

Phage Therapy: A Novel Approach to Overcome Antibiotic Resistance

Ruhollah Dorostkar^{1*}

¹ Applied Virology Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

*Corresponding Author: Ruhollah Dorostkar, Applied Virology Research Center, Baqiyatallah university of medical sciences, Tehran, Iran.
Email: r.dorost@yahoo.com

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Executive Summary

In this study, the focus was on *Pseudomonas aeruginosa*, a common bacterial agent in keratitis that exhibits antibiotic resistance. The aim was to isolate, identify, and assess the antimicrobial potential of a bacteriophage sample for treating keratitis caused by *P. aeruginosa*. The bacteriophage was isolated from wastewater at Baqiyatallah Hospital in Tehran, Iran, and identified using TEM imaging. Various methods, including plaque assays and host range determination, were employed to confirm the presence of the phage. The study evaluated the phage's minimum inhibitory concentration (MIC), biofilm degradation, cytotoxicity, and the safety of phage-containing eye drops on New Zealand rabbits, as well as their effectiveness in a mouse model. The results indicated that the isolated phage belonged to the Myoviridae family and exhibited anti-biofilm activity against clinical isolates. The MIC and IC₅₀ values for the phage were determined to be 10⁻⁸ and 10⁻¹, respectively. Notably, the combination treatment of ciprofloxacin eye drops with phage drops proved more effective in improving keratitis than either treatment alone. In conclusion, while the therapeutic effect of the isolated phage on clinical strains of *P. aeruginosa* was demonstrated, the combination of antibiotic and phage treatment showed synergistic effects.

Keywords: Bacteriophage, Phage therapy, Keratitis, Antimicrobial resistance, *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is a gram-negative bacillus widely found in soil and water. It is an opportunistic human pathogen that rarely causes disease in healthy hosts but is a major cause of infections in patients with underlying conditions or immune deficiencies.¹ This bacterium is one of the main causes of morbidity and mortality in cystic fibrosis (CF) patients, making it of particular importance.² It can also cause respiratory and urinary tract infections, skin and soft tissue infections, bacterial keratitis (eye infections), swimmer's ear, and similar conditions in individuals with underlying conditions.³ *Pseudomonas aeruginosa* infections can also cause conditions such as external otitis, middle ear infections, keratitis, and folliculitis in healthy individuals.⁴ The bacterium *Pseudomonas aeruginosa* poses a significant threat to public health due to its intrinsic resistance to many

antibiotics and its ability to easily acquire antibiotic resistance determinants.⁵ Bacteriophages, or phages, are viruses that attack bacterial cells and, in the case of lytic phages, disrupt the metabolism of the bacteria and cause bacterial lysis.⁶ Bacteriophages are highly specific to their hosts and typically infect only one species of bacteria or even a specific strain within a species. However, phages are very diverse in terms of size, morphology, and genomic organization.⁷ The effectiveness of phages in combating pathogenic bacteria depends on various factors, including the ratio of bacteriophages to target bacteria, the timing and conditions of treatment (such as pH and temperature), and bacterial resistance. Additionally, parameters such as the rate of phage adsorption, the latent period, the initial phage dose, and the clearance of phage particles from the body also play a significant role.⁸

Materials and Methods

This research was designed to isolate and evaluate specific bacteriophages against *Pseudomonas aeruginosa* as an alternative therapeutic method for antibiotic-resistant eye infections. In the first phase, various culture media including LB (liquid, semi-solid, and solid), Mueller Hinton agar (MHA), TSI agar, MacConkey agar (MAC), and tryptic soy broth (TSB) were prepared using standard protocols. Then, a clinical strain of *P. aeruginosa* was isolated from patients with corneal infections and confirmed using Gram staining methods, biochemical tests, and culture in specific media. To isolate phages, wastewater samples from the hospital treatment plant were collected and concentrated using centrifugation and filtration methods. Specific phages were identified and purified using the plaque assay and double-layer agar culture technique. In the next phase, the characteristics of the isolated phages, including morphology (using transmission electron microscopy, TEM), host range, impact on bacterial biofilm, and cytotoxicity (using the MTT assay), were examined. Additionally, the minimum inhibitory concentration (MIC) of the phages was determined, and the antibiotic resistance of the bacterial strains was assessed using the disk diffusion method. To ensure the safety of the phages, the Draize test was conducted on New Zealand rabbits. Finally, the efficacy of phage therapy was evaluated in a rat model infected with *P. aeruginosa* by comparing different treatment groups (control, antibiotic, phage, and combination therapy) using histopathological methods and colony counting. The results of this study could pave the way for the development of novel phage therapy methods to combat resistant eye infections.

Results

In this study, the bacterial strain *P. aeruginosa* from patients with eye infections was investigated. Initially, the strain's validity was confirmed through Gram staining and culturing on various media. Additionally, the presence of bacteriophage in the hospital wastewater was confirmed with lytic activity. Electron microscopy identified the isolated phage as belonging to the Myoviridae family, and it was found to infect 8 out of 9 clinical strains of *P. aeruginosa*. The results indicated that phage therapy could effectively degrade the bacterial biofilm and showed greater efficacy in reducing eye infections compared to antibiotic treatments. Moreover, the combination of phage and the antibiotic ciprofloxacin demonstrated a synergistic effect, contributing to a significant reduction in bacterial load and inflammation in the eyes. These findings highlight

the high potential of phage therapy as an effective method for treating eye infections caused by *P. aeruginosa*.

Discussion and Conclusion

Bacteria (such as *P. aeruginosa*) that are recognized as the main cause of eye infections worldwide are associated with various types of eye infections.⁹ Eye infections may damage the structures of the eye and lead to visual impairments, thus requiring therapeutic interventions.¹⁰ The bacterium *P. aeruginosa* is an opportunistic gram-negative pathogen capable of infecting and growing on catheters, respiratory tubes, and surgical instruments, leading to severe infections such as pneumonia, bacteremia, septicemia, and recurrent endocarditis, primarily in patients with compromised immune systems.¹¹ Recent reports indicate that the number of infections caused by resistant microbes is increasing globally, and the rise in bacterial resistance to various antimicrobial agents, such as antibiotics, limits the available treatment options for effective infection management, leading to challenges in managing infections.¹² The development of alternative methods for antibiotic therapy or antimicrobial biological treatments to prevent and control infections caused by *P. aeruginosa* has become a major challenge worldwide.¹³ In this study, a newly discovered lytic phage against antibiotic-resistant *P. aeruginosa* was isolated from wastewater samples at Baqiyatallah Hospital in Tehran. The results provided evidence for the phage's potential application in eliminating or reducing eye infections caused by *P. aeruginosa*. This phage formed clear plaques on *P. aeruginosa*-infected bacterial lawns and was identified as part of the Caudovirales order, likely belonging to the Myoviridae family. Most research on human phage therapy has reported on Myoviruses, Cytoviruses, Siphoviruses, and Podoviruses.¹⁴ The results of Rahimzadeh and colleagues, who examined a phage from the Cystoviridae family for the treatment of *P. aeruginosa* keratoconjunctivitis in a rabbit model, demonstrated the natural structure of the cornea.¹⁵ This study indicates that the method of gel formation at the site prolongs the release and durability of the bacteriophage. Additionally, the use of phage eye drops in the mouse model shows desirable antibacterial effects. Therefore, phage therapy should be formulated considering the type of bacteriophage, the route of administration, and the appropriate dosage. The results reported by Mapes and colleagues using a cocktail consisting of 4 phages demonstrated the antibacterial effect of phage therapy on levofloxacin-resistant *P. aeruginosa*, as well as the anti-biofilm activity of this bacterium and the expansion of the host range in the phage cocktail state.¹⁶ In the study by Amankwah and colleagues, it was shown that two phages

isolated from wastewater can disrupt the biofilm of multidrug-resistant *P. aeruginosa* on the surfaces of catheters and endotracheal tubes.¹⁷ The MTT analysis of the isolated phage determined an IC₅₀ near the 10⁻¹ phage dilution, which may have adverse effects on HCE-2 cells. Therefore, a lower phage dilution (10⁻²) is recommended for in vivo studies. The results from four groups in evaluating the effectiveness of phage eye drops on Wistar rats indicated that ciprofloxacin alone may not be sufficient for treating eye infections in this animal model. While phage therapy can be an effective treatment for such infections, the combination of ciprofloxacin and phage antibiotics shows a synergistic effect, making it more effective against *Pseudomonas* eye infections. Additionally, colony counting confirmed the effectiveness of the eye drops in the animal model. In a similar study, it was found that while phage therapy alone inhibited *P. aeruginosa* biofilm formation by about 50%, the combination of phage and ciprofloxacin inhibited all clinical strains of *P. aeruginosa*.¹⁸ In the present study, histological analysis results showed that phage therapy is effective in reducing inflammation. Additionally, the synergy between phages and antibiotics has a more favorable therapeutic effect compared to other groups studied. In the results of Rahimzadeh and colleagues, it was demonstrated that when an eye drop formulation containing phage was used as a gel to treat *P. aeruginosa* keratoconjunctivitis in a rabbit model, a natural corneal structure was observed, and inflammation, neovascularization, abscess, and conjunctival inflammation were significantly lower in the group treated with phage-containing eye drops.¹⁵

Conflicts of Interest Disclosures

The author declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent For Publication

Not applicable

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