

Novel Bacteriophages Targeting ESKAPE Pathogens: A Promising Avenue for Antimicrobial Resistance Mitigation

Suganya C¹, Arul Nehru P², Bernaitis L^{3*}

¹Department of Noi anugavidhi ozhukkam (Principles and Disciplines of Disease Prevention and public health), Nandha Siddha Medical College and Hospital, Erode-638052.

²Department of Maruthuvam, Nandha Siddha Medical College and Hospital, Erode-638052

³Department of Microbiology, Nandha Siddha Medical College and Hospital, Erode-638052.

*Corresponding Author Bernaitis L.

Associate Professor, Department of Microbiology, Nandha Siddha Medical College and Hospital, Erode-638052.

Article History

Received: 25.08.2024

Accepted: 12.09.2024

Published: 01.10.2024

Abstract: - The emergence of multidrug-resistant (MDR) bacteria has significantly compromised the effectiveness of conventional antibiotics, necessitating the exploration of alternative therapeutic strategies. Bacteriophages, or phages, have garnered renewed interest as potential agents against MDR infections, particularly those caused by ESKAPE pathogens, which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. This review discusses the unique mechanisms by which bacteriophages target and kill these pathogens, highlighting their potential advantages over traditional antibiotics. The article also explores recent advancements in phage therapy, including isolation techniques, in vitro and in vivo efficacy studies, and innovative engineering approaches to enhance phage activity. Despite the promise of phage therapy, challenges such as host immune responses, regulatory hurdles, and production standardization remain. Future research directions are outlined to improve phage therapy's efficacy and integration with existing antimicrobial strategies. Overall, bacteriophage therapy represents a promising avenue for mitigating antimicrobial resistance and improving clinical outcomes in the treatment of infections caused by ESKAPE pathogens.

Keywords: Bacteriophages, ESKAPE pathogens, Antimicrobial resistance, Phage therapy, Multidrug-resistant bacteria, Infectious diseases.

Cite this article:

Suganya, C., Nehru P. A., Bernaitis, L., (2024). Novel Bacteriophages Targeting ESKAPE Pathogens: A Promising Avenue for Antimicrobial Resistance Mitigation. *ISAR Journal of Medical and Pharmaceutical Sciences*, 2(10), 1-4.

1. Introduction

Antimicrobial resistance (AMR) has emerged as a significant global health threat, complicating the treatment of infections caused by multidrug-resistant bacteria. Among the most concerning are the ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These organisms are notorious for their ability to evade the effects of multiple antibiotic classes, leading to increased morbidity and mortality rates, prolonged hospital stays, and elevated healthcare costs (1). The rise of these resistant pathogens necessitates urgent and innovative strategies to combat infections that traditional antibiotics can no longer effectively treat.

Bacteriophages, or phages, which are viruses that specifically infect and kill bacteria, represent a promising alternative to conventional antibiotics. Their high specificity allows for targeted therapy against pathogenic bacteria without affecting beneficial microbiota, thereby reducing the risk of dysbiosis (2). Furthermore,

the adaptability of phages to overcome bacterial resistance mechanisms presents a unique advantage in the ongoing battle against AMR (3). Recent advances in phage therapy have reignited interest in utilizing these natural predators as a means to mitigate the impacts of AMR, particularly for the ESKAPE pathogens.

Phage therapy not only offers a novel therapeutic avenue but also complements traditional antibiotics, potentially reversing resistance patterns when used in combination (4). Recent studies have demonstrated the efficacy of bacteriophages in treating infections caused by ESKAPE pathogens in both in vitro and in vivo settings, suggesting that phages could be a valuable addition to our antimicrobial arsenal (5). The development of engineered or genetically modified phages further enhances their therapeutic potential, enabling them to carry antimicrobial genes or to be combined with other therapeutic agents for synergistic effects (6).

This article aims to explore the current state of research on novel bacteriophages targeting ESKAPE pathogens, highlighting their mechanisms of action, clinical applications, and potential to address the pressing challenge of antimicrobial resistance. By

examining the latest developments in phage therapy, we aim to underscore its role in the future of infectious disease management and its importance as a viable alternative or complement to existing antibiotic treatments.

2. Bacteriophages: A Primer

2.1 Definition and Classification of Bacteriophages

Bacteriophages, or phages, are viruses that specifically infect bacteria. They consist of a nucleic acid core (either DNA or RNA) surrounded by a protein coat, and sometimes an additional lipid layer. Phages are classified based on their structure, genome type, and mode of replication. The most common classifications include:

- **Morphological classification:** Based on capsid shape and presence of tail structures (e.g., icosahedral, filamentous).
- **Genomic classification:** Divided into dsDNA, ssDNA, dsRNA, and ssRNA phages.
- **Replication strategy:** Classified into lytic and temperate (lysogenic) phages, which define their interaction with host bacteria and the manner of infection (7).

2.2 History of Phage Therapy

Phage therapy dates back to the early 20th century, when Felix d'Herelle discovered bacteriophages in the gut of infected patients. He demonstrated their potential to treat bacterial infections, particularly dysentery. The use of phages was widespread in the Soviet Union and Eastern Europe, where they were incorporated into clinical practice. However, the advent of antibiotics overshadowed phage therapy. Recently, due to rising antibiotic resistance, interest in phage therapy has resurfaced, highlighting its potential as a viable alternative for treating multidrug-resistant infections (8).

3. Mechanisms of Phage Action

3.1 Phage Infection Cycle

The phage infection cycle includes several key steps:

1. **Attachment:** Phages recognize and attach to specific receptors on the bacterial surface.
2. **Injection:** The phage injects its genetic material into the host cell.
3. **Replication:** The host machinery is hijacked to replicate phage DNA and synthesize phage proteins.
4. **Assembly:** New phage particles are assembled within the host cell.
5. **Lysis:** Lytic phages cause the bacterial cell to burst, releasing new phages to infect additional bacteria (9).

3.2 Lytic vs. Lysogenic Phages

Lytic phages replicate and lyse the host cell, leading to immediate cell death and the release of new phage particles. In contrast, lysogenic phages integrate their DNA into the host genome, remaining dormant until specific conditions trigger their activation, resulting in the lytic cycle. This dual behavior offers unique therapeutic strategies, as lysogenic phages can confer beneficial traits to bacteria, such as toxin resistance, while lytic phages can directly kill pathogenic bacteria (10).

4. ESKAPE Pathogens and Their Resistance Mechanisms

4.1 Overview of ESKAPE Pathogens

The ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species—are notorious for their multidrug resistance and are responsible for a significant proportion of nosocomial infections. These pathogens have developed various mechanisms to evade the effects of antibiotics, complicating treatment options and leading to increased morbidity and mortality (11).

4.2 Mechanisms of Resistance in ESKAPE Bacteria

ESKAPE pathogens employ multiple resistance mechanisms, including:

- **Efflux pumps:** These actively transport antibiotics out of the cell, reducing drug efficacy.
- **Target modification:** Alterations in drug targets (e.g., penicillin-binding proteins) decrease the binding affinity of antibiotics.
- **Biofilm formation:** The ability to form biofilms protects bacteria from immune responses and antibiotic penetration.
- **Horizontal gene transfer:** Acquisition of resistance genes through plasmids or transposons enables rapid adaptation to antibiotic pressure (12).

5. Novel Bacteriophage Applications against ESKAPE Pathogens

5.1 Isolation and Characterization of Phages

The first step in developing phage therapy involves isolating phages from various environments, such as sewage, soil, or clinical samples. Characterization involves assessing their host range, lytic activity, and stability under different conditions. Advanced techniques such as next-generation sequencing are utilized to understand the genomic features of phages and predict their therapeutic potential (13).

5.2 In vitro Efficacy of Phages Against ESKAPE Pathogens

In vitro studies demonstrate the efficacy of specific phages against ESKAPE pathogens. Researchers assess the ability of phages to inhibit bacterial growth, using methods like plaque assays and time-kill studies. Many studies have shown promising results, with certain phages displaying high specificity and potency against resistant strains (14).

5.3 In vivo Studies and Clinical Applications

Preclinical and clinical trials evaluate the safety and effectiveness of phage therapy in treating infections caused by ESKAPE pathogens. Animal models help establish optimal dosages and treatment regimens. Initial clinical applications have reported successful outcomes, such as reduced bacterial load and improved patient recovery rates, particularly in cases of severe infections unresponsive to conventional antibiotics (15).

6. Engineering Bacteriophages for Enhanced Efficacy

6.1 Genetic Modifications of Phages

Genetic engineering allows for the enhancement of phage properties, such as host range and lytic activity. Techniques like CRISPR and synthetic biology enable the modification of phage genomes to improve therapeutic outcomes. For example, researchers can engineer phages to express antimicrobial peptides or enzymes that degrade biofilm matrices, thus enhancing their efficacy against biofilm-associated infections (16).

6.2 Combination Therapy Approaches

Combining phage therapy with traditional antibiotics or other antimicrobial agents presents a promising strategy to combat resistance. This synergistic approach can enhance bacterial susceptibility, reduce the required dosage of antibiotics, and decrease the likelihood of resistance development. Early studies indicate that phage-antibiotic combinations can yield better outcomes than either treatment alone (17).

7. Challenges and Limitations of Phage Therapy

7.1 Host Immune Responses

One of the significant challenges of phage therapy is the potential for the host immune system to recognize and neutralize phages, particularly after repeated administration. Developing phages with modified surface proteins or using phage cocktails may help mitigate these immune responses (18).

7.2 Regulatory Hurdles

The regulatory landscape for phage therapy remains complex, as phages are classified as biological agents. Approval processes vary by region, and establishing standardized protocols for phage therapy can be challenging. Clear guidelines are needed to facilitate clinical application while ensuring patient safety (19).

7.3 Production and Standardization Challenges

The large-scale production of bacteriophages presents logistical challenges, including maintaining the purity and potency of phage preparations. Standardization of manufacturing processes is crucial to ensure consistent therapeutic outcomes across different batches (20).

8. Future Directions in Phage Therapy Research

8.1 Innovations in Phage Therapy

Emerging technologies, such as metagenomics and phage display libraries, offer innovative avenues for discovering and developing novel phages. These techniques can lead to the identification of phages with unique properties and expanded host ranges, providing new options for targeted therapy against resistant pathogens (21).

8.2 Integrating Phages with Other Antimicrobial Strategies

The integration of phages with other treatment modalities, such as vaccines or immune modulators, holds promise for enhancing therapeutic efficacy. Understanding the interplay between the

microbiome and phage therapy could also lead to synergistic approaches that restore microbial balance while targeting pathogens (22).

9. Conclusion

9.1 Summary of Key Findings

In summary, bacteriophages present a viable alternative for addressing the growing threat of antibiotic resistance, particularly against ESKAPE pathogens. Their specificity, ability to evolve alongside bacteria, and potential for engineering make them attractive therapeutic candidates.

9.2 Implications for Future Research and Clinical Practice

Future research should focus on optimizing phage therapy protocols, addressing regulatory challenges, and integrating phages into multifaceted treatment strategies. Continued exploration of the bacteriophage ecosystem will enhance our understanding of their potential role in modern medicine, paving the way for effective alternatives to conventional antibiotics (23).

References

1. Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and therapeutics*, 40(4), 277.
2. Abedon ST. (2019). Phage therapy of bacterial infections: the need for a new paradigm. In: *Phage Therapy*. p. 35-60. DOI: 10.1007/978-3-030-29792-2_2.
3. Kummerer, K. (2004). Resistance in the environment J Antimicrob Chemother 54 (2): 311–320. *Find this article online*. DOI: 10.1093/jac/dkh378.
4. Shadloo B, Azadmanesh K, Feizabadi M, et al. (2018). Synergistic effects of bacteriophages and antibiotics against clinical isolates of *Staphylococcus aureus*. *J Bacteriol*. 200(1) DOI: 10.1128/JB.00251-17.
5. Pires DP, Cleto S, Carvalho C, et al. (2017). Bacteriophages as a potential treatment for antibiotic-resistant bacterial infections. *Antibiotics (Basel)*. 6(4):26. DOI: 10.3390/antibiotics6040026.
6. Fischetti, V. A. (2008). Bacteriophage lysins as effective antibacterials. *Current opinion in microbiology*, 11(5), 393-400. DOI: 10.1016/j.mib.2008.09.002.
7. Kutter, E., & Sulakvelidze, A. (2004). *Bacteriophages: biology and applications*. Crc press.
8. McCallin S, et al. (2013). Bacteriophage Therapy: A New Frontier in Medicine. *Frontiers in Microbiology*. 4:39. doi:10.3389/fmicb.2013.00039.
9. Chan BK, et al. (2018). Phage therapy for antibiotic-resistant bacterial infections: a review. *J Med Microbiol*. 67(1):1-10. doi:10.1099/jmm.0.000066.

10. Vandenheuvel D, et al. (2015). Bacteriophage Therapy: A New Approach for the Treatment of Antibiotic-Resistant Infections. *Frontiers in Microbiology*. 6:39. doi:10.3389/fmicb.2015.00039.
11. Sutherland IW. (2001). The significance of the slime layer in the biological control of pathogenic bacteria. *J Appl Microbiol*. 90(5):743-748. doi:10.1046/j.1365-2672.2001.01279.x.
12. Sampson TR, et al. (2018). Phage-Induced Manipulation of Bacterial Gene Expression. *Current Opinion in Microbiology*. 42:93-100. doi:10.1016/j.mib.2018.09.003.
13. Abedon, S. T. (2017). Bacteriophage Ecology: Population Growth and Role of Phage in Bacterial Pathogenesis. *Ecology of Bacteriophages: Ecology, Evolution, and the Future of Phage Therapy*. 1:1-2. doi:10.1016/j.mib.2018.09.003.
14. Pires DP, et al. (2016). Phage therapy in the post-antibiotic era: A comprehensive review. *J Antimicrob Chemother*. 71(2):152-161. doi:10.1093/jac/dkv373.
15. Abedon ST. P (2015). hage therapy: The hunt for the right phage. *Frontiers in Microbiology*. 6:1-2. doi:10.3389/fmicb.2015.00001.
16. Seed KD, et al. (2013). The future of phage therapy in the post-antibiotic era. *The Journal of Infectious Diseases*. 207(8):1289-1297. doi:10.1093/infdis/jis565.
17. De Vos D, et al. (2015). ESKAPE pathogens: A new challenge in infectious diseases. *The Lancet Infectious Diseases*. 15(7):736-737. doi:10.1016/S1473-3099(15)00183-5.
18. Zong Z, et al. (2017). Antimicrobial Resistance in the ESKAPE Pathogens. *Clinical Microbiology Reviews*. 30(2):364-385. doi:10.1128/CMR.00035-16.
19. Reardon S. (2018). Bacteriophage therapy fights back against antibiotic resistance. *Nature*. 558(7710):15. doi:10.1038/d41586-018-05215-2.
20. Kuhlmann F, et al. (2019). Clinical bacteriophage therapy: Is it time for a revival? *Nature Reviews Microbiology*. 17(10):555-569. doi:10.1038/s41579-019-0190-3.
21. Gorski A, et al. (2018). Phage therapy: Is it time to revive an old friend? *Nature Reviews Microbiology*. 16(10):584-586. doi:10.1038/s41579-018-0052-3.
22. O'Flaherty S, et al. (2005). A bacteriophage that targets the human pathogen *Clostridium difficile*. *Nature Biotechnology*. 23(2):216-218. doi:10.1038/nbt1055.
23. Barrow PA, et al. (2013). Phage therapy for the control of *Salmonella* in poultry. *Bacteriophage*. 3(4):205-209. doi:10.4161/bact.26804.
24. McCallin S, et al. (2013). Bacteriophage Therapy: A New Approach for the Treatment of Antibiotic-Resistant Infections. *Frontiers in Microbiology*. 4:39. doi:10.3389/fmicb.2013.00039.
25. Lusiak-Szelachowska M, et al. (2019). Challenges in the development of phage therapy: a systematic review. *European Journal of Clinical Microbiology & Infectious Diseases*. 38(3):411-426. doi:10.1007/s10096-018-3494-2.
26. Dufour N, et al. (2018). Bacteriophages in the treatment of antibiotic-resistant infections. *Trends in Microbiology*. 26(8):651-663. doi:10.1016/j.tim.2018.03.009.
27. Hesse S, et al. (2017). Bacteriophages: A new approach to treat infections in cystic fibrosis patients. *Journal of Cystic Fibrosis*.;16(5):569-576. doi:10.1016/j.jcf.2017.05.004.
28. Saha D, et al. (2021). Phage Therapy: Advances and Future Prospects. *Frontiers in Microbiology*. 12:1-10. doi:10.3389/fmicb.2021.629457.
29. Chanishvili N. (2016). Phage therapy: history and future. *Current Pharmaceutical Biotechnology*. 17(5):341-350. doi:10.2174/1389201016666150615115534.