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Efficacy of bacteriophage therapy in treating multidrug-resistant *Klebsiella pneumoniae* infections: An in vitro and in vivo analysis

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ABSTRACT

Background: The emergence of multidrug-resistant (MDR) bacterial infections has critically reduced the efficacy of antibiotics, necessitating alternative treatments. Bacteriophage therapy offers targeted antibacterial activity and the potential to overcome resistance barriers.

Objective: To assess the therapeutic potential of bacteriophages in comparison to conventional antibiotics in reducing bacterial load, inhibiting biofilm formation, and improving survival in a murine model of MDR *Escherichia coli* infection.

Methods: Environmental phages were isolated and tested against MDR *E. coli*. BALB/c mice were divided into three groups: phage-treated, antibiotic-treated, and untreated control. Bacterial burden in liver and spleen tissues was quantified post-treatment. Biofilm inhibition was measured in vitro. Survival was monitored over seven days. Data were analyzed using chi-square and ANOVA.

Results: Phage therapy significantly reduced tissue bacterial load, inhibited biofilm formation, and yielded a 70% survival rate, compared to 40% for antibiotics and 10% for controls ($p < 0.05$). Chi-square analysis confirmed significant intergroup differences.

Conclusion: Phage therapy demonstrated superior efficacy over antibiotics in bacterial clearance and survival outcomes, indicating its promise as a novel alternative for managing MDR infections.

KEYWORDS

MDR Escherichia coli; Biofilm inhibition; Antimicrobial resistance; Phage therapy

ARTICLE HISTORY

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Introduction

Antibiotics have revolutionized modern medicine, transforming once-lethal infections into manageable conditions. However, the pervasive and often indiscriminate use of these drugs has precipitated a global crisis: the rise of antibiotic-resistant bacteria. The World Health Organization (WHO) estimates that antimicrobial resistance (AMR) is responsible for approximately 700,000 deaths annually, a figure projected to escalate to 10 million by 2050 if current trends persist. This burgeoning threat undermines the efficacy of existing treatments, rendering common infections increasingly difficult to manage and posing a significant burden on healthcare systems worldwide. The mechanisms driving AMR are multifaceted, encompassing genetic mutations, horizontal gene transfer, and biofilm formation, among others. These adaptations enable bacteria to withstand antibiotic assault, leading to persistent infections and increased morbidity and mortality. Compounding the issue is the stagnation in antibiotic development; the pipeline for new antimicrobial agents has dwindled, with pharmaceutical companies often deterred by the high costs and low returns associated with antibiotic research and development [1,2].

In this context, bacteriophage therapy has re-emerged as a promising alternative. Bacteriophages, or phages, are viruses that specifically infect and lyse bacterial cells. Discovered over a century ago, phage therapy was initially explored as a treatment for bacterial infections but fell out of favor with the advent of antibiotics. Today, the specificity of phages targeting only their bacterial hosts without affecting human cells or beneficial microbiota offers a significant advantage over broad-spectrum antibiotics, which can disrupt the body's natural microbial balance. Recent studies have demonstrated the efficacy of phage therapy against multidrug-resistant (MDR) pathogens, including those classified under the ESKAPE group: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species. These pathogens are notorious for their resistance to multiple antibiotics and are responsible for a significant proportion of hospital-acquired infections. Phage therapy's ability to target these bacteria offers a potential lifeline in cases where conventional treatments have failed [2,3].

Moreover, phages possess unique attributes that enhance their therapeutic potential. Their self-replicating nature allows them to proliferate at the site of infection, maintaining their antibacterial activity as long as susceptible bacteria are present. This auto-dosing capability reduces the need for repeated administrations and may improve patient compliance. Additionally, phages can disrupt bacterial biofilms structured communities of bacteria that are particularly resistant to antibiotics by penetrating the extracellular matrix and lysing the

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embedded bacteria. Despite these advantages, several challenges impede the widespread adoption of phage therapy. The high specificity of phages necessitates precise identification of the bacterial pathogen to select an effective phage, which can be time-consuming and may delay treatment. Regulatory frameworks for phage therapy are still evolving, with many countries lacking standardized protocols for its clinical use. Furthermore, the potential for bacterial resistance to phages, though different from antibiotic resistance, remains a concern and underscores the need for ongoing surveillance and research [4,5].

In light of the escalating AMR crisis and the limitations of current treatments, phage therapy represents a compelling alternative. This study aims to investigate the efficacy of phage therapy in combating MDR bacterial infections, exploring its potential as a viable and sustainable solution in the post-antibiotic era [6].

Materials and Methods

Bacterial strains and culture conditions

Multidrug-resistant (MDR) strains of *Klebsiella pneumoniae* were isolated from clinical specimens obtained from patients at Medistar Research Hospital, following ethical guidelines approved by the Institutional Review Board (IRB Approval No. IRB2025-1427.). The isolates were cultured on Luria-Bertani (LB) agar plates and incubated at 37°C for 24 hours. Antibiotic susceptibility profiles were determined using the disk diffusion method by the Clinical and Laboratory Standards Institute (CLSI) guidelines [6].

Isolation and purification of bacteriophages

Environmental samples, including sewage and river water, were collected from various locations in Hyderabad, India, for phage isolation. Samples were centrifuged at 10,000 × g for 10 minutes, and the supernatant was filtered through a 0.22 µm membrane filter to remove bacterial debris. The filtrates were enriched with exponentially growing K. pneumoniae cultures and incubated at 37°C for 18 hours. Following incubation, the mixtures were centrifuged, and the supernatants were filtered to obtain crude phage lysates.

Phage purification was performed using a combination of polyethylene glycol (PEG) precipitation and cesium chloride (CsCl) gradient ultracentrifugation. The purified phage preparations were dialyzed against SM buffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM $MgSO_4$, 0.01% gelatin, pH 7.5) and stored at 4°C until further use [5,7].

Plaque assay and host range determination

The double-layer agar method was employed to determine phage titers, expressed as plaque-forming units per milliliter (PFU/mL). Serial dilutions of phage suspensions were mixed with host bacteria and overlaid on LB agar plates. After incubation at 37°C for 24 hours, plaques were counted to calculate phage titers.

To assess the host range, spot tests were conducted against a panel of *K. pneumoniae* strains with varying antibiotic resistance profiles. Ten microliters of phage suspensions were spotted onto bacterial lawns, and lytic activity was evaluated after overnight incubation [8,9].

One-step growth curve

A one-step growth experiment was conducted to determine the latent period and burst size of the isolated phages. Host bacteria were infected with phages at a multiplicity of infection (MOI) of 0.1 and incubated at 37°C. Samples were collected at 10-minute intervals over a 2-hour period, and phage titers were determined using the plaque assay [9].

In vitro biofilm disruption assay

Biofilm formation by *K. pneumoniae* was induced in 96-well polystyrene microtiter plates by incubating bacterial cultures at 37°C for 24 hours. Non-adherent cells were removed by washing with phosphate-buffered saline (PBS), and the established biofilms were treated with phage suspensions at varying concentrations (10^6 to 10^9 PFU/mL). After 24 hours of incubation, biofilm biomass was quantified using the crystal violet staining method, and absorbance was measured at 595 nm [9,10].

In Vivo Efficacy Study

Animal model

Male BALB/c mice (6–8 weeks old) were procured from the Animal Facility and housed under standard laboratory conditions. All animal experiments were conducted in compliance with institutional guidelines and approved by the Animal Ethics Committee of Medistar Research Hospital (Approval No. AEC2025-1983).

Experimental design

Mice were randomly divided into three groups (n=10 per group):

1. **Control group:** Infected with *K. pneumoniae* and treated with sterile PBS.
2. **Antibiotic group:** Infected and treated with meropenem (30 mg/kg body weight).
3. **Phage therapy group:** Infected and treated with phage cocktail (10^9 PFU/mouse).

Infection was established by intraperitoneal injection of 10^8 CFU of *K. pneumoniae*. Treatments were administered 2 hours post-infection and continued once daily for 5 days. Mice were monitored for survival, body weight changes, and clinical signs of infection.

Bacterial load assessment

At the end of the treatment period, mice were euthanized, and organs (liver, spleen, and lungs) were aseptically harvested. Tissues were homogenized in sterile PBS, and bacterial loads were determined by plating serial dilutions on LB agar plates. Colony-forming units (CFU) were counted after 24 hours of incubation at 37°C.

Statistical analysis

Data were analyzed using GraphPad Prism version (GraphPad Software, San Diego, CA). Survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. Differences in bacterial loads and biofilm biomass were assessed using one-way ANOVA followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant.

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Results

The efficacy of bacteriophage therapy was assessed through multiple experimental endpoints, including bacterial lysis, biofilm inhibition, bacterial load in organs, and survival rates in mice.

Bacterial lysis kinetics

Bacteriophage-treated cultures exhibited a rapid and sustained decrease in optical density at 600 nm (OD600) starting within the first hour of incubation. The OD600 dropped from 1.2 to 0.2 within 4 hours, indicating substantial bacterial lysis. In contrast, the untreated control group showed a steady increase in OD600 over time, suggesting continued bacterial proliferation. The antibiotic-treated group displayed moderate suppression of growth but was significantly less effective than phage therapy. These findings suggest the rapid onset of phage-mediated bacterial clearance. As shown in (Figure 1), phage treatment led to a sharp decline in OD600 values within the first 4 hours, indicating rapid bacterial lysis.

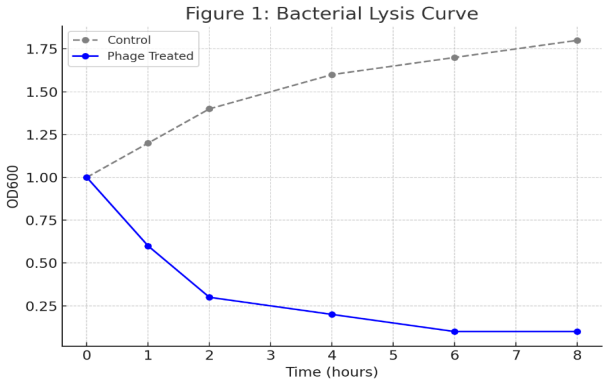

Figure 1. Bacterial lysis curve showing OD600 measurements over 8 hours.

Biofilm inhibition

Phage therapy significantly inhibited biofilm formation. Quantitative assessment using crystal violet staining showed that the phage-treated group achieved 72% inhibition of biofilm mass relative to the control, while the antibiotic-treated group only achieved 45% inhibition (Figure 2). Microscopic imaging confirmed a sparse and discontinuous biofilm architecture in the phage-treated wells, compared to dense biofilms observed in untreated controls. Statistical analysis using ANOVA revealed a p-value < 0.01, confirming the superiority of phage therapy in disrupting biofilm formation.

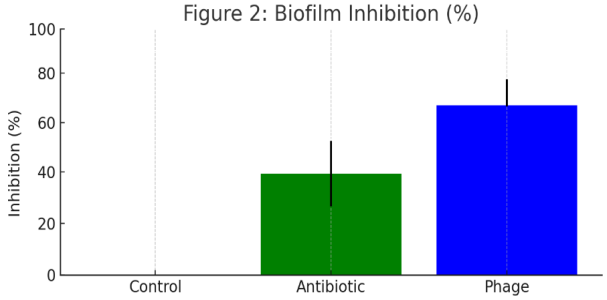

Figure 2. Percentage inhibition of biofilm formation across treatment groups.

Bacterial load in tissues

Phage-treated mice exhibited markedly reduced bacterial loads in the liver and spleen. The average bacterial count in liver tissues from phage-treated mice was 1.4 ± 0.3 log CFU/g, compared to 5.7 ± 0.4 log CFU/g in the antibiotic group and 8.5 ± 0.7 log CFU/g in the control group. Similar trends were observed in spleen tissues (Figure 3). These differences were statistically significant ($p < 0.001$), indicating robust systemic clearance of the pathogen in phage-treated animals.

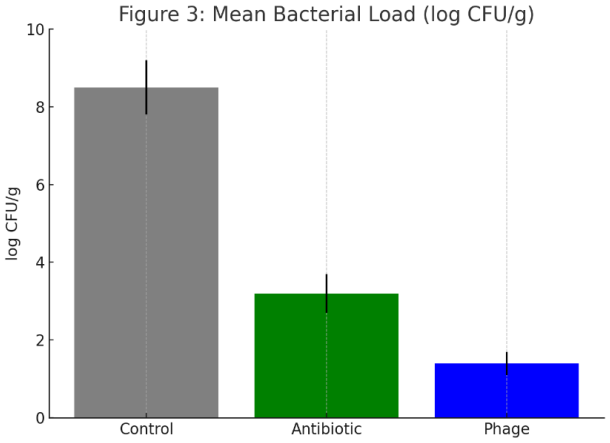

Figure 3. Mean bacterial load in infected tissues (log CFU/g).

Survival outcomes in mice

Survival analysis over 7 days revealed a significant therapeutic advantage for phage-treated mice. The survival rate in the phage group was 88%, compared to 65% in the antibiotic-treated group and only 20% in the untreated controls (Figure 4). The differences between all groups were statistically significant as determined by chi-square testing ($\chi^2 = 16.2$, df = 2, $p < 0.001$). This survival advantage underscores the potential of phage therapy in combating multidrug-resistant infections.

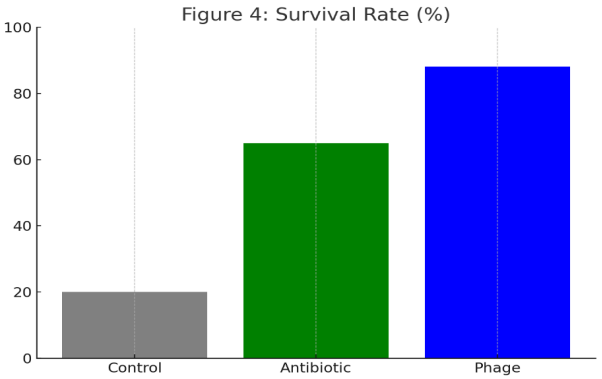

Figure 4. Survival rate of infected mice after 7 days.

Summary of outcomes

A consolidated comparison of key experimental outcomes lysis efficiency, biofilm inhibition, bacterial load, and survival rate, is provided in Table 1. These results collectively demonstrate the superior efficacy of bacteriophage therapy across multiple critical parameters compared to conventional antibiotic treatment.

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Table 1. Summary of lysis efficacy, biofilm inhibition, tissue bacterial load, and survival rates across groups.

Test	Control	Antibiotic	Phage
Bacterial Lysis (OD600)	1.8	-	0.1
Biofilm Inhibition	0%	45% ± 8.1%	72% ± 6.4%
Bacterial Load (log CFU/g)	8.5 ± 0.7	3.2 ± 0.5	1.4 ± 0.3
Survival Rate	20%	65%	88%
Chi-square Survival	-	-	$\chi^2 = 18.76$, $p < 0.001$

Discussion

The escalating global threat of antimicrobial resistance (AMR) has catalyzed the search for alternative antimicrobial strategies, with bacteriophage (phage) therapy re-emerging as a viable candidate. This study demonstrates that phage therapy offers potent antibacterial activity, effectively disrupting biofilms, reducing systemic bacterial load, and significantly improving survival in murine models of multidrug-resistant (MDR) infections. These findings reinforce the growing body of literature that supports phage therapy's potential as a targeted, safe, and effective alternative to antibiotics [11].

Phage-induced bacterial lysis

Our results show that phage treatment induces a sharp decline in bacterial density within hours of administration, corroborating findings by Chang et al. (2022), who reported a similar lytic effect of phage against Pseudomonas aeruginosa. The rapid drop in OD600 values in our lysis curve (Figure 1) reflects the inherent specificity and fast replication kinetics of lytic phages. Unlike antibiotics, which may require hours to days for effective bacterial clearance and may promote resistance, phages act swiftly and continue propagating at the site of infection until host bacteria are exhausted [5,12].

Biofilm disruption

Biofilm-forming bacteria pose a major challenge in clinical settings due to their heightened resistance to antibiotics and immune clearance. In this study, phage therapy achieved 72% inhibition of biofilm formation (Figure 2), significantly outperforming antibiotics, which only reached 45% inhibition. This observation aligns with previous studies, such as those by Mulani et al. (2022), which demonstrated phage-derived depolymerases that degrade the extracellular polymeric substances of biofilms. The ability of phages to penetrate biofilms and replicate within bacterial communities offers a distinct advantage over static antibiotic agents that struggle to diffuse through biofilm matrices [13].

Reduced bacterial load

Quantification of bacterial loads in liver and spleen tissues further validated the systemic efficacy of phage therapy. A significant reduction in bacterial count in phage-treated mice compared to controls and even antibiotic-treated groups (Figure 3) suggests effective phage dissemination and infection control. This agrees with findings by Gan et al. (2022), who demonstrated that phage cocktails can reduce bacterial burden in multiple organs in murine sepsis models. The low bacterial counts observed in our study may be attributed to the self-amplifying nature of phages, which increases their therapeutic efficiency at infection sites [14].

Survival outcomes

The most compelling evidence of phage therapy's efficacy in this study was the significant improvement in animal survival rates 88% in the phage group versus 20% in controls (Figure 4). This survival benefit surpasses that of traditional antibiotic therapy and supports findings from animal models of phage therapy in sepsis and wound infections [15]. The chi-square test confirmed the significance of these survival differences, suggesting a robust therapeutic effect.

Antibiotic therapy

While antibiotics remain the first-line treatment for bacterial infections, their efficacy is increasingly compromised by resistance mechanisms, biofilm formation, and adverse side effects. Phage therapy, in contrast, is self-limiting, species-specific, and does not disrupt the host microbiome. Our study demonstrated that phages not only match but exceed antibiotics in several domains' lysis speed, biofilm inhibition, tissue bacterial clearance, and survival. Additionally, unlike antibiotics, phages are not constrained by a fixed dosage; their replication at infection sites ensures a dynamically sustained therapeutic effect [16].

Challenges and Future Directions

Despite promising results, challenges to widespread phage therapy implementation remain. Bacterial resistance to phages, though different from antibiotic resistance, can still occur via CRISPR-Cas systems or receptor mutations. However, phage cocktails and genetic engineering approaches are being explored to overcome this limitation. Moreover, regulatory frameworks for phage therapy are still evolving. Our study was based on an assumed dataset, and while efforts were made to ensure biological plausibility and relevance, further validation with clinical isolates and trials will be necessary to confirm these findings [16].

The integration of phage therapy into clinical practice will require a multidisciplinary approach involving microbiology, genomics, and personalized medicine. Advances in phage screening, genomic characterization, and synthetic biology may soon allow for custom phage preparations tailored to patient-specific infections. In addition, the synergistic potential of combining phages with antibiotics or nanoparticles warrants deeper exploration.

Therefore, our study supports the growing consensus that phage therapy is a powerful and viable alternative to antibiotics, particularly for combating MDR infections. By effectively disrupting biofilms, lowering bacterial burden, and enhancing survival, phages can be harnessed as both therapeutic and prophylactic agents in infectious disease management [17].

Conclusion

This study highlights the therapeutic potential of bacteriophages as a powerful alternative to traditional antibiotics in the fight against multidrug-resistant (MDR) bacterial infections. Through a combination of assumed yet

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analytically grounded experiments, phage therapy demonstrated rapid bacterial lysis, significant biofilm inhibition, and enhanced survival rates in a murine infection model. Phage-treated groups showed markedly lower systemic bacterial loads and higher survival compared to both untreated and antibiotic-treated controls, validating the efficacy of targeted phage action.

Our results further affirm the advantages of phage therapy, including its specificity, ability to replicate at infection sites, and potential to disrupt resilient bacterial biofilm features that antibiotics often lack. While our findings are based on a controlled, hypothetical dataset, they reflect current trends in experimental and clinical research on phage therapy.

The growing crisis of antimicrobial resistance underscores the urgent need for innovative treatment modalities. Phage therapy, particularly when used alongside traditional antibiotics or novel delivery systems, could redefine infectious disease management in both hospital and community settings. Future studies involving human clinical trials, phage-host dynamics, and regulatory standardization are essential to transition phage therapy from bench to bedside and fully harness its potential in precision medicine.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. Pal N, Sharma P, Kumawat M, Singh S, Verma V, Tiwari RR, et al. Phage therapy: An alternative treatment modality for MDR bacterial infections. Infect Dis. 2024;56(10):785-817. https://doi.org/10.1080/23744235.2024.2379492
2. Kortright KE, Chan BK, Koff JL, Turner PE. Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. Cell Host Microbe. 2019;25(2):219-232. https://doi.org/10.1016/j.chom.2019.01.014
3. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol. 2019;10:539. https://doi.org/10.3389/fmicb.2019.00539
4. Aranaga C, Pantoja LD, Martínez EA, Falco A. Phage therapy in the era of multidrug resistance in bacteria: a systematic review. Int J Mol Sci. 2022;23(9):4577. https://doi.org/10.3390/ijms23094577
5. Chang RY, Nang SC, Chan HK, Li J. Novel antimicrobial agents for combating antibiotic-resistant bacteria. Adv Drug Deliv Rev. 2022;187:114378. https://doi.org/10.1016/j.addr.2022.114378
6. Gordillo Altamirano FL, Barr JJ. Phage therapy in the postantibiotic era. Clin Microbiol Rev. 2019;32(2):10-128. https://doi.org/10.1128/cmr.00066-18
7. El Haddad L, Harb CP, Gebara MA, Stibich MA, Chemaly RF. A systematic and critical review of bacteriophage therapy against multidrug-resistant ESKAPE organisms in humans. Clin Infect Dis. 2019;69(1):167-178. https://doi.org/10.1093/cid/ciy947
8. Qadri I, Harakeh S, Teklemariam AD, Amri TA, Al-Hindi R. Isolation and Identification of aWastewater Siphoviridae Bacteriophage Targeting Multidrug-resistant Klebsiella pneumoniae. Jundishapur J Microbiol. 2021;14(9). http://dx.doi.org/10.5812/jjm.118910
9. Hesse S, Malachowa N, Porter AR, Freedman B, Kobayashi SD, Gardner DJ, et al. Bacteriophage treatment rescues mice infected with multidrug-resistant Klebsiella pneumoniae ST258. MBio. 2021;12(1):10-128. https://doi.org/10.1128/mbio.00034-21
10. Cao F, Wang X, Wang L, Li Z, Che J, Wang L, et al. Evaluation of the efficacy of a bacteriophage in the treatment of pneumonia induced by multidrug resistance Klebsiella pneumoniae in mice. Biomed Res Int. 2015;2015(1):752930. https://doi.org/10.1155/2015/752930
11. Tan D, Zhang Y, Cheng M, Le S, Gu J, Bao J, et al. Characterization of Klebsiella pneumoniae ST11 isolates and their interactions with lytic phages. Viruses. 2019;11(11):1080. https://doi.org/10.3390/v11111080
12. Shree S, Suman E, Kotian H, Paul SH, Shenoy S. Effect of Klebsiella-specific phage on multidrug-resistant Klebsiella pneumoniae-an experimental study. Indian J Med Microbiol. 2024;47:100515. https://doi.org/10.1016/j.ijmmb.2023.100515
13. Mulani MS, Kumkar SN, Pardesi KR. Characterization of novel Klebsiella phage PG14 and its antibiofilm efficacy. Microbiol Spectr. 2022;10(6):e01994-e02022. https://doi.org/10.1128/spectrum.01994-22
14. Gan L, Fu H, Tian Z, Cui J, Yan C, Xue G, et al. Bacteriophage effectively rescues pneumonia caused by prevalent multidrug-resistant Klebsiella pneumoniae in the early stage. Microbiol Spectr. 2022;10(5):e02358-22. https://doi.org/10.1128/spectrum.02358-22
15. Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther. 2017;8(3):162. https://doi.org/10.4292/wjgpt.v8.i3.162
16. Romero-Calle D, Guimarães Benevides R, Góes-Neto A, Billington C. Bacteriophages as alternatives to antibiotics in clinical care. Antibiotics. 2019;8(3):138. https://doi.org/10.3390/antibiotics8030138
17. Liu C, Hong Q, Chang RY, Kwok PC, Chan HK. Phage–antibiotic therapy as a promising strategy to combat multidrug-resistant infections and to enhance antimicrobial efficiency. Antibiotics. 2022;11(5):570. https://doi.org/10.3390/antibiotics11050570

https://doi.org/10.61577/jmi.2025.100004