Evaluating the Efficacy of Bacteriophage Therapy against Multidrug-Resistant Klebsiella pneumoniae Infections

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ABSTRACT
Multidrug-resistant (MDR) Klebsiella pneumoniae poses a significant challenge to conventional antibiotic treatments. This study aims to assess the efficacy of bacteriophage therapy against MDR Klebsiella pneumoniae through a combination of in vitro assays, animal model experiments, and clinical case reviews. Phages isolated from environmental samples demonstrated potent lytic activity against MDR strains. In vitro assays showed that phages significantly reduced bacterial counts at a multiplicity of infection (MOI) of 1 or higher. Animal model experiments revealed that phage therapy, particularly using a cocktail of multiple phages, markedly improved survival rates and reduced bacterial loads in infected tissues compared to saline controls and standard antibiotics. Clinical case reviews indicated that patients receiving phage therapy experienced high rates of bacterial clearance and clinical improvement. These findings support the potential of bacteriophage therapy as an effective alternative or adjunct to traditional antibiotics in managing MDR Klebsiella pneumoniae infections.

1. Introduction

The rapid amplification of multidrug-resistant (MDR) bacteria is a great threat to public health all over the world. Among the wide range of MDR pathogens, Klebsiella pneumoniae (KP) is probably the most virulent one. KP is capable of causing pneumonia, urinary tract infections and septicaemia. The resistance of KP to all antibiotics in clinical use significantly increases morbidity and mortality. In addition, there is virtually no alternative therapies to treat infections of refractory MDR microorganisms. Therefore, the identification of new and effective antibacterial drug targets and drug discovery approaches are urgently needed(1,2).

Bacteriophage therapy is a treatment that uses bacteriophages, viruses that infect and lyse bacteria. Bacteriophages have a limited host range, meaning that they target a specific kind of bacteria with minimal effects on the native microbiota, reducing the chances of side effects. The aim of this paper was to examine whether bacteriophage therapy against MDR Klebsiella pneumoniae infections appears to be effective, looking at data from studies and randomised clinical trials(3,4). Klebsiella pneumoniae, a gram-negative, encapsulated bacterium common to the environment and the human gastrointestinal tract, is normally a commensal organism, but may become pathogenic, especially in the hospital where it is a major cause of healthcare-associated infections. The ability of K pneumoniae to acquire multiple antibiotic resistance genes via plasmids has led to the emergence of MDR strains with resistance to nearly all available antibiotics.(5,6). Antibiotic resistance in K pneumoniae, including MDR K pneumoniae, can occur through overproduction of extended-spectrum beta-lactamases (ESBLs), carbapenemases and other antibiotic-degrading enzymes, resulting in high levels of resistance to the majority of antibiotic therapy options. Consequently, therapy against infections caused by MDR K pneumoniae is difficult and requires alternative approaches, such as bacteriophage therapy(7,8).

Bacteriophages, also known as phages, are viruses that infect bacteria. They bind to the surface of bacterial cells, inject their own genetic material, and then use the bacterial machinery to replicate, eventually lysing, or destroying, the bacterial cell and releasing new phage particles that can go on to kill neighbouring bacteria.(9) Phage specificity to bacterial hosts is another advantage of phage therapy. Broad-spectrum antibiotics wipe out both the pathogen and the normal biota, but phage treatment affects only pathogenic bacteria, sparing the beneficial kind. This specificity also lessens chances for adverse effects and development of resistance(10,11) .Phages are naturally present in sewage, soil and water, and can be isolated from these environments. Once isolated, they must be

characterised, purified and formulated into a therapeutic preparation. They can be administered as a single agent, or combined (known as phage cocktails) to increase effectiveness and to prevent the development of bacterial resistance(12) .A number of in-vitro studies have shown efficacy of bacteriophagesiella pneumonia lytic phage isolation from environmental samples, followed by their testing for their in-vitro lytic activity against clinical isolates of Klebsiella pneumoniae(13).In one study, (14) identified a bacteriophage (named KPP-1) that showed potent lytic activity against a broad range of MDR Klebsiella pneumoniae strains. It reduced the abundance of bacteria rapidly in only several hours .Another study, published in the journal Scientific Reports earlier this year, tested a cocktail of three phages targeting different proteins against an array of MDR Klebsiella pneumoniae isolates. The cocktail was able to lysed every tested strain, including carbapenem- and colistin-resistant strains – two last-resort antibiotics (15). The authors note that phage cocktails could be prepared to broaden the spectrum of bacteria killed, thus may be an important way of circumventing phage resistance, which occurs when bacteria that have all the receptors for a particular phage mutate(16).Such in vitro studies are good evidence for the potential of bacteriophages against infections with antibiotic-resistant, highly pathogenic MDR Klebsiella pneumoniae, but the leap from in vitro efficacy to potential clinical application still needs to be tested in order to expand bacteriophage therapy to antibiotic-resistant infections. This could be done by animal models and in clinical trials (17,18).

2. Materials and Methods

2.1 Isolation and Characterization of Bacteriophages

Phages were isolated from sewage and hospital wastewater samples. Phage isolation involved filtering samples and enriching them with MDR *Klebsiella pneumoniae*. Plaque assays were used to identify and characterize lytic phages.

2.2 In Vitro Bactericidal Assay

MDR *Klebsiella pneumoniae* cultures were exposed to isolated phages at different MOIs. Bacterial viability was monitored by CFU counts over 2 and 4 hours.

2.3 Animal Model Experiments

Mice were infected with MDR *Klebsiella pneumoniae* and treated with either a single phage, a phage cocktail, or a standard antibiotic. Survival rates, bacterial loads in lungs and blood, and inflammatory markers were assessed over a 7-day period.

2.4 Clinical Data Analysis

Clinical data from patients treated with phage therapy for MDR *Klebsiella pneumoniae* infections were reviewed. Outcomes included bacterial clearance rates and clinical improvements.

3. Results

3.1 Phage Isolation and Characterization

Table 1 summarizes the characteristics of the isolated phages.

Phage ID	Host Strain	Plaque Morphology	Genomic Characteristics	Specificity
KPP-1	MDR KP1	Clear, circular	dsDNA, 60 kb	*K. pneumoniae*
KPP-2	MDR KP2	Turbid, irregular	dsDNA, 45 kb	*K. pneumoniae*
KPP-3	MDR KP3	Clear, round	dsDNA, 55 kb	*K. pneumoniae*

3.2 In Vitro Bactericidal Activity

Table 2 shows the reduction in bacterial counts in the presence of bacteriophages.

MOI	Initial CFU/ml	CFU/ml After 2 hours	CFU/ml After 4 hours
0.1	1.0 × 10^8	5.0 × 10^7	1.0 × 10^6
1	1.0 × 10^8	2.0 × 10^6	<1.0 × 10^3
10	1.0 × 10^8	<1.0 × 10^3	<1.0 × 10^2

3.3 Animal Model Outcomes

Table 3 presents the survival rates and bacterial loads in treated and control groups.

Treatment	Survival Rate (%)	Bacterial Load in Lungs (CFU/g)	Bacterial Load in Blood (CFU/ml)
Saline (Control)	30	1.2 × 10^6	1.5 × 10^5
Antibiotic	60	4.0 × 10^4	1.0 × 10^4
Single Phage	70	2.0 × 10^4	5.0 × 10^3
Phage Cocktail	90	1.0 × 10^3	1.0 × 10^2

3.4 Clinical Data Analysis

Table 4 summarizes the clinical outcomes of patients treated with phage therapy.

Case ID	Infection Type	Treatment Duration (Days)	Bacterial Clearance (%)	Clinical Improvement (%)
001	Pneumonia	10	80	75
002	Urinary Tract	14	70	70
003	Wound Infection	7	85	80

4. Discussion

4.1 Efficacy of Phage Therapy

These in vitro results confirmed that bacteriophage therapy kills MDR Klebsiella pneumoniae very effectively and significantly reduce bacterial count at MOI of 1 or more, which shows that bacteria's resistance mechanisms are overridden. The effectiveness of phage cocktails is also evident in preventing the emergence of resistant strains, since a single phage can't wipe out the entire population, while a cocktail of phages can attack multiple bacterial receptors simultaneously.(19,20).

For both doses of the phage cocktail, survival rates improved and bacterial loads in the lungs and blood were decreased compared with controls and antibiotic treatments, suggesting that phage therapy can significantly improve the clinical outcome of infected animals; the authors speculate that

this could have been due to its capacity to reduce bacterial loads and modulate the inflammatory response(21,22).

These findings are consistent with clinical case reviews, demonstrating consistently high bacterial clearance rates and clinical improvement in patients treated with phage therapy, yet variable outcomes between infection types, depending on the infection site and bacterial strain characteristics, requiring personalised treatment approaches.(23).

4.2 Challenges and Considerations

There are a number of challenges with phage therapy. First, phage-resistant mutants are ever-present, so phage cocktails or engineered phages are needed to counteract this. There's also a need to find ways to stabilise phages and deliver them into the body, perhaps through encapsulation and systemic methods(24).

There's also a question of regulatory and logistical hurdles. Standardising protocols for how phages are prepared, tested and administered are crucial to their consistent and safe use. Clear guidelines from regulatory agencies will help with this (25).

4.3 Future Directions

With these considerations in mind, future research should try to optimise phage therapy treatment protocols. This includes the development of personalised phage cocktails and combination therapies. In addition, a better understanding of combining phages with antibiotics or other targeted treatments could yield more beneficial synergistic effects for the management of MDR infections. In the end, large-scale clinical trials are needed to establish the effectiveness and safety of phage therapy in different patient populations. Collaborative endeavours will also play a vital role in moving the promising phenotypic therapeutic modality of phage therapy forward (26,27).

5. Conclusion

This study provides strong evidence for the usefulness of bacteriophages for treatment of MDR Klebsiella pneumoniae infections. The in vitro, in vivo and clinical analysis yielded solid results in favour of phage therapy as an alternative or adjunct to antibiotics. Overcoming obstacles towards the standardisation of phage therapy in the present day will be of vital importance.

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