Phages and phage therapy: past, present and future

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Summary

Bacteriophages are viruses that infect bacteria. Since their discovery in the last century, they have proved their effectiveness in biocontrol of bacteria. In this mini-review, we provide a brief history of bacteriophages, their life cycle and classification. We also discuss the potential use of bacteriophage in clinical therapy as an alternative to antibiotics, comparing their advantages and disadvantages.

Introduction

Bacteriophages, or simply phages, as their Greek name suggests (*phagein*, to eat or devour, and *bacterio*) are viruses infecting bacteria. Defined by Sulakvelidze as "the most ubiquitous organisms on Earth", they are abundant in all environments, including water, soil and air, occupying all those habitats where bacteria thrive. Indeed, the number of phages in aquatic systems lies within the range of 10^4 to 10^8 virions per milliliter and about 10^9 virions per gram in the soil, with an estimated number of 10^{31}-10^{32} phages in the world (27).

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History of bacteriophage and phage therapy

The first observation of bacteriophage dated back to 1896. It was the British chemist Ernest H. Hankin who first reported the presence of an antimicrobial activity in the Jumna and Ganges rivers, in India (27). However, it required 30 years for the scientific community to properly investigate phages. In 1915, Frederick Twort was the first to hypothesize that nonpathogenic viruses growing on bacteria were responsible for the transparent, glassy areas he observed in bacterial culture.

Still, the discovery of phages is officially attributed to the French-Canadian Félix d'Herelle who observed the same phenomenon of bacterial lysis two years later and coined the term bacteriophages. Contrary to Twort, who seemed to favor the notion that lysis was determined by an enzyme secreted by the bacteria itself, d'Herelle was quite certain that the phenomenon he observed was due to a virus capable of parasitizing bacteria. He had to wait until the year 1939, when the newly invented electron microscope confirmed the phage viral nature.

D'Herelle was also the first who developed the idea of 'phage therapy', pursuing the application of phages as therapeutic and prophylactic treatment in humans, exploiting phage selectivity towards pathogenic bacteria and investigating safety towards human host cells.

D'Hérelle founded the Bacteriophage Laboratory in France and began the production of the first commercial phage cocktails in what later became the great French company L'Oréal. At the same time, bacteriophages were also used for therapeutic purposes in the United States.

With the discovery of penicillin in 1940, the era of antibiotics started and phage therapy was abandoned in Western European Countries and North America. Nevertheless, phages continued to be used therapeutically in Eastern Europe and in the former Soviet Union states such as Poland and Georgia. In these countries, different institutions involved in the research and production of therapeutic bacteriophages were established. In particular, the Eliava Institute of Bacteriophages, Microbiology and Virology (EIBMV) of the Georgian Academy of Sciences (Tbilisi, Georgia) and the Institute Hirszfeld of Immunology and Experimental Therapy (HIIET) of the Polish Academy of Sciences (27).

Phage life cycle

Like other viruses, bacteriophages are obligate intracellular parasites. Phages, in order to reproduce, must come into contact

with a susceptible host capable of allowing replication. Based on their infectious cycle, the vast majority of phages can be distinguished into lytic or lysogenic. Lytic phages (also named virulent phages) carry out the lytic cycle in which the virus adsorbs to the surface of target bacteria, injects its genome into the host cytoplasm and induces the bacterial molecular system to produce virions that will eventually destroy the bacterial cell liberating themselves in the surrounding environment. This cycle will continue as long as the target bacteria are present, releasing hundreds of new virions within minutes or hours (12).

Lysogenic phages (also named temperate phages) are viruses that can adopt a lysogenic cycle, as an alternative to the lytic cycle. In the lysogenic cycle, phages integrate their genome in the host nucleic acid (or eventually linger as a plasmid) assuming the quiescent state of prophage, which guarantees the viral reproduction within the bacterial cells. In response to specific stimuli, the prophage will exit from its dormant state and enter into the lytic cycle. During viral genome excision, transduction may happen, resulting in horizontal gene transfer within the bacterial population (for example that of resistance determinants). For this reason, temperate phages are not suitable for phage therapy (7, 18). Potentially, transduction may occur also with obligate lytic phages; however, the likelihood is very low, given the rapid killing of bacterial host and the simultaneous increase of phage number. In addition to these two types, another kind of life cycle was observed in filamentous phages in which bacteriophages kill their host without lysing it (21).

Bacteriophage classification

The classification of bacteriophages is subject to continuous discussions and debates, particularly with the increasing volume of available genomic and proteomic data. Currently, both genomic and morphological information is used in their classification. The genetic material of phages consists of double-stranded (ds) or single-stranded (ss) DNA or RNA, and their genome sizes can range from very simple (*e.g.* -3.5 kb ssRNA genome in phage MS2) to highly complex (*e.g.* -500 kb dsDNA genome in *Bacillus* phage G) and can include modified nucleotides as protection against restriction enzymes. Morphologically, phages can be tailed (96% of phages), polyhedral, filamentous or pleomorphic, and some have lipid or lipoprotein envelopes. Most characterized phages belong to the *Caudovirales* order (ds DNA genome with a tailed morphology), divided into the three families: *Myoviridae* with contractile tail (for example, phage T4), *Siphoviridae* which possess non contractile tail (for example, phage λ) and *Podoviridae* which have very short tail (for example, phage T7) (3).

Advantages and disadvantages of phage

When compared to antibiotics, phages show several appealing properties that make their therapeutic use advisable. Table 1 summarizes some advantages and disadvantages of phages, and compares phage safety, specificity, bactericidal effect and ability to infect bacteria resident in biofilm with antibiotics.

A major problem that drawback phage application is the emergence of resistant bacteria which hamper the effectiveness of such therapy. Actually, bacteria can encounter phages using different strategies, including blocking phage adsorption or DNA entry into the bacterial cell or restriction-modification and CRISPR-Cas systems that are able to cut phage nucleic acids once they have been injected into the bacterial cell (15).

Even though bacteria could become resistant to phages, phage resistance is not nearly as worrisome as drug resistance. Like bacteria, phages mutate and can therefore evolve into counter phage-resistant bacteria (25). In addition, it appears that bacteriophage force a clinically relevant trade-off, during which organisms evolve one trait that improves fitness (a relative reproduction or survival advantage) while simultaneously suffering reduced performance in another trait. Therefore, phage therapy could be used as an 'evolutionary-based strategy' in which phages drive MDR bacteria to evolve resistance to them while recovering sensitivity to chemical antibiotics (6). Moreover, phage can be used in combination with antibiotics, as long as their mechanism of action does not interfere with phage infection and replication (16).

Phage therapy in 21st century

Antibiotic resistance, the hot topic of 21st century, leads to a renewed interest for phage therapy also in the Western world. In 2012, the widespread diffusion of multidrug-resistant (MDR) bacteria led the World Health Organization (WHO) to acknowledge a state of emergency all around the world, warning the possibility of entering an era where antibiotics lose their power over bacterial infections. In 2017, WHO published a list of bacteria urgently requiring new antibiotics, in order to guide and promote new antibiotics research and development; among the alternative approaches suggested, the use of clinical products containing bacteriophages was considered (26).

Over the last decades, phages have been applied to treat a great variety of bacterial infections. In particular, phage safety and efficacy have been investigated in animal models of both acute and chronic infection: for example, phages have been therapeutically used against abscesses and subcutaneous infections in mice, chronic otitis in dogs, chronic infected wounds in diabetic rats and pigs, cystic fibrosis lung infection in mice and cystic fibrosis related infection in larvae and zebra fish, mastitis in cows, osteomyelitis in rabbit (2, 4, 8, 23). In clinical human practice, phage therapy has been used for the treatment of longstanding, persistent, or chronic bacterial infections. Patients with abscesses, osteomyelitis, prostatitis, urinary tract infections, otitis, skin ulcers, venous leg and diabetic food, bed sores, suppurative fistulas and cystic fibrosis have been treated with phages, typically as last resort, when their bacterial infection did not respond to conventional treatments (2). The news of these days is of a 15-year-old patient with cystic fibrosis with a disseminated *Mycobacterium abscessus* infection following bilateral lung transplantation; this infection has been resolved by intravenous administration of engineered phage (20). Bacteriophage has also been used successfully in the treatment of a 68-year-old diabetic patient with necrotizing pancreatitis complicated by an MDR *Acinetobacter baumannii* infection (24).

Besides human therapy, bacteriophages have been applied in different fields, such as food bio-preservation and disinfection of medical devices. In the context of food safety, bacteriophages can be used at different stages of foodstuff production (from farm to fork) to: i) improve animal health (phage therapy), ii) decontaminate fresh-food and ready-to-eat products, iii) disinfect food-contact surfaces. Phages and their proteins were applied successfully against several pathogenic bacteria and their biofilms including *Escherichia coli*, *Bacillus* spp., *Salmonella* spp., *Campylobacter* spp., *Vibrio* spp., *Clostridium* spp., *Listeria* spp., *Staphylococcus* spp., and *Pseudomonas* spp (11). Lytic bacteriophages prove to be effective also in the treatment and formation prevention of bacterial biofilms

Table 1. Advantages and disadvantages of phage therapy versus antibiotic therapy.

Phage pros	Phage cons	Antibiotics cons
The omnipresence of bacteriophages in the environment implies a constant exposure of humans to phages, so that phages are not xenobiotic to our bodies. Our healthy microbiome includes a virome that is, in fact, largely phagome. Bacteriophages administered for therapeutic purposes are well tolerated by patients (13).	It has been demonstrated that bacteriophage therapeutic use could generate some immunological response (highly dependent on the way of administration). This can be attributed to endotoxin released from the bacteria in which phages are propagated; therefore, phage formulated products need to be highly purified (13).	Antibiotics have several side effects.
Phages are highly specific, with most of them infecting only a single bacterial species or even a estrict number of strains within a single species. This allows phages to target only pathogenic bacteria without disturbing the resident bacterial flora, earning them the epithet of "magic bullet" (13).	Phage strict specificity may pose a great problem for introducing phage therapy into clinical practice when facing variation and fast adaptation among bacteria (19).	Many chemical antibiotics tend to have broad spectrums of activity, target both pathogens and normal flora of patients, disrupting natural microflora and possibly causing secondary infections or superinfections (16).
Phages are antibacterial agents that grow exponentially in numbers, at the site of infection, where the host is present.	Bacteriophage pharmacology and pharmacokinetic can be relatively complex because of i) phage dimension and life cycle characteristics (*i.e.* adsorption rate latency time and burst size), ii) filtering organ activity, that can rapidly clear phage from circulation, iii) phage destruction caused by gut digestive enzymes (13, 19).	Antibiotics travel throughout the body and do not concentrate at the site of infection (16).
Obligatory lytic phages are bactericide able to infect the persister "dormant" cells populating the inner layer of biofilms, remaining dormant within them, and re-activating when they become metabolically active. Some phages express enzyme depolymerizing biofilm matrix constituent (13).	Extracellular polymers of biofilm matrix may slow phage penetration into bacterial surfaces. It has been suggested that, by slowing phage propagation within biofilms, bacteria may be able to escape from biofilms via standard dissemination-initiating mechanisms. Phage entrapment in the extracellular matrix and phage inactivation are also possible (10).	Certain antibiotics, that are bacteriostatic, do not kill bacteria. Antibiotics, requiring metabolically active cells, cannot exert their effect on persister cells. Biofilm bacteria can display up to 1,000-fold higher resistance to antibiotic than their planktonic counterpart (1,17).

commonly associated with infections of indwelling urological devices and catheter-associated urinary tract infections (5).

Example of clinical trail

Although bacteriophages were first used almost 100 years ago to treat infections, they were ignored in the Western world after the discovery of antibiotics. In other countries, however, such as Georgia and Poland, the use of phages for both preventive and therapeutic purposes continued (27).

Scientific reports on phage therapy in Eastern Europe include studies that do not always meet the criteria of modern evidence-based medicine (lack of control groups, ethics committee, etc.). For this reason, we will focus only on studies after the 1980s conducted in accordance with the Western regulation, in which treated patients suffered from antibiotic-resistant infections.

It is noteworthy to report the study of Wright *et al.,* 2009 (28) which is the first double-blind phase I/ II controlled clinical trial. In this study, bacteriophage cocktail was used to treat chronic otitis associated with *Pseudomonas aeruginosa*.

Recently, a number of clinical trials have been registered. For example, we can mention phase I safety set out in USA to treat venous leg ulcers infected by *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (22). Another example, the study of Jault *et al*.,2018 (14), which has been conducted in double blind phase using a cocktail of 12 bacteriophages to treat burn wounds clinically infected with *P. aeruginosa*.

Conclusions

We can distinguish four periods in the history of phage therapy: enthusiasm, skepticism, abandonment, and then a recent revival.

Based on clinical results, phage therapy seems to represent a promising alternative approach to antibiotics toward combating pathogenic bacteria.

However, advances in phage therapy need more robust evidence of clinical trials. Additional data are also required, such as bacteriophage formulation, dosing, efficacy and its effect on human immune response.

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