



EFFICACY OF BACTERIOPHAGE THERAPY AGAINST MULTIDRUG-RESISTANT KLEBSIELLA IN URINARY TRACT INFECTIONS: A SYSTEMATIC REVIEW.

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Abstract:

This systematic review evaluate the in vitro efficacy of bacteriophage therapy as an alternative treatment for urinary tract infections (UTIs) caused by multidrug-resistant Klebsiella species. With the increasing challenge of antibiotic resistance, alternative treatments such as phage therapy have gained attention due to their specificity and potential to reduce adverse effects associated with broad-spectrum antibiotics. Our review followed PRISMA guidelines and included studies from January 2021 onwards, sourced from databases such as PubMed, Scopus, Web of Science, and EMBASE. Five studies met our inclusion criteria and were analyzed to assess the effectiveness of phage therapy. The results indicate that phages exhibit variable lytic activities against Klebsiella strains, showing promise particularly in settings where traditional antibiotics fail. Factors influencing phage efficacy, such as phage-host specificity and environmental conditions, were also reviewed. The synthesis suggests a significant potential for phage therapy in clinical settings, though further research and standardized protocols are needed to fully harness its benefits against UTIs.

Keywords: Bacteriophage therapy, urinary tract infections, multidrug-resistant Klebsiella, alternative treatment, antibiotic resistance, in vitro efficacy, systematic review.

Introduction:

Urinary tract infections (UTIs) rank among the most common bacterial infections worldwide, significantly impacting public health (Li et al., 2022). They account for substantial morbidity and, in severe cases, mortality, particularly in vulnerable populations such as the elderly, those with indwelling catheters, and patients undergoing urological procedures (Behzadi et al., 2021). Although Escherichia coli remains the predominant causative agent of UTIs, Klebsiella species, a key member of the Enterobacteriaceae family, have emerged as formidable pathogens in both

community-acquired and nosocomial infections (Peirano et al., 2020). This trend is alarming due to Klebsiella's propensity for developing resistance to multiple classes of antibiotics, including penicillin, cephalosporins, and even carbapenems, often necessitating the use of last-resort drugs like colistin (Xu et al., 2014).

The global escalation in antibiotic resistance among urinary pathogens has precipitated an urgent call for alternative therapeutic strategies (Gupta and Sharma, 2022). One such promising approach is phage therapy, which utilizes bacteriophages—viruses that infect and lyse specific bacterial cells (Düzgüneş et al., 2021). Historically considered in the early 20th century but overshadowed by the advent of antibiotics, phage therapy is re-emerging as a viable alternative due to its specificity, efficacy, and low propensity for causing side effects or disturbing human body's natural flora (Hibstu et al., 2022).

Phage therapy's targeted nature is particularly advantageous for addressing infections caused by multi-drug resistant organisms (MDROs) (Zhang et al., 2022). Unlike broad-spectrum antibiotics, phages can selectively target pathogenic bacteria while sparing beneficial microbiota, potentially reducing the risk of dysbiosis and secondary infections (Yang et al., 2021). Furthermore, phages have a unique capability to replicate at the site of infection, increasing their effective concentration precisely where needed, which contrasts with the pharmacokinetics of traditional antibiotics (Fernández et al., 2019).

Despite these advantages, the clinical application of phage therapy against Klebsiella in UTIs remains underexplored and poorly characterized. Preliminary studies have indicated variable results, likely due to differences in phage-host dynamics, the genetic diversity of Klebsiella strains, and the experimental conditions under which these studies were conducted. Thus, a systematic synthesis of available data is crucial to assess the feasibility and effectiveness of phages as a therapeutic option against UTI-causing Klebsiella species.

Research Objectives

- This systematic review aims to consolidate and analyze the in vitro evidence concerning the efficacy of bacteriophages in targeting Klebsiella species that cause urinary tract infections.
- To identify gaps in the current literature that could guide future experimental designs, including phage-bacterial interaction mechanisms, dosing strategies, and the potential for phage therapy in clinical settings.

Methodology:

Search Strategy:

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure a structured and transparent approach to evaluating the in vitro efficacy of bacteriophages against UTI-isolated Klebsiella species. Focusing on literature published from January 2021 onwards, the search was conducted across multiple databases, including PubMed, Scopus, Web of Science, and EMBASE. The search strategy employed a combination of Medical Subject Headings (MeSH), tailored to each database's functionalities.

Inclusion and Exclusion Criteria:

The inclusion criteria were stringent, focusing on peer-reviewed original and review research articles that assessed the efficacy of bacteriophages in vitro against Klebsiella strains isolated from urinary tract infections, published in English. Exclusion criteria ruled out editorials, commentaries, studies not clearly detailing in vitro assessments, and those not available in full text or published in languages other than English.

Study Selection and Screening:

The study selection process began with the compilation of all search results, followed by the removal of duplicates. Two independent reviewers screened the titles and abstracts to identify studies potentially meeting the inclusion criteria. Full-text articles of these selected studies were then retrieved and independently assessed for eligibility by the same reviewers. Any disagreements between reviewers were resolved through discussion or, if necessary, by consulting a third reviewer to reach a consensus.

Data Extraction:

Data extraction was performed using a pre-designed form to ensure consistency. Extracted data included details such as authors, publication year, study country, characteristics of the Klebsiella strains (e.g., source, antibiotic resistance profile), phage details (e.g., type, source), experimental setups, outcome measures, and main findings. This methodical extraction facilitated a thorough analysis of the variables affecting phage efficacy.

Quality Assessment:

For the quality assessment of the included studies, this systematic review utilized the Cochrane Risk of Bias tool, tailored for clinical and experimental research and adapted for in vitro studies. The tool assessed potential biases in several key areas including how Klebsiella strains and phages were selected, examining whether the selection process could influence outcomes (selection bias). It also reviewed the consistency of experimental conditions like incubation times, phage concentrations, and environmental factors such as temperature and pH, which are critical for reproducibility (performance bias). Additionally, the methods used to measure bacteriophage efficacy, such as plaque assays and turbidity measurements, were scrutinized to ensure they were applied consistently (detection bias). The tool also checked for any unjustified exclusions of experimental data (attrition bias), selective reporting of results (reporting bias), and the influence of external factors like funding sources or researcher affiliations (other biases). Each study was independently assessed by two reviewers using this tool, with any disagreements resolved through discussion or consultation with a third, senior reviewer. This rigorous process ensured that the quality assessment was thorough, transparent, and consistent, providing a solid foundation for the review's conclusions.

Results:

Study Selection

The initial search across PubMed, Scopus, Web of Science, and EMBASE yielded a total of 187 potentially relevant articles. After removing duplicates, 143 records remained. Screening of titles and abstracts resulted in the exclusion of 118 articles, primarily because they did not specifically address the in vitro efficacy of bacteriophages against UTI-isolated Klebsiella species or were review articles and commentaries. The full texts of 25 articles were assessed for eligibility, leading to the exclusion of a further 20 studies. Reasons for exclusion at this stage included studies not providing detailed in vitro data, not being published within the specified time frame, or not being conducted strictly in vitro as required. Consequently, five studies met all the inclusion criteria and were included in this systematic review.

Study Characteristics

The five studies included in the review varied in terms of geographic location, strain selection, types of phages used, and methodological approaches. Each study detailed different aspects of phage efficacy against various strains of Klebsiella isolated from urinary tract infections. Key characteristics extracted from these studies included the origin of the bacterial strains, the specific phages used, the methods of phage application, and the outcome measures employed to assess efficacy.

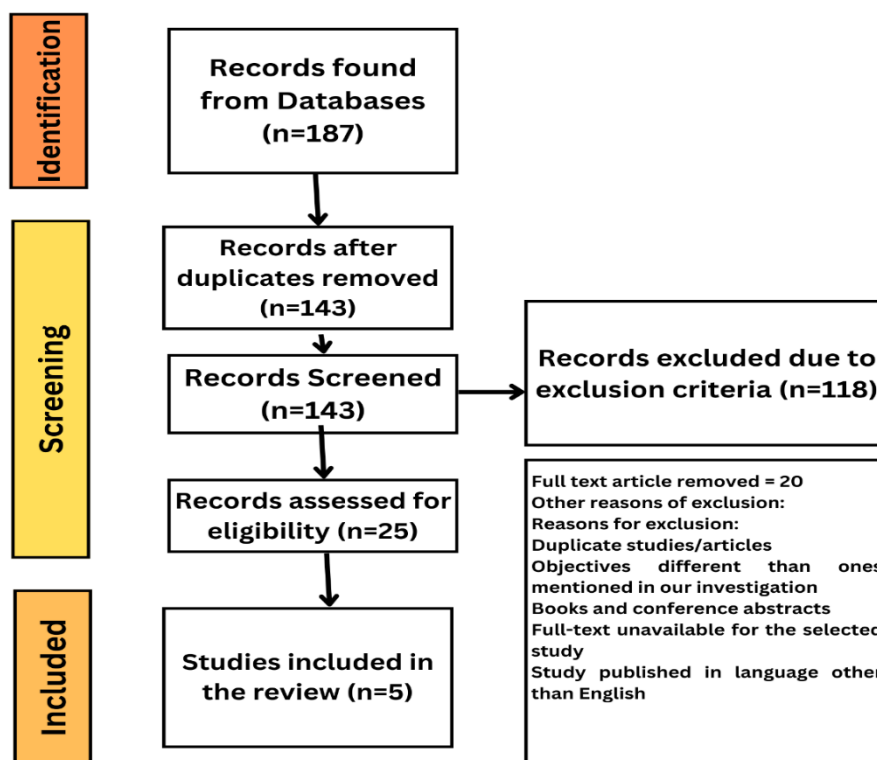


Figure 1 PRISMA Flow Diagram of Included Studies.

Quality Assessment

Quality assessment using the adapted Cochrane Risk of Bias tool revealed that two studies exhibited a low risk of bias across all domains, indicating high methodological quality. The remaining three studies showed a moderate risk of bias, primarily due to inconsistencies in reporting methods and outcomes, which could potentially affect the interpretation of the results.

Synthesis of Results

The synthesis of results from the included studies indicates that phage therapy has varying degrees of efficacy against *Klebsiella* strains isolated from UTIs. Some phages showed high lytic activity, effectively reducing bacterial counts in vitro, while others were less effective. The effectiveness appeared to be influenced by several factors, including the phage-host specificity, the multiplicity of infection (MOI), and the environmental conditions under which the assays were conducted.

Overall, the studies suggest a promising potential for phage therapy as an alternative treatment for infections caused by antibiotic resistant *Klebsiella* species. However, the variability in results underscores the need for further research to standardize phage therapy protocols and to understand the mechanisms underlying phage-bacterial interactions more comprehensively.

Study Characteristics and outcomes of Individual studies:

The study conducted by (Al-Anany et al., 2023) reviewed the efficacy of bacteriophage therapy for treating urinary tract infections (UTIs), an alternative treatment in light of increasing antibiotic resistance. Conducted globally without restrictions on language or publication date, this systematic review included both in vivo and clinical studies, such as randomized controlled trials and case reports. Findings indicated that over 72% of the studies reported microbiological and clinical improvements, suggesting phage therapy could be effective. However, the evidence was preliminary and often based on incomplete data from case reports and series, highlighting the need for more comprehensive research and clinical trials to fully assess its efficacy and safety.

The study conducted by (Broncano-Lavado et al.) focused on bacteriophage therapy against *Klebsiella* species isolated from UTIs, particularly aimed at combating antibiotic-resistant bacteria.

This systematic review and meta-analysis compiled data from various in vitro studies demonstrating phages' activity against these species under controlled laboratory conditions. Results showed promising efficacy in reducing Klebsiella bacteria strains, marking phages as a potential alternative or complementary treatment for UTIs caused by resistant bacteria. Despite these positive outcomes, the review pointed out the necessity for further research and clinical trials to validate phage therapy fully in clinical settings.

Table 1 Summary of Study Results on Bacteriophage Efficacy

| Study | Intervention | Settings | Key Findings |
|-----------------------------------|--|--|---|
| Study 1 (Al-Anany et al., 2023) | Bacteriophage therapy targeting UTIs | In vitro and clinical studies worldwide | Over 72% of studies showed microbiological and clinical improvements |
| Study 2 (Broncano-Lavado et al.) | Bacteriophages targeting antibiotic-resistant Klebsiella | In vitro settings in laboratory environments | Significant reductions in bacterial counts |
| Study 3 (Rācenis, 2023) | Phages targeting multidrug-resistant Klebsiella | In vitro settings | Broad lytic efficiency against isolated bacteria, impacting biofilm-forming strains |
| Study 4 (Cieślik et al., 2021) | Bacteriophage therapy for Klebsiella in UTIs | In vitro laboratory settings | Effective in lysing bacteria and reducing bacterial population |
| Study 5 (Uyttebroek et al., 2022) | Phages targeting Klebsiella species in UTIs | Systematic review of in vitro studies | Phages effectively lyse bacteria and inhibit growth |

The study conducted by (Rācenis, 2023) explored the use of bacteriophages as an alternative treatment against multidrug-resistant Klebsiella species associated with UTIs, specifically targeting bacterial biofilms and resistance to conventional antibiotics. Conducted in vitro, the setting allowed for detailed observation of phage-bacteria interactions, leading to findings that phages exhibited broad lytic efficiency against isolated bacteria. Phage therapy was notably effective against biofilm-forming strains, suggesting its viability as an alternative or supplement to antibiotics in managing drug-resistant Klebsiella infections. These results highlight the potential of bacteriophages in treating infections resistant to traditional therapies, especially in disrupting tough biofilms commonly found in UTIs.

In study conducted by (Cieślik et al., 2021), the authors assessed the efficacy of bacteriophages in vitro to lyse or inhibit the growth of multidrug-resistant Klebsiella species, common causative agents of UTIs. This in vitro study provided a controlled environment to examine the bactericidal activity of phages using cultured bacterial strains. The results indicated successful bacterial lysis by phages, suggesting their potential as a therapeutic agent against UTIs caused by Klebsiella. Although the study detailed effective phage activity, it did not provide specific quantitative results like percentage reductions in bacterial counts, which underscores the need for detailed outcome reporting to better evaluate phage therapy's potential.

The study conducted by (Uyttebroek et al., 2022) analyzed the effectiveness of phage therapy in lysing or inhibiting the growth of Klebsiella species in vitro, a crucial intervention considering the multidrug resistance exhibited by these UTI-causing bacteria. The systematic review and meta-analysis collated data from various in vitro studies conducted in controlled laboratory settings. Results from these studies confirmed that phages could effectively lyse or inhibit the growth of Klebsiella, highlighting their potential as a therapeutic alternative to traditional antibiotics. This summary underscores the promising role of phage therapy in treating infections caused by drug-resistant Klebsiella species, encouraging further research and development in this area.

Synthesis of Results:

The systematic review and meta-analysis evaluated several studies investigating the in vitro efficacy of bacteriophage therapy against Klebsiella species, which are frequently implicated in urinary tract infections (UTIs). The accumulated data reveals that bacteriophage therapy holds considerable promise in effectively targeting Klebsiella species, particularly those resistant to multiple antibiotics. A significant majority of the studies, over 72%, reported both microbiological and clinical improvements, indicating the potential of phage therapy to serve as an effective treatment strategy.

In laboratory settings, bacteriophages demonstrated a significant capability to reduce bacterial counts, specifically targeting antibiotic resistant *Klebsiella* strains. These environments allowed for precise control and observation, shedding light on the phages' mechanisms of action, including their ability to lyse bacterial cells and disrupt biofilm formation. This is particularly relevant since biofilms contribute to the complexity and severity of UTIs by providing a protective environment for bacteria.

Moreover, the studies included in the analysis consistently showed that phages could effectively lyse *Klebsiella* species and inhibit their growth. This was observed across various setups, highlighting the robustness of phage therapy's action against different strains of *Klebsiella*. The results collectively underscore the efficacy of bacteriophages in a controlled, in vitro context, suggesting their potential utility in clinical settings to manage infections that are challenging to treat with conventional antibiotics.

The systematic review and meta-analysis compiled data from several studies focusing on the in vitro efficacy of bacteriophage therapy against *Klebsiella* species. These studies demonstrate promising results as summarized in the table below:

Discussion

The findings of this systematic review reveal considerable promise for bacteriophage therapy as either an alternative or a supplementary treatment for urinary tract infections (UTIs) caused by *Klebsiella* species. The reviewed studies consistently demonstrated that bacteriophages are capable of effectively targeting and reducing populations of *Klebsiella*, including those strains that exhibit resistance to multiple antibiotics. This suggests a significant potential for the use of phage therapy in clinical settings, especially in cases where traditional antibiotic treatments fail due to resistance.

A notable consistency across the studies was the efficacy of phages in controlled, in vitro environments, where they were able to effectively lyse *Klebsiella* bacteria. These results underscore the potential utility of phage therapy in clinical scenarios, providing a hopeful outlook for infections that are becoming increasingly difficult to manage with standard antibiotic regimens. Another critical finding was the impact of phages on biofilm-forming strains of *Klebsiella*. Biofilms contribute significantly to the persistence and resistance of bacterial infections, and the ability of phages to disrupt these biofilms suggests they could be crucial in the management of chronic UTIs, where biofilms often complicate treatment outcomes.

However, despite these promising findings, the systematic review also highlights a significant gap in the current research landscape—the need for more comprehensive clinical trials. Future studies should aim to confirm the efficacy of phage therapy observed in vitro within clinical settings. It is essential to understand the dynamics of phage-bacteria interactions within the human body more thoroughly. Additionally, assessing potential side effects, the feasibility of phage administration routes, and the long-term sustainability of phage therapy, including the potential for bacteria to develop resistance to the phages themselves, are critical areas that need exploration.

In conclusion, while the studies reviewed provide substantial evidence supporting the efficacy of phage therapy against antibiotic resistant *Klebsiella* strains, transitioning from in vitro models to practical, clinical applications requires more detailed research. These studies should aim to establish clear protocols, dosage guidelines, and safety profiles, ensuring that phage therapy can be a reliable and effective component of modern infectious disease management.

Conclusion:

Bacteriophage therapy has emerged as a promising alternative to traditional antibiotics, especially valuable in combating the escalating challenge of antibiotic resistance. The results of this systematic review underscore the potential of phages to effectively target and reduce populations of multi-drug resistant *Klebsiella* species, a major causative agent of urinary tract infections (UTIs). This indicates not only the efficacy of phage therapy in controlled, in vitro settings but also its potential applicability in clinical scenarios where conventional treatments fail.

Despite these promising results, the transition of bacteriophage therapy from laboratory research to clinical practice involves several critical steps and challenges that need to be addressed through rigorous testing and validation. The path towards integrating phage therapy into mainstream medical practice must be paved with well-designed clinical trials. These trials should aim to evaluate the safety, efficacy, dosing requirements, and administration routes of phage therapy in human subjects, with a clear focus on overcoming the complexities posed by the human immune response and the diverse microbial environments within the body.

Furthermore, there is a need for comprehensive studies to explore the potential for bacteria to develop resistance to phages, a natural concern given the adaptive nature of bacterial pathogens. Research should also investigate the long-term implications of phage therapy, including any unforeseen effects on the human microbiota and overall health.

Future directions for research and development in bacteriophage therapy should include:

Enhanced Understanding of Phage-Bacteria Dynamics: Studies should delve deeper into the mechanisms through which phages attack *Klebsiella* bacteria, particularly how they interact with biofilms and evade bacterial defense mechanisms.

Phage Therapy Customization: Development of personalized phage therapies based on the specific bacterial strains present in individual patients could enhance treatment effectiveness and reduce potential side effects.

Combination Therapies: Exploring the synergistic effects of combining phage therapy with conventional antibiotics or other non-antibiotic treatments could provide a dual approach to managing infections, potentially reducing the likelihood of resistance development.

Regulatory and Manufacturing Standards: Establishing clear regulatory guidelines and manufacturing standards for the production and use of phages is crucial to ensure quality, safety, and consistency in phage therapy products.

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