ETHIOPIAN INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH

eISSN: 2349-5715 pISSN: 2349-5707

Volume: 12, Issue 02, February-2025 SJIF 2019: 4.702 2020: 4.737 2021: 5.071 2022: 4.919 2023: 6.980 2024: 7,662

Solomonnik Oksana Nikolaevna
Department of infectious diseases
Andijan State Medical Institute,Uzbekistan, Andijan

FEATURES OF BACTERIOPHAGE APPLICATION IN THE THERAPY OF INFECTIONS CAUSED BY OPPORTUNISTIC STRAINS OF KLEBSIELLA PNEUMONIAE

Relevance: In the context of the rapid rise of antimicrobial resistance among microbial pathogens, the need for alternative methods of treating bacterial infections has become increasingly evident. Klebsiella pneumoniae is one of the opportunistic pathogens that, under certain conditions (immunosuppression, chronic diseases, etc.), can cause severe forms of pneumonia, urinary tract infections, sepsis, and other pathological conditions. Conventional antibiotic therapy against resistant K. pneumoniae strains often proves insufficiently effective or is associated with significant side effects. In this situation, the use of bacteriophages appears to be a promising approach, as bacteriophages have the ability to selectively target and destroy bacterial cells without disrupting the body's normal microflora.
Keywords: Klebsiella pneumoniae, Bacteriophage, Antibiotic resistance, Phage therapy, Opportunistic microorganisms, Respiratory tract infections, Immunity

Introduction

Klebsiella pneumoniae is a Gram-negative rod from the family Enterobacteriaceae, widely distributed in the environment and comprising part of the normal human microflora [1]. However, in states of immunodeficiency or in the presence of comorbidities (diabetes, chronic lung diseases, oncological conditions, etc.), K. pneumoniae can cause severe nosocomial (hospital-acquired) and community-acquired infections [2].

Extensive antibiotic use has accelerated the development of resistance in K. pneumoniae. Nowadays, strains that are resistant to most available antibiotics, including last-resort drugs (carbapenems, extended-spectrum cephalosporins, etc.), are increasingly found [3]. Under such conditions, phage therapy has become one of the most promising alternative strategies.

Bacteriophages are viruses that selectively infect bacterial cells, leading to their lysis. Their mechanism of action is based on the specific recognition of receptors on the bacterial cell surface, allowing the phage to infect only certain bacteria without affecting other cells or the healthy microflora [4]. However, the success of phage therapy largely depends on the correct choice of phages, their sensitivity to specific K. pneumoniae strains, as well as the route of administration of the phage preparations.

The aim of this study was to investigate the features of bacteriophage application in the treatment of infections caused by opportunistic K. pneumoniae strains, to analyze the effectiveness of phages compared to conventional treatments, and to determine potential limitations of phage therapy.

Materials and Methods

Selection of K. pneumoniae strains.

We used 20 K. pneumoniae strains isolated from patients with various clinical manifestations of infection (pneumonia, urinary tract infections, wound infections). The strains were obtained from clinical specimens (sputum, urine, wound exudate) and cultured on standard media (MacConkey agar, blood agar).

Determination of antibiotic susceptibility.

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Each strain was tested for antibiotic susceptibility by the disk diffusion method on Mueller–Hinton agar using disks containing antibiotics from different classes (beta-lactams, fluoroquinolones, aminoglycosides, etc.). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Selection of bacteriophages and determination of their specificity.

Commercial and experimental bacteriophages active against K. pneumoniae were used, kindly provided by a microbiology laboratory (e.g., "Klebsiella polyvalent bacteriophage" and experimental phages with a narrow lytic spectrum). We used the spot test method to determine phage specificity and efficiency: a drop of phage lysate was applied to a lawn culture of K. pneumoniae (0.5 McFarland turbidity) on a Petri dish, followed by incubation at 37 °C for 24 hours. We then evaluated the presence of lysis zones (clear spots).

Experimental in vitro phage therapy.

Selected effective phages (polyvalent and specific) were used in in vitro experiments: in a test tube containing a growth medium with K. pneumoniae (concentration ~10^6 CFU/mL), phages were introduced in various titers (from 10^4 to 10^8 PFU/mL). Over 24 hours of incubation at 37 °C, the bacterial growth dynamics were regularly evaluated by measuring the optical density (620 nm) and/or by colony counts on culture media [5].

Statistical analysis.

The experimental results were expressed as mean values with standard deviations (M ± SD). Student's t-test was used to compare the groups; differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Antibiotic resistance profile of clinical K. pneumoniae strains.

Among the 20 strains studied, more than 70% showed resistance to multiple classes of antibiotics (multidrug-resistant strains). The most frequently observed resistance was to penicillins and second- and third-generation cephalosporins [6]. About 40% of the strains were also highly resistant to fluoroquinolones and aminoglycosides. This indicates considerable challenges in selecting conventional antibiotic therapy and underscores the need for alternative therapeutic approaches.

Assessment of bacteriophage efficacy by the spot test.

Out of all the phage preparations tested, the polyvalent Klebsiella bacteriophage demonstrated high activity against the majority (85%) of K. pneumoniae strains. In contrast, narrow-spectrum (monophage) preparations displayed strong activity only against certain strains, but the observed lysis was more pronounced in those cases [7]. This suggests that when selecting phage therapy, it is advisable to use combinations of both polyvalent and narrow-spectrum phages to achieve broader coverage of strains.

In vitro phage therapy results.

In test-tube experiments, phage treatment at a concentration of 10^6–10^8 PFU/mL led to a significant (2–3 log) reduction in K. pneumoniae counts within just 6–8 hours of incubation, and by 24 hours, the level of viable bacterial cells often dropped below the detection limit. Control samples (without phage) demonstrated the standard bacterial growth curve.

When the polyvalent phage was combined with a monophage, more pronounced bacterial lysis was observed than when either phage was used alone, possibly indicating a synergistic effect [8].

Limitations of phage therapy.

Despite the high specificity and effectiveness of phages against K. pneumoniae, there are several limitations: Phage resistance: bacteria can rapidly develop resistance to phages, requiring ongoing surveillance and the selection of new phage cocktails. Individual variability: phage activity can vary depending on the strain [9]. Up-to-date phage panels are needed for quick and effective

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results. Technological challenges: producing high-quality phage preparations demands strict adherence to biotechnological standards and stringent safety controls [10]. Bacterial defense mechanisms: some K. pneumoniae strains have a capsule with specific polysaccharides that can hinder phage penetration into the bacterial cell.

Despite these challenges, expanded research and clinical application of phages show great therapeutic potential for combating multidrug-resistant strains [11].

Conclusion and Recommendations.

High efficacy of phage preparations against most of the K. pneumoniae strains studied highlights the feasibility of using bacteriophages as an alternative or an adjunct to antibiotics.

Combining polyvalent and monophage preparations provides broader coverage of pathogenic strains and reduces the risk of phage resistance development.

Routine monitoring of phage sensitivity is essential to maintain treatment efficacy, as bacteria may evolve and acquire resistance to the phages in use.

Optimization of administration methods (local application for wound infections, inhalation for pneumonia, oral administration for intestinal infections) allows for the best therapeutic outcomes.

Wider clinical use of phage therapy requires further large-scale studies (including multicenter randomized trials) on the safety and efficacy of phage therapy, as well as the development of standards for the production and quality control of phage preparations.

References:

1. Kutter E., Sulakvelidze A. (Eds.). Bacteriophages: Biology and Applications. Boca Raton, FL: CRC Press, 2005.
2. Górski A., Miedzybrodzki R., Borysowski J. et al. Phage therapy: current status and perspectives. Med Res Rev. 2009;29(5): 1030–1064.
3. Nikolaevna, S.O., 2024. ETIOLOGY, PATHOGENESIS AND TREATMENT OF MRSA INFECTION. Ethiopian International Journal of Multidisciplinary Research, 11(10), pp.119-123.
4. Merril C.R., Scholl D., Adhya S.L. The prospect for bacteriophage therapy in Western medicine. Nat Rev Drug Discov. 2003;2(6): 489–497.
5. WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: World Health Organization; 2017.
6. Nikolaevna, S.O., 2024. CLINIC OF THE INITIAL PERIOD OF SALMONELLASIS CAUSED BY SALMONELLA TYPHIMURIUM IN CHILDREN. International Multidisciplinary Journal for Research & Development, 11(01).
7. Абдукодиров, Ш.Т., 2024, November. СОВРЕМЕННЫЕ МЕТОДЫ ЛЕЧЕНИЯ РОЖИ У ЛИЦ ПОЖИЛОГО ВОЗРАСТА. In Russian-Uzbekistan Conference (Vol. 1, No. 1).
8. Nikolaevna, Solomonnik Oksana. "ETIOLOGY, PATHOGENESIS AND TREATMENT OF MRSA INFECTION." Ethiopian International Journal of Multidisciplinary Research 11, no. 10 (2024): 119-123.
9. Nabijonovich, Kaxarov Abdukaxar. "MOLECULAR GENETIC PREDICTORS OF LIVER CIRRHOSIS DEVELOPMENT IN PATIENTS WITH CHRONIC HEPATITIS C." Ethiopian International Journal of Multidisciplinary Research 12, no. 02 (2025): 177-180.
10. Nabijonovich, Kaxarov Abdukaxar. "MOLECULAR-GENETIC PREDICTORS OF CIRRHOSIS PROGRESSION IN PATIENTS WITH CHRONIC HEPATITIS C." Ethiopian International Journal of Multidisciplinary Research 12, no. 02 (2025): 181-186.