Bacteriophage as a Promising Alternative or Complementary Therapy to Antibiotics

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ABSTRACT

The global issue of antibiotic resistance and the narrowing down of new antibiotic discoveries besides their cost has led to the reintroduction of bacteriophage as a focus of research projects. Phage therapy is mainly grounded on the discriminating ability of the bacteriophage to destroy the targeted bacterial cells, but not human cells, via lytic phages. Although there has been a number of life-sparing clinical trials and applications, this therapy is still facing challenging issues. This review gives an overview of the microbiology and history of the bacteriophages. It also reviews the past and current studies of phage therapy in humans and it's commercial production. The aim of this study is to shed the light on the rapidly growing field of phage therapy and the obstacles that appear ahead as antimicrobial science moves away from broad dependence on conventional antibiotics.

Keywords: bacteriophage, phage resistance, phage therapy, antibiotics.

أدت القضية العالمية لمقاومة المضادات الحيوية وتضييق الاكتشافات الجديدة للمضادات الحيوية إلى جانب تكلفتها إلى إعادة إدخال العاثيات كمحور تركيز المشاريع البحثية. يعتمد العلاج بالعاثيات بشكل أساسي على القدرة التمييزية للعاثيات على تدمير الخلايا البكتيرية المستهدفة ، ولكن ليس الخلايا البشرية ،عن طريق العاثيات اللاكتاتية. على الرغم من وجود عدد من التجارب والتطبيقات السريرية التي تحافظ على الحياة ، إلا أن هذا العلاج لا يزال يواجه تحديات صعبة. تعطي هذه المراجعة لمحة عامة عن علم الأحياء الدقيقة وتاريخ العاثيات. كما يستعرض الدراسات السابقة والحالية للعلاج بالعاثيات في البشر والإنتاج التجاري. الهدف من هذه الدراسة هو تسليط الضوء على مجال العلاج بالعاثيات سريع النمو والعقبات التي تظهر في المستقبل حيث يتحرك علم مضادات الميكروبات بعيدًا عن الاعتماد الواسع على المضادات الحيوية التقليدية.

INTRODUCTION

1 Bacteriophages

1.1. Definition

Following the announcement of the new *Bacteriophage* journal, launched in 2011, Alexander Sulakvelidze has given bacteriophages a definition as the most omnipresent organisms on the Globe, having a crucial role in preserving microbial balance on this globe (1). Basically, it can be any of a group of virus-like agents. These agents have the ability to infect not animal cells, but bacteria.

In terms of size, bacteriophages are generally smaller than the bacteria they invade and destroy- around 20-200 nm in size. Structurally, bacteriophages are composed of a protein head enfolding the hereditary material. The latter can be dsRNA, ssDNA,

or dsDNA in the range of 5-500 kilo base pairs long arranged circularly or linearly (2). Sighting of these viruses is honored to Frederick W. Twort (1915) and Felix d'Herelle (1917). Regarding terminology, "bacteriophage" is derived from the word 'bacteria' and the Greek word 'phagein', which means 'to eat' and its d'Herelle who was the one who came up with this term (3). With the aid of the electron microscope, the first description of particles that were rounded and sperm-shaped from a phage mixture has been made by Helmut Ruska (4). These particles were found attached to a bacterial membrane. One year later, Luria and Anderson (5), in Camden, New Jersey, were able to identify a variety of phages. These phages were non-homogenous, having round heads and delicate tails showing an odd sperm-like appearance. The two scientists were also able to define bacterial lysis following invasion with phages into the stage of adsorption which is a time-dependent stage followed by bacterial damage and the release of a huge quantity of bacteriophages (6).

1.2 Classification of phages

During the period from 1965 to 2010, more than 30000 publications related to phage have been reviewed by Ackerman (7).

Accordingly, over six thousand morphologically distinct bacteriophages have been reported, of these, the majority has been bacterial and only 88 archaeal phages (8). Categorization of phages has been made based on their range of host and the virion's physical characteristics such as shape, size of the capsid, being ineffective by organic solvents, in addition to the size and type of the genomic material. The latter could be, for example, single-stranded RNA [ssRNA], ssDNA, double-stranded RNA [dsRNA], and dsDNA (9). According to the morphological features and the nuclear material, the International Committee on the Taxonomy of Viruses (ICTV) phages' classification has been made (https://talk.ictvonline.org/taxonomy). This committee keeps updating its taxonomic system of classification (10). Although they have demonstrated differences in their sizes and morphology, the dsDNA tailed phages, or what's known as *Caudovirales*, has been shown to account for around 95% of all the reported phages in the scientific literature. They might also account for the majority of phages on the globe. There has been one order of bacteriophages of 13 families and 31 genera (11). Table 1 below states the principal features of the known phages classes.

Table 1. Bacteriophages classes and their basic characteristics (8).

Morphology (Symmetry)	Nuclear material	Order/families	Genera	Members	Particulars
Binary (tailed)	DNA, ds, L	*Caudovirales* *Myoviridoe* *Siphoviridoe* *Podoviridoe*	15 6 6 3	4950 1243 3011 696	Tail contractile Tail long, noncontractile Tail short

	DNA, ss, C C, ds RNA, ss, ds, L, S DNA. ss, C ds, L. ds.	*Micro vi ridae* *Corticoviridoe* *Tectiviridae* *Levi viridae* *Cystoviridue* *Inoviridoe* *Lipothrixviridae* *Rudiviridoe*	4 1 1 2 1 2 1 1	40 3? 18 39 1 57 6? 2	Complex capsid, lipids Internal lipoprotein vesicle Envelope, lipids Filaments or rods Envelope, lipids Resembles TMV
Cubic Helical					
Pleomorphic	DNA. ds. C. ds, C.	*Pkismoviridae* *Fuselloviridae*	1 1	6 8?	Envelope, lipids, no capsid Spindle-shaped, no capsid
C, circular; ds, double-stranded; L, linear; S, superhelical; ss, single-stranded.					

1.3 Structure of bacteriophages

There is a wide range of variability in the sizes and shapes of different bacteriophages. The majority of phages sizes fall in the range between 20-200 nm in length. Figure 1 illustrates the basic bacteriophages structural components. The head structure, composed primarily of repeat protein subunits and containing the viral genome, is known as the capsid (12). This structure can differ in size and shape (icosahedral or filamentous) and functions to protect the genetic material of the phage (13). The head is connected to the tail which helps passing the genetic material into the host cells during infection. The later process is initiated when the phage tail protein assemblies specifically recognize the target bacterial surface receptors (14, 15, 16). Roughly more than 90% of phages have tails attached to their heads. Sheath-enclosed tails contract when the phage invades the target bacterial cell (17).

More sophisticated phages, for instance the T4, possess additional structures such as the base plate and either one or more fibers attached to the tail.

These structures participate in phage binding to the host bacterium. In the absence of these structures in some phages, other structures might be involved in the process of phage-bacterium attachment (18, 13).
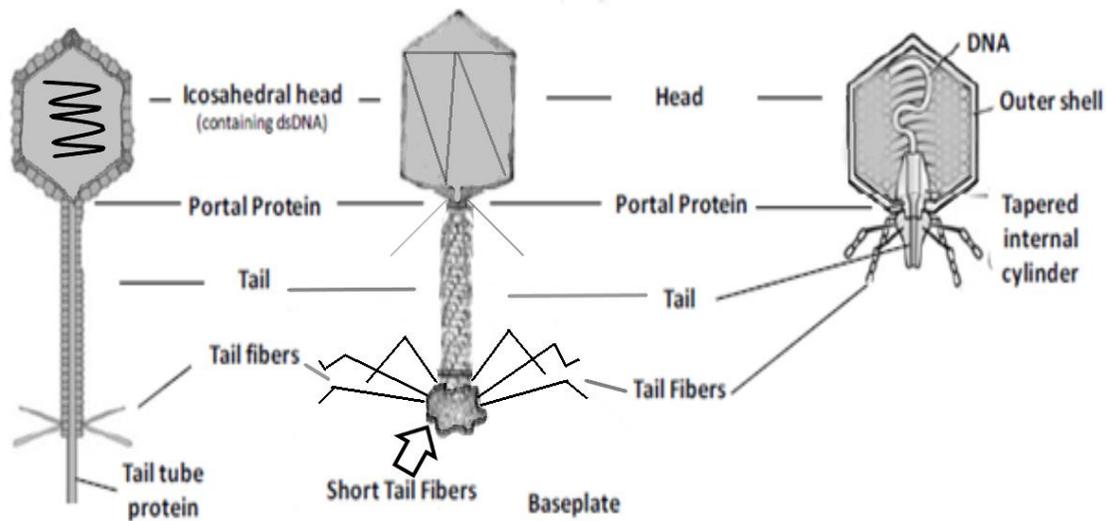
Figure 1: 2D-Graphic representation of the morphology of *Caudovirales* families. Starting from the left side, morphology of a *Siphovirus* (long, non-contractile tail), *Myovirus* (long, contractile tail) and *Podovirus* (short tail) are shown. See text for more details (adopted and modified from 5).

2. Phage therapy

2.1 Historical background

Phages had been used by d'Herelle to treat dysentery shortly after their discovery, this has been considered the first trial to employ the therapy of bacteriophages (19).

A piece of research was conducted in 1919 at the Hospital des Enfants-Malades in Paris. A phage formulation was given to a 12-year old age child suffering acute dysentery. Following one dose of the therapy, there was an improvement of the symptoms and complete recovery was achieved in a few days. Another three children with the same bacterial diarrhea recovered within one day period following administration of the single-dose phage mixture confirming the efficacy of the therapy (19). However, these discoveries were unpublished.

In 1921, phage therapy was used by Richard Bruynoghe and Joseph Maisin to treat skin infections caused by staphylococcus species.

It was found that the infectious lesions healed within 1-2 days following injecting the phage mixture into and around them (20). These findings were encouraging for more studies regarding the clinical application of bacteriophages (21, 22). Phage therapy as a treatment strategy was largely ignored in the West when penicillin was discovered, marketed, and widely spread in the early 1940s (23). However, some eastern countries continued to study and use bacteriophages, especially in Georgia and Poland. Because of publishing the literature in a non-English language, the concluded observations and results were limited to the authors' own countries. Several authors have just published a collection of this material, revealing that some authors have significant expertise with hundreds of treated patients (24, 25). Slopek et al. published a series of six publications (26, 27) considering the efficiency of phage-therapy against bacterial infection including multidrug resistance

superbugs. Five hundred fifty patients with bacterial septicemia, ranging in age from one week to 86 years, were treated at ten clinical departments and hospitals spread throughout three cities. In 518 of the patients, antibiotic treatment was found to be unsuccessful, prompting the introduction of phage therapy. *Staphylococci*, *Pseudomonas*, *Escherichia*, *Klebsiella*, and *Salmonella* were the causative agents in Slopek et al. studies and antibiotic therapy was found ineffective. The etiologic agents were isolated and specific bacteriophages were selected from around 250 lytic phages. Different routes of administration were used (oral and local) and phage suspensions were applied to the infected sites (eye, middle ear, or nose). The treatment course continued for 1-16 weeks and followed for another 2 weeks after negative culture. An overall success rate of 92% was obtained marked as absolute recovery and negative cultures. A higher success rate (94%) was reported for the 518 patients who showed resistance to the antibiotic therapy (27). Patients with purulent illnesses of the lungs and pleura were treated with *Staphylococcus aureus*-phages in another investigation. Briefly, two groups were included in the study; phage mixtures were administered to group A (223 patients) while group B (117 patients) were treated with antibiotics. It might be worth mentioning that in this clinical study, 48 patients in the first group obtained the phage mixture via injection. All of the patients were monitored healthy post-therapy using the conventional follow up criteria; X-ray examination, purulence decrease, and blood and sputum microbiological investigation. No side effects were reported for any of the study patients, even those who received the intravenous phage doses.

A recovery rate of 64% was reported for group B (antibiotic group) versus 82% for the phage-treated group A. Interestingly, 95% of the group that received the intravenous phage

therapy recovered completely (28). Phages have been shown to be successful in the treatment of cerebrospinal meningitis in newborns (29), *Pseudomonas*, *Staphylococcus*, *Klebsiella*, *Proteus*, and *E. coli* skin infections (30), recurrent subphrenic and subhepatic abscesses (31), and different chronic bacterial infections (32).

2.2 Phage therapy for bacterial infection prevention and treatment in humans

Several hundred papers on phage therapy in humans have been published worldwide (34-43). Most of these publications came from Eastern Europe and the former Soviet Union while other countries have published only a few studies (33). A randomized controlled trial was published first in the United States in 2009 (34). In this study, around 40 patients were presented with venous leg lesions caused by either *E. coli*, *S. aureus*, or *Pseudomonas aeruginosa*. Phage therapy was used but no favorable outcomes were detected as the rate of ulcer healing. However, no side effects were reported in any of the patients involved in the study.

Another randomized double-blind, placebo-controlled experiment was undertaken in the United Kingdom to see the effectiveness of a mixture containing six bacteriophages against chronic otitis of *P. aeruginosa* etiology (35). Following treatment, it has been shown that bacterial counts diminished significantly. Clinical improvement of symptoms such as itching, wetness and offensive smell were also observed.

The effectiveness of phage therapy for infection prophylaxis was comprehensively examined in a study conducted in Georgia (36). More than 30 thousand children aged between 6 months and 7 years were involved in the study. In one group, children (17,044 children) were given *Shigella* phages by mouth one dose every week, while children of the other group (13,725) were not. The

overall health status of the children in the two groups was checked weekly. Cases of gastrointestinal manifestations were subjected to stool examination for *Shigella* spp. and other diarrhea-causing bacteria. Clinically, there was a 3.8-fold higher incidence of dysentery in the placebo group in comparison to the group that received the phage therapy. However, laboratory findings based on culture-confirmed cases revealed a 2.6-fold higher incidence in the placebo group than the treated group. It has also been shown that the incidence of diarrheal disorders of unknown cause in phage-treated children was 2.3-time lower than in the untreated group. This might suggest that some cases were not able to be detected or it might propose an additional effect of *Shigella* phage against other infections of the gut (36). Another study was conducted on 9 patients treated at the Queen Astrid Military Hospital's Burn Wound Centre in Brussels, Belgium (37). Local application of phage therapy has been investigated. A phage mixture (BFC-1) is a cocktail of three lytic bacteriophages that are: Myoviridae A1, Podoviridae C1, and Myoviridae A1 of the host species of *P. aeruginosa*, *P. aeruginosa*, and *S. aureus* respectively. The cocktail was meant to be applied for the treatment of *P. aeruginosa*, and *S. aureus* wound infections. A substantial burned region of the wound was exposed to a single spray application, whereas a control piece of the wound was used. While the full data have yet to be released, no safety concerns have been raised and promising results are expected.

2.3 Review of commercial manufacturing of phages

The increased futuristic need of high quantities of bacteriophages for their proposed applications such as therapeutic replacement of antibiotics, phage gene vectors and vaccines (44) requires the development of a production platform such as the scalable Good Manufacturing Practice compliant (cGMP) (45).

Earlier, five commercial phage products were produced by d'Herelle's marketable laboratory in the city of Paris. These antibacterial phage formulations were so-called Bacté-coli-phage, Bacté-rhino-phage, Bactépyo- phage, Bacté-intesti-phage, and Bacté-staphy-phage. They were sold by what later converted to the large French company L'Oréal (19). The United States also played a role in the production of phages of therapeutic applications. For instance, the Eli Lilly Company (1940s) produced seven phage preparations for clinical use in humans. These products were aimed against bacterial pathogens causing varieties of infections, including abscesses, vulvovaginitis, oozing wounds, upper respiratory tract infections, and mastoid bone infections. The causative bacterial species included *Staphylococci, Streptococci, Escherichia coli*, and others (34). Despite these, phage preparations effectiveness has been controversial (34) as many of the technologies used for the small scale production are not applicable to the large scale industrial phage manufacturing. With the innovation of antibiotics and their broad spectrum of antimicrobial effect, the production of phage therapy has diminished in Western countries. However, countries of the Eastern Europe and the former Soviet Union continued to commercially manufacture phage to be used instead or in conjunction with antibiotics especially in cases resistant to conventional antibiotic therapy (41).

3.1. Antibiotics versus phage therapy

Antibiotics can be defined as any chemical substances produced by living microorganisms (bacteria or fungi) or synthesized *in vitro* possess the ability, in diluted form, to inhibit the growth or damage

bacterial cells and other microorganisms and can be safely used to treat infections *in vivo* (46, 47). Similarly, lytic phages have remarkable antibacterial activity (23). However, theoretically it has been proposed that phage therapy has some advantages over antibiotic use (table 2) with more effectiveness than antibiotics against some infectious agents in mammals; humans or animals (25, 26, 42, 43). For instance, in a study conducted by Meladze and colleagues (1982) it has been reported that patients with purulent disease of the lower respiratory system and pleura receiving *S. aureus* phages showed 82% complete recovery opposing to 64% of the patients received antibiotics (28). Although lysogenic and lytic phages have been studied thoroughly, it is only the lytic one (also referred to as the virulent phages) has shown promising results as a good choice for the use in evolving phage therapies (48, 49). However, it should be kept in mind that the *in vitro*-described lytic life cycle of

phages may not be sustained *in vivo* and that it might relapse to the lysogenic round (8).

Bacteriophages are characterized by high specificity against the causative pathogens. However, this specificity is disadvantageous since the disease-causing microbe must be identified prior to commencing phage therapy successfully (50) causing a delay in responding to critically ill patients. Generally, phage therapy can work successfully with no serious side effects contrary to most antibiotics. This is due to the fact that phages do not attack human or animal cells (6). However, minimal effects have been monitored due to the release of toxins from the target lysed pathogens (27, 30). Regarding the kinetic of phages, although they can be administered in any routes, their formulations require a neutralized niche. This is quite inapplicable in the digestive system of animals due to the secretion of the gastric juices (6).

Table (2): Main differences between prophylactic/therapeutic use of bacteriophages and antibiotic

Bacteriophages	Antibiotics
High specificity affecting the bacteria of the targeted species. Hence, there is no opportunity to develop secondary infection (50).	Low specificity affection both pathogenic and bacteria of the resident microbiota. This results in microbial imbalance and might lead to secondary infection.
Since phages are living biological agents, thus can be replicated at the infection site to provide the required dose where are needed (51).	Antibiotics are chemicals which are subjected to the kinetic of metabolisms and excretion from the body, thus might not be available at the desired concentration at the infected site.

Serious side effects are very uncommon.	Side effects due to alteration of the normal flora, allergic manifestations and opportunistic infections have been documented (52).
Bacterial resistance to phage therapy is not a threat since bacterial cells remain sensitive to other phages of the same board range.	Bacterial resistance to antibiotics is not limited to the embattled pathogens.
In case of bacterial-phage resistance, selection of alternative phage therapy is a relatively quick process (can be performed in days or weeks).	In case of bacterial antibiotic-resistance, the process of developing a new antibiotic agent is a time-laborious process (can take several years in average) (53, 54).

4. Phage resistance

The emergence of bacterial phage-resistance has been defined a century ago in an important publication by Luria and Delbrück (55). The author noticed a bacterial regrowth following initial phage-induced bacterial cell lysis. This was attributed to the emergence of sub-population that were phage-resistance. Phage-resistance can be acquired, resulting from *in vitro* or *in vivo* treatment or it may be primarily transmitted via different mechanisms. Three mechanisms of phage-resistance have been proposed by Levin and Bull (56). The first mechanism suggests that bacteria may develop resistance via de novo chromosomal mutations. Such mutation can result in a change or loss of specific surface receptors where phages attach and initiate infection of the bacterial cells. Consequently, phages cannot multiply in the target bacteria and "envelope resistance" ensue. Second resistance mechanism involved the mucoid colonies conferring bacterial cells to be partially resistant to phage invasion. Even though phages are still able to adsorb themselves and replicate but at a lower rate. The final mechanism proposed the plasmid-encoded restriction nucleases. The latter is found in the bacterial cells and functions to degrade infecting phage genome resulting in interrupting the lytic cycle (56).

Interestingly, phages have shown the ability to fight back and overcome bacterial resistance via what is called "evolutionary arms race". This ability has been considered advantageous over the traditional antibiotic therapy (56, 57). However, mutant phages are favored to attack and multiply in the multi-drug resistant bacteria (58). Therefore, the phage cocktail has been adopted as an interesting effective strategy to solve the problems of resistance and lower activity (59). The cocktail is usually composed of a variety of phages attacking different bacterial species or strains. Such phage combinations have been shown to play a role in combating biofilms and in decontaminating food (60).

It has been proposed that bacteria that are phage-resistant may offer less virulency as phage bacterial receptors might be capsules or other virulence factors (61). This phenomenon has been reported in fish pathogens where a phage-resistant bacterial mutant showed loss of bacterial virulence (62). On the other hand, the presence of bacteriophage as a prophage might lead to

broadcast resistance of bacteria against certain antibiotics (63, 64).

CONCLUSION

Bacteriophages can be considered as a promising alternative antibacterial therapy especially against multidrug-resistant bacterial pathogens (MDR) (65). In fact, numerous pros have been displayed by phage

therapy with minor reported adverse effects. Selectivity, self-propagation and lack of crucial adverse effects are examples of the reported advantages of phage-therapy. However, underreporting can be a reason and therefore further clinical studies in routine daily practice are needed to specify the applicability and safety of the old-new phage therapy.

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