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Analysis Of The Antimicrobial Activity Of Bacteriophages And Antibiotics Against Staphylococci And Streptococci

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ABSTRACT
Aims: *This study evaluated the in vitro antimicrobial activity of various antibiotics and a staphylococcal bacteriophage produced by the "Rufag" company (Russia) against Staphylococcus aureus and streptococcal bacteria.*
Methodology and results: *The study was conducted in 2025 at the multidisciplinary clinic of the Tashkent Medical Academy. Samples of pus were taken from 20 patients in the diabetic purulent surgery department and tested for the presence of staphylococci and streptococci. The lysis zone of the bacteriophage against S. aureus measured 25–27 mm, indicating a high level of antibacterial activity. Among the antibiotics tested, vancomycin (27 mm), levomycetin (27 mm), compound S (26 mm), and cefoperazone+sulbactam (25 mm) were the most effective against streptococci. Several other antibiotics demonstrated limited efficacy.*
Conclusion, significance and impact of study: *The findings underscore the importance of rational antibiotic selection, the ongoing challenge of antimicrobial resistance, and the potential role of bacteriophage therapy in modern infectious disease management.*
Keywords: Staphylococcus aureus, Streptococcus, Bacteriophage, antibiotic resistance.

INTRODUCTION

In recent years, there has been a notable increase in Group A streptococcal strains, which can lead to serious conditions such as toxic shock syndrome, a disease associated with a high mortality rate (Maucotel A. L. et al., 2024). Globally, approximately 200 million streptococcal infections are reported each year, with over 650,000 classified as severe invasive infections—of which around 25% result in death (Zhang F. et al., 2024). Another important pyogenic bacterium is Staphylococcus aureus, a pathogen responsible for a wide spectrum of diseases ranging from superficial skin infections to postoperative wound infections (Fernandes ÂR et al., 2024). Prior to the introduction of antibiotics, the mortality rate associated with S. aureus exceeded 80% (Kleine LM et al., 2025). The use of penicillin against S. aureus began in the 1940s, but resistance quickly developed, with penicillin-resistant strains emerging as early as 1947 (Zhang D et al., 2022). This phenomenon contributed to the broader crisis of antibiotic resistance (Ali Alghamdi B et al., 2023). One promising alternative to antibiotics is bacteriophage therapy (Sheykhsaran E et al., 2024). Bacteriophages—viruses that specifically target and destroy bacteria—offer a targeted and effective strategy for managing bacterial infections (Omar O.S. et al., 2024). Since their discovery in 1917 by Félix d'Hérelle, phages have been the subject of ongoing research at numerous scientific institutions and universities worldwide. Phages provide several benefits, including specificity, minimal disruption to the normal microbiota, and reduced likelihood of resistance development.

Advantages of Phage Therapy

1. **Effectiveness Against Resistance:** Unlike antibiotics, to which bacteria can develop resistance, bacteriophages co-evolve with their bacterial hosts, making them effective even against resistant strains.
2. **Naturally Occurring Agents:** Phages are naturally present in ecological environments and are considered safe and environmentally friendly.
3. **Targeted Action with Fewer Side Effects:** Phages selectively infect target bacteria, minimizing side effects compared to broad-spectrum antibiotics.
4. **Research Potential:** Phage therapy is an evolving field with untapped potential for developing innovative treatments.

Ongoing research continues to explore phage capabilities, offering new diagnostic and therapeutic avenues (Li X et al., 2023). In laboratory diagnostics, phages are viewed as rapid, low-labor tools applicable in diverse laboratory settings.

In surgical settings, phage therapy has shown significant promise in the treatment of infected wounds, particularly where infection control is critical for healing outcomes (Omar O.S. et al., 2024).

MATERIALS AND METHODS:

During the study, Staphylococcus bacteria were cultivated on nutrient media. Bacteriological examination of purulent material was performed prior to infection and on the 3rd and 5th days post-infection. To preserve bacterial viability, samples were inoculated into nutrient media within 1–2 hours of collection.

Pus samples were collected using sterile cotton swabs. Following preliminary microscopic examination, the samples were streaked onto solid nutrient media using the Gold method and also inoculated into sugar broth. In cases where growth was not detected on the solid media, re-inoculation from the sugar broth was performed.

The media used included 5% blood agar, mannitol salt agar, and Sabouraud agar for the isolation of aerobic microorganisms. After bacterial growth was established, colony counts were determined, and colonization levels were expressed in CFU/swab or CFU/ml. Cultures on Blood agar and SEYA (Salt Egg Yolk Agar) were incubated at 1–37°C for 18–24 hours. Growth of infection was typically observed on blood agar within 24 hours, while visible Staphylococcus growth appeared on JSA within 48 hours.

RESULTS AND DISCUSSION:

The growing use of antibiotics in the treatment of infections is accompanied by an increase in their adverse effects on the human body. Antibiotics may compromise immune function and progressively lose efficacy due to the rapid adaptability of bacteria (Ali Alghamdi B et al., 2023). In this context, the primary objective of this study was to isolate Staphylococcus bacteriophages from environmental and clinical samples. The research was conducted in the Department of Purulent Diabetic Wounds at the Tashkent Medical Academy. The procedure followed the Francis H. G. Huxley method (Maucotel A. L. et al., 2024). The bacterial strains identified from the collected samples are summarized in tabular form.

Table 1: Infections Identified in Samples Collected from the Department of Purulent Diabetic Wounds (n=20).

Sample Type	**Infection Type 1**	**Infection Type 2**
	Staphylococeus aureus	*Candida albicans*
Urine		+
Nasal cavity	+	
Ear		+
Pertussis (Whooping cough)		+
Stool		+
Intestinal flora from stool		+
Upper shin wound	+	
Sacral wound	+	
Lung abscess	+	
Sputum		+
Wound on the thigh	+	
Abdominal cavity	+	
Index finger wound	+	
Vaginal discharge		+
Breast mastitis	+	
Skin		+
Pus	+	
Big toe wound	+	
Cervical canal		+
Pharynx		+
Breast milk	+	

Prostate secretion		+
Cerebrospinal fluid (CSF)		+
Blood culture		+

Table presents data on patients from the Department of Diabetic Purulent Wound Surgery, in whom Staphylococcus aureus was identified—particularly from samples collected from the big toe. S. aureus was also found in mastitis cases during the postpartum period (Boddie RL et al., 1997), as well as in lung abscesses resulting from severe respiratory infections. Sampling locations, infection types, and culture growth results are detailed in Table and Figure 1.
Samples collected from patients were cultured using lawn techniques on nutrient media (Figure 1). After incubation, various antibiotic diffusion discs were applied, and inhibition zones were measured (Figure 2).

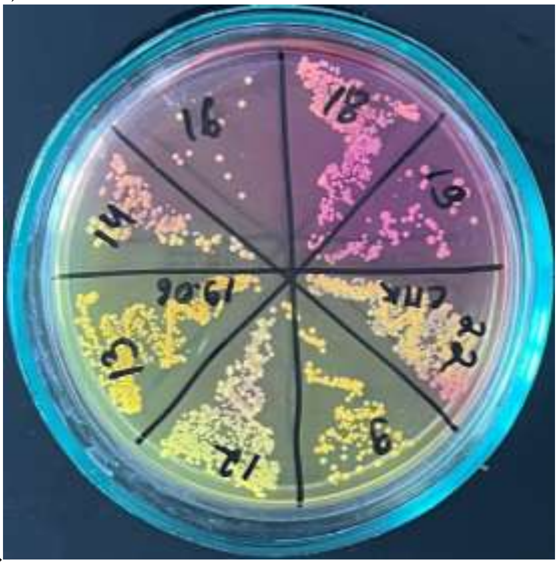


Figure 1: Appearance of *Staphylococcus aureus* bacteria on Endo nutrient medium.

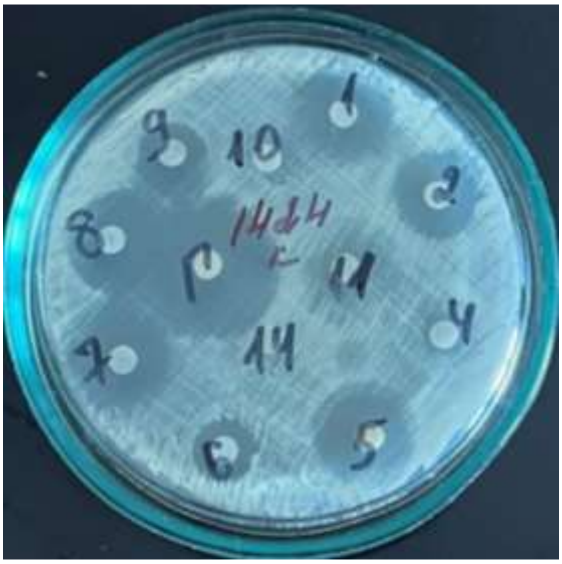


Figure 2: The effect of antibiotics on Staphylococcus infection.

Below the numbers, the discs indicate the zones of inhibition for the antibiotics that demonstrated the best effects. Cefoperazone/sulbactam showed the best effect at 25 mm, Ampicillin/sulbactam 16 mm, Tetracycline 14 mm, Chloramphenicol 11 mm, Cefepime 18 mm, Levofloxacin 13 mm, Ceftriaxone 10 mm, Ofloxacin/Ornidazole 11 mm, Azithromycin 9 mm, and Fosfomycin 13 mm. Rifampicin 10 mm, Penicillin 14 mm, Vancomycin 27 mm, Moxifloxacin 20 mm, Ceftazidime 16 mm, Cefepime 18 mm, Amikacin 16 mm, Levofloxacin 14 mm, S 26 mm, and Chloramphenicol 27 mm also demonstrated efficacy. All of these results (as shown in Figure 2) suggest that these antibiotics are 50-60% effective.

Currently, leading researchers and scientific centers involved in phage therapy include:

- Eliava Institute (Georgia): Operating since the 1920s, this institute is a prominent center for phage therapy in Georgia. Extensive research on phage-based treatments for various bacterial infections is still ongoing here (Zhang F et al., 2024).
- Texas A&M University (USA): This university actively conducts research on bacteriophage therapy, particularly focusing on the combined use of phages and antibiotics in collaboration with several other research centers.
- Yale University (USA): At Yale University, specifically within the field of engineering, research is being conducted to create effective phages against bacteria.
- Biotech Companies: Companies such as Phagelux, Adaptive Phage Therapeutics, and Armata Pharmaceuticals are actively developing alternative treatments to antibiotics through phage therapy in the pharmaceutical sector (Gu J et al., 2024).
- Institut Pasteur (France): This institute is also conducting significant research on phages and is renowned for developing new phage therapy strategies.

These centers and scientists are playing a leading role in advancing phage therapy as an alternative and effective treatment method compared to traditional antibiotics (Kleine L.M. et al., 2025).

Several scientific centers are conducting research on combating antibiotic-resistant infections using bacteriophages (Maucotel A. L. et al., 2024). For example, the University of California, San Diego School of Medicine (UCSD) is actively applying bacteriophages in clinical studies (Gebreyohans G et al., 2024). These long-standing studies, conducted for patients with limited treatment options, help to elucidate the impact of phage therapy on the immune system, optimal dosages, and durations. Research suggests that bacteriophages are not only effective against antibiotic-resistant bacteria but also exhibit a lower propensity for resistance development (Maucotel A. L. et al., 2024).

Furthermore, the World Health Organization (WHO) supports studying the potential of phage therapy as an alternative to antibiotics and has initiated several research programs to gather more scientific evidence in this area. Specifically, they are studying the effectiveness of using bacteriophages to reduce antibiotic-resistant bacteria in the human body. Additionally, Zhengzhou University in China is conducting research to expand the use of bacteriophages in fighting antibiotic-resistant bacteria such as Staphylococcus aureus (Aung MS et al., 2025). These studies demonstrate that bacteriophages can effectively target highly resistant bacteria and offer new treatment modalities (Maucotel A. L. et al., 2024). These findings indicate that bacteriophage therapy could become an important tool for fighting antibiotic-resistant infections in the future, and scientific research in this field is ongoing.

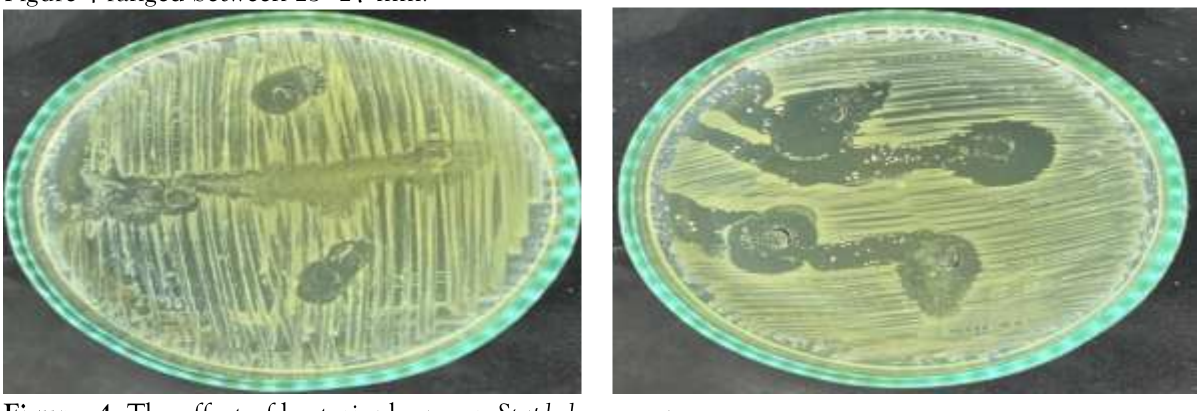
The properties of bacteriophages and their importance in the epidemiology of infectious diseases constitute a major area of research. The interaction between bacteriophages and bacteria significantly impacts disease treatment (Olivo G et al., 2024). The first studies in this area were conducted by D'Hérelle, who examined bacterial cultures grown with bacteriophages on solid media (such as agar). During these studies, D'Hérelle observed that bacteria, under the influence of bacteriophages, would dissolve and create sterile "spots." These spots are formed as a result of bacterial colonies lysing under the action of bacteriophages.

Once a bacteriophage infects a bacterium, it begins producing a special enzyme—lysin. This enzyme breaks down bacterial proteins, increasing the internal pressure of the cell. Consequently, the bacterium ruptures, releasing large quantities of new bacteriophages. After a bacterium bursts, 12 to 87 new bacteriophages can be produced. Research has shown that the replication rate of bacteriophages is dependent on their virulence (Omar O.S. et al., 2024). Highly virulent bacteriophages can double the number of new progeny in 10-20 minutes, while less virulent bacteriophages require 40-60 minutes. This duration depends on the specific type and properties of the bacteriophages (Silva-Santana G., 2025).

In our study, we investigated whether phage therapy is as effective as antibiotics in combating infections (Figures 3). After the infection was identified, the sample was taken to a petri dish, cultured using a lawn method, and Mediaphag Staphylococcus liquid bacteriophage was applied. After 12 hours, the phage exhibited a 14 mm effectiveness indicator, confirming the efficacy of phage therapy. When bacteriophages were observed under a microscope, swelling and rupture of bacterial cells were noted 30-35 minutes after their addition to the bacterial culture. After the cells ruptured, small particles and amorphous masses remained in the liquid instead of intact bacteria (Omar O.S. et al., 2024). Once the bacteria fully dissolved, the liquid became completely transparent, and ultramicroscopic bacteriophage progeny were present. The importance of phage therapy in combating diseases like plague, typhoid, and dysentery is significant.

Figure 3: The effect of bacteriophage on *Staphylococcus aureus*.

We tested the effect of bacteriophage on a cup containing a confirmed Staphylococcal infection, as shown in Figure 3. The phage activity was observed within 12 hours, and the inhibition zone reached up to 14 mm, as recorded in Figure 3.This clearly indicates the bacteriophage's effective impact and positive results. While antibiotics are used as an auxiliary to combat infections in the body, their side effects can weaken the immune system and reduce the body's ability to fight infections (Zhou W. et al., 2025). Antibiotics often provide only a temporary suppression, potentially leading to infection recurrence. In contrast, bacteriophages target only the infection without harming the host immune system (Dogruyol H., 2025).
Our observations showed that MediPhag's Staphylococcus aureus bacteriophage demonstrated high efficacy, yielding results comparable to or better than some antibiotics. For instance, Cefoperazone/Sulbactam (28 mm), Fosfomycin (26 mm), Cefepime (27 mm), Amikacin (26 mm), Levofloxacin (27 mm), Doxycycline (26 mm), and Chloramphenicol (27 mm) showed significant effects. The bacteriophage, within 12 hours, demonstrated an effect with a 14 mm efficiency rate. The observed effect of bacteriophages on Staphylococcus aureus bacteria suggests their potential use as natural predators in dry bacteriophage-based biocontrols, including antimicrobial therapy. This process, based on the growth and metabolism of bacteria, represents a potential solution to harmful factors associated with technological processes. The specific interaction between bacteria and bacteriophages, governed by defined spectrum criteria and ranges, generates extensive data that has been analyzed from a modern bacteriological and phage perspective.
This study evaluated the lytic potential of a bacteriophage preparation manufactured by the “Rufag” company (Russia) against Its appearance in Figure 4 Staphylococcus aureus using a standard in vitro bacteriological assay. The phage suspension was applied to The bacteriophage suspension was applied to *S. aureus* strains as shown in Figure 4, and its effect was evaluated by measuring the diameter of the inhibition zone. According to the results, the inhibition zone of the bacteriophage effect observed in Figure 4 ranged between 25–27 mm.



Figures 4: The effect of bacteriophages on *Staphylococcus aureus*

S. aureus is a clinically significant pathogen responsible for a broad range of purulent and systemic infections. The emergence of methicillin-resistant strains (MRSA) and the global rise in antimicrobial resistance underscore the urgency of developing alternative therapies. In this context, bacteriophage therapy offers a promising strategy due to its specificity, safety, and minimal impact on commensal microbiota.

The advantages of bacteriophage use include its low toxicity, high selectivity, and ecological compatibility, making it particularly suitable for vulnerable populations such as pediatric or immunocompromised patients.

Nevertheless, for phage therapy to become a widely accepted clinical tool, further studies are required. These should assess bacteriophage stability, genetic integrity, interaction with host immunity, and therapeutic efficacy through well-designed in vivo trials. These factors are particularly important in pediatrics and in treating immunocompromised patients. However, before the widespread clinical application of bacteriophage therapy, it is essential to study its stability, genetic consistency, and the potential for co-adaptation with bacteria. Furthermore, extensive *in vivo* clinical trials should be conducted to confirm the safety and efficacy of the preparation. Overall, this study indicates that bacteriophage preparations produced in Russia possess high therapeutic potential and open up the possibility of using them as an alternative to antibiotics in the fight against *S. aureus* infections.

This study analyzed the antimicrobial activity of various antibiotics against *Streptococcus* bacteria under in vitro conditions, as shown in Figure 5, using the disk-diffusion method (Kirby-Bauer) to determine inhibition zones and evaluate effectiveness. The results, as illustrated in Figure 5, showed significant differences in activity between the antibiotics. The highest activity was demonstrated by Vancomycin (27 mm) and Chloramphenicol (27 mm). Additionally, Cefoperazone/Sulbactam (25 mm) also formed large inhibition zones, indicating strong bactericidal effects against Streptococcus, which proves their clinical relevance in current practice.

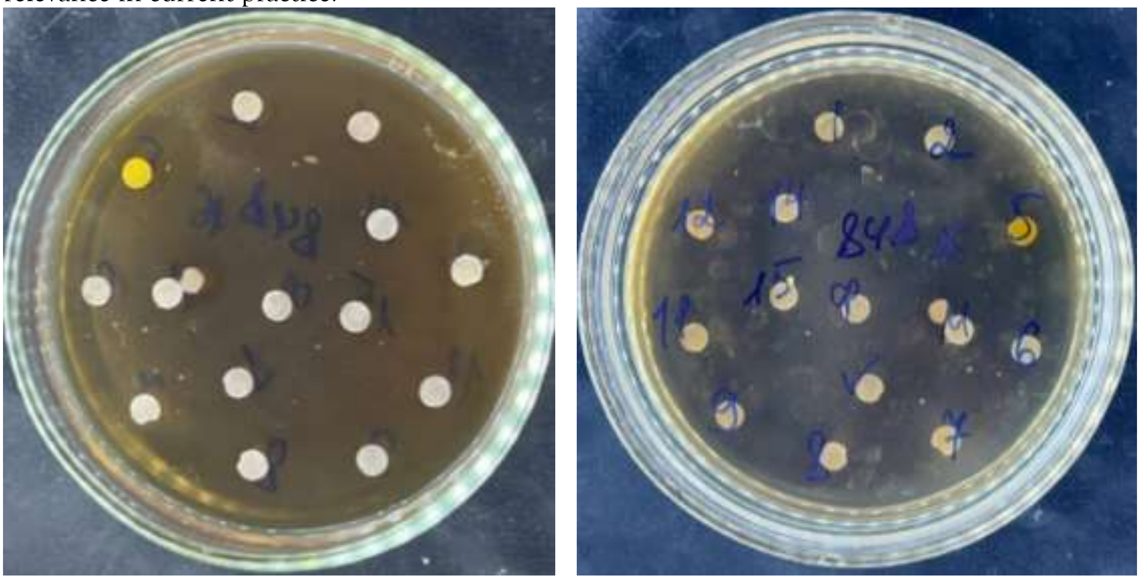


Figure 5: The effect of antibiotics on *Streptococcus* infections

Other antibiotics, such as **Moxifloxacin** (20 mm), **Cefepime** (18 mm), **Ampicillin/Sulbactam** (16 mm), **Ceftazidime** (16 mm), and **Amikacin** (16 mm), showed moderate effectiveness. These antibiotics often have a broad spectrum of action, making them suitable for treating *Streptococcus* infections, but their inhibition zones were slightly smaller than those of the more active agents.

Antibiotics such as **Levofloxacin** (14 mm), **Tetracycline** (14 mm), **Penicillin** (14 mm), **Fosfomycin** (13 mm), **Levofloxacin** (13 mm), **Chloramphenicol** (11 mm), **Ceftriaxone** (10 mm), **Rifampicin** (10 mm), and a combination of **Ofloxacin/Ornidazole** (11 mm) formed smaller inhibition zones, indicating limited efficacy against this *Streptococcus* strain. The observed differences in antibiotic effectiveness are mainly attributed to the specific resistance mechanisms of bacterial strains and the pharmacodynamic and pharmacokinetic properties of each antibiotic. For example, some beta-lactam antibiotics may be less effective against *Streptococcus* strains that produce beta-lactamase enzymes. However, combined antibiotics (such as Cefoperazone/Sulbactam, Ampicillin/Sulbactam) can expand the range of action by inhibiting beta-lactamase production.

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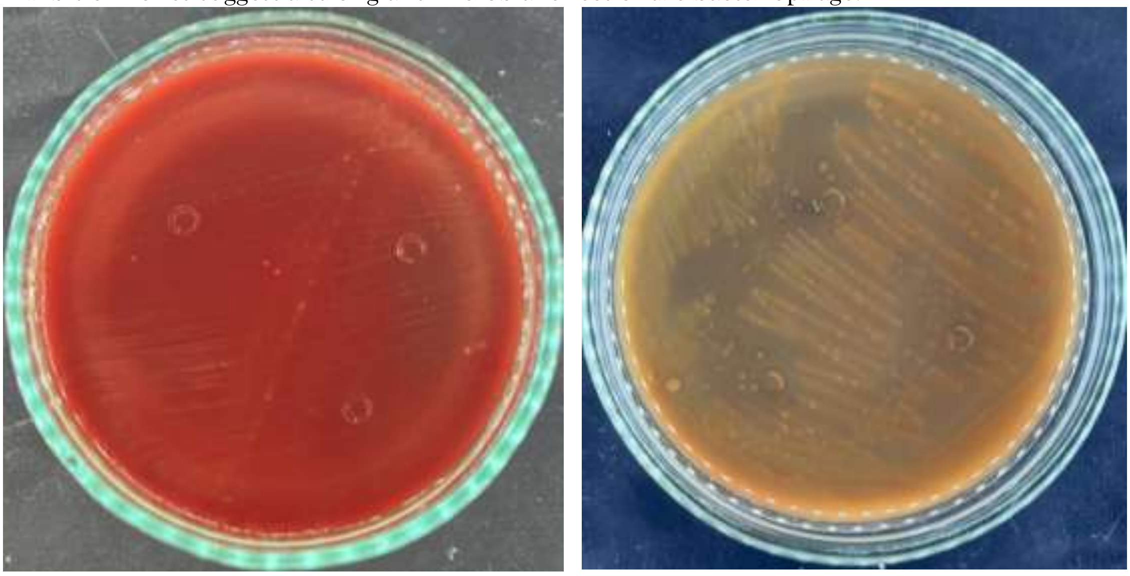
The findings highlight significant differences in antibiotic effectiveness, reinforcing the need for antibiotic selection based on individual strains in clinical practice. **Vancomycin** and **Chloramphenicol** can be recommended as highly effective agents for *Streptococcus* infections, but factors such as the patient's clinical condition, allergic reactions, pharmacological interactions, and existing resistance levels should be considered when choosing an antibiotic. Additionally, with the global rise in antibiotic resistance, selecting the appropriate antibiotic based on susceptibility testing for each microorganism has become a critical step in modern infectious disease therapy.

In this study, the antibacterial activity of a bacteriophage preparation obtained from the Rufag company in the Russian Federation against *Streptococcus* species was examined, as shown in Figure 6. The experiments demonstrated that the bacteriophage exhibited a significant lytic (destructive) effect against *Streptococcus* bacteria, as illustrated in Figure 6. Specifically, inhibition zones measuring 16–20 mm in diameter formed around the bacteriophage sample placed in the Petri dish, as seen in Figure 6. These inhibition zones suggest a strong antimicrobial effect of the bacteriophage.



Figures 6: The effect of bacteriophages on *Streptococcus* infections

Streptococcus species, particularly *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and other β-hemolytic *Streptococcus* bacteria, are responsible for numerous infectious diseases, including angina, tonsillitis, pharyngitis, pneumonia, and even sepsis. In recent years, increasing antibiotic resistance has made it more challenging to treat these infections. As a result, bacteriophages are being considered as an effective alternative therapy in this context. Bacteriophages are distinguished by their high specificity for bacteria, natural origin, ecological safety, and lack of harm to human cells.

Our research results indicate that the bacteriophage from the "Rufag" company can be used as an effective agent against *Streptococcus* infections. The inhibition zones of 16–20 mm are pharmacologically significant, demonstrating the bacteriophage's high lytic activity. Moreover, the results obtained in this study are consistent with other scientific works. For instance, a 2020 study (Ivanov et al.) showed inhibition zones of 14–18 mm for bacteriophages used against *Streptococcus* bacteria. In our experiment, slightly higher activity was observed, suggesting that the bacteriophage used was more potent or that there was greater sensitivity to the specific strain. An important aspect of our experimental results is that when used in combination with antibiotics, bacteriophages may have a synergistic (enhancing) effect. This opens the possibility for developing combined therapy strategies for combating bacterial infections. Additionally, using bacteriophages for treatment carries a lower risk of superinfection or damage to the normal microbiota.

Future research should explore the spectrum of action of this bacteriophage against various *Streptococcus* strains, its minimum lytic concentration (MLC), dosing regimens, and treatment durations. The bacteriophage's stability, storage conditions, toxicological safety, and long-term effects on the organism also require further investigation. In conclusion, the bacteriophage from the "Rufag" company is an effective tool for combating *Streptococcus* infections and holds promise as an alternative or adjunctive therapy to antibiotics. The results of this study provide fundamental data necessary for the clinical application of phage therapy.

CONCLUSION

This study assessed the in vitro antimicrobial efficacy of various antibiotics and a bacteriophage preparation produced by the “Rufag” company (Russia) against Staphylococcus aureus and Streptococcus species. The bacteriophage demonstrated high lytic activity against S. aureus, with inhibition zones measuring 25–27 mm, supporting its potential as an alternative to conventional antibiotics.

For Streptococcus infections, antibiogram analysis indicated that vancomycin (27 mm), levomycetin (27 mm), compound S (26 mm), and cefoperazone+sulbactam (25 mm) were the most effective agents. Other antibiotics, including moxifloxacin (20 mm), ceftazidime (16 mm), and amikacin (16 mm), showed moderate activity, while drugs such as azithromycin, rifampicin, and ceftriaxone had limited efficacy, suggesting potential resistance in the tested strains.

These findings underscore the necessity of individualized antibiotic selection based on microbial sensitivity testing. Furthermore, the results emphasize the therapeutic promise of bacteriophages as targeted, effective, and safe alternatives or adjuncts to antibiotics in the fight against resistant pathogens. The integration of susceptibility testing into clinical decision-making is crucial for optimizing antimicrobial therapy outcomes.

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