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Isolation, Characterization, And Therapeutic Potential Of Bacteriophages Against Multidrug-Resistant Bacteria

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Abstract **-** The global rise in multidrug-resistant (MDR) bacterial infections is driven by excessive and prolonged antibiotic use in human and veterinary medicine. This study aimed to isolate and characterize bacteriophages targeting ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacteriaceae*) —a group of bacteria responsible for the majority of hospital-acquired infections. Bacteriophages were isolated from clinical samples and wastewater. Morphological classification by electron microscopy revealed phages from the families Myoviridae, Podoviridae, and Siphoviridae. Genomic analysis confirmed the absence of toxin, integrase, or antibiotic resistance genes, supporting their therapeutic safety. The phages showed narrow host specificity, high lytic activity (up to 100% for some strains), low frequency of resistant mutants (10^{-7}–10^{-8}), and rapid adsorption and replication rates. Phage stability was maintained for 12 months under 4–25 °C. These findings support the potential of phage therapy as a targeted and safe treatment for MDR infections in clinical settings.

Keywords**:** bacteriophages, multidrug resistance, ESKAPE pathogens, phage therapy, electron microscopy, antimicrobial resistance

1. Introduction

The emergence and spread of multidrug-resistant bacteria have been fueled by the intensive use of antibiotics in medicine and agriculture [1], [2]. This resistance crisis calls for alternative strategies. One promising solution is the use of bacteriophages—viruses that specifically infect bacteria—for therapeutic purposes [3], [4].

A growing body of evidence supports the efficacy and safety of phage therapy against ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and members of *Enterobacteriaceae*), which are often resistant to antibiotics and associated with nosocomial infections [5], [6].

Given the urgent need for alternatives, this study evaluates the therapeutic potential of bacteriophages isolated from patients treated in hospitals across Kazakhstan.

2. Materials and Methods

Bacteriophages were isolated from clinical specimens collected from patients hospitalized in Astana, including throat and nasal swabs, sputum, urine, blood, pressure ulcer exudates, and wound secretions. Wastewater samples were also used.

The main biological properties of the isolated phages were characterized using standard methods, including plaque assays, adsorption kinetics, and one-step growth curves. Electron microscopy was used to determine morphological classification.

Molecular analyses (PCR and sequencing) were conducted to identify virulence, integrase, and resistance genes. Only phages lacking these markers were further evaluated.

3. Results

Bacteriophages were successfully isolated from clinical and environmental samples. Initial screening and purification yielded highly lytic phage strains. Electron microscopy classified the phages into the Myoviridae, Podoviridae, and Siphoviridae families, indicating structural and mechanistic diversity.

Genetic analyses revealed the absence of toxin genes, integrases, or antibiotic resistance markers, supporting their biosafety. Genes associated with replication and host lysis were present, confirming functional viability.

The phages exhibited narrow host specificity, lysing only selected strains. In some cases, up to 80–100% of isolates from a single species were susceptible. This specificity reduces the risk of disturbing the normal microbiota.

The frequency of phage-resistant mutants remained below 10^{-7}–10^{-8}, indicating high therapeutic durability.

Kinetic studies showed latent periods of 20–30 minutes and high adsorption rates. Burst sizes ranged from 50 to 200 virions per infected cell, reflecting high replication efficiency.

Phage formulations remained stable at 4–25 °C for 12 months. Freezing and overheating reduced titers, but activity was preserved under recommended storage conditions. Appelman and Grazia methods confirmed that lytic activity was retained throughout the shelf life, indicating suitability for clinical application and mass production.

4. Conclusion

The bacteriophages isolated in this study demonstrated high specificity, strong lytic activity, stability, and biosafety. These properties make them promising candidates for targeted therapy of infections caused by multidrug-resistant ESKAPE pathogens. This work contributes to the development of phage-based therapeutics for use in Kazakhstan and beyond.

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