the phage virion injects its genome, taking over most of the host metabolism as well as assembling the molecular machinery needed for phage replication and assembly (Clark and March 2006; Skurnik and Strauch 2006). Bacteriophages differ in their structural makeup. Phage virons can have filamentous, pleomorphic, polyhedral, or tails. The majority have single- or double-stranded RNA (ssRNA, dsRNA), double-stranded DNA (dsDNA), and single-stranded DNA (ssDNA) in lower amounts. Tailored phages make up around 96% of all phages and are the most common form of therapeutic phage (Ackermann 2001) shown in Fig. 1.

The potential of phages to destroy dangerous bacterial strains in situ and reproduce exponentially might be crucial for the treatment of infectious illnesses, as well as allow for shorter delivery times. While bacteriophage treatment offers several benefits, this method is not without its restrictions (Specificity of phage, phage-bacteria co-evolution and regulatorily hurdles). The limits of phage treatment resulting from the advent of phage resistance and the occurrence of bacteriophage insensitive mutants were also covered by (Hyman and Abedon, 2010). Like antibiotic resistance, phage resistance develops at a similar rate, and lytic, virulent, broad-spectrum phages that are ideal for treatment are hard to identify and cultivate. Phage treatment relies on a clear bacteriological diagnosis. However, there are concerns about potential side effects and unfavorable immune responses, particularly after repeated exposure.

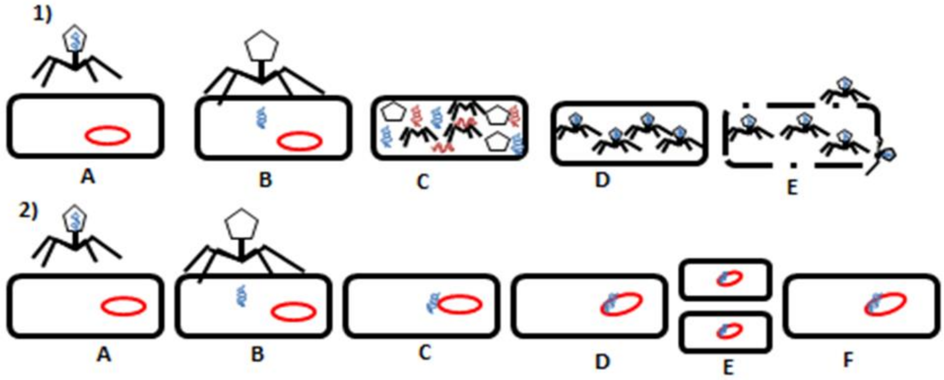

Fig. 1: Bacteriophage life cycle (1) lysogenic Cycle (2) Lytic Cycle

Understanding Bacterial Challenges in Poultry Production

The world's food production and sustainability are presently confronting an unprecedented challenge because of the expanding human population. Chicken health and safety continue to be serious problems that require prompt attention, even if it is acknowledged that the chicken industry is one of the maximum effective and quickly expanding food businesses to solve this challenge. Bacterial illnesses such necrotic enteritis, colibacillosis, and salmonellosis have become more prevalent in chicken farming. Similar to this, underdone poultry adulterated with zoonotic bacterial illnesses like Campylobacter, Salmonella, plus Listeria can cause outbreaks that are extremely dangerous for the public's health.

Bacteriophages are becoming more and more acknowledged as a desirable natural antibacterial substitute in light of the subject of antibiotic resistance or the limited usage of antibiotics in animals raised for food. Recently, bacteriophages have demonstrated encouraging results in the treatment of poultry illnesses, the reduction of carcass contamination, and the improvement of chicken product safety. Technologies that are crucial for bacteriophage interaction with bacterial hosts have been effectively used to precisely illustrate bacteriophages as well as its genes/proteins. This chapter explores the possibility of utilizing lytic bacteriophages to reduce the risk of main bacterial infections associated with poultry. The difficulties in getting companies to embrace this technology are also covered in this paper.

Public health concerns had drained more consideration to pathogens for example due to the risk that poultry poses as a source of such pathogens, *Salmonella enterica* subspecies enterica serovar *Enteritidis (S. Enteritidis*), *Salmonella enterica* subspecies of enteric serovar *Typhimurium (S. Typhimurium), Eschericha coli (E. coli), Listeria monocytogenes (L. monocytogenes*), as well as methicillin-resistant *Staphylococcus aureus* (MRSA) have been identified (Mor-Mur and Yuste, 2010). Most researches had examined the effectiveness of bacteriophages in reducing bacterial counts and controlling bacterial illnesses in poultry, that are zoonotic and ensure a substantial impact on public health (Żbikowska et al., 2020). As per the latest report by the European Food Safety Authority (EFSA) as well as the European Centre for Disease Prevention and Control (ECDC) (2019), and most commonly reported zoonosis in the European Union (EU) were campylobacteriosis, salmonellosis, yersiniosis, and E. coli infections that produce Shiga toxin (STEC) (EFSA, 2015). Individual the lytic bacteriophages are appropriate for phage therapy, which is used to treat bacterial illnesses, due to their limited capacity to destroy bacteria. Antibiotics are not nearly as specific as bacteriophages. It is important to remember that antibiotic therapy alters the normal gut microbiota in addition to eliminating harmful bacteria, which may cause dysbiosis, immunosuppression, moreover ensuing infections (Lin et al., 2017). Therefore, new bacteriophage therapies are a great way to treat bacterial infections in chickens because these therapies' have greater specificities, reduce antibiotic resistance and also involve in food safety (Fig.2).

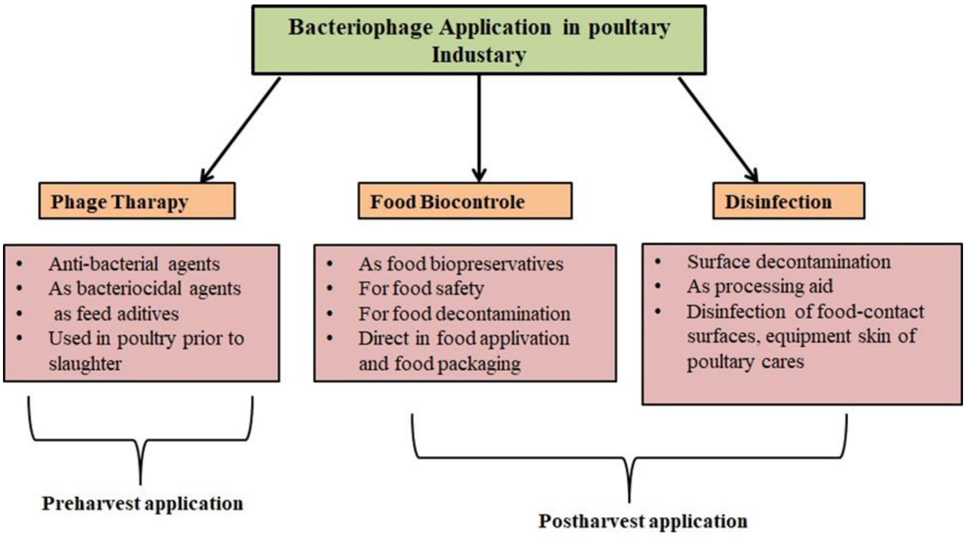

Fig. 2: Bacteriophage application

Role of Bacteriophages in Controlling Poultry Production

A phage attaches itself with a bacterium during infection, then introduces its genome into the cell. Resulting that, a phage usually goes through either the lytic (virulent) or lysogenic (temperate) life cycles. In order to produce phage components, lytic phages commandeer the cell's machinery. Afterwards, they lyse, or kill, the cell, releasing fresh phage particles. Lysogenic phages proliferate as a unit with the host cell by integrating their nucleic acid within its chromosome, all without causing the cell to die. It is possible to cause lysogenic phages to adhere to a lytic cycle in specific conditions (Dennehy and Abedon, 2021).

Here are more life cycles, for example persistent infection besides pseudolysogeny. A bacteriophage enters a cell during pseudolysogeny, but it neither permanently integrates into the host genome nor hijacks the machinery responsible for cell replication. When a host cell experiences unfavorable development conditions, pseudolysogeny takes place. Because it permits the phage genome to be maintained until the host's growth circumstances are favorable once more, this procedure appears to be essential for phage survival. Long-term, continual production of new phage particles occurs in chronic infections, yet there is no discernible cell death (Elois et al., 2023).

Bacteriophages are formed of basic genetic material, which could be either single- or double-stranded, then wrapped in a protein capsid. The three main phage structural forms are a filamentous form, an icosahedral head which has a tail, and an icosahedral head lacking a tail (Naureen et al., 2020).

The most popular meat consumed worldwide is poultry, especially chicken, which is also a significant source of high-quality protein and macronutrients without being associated with any social, religious, or cultural taboos. The demand for beef and pork can rise by 66 and 43%, respectively, between 2005 and 2050, but poultry is predicted to grow at the fastest rate—121%—becoming the most consumed meat globally over the next five years, corresponding to the Organization for Economic Cooperation and Development/Food and Agricultural Organization of the United Nations" (OECD/FAO, 2016).

While the poultry industry has seen impressive growth, this close relationship between humans and birds can also increase the risk of food-borne illnesses like salmonellosis and campylobacteriosis due to bacterial contamination. This contamination also shortens the shelf life of poultry meat, making it highly perishable.

The presence of pathogenic microorganisms in the animal prior to its slaughter at the farm of origin and cross-infection from the processing and production settings that come into touch with the contaminated animal or corpse can easily lead to contamination Moreover, inadequate pathogen control strategies and imprecise pathogen detection methods now in use at farms and/or processing facilities may be the root cause of the majority of diseases associated with poultry (Fister et al., 2019).

Furthermore, it is common to find pathogenic or spoilage bacteria residing on a variety of biotic and abiotic surfaces as sessile colonies embedded in biofilms. In industrial settings for poultry, biofilm development on work surfaces poses a severe risk, since the spread of these structures may allow dangerous germs to be released, which might contaminate and ruin carcasses.

As a result, the poultry sector has several difficulties in ensuring the safety of its products. The chicken industry has used a variety of pathogen-reduction intervention techniques over the past 20 years. Many compounds, such as chlorine and cetylpyridinium chloride (CPC), are rarely classified as generally recognized as safe (GRAS). Other tactics, like organic acids, while frequently successful, can have detrimental organoleptic effects (Hashem and Parveen, 2016).

Physical methods have been utilized extensively in the processing and production of chicken because they are successful in reducing the bacterial load on broiler carcasses. These methods rely on thermal treatment, ionizing irradiation, ultraviolet (UV), and high-pressure processing. They may, however, alter the meat's chemical and physical characteristics as well as bring about unfavorable alterations to its texture, flavor, and color.

To guarantee microbiological food safety, several biological treatments have been tried along with chemical and physical ones for inactivating harmful bacteria in chicken. Alternative approaches are emerging that utilize natural preservatives. These preservatives can be derived from plants or animals and can be either naturally occurring or artificially altered. One promising option is bacteriocins, produced by lactic acid bacteria. Bacteriocins offer an antibacterial effect without compromising food quality (Han et al., 2022).

The poultry industry faces a challenge: balancing food safety with consumer concerns. The overuse of antibiotics in animal production has led to the emergence of new strains of antibiotic-resistant bacteria. This, coupled with consumer anxieties about residues from detergents and disinfectants used in food processing, has driven the search for safer alternatives. In response, the industry is increasingly turning to natural antimicrobial agents for decontamination. These agents, derived from plants or animals, offer a promising solution. They can be as effective as traditional methods while addressing consumer concerns and potentially mitigating the rise of antibiotic resistance. Phages provide a unique chance to attack pathogens in a variety of foods without altering the typical microbiota, physicochemical properties, or organoleptic qualities because of their uniqueness. As a result, phages have received a lot of interest for their potential use as bio-preservatives and as an antibiotic substitute for the management of food-borne bacterial infections (Fig. 3).

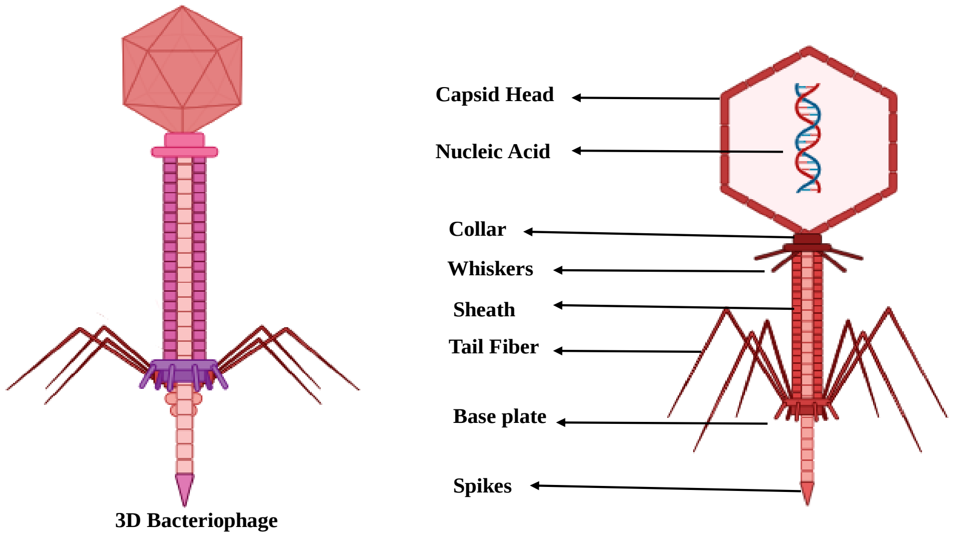

Fig. 3: Structure of bacteriophage.

Bacteriophage and Food Security/Safety

Foodborne infections found in the stomach and hide of agricultural animals that produce food are the main cause of cross-contamination in the food chain. Before cattle are slaughtered, target disease populations on and within them can be effectively decreased using on-farm phage-based control techniques. On the other hand, a target pathogen's incidence and persistence might change between animals, within a herd, or in various parts of the same animal.

In grill birds intended for slaughter, *C. jejuni* is common, while *E. coli* O157 super-shedders have been found in up to 20% of cow herds. It can be difficult to distinguish between transitory shedders and super-shedding animals within a herd, and it may take several significant sample episodes spread out over a lengthy time before phage therapy. A target pathogen may colonise the rumen, cecum, colon, and rectum, among other settings in the cow gut. The location of the colony within the digestive system affects the effectiveness of phage-based therapies.

This revision eliminates redundancy while still conveying the need to understand how phages remain stable throughout their use. It is necessary to optimize individual phages or cocktails in order to infect specific bacteria in a variety of settings and biofilms (Bumunang et al., 2023). Before phage application, comparative genomics may distinguish, monitor, and offer important information about likely bacterial variations within a specific pathogen population.

For phage applications to be used in agriculture and human therapy, a deeper comprehension of phage viability, stability, and survival in a variety of challenging conditions is needed. The Myoviridae or Siphoviridae families of phages were all employed in the experimental investigations. The majority of phages were extracted from their intended host and demonstrated to be stable and infectious during the course of therapy.

It is well known that certain phages belonging to these families exhibit exceptional resilience in harsh conditions, including desert surface sand that is subjected to extreme heat and cold (Zampara et al., 2017). The majority of research on phage-based L. monocytogenes management in food items has been on beef, pig, and poultry that are ready to eat. Food safety may be threatened by L. monocytogenes enrichment from cold storage. A potential remedy for managing Listeria monocytogenes in food items is the use of phages. Since silage is a frequent source of L. monocytogenes, using phages to target this source may be a useful strategy for stopping transmission of the infection on farms. It may be less expensive to target feed rather than to directly manage L. monocytogenes in cattle. Phage cocktails should be made with phages that are resistant to a broad variety of pH levels and temperatures for optimal effectiveness (Table 1).

Table 1: An overview of research on bacteriophages used to manage food-borne infections in/on animals used for food

Target specimen	Target Bacteria	Phage/Family	Phage/Mixture	Phage Dose	Phage Delivery Route	Efficacy	References
36-day-old chick	*C. jejuni*	NCTC12672, 12673, 12674, and 12678/Myoviridae	Cocktail	7.2 and 7.9 PFU/mL	Oral	A 3.2 $\log_{10}$ CFU/g	Kittler et al,2013
25-day-old chick	*C. jejuni*	CP220/*Myoviridae*	Single	10^7 and 10^9 PFU/mL	Oral	A 2.0 $\log_{10}$ CFU/g reduction 2 days post-treatment	El-Shibiny et al, 2009
24-day-old chick	*C. jejuni*	CP20 and CP30A/*Myoviridae*	Cocktail	10^7 PFU/mL	Oral	A reduction of up to 2.4 $\log_{10}$ CFU/g 2 days post-treatment	Richards et al,2019
4-day-old chick	*S. enterica* serotype Enteritidis	CNPSA1, CNPSA3, and CNPSA4/Nd[1]	Single	10^{11} PFU/mL	Oral	A reduction of 3.5 orders of magnitude of CFU/g 5 days post treatment	Fiorentin et al, 2005

The Reduction of Salmonella in Chicken Skin

The purpose of the study was to find out how bacteriophages and sanitizers affected chicken skin that had been experimentally infected with *S. enteritidis*. A randomized full block design with repetition was used in the trial, where treatments were organized into ten blocks, each including three duplicates. Using sterile, disposable spreaders, the chicken skin portions were equally disseminated over both sides after being injected with 105CFU/cm2. The parts were split into six batches, each including thirty portions, once they had dried. Three batches of samples were submerged in decontamination solutions, including lactic acid, peracetic acid, and sodium dichloroisocyanurate. In a separate batch, samples were submerged in sterile distilled water, phage cocktail, or untreated control (Oliveira et al., 2009). In order to replicate industrial settings, all decontamination chemicals were chilled to 6°C. Following treatment, slices of chicken skin were placed in sterile stomacher bags, placed in solutions designed to inactivate each agent, mechanically agitated (stomaching), and serially diluted. Sections treated with lactic acid were put to phosphate buffered saline, while sections handled with sodium dichloroisocyanurate or peracetic acid had been added to buffered peptone water. Saline was used to dilute skin samples. *S. enteritidis* counts were quantitatively determined using the droplet technique, which involved depositing successive dilutions onto XLT4 agar plates and incubating them for six to eight hours at 37°C. The numbers on S. enteritidis plates were given in CFU/cm^2.

Phage Sensitivity of Salmonella Recovered from Chicken Skin

S. enteritidis colonies were removed from chicken skin that had been phage-treated in order to determine whether there was any resistance to the five phages used in the decontamination treatment. The drop-on-lawn technique described before was used to evaluate the phage resistance of S. enteritidis isolates. After an 18-hour incubation period at 37 °C, the plates were examined for the presence of phage plaques, which shows phage sensitivity (Hungaro et al., 2013).

Bacteriophage Applications in Poultry Production

Food sustainability and safety are crucial challenges in the global food industry, as Western countries increasingly consume organic foods. The demand for food rises as a result of the expanding world population, which is predicted to reach 9.7 billion by 2050 and 11.2 billion by 2100. This puts pressure on the food business to adhere to food safety laws. Every year, 600 million individuals are afflicted with foodborne diseases, which lead to 420,000 fatalities and more than $110 billion in economic losses. Despite advances in technology, manufacturing practices, and hygiene, microbial safety problems persist, such as emergence of antibiotic resistance in bacteria and food borne illness. The industry is further burdened by the restricted use of specific antibiotics during the production of food animals and the dearth of novel antimicrobials. The FDA has approved food safety products from many commercial firms that use phage-based solutions to combat major food-borne diseases. The industry's faith in the effectiveness and security of phage-based preparations is demonstrated by this advancement.

Since bacteria, including phages, are naturally occurring, benign, and widely distributed in the environment, they are excellent choices for pathogen identification and management in the food production process. Salmonella serovars, Escherichia coli, and other major food-borne pathogens have recently been profitably controlled with phage-based products. These products have been approved by the FDA for food safety, and several commercial phage businesses have obtained the classification of Generally Recognized as Safe (GRAS). This study focuses on the most current developments in phage biocontrol in the food industry (Fig. 4).

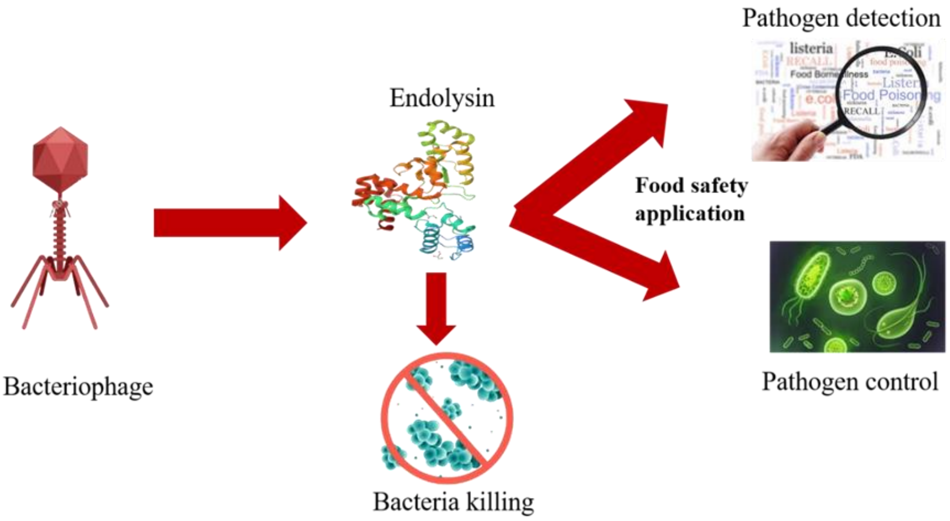

Fig. 4: Role of bacteriophage in food safety.

Since their discovery in 1915, bacteriophages—harmless viruses that infect bacteria—have found extensive application in both veterinary and human medicine as well as agriculture. They have the ability to incorporate genetic material into a bacteria's chromosome without causing cell death or lysis of cells to release viral particles. Because of their high specificity, replication by itself, self-limiting nature, ability to adapt continuously to modify host infrastructure, low inherent toxicity, ease of isolation and propagation at a low cost, resistance to environmental stresses during food processing, and extended shelf life, phages present advantages as biocontrol agents. They are widely distributed in food and have been shown to be absent from a number of processed, raw, fermented, and seafood goods. Phages are found in the same settings that their bacterial host(s) currently reside in or were formerly present, and people eat them on a regular basis. However, due to their potential to contribute to the decreasing effectiveness of antibiotics utilized for treating bacterial infections in humans and the development of superbugs like Salmonella DT104 or methicillin-resistant as well as multidrug-resistant Staphylococcus aureus, the use of antibiotics in farm animals has become a serious concern. Therefore, phages are a potential solution for food safety (Guenther et al., 2012).

Phage treatment, also known as minimizing pathogen colonization in livestock, is a key production technique that lowers the risk of cross-contamination with animal feces during food processing. It can be applied either during animal growth or prior to animal slaughter. For example, it is predicted that a two-log reduction in the quantity of Campylobacter in poultry intestines will be sufficient to lower the frequency of campylobacteriosis associated with chicken meat consumption by a factor of thirty. For a number of infections, phage treatment in animals has previously proven effective. Phages can be sprayed on to target pathogenic E. Coli in poultry, orally/rectally applied to control E. Coli in ruminants, orally administered to treat Salmonella and Campylobacter within poultry, and mixed into drinking water or food (Fig. 5) (Goodridge and Bisha, 2011).

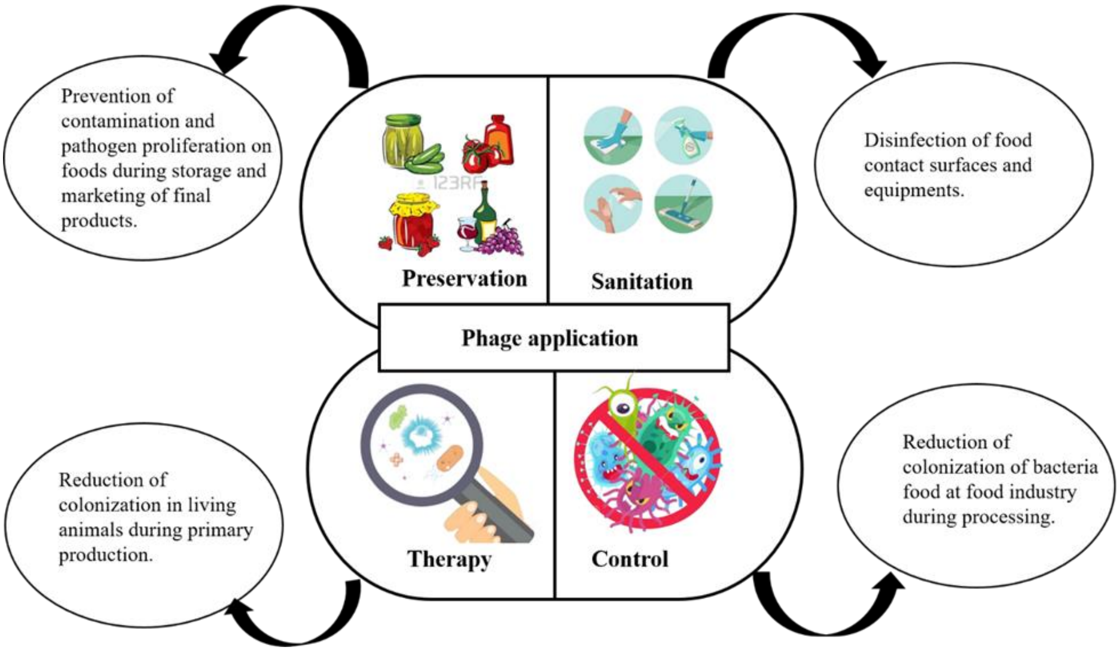

Fig. 5: Uses of phages to enhance food safety across the food chain.

Phages could be used to reduce colonization on food contact surfaces during industrial food processing. Their efficacy diminishes when applied to non-growing bacteria, but they remain powerful against actively developing ones. Phage titers that are high can be used to suppress infections that replicate as soon as food starts to warm up or that use "lysis from without" methods. Biofilms are frequently observed on surfaces used in the handling, storing, and processing of food, especially in hard-to-clean or sterilize locations, including tiny pipe systems, uneven surfaces, and complicated machinery crevices. Phages have demonstrated potential in mitigating viable cells against in vitro biofilms produced by spoilage and pathogenic bacteria in optimal conditions, which are defined as controlled environments with optimal temperature, pH, and nutrient availability; these attributes are indicative of those that facilitate biofilm formation in real-world scenarios. However, because bacteria vary widely in different environments, using them for bio-sanitation is difficult (Premaratne et al., 2021).

Phage lysing systems have been demonstrated to lyse hosts at as low as 1°C, making them suitable agents for food bio-preservation since they prevent the development of harmful and spoilage bacteria, especially psychrotrophic bacteria, on chilled foods. Phages can further regulate the growth of these bacteria once the meals are brought to room temperature (Sillankorva, Oliveira and Azeredo, 2012).

Bacteriophage Applications in Poultry Production

Viruses known as "bacteria eaters," as their name roughly translates, are known to attack and infect bacteria. Twort and D'herelle independently discovered bacteriophages in 1915 and1917, respectively (Duckworth, 1976). The most prevalent creatures on Earth are bacteriophages. Similar to other viruses, bacteriaphages need a host cell in order to multiply. Most phages are extremely selective and only have the ability to infect a small number of closely related bacteria. While bacteriophages are capable of killing bacteria, they are unable to utilize any resources from a deceased organism. Rather of being thought of as predators, bacteriaphages are really parasites. The bulk of gut viral genomes (97.7%) are composed of phage viruses, with eukaryotic (2.1%) and archaeal (0.1%) viruses following in order of prevalence (Abd-El Wahab et al., 2023).

Poultry, particularly chicken, is the most widely eaten meat in the world. It is also a substantial source of high-quality proteins or macronutrients and is not taboo in any religious, social, or cultural context.Most cases of Campylobacter and Salmonella are in chicken (Han et al., 2022).

Bacteriophages as Antibiotic Resistance

These bacteria *Salmonella enterica, Campylobacter jejuni, E. coli*, as well as *Staphylococcus aureus* causes diseases in poultry. In the chicken business, bacteriophage-based treatments have also been studied as an antibiotic substitute. Because of their extreme selectivity, bacteriophages could only be able to target a particular bacterial infection within the afflicted animal. However, in typical scenarios with many clinical strain infections, a specially blended complex cocktail of various bacteriophages might increase their antibacterial efficacy. Bacteriophages could be utilized as safe sterilizers in industrial settings to lessen adulteration on contact surfaces that come into contact with food or on chicken carcasses, in addition to their application in decreasing bacterial contamination in animals (Abd-El Wahab et al., 2023).

Examples in Industrial Use

Salmonella

Concerns from the public about strains that are resistant to antibiotics, particularly in zoonotic infections such as Salmonella, have prompted the chicken industry to find alternate forms of management. Because many of the ensuing food-borne illnesses are connected to chicken goods, minimizing microbial contamination during the manufacturing of poultry is essential. Salmonella Enteritidis recovery in broiler chicks treated with bacteriophage mixtures may be temporarily reduced; however, 48 hours later, there was no difference between the treated or untreated groups. Furthermore, there was no difference in the amount of Salmonella Enteritidis between the bacteriophage cocktail and a probiotic culture as compared to bacteriophages alone. A thorough investigation has been conducted to determine if bacteriophages in chickens have the ability to suppress paratyphoid Salmonella and cause illnesses associated with the bacteria.

Phage *S. Typhimurium* strains F98 [type 14], Beauville (type 40), and 1,116 (type 141) are examples of bacteriophages. birds challenged with *S. Typhimurium* at a dosage of 1012 plaque forming units (PFUs)/mL and found that the death rate linked to *S. Typhimurium* could be reduced to 20% compared with 56% in the untreated group. Six hours after therapy, S. Typhimurium returned to its pre-disease levels, but it was not completely eliminated. Moreover, if Salmonella was present, the bacteriophages do not survive in the gastrointestinal system. Bacteriophages often only survived as long as they were added to feed orally. For bacteriophages to be successful, they need to be administered in high amounts right away following S. Typhimurium infection. When bacteriophages are administered in excess, they have the potential to kill *S. Typhimurium*. The hens' death rate was lower when they received phage therapy, but not when they were subjected to the Salmonella challenge.

Numerous times, it was shown that using the bacteriophage combination in drinking water was safe. The behavior of the birds remained unaffected, as did the production metrics. In contrast to the control henhouses, where Salmonella was still found, the proportion of Salmonella in cloacal swabs at the end of the fattening phase (33 day) was nil.

Campylobacter

A prevalent cause of recorded food-borne enteritis is infection with campylobacter (Chinivasagam et al., 2020). Rarely, campylobacter is seen in birds under the age of two to three weeks. There are significant differences in the prevalence of Campylobacter species in chicken flocks, with values ranging from 2 to 100%. The study's findings show that at the time of slaughter, chickens and broiler flocks have a 42.5–100% prevalence of Campylobacter spp. There is an urgent need to reduce the prevalence of Campylobacter because there have been more reports of the bacterial pathogenicity and antibiotic resistance to erythromycin, gentamicin, tetracycline, and fluoroquinolones.

The majority of Campylobacter-specific bacteriophages in poultry are found in the Myoviridae family, with a smaller percentage occasionally found in the Siphoviridae family. This suggests that Campylobacter colonization in poultry can be effectively suppressed by phage therapy, which lowers the likelihood of Campylobacter getting into the food chain. Research indicates that pre-harvest phage treatment is more effective over Campylobacter loads in the feces and intestinal contents of experimentally diseased chickens without having a bad impact on the health of the animals. For instance, 28 hours after treating 47-day-old chicks with a mixture of Campylobacter phages orally, the number of bacteria in the ceca dramatically reduced (1-3 log10 CFUs/g).

In comparison to the negative control, phage CP14 (5 × 108 PFUs) treatment resulted in a reduction in 20-day-old chicks over the course of 31 days. After two days of therapy, the amount of *C. jejuni* or *C. coli* in chicken feces was reduced by almost 2 log10 CFUs/g, thanks to oral gavage and the in-feed administration of a three-phage cocktail. On the other hand, phage-treated hens have reportedly regenerated certain resistant bacterial phenotypes, but the phages did not prevent the decline of Campylobacter. The proper phage selection, optimization of the delivery method and dose, and research on chickens are essential components of an effective phage treatment regimen to cure Campylobacter. (Abd-El Wahab et al., 2023)

Campylobacter spp. successfully colonize the gut after infecting the bird, primarily the mucosa of the cecal crypts. In order to evade clearance, it has the ability to infiltrate the intestinal epithelium and grow quickly in the intestinal mucus. Furthermore, the animal's weakened immune system allows it to survive in commensal settings, allowing the bird to serve as a reservoir for human campylobacteriosis (de Mesquita Souza Saraiva et al., 2022).

Non-antibiotic Substitutes Include Bacteriophages, Antimicrobial Peptides and Bacteriocins

Bacteriocins are proteinaceous substances that only kill the type of bacteria that produce them. Bacteriocin synthesis and activity have, for the most part, only been shown in lab settings. Most of the evidence supporting the function of bacteriocins in natural systems like the digestive tract is indirect. When regularly added to the water supply, a genetically engineered strain of avian Escherichia coli that generates the bacteriocin microcin 24 decreased intestine Salmonella typhimurium levels in chickens. The potential of intestinal bacteria to generate bacteriocins in vitro is supported by the isolation of Fusobacterium mortiferum from chicken ceca. The bacteriocin-producing Enterococcus faecium strain J96 was also isolated from a chicken crop and had some protective effect on chicks infected with S. pullorum, indicating that bacteriocins might be beneficial for the survival of the digestive tract (Joerger, 2003).

Despite this, bacteriophages were superseded by antibiotics in the management of bacterial illnesses. Because bacteriophages are very selective to a particular strain or bacterial species, they safeguard the remainder of the microbiota, making bacteriophage treatment safe. Like "intelligent" or "active" medications, bacteriophages can be administered as a single dosage, proliferate while bacteria are still present and decompose in the same manner as their target bacteria until they are both eliminated from the body. Bacteriophages remain attached with their host bacteria, unlike other antibiotics that might trigger allergies, and the immune system typically identifies and tolerates them without endangering humans or animals. Bacteriophages are cheap and simple to replicate. Co-administration of antibiotics and bacteriophages enables synergistic and optimal therapeutic outcomes. *Salmonella* was also treated when bacteriophage treatment was used to treat bacterial infectious illnesses in poultry. The following are some highlights of the use of bacteriophages against Salmonella.

- Single dosages of high titer bacteriophages are preferable than repeated low titer doses.
- The effectiveness of using bacteriophages to prevent infections may have decreased due to the emergence of resistance.
- The capacity of the bacteria to produce resistance determines the efficacy of bacteriophage treatment.
- Bacteriophage cocktails are preferable to single bacteriophages
- By decreasing bacteria spread and death, the synergy between probiotics and bacteriophages may enhance healing.
- Bacteriophages are employed in food treatment even though they are considered "generally regarded as safe" (GRAS) goods; nonetheless, in order to utilize them in poultry farms, production methods must be in place.

Enzymes that Hydrolyze Peptididoglycans

Bacteriophages have two different types of enzymes:

- Endolysins
- Virion-associated peptidoglycan hydrolases

In order to get bacteriophage genetic material into the bacterial cell, the bacterial cell wall must be broken down by virion-associated peptidoglycan hydrolases, or VAPGHs. Bacteria are lysed by endolysins, which are the enzymes generated during the last phase of bacteriophage replication. Because these enzymes use peptidoglycan as their substrate and function as antibiotics by lysing out bacteria, they are categorized as enzybiotics, hydrolytic enzymes with antibiotic activity.One or more catalytic domains can be used to distinguish between endolysins and VAPGHs; endolysins also have a cell wall binding domain (CWBD), but VAPGHs do not have one.

For Salmonella in Poultry, What Is Left to Consolidate Bacteriophage/Endolysin Therapy?

Within the industry, bacteriophage formulations for commercial feed that may include Salmonella are generally accepted as acceptable for use with chicken by-products and other high-risk feed. Regulating the use of bacteriophages to cure illnesses in people or animals remains unrestricted. Treatment with Bacteriophage/Endolysin Differs A customized treatment should be developed by testing each pathogen isolate for the particular bacteriophages/endolysins. Personalized therapies such as autologous somatic cell therapy and tissue engineering, as well as potential uses like bacteriophage/therapy, are referred to as Advanced Therapy Medicinal Products (ATMPs) by the European legislation. As more clinical studies demonstrate the effectiveness of bacteriophages or their hydrolytic enzymes in treating multidrug-resistant infections of Salmonella, their popularity as pharmaceutical options will undoubtedly grow, and the data they give will help build regulatory frameworks (Ruvalcaba-Gómez et al., 2022).

A. Prevention of Bacterial Infection

Many professions and industries, including food preservation, aquaculture, animal husbandry, plant preservation, and medicine, need control of bacterial presence or populations. Conventional antimicrobial chemotherapy is frequently used to accomplish this. Phage treatment is one potential substitute for antibiotic therapy, which is becoming increasingly required due to the growth in antibiotic resistance. However, because stringent national and international standards would need to be followed, it could be difficult to apply phages in medicine on a large scale. (Rotman et al., 2020).

Eating poultry products typically exposes one to campylobacter, one of the primary food-borne bacteria. The frequent presence of Campylobacter as a component of the microbial population in the gastrointestinal tract of chicken still poses a challenge to optimizing intervention strategies. Immunoglobulin (IgY) is specific to SE on preventing colonization in broiler chickens that have oral infections. Whole cell antigens from SE were used to induce hyperimmunization in commercial Single Comb White Leghorn (SCWL) chickens. The enzyme-linked sorbent assay (ELISA) was used to measure the levels of anti-Salmonella antibodies, IgG and IgY, in egg yolk and serum, respectively (Rahimi et al., 2007).

In poultry medicine, the application of exogenous cytokines against infectious agents has focused on three main areas:

- Using them as adjuvants for vaccines
- Directly preventing infections and/or the undesirable consequences of immune responses that pathogens elicit
- Stimulating the ontogeny and activation of newborn host defences (Kogut, 2000).

B. Treatment of Bacterial Diseases

Phage treatment has a higher success rate and is safer than antibiotics, in part because it is more specific to certain bacteria and can only infect a single species, serotype, or strain. The commensal bacterial flora is not destroyed by this process. Targeted treatments using phages are now being successfully employed to treat infections that recover slowly in both people and animals. In the US, they are also used to remove germs from the surface of meals derived from plants and animals. Bacteriophages can offer an alternate method of getting rid of infections in an era where antibiotic resistance in bacteria is increasing and antibiotic use is being restricted (Tiwari et al., 2011).

Salmonella

In chicken farms, salmonella infection is a serious issue. Phages of Salmonella were isolated from chicken feces. Once the host range of the phages was established, morphological characterization was performed using transmission electron microscopy inspection. One-step growth curves were then used to calculate the replication parameters and adsorption rates. Following that, the phage cocktail was made and evaluated for efficacy in three different settings: shavings, plastic surfaces, and drinking water. The findings show that the phage cocktail can reduce the quantity of Salmonella by up to 2.80 log10 units in drinking water, up to 2.30 log10 units in shavings, and up to 2.31 log10 units in plastic surfaces. It has been discovered that phage combinations are an effective alternative for reducing Salmonella infection in situations including chickens (Evran et al., 2022).

Hundreds of thousands of individuals worldwide suffer from salmonellosis, which can be fatal and cause severe fever and diarrhea. Due to its widespread significance, Salmonella has been the subject of monitoring systems in many nations, which have made it possible to gather crucial data regarding antibiotic resistance. More than 2,650 serovars of Salmonella enterica have been discovered too far, and a number of them are linked to major sources of illness and public health concerns, including chicken meat and eggs. Resistant Salmonella spp. in hens have the potential to induce occupational salmonellosis in farmers and keepers, in addition to causing financial losses.

Phage Treatment

Phage treatment was also found to be effective in preventing horizontal infections in flocks of laying hens caused by strains of *S. Gallinarum*. After being in contact with infected persons, hens treated with bacteriophages added to their feed saw a 5% death rate, but the group not treated with phage treatment experienced a 30% mortality rate (Tiwari et al., 2011).

Antibacterial Treatment

As demonstrated by a significant (about 80%) synergistic antibacterial effect of a commercial oral probiotic preparation applied in conjunction with a bacteriophage "cocktail" of phages S2a, S9, and S11 (5.4 × 106 PFU/0.5ml/bird) at 4, 5 and 6 days of age as well as at 8, 9 and 10 days of age to combat *S. Typhimurium* infections in poultry, bacteriophages may be used in combination with other preparations. Compared to challenged birds who were not given treatment, treated chickens with bacteriophages and a probiotic had ten times fewer bacteria in their spleen, liver, and caecum (Tiwari et al., 2011).

Challenges and Limitations

The commercial poultry business is always looking for innovative ways to fight avian flu. The first week saw an increase in bird mortality due to many bacterial illnesses, including coli septicemia, involving around 10 different bacterial species. Because of the persistent illnesses, this has an impact on the flock's output, consistency, and appropriateness for slaughter. Poultry disease syndromes caused by *Escherichia coli (E. coli)* include septicemia, respiratory tract infections, and infections of the yolk sac (omphalitis). Septicemia is the defining feature of E. coli infections in young chickens. While pericarditis, air sacculitis, and perihepatitis may be symptoms of the subacute type of septicemia, acute septicemia may be fatal. O1, O2, and O78 serogroups comprise a large number of *E. coli* isolates that are often recovered from commercial broiler chicks (Swelum et al., 2021).

Harmful bacterial infections cause significant mortalities, poor weight increase, and poor flock homogeneity, especially in the first week of the birds' life. Producers suffer financial losses as a result. Antibiotics that promote in-feed development and are prophylactic have long been used as a preventive measure to address persistent issues. (Swelum et al., 2021)

1. In addition to causing significant financial losses, these bacteria's pathogenicity also endangers public health. Scientists are now again interested in employing bacteriophages as antimicrobial agents due to the growing incidence of bacterial infections that are resistant to the majority of traditional antibiotics (Rotman et al., 2020).
2. The length of the bacteriophage's in vivo activity is known as the limitation of the therapy. Additionally, the lytic activity of bacteriophages declines. It takes 60 minutes for intravenously administered bacteriophage (T7 phage) to clear, while the half-life of λ-phage was found to be around 6 hours. Delivery mechanisms need to be designed to protect the phage from serum inactivation or acidic/alkaline pH in order to stop in vivo bacteriophage decay. Sustained bacteriophage releases by any biomaterial matrix locally implanted in the place of infection site could increase the effective therapeutic duration by eliminating the requirement for repeated phage infusion. This might be helpful when treating non-topical tissues like bone, where it is generally believed that intravenous phage injection will not result in surgical site closure (Rotman et al., 2020).

Future Perspective

Furthermore, as the world's population is expected to reach 9.7 billion in 2050 and10.9 billion in 2100, there will be a growing need for meat protein worldwide, which is linked to the rising demand for chicken meat. By 2030, South Asia's chicken meat demand is expected to rise dramatically (75%), particularly in nations like India, where intake is expected to rise from 1.05 to 9.92 million tons yearly during the next three decades. (de Mesquita Souza Saraiva et al., 2022)

Concerns over animal care, cleanliness and disease prevention that may arise from strong genetic pressure to increase meat and egg output are now quite high. The natural immunity and consequently illness tolerance of animals are negatively impacted by genetic pressure to increase their productivity. Improved illness prevention, dietary management, and husbandry techniques lead to genetic selection. Reduction of the market age by around 4 weeks, improved growth rate, increased breast yield, increased laying rate, and increased daily egg mass have all been achieved. There is, however, great concern that the above-mentioned selection pressure may have already sparked major animal welfare and disease issues. Increased selection forces also impede the freedom of animals.

Conclusion

Public health is also threatened by antimicrobial resistance, resulting in a reduction in production. Due to the elimination of pathogens by bacteriophages, antibiotics have been replaced as an effective alternative solution to treat infections. As a result, meat production has increased. The use of these phages in general and economically requires a lot of research, since several formats are used. The application of this novel technique has resulted in considerable economic losses being reduced. Some limitations do, however, warrant attention, including adverse reactions, bacteriophage infections themselves, eliminating beneficial bacteria, and dose standardization.

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