THE USE OF BACTERIOPHAGES IN THE TREATMENT OF STAPHYLOCOCCAL INFECTION

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DI conducted literature review, and wrote the first manuscript; LG revised, added and completed the final text; AF conducted literature review; EC conducted literature review; GB conceptualized the idea and revised the article critically. All the authors approved the final version of the manuscript.

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Abstract

Aim of study. The current rate of increase in the number of antimicrobial-resistant infections is poised to become the leading global cause of loss of human life. The limited discovery of new antibiotics necessitates investment in alternative solutions. Bacteriophages, viruses that target bacteria, offer a potent alternative approach to combating bacterial infections. One area of modern microbiology and biotechnology that has garnered significant interest is the study of bacteriophages, owing to their potential practical application in various branches of medicine.
Material and methods. This study conducted a comprehensive synthesis of specialized reference sources from international open-access databases, examining the impact of bacteriophage therapy on staphylococcal infections and antimicrobial resistance.
Results. The synthesis is based on studies in human clinical trials, including randomized clinical trials, based on data from recent years. Multiple successful cases regarding the use of phages in the treatment of human infections have been reported. The studies conducted provide evidence that the development of phage therapy is a promising alternative for combating bacterial resistance to antibiotics. The effective action of antistaphylococcal phage cocktails against the most common strains has been demonstrated. The mechanisms underlying the phage-antibiotic synergy effect, as well as the advantages of bacteriophages over other forms of treatment, are presented.
Conclusions. Therapy methods using bacteriophages in combination with antibacterial drugs can provide a higher therapeutic effect.

Keywords: Staphylococcal infections, resistance to antibiotics, bacteriophages, phage therapy.

Introduction

In recent years, there have been practically no new antibacterial drugs (ABDs), while resistance to the "old" ABDs has been steadily growing and, in many cases, has already reached a critical level. WHO draws attention to the fact that many discoveries in the field of drug treatment made in the 20th century may lose their significance due to resistance to ABD. As a result, most infectious diseases can simply get out of control (Cisek, Dąbrowska, Gregorczyk, & Wyżewski, 2017). Healthcare-associated infections (HAIs) pose the greatest danger. According to the European Center for Disease Prevention and Control (ECDC), between 3.1 and 4.6 million people hospitalized in acute care health facilities develop an HAI each year, which is consequently responsible for increasing morbidity and mortality rates as well as costs associated with health services. HAI is the most expensive and deadly adverse event, with the expenses generated by it amounting to 6% of the budget of public hospitals (Organisation for Economic Cooperation and Development, 2020). The burden associated with HAI is closely related to the emergence of the phenomenon of microbial resistance to antibiotics (AMR), with most HAIs being caused by resistant microorganisms (Iaconi et al., 2023; European Centre for Disease Prevention and Control, 2022; Balan et al., 2017). The prevalence of staphylococci in the occurrence of bacterial infections, the annual increase in the number of strains of staphylococci resistant to methicillin, and the emergence of strains resistant to antistaphylococcal reserve antibiotics place this pathology among emerging infectious diseases. One of the options for the treatment of multiresistant strains is the use of bacteriophages.

The aim of this synthesis was to study the modern aspects of using bacteriophages for treating infections caused by antibiotic-resistant strains of *Staphylococcus aureus*, as well as successful methods for their application as of early 2022.

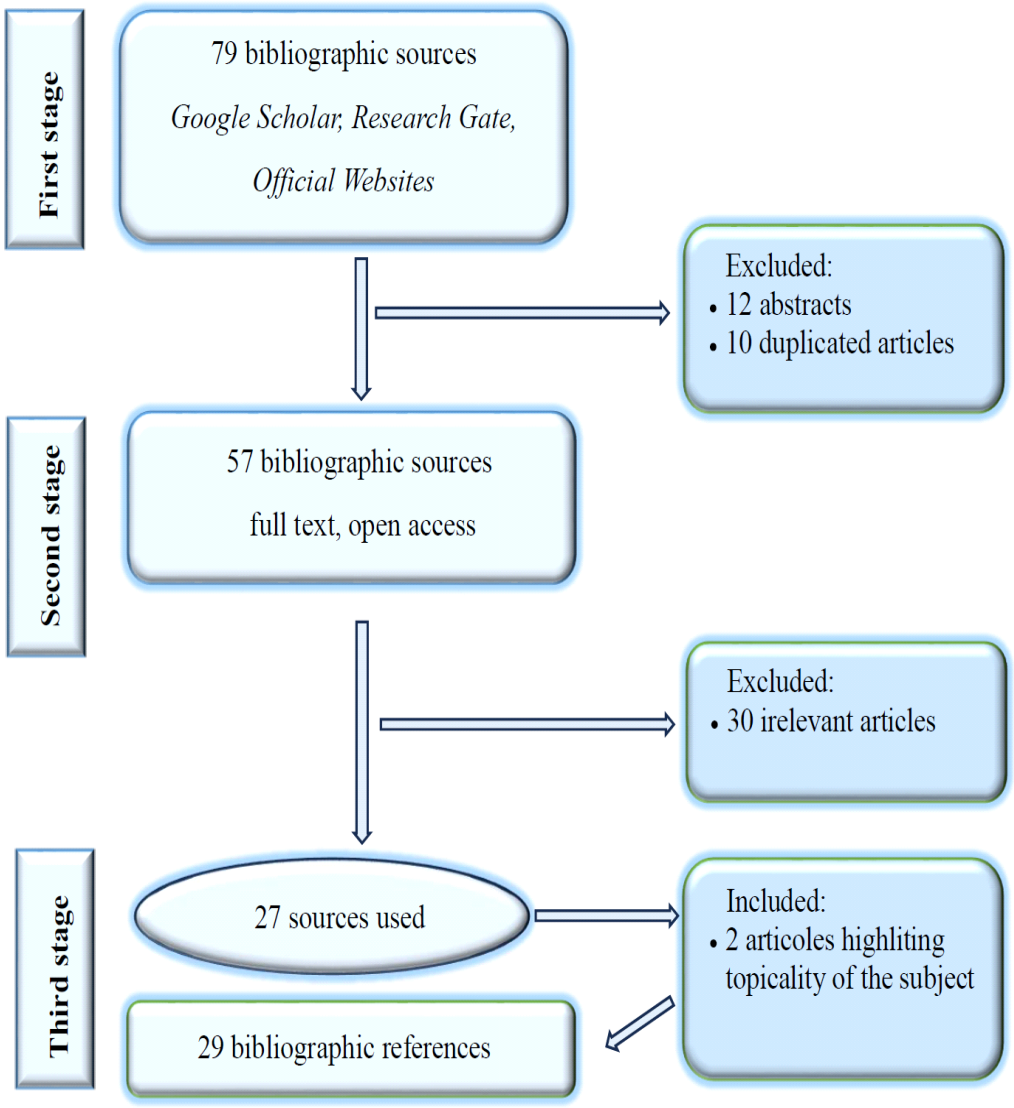
Material and methods

A comprehensive synthesis was conducted by compiling specialized reference sources from international open-access databases. This involved a meticulous examination of publications using keywords such as "bacteriophage therapy," "the phenomenon of antimicrobial resistance," and "staphylococcal infections." The selection criteria for the literature encompassed several key facets, including topic relevance, availability, outcome measures, strain specificity, type of bacteriophage, and comparative studies. The exclusion criteria involved off-topic articles, duplicate studies, and studies that did not focus on bacteriophage treatment.

A total of 79 scientific papers available through open access from both international databases and official websites were reviewed and analyzed. After applying specific selection criteria, 50 publications were deemed unsuitable for inclusion. Consequently, the in-depth research focused on 29 carefully chosen scientific publications (Figure 1).

This study delves into the realm of scientific evidence, aiming to shed light on the profound implications of bacteriophage therapy in the context of infectious diseases caused by staphylococci and its influence on the development of resistance to antimicrobial agents.

Figure 1.
Article selection algorithm



Results

In 1921, J. Meisin and R. Bruynoghe first described a successful method for treating staphylococcal skin infections using staphylococcal phages. Some phages have the ability to selectively lyse only a specific strain of pathogenic bacteria while leaving the rest of the symbionts intact. Additionally, bacteriophages can only circulate as long as bacteria sensitive to them are present in the biotope. Some phages have polysaccharide depolymerases on their tail structures, which can act as an adjuvant to phage infection by disrupting the extracellular matrix of bacterial biofilms. Phages are considered to be quite harmless in relation to human tissues, so the normal human bacterial flora remains intact, which can be explained by their high specificity and rapid

inactivation and elimination from the human body as soon as their host (bacterium) is no longer present in it (Bruynoghe & Maisin, 1921).

In addition, multiple studies have revealed a minimal number of side effects, which include individual intolerance to the components of phage preparations.

There are numerous reasons why phage therapy is increasingly being utilized for therapeutic and prophylactic purposes, and it is likely that it will continue to be used more widely in the future. The situation with this type of therapy is in contrast to antibiotics, the effectiveness of which has drastically decreased, while new antibiotics are quite scarce or are in the early stages of development (Bruynoghe & Maisin, 1921; Ling, Schneider, & Peoples, 2015).

Staphylococcus aureus is ubiquitous, abundantly colonizing the skin and mucous membranes, and can cause respiratory and wound infections, osteomyelitis, sepsis, arthritis, and endocarditis. *S. aureus* is known to possess a high variability of biochemical properties, which is why it often acquires polyresistance to modern chemotherapeutic drugs (Cisek, Dąbrowska, Gregorczyk, & Wyżewski, 2017).

Bacteria can develop resistance to the effects of a particular antibiotic through several mechanisms simultaneously. The selective pressure caused by antibiotics, through mutations and recombinations, can quickly lead to the emergence of multiresistance in strains that were previously sensitive to a wide range of drugs. In general, resistance results from mutational changes or the acquisition of genetic material encoding resistance. In one study, 35 different vancomycin-resistant mutants were sequentially isolated and identified during a 3-month vancomycin treatment of a patient with hospital-acquired *S. aureus* infection (Герасименко, 2022).

Bacteriophages are viruses with a DNA or RNA genome encapsulated in a protein capsid, which sometimes ends with a tail and more or less complex appendages. Bacteriophages can be cubic, filamentous, or tadpole-shaped. The bacteriophage head contains a nucleic acid (DNA or RNA) enclosed in a protein shell (capsid), and below is the tail process, consisting of a contractile sheath and an internal rod.

The bacteriophage attaches to the receptors of the bacterium's cell wall with the help of fibril legs, fastened in the center with a basal plate. The size of a bacteriophage is tens to hundreds of times smaller than that of microbial cells. They attach to specific receptors on the surface of bacteria and subsequently introduce their genomes into bacterial cells, after which one of two outcomes can occur. The first outcome involves the manipulation of the bacterial metabolic machinery to produce viral proteins and copy the viral genome. Subsequently, the viral particles are assembled, and the bacterial cell is lysed, releasing many new phages. This situation is similar to that of virulent phages, which only perform lytic cycles and, as a result, form clear halos (plaques) on bacterial lawns. The second possibility is the lysogenic cycle, during which phages insert their DNA into the host cell, and then such a cell may undergo lysis (Sattar, Ullah, & Khanum, 2022; Peng et al., 2022).

In some cases, the entire pathogenic potential of the causative agent of an infectious disease is determined by a specific interaction with only one prophage (as seen in diphtheria, cholera, and botulism). However, for the majority of pathogenic and opportunistic microbes, the realization of pathogenic potential depends on horizontal genetic exchange involving temperate phages. Furthermore, each of the prophage genes can contribute to an increase in the pathogenic potential of a lysogenic bacterium (as observed in staphylococcal, *Pseudomonas aeruginosa*, and *Proteus* nosocomial infections) (Cisek, Dąbrowska, Gregorczyk, & Wyżewski, 2017).

Other disadvantages of using bacteriophages include a special dosing regimen, a longer treatment course, and a longer onset time for the effect (Kuptsov et al., 2022; Brix et al., 2020).

Bacteriophage treatments have been infrequently described for different infections in case studies (table 1).

Table 1.
Summary of recent published clinical reports of phage therapy in staphylococcal infections

Case report	Phage treatment and route of administration	Outcomes	References
Staphylococcal enterocolitis	combined therapy: nifuroxazide + "bacteriophage 1" or azithromycin + "bacteriophage 2", orally (10-12 days)	the duration of symptoms of staphylococcal enterocolitis in children receiving combination therapy was 1.2-1.4 times less than in the group of children receiving only antibacterial drugs	[Ayzenshtadt A., et al., 2018]
Ulcerative foot osteomyelitis	Staphylococcal bacteriophage suspension, injectable (once a week during 7 weeks)	ulcer healing	[Kutter E., et al., 2018]
Recurrent prosthetic joint infection caused by *S. aureus*	Cocktail of 6 bacteriophages, topically	no clinical signs of persistent infection were observed	[Nepal R., et al., 2021]
Diabetic foot ulcer, monoinfected with MRSA and MSSA,	AB-SA01 phage cocktail (J-Sa36, Sa83, and Sa87) intravenously	led to positive changes in 83% of patients	[Goerke C., et al., 2009]
Chronic dermatoses of staphylococcal etiology	Bacteriophage „Staphefekt"	Clinical response for elimination of *S. aureus*	[Lysko K. A., et al., 2013]
Prophilaxis of respiratory infections	Staphylococcal bacteriophage, orally (21 days)	The dynamics of the incidence of acute respiratory infections decreased by 1.7 times	[Akimkin V. G., et al., 2016]
Prophilaxis of respiratory infections (staphylococci were isolated in 25% of people includet in study)	"Sexta-phage", orally (21 days)	No staphylococci were isolated from any person	[Akimkin V. G., et al., 2016]

In a study conducted between January 2017 and December 2018 at an infectious diseases hospital in the city of Astrakhan, the results of treating 158 children aged 1 to 12 months diagnosed with staphylococcal enterocolitis were analyzed. The elimination of staphylococcus on days 10-12 of combined therapy (nifuroxazide + "bacteriophage 1" or azithromycin + "bacteriophage 2") was

achieved in 21 (51%) and 24 (64%) patients, respectively, while with monotherapy (nifuroxazide or azithromycin), it was achieved in 12 (31%) and 14 (35%) patients, respectively. It was found that the efficiency of staphylococcus elimination increased during azithromycin therapy when combined with "bacteriophage 2". At the same time, the duration of symptoms of staphylococcal enterocolitis in children (fever, diarrhea) who received combination therapy was 1.2-1.4 times shorter than in the group of children who received only antibacterial drugs. As a result of combination therapy, by 10-12 days from the start of treatment, there was also a significant (1.7-2 times) decrease in the number of patients with lactase deficiency, while with monotherapy with antibacterial drugs it was 1.3-1.4 times ($p<0.05$). By 10-12 days from the start of treatment, the proportion of patients with intestinal dysbiosis who received combination therapy decreased by 1.4-1.8 times, and with monotherapy it decreased by 1.3 times ($p<0.05$) (Ayzenshtadt & Sadovnikova, 2018).

In the presence of *S. aureus* in the body, a number of studies have demonstrated the effectiveness of phages specific to it and endolysins through various animal experiments, followed by clinical trials. For instance, animal models of joint prosthesis infection have been utilized to evaluate the effectiveness of the φMR-5 phage in combination with linezolid to reduce bacterial adherence, resulting in a quicker recovery of lower limb motor function (Gupta & Prasad, 2011). A recent publication documented the successful use of a highly purified staphylococcal bacteriophage for treating ulcerative foot osteomyelitis in a diabetic patient who declined amputation and/or long-term antibiotics. Injections (0.7 mL) containing the phage suspension were administered once a week for 7 weeks until the ulcer healed (Kutter et al., 2018). Similarly, topical injection of a selected cocktail of 6 bacteriophages infecting *P. aeruginosa* and *S. aureus* was employed in a patient with a recurrent *S. aureus* prosthetic joint infection. In this instance, the treatment was deemed safe, and no clinical signs of persistent infection were observed (Nepal et al., 2021).

AmpliPhi Biosciences Corporation is also advancing an expanded *S. aureus* control program through the development of the bacteriophage drug AB-SA01, specifically designed to treat severe infections. Their studies have demonstrated that AB-SA01 therapy was well tolerated, with virtually no side effects, and resulted in positive changes in 83% of diabetic foot ulcer patients monoinfected with both methicillin-resistant (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) (Goerke et al., 2009). The treatment of chronic dermatoses of staphylococcal etiology with Staphefekt phage endolysin and *Pseudomonas aeruginosa*, sensitive only to colistin, along with a bacteriophage preparation, was also highly successful. The gold standard for clinical trials is randomized clinical trials (RCTs). Thus far, only 1 RCT (the PhagoBurn study) has been published using a phage cocktail according to GMP ("good manufacturing practice") and GCP ("good clinical practice") guidelines against *E. coli* and *P. aeruginosa* burn wound infections (Lysko et al., 2013).

The PhagoBurn study was conducted at burn wound centers in France and Belgium. This study highlights the importance of phage stability and composition, as phage titers decreased after formulation, and patients in the experimental group received formulations with lower titers than expected. Despite the slow clinical effect in the phage therapy group compared to the group receiving standard treatment (sulfadiazine, silver cream), there was a corresponding decrease in bacterial load and fewer side effects (Lee et al., 2018). The development of anti-staphylococcal phage cocktails has shown that mixtures with six different phages are effective against the most common resistant strains (Alvarez et al., 2019).

For the treatment of multiresistant staphylococci in life-threatening cases, antibiotics based on methicillin and vancomycin are typically used, but strains resistant to them have recently emerged. Fortunately, there is both old and new clinical data confirming the existence of numerous

virulent staphylococcal phages, such as the φ812 phage, capable of infecting hundreds of strains of *S. aureus* (Alvarez et al., 2019; Brix et al., 2020). Such sensitivity to phages is not characteristic of other bacteria and makes staphylococcal infection particularly suitable for phage therapy (Luong et al., 2020).

It has recently been demonstrated that, irrespective of a bacterium's antibiotic resistance status, sublethal doses of some antibiotics increase the degree of phage virulence. In particular, they synergistically increase the number of viruses produced and reduce the density of bacteria. It has been shown that the mechanism underlying the phage-antibiotic synergy (PAS) effect is associated with triggering cell elongation under the action of antibiotics, which promotes phage replication and potentially facilitates external attachment to bacteria due to increased cell surface area (Nepal et al., 2021).

Let us consider in detail another study on the antibacterial efficacy of two commercially available compositions of *Staphylococcus aureus* bacteriophages, specifically strain MRSA ATCC 43300. Following calorimetric analysis, 50 μL of culture, along with corresponding 10-fold serial dilutions, were plated on blood agar for colony counting. The minimum bactericidal titer of phages was defined as a decrease in the number of bacteria by more than 3 log10 CFU/mL (colony-forming units per mL) compared to the initial amount of inoculum in CFU/mL. Phage lytic activity against methicillin-resistant *Staphylococcus aureus* biofilms was determined using isothermal microcalorimetric (IMC) testing and sonication. IMC analyses were recorded for 48 hours at 37°C. The minimum heat inhibitory concentration of phage (MHICP) was defined as the lowest titer inoculated with bacteria at the starting point of the experiment that inhibited growth-related heat production during 24 hours of microcalorimeter incubation by more than 90% compared to the untreated control (growth control) (Kondo et al., 2021).

The viability of MRSA was examined in real time for 24 hours using IMC, measuring the heat generated by MRSA in the presence of phages and counting the CFU after treatment with phages. An untreated growth control was also added. Sb and PYO bacteriophages rapidly inhibited MRSA growth in a titer-dependent manner compared to untreated growth control. Indeed, heat generation was not observed in the presence of 107 plaque-forming units per mL (PFU/mL) of both phage formulations during 24 h of incubation, indicating that the 107 PFU/mL titer corresponds to MHICP (Kondo et al., 2021).

The interaction between bacteriophages and biofilms

S. aureus was also analyzed in real time using microcalorimetric measurements. Both Sb (monophage) and PYO (polyphage) inhibited bacterial replication in a titer-dependent manner compared to the growth control, resulting in the suppression of heat production during 48 hours of incubation. Simultaneously, a decrease of more than 90% in the total amount of heat generated by cells embedded in the MRSA biofilm was observed at titers of 107 PFU/mL for both phage preparations. This study strongly suggests that PYO and Sb bacteriophages show promise for preventing bacterial colonization and disrupting previously formed biofilms. In the future, further research should explore the potential of phages against other clinical strains in more detail and evaluate the effectiveness of treating systemic infections using complex models involving laboratory animals (Cisek, Dąbrowska, Gregorczyk, & Wyżewski, 2017).

Within the framework of this study, military personnel were divided into groups. The first group comprised individuals who received streptococcal bacteriophage for 21 days in the form of a solution for oral administration, as well as for local and external use. The second group consisted of

individuals who received staphylococcal bacteriophage in the form of a solution for oral administration. In the third group, subjects received "Sextaphage," also in the form of a solution for oral administration, over the same period. The fourth group included individuals who received a single injection of bicillin-5 (Akimkin et al., 2016).

It was observed that after the prophylactic course, the number of isolated streptococci in Group No. 1 decreased by 2.4 times. In the second group, streptococci predominated in the structure of isolated cultures, being isolated in 23 (71.9%) individuals: *S. pneumoniae* in 5 (15.6%) and *S. pyogenes* in 18 (56.3%). *S. aureus* was isolated from 9 (28.1%) individuals. However, after the prophylactic course, the number of isolated staphylococci in the second subgroup decreased by 1.8 times, while a combined carriage of *S. pneumoniae* and *S. aureus* (8.2%) was detected. The incidence of acute respiratory infections in the second group significantly decreased by 1.7 times after the prophylactic course of staphylococcal bacteriophage. In the third group, streptococci dominated initially in 12 (75.0%) individuals, while 4 (25.0%) had *S. aureus*. After a prophylactic course, *S. pneumoniae* was isolated only in 8 servicemen, and other pathogens were not detected. Meanwhile, no statistically significant differences were found in the structure of pathogenic cultures isolated from individuals of groups 4-1 before the use of bicillin-5 and after its single administration (Akimkin et al., 2016).

Discussions

This synthesis emphasizes the importance of phage therapy in the treatment of infections caused by *S. aureus*. *S. aureus* is a common pathogen responsible for both healthcare-associated and community-acquired infections worldwide. It is one of the most significant pathogens in terms of antimicrobial resistance, as it has developed resistance mechanisms against nearly all agents used against it. This species can readily demonstrate adaptive evolution to antimicrobials, as it has shown a unique ability to rapidly respond to each new antibiotic by developing resistance mechanisms, from penicillin and methicillin to the latest ones like linezolid and daptomycin (Foster, 2017).

Given the increasing number of documented studies on bacteriophage therapy, it emerges as a promising alternative for treating bacterial infections caused by multidrug-resistant or antimicrobial-sensitive microorganisms. However, due to the lack of data from controlled randomized trials, phages are currently primarily used in the treatment of severely ill patients who have not responded to conventional therapies. Nevertheless, the potential applications of phages are diverse, and they may serve as substitutes for antimicrobials. By employing phages in the treatment of infections caused by antibiotic-resistant bacteria and limiting the use of antibiotics, we can potentially prevent the emergence of further resistant strains. Bacteriophages have applications not only in human health but also in animal health and environmental contexts (Fernandez et al., 2018; Buttimer et al., 2017; Chan & Abedon, 2015).

Multiple studies have demonstrated the high efficacy of bacteriophages against various strains of *S. aureus*, including MRSA. Despite the use of different phage doses, administration methods, and infection forms in these studies, no negative side effects have been described. It is worth noting that the analyzed studies have shown that the antibacterial effect can be enhanced through improved phage administration methods, the use of phage cocktails, or their combination with antibiotics, as well as through their use in prophylaxis. Additionally, regulated procedures for phage production are necessary to ensure their safe clinical use, as the effectiveness of phage therapy largely depends on maintaining phage stability.

The development of bacterial resistance to bacteriophages is indeed possible, as bacteria have the capacity to evolve various mechanisms to prevent viral infections (Seed, 2015). For example, the *S. aureus* protein A located on the surface of the bacterial cell inhibits the adsorption of bacteriophages (Nordström & Forsgren, 1974). Combining antibiotics with bacteriophages, using phage cocktails, or administering a higher quantity of phages can minimize the development of bacterial resistance to phages. If bacteriophages can kill pathogens faster than they can reproduce, using a large inoculum is associated with a lower risk of bacteria developing resistance to phages. Therefore, when selecting therapeutic phages, it is essential to consider each phage's ability to create bacterial resistance and estimate the necessary dosage to prevent the formation of bacterial resistance.

Another significant issue regarding phage therapy for *S. aureus* infections is the lack of clinical studies, specifically the absence of double-blind randomized trials. Current treatments primarily rely on case reports or clinical studies with a small number of patients.

Furthermore, studies are needed to evaluate whether phages should be used alone, in cocktails, or in combination with antimicrobials. Due to the potential synergistic effect of combined treatment demonstrated in some studies, it has the potential to be used in medical practice to mitigate the development of antimicrobial resistance. The therapeutic outcome of combined therapy applied to *S. aureus* infections has, in most cases, shown synergy, as demonstrated by a decrease in bacterial concentrations and a substantial increase in survival rates. This underscores that the dosage, administration method, and choice of antimicrobial can influence treatment effectiveness.

Conclusions

The antibiotic resistance of *Staphylococcus aureus* remains a significant problem in modern medicine, the relevance of which continues to increase every day with the emergence of new resistant strains. Methods of therapy using bacteriophages against antibiotic-resistant strains of *Staphylococcus aureus* have recently gained increasing usage both in our country and worldwide, serving as one of the main alternatives to antibacterial drugs.

Bacteriophages destroy pathogens without interacting with human body cells at all; therefore, patients do not require subsequent intake of drugs for intestinal dysbacteriosis. Additionally, phages do not suppress immunity and do not have a toxic effect on the liver and kidneys.

It was found that regardless of the bacteria’s resistance state to antibiotics, sublethal doses of some antibiotics increase the virulence of phages. Specifically, they enhance the production of viruses and decrease bacterial density synergistically. It is also important to note that the use of bacteriophages in combination with antibacterial drugs can yield an even greater effect in treating these diseases, highlighting the need for a further search and comprehensive study of bacteriophages with inhibitory effects on *S. aureus*.

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