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Phage Therapy in Poultry: Alternative Non Antibiotic Strategy

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A B S T R A C T

There is a global crisis of antibiotic resistance, which is among the world's most serious health problems. Antibiotic growth promoters (AGPs) have been implicated in the development of antimicrobial resistance (AMR) in bacteria that cause common infections over the past 70 years due to their use in animal agriculture. The use of phage therapy is a promising approach for combating bacterial infections and encouraging healthy poultry growth.In addition to being an excellent vehicle for foodborne pathogens, poultry and poultry meat usually contain millions of bacteria, particularly Campylobacter and Salmonella. There are multiple current strategies for phage therapy, including phage cocktails, enzymes derived from phages, phages combined with antibiotics, phage engineering, the combination of phages and clustered regularly interspaced short palindromic repeats (CRISPR-Cas). Poultry phages are used for phage therapy, phage biocontrol, and phage biosanitization. As foodborne pathogens increase, biocontrol has the potential to become the most effective way to reduce them in animals as well as in processed foods. Phage biocontrol may decrease the use of antibiotics in poultry production, thus paving the way for the use of an essential part of our health care system that has been forgotten.

Introduction

Numerous antibiotics used by the poultry industry are less effective against pathogenic microorganisms in recent years. Antibiotic resistance has additionally been linked to the use of antimicrobial agents in animal husbandry. In the absence of restrictions on antibiotic use, it is possible that antibiotics can enter the final products of poultry, such as eggs and meat. The result will be an increase in antibiotic resistance. As all microorganisms are capable of resisting some antibiotics, it has caused a rapid rise and development of antibiotic resistance from excessive use of antimicrobial agents. In order to combat antibiotic resistance on a global scale, a complementary strategy must be implemented. The use of Phage Therapy for the control of bacterial infections and promoting the healthiness of poultry is one such remedy that is now emerging as a useful method for controlling bacterial infections.

Background

Bacteriophages (phages) are viruses that specifically target and infect prokaryotes (bacteria) or archaea. Bacteriophage term is derived from the Greek word phagein, which means devour (Sulakvelidze *et al.*, 2001). They are known as bacterial parasites because they lack the cell structure and enzyme systems necessary for food uptake, protein synthesis or construction of new particles.

According to Ernest Hanbury Hankin, something was found in the waters of the Ganges and Yamuna rivers in India that could pass through very fine porcelain filters. He pointed out that this led to the limited spread of the cholera epidemic (Sulakvelidze *et al.*, 2001).

Hankin in 1896 described the phenomenon without identifying the bacteriophages. Bacteriophages were discovered by Twort in 1915 as unidentified molecules that inhibit bacterial growth (Haq *et al.*, 2012). In 1915, The Lancet published an article written by Frederick Twort about ‘‘the transmissible bacterial lyses’’. It was the first publication on bacteriophages (Twort, 1925).

It was D'Herelle in 1917 who isolated and characterized the first phages, and he developed the first phage therapy to treat fowl typhoid caused by Salmonella Gallinarum. Felix d'Hérelle noted a novel kind of microbe had been described as "a parasite of bacteria" in a 1917 article in the prestigious journal "Comptes Rendus de l'Académie des Sciences". The term "bacteriophage" was first coined by Felix d'Herelle. While controversy surrounds the claim of priority, the discovery of bacteriophages is independently credited to both Twort (1915) and D’Herelle (1917). (Gigante and Atterbury, 2019).

Research into the therapeutic potential of bacteriophages slowly declined amid the production and massive use of antibiotics, especially in western institutions (Gigante and Atterbury, 2019). Since 1970, drug development and implementation have taken place both in human and animal health and agriculture. In spite of their success, antimicrobials are often overused and often indiscriminately used by patients, leading to antimicrobial resistance (Aminov, 2010).

World Health Organization (WHO) has been warning that “the world is on the brink of losing these miracle cures -the antibiotics” and that “in absence of urgent protective actions, the world is heading toward a post-antibiotic era, in which many common infections will no longer have a cure” (Liljeqvist *et al.*, 2012).

Alternate non-antibiotic strategies

The possible alternative non-antibiotic strategies would be antimicrobial peptides, faecal microbiota transplant, probiotics, nanobiotics, immunotherapeutic and vaccines, gene editingtools, enzybiotics and phages (Kumar *et al.*, 2021).

Phage Therapy is one such alternative now emerging as a useful tool in controlling bacterial infections and at the same time is encouraging the growth of healthy poultry.

Taxonomy and structure of phages

Phages have been classified into 873 species, 204 genera, and 14 subfamilies by the International Committee on Taxonomy of Viruses (ICTV). The electron microscope has been used to examine over 5000 phages, and most of them (96%) had tails. Caudovirales include a vast majority of known phages that have wide isometric heads ranging from 20 to 200 nm, making them 1000 times smaller than the average bacterium (0.5-20 m). Three families are found in this order: *Myoviridae* with long, flexible tails, *Siphoviridae* with long, non-contracted tails, and *Podoviridae* with short, stubby tails and a striking lack of features (Fauquet *et al.*, 2005). The Body is mainly divided into 3 portions- head, neck and tail portion. They all contain nucleic acid and protein. A bacteriophage particle or virion consists of a single or double-stranded (ss or ds) DNA or

RNA molecule, encapsulated inside a protein or lipoprotein coat.

Sources

Bacteriophages are among the most common and diverse entities in the biosphere. It is usually present inhuman and animals intestines, running water, soil effluent outlets, sewage.

Replication strategies

Based on phage interactions with bacteria and their life cycle, they are classified into two types: lytic (virulent, productive) and lysogenic (temperate, dormant).

When infected with a lytic phage, cells are killed, lysed, and progeny phage particles are released. Phage genomes are integrated into bacteria's chromosomes during the lysogenic cycle (most common) or remain separate, as plasmids. Temperate phages have a choice between the lytic pathway and an alternative lysogenic pathway. In the latter pathway, phages integrate their genetic material into the host's chromosome, replicate, and co-exist stably with the host bacteria as prophages.

There is another type of temperate phage that replicates in bacteria by permanent slow release of progeny without cell lysis, which is designated as permanent infection. Certain conditions such as induction with DNA-damaging agents (such as UV radiation and chemicals) will trigger most prophages in a population to re-activate; thus, prophages will stop its dormancy and enter the lytic cycle, further replicating phages and lysing the host.

Nonetheless, temperate phages are often not used for phage therapy because they tend not to cause host bacterial lysis until the right conditions are met, and they will prevent subsequent phage entry once a host is infected. Additionally, they are often vectors of horizontal gene transfer, resulting in the transfer of virulent or resistant genes as well as other risks (Wei *et al.*, 2020).

The life cycle of lytic phages

Attachment

The phage attaches itself to the surface of the host cell in order to inject its DNA into the cell.

Penetration

The phage injects its DNA into the host cell by penetrating through the cell membrane.

Transcription

The host cell's DNA is degraded and the cell's metabolism is directed to initiate phage biosynthesis.

Biosynthesis

The phage DNA replicates inside the cell, synthesizing new phage DNA and proteins.

Maturation

The replicated material assembles into fully formed viral phages (eachmade up of a head, a tail and tail fibers).

Lysis

The newly formed phages are released from the infected cell (which is itself destroyed in the process) to seek out new host cells to infect.

Characteristics of phages

Bacteriophages are natural micro-organisms made up of only genetic material namely DNA and RNA plus protein (Haq *et al.*, 2012). The beneficial microbiome remains unaffected by their presence. These naturally occurring organisms only eliminate selected bacteria. More and more companies are embracing bacteriophages instead of antibiotics when it comes to poultry production. This is because bacteriophages are safe as they are only able to infect bacterial cells, not human or

animal cells. If they are not in contact with their bacterial host, they become inactive after 48 hours. A single phage can destroy a multitude of bacteria by multiplying within the bacteria itself, which is why fewer doses per administration are needed compared to antibiotics. In addition, once adsorbed irreversibly, phages do not separate from bacterial targets**.**

Strategies for phage therapy

As of today, many approaches to phage therapy use phage cocktails, phage-derived enzymes, antibiotics and phage engineering, as well as phages combined with the clustered regularly interspaced short palindromic repeats-associated (CRISPR-Cas) system emerging today (Wei *et al.,* 2020).

Phage cocktail

Phage infections are sometimes limited to a single or few strains of a particular pathogen species. For genetically diverse bacterial pathogens, using phage mixtures or phage cocktails is often necessary. Phage cocktails are unique in preventing the development of phage-resistant bacterial pathogens because a bacterial pathogen does not have the opportunity to develop resistance to multiple phages concurrently (Liu *et al.,* 2020).

Phage derived enzymes

Phage genomes encode a number of enzymes that are capable of breaching bacterial cells, such as virion-associated peptidoglycan hydrolases (VAPGH), endolysins, and polysaccharide depolymerases (Liu *et al.,* 2020). By degrading the peptidoglycan of the bacterial cell wall, VAPGH which is present in the base plate creates holes that allow the phage genes to enter the bacterial cell during the adsorption of the phages (Rodriguez-Rubio, 2013).

Their antimicrobial properties make them an attractive option. The peptidoglycan hydrolase phage lysin is a class of enzymes capable of destroying peptidoglycan directly on a bacterial cell wall (Cahill and Young, 2019).

Phage-encoded depolymerases hydrolyze polysaccharide compounds of bacteria, such as capsule, lipopolysaccharide (LPS), or extracellular polysaccharides of biofilms (Latka *et al.,* 2017). The destruction of these protective barriers of the pathogens by depolymerases can therefore improve the therapeutic effect of bacterial infection.

Phage and phage enzymes combined with antibiotics

Phage-antibiotic synergy (PAS) was first proposed in 2007, referring to the phenomenon that "the sub-lethal concentrations of certain antibiotics can significantly stimulate the propagation of lytic bacteriophages in the host bacteria, thus leading to accelerated cleavage of host cells and rapid diffusion of progeny phages" (Comeau *et al.,* 2007). Antibiotics and phages work in synergy to effectively remove bacterial biofilms.

Phage engineering

By changing the lysis spectrum, or delivering exogenous genes and proteins, phage engineering can enhance the therapeutic potential of phages. A common method is to engineer genes that encode receptor-binding proteins (RBPs) into the tail fibers or spikes of phages to obtain a broad spectrum (Dams *et al.,* 2019).

Phage combined with Crispr Cas system

Using phages to deliver the CRISPR-Cas system specifically to the bacterial genome in order to eliminate pathogenic bacteria and prevent the spread of resistance. To express the artificial crRNA matching the drug-resistant bacterial genome in target bacteria that contain an active endogenous CRISPR-Cas system, a mini-CRISPR array is embedded into the phage genome. An endogenous CRISPR-Cas system is exploited by a bacteriophage to attack its host cell (Li and Peng, 2019).

The use of bacteriophages in poultry

Bacteriophages are now being used in the poultry sector in many ways from farm to fork.

Phage therapy

In bacteriophage therapy, bacteriophages are used as bioagents to treat or prevent bacterial infections using their products or whole phages.

Public health concerns have increased the attention paid to the pathogen, such as *Campylobacter jejuni* (*C. jejuni*), *Salmonella enterica* subspecies enterica serovar Enteritidis (*S. Enteritidis*), *Salmonella enterica* subspecies enterica serovar Typhimurium (*S. Typhimurium*), *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*) and methicillin-resistant *Staphylococcus aureus* (MRSA) because of the risk posed by the poultry as a source of these pathogens. Most works have addressed the efficacy of bacteriophages in reducing bacterial count and in the control of bacterial infections in the poultry which are zoonotic and have a substantial impact on public health (Żbikowska *et al.*, 2020).

Phage therapy for campylobacteriosis

Campylobacter spp. is ubiquitous in various environments but prefers the gut of birds, which they colonize as commensals. Due to the conducive (optimal) body temperature, poultry has become a natural reservoir for *Campylobacter* species, representing the major source of human infections. Recent experimental studies have provided evidence for the efficacy of phage treatment in reducing the *Campylobacter* colonization in chickens and thus in minimizing the risk of its entrance into the food chain (Żbikowska *et al.*, 2020).

A phage cocktail containing virulent *Campylobacter* phages was used by oral route to treat broiler chickens colonized with *C. jejuni*. Bacteriophage predation of *C. jejuni* did not affect the microbiota but selectively reduced the abundance of *C. jejuni*. The bacteriophage controlled to reduce *C. jejuni* levels in chickens can reduce human exposure and disease acquired through the consumption of contaminated poultry products (Richards *et al.*, 2019).

Two phages (CP8 and CP34) from a panel of 53 isolated from chicken faeces were selected to use as candidates to reduce *Campylobacter* in chickens. The phages were selected based on favourable invivo replication kinetics and a broad host range. Ross broiler chickens were experimentally infected with *C. jejuni* isolates HPC5 and GIIC8 at various doses (from 2.7 to 7.8 log10 CFU) by oral gavage at 18 to 20 days of age. A single phage treatment (5–9 log10 PFU) was administered at 25 days of age by oral gavage. *C. jejuni* counts in the upper intestine and ceca of phage-treated birds were reduced by between 0.5 and 5 log10 CFU/g when phages were applied at $\geq 10^7$ PFU (Loc Carrillo *et al.*, 2005).

Phages isolated using *C. jejuni* NCTC 12662 isolate as a host strain are almost exclusively Group III phages that target a particular receptor, the capsular polysaccharide. In contrast, phages isolated on *C. jejuni* RM1221 are typically Group II phages that utilize the flagella as a route of entry. Hence, a phage cocktail consisting of phages that target different receptors could potentially lead to a broader target range and more effective cocktails (Sorensen *et al.*, 2015).

Phage therapy for salmonellosis

Salmonella is one of the main bacteria affecting commercial poultry and the second (after *Campylobacter*) of the most important zoonotic foodborne pathogens.

An experiment was demonstrated where a group of day-old chick was, challenged with 100 µl fresh *S.* Enteritidis PT4 culture at 10^8CFU/bird via oral inoculation and treated with single oral application of phage cocktail (CNPSA1, CNPSA3 and CNPSA4) at 10^{11} PFU observed that a single dose of a bacteriophage suspension with a high titer was highly effective in reducing the population of

pathogenic bacteria in the digestive tract and reduction of 3.5 orders of magnitude in colony-forming units of *S.* Enteritidis PT4 per gram of caecal content (Fiorentin *et al.,* 2005).

Lim *et al.,* in 2011 challenged 6-week-old chicken with *S.* Gallinarum at 5 × 10^8CFU/mL via oral inoculation and used bacteriophage CJø01 as feed additive at 10^6 PFU/kg observed that treatment using bacteriophages as a feed additive for chicken having contact with infected individuals led to a mortality rate of only 5%, as compared to 30% in the group that did not receive phage therapy (Lim *et al.,* 2011).

Atterbury *et al.,* in 2007 challenged 36-day-old chickens with 1 ml of an 8.0-log 10 CFU/ml suspension of *S. Enteritidis* and used a cocktail of phage consisting of φ151 against *S.* Enteritidis, φ25 against *S.* Hadar, φ10 against *S.*Typhimurium. Cocktail administered by oral gavage at 10^9 PFU/ml and 10^{11} PFU/ml. Significant reduction in the concentration of *S.* Enteritidis, *S.* Typhimurium by 2–4 log units after administration of a bacteriophage suspension with a density of 10^{11} PFU (Atterbury *et al.,* 2007).

Ahmadi *et al.,* in 2016 orally challenged 33-day-old quails with 100 ml of S. Enteritidis at 1.2 × 10^9CFU/mL and used Single *Salmonella*-lysing phage (PSE) at 10^9 PFU/ml in 100 µl aliquot by oral gavage for 2 days observed 100% efficacy in eliminating *S.* Enteritidis strains from the tonsils, 6 h after application of bacteriophage suspension and PSE phage was more effective when administered prophylactically before*S.* Enteritidis infection than as a treatment for established *S.* Enteritidis infections (Ahmadi *et al.,* 2016).

Phage therapy for colibacillosis

Escherichia coli is a Gram-negative bacillus, a normal inhabitant of the digestive tract of birds, which is widely disseminated with faeces. Most strains are non-pathogenic, however, certain pathogenic serotypes (avian pathogenic *Escherichia coli*—APEC) may induce disease, leading to mortality and condemnations. Some strains, such as enterohemorrhagic *E. coli* (EHEC) and its subgroup of Shiga toxin (Stx)-producing *E. coli* (STEC), are food-borne pathogens responsible for serious human diseases worldwide.

Barrow *et al.,* (1998) used bacteriophage R for treating septicemia and cerebritis or meningitis in chickens. Chickens were challenged with *E. coli* O1:K1 or O2:K1 10^6CFU/mL by intracranial or intramuscular inoculation whereas phage preparations were administered by intramuscular injection. Phages reached the brain in chickens, which were earlier intracranially infected with *E. coli*. They were able to multiply rapidly and decline the bacterial count (Barrow *et al.,* 1998).

Huff *et al.,* (2002) challenged 3-day-old birds and 7-day-old birds with *E. coli* by injection of 10^4 CFU/mL into the thoracic air sac at 7, 8, or 10 days of age and applied Bacteriophages SPR02 directly to air sac in a range of titers from 10^8 to 10^3 PFU and also Bacteriophage suspension applied to drinking water (10^3 or 10^4 PFU/ml) and observed reduced mortality rates to 5% and 25% and 100% depending on the titer of bacteriophage suspensions (Huff *et al.,* 2002).

El-Gohary *et al.,* (2014) challenged three-week-old chickens with 0.5 mL *E. coli* culture containing 4.5 × 10^8CFU/mL or 5.5 × 10^8CFU/mL/bird and used Bacteriophage preparation by applying to litter at titer 10^8 PFU/ml - 200 mL sprayed on the surface of 3.9 m^2 pens after *E. coli* challenge and observed that mortality was significantly reduced by spraying bacteriophage on the litter and also reduction in shedding of *E. coli* among poultry flocks (El-Gohary *et al.,* 2014).

Phage therapy for clostridium

Miller *et al.,* (2010) challenged one-day-old chickens with *C. perfringens* CP-6 strain 10^8CFU/mL at 1.0 mL/bird by oral gavage and used bacteriophage cocktail (CPAS-7, CPAS-12, CPAS-15, CPAS-16, and CPLV-42 at titers of 10^5

PFU/mL) with feed or water or oral gavage and spray application and observed significantly reduced mortality of *C. perfringens*-challenged birds by 92% and also weight gain and feed conversion ratios were better in *C. perfringens* challenged chickens treated with the bacteriophage cocktail (Miller *et al.*, 2010)

The phage-encoded endolysins, enzymes that target and hydrolyze specific bonds within the peptidoglycan mesh, have been reported to suffice to achieve bacterial lysis. The usage of purified endolysins from phages that target *C. perfringens* is shown as a promising route to reduce the colonization or treat the infection (Fischetti, 2010).

Phage Biocontrol

Phage biocontrol can greatly reduce harmful bacteria entering the food chain at the farm level when produced with virulent phages that can survive extreme environments and have a broad host range for the target genus, without displaying bacterial virulence genes (Jassim and Limoges, 2014).

The phages are normally administered directly to live 'food animals' before they are processed into the meat during pre-harvest interventions. In such a method, phages may prevent the spread of pathogenic bacteria on livestock before slaughter and carcass processing to ensure that processed meat is free from pathogens. Additionally, post-harvest interventions aim to ensure food safety by using phages to eliminate or reduce foodborne bacterial contamination, thus ensuring the foods are safe to consume. Consumers could benefit from both pre- and post-harvest intervention strategies, reducing medical costs associated with salmonellosis and campylobacteriosis, and improving their quality of life.

Using phages in biocontrol applications offers advantages for several reasons

When used for biocontrol purposes, a single bacterial species or strain can be used without harming the normal flora, allowing direct targeting of pathogenic bacteria.

Self-replication and self-limitation mean that even low dosages will multiply as long as there is still a host threshold, increasing their overall antimicrobial activity.

Phage defense mechanisms develop in bacteria for their survival, but phages adapt to altered bacterial systems continuously, and phage programming technology can also overcome such defense mechanisms in bacteria.

A general ability to withstand food processing environmental stresses (such as food physiochemical conditions) is also among their main benefits.

They have been shown to have an extended shelf life.

Phage Bio sanitation

Phage egg sanitizing

Jassim and Limoges described a method where the eggs are cleaned with the help of a phage solution. It contains a concentrated appropriate phage stock suspension in Lambda (k)-buffer [6 mmol l^{-1} Tris pH 7.2, 10 mmol l^{-1} Mg $(SO4)_2$. $7H_2O$, 50 µg ml^{-1} gelatin (Oxoid, UK)] titer 10^{10} pfu ml^{-1} diluted in warm (30–37 °C) clean fresh water to give a final phage titer of 10^7 pfu ml^{-1}. Eggs are carried with the help of a conveyor belt where they are first washed by water to remove dirt, soil, stains, etc., then on to the phage sanitizing section. A spray of the solution containing millions of phage particles will be used to cover the whole eggshells during phage treatment. In the presence of the target pathogen on the eggshell, the phage will infect the pathogen within 3–5 minutes (i.e., after the egg leaves the washing facility) and kill the bacteria within 45 minutes. Following egg cleaning and phage disinfection, the phage populations on the infected eggshells will increase due to replication and transmission from nearby organisms. This will increase protection

against further contamination by the target bacteria after the eggs depart from the facility. It is only for a few days that the phage is active on eggshells during egg packing, shipping and storage (Jassim and Limoges, 2017).

Phage disinfection

Jassim and Limoges proposed that poultry manure, poultry barns, and egg production be decontaminated by phage sanitization. The phage solution is comprised of smart phages (cocktail) at titer of 10^7 pfu ml^{-1} in clean warm water (30–37 °C). The water is supplemented with Lambda-buffer [6 mmol l^{-1} Tris pH 7.2, 10 mmol l^{-1} $Mg(SO4)_2$. 7H2O, 50 µg ml^{-1} gelatin (Oxoid, UK)] by using a sprayer or an electric mist fan with ultrasonic vibration, water is atomized to1–5 µm ultra-particles and the phage mist is spread. Direct disinfection of floors, walls, equipment, and walls is possible with the phage. Decontaminated manure and dust are removed after 2 to 3 hours. To ensure that each flock starts its life in a clean environment, phage barn cleaning can be implemented after each flock and as needed (Jassim and Limoges, 2017). In addition, they recommended installing an automatic biohazard detector known as a 'Phage Alarm and Detector' (PAD) that would alert the meat manufacturer if pathogenic bacteria were detected in the processing room of a meat brand so steps could be taken to prevent food contamination. One or more automatic airborne contamination checking systems can be set to pull air from poultry houses, hatcheries, slaughterhouses, and meat processing rooms once or twice each day or as needed.

Reporter phage signal

Adenosine triphosphate (ATP) bioluminescence is extremely sensitive to levels of ATP isolated from bacterial lysis (Jassim *et al.,* 1990; Jassim *et al.,* 1993). Phages are used as probes to bind and lyse 10^4 CFU ml^{-1} *E. coli* or *Salmonella* in the sample and the released ATP can be detected after 60 min (36). A thin chip is placed which contains 10^9 pfu phages on 1 cm^2, if the target bacteria are present in the air, the phage will rapidly infect bacterial cells and lyse them within 60 min. Following 61 minutes (the time it takes for 120 ml of air to collect and the time it takes for the phage to lyse 60 min), the chip can be subjected to an auto detector and measured for ATP release with ATP-monitoring reagents (luciferase and D-luciferin). Luciferin-luciferase enzymes can also be added to the smart chip in this regard. An auto-measurement can be used to monitor the luminescent signal. The PAD is connected to a luminescence reader.

Firefly luciferase catalyzes bioluminescent reactions. Luciferin is oxidized in two steps by firefly luciferase using ATP, which yields 560 nm light. The light output is expressed in the form of relative light units (RLUs) (Jassim and Limoges, 2017).

Phage decontamination techniques

Roy *et al.,* (1993) studied the effectiveness of different phages to remove *Listeria* from stainless steel and polypropylene surfaces. They found that phage treatment alone was able to achieve approximately a 3-log cycle decrease in cell number (Roy *et al.,* 1993). Apart from controlling *Listeria* biofilms, *Campylobacter* biofilms were successfully removed from the surface of the glass (Siringan *et al.,* 2011) and growth of *E. coli O157:H7* was controlled using phage mixture *BEC8* on stainless steel and ceramic tiles (Viazis *et al.,* 2011).

Chemicals, physical disrupting agents and irradiation are traditional decontamination methods that have significant disadvantages in food-processing facilities. It is known that they cause corrosion of the equipment used for food processing, damage to food, and leave toxic residue behind (Sulakvelidze, 2013). Bacteriophages, which are both non-toxic and environmentally friendly, do not display these deleterious effects. Upon entering and leaving a poultry farm, phage wash stations can be set up at the gate to clean vehicles, phage hand-sanitizing stations (phage-based hand rubs) and boot dips at barn entrances to prevent bacterial pathogens

from entering the barn on hands and feet (Jassim and Limoges, 2017). In all poultry industries, including those associated with high prevalence and/or risk of bacterial infection, phage-based biocontrol/disinfectant treatment of broiler and laying hen flocks is safe to use. In addition to reducing *Salmonella, Campylobacter*, and other important foodborne pathogens in poultry populations, they also help balance commensal intestinal bacteria. The effect of this method is to gradually reduce the percentage of positive flocks until an acceptable number is reached and simultaneously reduce the need for antibiotics. The safety level can then be maintained from farm-to-fork using phage-based biocontrol and disinfectants.

Commercial preparations

There are many phage companies all-over the world selling their products at different pricing, organisms, services. Few companies of such kind areOmnilytics, Proteon pharmaceuticals, Intralytix, Phageguard,Gangagen, etc.,

Challenges

Phages do not have a long residence time in the chicken intestine due to their acidic environment and their unstable pH.

Three protocols have been developed for stabilizing phages in acidic media:

using alkali to phage suspensions.

the addition of antacids (e.g., $CaCO_3$) to phage suspensions.

the encapsulation of phage polymeric microcapsules.

Anti-phage antibodies

In treating chronic infections it may be possible to administer a higher dose of phage, to compensate for those that are cleared by interaction with neutralizing antibodies. Phages are relatively large in comparison to chemical molecules. For this reason, the sites in the body that can be reached by them must be carefully clarified.

Many phage experiments done in vitro need to be extrapolated to in vivo growth.

Phage efficacy in humans or animals is unknown and needs to be tested in the lab before use in each treatment.

Producing phages at a cost that increases food prices may not be well accepted by producers and consumers. To educate the public about phage-based biocontrol, an educational campaign could be implemented.

Anti-phage antibodies

In treating chronic infections it may be possible to administer a higher dose of phage, to compensate for those that are cleared by interaction with neutralizing antibodies.

Increasing drug-resistant bacteria have opened a second window for phage therapy. Phage preparations adapted to combat bacteria pathogenic to humans and animals could be one of the most effective ways to fight bacteria in the future. This would eliminate the need for antibiotics.

With today's health-conscious consumers, animal products raised in a safe environment are more popular. The most effective way to prevent and control infectious diseases in poultry farms is to keep them clean and feed them healthily. Phage therapy is seen as a challenging process from a clinical and economic perspective, but the benefits to commercial, economic, and societal stakeholders far outweigh its challenges.

Use of phage preparations must be used responsibly if their use is soon approved, so as not to repeat the mistakes that led to antibiotic resistance.

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