Title:

Advancing Bacteriophages as a treatment of antibiotic resistant bacterial pulmonary infections

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Abstract

Purpose of review: This paper summarises the recent advances in the use of bacteriophages to treat pulmonary infections, particularly those caused by gram-negative drug-resistant bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Klebsiella pneumoniae* and *Burkholderia* species. It provides an updated overview of the current available evidence, with a summary of published clinical cases, case series and clinical trials currently underway.

Recent finding: Personalized treatment with bacteriophages is still in its infancy in Europe and the USA, despite extensive experience in Eastern countries. However, more patients are expected to be treated with clinical trials in progress and others planned.

Summary: Despite very promising initial results and the confirmation of phage safety, there are still many ethical and practical implications to be considered, from the necessary regulatory approval to optimization of dose and route of administration, to developing strategies to tackle bacterial resistance. Patients with cystic fibrosis are a group where phage therapy, if successful, could have a major impact.

Keywords:

Bacteriophages, respiratory infections, pneumonia, multi-drug resistant gram negatives

Introduction

The rapid spread of multidrug-resistant (MDR) bacterial pathogens represent a serious public health concern worldwide (1). Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Burkholderia cepacia* complex and *Escherichia coli* are recognised as being very difficult to treat due to their ability to rapidly develop antibiotic resistance (2). In addition, the ability of bacteria to form biofilm, sessile communities of microorganism attached on a biotic or abiotic surface and encased in a self-produced matrix, makes them extremely tolerant to a high concentration of antibiotics even if they are susceptible in their planktonic form (3, 4).

Pulmonary infections are the fifth leading cause of mortality for all ages (5). Each year it is estimated about 156 million children worldwide will develop an acute chest infection, leading to 4 million deaths (6). Patients with chronic pulmonary disorders, such as cystic fibrosis (CF), primary ciliary dyskinesia and bronchiectasis are also at considerable risk of chronic respiratory infections due to MDR gram-negative bacteria such as *Pseudomonas aeruginosa* and *Burkholderia cepacia* (7).

As current antibiotics are becoming ineffective and investments into new antibiotic development is poor, there are few alternative treatments for MDR bacterial infection under development. The use of bacteriophages (phages) as antibacterials has been receiving greater attention as a promising and safe therapeutic addition to the treatment of bacterial infections, including pulmonary infections caused by MDR bacteria (8). Bacteriophages are natural viruses of prokaryotes, infecting and exclusively killing host bacterial cells in a species-specific manner (9). As they are viruses, phages replicate inside their bacterial hosts and lyse them, propagating the killing activity to the other bacterial cells by releasing the viral progenies that shed the phage infection until all bacteria have been killed (9) (**Figure 1**). Phages can be isolated from different sources (environmental and human-associated samples), in which both virulent (strictly lytic) and temperate (lysogenic) phages can be found. However, it is generally recommended to use strictly lytic (virulent) phages which are not able to integrate their genome into bacterial cells (as lysogenic phages do) (10). Indeed, temperate phages are considered inappropriate, as integration of temperate phage DNA into the bacterial genome could contribute to unwanted virulence and also because antibiotic resistance genes can be stably transferred between bacterial hosts (10).

Most published papers describe patients treated with natural phages (11-15). However, employing engineered phages is also an option (16). Several new technologies such as synthetic biology and genetic engineering now exist and have the potential to make temperate phages suitable for therapeutic purposes. For instance, they have been engineered to eliminate lysogenic genes responsible for the genome integration, which at least in theory gives them similar properties to virulent phages (although there are concerns that their biology may still differ) (17).

Phages are able to kill antibiotic resistant bacteria and target biofilm-embedded populations (18, 19). They can be also employed as adjunct to antibiotics, with synergistic effects observed between some phages and different classes of antibiotics (19). This might prevent phage resistance occurring in bacteria during treatment and *in vitro* studies have shown phage therapy may even restore susceptibility of bacteria to antibiotic. More *in vivo* data is needed to evaluate this effect (20).

In this review, we discuss recent advances in phage therapy for the treatment of lung infections caused by gram-negative drug-resistant bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Klebsiella pneumoniae* and *Burkholderia cepacia* complex. First, we will critically discuss the efficacy of phage therapy in pre-clinical models and move on to the evaluation of clinical experience of phage treatment of respiratory infections. This paper will also cover safety issues and the regulatory considerations related to phage therapy.

Animal models of respiratory infections and phage therapy

According to a standard approach of drug development, the safety and efficacy of new compounds (including biologics) should be tested in an *in vivo* model before clinical study (8). Although phages have already been safely administered to humans, animal models can still provide additional important data to elucidate the best route of administration, therapeutic phage dose and timings of application and interaction of phages with mammalian immune systems.

Currently, mice are the most common animal model of pulmonary infection for testing the anti-bacterial efficacy of phage therapy. In the last 15 years, different studies have been performed showing the successful treatment of *P. aeruginosa* in both acute and chronic lung infections in the mouse (21-25). Much of this work was pioneered by Debarbieux and colleagues (26) who showed the rapid efficacy of phage PAK_P1 in killing *P. aeruginosa* when administered as an aerosol for the treatment of acute lung infection in mice (8-week-old Balb/c males). The survival of all animals treated with phages was observed when a ratio (or multiplicity of infection, MOI) PAK_P1-to-bacterium of 10:1 was used, while mice either treated with an MOI of 0.1 phage or no phage died within 5 and 2 days, respectively. This suggested a phage-dose-dependent efficacy which was in agreement with previously reported data (27).

As pressures from the host immunity versus the interaction phage-bacteria might influence both susceptibilities to bacterial infection and the effectiveness of phages in a therapeutic setting, Roach and collaborators (27) evaluated the efficacy of PAK_P1 phages to prevent and treat acute pneumonia caused by *P. aeruginosa* in mice with innate and/or adaptive immunity deficiencies. Comparing efficacies of prophylactic treatments versus phage-curative in healthy immunocompetent, MyD88-deficient, lymphocyte-deficient, and neutrophil-depleted murine hosts, they found that *P. aeruginosa* lung-infected wild-type mice, depleted of their neutrophils, were completely unresponsive to inhaled phage treatment and ultimately died because of a resurgent bacterial outgrowth. These results suggested an essential synergy between phage lysis and the host immune defences (such as neutrophils) for the success of the therapy, called *immunophage synergy* (27). Moreover, it was observed that a high phage dose (10^9 PFU/ml) did not significantly increase the production of cytokines in mouse lung tissues when compared to the PBS control, indicating that phage PAK_P1 was also immunologically well tolerated. By contrast, the value of cytokines produced after exposure to phages was lower in comparison to that produced after *P. aeruginosa* LPS exposure (27) and a reduced inflammatory response in murine lung was also reported by Pabary et al (28) . Whilst these results are encouraging it cannot be assumed results will be similar in humans.

Chronic lung infections caused by *P. aeruginosa*, associated with chronic respiratory diseases such as CF, are often recalcitrant to antibiotic treatment, due to the development of drug-resistance and/or biofilm formation. However, the efficacy of phage therapy has also been shown in a mouse model where *P. aeruginosa* established a natural long-term chronic lung infection (17).

Although *P. aeruginosa* is the reported bacterial target of phage therapy in most of the *in vivo* pre-clinical experiments, the therapeutical efficacy of phages in pulmonary infections has also been investigated for other gram-negative bacteria, such *B. cenocepacia* (29), *K. pneumoniae* (30) and *A. baumannii* (31). For example, Carmody et al (29) showed that intraperitoneal phage administration was more effective than intranasal administration for the treatment of an acute *B. cenocepacia* in a respiratory infection model, suggesting that circulating phages have better access to bacteria in lungs than topical phages. However, a single phage intranasal instillation (MOI 10) was also able to rescue 100% of mice after a lethal challenge with *A. baumannii* and to strongly reduce the inflammatory response, resulting in the recovery of mouse lung tissue to a healthy status (31). Even though different administration routes based on various bacterial infection models were shown to be effective, the combination of intravenous and local phage treatment might represent the best therapeutical option for treating pulmonary infection.

Case reports and case series of respiratory infections treated with bacteriophages

Despite promising results from animal models, clinical use of phages to treat pulmonary infections caused by multi-drug resistant pathogens has been limited to a few case reports and case series (11, 14, 15, 32-34). Although phages have been extensively used therapeutically in former Soviet Union countries (35) to treat a wide range of other infections, their clinical use in the Western world is generally case-by-case, under the compassionate use route, when all other treatment options have failed.

The first patient treated with intravenous phage therapy (but not for pneumonia) in the US suffered from a systemic infection caused by a multidrug-resistant *Acinetobacter baumannii*. He successfully recovered, paving the way for more patients to be considered for personalized phage therapy (15). Other case series on the compassionate use of phage therapy in Europe (covering a variety of different infections) have also demonstrated significant clinical improvement after phage treatment (36, 37).

Strikingly, there are no clinical trial outcome results to date on the use of phages for the treatment of antibiotic-resistant bacterial pulmonary infections. This may change in the near future as a simple search on the ClinicalTrials.gov website (on the 1st December 2021) found 60 different clinical

trials worldwide using phages, aimed at treating from tonsillitis to prosthetic joint infection. Out of these trials, five are focused on the use of phages in respiratory infections in cystic fibrosis (CF) patients and one commercially sponsored trial is on the use of phages in Covid-19 patients with superadded bacterial pneumonia or bacteraemia.

The interest in phages among CF patients should not come as a surprise. Because of the recurrent chest infections and exacerbations, the CF population have always been troubled by increased antimicrobial resistance, in particular in *Pseudomonas aeruginosa*, *Burkholderia species* (7, 38) and atypical mycobacteria. Some of the already mentioned case reports included the use of phages in different CF patients with infections caused by those gram-negative bacteria (11, 32-34). Much of the early and indeed contemporary literature from Georgia and Russia is not published in English and is thus difficult to access. However, in one CF case reported by the Eliava Institute in Tbilisi (Georgia) (34), a paediatric patient received nebulized phages against *Staphylococcus aureus* and *P. aeruginosa* with a reduction in bacterial titers and a 50% reduction in the total amount of antibiotics needed for treatment over a 9 months period. In the other cases from USA (11, 32, 33), phages have been administrated intravenously to treat *Burkholderia dolosa*, *Burkholderia cenocepacia*, *Burkholderia gladioli*, *Achromobacter xylosoxidans* (in this case only, also nebulized) and *P. aeruginosa* infections. In all cases (apart from one patient that died during phage treatment without antibiotics), phages were always used in combination with antibiotics and for a prolonged course (from 2 to 8 weeks). However, a deep clinical knowledge based on experience of using phages to treat respiratory infections caused by other resistant gram-negative organisms (i.e., *Enterobacterales*, such as *Escherichia coli* and *Klebsiella* species) is still missing. This lack of information is largely due to a combination of several factors and it includes: the laborious process of isolating the active phages; the time required for the necessary regulatory and ethical approvals; the availability of some antibiotics that are still partially active (such as colistin and cefiderocol) and also the higher mortality rates caused by multi-drug resistant *Enterobacterales* infection (39).

These individual reports seem to suggest the potential of some clinical benefit from the use of bacteriophage treatment for bacterial respiratory infections, but we can only speculate on genuine efficacy until data from randomized-controlled clinical trials become available. Some authors have also highlighted the need for basic research to accurately predict the different responses of target bacterial pathogens when phages are administered as a stand-alone treatment, sequentially, or as cocktails (of active phages with and without antibiotics), and whether there are wider consequences on the efficacy of phage therapy in the long run, in particular with the development of resistance (40).

Ethical and practical implications to be considered

While phage therapy remains experimental, each clinical case currently requires multiple local approvals (i.e., ethical committee, FDA in USA, MHRA & NHSE in UK and other national bodies in different European countries). Other practical implications will inevitably extend the timeline from when the decision of using phage therapy is initiated till the actual administration to the patient (Table 1).

One important reassurance from the few case reports and small series already mentioned in this review is about the safety of phage treatment. Some authors (41) also argue that exposure to phages occurs in humans every day and this is further evidence of their safety. Sterility and minimal endotoxin and LPS concentration of the final phage preparation is essential and phage production must follow good manufacturing practices in line with other commercial pharmaceutical products. Various authors have already presented protocols on how to achieve this, although there is debate about what constitutes GMP production and on how to best achieve high titre/high purity phage stocks (42, 43).

Due to phage specificity to different bacterial strains, personalized treatments are likely to be needed at least in some cases and very few centres in the world (but likely to increase in the next few years) are able to perform this tailored manufacturing, causing significant delays from request to administration. There are an estimated 10^{31} phage particles on the planet (44) and it is believed that at least one type of phage can potentially infect every strain of bacteria (45). Centres like the Eliava Institute in the Republic of Georgia or the Hirszfeld Institute in Poland have several phage preparations readily available to treat a range of clinical infections but they can also provide more personalized phage products directly to physicians if needed (46, 47). For this approach though, there have not been rigorous safety studies on all phages used. In other parts of the world, such Western Europe and USA, however, phage therapy presents a unique regulatory challenge, which is being addressed for by the European Medicine Agency (EMA) and Food and Drug Administration (FDA). As a result of various efforts in this area, it is foreseeable that more biotechnology companies will develop in the next few years to produce phages.

At present, there are no clinical data and very little experience in clinical practice on what constitutes the most suitable phage formulation to treat respiratory infections. Delivery of phages to the lungs could benefit from aerosolization, but most of the formulations used in the case reports outlined were given intravenously. Theoretically, liquid phage formulations can be easily aerosolized into fine droplets using commercially available nebulizers (48), but this also needs to researched as delivery has been shown to vary greatly depending on which nebuliser is used to delivery phages (49). Challenges remain as the most commonly used phage stabilizers, including phosphate and Tris, are not yet approved for inhalation (50). A clinical trial is currently ongoing at Yale in CF patients with

P.aeruginosa chest infection and treated with inhaled (nebulized) phage therapy versus placebo, with planned completion date at the end of 2022 (ClinicalTrials.gov Identifier: NCT04684641).

Phage pharmacokinetics is still largely an uncharted territory and little is known about the optimal dosage (51). In this regard, Schooley et al. achieved a good clinical outcome with a concentration of 10^4/mL for *A. baumannii* (15), whilst Dedrick et al. reported serum concentrations of at least 10^9/mL to treat more resistant infection with *Mycobacterium abscessus* (17). Recent experience from California has shown the development of side effects at concentrations of 10^{11}/mL (13), and the production of neutralizing antibodies to the phages has already been observed in humans (limiting their efficacy and bioavailability) (52, 53). In addition to human trial, more data is increasingly likely to come from the use of phages within agricultural arenas, such as when they are used to treat swine or poultry. We will not cover in this review the potential development of resistance to phages as this has been recently covered elsewhere (54) although it should be stated that phage resistance is significantly reduced when phage mixtures or cocktails are used (55).

Conclusion

Phage therapy represents a very attractive solution to combat the emergence of multi-drug resistant infections. Whilst numerous studies highlight the *in vitro* and *in vivo* potential of therapeutic phages, there is still a paucity of clinical experience in their use against pulmonary infections due to gram-negative bacteria. Some clinical trials are currently ongoing (in particular for CF patients) and further research will have to address the best formulation, combination (with or without antibiotics) and dosage for the treatment of such bacterial respiratory infections. The wider impact on the potential development of resistance will also need careful surveillance over time.

Key points

– Bacteriophages are natural viruses, that infect and exclusively kill their host bacterial cells in a species-specific manner, including bacteria present in biofilm.

– Phages can be isolated from different sources (environmental and biological samples), in which both virulent (strictly lytic, generally preferred for treatment) and temperate (lysogenic) phages can be found.

– Clinical interest is rapidly developing for the phage treatment of infections (including pneumonia) caused by multi-drug resistant bacteria.

– Standardized protocols are needed to obtain good manufacturing practices in a manner comparable to other commercial pharmaceutical products.

– Some clinical trials are currently ongoing, but there are still many unanswered questions to be addressed by research studies.

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Figures and tables

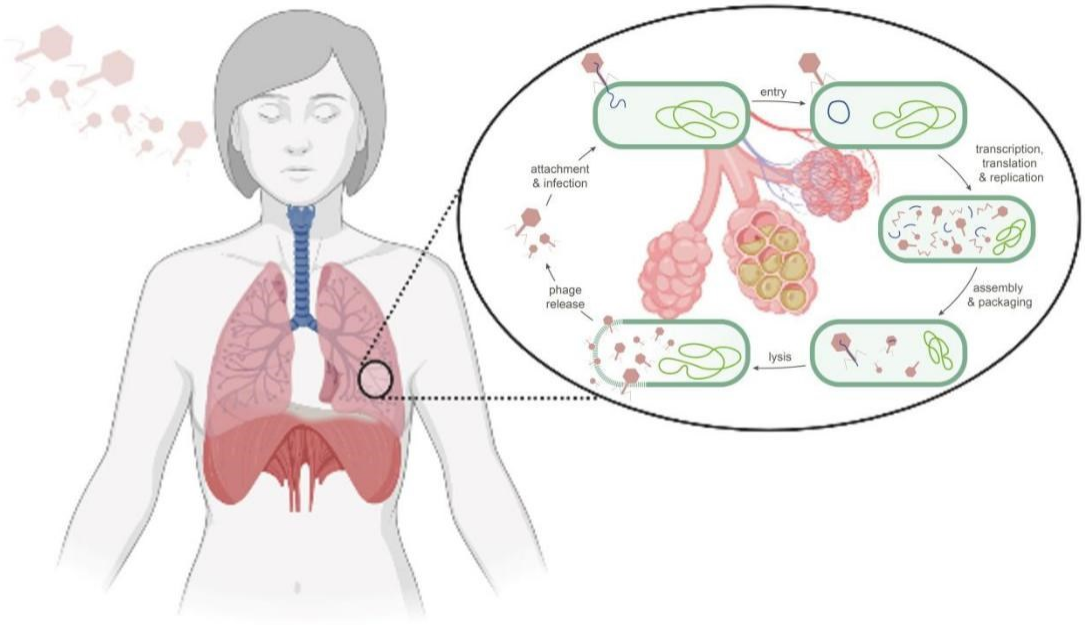

Figure 1: Picture showing the different stages of a phage lytic cycle. These stages include attachment, entry, biosynthesis (transcription, translation and replication), assembly, and final lysis of the bacterial cells with phage release (Created with BioRender.com).

Ethical and practical implications to be considered in bacteriophages treatment of respiratory infections	
Ethical approval for compassionate use	- Each clinical case requires multiple local approvals, including ethical committee and national approval body depending on the country - Sterility and minimal endotoxin concentration of the final phage preparation is essential
Selection of bacteriophages	- Laborious screening process of thousands of different phages - Few centres in the world are able to perform this personalized manufacturing - Some commercial companies are becoming available and they offer emergency access via the compassionate route
Administration	- Intravenous route for disseminated infection is required - Serum concentrations from 10^4 to 10^{10}/mL, but more data needed to confirm

	- Nebulized formulations still under development and need to confirm stability and penetration in lung parenchyma
Safety profile	- General safety of phage treatment has been demonstrated but there is the need of large trials to rule out rare side effects - Concentrations of 10^{11}/mL more associated with side effects
Development of resistance	- Acquired resistance after treatment/ bacterial defence mechanisms need further evaluation in long term studies - Production of neutralizing antibody against phage already described

Table 1: Summary of main challenges during phage therapy, from the selection of phages to the necessary regulatory approvals and treatment considerations.

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