Bacteriophage therapy of urinary tract infections

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Abstract

The search for antibiotic alternatives, due to the growing level of antibiotic resistant microorganisms against the background of a decrease in the development and introduction of new antibiotics, draws our attention to bacteriophages (phages). The chapter briefly presents the history of bacteriophage therapy (phage therapy), the principles of phage treatment, modern knowledge about genetically engineered phages, phage lytic proteins – lysine and endolysins. Prospects of using phages in treatment of biofilm infection and joint use of phages and antibiotics are given. The mechanisms of resistance of bacteria to phages, the kinetics of phages in the human body during oral and rectal administration are described. Studies on the clinical use of phages are given, the problems of phage therapy and further prospects for the use of phages are discussed.

Keywords: urinary tract infection, bacteriophages, bacteriophage therapy, antibiotic resistance

Abbreviations

- CDC: Centers for Disease Control and Prevention
- CFU/ml: colony forming units per ml
- CRISPR: clustered regularly interspaced short palindromic repeats
- DNA: deoxyribonucleic acid
- EPS: extracellular polymeric substances
- ESBL: extended spectrum beta lactamases
- EU: European Union
- IgG: immunoglobulin G
- KT: kidney transplantation
- ml: millilitre
- NIH-CPSI: National Institutes of Health – Chronic Prostatitis Symptom Index
- PAS: phage-antibiotic synergy
- PG: peptidoglycan
- pH: hydrogen ion exponent
- PLEs: phage lytic enzymes
- RNA: ribonucleic acid
- UN: United Nations
- UTI: urinary tract infection
- UPEC: uropathogenic *Escherichia coli*
- USA: United States of America
- USSR: Union of Soviet Socialist Republics
- VALs: virion-associated lysins
- WHO: World Health Organization

Summary of findings

- Phage therapy is about creating viral infections in bacteria
- Bacteriophage preparations contain virulent clones of bacteriophages with high specificity that do not inhibit normal microflora. Safe and non-toxic to humans. Highly stable, can be stored for a long time
- The principles of phage therapy are based on determining the sensitivity of the pathogen to the bacteriophage preparation

- Use of updated phage cocktails helps overcome resistance and anti-phage immunity
- Use of monotherapy with phages or in combination with antibiotics
- Oral and topical application of bacteriophage preparations are effective
- Effectiveness of individual phages and cocktails has been demonstrated when used against biofilm-forming uropathogenic strains *in vitro*
- Genetically engineered phage contributed to the lysis of biofilm-forming bacteria

1 Introduction

A wide and uncontrolled use of antibiotics not only in medicine, but also in agriculture and animal husbandry has resulted in the spread of multiresistant strains of microorganisms with genes encoding bacterial resistance to the most common antibiotics, including β-lactams, fluoroquinolones, aminoglycosides, and chloramphenicol, which is a great threat to the treatment of infectious inflammatory diseases in general and urological infections in particular. Such regulatory authorities as the WHO, the UN, and the CDC have declared antibiotic resistance to be a threat to global health [1], [2].

When searching for alternative strategies for bacterial infection prevention and control, one of the most popular suggestions is using phage therapy. The major advantages of phages over antibiotics are their host specificity, self-replication, biofilm degradation, and low toxicity to humans [3], [4].

2 Methods

A systematic literature search was performed in PubMed, Medline, Embase, the Cochrane database and in books, journal articles (in English and Russian) with the following keywords: “bacteriophage; bacteriophage therapy; phages; bacteriophage therapy of urinary tract infections” and without limitation on gender, age, or clinical studies.

By reviewing English-language literature published after 2000 a total of 89 publications in English were identified, which were screened by title and abstract. By reviewing articles, dissertations and books on the topic of phage therapy published in Russian over the past 30 years 3 monographs and 106 articles were found and screened by title and abstract. After reviewing, 76 publications out of the English literature and 10 publications out of the Russian literature were found suitable to be used for this review article.

3 Results

3.1 Background information on bacteriophages

Bacteriophages (phages) are bacterial viruses and the most abundant life form on earth; they are estimated to be 10 times more numerous than bacteria and can be found in all environments, especially in the aquatic medium [5], [6], [7].

Twort and d´Hérelle, working separately, discovered the bacteriophages in 1915 and 1917, respectively [8], [9]. The nature of their existence had been a matter of dispute until they were visualized in the 1940s after the invention of the electronic microscope [10]. Phage virions have a different size and morphology: they are tailed (95% of all the phages), polyhedral, filamentous, or pleomorphic [6], [11]. Most phages contain double-stranded (ds)DNA, but there are groups with single-stranded (ss)DNA, ssRNA, or dsRNA, and their genetic diversity is remarkable [12].

Phages infect the host bacterial cell by binding to specific receptors located on the bacterial cell surface and releasing their genetic material (DNA or RNA) into it. Phages may choose a lytic or a lysogenic cycle to replicate in the host bacteria [13], [14], [7]. A lytic phage would use the host cells’ replication enzymes to make copies of itself and promote the bacterial lysis, which would release new infective viral particles. One lytic cycle (from the moment of phage adsorption to their release from the cell) takes 30 to 40 minutes. The process of lysis of bacteria by phages comprises several cycles until all bacteria susceptible to this phage are lysed [15], [16]. A lysogenic phage would integrate its genetic material into the bacterial genome as prophage, which is then replicated as part of the bacterium’s genome, resulting in a temperate phage. At the end, both cycles lead to the destruction of the host cell, so bacteriophages can be used against pathogenic bacteria [10].

One of the problems faced by phage therapy is the same as for antibiotics: the appearance of resistance.

A promising strategy to avoid this phenomenon is to produce a cocktail of several phages, each one attaching to a different receptor on the cell, so that if one receptor is mutated there is another phage pointing to a different target. The more phages a cocktail contains, the less likely resistances will occur. The use of cocktails broadens the spectrum of activity and would allow the targeting of different bacterial strains responsible for UTIs [17].

By acting as a 'mobile gene bank', phages help their hosts (bacteria) to quickly adapt to nutrition changes, high temperatures, pressure, and chemical exposure. These genes can be useful for the bacterial host and can encode factors of virulence, metabolic genes, and antibiotic resistance genes (e.g. resistance to β-lactamase) [18], [19]. Most phages are only transmissible to the bacteria carrying the complementary receptor, which in turn determines the spectrum of a lytic phage [20].

Since the phages' mechanism of action is completely different from that of all antibiotics, they are effective even against multi-drug resistant bacteria [7]. They can also be prescribed to patients in whom antibiotics are contraindicated. Since the antibacterial spectrum of phages is much narrower than that of antibiotics, they can act on pathogens without significantly affecting the normal bacterial flora [21], [22]. An important factor of the treatment efficacy with bacteriophage preparations is the determination of the pathogen's susceptibility. The spectrum of activity is determined by the spot test, and the activity by the Appelman method [23].

For therapeutic purposes new phages can be isolated from the environment or, in some cases, by selective passage of a set of phages presenting weak lytic activity in the target pathogenic bacteria. There are also methods that enable obtaining active phages by genetic modification [24], [25].

3.2 Genetically engineered phages

Using synthetic phages obtained by genetic engineering can help overcome many limitations associated with the use of natural phages. Genetic engineering methods include homologous recombineering, bacteriophage recombineering of electroporated DNA, *in vivo* recombineering, clustered regularly interspaced short palindromic repeats (CRISPR) -Cas-mediated genome engineering, *in vivo* manipulations with phage genomes, whole genome synthesis and assembly from oligonucleotides, yeast-based phage genome assembly, and cell-free transcription/translation systems [26]. An engineered phage promoted the lysis of biofilm-forming bacteria while disrupting extracellular polymeric substances (EPS), which facilitated the penetration of phage particles into other bacterial cells [27]. Engineered synthetic phages also proved to be highly effective in destroying pathogens inside human cells [28].

3.3 Phage lytic proteins

An interesting approach to control UTIs is the use of isolated phage lytic enzymes (PLEs) as antimicrobial molecules. These can be divided into two categories – endolysins and virion-associated lysins (VALs). Endolysins are lytic enzymes that destroy the bacterial cell by attacking the peptidoglycan (PG) from within, allowing the virus progeny to spread. On the contrary, VALs are attached to the virion particle and degrade the cell surface from outside allowing the phage to inject its genetic material into the infected bacterial cell [29].

3.4 Phages and biofilm

Some studies have revealed the effectiveness of single phages and cocktails when used against biofilm-forming uropathogenic strains *in vitro* [30], [31]. However, they observed that the appearance of resistant strains took place after 24–48 h with the consequential re-growth of the biofilm. When a five-phage cocktail was used, the density of the biofilm formed at 48 h was reduced to 99.9%, thus indicating a remarkable delay in the emergence of resistance [31]. Biofilm polysaccharide normally protects the bacteria against the majority of phages. However, if phages produce the specific polysaccharide depolymerase, they may be able to degrade the biofilm's extracellular polysaccharide matrix and gain access to the bacterial surfaces [32].

The biofilm reduction by the phages is not dose-dependent, it would mean that low titers of the phages were as effective as using a higher titer in eradicating established biofilms. Re-establishment of biofilms after 24 h exposure to the phages *in vitro* may be attributed to development of bacterial resistance against the phages. This could be overcome by using a cocktail of phages [33]. The candidate phages with adequate lytic spectrum for therapeutic purposes (including efficacy for disruption of existing

biofilms) were characterized in detail. The phages have a promising potential for phage therapy of UTIs caused by biofilm-forming UPEC. Although the phages have been proposed as good candidates to treat UTIs and catheter-associated UTIs, there is concern as to whether they would be able to keep their activity *in vivo* due to the flow caused by voiding or saline flush, or on the contrary whether they would be washed out of the system.

3.5 Phagotherapy in combination with antibiotics

For more than a decade it has been known that the phage infection of bacteria cultured with sub-lethal doses of antibiotic leads to an enhanced production of virulent phages. This phenomenon, first described and named phage-antibiotic synergy (PAS) by Comeau et al., can be observed as an enhanced size of the phage plaques. Delayed lysis of the bacterial cell is associated with a decrease in holin, elongated cells and, as a result, an increase in the number of phages inside them [34]. Results of the Kumaran et al. study showed that infecting the biofilm cells with the phage prior to treatment with antibiotic causes the maximum reduction in the size of the biofilm [35].

3.6 Phage resistance mechanisms in bacteria

Phage resistance in bacteria includes modification of phage surface receptors on a bacterial cell, integration of phage genome into the bacterial genome, loss of genes specific for phage replication and assembly, and production of extracellular matrix – an important component of bacterial biofilm that provides a physical barrier between phages and their receptors [36]. The mechanisms of phage resistance can be classified into prevention of phage adsorption, prevention of phage DNA integration, cutting phage nucleic acids, and abortive infectious processes [37], [29]. The most common types of phage resistance are prevention of phage adsorption by point mutations and/or changes in the expression of genes encoding phage-binding receptors [29]. Although the molecular mechanisms remain unclear, a CRISPR/Cas system has been revealed; it acts as a bacterial acquired immune system, which remembers the viral genetic material to prevent future infection attempts [37]. The last instrument of bacterial resistance mechanism is an abortive infection system. This system causes death of infected host bacteria, thus preventing phage replication and further infection of other bacteria [37].

A promising strategy to avoid resistance is to produce a cocktail of several phages, each one attaching to a different receptor in a cell, so that if one receptor mutates, another phage points to a different target. The more phages the cocktail includes, the less likely the development of resistance is to all of them [38]. Bacteriophages have also developed strategies to counteract and override these resistance mechanisms. For example, phages can change the life cycle (by correcting the rate of explosion-harvesting, lysis time, etc.) [39], and can encode CRISPR-Cas protein inhibitors (i.e. anti-CRISPRs) [40]. Moreover, mutations in phage receptor binding proteins can occur and cause phages to recombine (unite) with other viruses [39].

3.7 Phage kinetics in the body

After an intravenous administration, phages rapidly localize in the liver, spleen, lungs, kidney, and urine [41], [42] as is the case after oral administration [43]. However, it is important to protect them against gastric acid because therapeutic phages are rather sensitive to low pH [44]. One hour after oral administration in humans, phage particles were detected in the blood, the bronchial content, the cerebrospinal fluid, on the surface of burn wounds, and in urine. After a single dose, bacteriophages were identified in the body for 7 and more days [45].

If there is a bacterial infection, homologous bacteriophages replicate and can be identified in urine for up to 6–7 days. In healthy people, bacteriophages are excreted from the body within 24 hours. Rectal administration may also be an efficient route of phage delivery. It was shown in rabbits and mice that it only takes a few minutes for phages to penetrate from the rectum through the intestinal wall into the circulation [46], [47]. The blood phage level may be about two orders of magnitude higher than that with oral feeding. This may result from the lack of phage inactivation by gastric juice.

E. coli T4 phage can penetrate rat prostate tissue after their intravenous administration and after rectal application [48]. Noteworthy is the effect of acidic urine pH on phages. Tan et al. [49] did not observe any negative effect of urine on suspended phage particles when testing two *Klebsiella* phages against nine carbapenemase-producing *K. pneumoniae* isolated from two elderly patients with UTI. Pereira et al. [50]

suggested that phages acting against urinary tract bacteria are well adapted to survive in the urinary tract niche, which is characterized by pretty low pH levels. In an experiment conducted by the authors, *E. cloacae* phages remained stable with preserved lytic activity for at least 12 hours in urine samples.

3.8 Clinical use of bacteriophages

The use of bacteriophages to combat bacterial infections dates back to the early twentieth century, and research and clinical practice on the topic has been performed continuously up until today in some countries of the former Soviet Union like Georgia and Russia. Nevertheless, this therapy was not popular in other parts of the world, mainly due to the discovery and great success of antibiotics as chemotherapy agents [51].

Only few reports address the results of phage applications in urology. Boratynska et al. [52] summarized the results of treatment with phage lysates of 15 patients (24–77 years old) with recurrent UTI (a few acute exacerbations per year) during the course of chronic pyelocystitis, nephrolithiasis, vesicoureteral reflux, or floating kidney and UTI after kidney transplantation (KT). UTI was caused by *E. coli* (n = 9), *P. aeruginosa* (n = 2), *Klebsiella pneumoniae* (n = 2), *Proteus vulgaris* (n = 2), Enterobacter aerogenes (n = 2), and *S. aureus* (n = 2), which were all resistant to the available chemotherapeutics. The patients received 10 ml of specific phage orally three to four times daily after neutralization of gastric juice. The patients who had KT (n = 3) were simultaneously treated with antibiotics. The treatment lasted 3–11 months (mean: 5.4 months). Long-lasting (12–36 months) abatement of symptoms of UTI and pathogen eradication were achieved in five patients (including one with KT). In three cases, remission was observed after 3–6 months. Phages were present in the urine of some patients. No changes were observed in blood morphology, serum proteins, electrolytes, or renal and liver function. Researchers from a leading medical institute in Bucharest reported the results of phage treatment of a much larger group (87 patients) with UTI [53]. The phages, applied as the only antibacterial treatment in the case of infection with multidrug-resistant bacteria, exerted remarkable effects in acute UTI, resulting in rapid temperature decline and retreat of leukocyturia. A synergistic effect with simultaneous antibiotic treatment was observed. Similar results were obtained by Russian physicians [54]. In a group of 46 patients with acute or chronic urogenital inflammation, treated both locally (direct administration into the urinary bladder) and orally with phages targeting *P. aeruginosa* , *Proteus sp.*, *Staphylocccus sp.*, or *E. coli* or with combined pyobacteriophage (in monotherapy and in combination with antibiotics), a clinical improvement was observed in 92% of the cases.

Nowadays, bacteriophages are being used in the USA and EU as a compassionate therapy under the regulation of the article 37 of the Helsinki Declaration [55], which limits its use to cases where there is no other possibility of intervention or all previous attempts were unsuccessful. Nowadays, in the era of antibiotic resistance the Western world has turned its attention to this forgotten therapy, as an alternative to treat some infections, for which antibiotics are starting to fail. There are two main approaches in the phage therapy – the one that uses the entire phage (either natural or genetically-engineered) to attack the pathogenic bacteria and the one that uses isolated phage lytic enzymes to promote cell death [56].

In Russia, bacteriophage preparations are obtained at phage production facilities that were created in the 1960s in the USSR by selecting highly virulent phages that possess a wide antibacterial activity spectrum. The preparations do not contain moderate phages capable of transduction or lysogenic conversion. The clearance rate of lysed bacterial cells, bacterial antigens, and toxins is 98 to 99% [57]. Zorkin and Skakhnovsky [58] studied the efficacy of bacteriophages vs. antibiotics in children with complicated urinary tract infections due to congenital urinary tract abnormalities. Although this method cannot be recognized as safe, the authors conducted local irrigation of the pelvicalyceal cavity, the bladder cavity, or the ureter lumen with 5–10 ml of a sensitive bacteriophage with drainage clamping for 15–20 min within the subsequent 5–7 days, after which the drainage was removed. 6 to 12 months after the urodynamics normalization, the combination of antibiotic with bacteriophage was noted to have the best clinical and bacteriological efficacy.

In a systematic review devoted to the phage therapy of infections caused by multiresistant microorganisms, out of the 30 studies enrolling more than 1152 patients [59], only 4 publications were about phage therapy of UTI.

- Khawaldeh et al. [60]: 1/female/67 years old Cocktail Pyophage. Urinary tract infection instilled directly into bladder and intravenous antibiotic administration + meropenem + colistin/every 12 h/10 days. Reduction of *P. aeruginos*a from 107 CFU/ml to 103 CFU/ml on day 21
- Uimajuridze et al. [61]: 170 patients with UTI. Ongoing study. Significant decrease of the concentration of pathogens in the urine in the treatment of pyophage

- Perepanova et al. [54]: 106 patients with acute and chronic UTI. Local and oral administration of phages. Phage preparations were adapted to uropathogens. Phage clinical and microbiological efficacy of 92%
- Letkiewicz et al. [62]: 22 males with chronic bacterial prostatitis. Application of phages rectally, orally, and/or topically. Eradication of the target bacteria was observed in 50% of the cases. In other cases, patients' condition improved.

The most efficient administration routes of phage preparations in 22 women with reproductive and urinary tract infections were intravaginal and oral, both of which resulted in a good microbiological effect in 50% of the cases. A relatively high percentage of patients with a favorable outcome following oral administration suggests that the phages effectively penetrated the focus of infection from the gastrointestinal tract [63].

Intrarectal administration of phage-based preparations turned out to be highly efficient in a recent study in Poland [64] that described the treatment of UTI in a 60-year old kidney transplant recipient. A clinical symptom remission was achieved and maintained for 4 years after the extraction of the left kidney and for 5.5 years after kidney transplantation. However, it should be noted that the patient received a combination therapy with meropenem, to which pathogenic ESBL (extended spectrum beta lactamases)-producing *K. pneumoniae* bacteria were totally susceptible.

Letkiewicz et al. [65] recently described in detail three cases of prostatic patients treated with phages in whom successful pathogen eradication was achieved. Specific phage preparations active against *E. faecalis* isolated from the patients (107–109 CFU/ml) were prepared and applied rectally, 10 ml two times daily, for 28–33 days. The treatment caused bacterial eradication (confirmed by two negative cultures of EPS conducted 7–17 weeks apart), improvement in the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI; analyzed in two cases), reduction in prostate size and pain, and significant increases in the maximum urinary flow rate in all cases.

Many authors used phage treatment for biofilms formed in urinary catheters that are a unique environment for microorganisms [66], [67], [68]. A significant reduction of biofilm formation was detected after covering permanent catheters with bacteriophages specific to *P. aeruginosa*, *E. coli*, and *P. mirabilis*. Studies have shown that phages can reduce biofilms formed by uropathogenic *E. coli* regardless of the dose, which means that low titers of phages are as efficient as higher titers for the destruction of formed biofilms [33]. However, our data demonstrated that low doses of phages stimulated the growth of biofilms on catheters [69].

3.9 Problems associated with phage therapy

Clinical trials demonstrate the efficacy of phages for the treatment of infectious inflammatory diseases; however, trial methods vary between different countries. The trials predominantly focus on monomicrobial infection and do not include mixed infection. Although many trials have demonstrated a high efficacy of infection treatment with phages, they do not comply with the modern requirements of evidence-based medicine.

Phages are essentially protein structures, which explains their susceptibility to all the environmental changes causing protein denaturation, such as acidic pH, high temperatures, exposure to organic solvents (e.g. disinfectants), and mechanical stresses [70], [71]. Phage clearance by the immune system can affect the efficacy of phage therapy. Since phages are found ubiquitously and permanently (e.g. in different food products), low phage-specific antibody titers can often be encountered in patients, but the titers may rise during phage therapy [71]. However, it is possible that repeated administration of phages, increased phage concentration, or using different phages or a phages cocktail can compensate for this phenomenon [29], [72]. Moreover, immunity stimulation with phages can even improve the treatment outcome [73].

Aleshkin et al. [74] studied anti-phage immunity in 42 patients of the Neurointensive Care Unit by enzyme-linked immunosorbent assay performed prior to and after phage therapy. While during the first course of phage therapy microbiological efficiency was confirmed in 54–62.5% of the cases, repeated courses with the same strains of phages did not result in a significant eradication of pathogens. Anti-phage immunity following a single administration of bacteriophage preparations was confirmed by the presence of *IgG* antibody titers in the range from 1/16 to 1/4096. During further study, the authors found out that neutralizing *IgG* antibodies appear two to three weeks after the initial course of phage therapy and are strain-specific. Thus, in case of repeated phage therapy, the authors recommend changing the

composition of the phage cocktail to maintain the necessary level of its lytic activity spectrum by individually selecting the strain composition, the dosage, and the administration route of the bacteriophage preparation.

Although no serious clinical immunological complications associated with phage therapy have been reported so far [29], a question may arise on whether there is a risk of indirect immunogenicity due to cell lysis. Yet, beta-lactam antibiotics also cause bacterial cell wall lysis. However, clinical situations requiring antibacterial therapy or phage therapy are generally associated with a systemic inflammatory reaction and bacteriemia, i.e. the conditions that are already life-threatening. When bacteria, phages, and the human body interact, especially in the presence of an infection, the immune system becomes a set of complex interactions that are still difficult to accurately model/predict [75].

4 Further research

4.1 Advantages of bacteriophages over antibiotics

Phages have several important properties that facilitate their therapeutic potential. Firstly, phages can self-enhance (replicate), which is an asset facilitating its efficacy and contrasting them with antimicrobial drugs [76]. Secondly, some phages have polysaccharide depolymerases on their tail structures that can act as an adjuvant to phage infection by destroying the extracellular matrix of biofilm infection [77]. Thirdly, phages are considered to be safe for human tissues and do not affect the human normal bacterial flora, which can be due to their high specificity (they frequently infect only a subpopulation of strains within the same species) and rapid inactivation and clearance as soon as the host (bacterium) is no longer present [76]. It also implies that each new bacterial strain can require a specific phage [77], [78]. Luckily, phages are abundant in nature and can be easily extracted and fully characterized. Finally, the phages' mechanism of action differs from that of antibiotics, so they are usually not affected by bacterial antibiotic resistance mechanisms [79], which is the main reason of the keen interest in phage therapy in parallel with the upsurge of antibiotic resistance in the recent decades.

5 Conclusion

Increasing evidence on the emergence of multidrug-resistant strains of uropathogenic bacteria is making the scientific community look for clinical solutions, other than the search for new antibiotics, to this problem. Phage therapy is a promising alternative that has resulted in proved efficacy against UTIs both *in vitro* and *in vivo* using all the approaches discussed above: natural phages, phage cocktails, phage lytic enzymes (PLEs), engineered phages or PLEs, and phage therapy in combination with antibiotics. Nevertheless, an indispensable step for this therapy to be used in clinical practice is the evidence provided by validated clinical trials. Unfortunately, many clinical trials on phage therapy carried out so far have either been not adequately controlled or with a reduced sample size. Well-planned evidence-based studies are needed to present reliable results supporting the clinical use of phage therapy.

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