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# Review on Bacteriophages and its Antimicrobial Uses

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**Abstract:** Bacteriophages, also called phages, are obligate intracellular parasite that infect bacteria and reproduce by taking their host's biosynthetic pathways. They are the most abundant living entities on earth which affect the abundance and evolution of bacterial species. Two categories of bacteriophages are recognized; temperate and virulent, which propagate in two possible ways; lytic life cycle and lysogenic life cycle. They have been characterized by their host range and the physical characteristics, including capsid size, shape and structure, as well as genome size and type. These advances can have a profound impact on the development of safe therapeutic phage preparations having optimal efficacy against their specific bacterial hosts in humans and animals. Bacteriophages have been reported to be effective in treating various bacterial diseases such as cerebrospinal meningitis, skin infections, hepatic abscesses, lung infections, eye infections and urinary tract infections. Although phage therapy has been historically associated with the use of bacteriophages in human medicine, phages also have been extensively used in veterinary medicine. In conclusion, use of bacteriophages as antimicrobial is an excellent alternative for the treatment of bacterial infections. Therefore, optimization of formulations and long-term stability data is required before it can be widely used within a clinical setting.

**Key words:** Animal · Antimicrobial · Bacteriophage · Human

## INTRODUCTION

Bacteriophages are viruses that infect bacteria. They are the most abundant living entities on earth which affect the abundance and evolution of bacterial species, since several bacteriophages exist on the planet [1]. Bacteriophages were discovered by Fredrick Twort and Felix d'Herelle, whom d'Herelle called bacteriophages (eaters of bacteria) [2].

Bacteriophages, also called phages, are obligate intracellular parasite that infect bacteria and reproduce by taking their host's biosynthetic pathways [3]. It depends on the host for its propagation, which is influenced by a variety of factors such as temperature, nutrients, light and other environmental forces [4]. These bacterial viruses have genetic material in the form of either DNA or RNA, encapsidated by a protein coat. Bacterial genomes have revealed that phage genome elements are an important source of sequence diversity and can potentially influence pathogenicity and evolution of bacteria [5].

In theory, all bacteria are susceptible to viral infection, often by several types of phages [6]. Two categories of bacteriophages are recognized; temperate and virulent, which propagate in two possible ways; lytic life cycle and lysogenic life cycle [7]. During lytic infection, virulent phages inject their nucleic acid into the host cell following attachment. Expression of the phage genome directs the cellular machinery of the host to synthesize new phage capsule material. The resulting phage progeny are released by fatal cell lysis enabling the lytic cycle to continue as new cells are infected. The number of progeny released (burst size) varies from 50-200 new phage particles [1]. In contrast, during lysogenic infection, temperate phage nucleic acid recombines with the host cell genome forming a dormant prophage. The prophage is reproduced in the host cell line and confers immunity from infection by the same type of phage. Stress conditions such as ultraviolet light or chemical mutagens can induce a switch to the lytic cycle [8].

The number of phages that has been isolated and characterized likely represents only a tiny fraction of the phages existing on earth and phages with new characteristics are continuously being isolated from different parts of the world [9, 10, 11]. Nowadays when

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everything is much more advanced than ever before, scientists begin paying attention to bacteriophage due to their potential to be used as antibacterial, phage display systems and vehicles for vaccines delivery [5]. In addition, the evolution of antibiotic-resistant bacteria has aggravated curiosity in development of alternative therapy to conventional drugs. One of the emerging drugs that can be used as alternative to antibiotics is bacteriophage therapy. The use of living phages in the cure of lethal infectious life threatening diseases caused by gram positive and gram negative bacteria has been reported [12, 13]. The emergence of modified pathogens such as *Mycobacterium tuberculosis*, *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and Methicillin-resistant S.aureus (MRSA)* has created massive problems in treating patients in hospitals [14, 11, 15]. Since phages have already been proven to be a good natural antimicrobial treatment, the use of phages as alternative bacterial therapeutics may have promise [16, 11].

Bacteriophages are very specific to their hosts which minimize the chance of secondary infections unlike antibiotics which can cause secondary infection or super infection. They are environmentally friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process compared to new antibiotic development, which may take several years, cost millions of dollars for clinical trials and may not be very cost effective [17]. Knowledge of the bacteriophage in general would allow the design of phage specific for bacterial illness. Unlike the previous generation of antimicrobial preservatives, bacteriophages are viruses that are the natural predators of bacteria [3]. The antibiotic resistance of the bacteria does not affect the infectious activity of a phage. Therefore, the objective of this seminar paper is to overview the bacteriophages and its antimicrobial uses.

## Overveiw of Bacteriophage

**Historical Background:** Bacteriophages were discovered more than a century ago by observing unexpected antibacterial properties against cholera in the water of Indian River and similar phenomenon was observed while working with *Bacillus subtilis* [18]. However, bacteriophages were discovered independently in 1915 by Frederick Twort (1877–1950), an English bacteriologist and physician and in 1917 by Félix d'Herelle (1873-1949), a self-taught French-Canadian bacteriologist who proposed the name "bacteriophage". Félix d'Herelle published his discovery of "an invisible, antagonistic microbe of the dysentery bacillus" [19].

Historically, phage therapy has been practiced in France since 1919, when d'Hérelle's preparations were given to patients with dysentery at the Hospital [20]. His bacteriophage laboratory produced the first commercial phage cocktails. These preparations were produced commercially in France until approximately 1978. Through the mid 1990's, the Pasteur Institutes of Paris and of Lyon continued to produce small amounts of bacteriophage preparations on demand. Intensive studies on the therapeutic use of phages for treating infectious diseases were taken up in 1920 [3] and continued to be reports in the literature of phage therapy until 1979 [21]. Successful Phage therapy trials were reported from Baylor University's College of Medicine in 1923 and said that "bacteriophage holds enormous possibilities as a new weapon for fighting infectious disease" [22]. Not long after their discovery, bacteriophages were successfully used to treat certain bacterial diseases, such as dysentery [23]. However, a number of factors led to the decline of bacteriophage usage for medical applications. The most serious challenge came when antibiotics were discovered. Other contributing factors included poorly designed clinical trials, clinical failures (often due to the lack of recognition that phage are specific for a given host bacteria) and overzealous claims for efficacy. However, large pharmaceutical Companies in some countries recognize the effect of bacteriophage treatment (e.g.USA) and commercialization of bacteriophage preparation for the treatment of Staphylococcus infections was begun [3].

**Morphology:** The basic structural features of bacteriophage are coats or capsids that protect the genome hidden inside a capsid and additional structure providing interface with a bacterium membrane for the genome release. Different phage species can vary both in size from 24-400 nm in length and genome length. All phages contain a head, which stores the genetic material and forms part of the overall structure of a bacteriophage. Most have 20 sides of capsid and are called icosahedral and other bacteriophages may be filamentous. The capsid is usually in a geometric shape and it consists of one or two different proteins. The main function of the capsid is to protect the genetic material from the environment. Specifically, the capsid prevents enzymes from breaking down the genetic material [24].

Some phages have tails attached to the phage head. The tail is a hollow tube that serves as a passageway for the genetic material to pass from the capsid to the host bacteria. A bacteriophage tail is attached to the capsid through a connector which serves as an adaptor between these two crucial components of the phage.

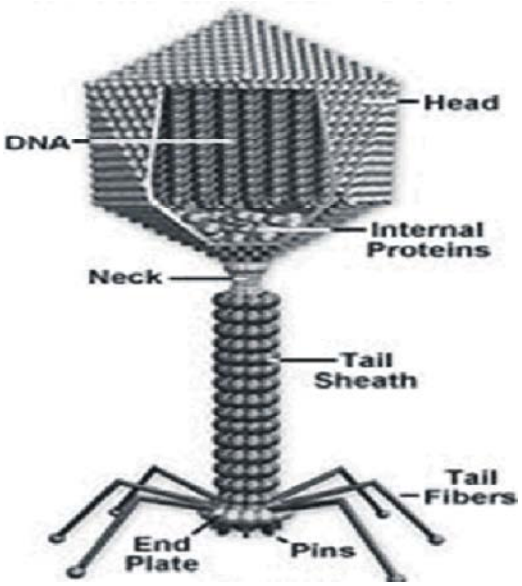


Fig. 1: Structure of bacteriophage [27].

The connector is a hetero-oligomer composed of several proteins [25]. At its distal end is a hexagonal baseplate, bearing the tail fibers. A thin tail tube is built up on the base plate and this is surrounded by a sheath, which contracts upon infection and becomes demonstrably shorter and thicker. The base plate and tail fibers are involved in the binding of the phage to the bacterial outer membrane that makes the attachment irreversible [26]. Structure of bacteriophage (e.g $T_4$ page) is shown below (Fig-1).

**Nature of Bacteriophages:** Phages, like all viruses, are obligate intracellular parasites. Although they carry all the information to direct their own reproduction in an appropriate host, they have no machinery for generating energy and no ribosome for making proteins. They are the most abundant living entities on earth, found in very large numbers wherever their hosts live, in sewage and feces, in the soil, in deep thermal vents and in natural bodies of water. Their high level of specificity, long-term survivability and ability to reproduce rapidly in appropriate hosts contribute to their maintenance of a dynamic balance among the wide variety of bacterial species in any natural ecosystem [28].

**Classification of Bacteriophages:** At present, more than 5000 bacterial viruses have been examined by electron microscope [29]. Historically, phage have been characterized by their host range and the physical characteristics of the free virion, including capsid size, shape, resistance to organic solvents and structure, as well as genome size and type. Currently, there exist three basic means of characterizing phages into types: first one is infection which can be either lytic or lysogenic phage. The second one is morphology in which bacteriophage can be tailed, isometric (isosahedral), helical (filamentous or rod-shaped) and pleomorphic phages. The third one is genetic makeup (genome) in which they can be represented by DNA or RNA in the form of dsDNA (Helical and Pleomorphic phages), ssRNA, dsRNA, or ssDNA. The vast majority of phages contain double strand DNA [6].

Phages can be divided into two classes based on lifestyle: virulent or temperate. Virulent phages (e.g T4 phage) can only multiply by means of a lytic cycle; the phage virion adsorbs to the surface of a host cell and injects its genome, which takes over much of host metabolism and sets up molecular machinery for making more phages. The host cell then lyses minutes or hours later, liberating many new phages. Temperate phages, in contrast, have a choice of reproductive modes when they infect a new host cell. Sometimes the infecting phage initiates a lytic cycle, resulting in lysis of the cell and release of new phage. The infecting phage may alternatively initiate a lysogenic cycle; instead of replicating, the phage genome assumes a quiescent state called a prophage, often integrated into the host genome [28, 30].

**Bacteriopage Genetics:** Bacteriophages are the viruses with either DNA or RNA as the genetic material and both single and double stranded forms of each are known [31]. The structure is same as that found universally among living organisms: a polynucleotide chain consisting of a deoxyribose (or ribose) phosphate backbone to which are attached specific sequences of the four nucleotides adenine, thymine (or uracil), guanine and cytosine; in all, except the single stranded phages, two such complementary chain are paired together in a double helix [32]. Each phage particle (virion) contains its nucleic acid genome (DNA or RNA) enclosed in a protein or lipoprotein coat, or capsid; the combined nucleic acid and capsid form the nucleocapsid. Most known bacteriophages contain dsDNA genomes and this type of bacteriophage is thought to be the most common in nature. However, many other kinds are known, including those with ssRNA genomes, dsRNA genomes and ssDNA genomes [28].

**Bacteriophage Life Cycle:** A bacteriophage reproduces by one of two types of life cycles. These cycles are the lyticlife cycle and the lysogenic life cycle [33].

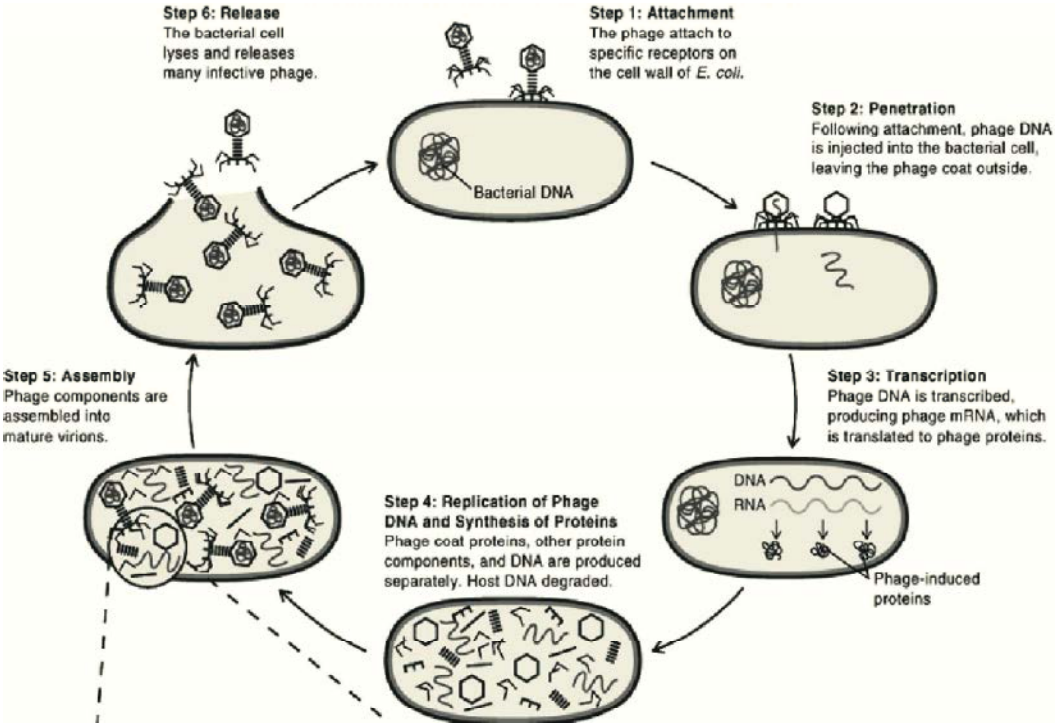


Fig. 2: Lytic life cycle of bacteriophage [36].

**Lytic Cycle:** Lytic or virulent phages are phages, which multiply in bacteria and kill the cell by lysis at the end of the life cycle.In the lytic life cycle, the virus breaks open or lyse the host cell in which it results in the death of the host. The host cell undergoes lysis and dies, simultaneously liberating a large number of progeny phages, which are each then ready to start another cycle by infecting new neighboring bacterial cells [4]. Soon after the nucleic acid is injected, the phage cycle is said to be in eclipse period [34, 35]. During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell. Eclipse phase represents the interval between the entry of phage nucleic acid into bacterial cell and release of mature phage from the infected cell. The phage nucleic acid takes over the host biosynthetic machinery and phage specified m-RNA and proteins are made. In some cases the early phage proteins actually degrade the host chromosome structural proteins (head, tail) that comprise the phage as well as the proteins needed for lysis of the bacterial cell are separately synthesized. Nucleic acid is then packaged inside the head and then tail is added to the head. The assembly of phage components into mature infective phage particle is known as maturation. In lysis, the bacteria begin to lyse due to the accumulation of the phage lysis protein and intracellular phage are released into the medium. It is believed that phage enzymes weaken the cell wall of bacteria [33].

Generally, lytic phages have distinctive phases in the lytic developmental cycle (Fig-2):Adsorption of the phage on the host cell by binding to a specific receptor, nucleic acid injection in to the bacterial cell, expression of the phage early genes, synthesis of early proteins and phage genome replication followed by expression of the phage late proteins involved in the formation of new phage particles and lysis of the host bacterium, Assembly of the phage heads and tails and packaging of the genome and finally lysis of the host bacterium and release of the new phage progeny [36].

**Lysogenic Cycle:** Lysogenic bacteriophages are those that can either multiply via the lytic cycle or enter a dormant state in the cell. The lysogenic cycle is a cycle in which bacteriophages reproduce without killing the host. Genetic recombination occurs between the viral DNA and the bacterial genome as the viral DNA is inserted into the bacterial chromosome. Bacteriophages which have this life cycle are lysogenic phages, or ‘temperate’ or dormant phages which may take the form of a ‘prophage’ by integrating with the viral DNA in the host chromosome [37].

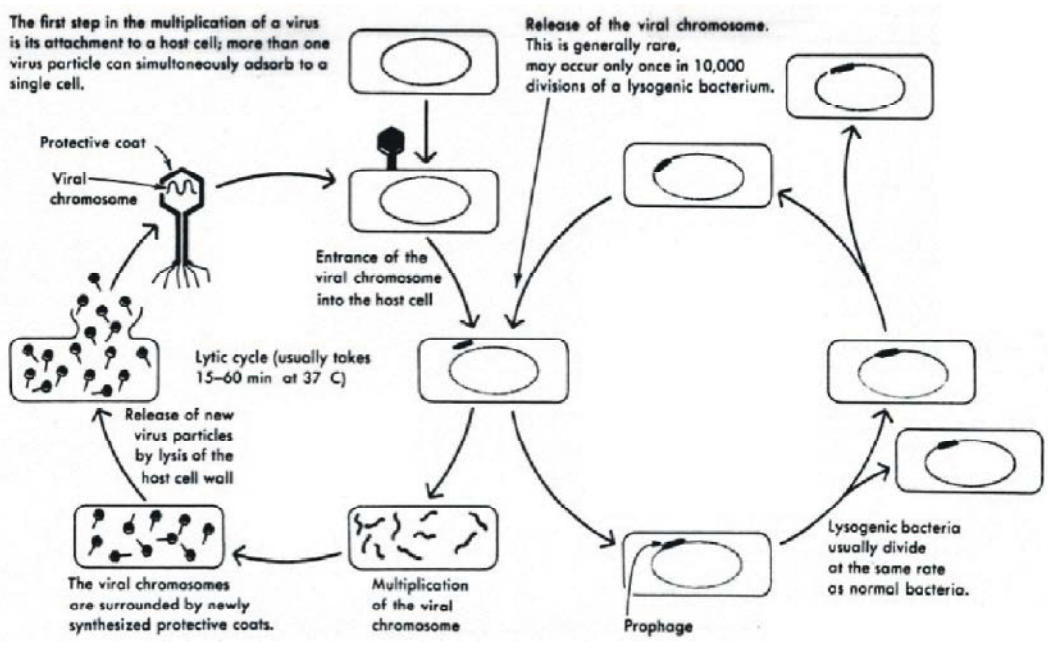


Fig. 3: Two cycles of bacteriophage reproduction [24].

In most cases the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. This integrated state of phage DNA is termed prophage. This process is known as lysogeny and the bacteria harboring prophage are called lysogenic bacteria. Since the prophage contains genes, it can confer new properties to the bacteria. When a cell becomes lysogenized, occasionally extra genes carried by the phage get expressed in the cell. These genes can change the properties of the bacterial cell [37]. This process is known as lysogenic conversion or phage conversion. The lysogenic and lytic life cycle is shown below (Fig-3).

**Application of Bacteriophage as Antimicrobial Agents:** Since their discovery by Twort (1915) and independently by d'Herelle (1917), the bacteriocidal properties of phage have raised interest in their potential use in the control of medical conditions. In the pre-antibiotic era of the early twentieth century, the potential of bacteriophages to be a powerful tool in dealing with infectious diseases of bacterial etiology also captured the imagination of the scientific and non-scientific communities [22]. Phages were used widely in the early 20$^{th}$century to treat human and animal illness with varying degrees of success. In the West and US, research into phage therapy declined following inconsistent results and as a consequence of the discovery of antibiotics in the 1940s. Phage therapy research continued in Eastern Europe where phage treatments against a wide array of bacteria including Staphylococci, *Pseudomonas*, *Proteus spp*. and enteric pathogens were produced [17, 38]. There has been a renewed interest in phage therapy over the past two decades, partly as a consequence of increasing antibiotic resistance in bacteria. Good work has focused on animals as models for human infection or veterinary applications [39, 40].

**Phage Products as Antimicrobial Agents:** With the increasing worldwide prevalence of antibiotic resistant bacteria, bacteriophage endolysins represent a very promising novel alternative class of antibacterial in the fight against infectious disease. Phage endolysins, or lysins, are enzymes that damage the cell walls' integrity by hydrolyzing the four major bonds in its peptidoglycan component [41]. Vincent Fischetti (1940) was the first, however, to focus on the deadly weapons, the potent and specific enzymes called lysins produced by these viruses. These lysins create lethal holes in bacterial cell walls that can kill a wide range of gram-positive pathogenic bacteria and have proven their effectiveness in both preventing and treating infections in mice, an important step towards their potential application in human disease For nearly a century, scientists have attempted to treat bacterial infections with whole phages [12].

**Bacteriophage as Antimicrobial Agents**

**Bacteriophage Therapy in Humans:** Understanding of biological properties of phages and the basic mechanisms of phage-bacterial host interaction has improved dramatically since the days of early therapeutic uses of bacteriophages. These advances can have a profound impact on the development of safe therapeutic phage preparations having optimal efficacy against their specific bacterial hosts. Phages as therapeutic agents in humans were first used in 1919 just when they were discovered [42, 43]. Phages have high specificity for specific bacterial strains, a characteristic which requires careful targeting [44, 45]. The majority of human infections such as viral or bacterial start at a mucous membrane site (upper and lower respiratory, intestinal, urogenital and ocular) which are the reservoir for many pathogenic bacteria found in the environment (i.e., P*neumococci, Staphylococci, Streptococci*), many of which are reported to be resistant to antibiotics [46]. Phage lytic enzymes have recently been proposed for the reduction of nasopharyngeal carriage of *pathogen* and also as a therapeutic use for resistant bacteria [47].

Phages have been reported to be effective in treating various bacterial diseases such as cerebrospinal meningitis in a newborn [48], skin infections caused by *Pseudomonas, Staphylococcus, Klebsiella, Proteus, E. coli* and sub hepatic abscesses, *Staphylococcal* lung infections, *Pseudomonas aeruginosa* infections in cystic fibrosis patients [49], eye infections, neonatal sepsis, urinary tract infections and cancer [50]. Clinical improvement was associated with rapid (7 days) elimination of the *S. aureus* resistant to many antibiotics (including ciprofloxacin) [51]. Oral phage therapy was used for targeting MRSA [52]. The first controlled clinical trial of a therapeutic bacteriophage preparation showed efficacy and safety in chronic otitis because of drug resistant *P. aeruginosa* [53]. Phage lytic enzymes also effective in post-exposure cases of anthrax, in which individuals can be treated intravenously to control the bacilli entering the blood after germination because higher doses of phage lysin or multiple doses will result in nearly 100% protection [54]. Several clinical trials on phage therapy in humans were reported with the majority coming from researchers in Eastern Europe and the former Soviet Union [55].

**Bacteriophage Therapy in Animals:** Since the discovery of bacteriophages in 1915–1917, they have been used to prevent and treat various bacterial infections. Although phage therapy has been historically associated with the use of bacteriophages in human medicine, phages also have been extensively used in veterinary medicine. One of the earliest animal models used in several phage therapy studies was murine salmonellosis, a systemic disease produced in susceptible mice by several serotypes of Salmonella. Phages are ubiquitous in the environment and their use in livestock is likely to provide one of the most environmentally friendly antibacterial approaches available today [43].

In Britain, Smith and Huggins carried out a series of excellent, well-controlled studies on the use of phages in systemic *E. coli* infections in mice and then in diarrheic disease in young calves and pigs. Bacteriophages might be useful for the prevention of *P. aeruginosa* infections in patients with burn wounds [56]. Phage therapy has been successfully used to remove *E. coli 0157:H7* from livestock. The protective effect of bacteriophage was assessed against experimental *S. aureus* infection in mice [57].

The sewerage-derived bacteriophage reduced the abscess area and the count of S. aureus in the abscess was lowered in a bacteriophage dose dependent way [58]. It was reported about treatment of a dog with chronic bilateral otitis external that had consistently grown *P. aeruginosa*. In recent years the phage therapy has received lot of attention due to an increase in the prevalence of antibiotic resistant strains in clinical settings. A numbers of recent experimental studies have proved the efficacy of phages in treating different infections. When the animals were colonized with their respective bacteria and treated with a small amount of lysin specific for the colonizing organism, the animals were found to be free of colonizing bacteria two to five hours after lysin treatment [59].

**Safety of the Therapeutic Bacteriophages:** During the long history of using phages as therapeutic agents through Eastern Europe and the former Soviet Union, there has been no report of serious complications associated with their use [60]. Phages are extremely common in environment and regularly consumed in foods [61]. They have been commonly found in human gastrointestinal tract, skin and mouth, where they are harbored in saliva and dental plaques [62]. Phages are also abundant in environment including saltwater, freshwater, soil, plants and animals and they have been shown to be unintentional contents of some vaccines and sera commercially available in United States Therefore, phage therapy can be used to lyse specific pathogens without disturbing normal bacterial flora and phages pose no risk to anything other than their specific bacterial host [63].

From a clinical standpoint, phage therapy appears to be very safe. However, in the last few years, modified phages are being explored increasingly, due to the limitations of phage therapy using lytic phages. The safety concerns regarding spontaneously propagating live microorganisms and the inconsistency of phage therapy results in the treatment of bacterial infections specifically induced scientists to explore more controllable phages [64]. This phage had the benefit of minimizing the release of membrane associated endotoxins during phage therapy [13]. In order not to compromise on the issue of the safe use of therapeutic phage preparation, rigorous characterizations of each phage to be used therapeutically should be done, in particular, especially looking for potentially harmful genes in their genome [65].

**Benefits of Bacteriophage Therapy:** Phages appear to be better therapeutic agents as they have several advantages over traditional antibiotics. Although bacteriophage therapy can probably be used to prevent or treat most bacterial infections, it is likely that antibiotic-resistant bacteria will become the first clinical targets of phage therapy technology. One of the main differences between phages and antibiotics, as far as their antimicrobial activity is concerned, is their target range. Phages are very specific and they will normally only lyse strains of one species or a subset of strains within a species, it permits targeting of specific pathogens without affecting desirable bacterial flora. Several studies in which the efficacy of phage preparations and antibiotics were evaluated side-by-side suggest that, when phage preparations are carefully selected, they can be as effective as and sometimes more effective than, antibiotics in treating certain infections in humans [66, 67] and experimentally infected animals. On the other hand, several studies in which phages and antibiotics were used simultaneously (e.g., to treat chromic pulmonary suppurations) reported that the combined use of phages and antibiotics improved the outcome of therapy [67]. Moreover, bacteriophages replicate at the site of infection where they are mostly needed to lyse the pathogens, but antibiotics travel throughout the body and do not concentrate at the site of infection. No side effects have been reported during or after phage application, but resistant bacteria, allergies (sometimes even fatal anaphylactic reaction) and secondary infections are the common side effects of antibiotics treatment [63].

Bacteriophages are environmentally friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process compared to new antibiotic development, which may take several years, may cost millions of dollars for clinical trials and may also not be very cost effective [17]. Furthermore, the development of phage resistance can be forestalled altogether if phages are used in cocktails (preparations containing multiple types of phages) and/or in conjunction with antibiotics [68]. In fact, phage therapy and antibiotic therapy, when co-applied, are synergistic [69]. Bacteriophage production is simple despite complex and expensive antibiotic production. The pharmacokinetics of bacteriophage therapy is such that the initial dose increases exponentially if the susceptible bacterial host is available. In such cases, there is no need to administer the phages repeatedly, however; a repeated dose of antibiotic is required to cure the bacterial disease [70].

## CONCLUSION AND RECOMMENDATION

Numerous studies performed in human and different species of animal have demonstrated that the bacteriophage therapy is important in the treatment of bacterial infection. Intensive studies on the therapeutic use of phages for treating infectious diseases were started long time since the pre-antibiotics era and continued to be reported as a new weapon for fighting against bacterial infection. Currently, there has been an increasing interest to use bacteriophages as antimicrobial over the past two decades, partly as a consequence of increasing antibiotic resistance in bacteria Bacteriophage therapy has been practiced in worldwide in both animals and humans. With regard to antimicrobial, Phages are very specific and effective than antibiotics and act without affecting desirable bacterial flora. Moreover, bacteriophages are environmentally friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process compared to new antibiotic development activity. Surprisingly, phages and antibiotics were used simultaneously (e.g., to treat chromic pulmonary suppurations) in which they boost the outcome of therapy.

On the above conclusion, the following recommendations are forwarded:

- More research should be done on the properties and behavior of the specific phage–bacterium system for safe and controlled use of phage therapy.
- New technologies should be developed and commercialized to analyze phage and their antimicrobial usage.

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