

## Original article

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## Comparative evaluation of commercial bacteriophages and prospects for their application in the treatment of orthopedic MRSA-infection

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### Abstract

**Introduction** Phage therapy is a promising approach to addressing the problem of antibiotic resistance in orthopedic infection pathogens. Staphylococci are the leading etiologic agents of implant-associated infections, with 15 % of *S. aureus* strains being methicillin-resistant (MRSA). Bacteriophage preparations are available on the Russian pharmaceutical market, and the concentration of phages active against the microbial agent influences their effectiveness.

The **objective** was to compare the activity of commercial bacteriophage kits against methicillin-resistant *Staphylococcus aureus* isolated from patients with orthopedic infections.

**Material and methods** Clinical strains of *S. aureus* ( $n = 25$ ), consecutively isolated from patient biomaterial in 2025 were examined. Identification was performed using MALDI-TOF MS, and antibiotic susceptibility assessed according to EUCAST v.15. Phage lytic activity was evaluated using meat-peptone agar and a five-point scale with the strain sensitivity to a specific drug determined as sensitive, weakly sensitive, or resistant. Statistical analysis was performed using IBM SPSS Statistics v.26.

**Results** The *S. aureus* strains included in the study were resistant to ceftazidime. Of the MRSA strains tested, the majority (76 %) were sensitive to PBP 1. A larger number of strains (60 %) were classified as "weakly sensitive" to PBP 3. There were less variations in "non-susceptible" cultures, with only one strain demonstrating resistance to the three bacteriophage preparations. A comparative analysis of antistaphylococcal drugs from various manufacturers revealed differences in the activity against clinical MRSA isolates. There were 84 % isolates being sensitive to PBP 4 and 36 % to PBP 5. One isolate was resistant to the phages tested.

**Discussion** The differences in the activity of commercial phages could be associated with the composition of the resulting preparations, which had lower affinity for strains isolated from patients in other regions. Given the wide geographical distribution of patients with orthopedic infections hospitalized in federal centers, the ability to choose bacteriophages from a wide range of commercial kits available on the market increases the likelihood of their successful use.

**Conclusion** Commercial drugs presented on the Russian market were characterized by different lytic activity against clinical strains of MRSA, with Pyophag<sup>®</sup> and Staphylophag<sup>®</sup> exhibiting greater activity.

**Keywords:** bacteriophages, orthopedic infection, *S. aureus*, MRSA

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## INTRODUCTION

Since their discovery in the early 20th century, antibiotics have revolutionized medical practice, enabling the control and cure of many infectious diseases [1]. The widespread use of antibacterial drugs has stimulated the development of adaptive resistance mechanisms among bacteria [2–4]. The World Health Organization has recognized antibiotic resistance as one of the top ten public health threats [5]. The need to find alternative treatments that can effectively combat bacterial infections is a priority for the modern healthcare system [6].

The increasing number of orthopedic surgeries involving the use of various metal constructs leads to a corresponding increase in the incidence of infectious complications associated with implants. The incidence of infections associated with orthopedic implants reaches 0.3–5.0 % across all types of surgeries (internal osteosynthesis, joint replacement) [7]. Surgical removal of infected implants and long-term treatment with antibiotics active against the isolated pathogens is the gold standard for treating patients with periprosthetic joint infection. However, problems associated with pathogen resistance to antibiotics and the persistence of biofilms on the surface of implants can lead to recurrent or difficult-to-treat infections, which poses a serious threat to the health and lives of patients [8].

Bacteriophage-based therapy represents a promising approach to addressing the problem of bacterial antibiotic resistance [9–11]. Phage activity is determined by the ability to complete all stages of their life cycle, including adsorption, penetration of genetic material into the cell, replication of genetic material, assembly of phage particles, and lysis of the bacterium [12]. Unlike broad-spectrum antibiotics, which can act on a wide range of bacteria, phages are highly specific, typically targeting only one species or even a specific strain of bacteria [12]. The specificity of phages represents both strengths and challenges in their therapeutic application [13, 14]. The advantage is the specific targeting of pathogenic bacteria while preserving beneficial human microbiota. However, such specificity requires precise microbiological judgment to select an active phage, making administration more challenging compared to broad-spectrum antibiotics [15].

The Russian pharmaceutical market offers a variety of bacteriophage preparations, including those for topical and/or systemic use. The concentration of bacteriophages active against the infectious agent in a preparation directly impacts its efficacy [16], and broader spectrums of activity, allowing for a reduced diversity of phages in cocktails while maintaining the overall range of activity, are a preferred property for therapeutic bacteriophages [17, 18]. Of particular interest is a comparative analysis of the lytic activity of domestic bacteriophage preparations against staphylococcal strains isolated from patients with orthopedic infections.

The **objective** was to compare the activity of commercial bacteriophage kits against methicillin-resistant *Staphylococcus aureus* isolated from patients with orthopedic infections.

## MATERIAL AND METHODS

*S. aureus* cultures were prospectively isolated in 2025 from biomaterial of patients with periprosthetic joint infection and/or osteomyelitis in accordance with international microbiological research standards. Species identification was performed with MALDI-TOF MS using the FlexControl system and MBT Compass 4.1 software, Score  $\geq$  2.0. The susceptibility of clinical isolates to antibacterial agents was explored in accordance with EUCAST v.15 requirements.

According to the local committee for the ethical review of clinical and experimental studies, this work is based on microbiological analysis of pathogen susceptibility, which is not subject to review by the ethics committee.

The susceptibility of MRSA ( $n = 25$ ) to five domestic bacteriophage preparations (DBP):

- DBP 1 – Pyophag® (Mikrogen, Nizhny Novgorod, IMP RU LP-No. (002513)-(RG-RU) of the Russian Ministry of Health),
- DBP 2 – Pyobacteriophag® (Mikrogen, Ufa, IMP RU No. LS-002031 of the Russian Ministry of Health),
- DBP 3 – Sextaphag® (Mikrogen, Perm, IMP RU No. LS-001049 of the Russian Ministry of Health),
- DBP 4 – Staphylophag® (Mikrogen, Nizhny Novgorod, IMP RU No. R N001973-01 of the Russian Ministry of Health);
- PBF 5 – Staphylophag® (Microgen, Perm, IMP RU No. R N001973-01 of the Russian Ministry of Health).

The lytic properties of BP were assessed using meat-peptone agar (MPA). A bacterial suspension (0.5 % McF) was applied to the MPA with a cotton swab, and after 10 minutes, 10 µl of PBP were dispensed in duplicate onto the agar surface. The plates were incubated at 37 °C. After 24 hours, BP activity was assessed on a five-point scale in accordance with the Methodological Recommendations "Rational Use of Bacteriophages in Therapeutic and Anti-Epidemic Practice" (2023). Based on the findings, the strain's sensitivity to a specific drug was determined as sensitive, slightly sensitive, or resistant.

The nonparametric Kruskal-Wallis test in IBM SPSS Statistics Version 26 was used for statistical analysis of differences in the findings between the groups of explored susceptibility and commercial preparations. Dunn's test with correction for multiple comparisons was used for pairwise comparison of groups (post-hoc analysis) with statistically significant differences in the analysis of the activity of the three bacteriophage preparations. With multiple comparisons, a lower critical significance level was used and calculated using the formula:  $p = 1 - 0.95^{1/n}$ , where  $n$  was the number of comparisons performed. For this study,  $p$  values  $< 0.017$  were considered statistically significant.

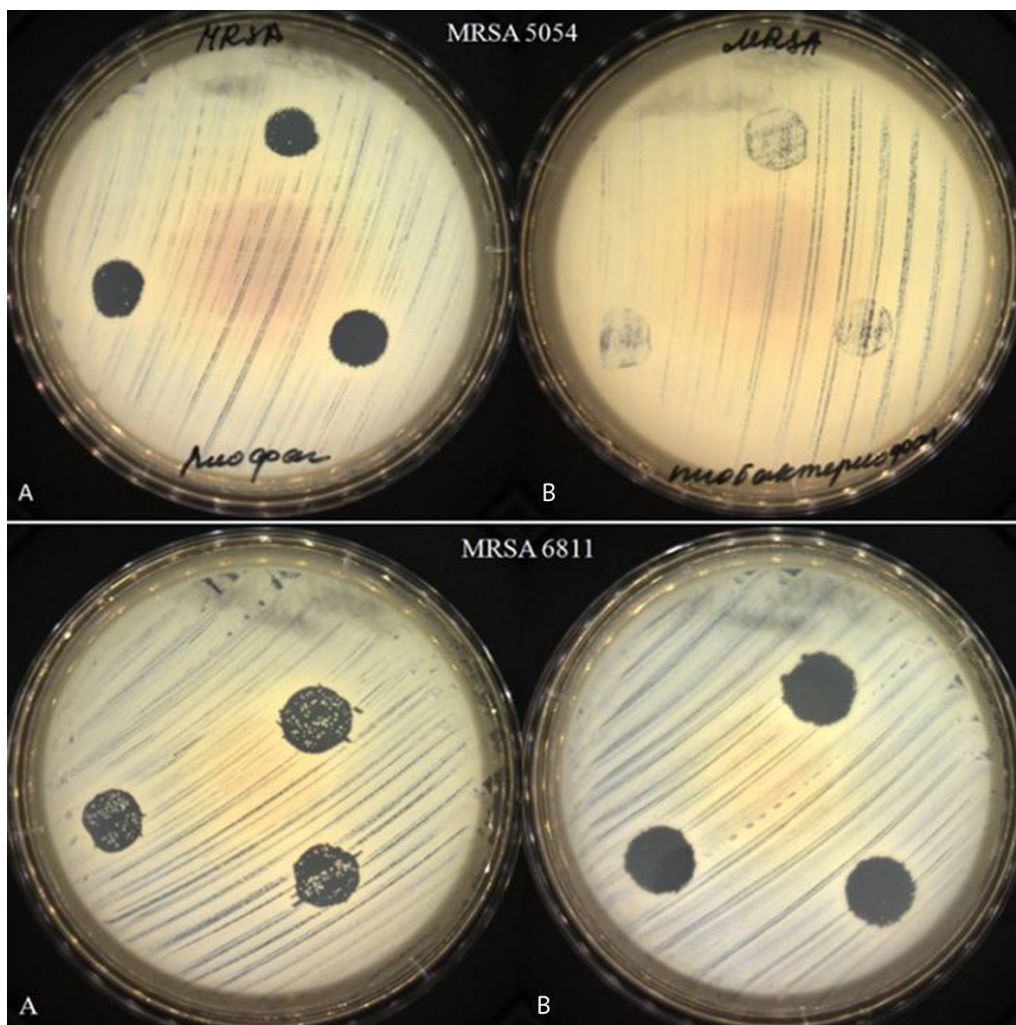
## RESULTS

The *S. aureus* strains included in the study ( $n = 25$ ) were resistant to cefoxitin (MRSA). The bacteriophage preparations tested were active against most clinical MRSA isolates from patients with orthopedic infection; however, differences were revealed when comparing the BPs (Table 1, Fig. 1).

Table 1

Evaluation of the antibacterial activity of bacteriophage preparations against methicillin-resistant *S. aureus*

Attitude to the BP	Tested preparations						$p$
	BP 1		BP 2		BP 3		
	abs.	%	abs.	%	abs.	%	
Sensitive	19	76	11	44	8	32	0.01
Slightly sensitive	5	20	11	44	15	60	0.01
Resistant	1	4	3	12	2	8	0.05



**Fig. 1** Photographs of Petri dishes with zones of complete and partial lysis of MRSA cultures: A, BP 1; B, BP 2

Of the MRSA strains tested, a greater number (76 %) were sensitive to BP 1 ( $p = 0.004$ , pairwise comparison with BP 2). The opposite trend was recorded for the remaining susceptibility gradations: a greater number of strains (60 %) were classified as "slightly sensitive" to BP 3 ( $p = 0.003$ , pairwise comparison with BP 1,  $p = 0.009$ , overall). There were less variations in resistant cultures, with only one strain demonstrating resistance to the three bacteriophage preparations tested.

A comparative analysis of antistaphylococcal drugs from various manufacturers revealed differences in their activity against clinical MRSA isolates isolated from patients at our Center (Table 2, Fig. 2). There were 84 % isolates sensitive to BP 4 and 36 % to BP 5 ( $p = 0.008$ ). One isolate was not susceptible to the phages tested.

Table 2

Evaluation of the activity of antistaphylococcal drugs against methicillin-resistant *S. aureus*

Attitude to the BP	Preparations tested				p
	BP 4		BP 5		
	abs.	%	abs.	%	
Sensitive	21	84	9	36	0.008
Slightly sensitive	3	12	15	60	0.004
Resistant	1	4	1	4	0.899

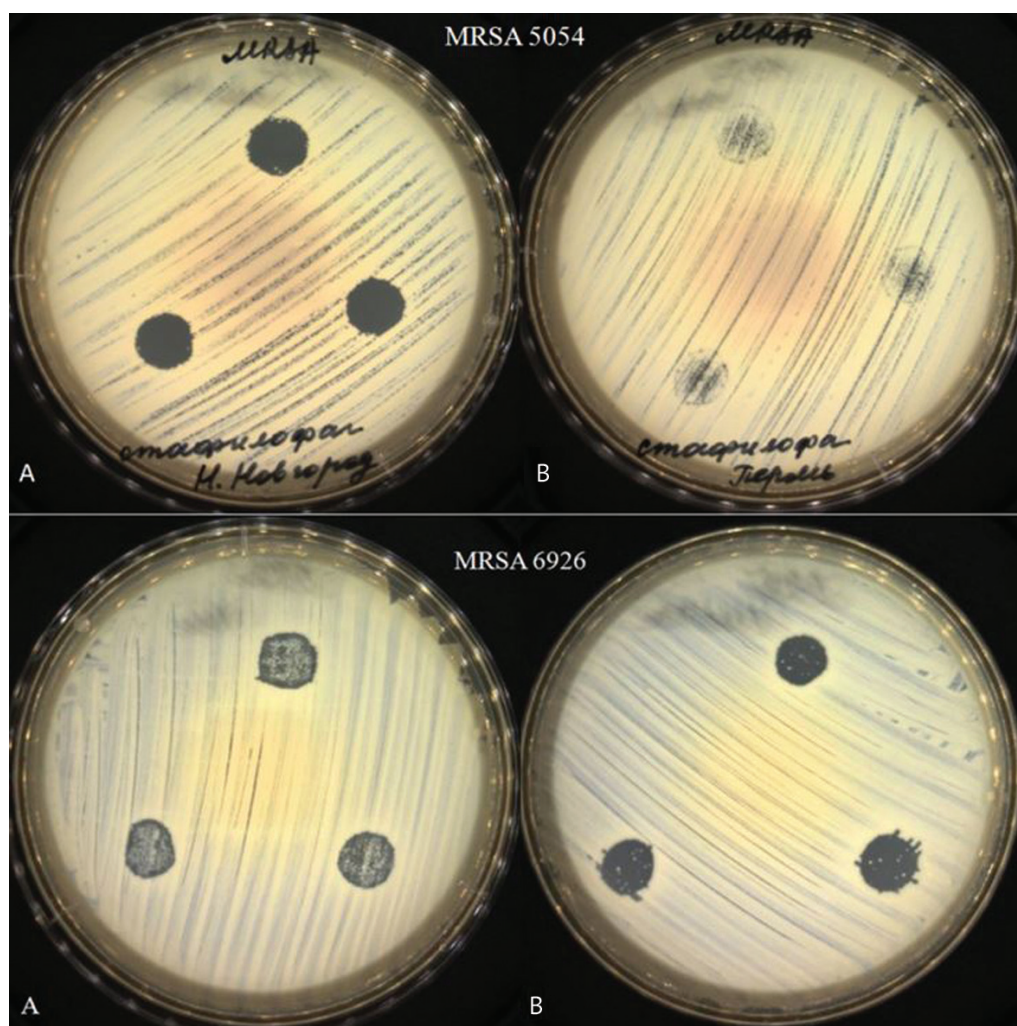


Fig. 2 Photographs of Petri dishes with zones of complete and partial lysis of MRSA cultures: A, BP 4; B, BP 5

#### DISCUSSION

The presence of antibiotic resistance mechanisms in infectious disease pathogens worsens the prognosis of disease outcome, prolongs treatment periods, significantly increasing healthcare costs. Our 2024 study showed that over a 12-year period, *S. aureus* was the causative agent of orthopedic infection in 33 % of cases with the proportion of methicillin-resistant *S. aureus* strains not decreasing below 15 % throughout the entire period of the study [19]. Tsiskarashvili et al. [20] and Sokolovsky et al. [21] reported *S. aureus* as the most common causative agent of periprosthetic joint infection, in 32 % and 38 %, respectively.

The ability of *S. aureus* to resist a broad spectrum of antibacterial drugs makes it an ideal subject for research using bacteriophages. In recent years, approaches to the selection, production, and delivery of phages have expanded significantly, and improvements in the technologies for their production allow for personalized phage therapy. The promising potential of phages against MRSA has been demonstrated in some trauma and orthopedic cases [22]. Fedorov et al. reported that the addition of a solution of commercial staphylococcal bacteriophage (6 mL per 40.0 g of dry polymethyl methacrylate substance) followed by its introduction through the drainage or by puncture into the periprosthetic area for 10 days increased the effectiveness of one-stage treatment of staphylococcal periprosthetic hip joint infection from 69 % to 96 % [23]. Ferry et al. reported an improvement in three patients with chronic periprosthetic joint infection with use of a phage

cocktail combined with antibiotics [24]. Similar results were reported by Ramirez-Sanchez et al. who described the successful treatment of patients with MRSA infection of the knee joint by intra-articular administration of a phage cocktail [25]. The variety of methods of the use suggested safety of bacteriophages with the significant advantage over antibiotics. The sensitivity of a specific pathogen to a specific bacteriophage preparation (to a specific batch of a commercial kit) is essential for successful phage therapy to ensure elimination of the pathogen and prevent bacterial resistance to the bacteriophage.

Phage susceptibility testing was performed at our Center for the isolated MRSA strains using different commercial preparations. Treatment of patients with periprosthetic joint infection caused by a pathogen sensitive to the available phages was produced with a drainage system inserted from the joint cavity after surgery. Twenty mL of the bacteriophage preparation was injected daily into the periprosthetic area for three to five days using the drainage system, then the drainage system was closed to increase the exposure time of the phages. The drainage system was removed upon completion of phage therapy. In accordance with clinical guidelines, the patients with periprosthetic joint infection received high-dose, etiotropic antibacterial therapy, since bacteriophage therapy did not replace antibiotics but was administered in combination with other antibiotics. A 2023 review [26] described the results of studies on the combined use of lytic bacteriophages and antibiotics for infections caused by resistant *S. aureus* strains.

The authors emphasized that the combined administration of phage and antibiotic could lead to a significant reduction in bacterial growth in many cases, while the reverse antagonistic effect was less common [26]. Treatment outcomes could be influenced by the sequence of therapeutic agents administered; the best results were recorded in cases with the phage therapy preceding antibiotics [27]. It has been established that the use of bacteriophages can alter the sensitivity profile of bacteria to antibacterial drugs and lead to the inclusion of the drug in the treatment regimen [28].

Understanding the pharmacokinetics (PK) and pharmacodynamics (PD) of phages is crucial for optimizing therapeutic efficacy in clinical settings [29]. However, achieving a comprehensive PK/PD understanding for phage therapy is challenging due to the three-way interactions between phages, bacteria, and humans. Every phage–bacteria–patient combination can exhibit a unique PK/PD profile and development of standardized models applicable across diverse clinical settings remains challenging. To date, there is no validated approach for the clinical use of phage therapy, requiring comparative clinical trials or the accumulation and analysis of real-world clinical data on the use of bacteriophages in the treatment of patients with orthopedic infection.

Our comparative study of the activity of commercial bacteriophage kits against clinical MRSA strains isolated from patients with orthopedic infections demonstrated a high level of lytic properties of the phage preparations. It is important to note different levels of antibacterial activity of kits produced at different regional facilities. Pchelin et al. suggested that the search for active bacteriophages planned for use as antimicrobial agents should probably be conducted in habitats geographically close to pathogenic bacterial populations [30].

The differences in the activity of commercial kits may be related to the composition of the resulting preparations, which have lower affinity for strains isolated from patients in other regions. However, the search for active phages is necessary for the prescription of personalized phage therapy selecting a specific bacteriophage against a specific pathogen strain. Given the wide geographical distribution of patients with orthopedic infections hospitalized in Federal Centers, the ability to choose bacteriophages from a wide range of commercial kits on the market increases the likelihood of their successful use.

## CONCLUSION

Commercial domestic bacteriophage preparations available on the market exhibited varying lytic activity against methicillin-resistant *S. aureus* isolated from patients with orthopedic infections. Pyophag® and Staphylophag® (Nizhny Novgorod) demonstrated high efficacy compared to other preparations tested. The phage therapy should be considered a promising method for combating infections caused by antibiotic-resistant pathogens in trauma and orthopedic patients in conjunction with established treatment methods after mandatory testing of the pathogen's susceptibility to bacteriophage preparations.

**Conflict of interest** None of the authors has any potential conflict of interest.

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