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O.V. SHEVCHENKO[1*], A.V. KHARINA[1], N.O. KORNIIENKO[1], I.H. BUDZANIVSKA[1], O.M. ANDRIICHUK[1], I.M. POZHYLOV[1], H.O. SNIHUR[1], O.B. LIUTKO[2], O.M. LINENKO[3], I.I. MOYSA[3], V.A. PONIATOVSKYI[4]

[1] Virology Department, ESC «Institute of Biology and Medicine», Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska Str., Kyiv, 01601, Ukraine
[2] The Laboratory of Microbiology and Chemotherapy, State Institution «The Institute of Traumatology and Orthopedics» by NAMS of Ukraine, 27 Bulvarno-Kudriavska Str., Kyiv, 01601, Ukraine
[3] The Department of Bone and Purulent Surgery, State Institution «The Institute of Traumatology and Orthopedics» by NAMS of Ukraine, 27 Bulvarno-Kudriavska Str., Kyiv, 01601, Ukraine
[4] Department of Microbiology and Parasitology with the Basics of Immunology, Bogomolets National Medical University, 34 Peremohy Avenue, Kyiv, 03057, Ukraine
* Author for correspondence; e-mail: alexshevchenko@ukr.net, alexshevchenko@knu.ua

PHAGE THERAPY IN TRAUMATOLOGY: A REVIEW ON PERSPECTIVES FOR TREATING ACUTE WOUNDS AND POST-SURGICAL COMPLICATIONS

Full-scale hostilities in Ukraine led to an unprecedented number of victims with serious injuries, including gunshot wounds, broken bones, and mine-explosive injuries. Wound infections are one of the main causes of non-combat losses of personnel. A prerequisite for the development of a wound infection, among other things, is high microbial contamination of combat wounds. Major bacteria causing such infections are staphylococci (Staphylococcus aureus, S. epidermidis), streptococci (Streptococcus pyogenes, St. agalictiae), enterococci (Enterococcus faecalis), gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii), anaerobic bacteria (Clostridium perfringens, Bacterioides spp.), etc. Modern data indicate a change in the current species composition of causative agents of wound infections, an increase both in the polyresistance of the microbiota to antibacterial drugs and in the frequency of biofilm formation protecting pathogenic microorganisms from antimicrobial therapy and the patient's immune response. Such purulent bacterial infections require new approaches to therapy. Taking into account the large

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number of combat injuries in Ukraine, the use of bacteriophages as specific agents for the biological control of pathogenic microbiota is of particular importance. In view of the nature of injuries, phage preparations intended for the treatment of purulent-surgical infections of wound and burn surfaces are considered the most promising and in demand today. This review summarizes modern data on the use of phage preparations in clinics and in orthopedics and traumatology in particular. Issues of the delivery of phages to the focus of infection, their effective dose, duration of therapy, and the possibility of combining it with antibiotic therapy are discussed. Information about phage therapy programs that have already been implemented in some countries is presented. The advantages and disadvantages of the use of bacteriophages for personalized therapy of severe patients are highlighted, and the prospects for further research are indicated.

***Keywords**: pathogenic microbiota, bacteriophages, phage therapy, bacterial complications, purulent wounds, gunshot and mine-explosive injuries, antibiotic resistance.*

The last two years of full-scale hostilities on the territory of Ukraine witnessed an unprecedented increase in the number of victims with severe wound infections or bacterial complications. Such purulent complications are difficult to treat and often cause not only a prolonged stay of patients in hospital treatment due to the chronicity of the pathological process but also fatal consequences, which determines the need for new approaches to their therapy.

In the case of a complicated course of such pathologies, antibiotics must be included in the treatment regimen. The factors that reduce the therapeutic value of antibiotics are the toxic effect on the patient's body, the development of allergic reactions, the negative effect on the normal microbiota, the decrease in the sensitivity of bacteria, and the appearance of their resistant forms.

Currently, the efforts of many scientists and medical workers are aimed at developing strategies to reduce the use of antibiotics. On the other hand, the problem of gunshot and mine-explosive injuries with the subsequent development of septic complications is far from being solved today, and against the background of the armed aggression of the russian federation (RF), it requires the involvement of all possible therapeutic means.

In this context, bacteriophages are considered as an alternative or addition to antibiotics, and sometimes even as the only possible means of controlling the causative agents of post-traumatic complications (due to the massive development of antibiotic resistance). Bacteriophages are viruses that specifically (via receptor-mediated process) infect bacterial cells, which makes them attractive agents for designing antibacterial agents. Due to the high specificity of phages, the emergence of antibiotic-resistant strains of bacteria is unlikely or delayed. Unlike antibiotics, phage drugs can be rapidly modified in response to changes in the consortium of pathogenic bacteria and their sensitivity. Finally, bacteriophages can be used even along with antibiotics to combine the characteristics of personalized (targeted) and mass medicine (Gu Liu et al., 2020).

Bacteriophages have been successfully used for treatment as early as during the World War II, but then they were practically not used in surgical practice after the discovery of antibiotics (Summers, 2012). The high specificity of bacteriophages, surprisingly, is also the main problem of their effective use. Unlike antibiotics, which have a broad spectrum of action and are generally not characterized by high specificity for microorganisms (which at the same time ensures their effectiveness and ease of use), bacteriophages will be effective only if they are used against specific species (and sometimes strains) of bacteria (Gordillo Altamirano & Barr, 2019). Because of this, screening of patients and identification of pathogenic microbiota present in most of them are important prerequisites for effective phage therapy, which could replace or amend antibiotic therapy. Actual bacterial isolates should also be identified and tested for antibiotic susceptibility. This will allow the identification of bacterial pathogens dominating the microbial consortium for the subsequent search for highly specific lytic

bacteriophages for each (or most) of such pathogens. Isolation of bacteriophages can be carried out from various sources: clinical samples from patients, washings from their wounds or hospital surfaces, or even sewage. Ideally, for each bacterium, it is desirable to select several isolates of bacteriophages with their subsequent detailed study, since not all bacteriophages are lytic and/or effective in a specific field of application.

Bacteriophages isolated in this way can become components of rational treatment of purulent-inflammatory processes after gunshot wounds, bone fractures, and mine-explosive injuries of various nature, as well as other postoperative complications. In addition, bacteriophages can be safely used prophylactically to effectively prevent complications during the development of purulent-septic processes.

Injuries and wound infections as the main cause of hospitalization and mortality. Injuries of any nature are often accompanied by chronic complications, remain the leading cause of death (Cornett et al., 2016), and cause significant economic losses, amounting to billions of US dollars per year in the United States alone (Peterson, 2021). Similarly, the annual cost to the UK public health system (NHS) of wound care alone was £8.3 billion per year (Guest et al., 2020). In Ukraine, monthly insurance payments due to injuries and loss of working capacity as of 2023 exceed UAH 1 billion (Kyrylenko, 2023). This figure includes exclusively injuries of 'peaceful life' without taking into account combat wounds, the treatment of which is currently extremely relevant for Ukraine.

There is no publicly available up-to-date information on the number of military and civilian people with gunshot or mine-explosive injuries in Ukraine. However, according to indirect estimates, as of August 2023, during the full-scale war, at least 200,000 people were seriously injured, and 20,000—50,000 people needed limb amputations (Pancevski, 2023). It is obvious that these numbers are currently only growing, fully demonstrating the global nature of this problem and the urgency of its solution.

In the structure of medical and sanitary losses during hostilities in Ukraine, injuries to the limbs accounted for 57—62%, burns — for 2,7% of all injuries (Khomenko et al., 2021), and 40—43% of this amount was injuries to the soft tissues of the limbs. The full-scale hostilities on the territory of Ukraine, which have been ongoing since February 2022, led to an unprecedented increase in the number of victims with serious injuries, including gunshot wounds, broken bones, and mine-explosive injuries of various types.

Primary surgical treatment of the wounds has an important role and involves wound sanitation, antibiotic therapy, and installation of drains when the wound is not sutured but covered with dry bandages or wetted antiseptic solutions to maintain access to the deep parts of the wound (Zavhorodnii et al., 2023).

Traumatic wounds, superficial burns, and the consequences of surgery are usually considered acute wounds. Wound infections complicate the postoperative recovery of patients and are the main reason for increasing the cost of treatment (Sen, 2021). The development of new and practical concepts for the prevention and treatment of these wound infections is the key to the effective treatment of wounds, including combat injuries.

Wound infections are one of the main causes of non-combat loss of personnel. A prerequisite for the development of a wound infection, among other things, is the high microbial contamination of a gunshot or mine-explosive wound of bone and muscle tissue. Bacteria quickly colonize open wounds after trauma (Roy et al., 2014, Chaney et al., 2017, Roy et al., 2020) or surgery. Such organisms are usually the normal biota of the patient or may be transmitted through contact with contaminated water, fomites, or the hands of healthcare workers, instruments, etc. (Abbas et al., 2020). Common pathogens that can cause acute wound infections include gram-positive and gram-negative bacteria, as well as

microscopic fungi (*Candida* spp. and *Aspergillus* spp.). Some of these bacteria may be resistant to antibiotics (Flowers & Grice, 2020; Mosselhy et al., 2021). Medical and scientific reports indicate a change in the actual species composition of causative agents of wound infections and a threatening rate of selection of antibiotic-resistant microorganisms (Sandar et al., 2021; Klyackiy et al., 2022; Kharina et al., 2023).

Difficulties in the treatment of patients with purulent surgical infections are caused by the increase in multidrug resistance (MDR) of pathogenic microbiota, and by the frequent occurrence of microbial associations forming biofilms. The biofilm protects pathogenic microorganisms from antimicrobial therapy and the patient's immune response. Bacterial consortia of wound surfaces are often associated with so-called chronic wounds (Roy et al., 2020; Kharina et al., 2023) increasing the length of stay of patients in the hospital.

Such purulent bacterial infections of wound surfaces are the cause of increased morbidity and mortality after gunshot and blast injuries (Wu et al., 2019; Stewart et al., 2020; Infectious Disease Clinical Research Program, 2020), are difficult to treat, and require new therapeutical approaches. Some promising new approaches to treat such injuries and bacterial complications include nanotheranostics (the use of nanoparticles and fibers as a combined diagnostic and therapeutic agent against antibiotic-resistant and biofilm-forming bacteria) (Mosselhy et al., 2021) and electrotherapy (to physically disrupt bacterial associations) (Barki et al., 2019). In the context of the existing problem and taking into account the large number of combat injuries in Ukraine, the use of bacteriophages as specific and promising agents for the biological control of pathogenic microbiota is gaining particular importance (Secor et al., 2020; Taati Moghadam et al., 2020).

Bacterial pathogens dominating wound consortia. The development of pathological processes of soft tissues and bones can be caused by various microorganisms. According to the literature, the main bacteria that are often identified as the cause of soft tissue and bone infections are staphylococci (*Staphylococcus aureus*, *Staphylococcus epidermidis*), streptococci (*Streptococcus pyogenes*, *Streptococcus agalictiae*), gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*), and anaerobic bacteria (*Clostridium perfringens*, *Bacterioides* spp.). However, this list is not exhaustive.

S. aureus and coagulase-negative staphylococci (CoNS), such as *S. epidermidis* and *S. lugdunensis*, account for a significant proportion of bacterial complications in traumatology and orthopedics, with *S. aureus* considered the most common individual pathogen (Motififard et al., 2021). *S. aureus* is capable of causing osteomyelitis and abscesses and is often found in the form of methicillin-resistant strains (MRSA), making treatment difficult (Urish & Cassat, 2020). *S. epidermidis* is frequently associated with implant-related infections (Lu et al., 2022).

Among the agents inducing infections of the musculoskeletal system, *P. aeruginosa* (a significant cause of osteomyelitis) remains one of the most important pathogens endangering human life due to its high virulence and adaptability to environmental effects, in particular to antibacterial drugs, as evidenced by the development infectious processes of varying severity (Pliska, 2020). *P. aeruginosa* is commonly found in hospital settings and poses a particular problem for patients with weakened immune systems or chronic wounds, which is of specific importance for Ukraine today.

Not the last role in the development of infections of the musculoskeletal system is attributed to *E. coli* — one of the most common commensal human inhabitants. Adaptation of *E. coli* under the influence of stressful conditions (for example, in bone tissue cells) may involve changes in genome implementation and some important changes in the metabolism and balance of virulence/resistance of the bacteria (Lienard et al., 2021).

Acinetobacter baumannii has become a common cause of infection specifically associated

with military traumas (Sebeny et al., 2008) and can cause a variety of infections. In traumatology, this microorganism is often associated with wound infections, especially after surgical interventions. One of the biggest problems with *A. baumannii* is its quick adaptation and development of wide antibiotic resistance.

Data on the prevalence of certain bacteria causing complications in various injuries may vary, but the list of the main bacterial agents in infectious processes of soft and bone tissues is only occasionally enlarged with new representatives. As an example, Jain et al. have shown that the most common pathogens were *S. aureus* (28,9%), *P. aeruginosa* (9,4%), *K. pneumoniae* (7,5%), mycobacteria (3,8%), coagulase-negative staphylococci (1,3%), MRSA (1,3%), and other bacteria (4,3%) (Jain et al., 2015). In contrast, according to Eisner et al., the following bacteria were identified in patients after open or arthroscopic surgery due to recent trauma with subsequent surgical site infection: *S. aureus* (27,1%), *S. epidermidis* (20,6%), *Enterococcus faecalis* (13,6%), *E. coli* (5,1%) and *P. aeruginosa* (3,7%) (Eisner et al., 2020). Hence, different dominant pathogens have been noted in various studies, which affects the choice of both treatment regimens and active antibacterial drugs. Such differences in the bacterial consortium will also affect the selection of sensitive bacteriophages.

According to various estimates, infection in the area of surgical intervention in traumatology under civilian conditions is observed in 2—4% of cases (Dash & Mohapatra, 2020), but it can reach 50%. This problem is significantly aggravated in case of combat injuries. Combat wounds have several unique characteristics that distinguish them from civilian injuries. From the microbiology point of view, there are several key aspects characterizing combat wounds:

1. Mechanisms of injury. Combat wounds are often resulted from the blast injuries, small arms, shrapnel, and melee combat. These factors can lead to particularly severe and complex injuries.

2. Multiplicity and complexity of wounds. In combat conditions, multiple wounds often occur, which can be of different types and located in various parts of the body, which complicates diagnosis, assessment, and treatment.

3. High risk of infection. Combat wounds are often contaminated with debris, clothing, and dirt, which increases the risk of developing infections, including severe and resistant to treatment.

The main principle of military field surgery is based on the fact that a gunshot wound always contains an initial mixed microbiota (initial microbial contamination), as well as necrotic tissues creating favorable conditions for the development of an infectious process (Zarutskyi & Shudrak, 2014). Therefore, combat injuries require a special approach to the treatment and prevention of infections, taking into account their unique nature and potential complications.

In view of the RF's military aggression since 2014, studies of the microbial landscape of combat wounds have been conducted in Ukraine, and dynamic changes of different bacteria inducing the development of infectious processes have been recorded. Accordingly, studies of infection of limb wounds received in combat conditions showed that the main pathogens in the first stages of the development of the pathological process were rod-shaped gram-negative non-fermenting bacteria (68%), of which *Acinetobacter* spp. accounted for 53% of cases and *Pseudomonas* spp. — 15%. Gram-positive cocci were found in 24% of cultures. Among all bacteria, regardless of the time of sampling after wounding, most isolates (79,5%) showed polyresistance to antibiotics, which was especially dramatic for gram-negative non-fermenting bacilli (Zheliba et al., 2019). The authors also noted changes in the microbial landscape at various stages of the development of the infectious process.

Recently published data on patients with purulent-inflammatory processes of soft tissues showed that staphylococci were the most common among the identified microorganisms:

S. aurerus was found dominant in 55.6% of patients. The authors also noted the increasing role of *S. epidermidis* in 30.8% of patients and *S. faecalis* in 15.5% of patients. Among gram-negative bacteria, *E. coli* prevailed (76.4%), whereas *Proteus* sp. (11.7%), *P. aeruginosa* (3.7%), and *K. pneumoniae* (1.9%) were much less common. Importantly, only a single type of bacteria was identified in 44.7% of patients suggesting monoinfection, while associations of microorganisms were common in the wounds of the remaining patients, which greatly complicated their therapy (Solomenny, 2021).

The high frequency of development of purulent-inflammatory processes and the significant prevalence of postoperative purulent complications put the issue of their prevention and treatment at the forefront in the field of modern military and civil surgery. Difficulties in treating patients with purulent surgical infections are associated with both the spread of microorganisms resistant to many antibacterial drugs and the increase in cases of detection of microbial consortia. This highlights the critical need to study the species composition of pathogens causing surgical and post-traumatic infections, analyze their susceptibility to antibiotics, and develop new approaches to combat bacterial infections.

Phage therapy as an alternative or addition to antibiotics in the treatment of purulent bacterial complications of soft and bone tissues. Phage therapy traditionally focuses on the use of phages to eliminate disease-causing bacteria. Phages have been used as antimicrobial agents since the 1900s (D'Herelle, 1917). The first proposals and reflections on the use of bacteriophages for the treatment of purulent joint infections and osteomyelitis appeared as early as 1933 (Albee, 1933), when a protocol for treating a wound with a suspension of bacteriophages through a rubber catheter once or twice a week was described. Phages were widely used in the treatment of infections caused by various wounds during World War II (Chang et al., 2020).

The experience of using phage preparations as therapeutic agents is very extensive. It should be noted that historically phage drugs were most widely used in the treatment of acute intestinal infections (particularly in children) and purulent-inflammatory diseases of various localization, especially bacterial insemination of burn wounds (van der Vlugt & Verbeek, 2008).

Nevertheless, after the discovery of antibiotics (penicillin), the research and use of bacteriophages in therapy gradually lost popularity (Fleming, 1929; Fujiki et al., 2023). However, as mentioned above, the widespread use of antimicrobial drugs over time has led to a number of problems related to their negative impact on normal human microbiota and the rapid emergence and spread of resistant forms of bacteria.

Today, bacterial infections that are resistant to one or a range of antibiotics are becoming more and more common. This has led to the renewed interest of scientists in personalized therapy of difficult-to-treat bacterial complications using bacteriophages.

Phage therapy programs have been developed and approved in many countries, including the United States, Belgium, France, Sweden, Poland, and Georgia (Jikia et al., 2005; Petrovic Fabijan et al., 2020; Strathdee et al., 2023). For example, phage therapy is approved in Belgium as an alternative when antibiotics and surgery are not enough to eradicate a bacterial infection (Pirnay et al., 2018; Verbeken & Pirnay, 2022). In Ukraine, the «Pyophage» and «Intestiphage» preparations are registered and used. These preparations include bacteriophages specific for such opportunistic microorganisms as *S. pyogenes*, *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, and *P. mirabilis* (https://bacteriophages.info/).

Below is a brief overview of worldwide trends in phage therapy. One of the biggest problems in clinics is controlling purulent inflammatory infections resulted from trauma or surgical complications, as well as from burn wounds and, in today's realities, combat injuries. The study of

the etiology of purulent inflammatory processes in orthopedic/trauma patients and patients with burns indicated that the wounds are usually contaminated with microbial associations, most of which are resistant to widely used antibiotics. Based on this, phage preparations intended for treatment of purulent surgical infections of wounds and burn surfaces caused by *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Proteus*, etc., are considered the most promising and in demand, as discussed above (Motififard et al., 2021; Urish & Cassat, 2020; Lu et al., 2022; Pliska, 2020; Lienard et al., 2021; Sebeny et al., 2008; Jain et al., 2015; Eisner et al., 2020; Dash & Mohapatra, 2020; Zarutskyi & Shudrak, 2014; Zheliba et al., 2019; Solomeny, 2021) primarily due to the difficulty of their effective control by other means alone.

The widespread complex bacterial nature of various pathologies (presence of different strains and often different species of pathogens) necessitates the development of complex preparations, so-called phage cocktails, which would contain a mixture of different phages against one or different strains/species of microbial agents causing diseases of a complex nature.

Typically, the major problems are therapy of open wounds and healing of postoperative interventions. Given the study of the microbiological profile of infected surfaces, most of the effort in phage therapy has historically been directed at fighting staphylococcus. For instance, Slopek et al. reported 92.4% positive cases of phage therapy of 550 cases of infections of mono- and mixed etiology involving *S. aureus*. The effectiveness of such treatment of purulent staphylococcal infections in adults was 93% and in children 95.5% (Slopek et al., 1987). Similar results were obtained by other scientists in the treatment of chronic infections caused by *S. aureus*. Clinical improvement was observed in half of the patients after using the phage preparation without concomitant antibiotic treatment, indicating high efficiency of phages (Lecion et al., 2013; Abedon et al., 2021).

In 2005, Jikia et al. published the first results of the use of bacteriophage-based preparation 'PhagoBioDerm' (Intralytix Inc., USA) for the treatment of various bacterial infections of wounds, burns, and bedsores. A phage-based antimicrobial wound dressing was used, resulting in rapid clinical improvement within a week in patients suffering from antibiotic-resistant *S. aureus* infections (Jikia et al., 2005). The use of phage therapy for treating severe infections caused by *S. aureus* led to clinical improvement in 62% of patients without any side effects (Petrovic Fabijan et al., 2020).

In addition to phages active against *S. aureus*, similar preparations were also developed for eliminating other associated bacteria. In particular, in Belgium and France, phages were used to treat burn wound infections caused by *P. aeruginosa*. A cocktail of 12 phages (PP1131) was used for local application. A stable decrease in bacterial load was observed during treatment with phages. The results of the study indicated the effectiveness of phage antibacterial therapy, despite the problems associated with the instability of phages during their storage before treatment (Jault et al., 2019).

Similarly, treatment of antibiotic-resistant *P. aeruginosa* on burn wound surfaces has been reported. Bandages impregnated with phage preparations were used for therapy. In half of the clinical cases, an «effective action» was registered. In some cases, the infection was eliminated (Abdul-Hassan et al., 1990).

A serious concern with burn wounds is infection that often prevents skin transplantation. Using an animal model, it was shown that the application of phages to wounds before transplantation can prevent their contamination with *P. aeruginosa*. Moreover, it was found that phages did not prevent wound healing and even promoted healing (Soothill, 1994).

In 2008, a successful trial was completed evaluating phage therapy against skin ulcers and other wounds using the complex «Pyophage» prepara-

tion developed at the George Eliava Institute of Bacteriophages (Georgia). A set of phages from Intralytix (containing two phages against *S. aureus*, five phages against *P. aeruginosa*, and one phage against *E. coli*) was used for treating chronic infections. The high efficiency of this phage cocktail was confirmed by the absence of side effects (Wright et al., 2009; Bruttin & Brüssow, 2005; Abedon et al., 2011).

Since monotherapy using exclusively phages revealed a number of potential shortcomings in practical application (narrow range of hosts, emergence of resistant phenotypes, instability of phage preparations, etc.), combined phage-antibiotic therapy (PAS) was proposed as an alternative. In such a study, a model PAS containing phage vB_AbaM-IME-AB2 and colistin was used to develop stable wound dressings and reduce in-

Table 1. **Clinical studies of phage therapy in patients with bone, joint, and post-prosthetic infections**

Diagnosis	Etiological factor	
Recurrent infection of the prosthetic joint of the right hip	MDR *P. aeruginosa,* methicillin-sensitive *S. aureus*, *E. faecalis* and *S. lugdunensis*	
Distal phalanx osteomyelitis, diabetes	Methicillin-sensitive *S. aureus*	
Infection in the right sacroiliac joint after cementoplasty in a patient with lung cancer	Methicillin-sensitive *S. aureus*	
Postoperative infection with cerebritis and subdural and epidural empyema	MDR *A. baumannii*	
Recurrence of periprosthetic infection of the right knee joint and chronic osteomyelitis of the femur after a gunshot wound ?	MDR *P. aeruginosa*	
Severe infections of the musculoskeletal system (pelvis/femur), osteomyelitis	*P. aeruginosa, S. epidermidis, S. agalactiae, S. aureus, E. faecalis*	
Infection of the left tibia due to trauma	High-resistant *A. baumannii,* MDR *K. pneumoniae*	
Recurrent infection of the knee prosthesis caused by *S. aureus*	*S. aureus*	
Chronic osteomyelitis, diabetic foot ulcer, severe infectious complication after skin transplantation	*S. aureus, Burkholderia cepacia, E. faecalis, P. aeruginosa*	
Infection of the knee joint prosthesis	Methicillin-sensitive *S. aureus*	
Recurrent infection of the knee prosthesis	*P. aeruginosa*	
Persistent infection of the knee joint prosthesis	*K. pneumoniae*	

*MDR — Multidrug-Resistant

fections associated with *A. baumannii*. The obtained positive results confirmed the perspective of further use of PAS in the form of a local wound dressing (Mukhopadhyay et al., 2024).

In orthopedics, purulent inflammatory infections and complications become even more important due to the complex process of drug delivery to the site of the lesion, which often is not superficial. Such pathologies include bone infections (osteomyelitis), infections of joints (septic arthritis) or implants associated with these structures (periprosthetic joint infections), and infections associated with metal osteosynthesis. The ineffectiveness of treatment of such infections occurs in 10—20% of cases (Al-Mayahi et al., 2013; Senneville et al., 2011), reaching 28% among patients with osteomyelitis (Barshes et al., 2016). The mortality of such patients remains

Treatment	Phage therapy results	Reference, country
Administration of phage solution into the joint; antibiotic therapy	Full recovery of the patient	Ferry et al., 2018a, France
Administration of bacteriophage into the soft tissue surrounding the distal phalanx; antibiotics were not used during phage therapy	Improvement of the patient's condition, reduction of inflammation	Fish et al., 2018, USA
Single injection of a phage cocktail into the bone cavity, followed by application of preparation-impregnated dressings and waterproof dressings, antibiotic therapy	Fast healing within 14 days without further release of bacteria	Ferry et al., 2018b, France
Intravenous administration of 98 phage doses via a catheter every 2 hours with lactate-enriched Ringer's solution for 8 days	Full recovery of the patient	LaVergne et al., 2018, USA
Single dose of phage (100 ml) at the time of surgery followed by 5 ml through the drainage every 8 hours for 5 days; antibiotic therapy	Absence of bacterial growth in periprosthetic tissue samples after reimplantation of the prosthesis, full recovery of the patient in 10 months	Tkhilaishvili et al., 2019, Germany
Phage administration through drainage in close contact with the infected bone, 3 times a day for 7-10 days; antibiotic therapy	No recurrences of infection at check-ups 8 and 16 months after phage therapy	Onsea et al., 2019, Belgium
Intravenous administration of phage for 35 min for 5 days; 2 courses of treatment with an interval of 6 weeks; antibiotic therapy	Full recovery of the patient	Nir-Paz et al., 2019, Israel
Administration of phage solution directly into the joint	Improvement of joint functions in all patients	Ferry et al., 2020, France
Oral administration of phages, in some cases local administration	Complete wound healing a few months after treatment	Nadareishvili et al., 2020, Georgia, USA
Single intra-articular administration of phage with subsequent intravenous administrations with an interval of 12 hours for 2 weeks; antibiotic therapy	Full recovery of the patient	Ramirez-Sanchez et al., 2021, USA
Phage administration through an arthroscope; antibiotic therapy	Disappearance of heart failure signs after treatment, restoration of mobility and general condition of the knee joint	Ferry et al., 2021, France
40 intravenous doses of one phage, parallel course of minocycline	Disappearance of symptoms and signs of infection, full recovery without side effects	Cano et al., 2021, USA

high. In cases of ineffective treatment, there are several further options, and amputation here is not uncommon (Peel & de Steiger, 2020).

Phage therapy has brought new hope for treatment of patients with such infections, particularly those caused by MDR bacterial strains. Over the last decade, the number of clinical trials using bacteriophages has greatly increased in response to the spread of MDR bacteria. A far from exhaustive list of indicative recent achievements in this field is given in Table 1. However, with regard to infections occurring at burn sites (Azevedo et al., 2022) or resulted from gunshot wounds (Shelton et al., 2023), clinical studies of bacteriophage activity have been few, despite the promising previous results of their use in severe cases, as described above (Jikia et al., 2005; Jault et al., 2019; Abdul-Hassan et al., 1990; Soothill, 1994). Individual preclinical experiments do not sufficiently describe the phage therapy approach in case of such injuries. One of the main future tasks of virologists and clinical microbiologists is to conduct clinical research.

In general, when treating superficial wound infections, bacteriophages are applied in liquid or semi-liquid (gel) form. In most cases, a solution of phage(s) in physiological saline (NaCl 0.9%) or phosphate-buffered saline (PBS) is used. Such phage preparations are easier to make, and they are stable for up to two years if the storage conditions are observed. However, the use of phages in this form for treating open wounds remains problematic. In this case, phages are applied directly as a solution in drops to the wound, or by applying a wet gauze dressing (Fish et al., 2016). With such application, it is difficult to control the phage dose which gets to the focus of the infection. The liquid preparation can flow away from the treatment site without reaching the infected areas in the required doses. This problem is often solved by the use of gauze dressings impregnated with a phage preparation, but there is no data in the literature regarding the efficiency of phage release with this treatment method or the final dose of phages ultimately reaching the site of infection. The release of phages from the tissue can be complicated preventing further lysis of bacteria. Treatment with a phage solution in the form of a spray, although it has been studied mainly on food products (Leverentz et al., 2004; Arthur et al., 2017), can also be used for wound treatment possibly favoring better distribution of phage particles.

There is increasing evidence that hydrogels represent a more reliable and effective way to treat wounds compared to conventional dressings (Kim et al., 2021). Hydrogels effectively absorb liquid and can hold a large amount of water. They are made from water-soluble polymers such as hydroxyethyl cellulose, hydroxypropyl methylcellulose, carbomer, and agarose (Kumari et al., 2010; Bean et al., 2014). Among them, thermosensitive hydrogel wound dressings, which become semi-solid at skin temperature and take the shape of a wound, have been studied (Yan et al., 2021). Such dressings provide a slow and long-term release of phages at the site of wound infection increasing their effectiveness compared to the use of phages as a liquid. Nonionic polymers contribute to phage stabilization, minimizing their inactivation. However, the stability of phages in hydrogels has not been sufficiently investigated. Various systems have been developed to more effectively control the process of phage release from the gel, for example, polymer phage release systems (Hathaway et al., 2015), the effectiveness of which was proven using the phage Staphylococcus K. The possibility of introducing phages into the composition of special creams has also been demonstrated (O'Flaherty et al., 2005), which is appropriate in the treatment of burn wounds.

Summarizing the information on methods of phages' delivery, it should be noted that the main condition for effective treatment of wounds or injuries with phages is their immobilization in the infection site as long as possible without damaging soft tissues and ensuring the steril-

ity of the process. The chosen method of phage application depends on the complexity of the wound, its location, and the patient's condition. Orthopedic infections in general and combat injuries of bone tissues in particular are extremely difficult for therapy due to the frequent inaccessibility of the focus of infection. In this case, oral administration of phages or intra-articular or even intravenous injections are also practiced (the latter require guaranteed microbial sterility of the phage preparation). Several clinical studies indicated in Table 1 provide data on phages' practical use in orthopedics and traumatology. In particular, in most of these studies, phages were injected directly into the joints or bones of patients, which accelerated and ensured the delivery of phage preparation to the source of infection. Intravenous injections also contributed to better results than oral or topical administration. The duration and doses of treatment also turned out to be important. Courses of phage therapy lasting a week or less, in which phage(s) was administered by only one route, were ineffective (Jault et al., 2019). The best effect was achieved with several courses of phage therapy with small breaks, and by using several routes of phage administration.

A noticeable modern trend is the combined use of phages and antibiotics, which is also reflected in Table 1. This is justified by several reasons:

1. Recent studies of the synergy of phages and antibiotics have shown that the combined use of them can not only restore the sensitivity of a particular strain of bacteria to the appropriate antibiotic and, as a result, reduce its dosage but also reduce the number of phage-resistant bacterial cells, which solves one of the current problems of phage therapy (Engeman et al., 2021). It has been proven that the effectiveness of this approach increases if phages are added before the use of antibiotics (Pirnay et al., 2022).

2. Often, the difficulty of treating infectious diseases is complicated by the ability of bacteria to form biofilms (Del Pozo & Patel, 2007). The surgical way to overcome this problem is to remove necrotic tissues serving as the point of attachment of persistent biofilm cells. In some clinical studies, phages were used primarily for the destruction of biofilms, which greatly increased the effectiveness of the subsequent use of antibiotics (Ramirez-Sanchez et al., 2021; Cano et al., 2021). Recently, it was found that phages active against *A. baumannii* effectively destroyed the biofilm and prevented the formation of a new one (Luo et al., 2023). It was also demonstrated that an excessively high concentration of bacteriophages, although this had a pronounced synergistic effect in the first 8 hours of use, may negatively affect the result in the future due to the rapid adaptation of the bacterial culture and emergence of phage resistance (Tagliaferri et al., 2019).

An important factor for the successful treatment of patients is the individual selection of bacteriophages when researchers isolate one or two phages specific to a particular strain of bacteria isolated from a patient. The use of a cocktail of more than ten types of bacteriophages may lead to a decrease in their concentration over time (Jault et al., 2019), although it will have a positive effect at the beginning of the treatment. The concentration, dosage, alternation with antibiotics' course, and the method of treatment should be selected , taking into account the history and condition of a particular patient, antibiotic type, and pathogen specificity. The above indicates the high potential of such phage therapy, which, however, is accompanied by the probable need to adapt the phage cocktail to a specific patient or a group of them.

Another issue to consider is the type of phages used in practice, i.e. lytic or temperate (lysogenic). Lytic phages are considered preferable for phage therapy as they can only kill bacteria. Many authors currently argue for the exclusive use of obligate lytic phages, as temperate phages have a set of characteristics that

make them problematic for therapeutic use. Such phages may not rapidly eliminate all of their hosts (some may induce lysogeny in a part of host cells) and hence may be ineffective in eliminating bacterial infections. In addition, genomes of lysogenic phages may contain genes that increase antibiotic resistance or virulence when expressed in lysogen bacteria. Also, bacterial immunity against superinfection conferred by the prophage may increase resistance to other phages, possibly producing an even more virulent pathogen and exacerbating infection (Mavrich & Hatfull, 2019). The integrated genomes of temperate phages are also capable of transducing nearby bacterial genes, including those capable of enhancing virulence or antibiotic resistance, into new host cells, thus spreading these characteristics within the bacterial population or even between species (Ronayne et al., 2016). However, given the evolutionary advantages of the lysogeny for phages, it can be argued that obligate lytic phages, which lack lysogenic genes altogether, may be greatly outnumbered (and outcompeted) in the environment, and therefore the exclusive use of such phages for therapy may not be a practical approach. Nowadays, genomes of the candidate phages are often fully sequenced, which allows one to determine whether such viruses are lytic or temperate. Despite the challenges associated with temperate phages, the efficacy of such phages in both *in vitro* and *in vivo* therapeutic settings has been demonstrated (Mavrich & Hatfull, 2019; Ronayne et al., 2016), suggesting that at least some temperate phages may be useful in therapy.

Benefits and disadvantages of phage therapy, prospects for further research. The reviewed information allows us to formulate some inherent advantages of bacteriophages as compared to antibiotics.

The benefits of phage preparations are: 1) high specificity (due to the biological properties of individual phages in the composition of the preparation); 2) typically wide spectrum of activity (resulted from the complexity of preparations); 3) reproductive capacity of phages as biological agents; 4) elimination from the macroorganism after the disappearance of susceptible bacteria; 5) theoretical harmlessness for the macroorganism and the absence of side effects (remains the subject of research today); 6) possibility of combined use with other forms of therapy (antibiotics, immunotherapy), and 7) possibility of preventive use.

Despite the great prospects of using phages in the therapy of bacterial complications, there are issues limiting that: 1) instability of phages during storage (Jault et al., 2019); 2) high specificity of phages to specific strains of bacteria and the resulted need to select a suitable phage for each new strain/species of bacteria, which can complicate treatment (Lauman & Dennis, 2021; Mäntynen et al., 2021); 3) own antigenic properties of phages and the formation of specific antiphage antibodies and other effects on the immune system in humans upon repeated administration of the preparation; 4) probability of individual non-acceptance of phage preparations (allergic reactions); 5) a short-term increase in patient's temperature up to 38—39°C, caused by absorption into the blood of the decomposition products of lysed microorganisms (can be observed during phage therapy of severe cases of generalized infections); 6) possibility of bacteria's developing resistance to phages (as in the case of antibiotic therapy), which can be problematic during long-term treatment or in the case of insufficient specificity of phages (Labrie et al., 2010); 7) probability of complex interactions (synergistic or antagonistic) when using a cocktail of phages, which can complicate the development of effective therapeutic regimens (Dedrick et al., 2023); 8) a limited number of scientific data and clinical studies confirming the effectiveness and safety of phage therapy (Chanishvili, 2012).

In this context, own immunogenic properties

of phages can be an issue preventing efficient long-term therapy, as discussed above (see Table 1). Overall, a combination of different delivery ways of the phage preparation coupled with shorter periods of more effective (for instance, combined phage-antibiotic) therapy is supposed to help either reduce immune reactions of human body on phages or delay such responses until the therapy is done (i.e., bacteria have been eradicated).

Recent studies indicate as well possible synergy among the phages and mammal immune systems. For instance, using an acute pneumonia model induced by MDR *P. aeruginosa* in mice, Roach et al. showed that neutrophils were required to control both phage-sensitive and emergent phage-resistant bacteria to clear infection, indicating the importance of neutrophil-phage synergy for the recovery. Moreover, therapeutic phages were also immunologically well tolerated by lung tissues (Roach et al., 2017). This is in agreement with another research reporting the reduction in the level of inflammatory factors after phage therapy, possibly resulted from the reduction in the number of bacterial pathogens *in vivo* (Van Belleghem et al., 2018).

The question of how phages affect the human body is not fully understood. Although phage therapy has traditionally focused on the use of phages to eliminate disease-causing bacteria, new areas of phage use have emerged, for example, to modify the composition and activity of the microbiome. A separate area of research is the development of phages for «engineering» the microbiome by selective elimination of certain target species (Duan et al., 2022).

It should also be noted that using phages in treatment is subject to the regulation and ethical standards, creating additional challenges.

A promising innovative direction of phage therapy, which can expand the possibilities of this treatment method, is recombinant phages and/or their enzymes (for example, lysins) (Schmitt et al., 2023).

Recombinant phages are bacteriophages that have been genetically modified to increase their specificity and effectiveness against specific strains/species of bacteria. Recombinant phages can be developed to combat antibiotic-resistant or emerging pathogens, or to treat severe infections. This approach can increase the specificity and effectiveness of treatment, although it raises many ethical and legal questions.

Another engaging way is the use of phage lysins (Jeong et al., 2023). Phage lysins are proteins produced by bacteriophages for natural lysis of bacterial cell walls to release the progeny phages from the cell after replication. Phage lysins can be used to kill bacteria without using the phage itself. Potentially, they can be directly applied to the focus of infection. Phage lysins can be more stable and less specific than phages, allowing their use against a wider range of bacteria. Also, they can be useful when used in sites where phage access is limited (Haddad Kashani et al., 2018; Murray et al., 2021; Rahman et al., 2021).

Therefore, the consequence of continuing Russian aggression in Ukraine was the development of additional conditions for the evolution and spread of antibiotic-resistant strains of bacteria. This problem is especially acute in traumatology, where there is an unprecedented increase in the number of severe injuries, wound infections, and cases of concomitant osteomyelitis. Considering this, today, phage therapy is not only a promising alternative and additivebut also, in some cases, the only possible means for combating antibiotic-resistant bacteria.

The introduction of phage therapy into clinical practice requires further research, regulatory policy, and resolution of issues related to bacterial resistance to this treatment. Each species of bacteria requires the appropriate phage(s), complicating phages' selection for therapy. In addition, like antibiotics, bacteria can develop resistance to phages. This can become a challenge during long-term treatment or in case of insufficient phage specificity. Thus, the activity of

phages against relevant bacteria should be carefully monitored, and the arsenal of new phages should be constantly replenished with new representatives.

Currently, only two phage-based preparations are registered in Ukraine («Pyophage» and «Intestiphage», produced by the Neoprobiocare-Ukraine LLC), which is insufficient. Further developments in this field should take into account the disadvantages of bacteriophages due to their biological features or methods of application. Taking into account the innovative approaches to using recombinant phages and their lysines, phage therapy may become more effective in the treatment of bacterial infections in the coming years. Therefore, further large-scale research and clinical trials are needed.

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О.В. Шевченко[1], А.В. Харіна[1], Н.О. Корнієнко[1],*
І.Г. Будзанівська[1], О.М. Андрійчук[1], І.М. Пожилов[1], Г.О. Снігур[1], О.Б. Лютко[2],
О.М. Ліненко[3], І.І. Мойса[3], В.А. Понятовський[4]

[1] Кафедра вірусології, ННЦ «Інститут біології та медицини»,
Київський національний університет імені Тараса Шевченка,
вул. Володимирська, 64/13, Київ, 01601, Україна
[2] Лабораторія мікробіології та хіміотерапії, ДУ Інститут травматології та ортопедії НАМН України,
вул. Бульварно-Кудрявська, 27, Київ, 01601, Україна
[3] Відділення кістково-гнійної хірургії, ДУ Інститут травматології та ортопедії НАМН України,
вул. Бульварно-Кудрявська, 27, Київ, 01601, Україна
[4] Кафедра мікробіології та паразитології з основами імунології, Національний медичний університет імені О.О. Богомольця,
пр. Перемоги, 34, 03057, Україна

ФАГОТЕРАПІЯ В ТРАВМАТОЛОГІЇ: ОГЛЯД ПЕРСПЕКТИВ ЛІКУВАННЯ ГОСТРИХ РАН ТА ПІСЛЯХІРУРГІЧНИХ УСКЛАДНЕНЬ

Травми будь-якої природи є основною причиною смерті людей, часто супроводжуються хронічними ускладненнями і спричиняють значні економічні втрати. Повномасштабні бойові дії на території України зумовили безпрецедентне збільшення кількості постраждалих з важкими травмами, зокрема вогнепальними пораненнями, переломами кісток та мінно-вибуховими травмами різного характеру. Ранові інфекції ускладнюють післяопераційне відновлення пацієнтів, призводять до збільшення вартості лікування і є однією з основних причин небойових втрат персоналу. Передумовою розвитку ранової інфекції, серед іншого, є високе мікробне забруднення вогнепальної кістково-м'язової рани. Такі мікроорганізми зазвичай є нормальною флорою пацієнта або можуть передаватися через контакт із контамінованою водою, фомітами чи руками медичних працівників, інструментами, тощо. Основними бактеріями, які часто ідентифікуються як причина розвитку інфекцій м'яких тканин та кісток, є стафілококи (*Staphylococcus aureus*, *Staphylococcus epidermidis*), стрептококи (*Streptococcus pyogenes, Streptococcus agalictiae*), ентерококи (*Enterococcus faecalis*), грамнегативні бактерії (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii*), анаеробні бактерії (*Clostridium perfringens, Bacterioides* spp.) та ін. Сучасні дані свідчать про зміну актуального видового складу збудників ранових інфекцій. Також труднощі лікування хворих із гнійною хірургічною інфекцією зумовлені зростанням полірезистентності мікрофлори до антибактеріальних препаратів та підвищенням частоти висівання мікробних асоціацій, які можуть утворювати біоплівку, що захищає патогенні мікроорганізми від антимікробної терапії та імунної відповіді пацієнта. Такі гнійні бактеріальні інфекції ранових поверхонь складні у лікуванні і потребують нових підходів до терапії. З урахуванням великої кількості бойових травм в Україні особливої ваги набуває використання бактеріофагів як специфічних та багатообіцяючих агентів для біологічного контролю патогенної мікрофлори. Фаготерапія традиційно зосереджена на використанні фагів для елімінації хвороботворних бактерій. З урахуванням характеру травм, зокрема бойових, найбільш перспективними та затребуваними сьогодні вважаються фагові препарати, призначені для лікування гнійно-хірургічних інфекцій ранових та опікових поверхонь. У даному огляді узагальнено сучасні дані щодо використання фагових препаратів у клініці загалом і в ортопедії та травматології зокрема. Обговорюються питання способу використання (доставки) фагів до вогнища інфекції, їх ефективної дози, тривалості терапії та можливості її комбінування з антибіотикотерапією. Представлено інформацію стосовно програм фаготерапії, запроваджених в окремих країнах. Виокремлено переваги й недоліки використання бактеріофагів для таргетної терапії важких пацієнтів та зазначено перспективи подальших досліджень.

Ключові слова: *патогенна мікробіота, бактеріофаги, фаготерапія, бактеріальні ускладнення, гнійні рани, вогнепальні і мінно-вибухові травми, антибіотикорезистентність.*