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Review Article

Bacteriophages (the living drugs): novel applications and alternative to antibiotics in poultry production

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ABSTRACT

Infections in poultry cause economic loses and in some cases human health problem worldwide. The most common infections are associated with salmonellosis, colibacillosis, campylobacteriosis, and others. Infections caused by these pathogens, often through poultry products, are also serious public health issues. The practice of phage therapy, which uses bacterial viruses (phages) to treat bacterial infections, has been around for almost a century. The universal decline in the effectiveness of antibiotics has generated renewed interest in revisiting this practice. The high success rate and safety of phage therapy in comparison with antibiotics are partly due to their specificity for selected bacteria and the ability to infect only one species, serotype or strain. Conventionally, phage therapy relies on the use of naturally-occurring phages to infect and lyse bacteria at the site of infection. This mechanism does not cause the destruction of commensal bacterial flora. In this review, we highlighted recent diverse advances in the field of phage research, going beyond bacterial control using whole phage, diagnostics, novel drug delivery systems, phage-based vaccine, bio-nanotechnology and application of bacteriophages in poultry production as an alternative means of eliminating pathogens.

Keywords: Bacteriophage, Bacteriophage-based tools, Bacteriophage therapy, poultry

INTRODUCTION

Phages, short for bacteriophages, are bacteria-specific viruses that have been used as a treatment against pathogens such as *Shigella dysenteriae* as early as 1919 (Chanishvili, 2012). With an estimated 10^{31}-10^{32} phages in the world at any given time (Suttle, 2007), they make up the most abundant biological entity on the earth and play a crucial role in regulating bacterial populations; phages are responsible for the death of approximately 20%-40% of all marine surface bacteria every 24 h (Wittebole et al., 2014). Positive results of the use of bacteriophages in fighting bacterial infections have contributed to the development of research on the potential use of viruses that destroy bacteria in treatment of diseases in both human and animals (Sulakvelidze et al., 2001; Summer et al., 2007). Much of the controversy surrounding phage therapy was due to poor documentation of use and variable success. In fact, the nature of their existence was a topic of contention until they were visualized in the 1940's after the invention of electron microscopy (Ackermann, 2011). A number of logistical and technical obstacles in developing phage therapy led to its widespread abandonment after the discovery of antibiotics.

Almost a decade before the discovery of penicillin, the controversial practice of phage therapy was being developed as a treatment for bacterial infections. Generally, bacteriophages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. Implication of BP has been based on its specificity targeting at particular species or strains of pathogenic

bacteria (Monk et al., 2010). There has been increasing evidence to suggest that the applications of a single or mixture of specific BP by aerosol spray (Borie et al., 2008; Borie et al., 2009), muscle injection, drinking water (Borie et al., 2008), or oral gavage (Atterbury et al., 2007) to chickens challenged with specific pathogens ameliorate clinical symptoms of infection and decrease mortality (Johnson et al., 2008). Recently, it has been reported that the inclusion of bacteriophages could also benefit the laying hens in egg production and egg quality (Zhao et al., 2012) and also the broiler in improving feed conversion ratio.

In many developing countries including Bangladesh, antibiotics at low dosages are used in poultry feed as feed additives to promote performance of poultry and to control common bacterial diseases. However, random use of antibiotics in poultry feed may create serious human health hazard by generating antibiotic resistance in bacteria and transferring those resistant bacteria to human through poultry meat and eggs. Therefore, searching for a natural alternative to use as growth promoter in poultry feed is of great interest in current days in developing countries.

1. Bacteriophage

Bacteriophages are viruses that infect and replicate in bacteria and, as such, are not pathogenic for animals, including humans. The term was derived from "bacteria" and the Greek: (phagein), "to devour". Bacteriophages are among the most common and diverse entities in the biosphere composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures (Grath and Sinderen, 2007). These ubiquitous viruses can be found in every environment where their bacterial hosts are present. It is estimated that there are over 1031 bacteriophages on the planet which are more than every other organism on the Earth, including bacteria. Phages are isolated from all natural environments, including wastewater, human and animal waste, natural water bodies, soil, forest groundcover, food products, and other microorganisms (Ackermann, 2001; Brüssow and Kutter, 2005; Hyman and Abedon, 2009). Approximately 104 – 108 bacteriophages per ml in aquatic systems and 109 bacteriophages per 1g in soil and sediment particles were detected (Weinbauer, 2004). At present, over 5500 different bacteriophages have been discovered (Ackermann, 2007) and more than 25,000 bacteriophage nucleotide sequences had been deposited in INSDC databases (Ackermann, 2001; Adriaenssens and Brister, 2017), each of which being able to infect one or several types of bacteria.

1.2. Classification of bacteriophages

The most up-to-date classification of bacteriophages is given by Ackermann, 2006. The key classification factors are phage morphology and nucleic acid properties. The genome can be represented by either DNA or RNA. The vast majority of phages contain double strand DNA (dsDNA) such as Plasmaviridae, Fusselovirida, Lipothrixviridae, Rudiviridae, Guttavirus etc., while there are small phage groups with ssRNA (Leviviridae), dsRNA (Corticoviridae), or ssDNA (Inoviridae) (ss stands for single strand). There are a few morphological groups of phages: filamentous phages, icosahedral phages (Microviridae, Cystoviridae and Tectiviridae) without tails, phages with tails, and even several phages with a lipid-containing envelope (Lipothrixviridae) or contain lipids in the particle shell. Pleomorphic and filamentous phages comprise ~190 known bacteriophages (3.6% of phages) (Ackermann, 2004) whereas, tailed phages can be found everywhere and represent 96% of known phages. The tailed phages were classified into the order Caudavirales (dsDNA) (Ackermann, 2006) and are separated into three main phylogenetically related families: A - Myoviridae with contractile tails consisting of a sheath and a central tube (25% of tailed phages); B - Siphoviridae , long, noncontractile tails (61%); C - Podoviridae, short tails (14%). Since the tailed phages represent the biggest population of bacteriophages they are easy to find and purify (Ackermann, 2006).

1.3. Mode of Action of Bacteriophages

Depending on environmental conditions and the type of bacterial cell, there are several different pathways of bacteriophage infection, including chronic infection, pseudolysogeny, and abortive infection. Not all of these cycles end with the death of the bacterial cell and replication of phage particles. In many cases,

daughter virions are produced without induction of lysis of the bacterial cells, and thus viral particles are not released outside the cell (Hagens and Loessner, 2010; Urban-Chmiel et al., 2015; Weinbauer, 2004). When a virulent phage infects a host bacterium, it replicates much faster than the host cell. The whole cycle can be completed in 30–40 min.

In lytic form of infection, the release of DNA induces switching of the protein machinery of the host bacterium for the benefit of infectious agents to produce 50-200 new phages. To make so many new phages it requires nearly all the resources of the cell, which becomes weak and bursts. In other words, lysis takes place, causing death of the host bacterial cell. As result, new phages are released into the extracellular space and can infect new neighboring bacterial cells (Weinbauer, 2004). This cycle is known a lytic 'virulent' cycle **(Figure 1)**.

In case of lysogenic phage, they become a part of the host cell and replicate along with the host chromosome for many generations, coexisting as opposed to lysing the host cell (Jassim and Limoges, 2013). This phenomenon is called 'lysogeny', which also provides immunity against infection by further phage particles of the same type, ensuring that there is only one copy of phage per bacterial cell. Lysogenic infection is characterized by integration of the phage DNA into the host cell genome, although it may also exist as a plasmid. Incorporated phage DNA will be replicated along with the host bacteria genome and new bacteria will inherit the viral DNA **(Figure 1)**. Such transition of viral DNA could take place through several generations of bacterium without major metabolic consequences for it. Eventually the phage genes, at certain conditions impeding the bacterium state, will revert to the lytic cycle, leading to release of fully assembled phages.

2. Bacteriophage-based tools: recent advances and novel applications

After the discovery of bacteriophages in 1915 by Twort and 1917 by d'Herelle, these agents were initially used to treat bacterial infections, although widespread acceptance was limited owing to lack of understanding of phage biology and the development of antibiotic therapy in the 1940s (Wittebole et al., 2014). With antibiotic resistance becoming problematic in the late twentieth century (Davies and Davies, 2010), there was a renewed interest in phage therapy research. Alongside with the application of bacteriophages as antibiotic therapy and biocontrol, phage research has led to the development of new technologies for bacterial detection, drug delivery, drug discovery, and nanotechnology.

A recent advance in the area of antibiotic therapy has been the exploitation of phages to control antibiotic-resistant bacteria. Phages have been engineered to deliver CRISPR-Cas nucleases into antibiotic-resistant bacterial cells, and, in doing so, researchers have been able to harness the specific DNA-cleaving capacity of CRISPRs to knock out antibiotic resistance sequences, rendering resistant organisms antibiotic sensitive (Bikard et al., 2014).

Phage virions and their encoded proteins can also be useful for the detection and specific identification of bacteria. The simplest of these is where a standard number of specific phages are incubated with a food material or some other test sample. If the bacterial target is present and viable, detectable phage numbers will increase through amplification on the pathogen. Modifications of this method can generate results more rapidly, and in the case of *Yersinia pestis*, Sergueev *et al.*, for example, developed a quantitative real-time PCR to detect the increase in phage DNA instead of traditional plaque assays (Sergueev et al., 2010). Reporter phage assays have also been adapted to assess drug sensitivity in the target bacterium (Jain et al., 2012). Phage receptor-binding proteins (RBPs) have also been used successfully in bacterial detection and identification through fluorescent microscopy (Javed et al., 2013).

Phage display is now allowing the modification of phages into vehicles (nanocarriers) for chemotherapeutic drug delivery by the attachment of a drug to the phage surface and presentation of peptides on the surface of that phage with specificity to a ligand of interest. Such constructs have even been designed to target non-host bacteria, including mammalian cells (Vaks and Benhar, 2011). These phages, displaying therapeutic peptides, can even be designed to pass the blood–brain barrier

(Ksendzovsky et al., 2012), and such constructs could thus have potential in the treatment of diseases such as Alzheimer's and Parkinson's. Phages with an affinity to specific cell receptors, such as those overexpressed in cancer cells, may be exploited beyond drug delivery to allow for simultaneous target detection by displaying diagnostic reporter molecules or by detection of bound phage DNA by real-time PCR (Brasino and Cha, 2016; Hosoya et al., 2016).

Filamentous phages are viable platforms for vaccine development that can be engineered with molecular and organismal specificity. Phage-based vaccines can be produced in abundance at low cost, are environmentally stable, and are immunogenic when administered via multiple routes (Samoylova et al., 2017).

The increased use of treatment with bacteriophages is determined by their ability to lyse infected bacteria and mutate resistant bacteria, as well as by the high specificity of phages for particular bacteria. A vast number of infections in humans are induced by multi-drug resistant hospital strains of bacteria and by bacteria which have acquired resistance traits in the natural environment. Phage therapy has found application in treating bacterial infections in dermatology, stomatology, otolaryngology, ophthalmology, gynaecology, paediatrics, gastroenterology, urology and pulmonology (Kutateladze and Adamia, 2010). The use of bacteriophages in treating infections in humans has had a high success rate (about 85%), particularly in the case of mixed infections induced mainly by *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli*, *Proteus*, *Pseudomonas*, *Enterobacter* and vancomycin-resistant *Enterococci* (Biswas et al., 2002).

Genetically modified filamentous phages have also been used in material synthesis to construct nanowires and films for semi-conductor applications (Mao et al., 2003), piezoelectric energy generation (Lee et al., 2012a), and photo-response properties (Murugesan et al., 2013). These materials have been used to create devices such as ion batteries and catalysts (Lee et al., 2012b), with phage M13-based nanowires also being constructed into scaffolding to allow guided cell growth for human tissue formation (Yoo et al., 2011).

3. Use of bacteriophage in poultry production

In the past, antibiotics were used widely in the poultry industry as growth promotants (Castanon, 2007) and as prophylactics to minimize the risk of colonization by enteric pathogens. The use of antibiotics for the control of pathogens of public health significance has not been routine in Australia and has been banned more recently in Europe and the United States. Even therapeutic use is very carefully considered, given concerns over the selection or generation of antibiotic-resistant strains and evidence that some antibiotics may facilitate colonization and increase shedding and prolong carriage of *Salmonella* (Plym and Wierup, 2006).

Bacteriophages have been used widely in old Eastern Bloc countries (e.g. since 1918 in the USSR) as therapeutic agents, in place of antibiotics (Hanlon, 2007). Phages were shown to be effective in reducing carriage of *Salmonellae* in live birds (Bielke et al., 2007; Higgins et al., 2005). Phages and their lysins can serve as an alternative to chemical agents in processing and reducing pathogen populations after spraying onto the surface of the carcass (Hugas and Tsigarida, 2008). It is likely that interest in the use of phage in the poultry industry will increase with increased consumer demand to reduce or eliminate use of antibiotics and chemical treatments.

3.1. Bacteriophages as feed additives

Dietary anti-*SE* (anti- *S. enteritidis)* bacteriophage from 0.05 to 0.2% of total diet can be useful to prevent *Salmonella enteritidis* infection and subsequently reduce mortality without any detrimental influences on broiler productivity. After consideration of their safety, stability and therapeutic efficacy in broiler production, the use of anti-*SE* bacteriophage in broiler diet can be an alternative feed additive instead of antibiotics (Kim et al., 2013). Similar results were obtained in inhibiting horizontal infection with *Salmonella* following application of a bacteriophage suspension in the amount of 10^5 and 10 PFU/g as a feed additive for chickens challenged with 5×10^7 CFU of bacteria. Different groups of birds were treated with

different titres of bacteriophage contained in the feed additive for 21 days after *Salmonella enteritidis* challenge. These preventive measures significantly inhibited the replication of pathogens in the digestive tract of the chickens; however, this effect was observed mainly in chickens treated with bacteriophages at concentrations of 10^9 PFU/mL, which were compared only with the positive control groups (Lim et al., 2012).

3.2. Bacteriophages for control of pathogens

The most common bacteria inducing food borne infections in humans include bacteria of the genera *Salmonella* and *Campylobacter* and *E. coli*. According to the 2015 EFSA report on resistance to antibacterial agents in selected zoonotic bacteria (*Salmonella* and *Campylobacter*), indicator bacteria (*E. coli* and *Enterococcus* spp.), and other bacteria isolated from poultry and from food, a considerable percentage of the isolates posing a threat to humans and animals are resistant to available antibiotics, partially as a result of their widespread use in treatment of disease in humans and animals. The use of bacteriophages to eliminate pathogens seems quite promising, especially as they are present in every ecosystem and number 10^{31}, which is more than 10 times the number of characterized bacteria (Brüssow and Kutter, 2005; Urban-Chmiel et al., 2015).

The effectiveness and safety of phage therapy in comparison to antibiotics is partially due to the specificity of bacteriophages for particular bacteria, manifested as the ability to infect only one species, serotype or strain. This mechanism of action does not cause destruction of the commensal intestinal flora. Self-replication of bacteriophages takes place during treatment, which eliminates the need to apply them repeatedly. Another advantage of phages is that they cannot bind to and replicate in eukaryotic cells, which causes a decrease in their titre, correlated with a marked reduction in the number of pathogenic bacteria inducing a given infection in the organism. An equally important advantage is that phages are not toxic because most of them are composed mainly of proteins and nucleic acids (Loc-Carrillo and Abedon, 2011).

3.3. Application of phages in bio-control and therapeutic design

Bacteriophages therapies are effective tool in eliminating bacterial infections in various species of animals and have also proven successful in treating diseases in poultry. One of the objectives of phage therapy in animals is to assess the suitability of bacterial viruses for control of pathogens having an important influence on animal productivity and health. Phages used in treatment have been effective in preventing infections and in treatment of colibacteriosis in poultry. Positive results, with a high success rate in eliminating pathogens, have also been obtained in combating infections induced by various *Salmonella* serotypes in gamefowl, such as Enteritidis and Typhimurium (Fiorentin et al., 2005; Lim et al., 2011; Sklar and Joerger, 2001), as well as campylobacteriosis in poultry, particularly infections induced by *Campylobacter jejuni* and *C. coli* (Wagenaar et al., 2005). The effectiveness of phage therapy has also been confirmed in infections of broiler chickens by anaerobic *Clostridium perfringens* during the course of necrotic enteritis (Miller et al., 2010). A list of important bacteriophages having antibacterial effect against food borne pathogens has been given in Table 1 with their target organisms.

3.3.1. Salmonellosis

Long-term use of phages in poultry has proved to be moderately effective in reducing the number of *Salmonella* pathogens colonizing the digestive tract (Sklar and Joerger, 2001). However as shown by Fiorentin et al. (2005), single oral application of a cocktail of phages (CNPSA1, CNPSA3 and CNPSA4) at a dosage of 10^{11} PFU decreased the occurrence of *Salmonella enteritidis* strains by 3.5 log units. The authors confirmed that applying a single dose of a bacteriophage suspension with a high titre was highly effective in reducing the population of pathogenic bacteria in the digestive tract, in contrast with long-term application of a lower titre.

A positive effect of phage therapy was also observed in combating horizontal infections induced by strains of *S. gallinarum* in flocks of laying hens. Treatment using bacteriophages as a feed additive for chickens 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).

The effectiveness of phage therapy may also depend on the individual antibacterial properties of a given bacteriophage and on the adaptive mechanisms of the bacteria. A study by Andreatti Filho et al. (2007) showed that the use of selected bacteriophages in an orally administered cocktail to prevent colonization by *S. enteritidis* strains in poultry was only effective for a short time (about 48 h), with no long-term protective effect, which was partly due to acquisition of resistance to the bacteriophage by the bacteria.
It seems promising that a wide range of lytic activity against three serovars of *Salmonella* – Enteritidis, Typhimurium, and Hadar – were obtained in 36-day-old broiler chicks, in which a significant reduction in the concentration of bacteria was noted following experimental infection with these serovars, by 2–4 log units (Atterbury et al., 2007). The authors suggest that adjustment of treatment conditions may make it possible to use just one or two bacteriophages rather than many.

Other studies suggest that bacteriophages may be used in combined treatment with other preparations, as indicated by a significant (about 80%) synergistic antibacterial effect of a commercial oral probiotic preparation applied together with a bacteriophage 'cocktail' of phages S2a, S9, and S11 (5.4×10^6 PFU/0.5 ml/bird) at 4, 5, and 6 days of age and at 8, 9, and 10 days of age to combat *S. typhimurium* infections in broilers. The authors showed that chickens treated with a probiotic and bacteriophages showed 10 times fewer bacteria in the ileum, caecum, liver and spleen than did untreated challenged chickens (Toro et al., 2005).

In another study, simultaneous application of three phages (MOI 103) at 10^8 PFU/ml/dose at 6 days of age (two daily doses) by aerosol spray and probiotics administered at 1 day of age by coarse spray, followed by oral inoculation with 2.95×10^5 CFU/ml in seven-day-old chickens, reduced *Salmonella* incidence and *Salmonella* intestinal colonization, leading to complete elimination of deaths in broiler chickens caused by infection with *Salmonella enteritidis* (Borie et al., 2009).

3.3.2. Colibacillosis

Phage therapy has also proven to be an effective therapeutic tool in fighting pathogenic strains of *Escherichia coli*, particularly in preventing the development of colibacillosis, which initially develops in the respiratory tract and air sacs and then takes the form of sepsis, causing considerable mortality in poultry.
Phage suspensions applied directly to the air sac in 3-day-old birds in a range of titres from 10^6 to 10^3 PFU to treat *E. coli* infections substantially reduced mortality rates to 5% and 25%, respectively. Similar results were obtained after inoculation of a bacteriophage suspension in the drinking water of birds at 1 week of age (10^3 or 10^4 PFU of bacteriophages per mL) followed by air sac challenge with 10^3 CFU of *E. coli* phages. Mortality was decreased to 25% and 5%, respectively. No mortality was observed in chickens treated with 10^8 PFU of an *E. coli* bacteriophage mixture (Huff et al., 2002b). Bacteriophages have also been shown to be highly effective in treating sepsis and meningitis in newly hatched and 3-week-old chicks infected intramuscularly and intracranially with a strain of *E. coli*. Administration of lower doses from 10^4 PFU of the phage after challenge with *E. coli* also provided significant protection, indicating that the phage had multiplied in vivo. However, the application of phages in lower doses, e.g. 10^2 PFU, produced no statistically significant protection against *E. coli* infection.

The authors suggest also that similar effects preventing early development of colibacillosis in chicks are obtained by applying a bacteriophage suspension *in ovo* (Huff et al., 2009). The authors also demonstrated that the effect of this kind of bacteriophage treatment is comparable to enrofloxacin treatment, and suggest that a combination of enrofloxacin and bacteriophage treatments could be efficacious and beneficial in controlling colibacillosis.

Apart from bacteriolytic activity, the effectiveness of bacteriophages is also determined by the site and route of administration of the preparation. According to Huff et al. (2003) bacteriophages should be applied directly to the site of infection, which was confirmed during treatment of *E. coli* infections in the air sacs of chickens.

The direct or aerosol administration of bacteriophages in poultry and evaluation of their therapeutic effect has been a subject of study at many research centers. A study by El-Gohary et al. (2014) demonstrated that bacteriophage treatment of litter by spraying 200 mL of a bacteriophage preparation at a titre of 8×10^8 PFU/mL on the surface of 3.9 m^2 pens significantly reduced the mortality of male broiler chickens (about 2–3 weeks old) with colibacillosis resulting from exposure to *E. coli* in the litter, even when the birds were exposed to cold stress, and furthermore reduced shedding of the pathogen among flocks.

3.3.3. Campylobacteriosis

The potential uses of phage therapy against *Campylobacter* bacteria may offer an alternative means of eliminating bacteria in the digestive tract of birds. This pertains in particular to infections induced by *Campylobacter jejuni* and *C. coli*, which constitute 80% of the bacteria colonizing the digestive tract in poultry. One of the first attempts to use bacterial viruses against *Campylobacter* bacteria was a study by Wagenaar et al. (2005), in which colonization by *C. jejuni* was inhibited in 10-day-old chicks and adult birds, first by 2 and then by 1 log unit in broiler caeca.

The use of a suspension of bacteriophages specific for *Campylobacter jejuni* and *C. coli* bacteria in the water or feed of broiler chickens caused a significant decrease of nearly 2 $\log_{10}$ CFU/g in colonization by both species of bacteria. Moreover, in contrast with earlier research, the bactericidal effect of the phages was maintained for over 7 days, enabling application of the suspension at each stage of the production cycle (Carvalho et al., 2010). Preventive treatment delayed but did not prevent colonization. Levels of *C. jejuni* were initially 2 log units lower than in controls, and then stabilized at 1 log unit lower than in the controls. On the other hand, the use of bacteriophages to prevent colonization by *Campylobacter* spp. bacteria in newly hatched broiler chicks was only partially successful. Application by oral gavage of a phage suspension with 0.4 to 2×10^{10} PFU/mL of phage 71 in 10-day-old broiler chickens initially reduced the total number of bacteria, but colonization by pathogens re-occurred within 24 h (Wagenaar et al., 2005). The studies cited also showed that resistance of *Campylobacter* spp. to particular phages was about 4%. For this reason the authors suggest creating a combination of several bacteriophages specific for *Campylobacter*, which in vitro research has shown to improve the effectiveness of phage therapy (Johnson et al., 2008).

3.3.4. Clostridiosis and listeriosis

Phage therapy was shown to be effective in the case of infection of broiler chickens with anaerobic *Clostridium perfringens* inducing necrotic enteritis (Miller et al., 2010). Bacterial toxins produced by this bacterium are responsible for generalization of the disease process, cause a decrease in feed intake, and inhibit growth. Oral administration to chickens of various ages of a suspension of a cocktail (INT-401) of 5 different *C. perfringens* phages (CPAS-7, CPAS-12, CPAS-15, CPAS-16, and CPLV-42) at titres of 10^5

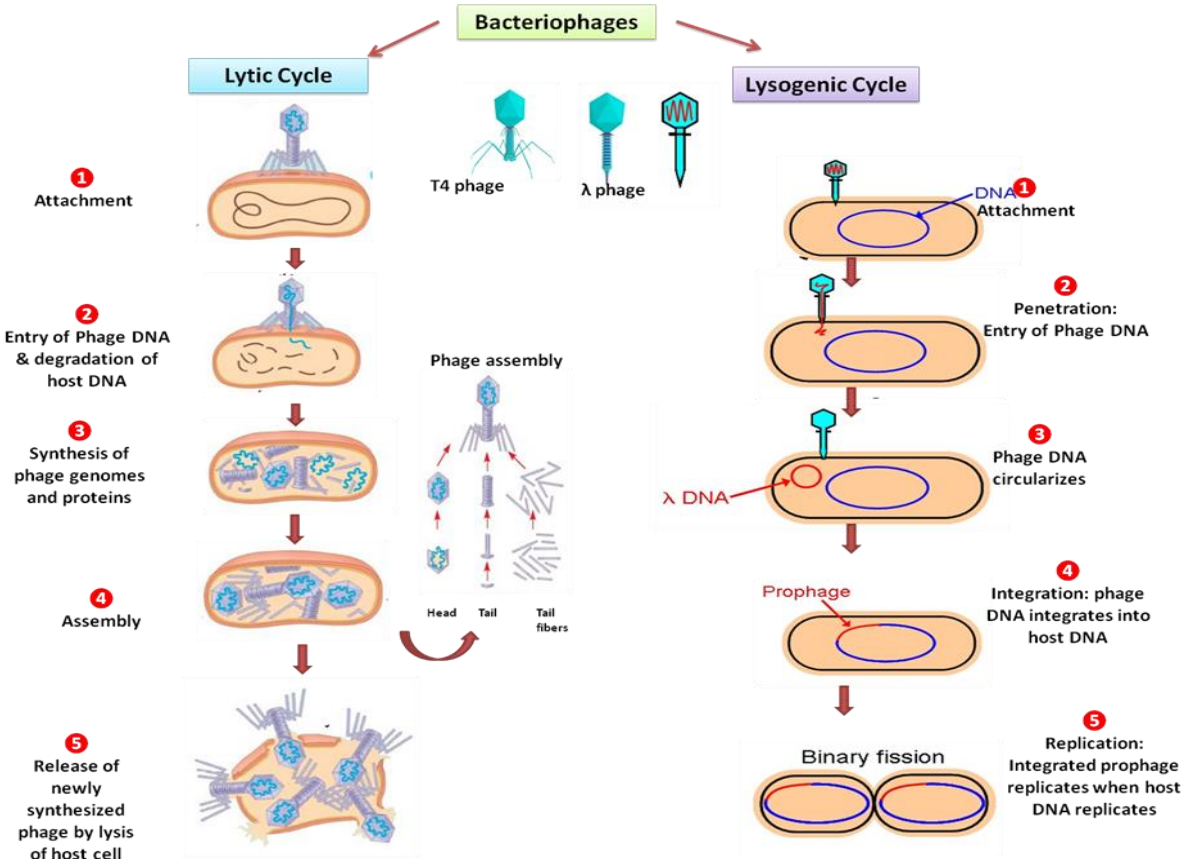

Figure 1. Life cycle or mode of action of bacteriophages: Lytic cycle of bacteriophages (T4) consists of adsorption, which involves adhesion to the bacterial cell, and binding of phage proteins to previously recognized receptors on the bacterial cell surface. The penetration phase involves rupture of the cell wall by the bacteriophage enzymes and penetration of the genetic material into the host cell. Next is the eclipse phase, involving replication of nucleic acid and proteins constitute the structural part of the capsid, while replication of the bacterial DNA is inhibited. This is followed by the formation and maturation of the bacteriophage, lysis of the bacterial cell and the release of daughter phages capable of infecting other cells. In case of lysogenic cycle, phage (λ phage) directly injects its genetic material into the host cell and integrates with host genome to produce prophage. Integrated prophage replicates when host genomes replicates.

Table 1. List of important bacteriophages having antibacterial effects against food borne pathogens with their target organisms**.**

Name of Bacteriophages	Target Organisms
ØVC8	*Vibrio cholera* (Solis-Sanchez et al., 2016)
SPN9CC	Gram-negative bacteria (Lim et al., 2014)
T5	*Escherichia coli* (Shavrina et al., 2016)
P2	*Escherichia coli* (Walmagh et al., 2013)
PsP3	*Salmonella enterica* (Walmagh et al., 2013)
K11 and KP32	*Klebsiella pneumonia* (Walmagh et al., 2013)
OBP	*Pseudomonas fluorescens* (Walmagh et al., 2012)
PVP-SE1	*Salmonella enterica* serovar Enteritidis (Walmagh et al., 2012)
201φ2-1	*Pseudomonas chlororaphis* (Walmagh et al., 2012)
phiKZ	*Pseudomonas aeruginosa* (Briers et al., 2007)
EL	*Pseudomonas aeruginosa* (Briers et al., 2007)
F2	*Campylobacter jejuni* (Atterbury et al., 2005)
SJ2	*Salmonella* (Modi et al., 2001)
phi29 (Φ29 phage)	*Bacillus sp.* (Grath and Sinderen, 2007)

PFU/mL, with feed or water or by oral gavage and spray application, led to a significant decrease ($P \leq 0.05$) in mortality during the 0-to-42-day of experiment in comparison with the group of untreated birds. These measures also improved weight gain in the chicks. It should also be emphasized that the treatment proved more successful in reducing mortality than a formalin-inactivated vaccine containing *C. perfringens* alpha toxin. However, the study cited confirmed the high efficacy of the bacteriophages in controlling necrotic enteritis in poultry.

The bactericidal effectiveness of phages has also been confirmed in fighting infections induced by *Listeria monocytogenes*, which like *Campylobacter* spp. or *Salmonella* is included among zoonotic pathogens inducing food poisoning in humans, with a high mortality rate of 30%. Application of bacteriophages on the surface of poultry products ready for consumption reduced the number of bacteria by 2.5 log units on a product stored at 30 °C after just 5 h. Later testing for *Listeria monocytogenes* in food samples kept in cold storage also yielded positive results, as the pathogen was not detected for a period of 21 days or use of a bacteriophage mixture on poultry carcasses could completely eliminated *L. monocytogenes* (Bigot et al., 2011). Due to the risk posed by the occurrence of poultry infections induced by *L. monocytogenes*, as well as their increasing drug-resistance and efforts to limit the use of antibiotics, international and American health organizations are attempting to replace antibiotics with other preparations. This resulted in FDA approval on 18 August 2006 of 102-LMP™, a suspension of bacteriophages specific for *L. monocytogenes*, as an antibacterial agent against *L. monocytogenes*. This product has been estimated to successfully kill over 170 strains of *Listeria* spp. (Housby and Mann, 2009).

4. The main problems to the use of phage therapy in poultry

Despite the significant positive aspects of phage therapy, there are also some limitations in the widespread use of bacteriophages to eliminate pathogens. One of the main obstacles to elimination of bacteria from poultry is that significant numbers of phages are needed to adsorb individual host cells (Zimmer et al., 2002). Some authors (Huff et al., 2002a) have shown that the application of phages in lower doses, e.g. 102 PFU, provided no statistically significant protection against *E. coli* infection. Moreover, preventive treatment in phage therapy did not prevent colonization (Carvalho et al., 2010). In some cases, a protective effect was obtained only in younger birds after high (106 PFU) doses of phage administration. In many cases, the efficacy of phage therapy should be maximized by the use of a high titre of bacteriophages to reduce *Salmonella* colonization by passive inundation. An additional obstacle in the use of phage therapy is that colonization of chicken caeca by *S. enterica* serotypes enteritidis and typhimurium is inhibited for only 24 to 48 h after phage treatment. For this reason it seems necessary to determine the optimal timing and delivery of bacteriophages in a real-life poultry industry setting (Lim et al., 2012). An important problem in phage therapy is that only strongly lytic phages are suitable. An area of safety concern is the potential release of toxic proteins from the lysing bacteria. In some cases, lysing bacteria inside a patient are known to release endotoxins that cause fever, and sometimes toxic shock. All phages contain foreign proteins which could induce an immune response potentially reducing the effectiveness of the therapy, or even cause death as a consequence of anaphylactic shock (Kutter et al., 2010; Wright et al., 2009).

CONCLUSION

Since the discovery of phages a century ago, their research focus has diversified from applying these agents to simply treat bacterial infections to a broad range of useful functions including biocontrol, diagnostics, drug discovery, and drug delivery as well as several applications in nanomedicine. In poultry products decreasing the load of bacteria decrease the risk of people getting sick, and in that sense, phage therapy may also be considered to have an indirect "probiotic" activity. On the other hand, the increasingly observed acquisition of antibiotic resistance by bacteria necessitates new strategies for combating drug-resistant bacteria. Based on our recent review on bacteriophages, we conclude that they can be a natural, environment friendly alternative means of using antibiotics to eliminate pathogens posing threat to humans and animals. The development of adequate phage preparations in the future may prove to be one

of the most effective methods for fighting bacteria that are pathogenic for humans and animals, and will also make it possible to obtain products that are safe and free of antibiotics.

AUTHORS' CONTRIBUTION

Nurjahan Yasmin Runa, Asmaul Husna and A.T.M. Badruzzaman prepared the first draft. Mohammad Rafiqul Islam revised the first draft. Md. Masudur Rahman designed and critically reviewed the article. The authors declare that no conflict of interest exists. No writing assistance was utilized in the production of this review article.

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