Chapter

Bacteriophages: The Good Side of the Viruses

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Abstract

Bacteriophages or phages are bacterial viruses that are known to invade bacterial cells and, in the case of the lytic phages, impair bacterial metabolism, causing them to lyse. Since the discovery of these microorganisms by Felix d'Herelle, a French-Canadian microbiologist who worked at Institut Pasteur in Paris, Bacteriophages begin to be used in the treatment of human diseases, like dysentery and staphylococcal skin disease. However, due to the controversial efficacy of phage preparations, and with the advent of antibiotics, commercial production of therapeutic phage preparations ceased in most of the Western world. Nevertheless, phages continued to be used as therapeutic agents (together with or instead of antibiotics) in Eastern Europe and in the former Soviet Union. Therefore, there is a sufficient body of data that incite the accomplishment of further studies in the field of phage therapy.

Keywords: Bacteriophages, therapy, antimicrobial, viruses, phages

1. Introduction

The resistance of pathogenic bacteria to most, if not all, currently available antimicrobial agents, has become a major problem in modern medicine, especially because of the increased numbers of immunosuppressed patients. The concern that humankind is approaching the "preantibiotics" era is becoming realer day by day, and this scenario increases the demand for the development of new antibiotics that can be used to treat these life-threatening diseases to human life [1].

Before the discovery and the wide spread use of antibiotics, it was suggested that bacterial infections could be prevented and/or treated with the administration of bacteriophages. Despite the fact that the clinical studies with bacteriophages were discontinued in United States and Western Europe, phages continued to be utilized in the former Soviet Union and in Eastern Europe. The results of the studies were extensively published in non-English journals, and, therefore, were not available to the western scientific community [1]. In this book chapter, we describe the history of bacteriophage discovery, the first clinical studies with phages, the application of phages in different bacterial diseases, the reason why its usage failed to prevail in

the Western World, and last, but not less important, the future prospects of the use of Bacteriophages as therapeutical agents in bacterial diseases.

1.1 The discovery of bacteriophages and the first phage therapy research

Bacteriophages or phages are bacterial viruses that are known to invade bacterial cells and, in the case of lytic phages, impair bacterial metabolism, causing them to lyse. Since the discovery of bacteriophages, there has been a debate over claims for who really first discovered these microrganisms. Ernest Hankin, a British bacteriologist, reported in 1896 an antimicrobial activity against *Vibrio cholerae* in samples of water of Ganges and Jumna rivers in India, and he suggested that this phenomenon could be possible by the presence of an unidentified substance that passed through the fine porcelain filters and was heat labile, limiting the spread of cholera epidemics [2].

Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with another bacterial species: *Bacillus subtilis* [3]. Other scientists also observed this event, but with other bacteria. However, none of them further explored their findings until Frederick Twort, a medically trained bacteriologist from England, reintroduced the subject almost 20 years after Hankin's observation by reporting a similar phenomenon and hypothesizing that it may have been due to, among other possibilities, a virus [4]. However, Twort did not continue his research because of many reasons, including financial difficulties [4–6] and only two years later, bacteriophages were "officially" discovered by Felix d'Herelle, a French-Canadian microbiologist at the Institut Pasteur in Paris [1].

Unlike Hankin and Twort, d'Herelle had almost no doubt about the nature of the observed phenomenon, and he proposed that it was caused by a virus capable of parasitizing bacteria. He and his wife Marie, on 18 October 1916, then decided to name this microorganism as "bacteriophage" [5]. The name derived from the words "bacteria" and "phagein" (to eat or devour, in Greek), implying that phages "eat" or "devour" bacteria. D'Herelle considered himself to be the discoverer of bacteriophages, but he acknowledged that his discovery was different from Twort's discovery. Also, in contrast to Twort, d'Herelle carried on studies of bacteriophages and strongly supported the idea that phages were live viruses – and not "enzymes" as many of his fellow researchers thought. The fight for the priority ceased eventually and many scientists accepted the independent discovery of bacteriophages, naming it as the "Twort-d'Herelle phenomenon" and later, the "bacteriophage phenomenon" [1].

2. First studies of phage therapy

After his discovery, d'Herelle used phage to treat dysentery, representing the first attempt to use bacteriophages to treat a bacterial disease. The study was conducted at the Hospital des Enfants-Malades in Paris in 1919 [5] under the supervision of Professor Victor-Henri Hutinel, the Hospital's Chief of Pediatrics. The phage preparation was ingested by d'Herelle, Hutinel and several hospital interns in order to test its safety before its usage by humans, more specifically, a 12-year-old-boy with severe dysentery. The patient's symptoms disappeared after a single administration of d'Herelle's antidysentery phage, and the boy fully recovered after a few days. The phage preparation proved its "efficacy" shortly after, when three other patients presenting bacterial dysentery that were treated with one dose of the preparation recovered within 24 hours of treatment [1].

However, the results of these studies were not published and the first reported application of phages used in the treatment of bacterial diseases happened only in 1921 in a study performed by Richard Bruynoghe and Joseph Maisin [7], who used bacteriophages to treat staphylococcal skin disease. The bacteriophages were injected into and around surgically opened lesions and it was observed a regression of the infections within 24 to 48 hours. In view of these promising results, several companies began commercial production of phages against various bacterial pathogens [1].

2.1 Marketing of phages

D'Herelle's commercial laboratory in Paris produced five phage preparations against various bacterial infections: Bacte-coli-phage, Bacte-rhinophage, Bacte-intesti-phage, Bacte-pyo-phage, Bacte-staphy-phage, and they were marketed by what later would become the large French company L'Oreal [5]. The production of therapeutic phages also began in the United States at that time. In the 1940s, the Eli Lilly Company (Indianopolis, Ind.) produced seven phages for human use against staphylococci, streptococci, *Escherichia coli*, and other bacterial pathogens, which consisted of phage-lysed, bacteriologically sterile broth cultures of the targeted bacteria (e.g., Colo-lysate, Ento-lysate, Neiso-lysate, and Staphylo-lysate) and the same preparations in a water-soluble jelly base (e.g., Colo-iel, Ento-iel, and Staphylo-jel). They were used to treat various infections, including abscesses, suppurating wounds, vaginitis, acute and chronic infections of the upper respiratory tract and mastoid infections. However, due to its controversial efficacy, and with the advent of antibiotics, commercial production of therapeutic phages ended in most of the Western World [8, 9]. Even so, phages continued to be used therapeutically (together with or instead of antibiotics) in Eastern Europe and in the former Soviet Union.

The institute, during its best times, employed approximately 1,200 researchers and support personnel, resulting in a production of phages of several tons a day, against a dozen bacterial pathogens, including *Staphylococci*, *Pseudomonas*, *Proteus*, and many enteric pathogens [1].

The bacteriophage laboratory of the Institute then began to produce phages for the treatment of many diseases, such as septicemia, furunculosis, and pulmonary and urinary tract infections and for the prophylaxis or treatment of postoperative and posttraumatic infections. In most of the cases, the phages were used against multi-drug resistant bacteria that were refractory to the conventional treatment with the majority of the antibiotics used in the clinical setting [10–16].

2.2 Experimental studies in animals

The first experimental studies that utilized animals in laboratories on the treatment of bacterial diseases using bacteriophages came from the Laboratory of William Smith and Smith and his colleagues [17–20] at the Institute for Animal Disease Research in Houghton, Cambrigeshire, Great Britain. In one of their first published papers, the authors reported the successful use of phages to treat *E. coli in vitro* infections in mice. In the next studies, [18–20] the authors found that a single dose of specific *E coli* phage reduced, by many orders of magnitude, the number of targeted bacteria in the digestive tract of calves, lambs, and piglets previously infected with a strain of *E. coli* that caused diarrhea. The treatment also ceased the associated fluid loss, and all the animals that were treated with the bacteriophages survived the bacterial infection. Furthermore, such positive results rekindled the interest in phage therapy in the West World and stimulated other researchers to

investigate the possibility of using phages on the treatment of bacterial diseases caused by antibiotic resistant bacteria capable of causing human infections.

Another *in vivo* study performed by Soothill et al. [21] reported the importance of the phages in preventing and treating diseases induced experimentally in mice and guinea pigs infected with *Pseudomonas aeruginosa* and *Acinetobacter*, suggesting that its usage might be efficacious in preventing infections of skin grafts used to treat burn patients. However, it is uncertain if these "preclinical" studies preceded human clinical trials. Indeed, although many human trials were preceded by at least some *in vitro* studies using laboratory animals, the scientific literature regarding this topic is scarce.

Since the history of the discovery of the bacteriophages and some pioneer studies regarding this subject was already explored, the next section of this book chapter will explore the lytic and lysogenic cycles of phages, mode of action of these microrganisms when used in the therapy to treat bacterial diseases as well as some specific advantages and disadvantages in such use in the clinical settings.

2.3 Lytic and lysogenic life cycles of phages

Recent publications have provided interesting evidence that questions the notion that viruses are non-living organisms [22]. Erez et al., in their recent publication, identified a communication between viruses. They found a unique small-molecule communication system that controls lysis-lysogeny life cycles in a temperate phage [23]. Another study described the assembly of a nucleus-like structure during the viral replication of phage 201Φ2–1 in *Pseudomonas chlororaphis*, which suggested that phages have evolved a specialized structure to compartmentalize viral replication [24].

Phages can go through two different life cycles: the lytic and the lysogenic cycle. First, phages bind to the bacterial host specifically on a receptor found on the bacteria's surface and then injects its genetic material into the cell. The phage then takes advantage of the bacterium's biochemical machinery and replicate its genetic material, producing progeny phage. Subsequently, the phage synthesizes proteins such as endolysin and holin, which lyse the host cell from within. Holins are small proteins that accumulate in the cytoplasmic membrane of the host, allowing endolysin to degrade peptidoglycan and the progeny phage to escape the bacterial host. In the external environment, lytic phage can infect and destroy all bacteria nearby its initial bacterial host (**Figure 1**). The rapid proliferation and the large number of lytic phages are advantageous when they have therapeutic purposes. However, lytic phages have narrow host ranges and infect only specific bacterial species. Though, it can be overcame by giving a cocktail of different phages to patients afflicted by bacterial infections [25].

In the lysogenic cycle, the temperate phages do not immediately lyse the host cell, instead, they insert their genome into the bacterial chromosome at specific sites. This phage DNA now inserted into the host genome is called prophage, while the host cell containing the prophage is called a lysogen. The prophage then replicates along with the bacterial genome, establishing a stable relationship between them. The disadvantage of using temperate phage in phage therapy is that once the phage DNA is inserted into the bacterial genome, it can remain dormant or even alter the phenotype of the host [25].

Another advantage of using temperate phages in phage therapy is that the lysogenic cycle can continue indefinitely unless the bacteria are exposed to stress or adverse conditions. The signals that triggers such event vary from phage to phage, but prophage are commonly induced when bacterial stress responses are activated

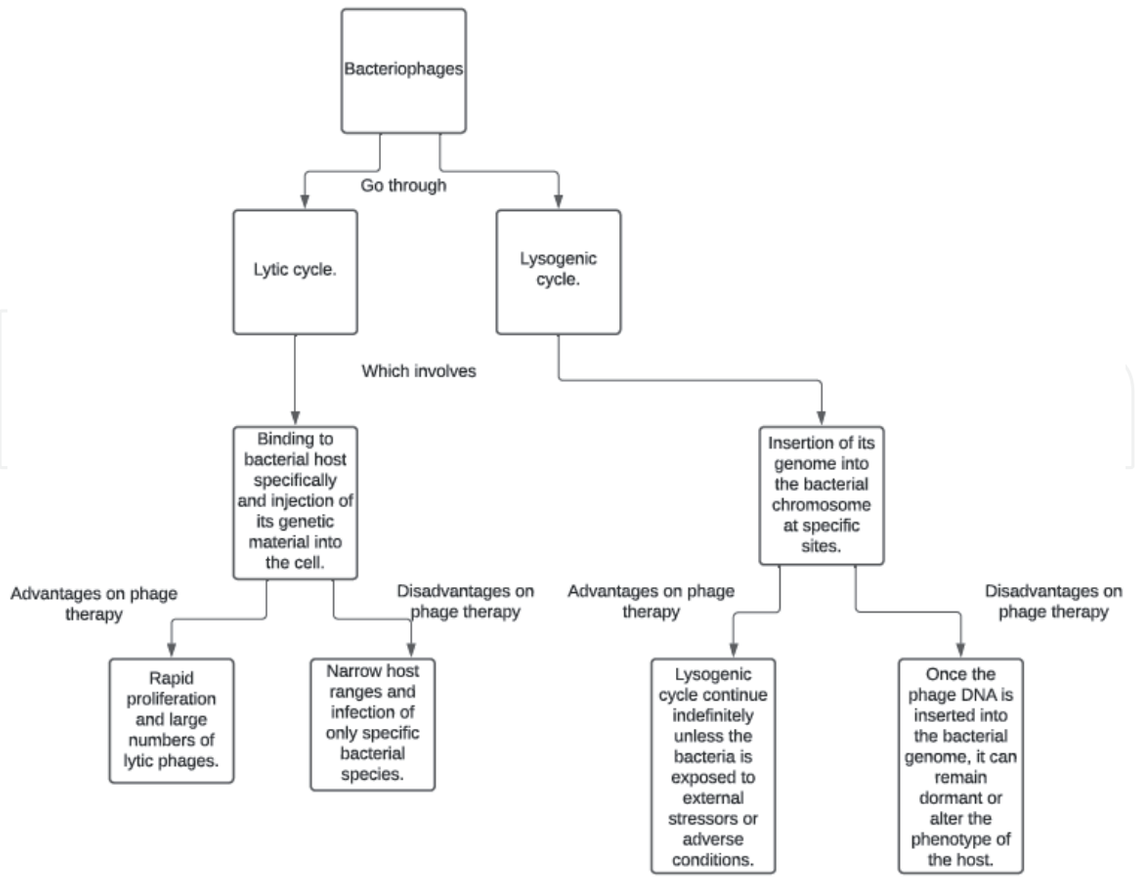

Figure 1.
Diagram representing the lytic and lysogenic cycle of the bacteriophages, as well as their advantages and disadvantages on phage therapy.

due to antibiotic treatment, oxidative stress, or DNA damage [26]. Once the lysogenic cycle finishes, expression of phage DNA starts and lytic cycle begins. In recent studies, it was found that phages that infect *Bacillus* species depends on small molecules called "arbitrium" to communicate to each other and make lysis-lysogeny decisions [23].

The biological implication of this phenomenon is very significant and explains why when phages encounters a large numbers of bacteria colonies, therefore, finding plenty of hosts to infect, they activate the lytic cycle. If host numbers is limited, the progeny phage then activates the lysogenic cycle and enters in a dormancy state. These recent findings stimulate other researches to be done to determine if there are other peptides also implicated in this phenomenon or if cross-talk is evident among different bacteriophage [25].

Furthermore, recent study regarding the full genetic sequence of the T4 phage (GenBank accession number AF158101) showed that the lysis of the bacteria by a lytic phage involves a complex process consisting several structural and regulatory genes. Besides, it is also possible that some therapeutic bacteriophages have some unique and unidentified genes or mechanisms responsible for effectively lysing their targeted bacteria. This led scientists to identify and clone, years later, an anti-*Salmonella* phage possessing a potent lethal activity against *Salmonella enterica* serovar *Typhimurium* host strains. Another study showed an unique mechanism for protecting phage DNA from the restriction-modification defenses of an *S. aureus* host strain. Further studies are necessary to gather information that are going to be useful to genetically engineer therapeutic phage preparations [27].

2.4 Mode of action of the bacteriophages

The first studies regarding the pharmacokinetics of bacteriophages showed that phages got into the bloodstream of laboratory animals after a single oral dose within 2 to 4 hours and that they were found in the following organs of the human body: liver, spleen, kidney, etc. in approximately 10 hours. Additionally, data concerning the period of time that the phages can remain in the human body indicate that it can happen for a long period of time, i. e., for up to several hours [28].

Despite the efforts in better understanding the pharmacokinetics of phages, their self-replication creates a complex scenario influenced by both decrease and proliferation. Although *in vivo* amplification of phages has been already performed, the topics are dominated by mathematical models of *in vitro* infections, which does not necessarily corresponds to *in vivo* amplification [29]. On the other side of it, phage lytic enzymes are considered as standard drugs in terms of pharmacokinetics. SAL200, a *S. aureus*-specific endolysin, has a $t_{1/2}$ between 0.04 and 0.38 hours after intravenous administration in healthy volunteers. The authors stated that, based on the molecular weight, renal clearance and drug distribution from the intravascular to the extravascular space should be minimal. Therefore, the presence of plasma proteases can explain the decay of this endolysin [30]. Other endolysins have a longer half-life (e.g., CF-302 has a half-life of 11.3 hours, while P128 has a half-life of 5.2 and 5.6 hours for the highet doses, 30 and 60 mg/kg, respectively) [31, 32]. Thus, as lytic enzymes in pre-clinical analyses shows an easier determination of its dosing regimen when compared to dosing regimen of phages, lytic enzymes are currently preferred to be used on patients [33]. In this sense, further studies are needed to better evaluate the pharmacological data concerning the lytic phages, including full-scale toxicological researches, before they can be used therapeutically in the West World [1].

2.5 Safety in the usage of phage preparations

From a clinical perspective, phages are apparently harmless. During the long period of usage of the phages as therapeutic agents in Eastern Europe and in the former Soviet Union (and before the antibiotic era, in the United States), phages have been administered to humans (i) orally, in tablet or liquid formulations (10^5 and 10^{11} PFU/dose) (ii) rectally (iii), locally (skin, eye, ear, nasal mucosa, etc.), in tampons, rinses and creams, (iv) aerosols or intrapleural injections, and (v) via intravenous access, though less frequently than the first four cited methods, and there are no reports of serious complications associated with their use [1].

Another aspect regarding safety of the bacteriophages usage is that they are extremely common in the environment (e. g., nonpolluted water has been reported to contain ca. $2x10^8$ bacteriophage per ml) [34] and are usually consumed in foods, highlighting their potential to be used as bioremediation agents on polluted environments. However, it would be prudent to ensure the safety of these microrganisms before using them as therapeutic agents, making sure, for example that: (i) they do not carry out generalized transduction and (ii) have genetic sequences possessing considerable homology with some genes related to antibiotic resistance, genes for phage-encoded toxins, and genes for other bacterial virulence factors [1].

2.6 Advantages in the use of bacteriophage therapy

Bacteriophage therapy presents many advantages such as high host specificity, preventing damage to normal intestinal flora, thus not infecting eukaryotic cells, low

dosages required for the treatment, rapid proliferation inside the host bacteria, making them ideal candidates to treat bacterial infections [35]. Unlike antibiotics, another advantage in the usage of bacteriophages is that they reinfect the bacteria host and mutate alongside them [36].

However, high specifity of the phages can be both advantageous and a limiting factor. To use a monophage therapy it is necessary to check the efficacy of the phage by performing *in vitro* assays against the disease-causing bacteria before applying it in the patient, which can be a laborious task to do. The solution to this problem would be to use phage cocktails, which comprises a wide range of phages acting against different bacterial species or strains [37]. According to experts all around the world, an ideal phage cocktail consists of phages belonging to different families or groups so that it would target a broad range of hosts. Also, they would have to possess a high absorption ability to the highly conserved cell wall structures of the bacterial hosts. Additionally, the usage of phage cocktails may reduce the emergence of phage resistant bacterial population. On the other side, other researchers defend the sequential use of individual active phages to the patient, though, in clinical practice, it appears to be a difficult strategy to perform [38].

Not only bacteriophages *per se* can be used to treat bacterial infections. Their by-products can also do the trick. It was already reported that lytic enzymes showing function similar to lysozyme can also be used as an antibacterial agent or can be used in synergy with other antimicrobials like antibiotics to improve the efficacy of the treatment [39]. A phage derived protein, "endolysin", also possesses antibacterial and antibiofilm activity against ESKAPE pathogens [39–43]. V12CBD, a recombinant protein derived from bacteriophage lysine, PlyV12, was also able to attenuate virulence of *S. aureus* and also enhance its phagocytosis in mice [44].

2.7 Disadvantages in the usage of bacteriophage therapy

It is widely known that phages can be vector for horizontal gene transfer in bacteria, and in this process, bacteria can exchange virulence or antibiotic resistance gene, making these microrganisms resistant to a wide range of antibiotics [45]. Therefore, phages cannot harbor virulence factors or antibiotic resistance genes like integrases, site-specific recombinases, and repressor of the lytic cycle that may accelerate the integration of these genes in the bacterial hosts. Algorithms that can predict the mode of action of the phages as well as their virulent traits are available but their database needs to be constantly updated with a greater amount of genome sequence of phages [46].

Recent studies demonstrated an *in vivo* efficacy of phages against infections caused by ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa* and *Enterobacter* spp.), and their authors used fully characterized phages that showed no virulence factors or antibiotic resistance genes, therefore, they were considered safe as they do not provoked any allergic or immune response in the patient, and they were also stable at varied pH and temperature, making them ideal candidates for bacteriophage therapy [47–50].

Another limitation is the relatively weak stability of phages and their proper administration in order to reach the site of action. Phage preparations can be applied orally, nasally or topically [51, 52]. To overcome this limitation, studies were conducted and they have shown that phage's efficacy is improved when they are entrapped with liposomes [51, 53–55]. They can also reach the infection site in the form of a powdered formulation [56].

3. Future perspectives on phage therapy

There is an increasing urge to restock our ammunition of antimicrobials to combat the ever rising drug resistant bacterial pathogens. Effective antibiotic combinations are scarce and to add to the problem, the incoming of new drugs is also very low and happens in a very slow pace. Phages are a promising source of new antimicrobial drugs and they have been sparking up an interest on researchers all over the world, but still, their use is not approved on the United States and in Europe. But once limitations on their use is overcame, like preventing the phages to insert genes on their bacterial hosts that could confer them resistance to antibiotics and also the production of toxins, for example, the use of bacteriophages to treat bacterial diseases will be extremely helpful to treat patients affected by these bacterial diseases.

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Conflict of interest

The authors declare no conflict of interest.

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