BIL, Katarzyna, KIEŁBASA, Justyna, KOWALCZYK, Agata, ZOZULA, Natalia, LATAŁA, Aleksandra, ŚLESICKA, Iga, RYKUCKA, Aleksandra, WĄS, Marcin, PRZESTRZELSKA, Magda and TOMCZEWSKA, Zuzanna. The treatment of chronic wounds using bacteriophages. Journal of Education, Health and Sport. 2024;74:52572. eISSN 2391-8306.
https://dx.doi.org/10.12775/JEHS.2024.74.52572
https://apcz.umk.pl/JEHS/article/view/52572

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences); Health Sciences (Field of medical and health sciences).
Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).
The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 25.04.2024. Revised: 10.05.2024. Accepted: 17.06.2024. Published: 26.06.2024.

The treatment of chronic wounds using bacteriophages

Katarzyna Bil [KB]

Independent Public Specialist Western Hospital named after St John Paul II, Daleka 11, 05-825 Grodzisk Mazowiecki, Poland, katarzynabil2503@gmail.com, https://orcid.org/0009-0003-6518-1711

Justyna Kiełbasa [JK]

Independent Public Healthcare Complex in Pruszków, 2/4 Armii Krajowej Avenue, 05-800 Pruszków, Poland, justynakielbasa1b@gmail.com, https://orcid.org/0009-0002-0616-7402

Agata Kowalczyk [AK]

Mazovian Specialized Hospital in Ostrołęka of Józef Psarski, Jana Pawła II 120A street, 07-410 Ostrołęka, Poland, agata.kowalczyk95@gmail.com, https://orcid.org/0009-0007-9497-0970

Natalia Zozula [NZ]

District Medical Center in Grójec, Piotra Skargi 10 street, 05-600 Grójec, Poland, nataliazozula0@gmail.com, https://orcid.org/0009-0009-9741-5061

Aleksandra Latała [AL]

District Medical Center in Grójec, Piotra Skargi 10 street, 05-600 Grójec, Poland, aleksandra.latala10@gmail.com, https://orcid.org/0009-0007-1701-2728

Iga Ślesicka [IŚ]

Mazovian Specialized Hospital in Ostrołęka of Józef Psarski, Jana Pawła II 120A street, 07-410 Ostrołęka, Poland, igaslesicka@gmail.com, https://orcid.org/0000-0001-7282-4875

Aleksandra Rykucka [AR]

Rear Admiral Professor Wiesław Łasiński 7th Military Navy Hospital with Outpatient Clinic named after in Gdańsk, Polanki 117 street, 80-305 Gdańsk, Poland, aleksandra.rykucka@wp.pl, https://orcid.org/0009-0003-0171-8647

Marcin Wąs [MW]

District Medical Center in Grójec, Piotra Skargi 10 street, 05-600 Grójec, Poland, mos1@vp.pl, https://orcid.org/0009-0004-8645-0121

Magda Przestrzelska [MP]

Faculty of Medicine Collegium Medicum Uniwersytet Kardynała Stefana Wyszyńskiego w Warszawie, Wóycickiego 1/3, 01-938 Warszawa, Poland, magda.przestrzelska123@gmail.com, https://orcid.org/0009-0009-8688-7047

Zuzanna Tomczewska [ZT]

Provincial Specialist Hospital named after Blessed Father Jerzy Popiełuszko in Włocławek, Wieniecka 49, 87-800 Włocławek, Poland, ztomczewska@gmail.com, https://orcid.org/0000-0002-4327-3311

ABSTRACT

Introduction and aim of study: Chronic wounds are increasingly challenging global healthcare. These wounds, which take over 3 months to heal, are complicated by untreated infections and the formation of biofilm, hindering healing and antibiotic effectiveness. To tackle these issues, new treatments like bacteriophage therapy are being explored. Bacteriophages, viruses that target bacteria, offer promise in overcoming antibiotic resistance. However, their use presents challenges that need to be addressed.

Material and methods: Our review is based on the analysis of materials collected in Pubmed, Elsevier and other scientific articles using keywords: "chronic wound", "chronic wounds infection", "biofilms", "MDR", "bacteriophage", "phage therapy".

Conclusions: The rise of chronic wounds due to resistant infections poses a significant challenge for patients and healthcare systems. Multidrug-resistant bacteria, often forming biofilm, evade current treatments, urging the search for alternatives. Phage therapy, showing efficacy against various stubborn infections, including those from surgery or diabetes, gains attention. Advanced delivery systems enhance targeted treatment, while phage cocktails improve effectiveness, especially against multiple resistant strains. Safety is generally observed, but larger trials are needed. Though not a replacement for antibiotics, phage therapy offers hope, needing robust clinical validation. While challenges exist, its societal, commercial, and economic benefits suggest a promising future beyond clinical use.

KEY WORDS: chronic wound; chronic wounds infection; biofilms; MDR; bacteriophage; phage therapy.

INTRODUCTION

The occurrence of chronic wounds presents an increasing challenge for global healthcare. This is influenced, among other factors, by an aging population, an increase in the incidence of diabetes, and the growing resistance of bacteria to available antibiotics [1]. A chronic wound is a wound that does not heal in a typical manner, takes more than 3 months to heal, and is associated with many potential complications [2]. An untreated wound infection is

a major factor that can cause wounds to stagnate and turn chronic [3]. Bacteria complicate the wound healing process by increasing inflammation and damaging tissue. Additionally, the rising antibiotic resistance of bacteria and the formation of biofilm present further challenges. Biofilm is described as "a structured community of microbial cells encased in a self-generated polymeric matrix that adheres to either an inert or living surface." Biofilm further impairs healing processes and hinders the penetration of antibiotics and antiseptic agents [4]. These problems have necessitated the development of new treatment approaches for chronic wound infections. One of these methods is bacteriophage therapy, first utilized nearly a century ago and currently experiencing a renaissance, mainly driven by the antibiotic resistance crisis. This review includes a description of chronic wounds, the role of biofilm, and primarily focuses on the role of bacteriophages as a potential solution to the impending era of antibiotic resistance. Different ways of delivering phages have been explored, including traditional methods and newer strategies like combining phages with antibiotics, using enzymes from phages, and harnessing phage resistance mechanisms. The review also touches on the challenges and difficulties encountered in the use of bacteriophages.

CHRONIC WOUND

A chronic wound is a type of wound that does not heal in an expected timeframe, typically over three months, and fails to progress through the normal stages of healing. Unlike acute wounds, which heal relatively quickly and follow a predictable course, chronic wounds remain stuck in a prolonged inflammatory phase and do not respond to standard treatment [5]. There is no universally accepted definition of a chronic wound. Generally, wounds are considered chronic if they haven't healed within 4 to 6 weeks. Some definitions specify chronic wounds as those that do not show a 20% to 40% reduction in size after 2 to 4 weeks of optimal treatment. Surgical textbooks often define chronic wounds as those that remain unhealed after 3 months. Regardless of the timeframe, wounds that do not follow a normal healing process to achieve proper anatomical and functional outcomes are classified as chronic [2]. They are typically categorized as vascular, diabetic, or pressure ulcers. They often arise from wound characteristics, patient physiology, or complications from diseases, which extend or intensify the inflammatory phase and hinder dermal or epidermal cells from responding to regenerative signals [6].

The normal wound healing process begins immediately and involves four overlapping phases in a specific sequence and duration at optimal intensity. Hemostasis involves blood vessel constriction, platelet aggregation, and fibrin clot formation, restoring the skin's protective barrier and supporting cell migration and fibroblast proliferation. Inflammation starts right after the injury, lasting 4–6 days, with neutrophils, monocytes, and lymphocytes infiltrating the site, and monocytes differentiating into macrophages that phagocytize debris and produce healing factors. The proliferative phase includes angiogenesis, fiFbroplasia, and reepithelialization, where granulation tissue fills the wound, fibroblasts contract the wound edges, and keratinocytes migrate and proliferate to cover the lesion. Tissue remodeling, lasting up to a year, involves the reorganization, degradation, and resynthesis of the extracellular matrix, replacing collagen III with collagen I, and forming scar tissue rich in collagen fibers [1]. Chronic wounds are characterized by their inability to progress through the typical stages of wound healing in a coordinated and prompt manner. Frequently, these wounds become stuck in the inflammatory phase of healing. Although the molecular causes may vary, chronic wounds exhibit consistent characteristics such as elevated levels of proinflammatory cytokines, proteases, reactive oxygen species (ROS), and senescent cells. Additionally, they often harbor persistent infections and lack functional stem cells, exacerbating the healing process [7].

Chronic wounds have become significantly more prevalent over the past few decades. This rise is largely attributed to an aging population, which is more susceptible to conditions that impede healing. Additionally, increasing rates of obesity and diabetes contribute to the persistence of chronic wounds. These conditions often maintain wounds in a state of low-level inflammation, which hinders the normal healing process by preventing the proper function of dermal and epidermal cells. Consequently, the management and treatment of chronic wounds have become more challenging, necessitating a comprehensive approach to address these underlying factors and promote effective healing [1]. These wounds are considered a global problem [8]. An estimated 1% to 2% of the populace in developing countries will experience a chronic wound during their lifetime. These wounds predominantly affect patients aged older than 60 years [2].

CHRONIC WOUND INFECTION

One of the primary reasons wounds may not heal and become chronic is due to untreated infections [3]. Bacteria in wounds can delay healing by causing infection, triggering

inflammation, forming biofilms, damaging tissue, inhibiting angiogenesis, weakening the immune response, and increasing the risk of secondary infections [9]. Differentiating between a chronic uninfected wound and an infected one can be difficult. Chronic wound infections often present non-traditional signs such as heightened pain, fragile granulation tissue, delayed healing, wound deterioration, and a foul odor, which may not be easily recognized by non-experts [10].

Most chronic wounds contain more than one bacterial species and produce a synergetic effect that results in previously non-virulent bacterial species becoming virulent and causing damage to the host. Studies on bacterial diversity in chronic wounds have found that Staphylococcus, Pseudomonas, Peptoniphilus, Enterobacter, Stenotrophomonas, Finegoldia, and Serratia are the most frequently present species [11]. Bacterial endotoxins and exotoxins can trigger both non-specific and specific immune responses, with immune cells like neutrophils and macrophages playing key roles. Chronic wounds often have a high number of neutrophils, leading to pathological inflammation and delayed healing [12]. Additionally, neutrophils and other immune cells recruited to the wound bed can produce excessive protease, which degrades the extracellular matrix, causing further tissue damage and slowing reepithelization [13, 14]. Another problem is the increasing antibiotic resistance of bacteria. Patient hand contamination with MDROs is common and correlates with contamination on high-touch room surfaces. Patient hand hygiene protocols should be considered to reduce transmission of pathogens and healthcare-associated infections [15]. Contaminated surfaces play a significant role in transmitting hospital pathogens. Evidence shows that patients admitted to rooms previously occupied by infected individuals have a higher risk of acquiring those pathogens. However, this risk can be reduced with better environmental decontamination, highlighting the need for improved cleaning practices. Strategies to address this issue include reducing the shedding of pathogens into the environment and enhancing the effectiveness of cleaning and disinfection. The best approaches will vary based on the specific setting and local epidemiology [16].

THE ROLE OF BIOFILM IN THE WOUND HEALING PROCESS

A biofilm is an organized cellular structure embedded in a self-produced matrix, exhibiting adhesion to both biological and abiotic surfaces [4]. Bacterial cells within a biofilm are encased in an extracellular polymeric substance (EPS) composed primarily of

exopolysaccharides, secreted proteins, lipids, and extracellular DNA. This matrix significantly challenges standard treatments due to its high level of antibiotic resistance [17]. The highly organized structure of the biofilm reduces the penetration of antibiotics into the wound. The use of antibiotics on chronic wounds is controversial because it causes the selection of multi-drug-resistant strains [18].

In a mature biofilm, bacteria grow slowly due to deficiency of nutrients that results in the resistance of bacteria to antibiotics [19]. Biofilm is composed of multiple closely cooperating species of bacteria, which interact similarly to a multicellular organism [20]. Diffusion is the predominant solute transport process within cell clusters. Water channels can carry solutes into or out of the depths of a biofilm, but they do not guarantee access to the interior of cell clusters [21]. The population in biofilms is diverse and includes cells at different growth stages and also antimicrobial-resistant and persister cells [22]. These cells evade antimicrobial treatment by maintaining an inactive metabolism with limited protein synthesis. They sustain reduced ATP levels and enter a dormant state during treatment, rendering them resistant. Persister cells persist in a viable but inactive state, capable of resuming growth when antimicrobial levels decline. As a result, they frequently contribute to the stubbornness of chronic infections [23].

Biofilms also provide an ideal niche for the exchange of extrachromosomal DNA (plasmids). Conjugation (the mechanism of plasmid transfer) occurs at a greater rate between cells in biofilms than between planktonic cells [24, 25]. Quorum sensing is a cellular communication mechanism where cells produce specific molecules released into their environment. Nearby cells detect these molecules, leading to changes in gene expression, virulence, microbial competence, and even antibiotic resistance [25].

In 2017, the World Health Organization (WHO) issued a global priority roster of antibiotic-resistant bacteria to steer research, discovery, and the creation of new, potent antibiotics. This compilation encompasses 12 bacterial species classified into critical (three species), high (six species), and medium (three species) categories based on their resistance levels. Critical species include Acinetobacter baumannii and P. aeruginosa, both resistant to carbapenems, and Enterobacteriaceae (comprising K. pneumoniae, E. coli, Enterobacter spp., Serratia spp., Proteus spp., and Providencia spp., Morganella spp.), resistant to carbapenems and third-generation cephalosporins. These bacteria, highlighted by the WHO, are renowned for their biofilm-forming capabilities. For instance, P. aeruginosa harbors abundant DNA in its

EPS matrix, fostering genetic diversity within the biofilm, thus elevating the likelihood of some individuals resisting environmental changes, such as antibiotics [25, 26, 27].

PHAGE TRANSFER

One of the modern methods of treating wounds covered with biofilm is bacteriophage therapy. In the slowly approaching postantibiotic era and a time of limited therapeutic options, it has become crucial to find an alternative treatment for bacterial infections [28]. Bacteriophage (phage) therapy, originally identified in the early 1900s, has experienced a resurgence in recent decades because of its effectiveness against antibiotic-resistant bacteria. Utilizing it for non-healing wound treatment has demonstrated encouraging results [23].

Bacteriophages (phages) are viruses that specifically target and infect bacteria, making them one of the most abundant microorganisms worldwide. Similar to other viruses, they can only replicate within a host cell. Their life cycle is characterized by two main types: lytic bacteriophages and lysogenic bacteriophages. From a therapeutic standpoint, only lytic phages are employed in biofilm therapy due to their ability to directly destroy bacterial cells [29].
Phage therapy involves isolating naturally occurring phages from the environment. Once isolated, these phages are tested against common pathogens, including drug-resistant and multidrug-resistant bacteria, and assessed using in vitro and in vivo models, which include animal studies and some human clinical trials [23].

Bacteriophages exhibit group specificity, meaning a single phage strain typically cannot infect all strains of a bacterial species. This challenge can be addressed by creating phage cocktails, which include multiple phages with varying lytic spectra that can infect different strains of the same species [30].

Phage cocktail can be administered using various routes (e.g., parenteral, oral, or local). The acidic conditions of the stomach, along with enzymes and digestive compounds like bile, can quickly diminish the viability of orally administered phages [31]. Without adequate protection, phages may not survive the passage through the stomach, rendering them ineffective in the intestine. Consequently, it is crucial to develop an efficient delivery system to shield orally administered phages from the harsh gastrointestinal environment until they reach the infection site in the intestine. One potential method for protecting phages involves encapsulating them in microspheres or microparticles made of pH-sensitive polymers [32].

At present, liquid formulations are preferred for delivering phages to wound infection sites. In theory, creating liquid phage formulations is more straightforward and requires relatively little development to ensure phage stability [33]. Phages are commonly formulated in sterile buffered solutions, such as phosphate-buffered saline or Tris-buffered salt-magnesium buffer. Addition of divalent ions, including $Mg2+$ and $Ca2+$ (10 mM each) further aids in promoting phage stability during storage [34]. These cations interact with negatively charged moieties on the surface of phages, which helps with phage stabilization in aqueous buffered systems [33].

Another method of delivering phages to a wound is by using semi-solid preparations such as gels, creams, and ointments. They are easy to apply, minimally irritating on the skin and often easily washable with water. Hydrogels are water-based semi-solid formulations. They are highly absorbent and are capable of retaining a large amount of water, which help protect the skin against excessive loss of body fluids while absorbing wound excreta. Hydrogel formulations enhance wound site hydration and facilitate hydrogen bonding between water and phage proteins, aiding in phage stabilization. Water-based hydrogels are preferred in phage therapy because phages are inactivated in alcohol [33, 35].

Since the method of administration can influence disease progression, understanding the pharmacokinetics of bacteriophages is crucial. Research has demonstrated that various administration routes are more suitable for different scenarios and pathogens. Good results are also observed when using a combination of different methods. Often, both oral and topical phage therapy are used simultaneously. The liquid form of the phage can be sprayed onto sterile gauze and applied to the wound. Another method is to insert a sterile catheter into the ulcer, through which the phages are directly introduced into the wound [28].

The pharmacokinetics of phage therapy significantly differ from those of antibiotics, providing several benefits such as exponential replication, efficient penetration to target sites, a specific host range, and minimal toxicity. Due to their persistence and ability to evolve, phages might be more effective than antibiotics in certain situations, particularly when dealing with antibiotic-resistant bacteria. However, combined therapy with both agents sometimes proves to be significantly more effective in halting the entire infection process. The overall tissue healing process is also accelerated. It is known that the use of combined agents reduces the frequency of resistant mutants, making this approach a potentially effective strategy for treating infections [36].

Another method of delivering phages is the use of nanostructured lipid-based carriers, such as transfersomes, as transdermal delivery systems for encapsulation. Results from in vitro

stability and in vivo phage titer experiments demonstrated that phages encapsulated in transfersomes exhibited greater persistence and stability compared to free phages. These findings endorse the use of transfersomes as delivery agents to improve the stability and in vivo persistence of encapsulated phages. Additionally, the study underscores the benefits of transfersome-encapsulated phages in offering superior therapeutic options for treating skin and soft tissue infections compared to free phages. These vesicles disperse the substance throughout the body, avoiding fast breakdown, and increasing cellular absorption [37].

The use of whole phages has some drawbacks, including the potential transfer of virulence or resistance genes through transduction. Additionally, as phages and bacteria continuously evolve, many historically significant phages may lose effectiveness against evolving pathogenic bacteria. Another concern is the rapid clearance of phages from the bloodstream due to phage-neutralizing antibodies, which can impede phage therapy. These disadvantages have prompted the exploration of phage-encoded enzymes. These enzymes offer several benefits; for instance, lytic enzymes eliminate the risk of transferring resistance genes. While improperly purified phages can be toxic, phage lytic proteins produced via recombinant DNA technologies are highly purified. Moreover, resistance to recombinant endolysins is not widely reported [38].

PHAGE IN WOUND TREATMENT

Bacteriophages can serve as an effective topical treatment for S. aureus biofilm-infected wounds, particularly when the biofilm structure is either deficient (mutant) or disrupted (through debridement). A combination therapy that targets the disruption of the extracellular biofilm matrix to enhance the penetration of species-specific bacteriophages offers a novel and potentially effective strategy for managing chronic wounds [39]. In another study, it was demonstrated that topical treatment with a phage cocktail could be effective in treating diabetic foot ulcers infected with multidrug-resistant (MDR) Staphylococcus aureus. The phage cocktail used, AB-SA01, consists of three S. aureus Myoviridae phages and was produced according to current Good Manufacturing Practices (cGMP). It has undergone two Phase I clinical trials. In mice treated with the phages, wound healing was comparable to treatment with vancomycin. The treatment resulted in reduced bacterial load and wound closure [40].

Phage therapy was evaluated in 20 patients (aged 12 to 60) with chronic non-healing wounds unresponsive to conventional debridement and antibiotic treatment. The wounds were

infected with E. coli, S. aureus, and P. aeruginosa. A customized bacteriophage cocktail was applied topically to the wounds on alternate days until the wound surfaces became microbiologically sterile. Significant improvement in wound healing was observed, with no clinical or microbiological signs of infection after 3 to 5 doses of bacteriophage therapy. By day 21 of follow-up, seven patients had achieved complete healing, while others showed healthy margins and granulation tissue. No side effects of the therapy were observed [41].

The observed increase in antibiotic resistance of A. baumannii is increasingly leading to treatment failures for infections caused by this bacterium. In early 2019, the World Health Organization released a list highlighting the ten most severe threats to public health, which included antibiotic resistance. A. baumannii was categorized within the highest-risk critical priority group of multidrug-resistant (MDR) bacteria. Therefore, the use of phage therapy in the course of such infections could be a promising approach [42]. The potential of phi G7 phage application was examined in a rat wound model. Phage application effectively decreased the number of bacteria isolated from the wounds of successfully treated animals [43]. Studies conducted on a rat model of diabetes have demonstrated the effectiveness and safety of bacteriophage therapy in treating wounds infected with A. baumannii [44].

Non-healing wounds, a frequent complication of diabetes, stand as the leading non-traumatic reason for lower limb amputations. Traditional treatment for infected diabetic wounds often proves ineffective due to insufficient tissue blood flow, inadequate antibiotic levels locally, and the escalating issue of antibiotic resistance. A study was conducted on animal models of diabetes (rats and pigs). Chronic wounds were infected with S. aureus, P. aeruginosa, and A. baumannii. It was found that local treatment with a bacteriophage cocktail effectively reduced the number of bacterial colonies and improved wound healing, as indicated by smaller epithelial and cutaneous fissures. The results suggest that locally administered bacteriophage therapy may be effective in treating chronic infections, especially when used in conjunction with wound debridement [45]. Other studies on animal models have also demonstrated effectiveness and safety of bacteriophage therapy in treating chronic ulcers recurring in the course of diabetes [36, 40, 44]. The study involved nine patients with diabetes and foot ulcers infected with S. aureus (one MRSA, the rest MSSA). All admitted patients did not respond to conventional therapy for a period ranging from 10 days to seven weeks before phage treatment. Topical application of the Staphylococcal phage Sb-1 on ulcers once a week, combined with standard wound care, healed the ulcers within about seven weeks and severe ulcers within 18

weeks. Despite poor vascularity and inadequate response to previous antibiotic treatment, local phage administration effectively healed S. aureus-infected ulcers [23].

Another significant problem is infections caused by Pseudomonas aeruginosa bacteria. Their ability to rapidly adapt is the primary reason for identifying them as opportunistic pathogens. They most commonly cause infections in patients with compromised immunity and have acquired the status of hospital pathogens. They are frequently isolated from wounds. Infections with multidrug-resistant (MDR) Pseudomonas aeruginosa pose a serious health threat. Phage–antibiotic combination therapy is a promising candidate for combating MDR P. aeruginosa infections. The studies described here show that using P. aeruginosa phages in combination with different classes of antibiotics was not only efficacious, but synergistic in the reduction of bacterial populations and resulted in the re-sensitization of MDR P. aeruginosa to antibiotics [46]. Other studies have also demonstrated the effectiveness of bacteriophages in treating wounds infected by P. aeruginosa [47, 48].

Formulated phage ointment could be a promising approach for treating infected burn wounds [49]. Multidrug-resistant (MDR) Acinetobacter baumannii (A. baumannii) is one of the major pathogens present in burn wound infections. A formulation of a bacteriophage specific to A. baumannii (BPABΦ1), encapsulated in chitosan microparticles, has been created. This preparation showed outstanding potential for anti-biofilm eradication in vitro and promoted effective wound healing when used topically [50]. Phage therapy has also proven effective in treating burn wounds infected with Pseudomonas aeruginosa, Klebsiella pneumoniae, or Staphylococcus aureus [47, 51].

PROBLEMS AND CHALLENGES

One of the issues in phage therapy is the specificity of phages to their hosts, which has both pros and cons for the treatment. High specificity necessitates precise diagnosis and identification of the infectious bacteria for phage therapy to be effective. This process can be difficult, time-consuming, and resource-intensive. Additionally, many wounds are co-infected with multiple bacteria or various subtypes of a single pathogen, meaning the high host specificity of phages can significantly limit their applicability [52]. It seems that these issues can be addressed by employing genome engineering, synthetic biology, structure-guided design, and machine learning. This will allow the full realization of the potential of phage therapy [53]. Another significant problem is the emergence of bacterial resistance during phage treatment,

especially after prolonged phage use [54]. A solution to this problem may be the use of bacteriophages together with antibiotics [36, 55] or the use of a cocktail composed of several phages characterized by different mechanisms of action [37, 45]. Access to phage therapy is limited. Medical tourism to recognized phage therapy clinics is possible, typically at the patient's expense. In some countries, there are expanded access programs; however, these are limited to life-threatening cases, and there is a shortage of medical personnel specializing in phage therapy. Recruitment processes for clinical trials are highly selective, and the number of studies is limited [53]. In the survey, it was found that the majority of patients with diabetic foot ulcers are positively inclined towards bacteriophage therapy. Patients are concerned about antibiotic resistance and support "new" antimicrobial agents. A significant majority of patients would accept phage therapy if suggested by their doctor [56].

CONSLUSIONS

The growing occurrence of chronic or slow-healing wounds caused by stubborn infections has become a significant challenge for both patients and healthcare systems. This is influenced by the continuously increasing number of infections caused by multidrug-resistant bacteria that do not respond to available antibiotics. These bacteria, by forming biofilm, evade available therapeutic options. The escalating concern regarding antibiotic resistance puts significant pressure on all parties involved in infectious disease management to discover novel antibiotics and seek out safe alternatives. Phage therapy has regained prominence due to its proven effectiveness in treating various stubborn wound infections, such as those resulting from surgery, burns, and diabetic foot issues. Phages have demonstrated effective bactericidal activity, even against MDR bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii or Klebsiella pneumoniae. Advancements in phage delivery systems, such as hydrogels, liposomes, nanospheres, emulsions, ointments and creams, facilitate the targeted delivery of viable phages to specific sites, thereby amplifying the efficacy of phage therapy for managing wound infections. The use of cocktails composed of several different phages increases the effectiveness in combating infections, particularly because the majority of chronic wounds are colonized by multiple different strains of antibiotic-resistant bacteria. In many studies, the safety of phage therapy has been demonstrated, with no observed side effects; however, further research on larger patient groups is needed. Nevertheless, for many patients

with untreatable infections, the use of bacteriophages may be the last available therapeutic option.

The goal of phage therapy applications should not be to substitute antibiotics but rather to supplement their effects in combating infections. It is crucial to establish the effectiveness and safety of phage application through rigorous clinical trials. While implementing widespread phage therapy may present challenges, it promises to yield societal, commercial, and economic advantages that extend beyond the clinical realm.

Author`s contribution:

Conceptualization: Katarzyna Bil and Justyna Kiełbasa.

Methodology: Zuzanna Tomczewska and Marcin Wąs.

Software: Agata Kowalczyk.

Check: Aleksandra Rykucka and Iga Ślesicka.

Formal analysis: Magda Przestrzelska and Iga Ślesicka.

Investigation: Katarzyna Bil and Aleksandra Latała.

Resources: Zuzanna Tomczewska and Magda Przestrzelska.

Data curation: Justyna Kiełbasa and Natalia Zozula.

Writing - rough preparation: Katarzyna Bil and Agata Kowalczyk.

Writing - review and editing: Justyna Kiełbasa, Aleksandra Latała and Marcin Wąs.

Supervision: Natalia Zozula.

Project administration: Natalia Zozula and Aleksandra Rykucka.

All authors have read and agreed with the published version of the manuscript.

Founding Statement: The study did not receive funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflict of Interest Statement: The authors declare no conflicts of interest.

Acknowledgments: Not applicable.

REFERENCES

1. Pence BD, Woods JA. Exercise, Obesity, and Cutaneous Wound Healing: Evidence from Rodent and Human Studies. Adv Wound Care (New Rochelle). 2014 Jan 1;3(1):71-79. doi: 10.1089/wound.2012.0377. PMID: 24761347; PMCID: PMC3900100.

2. Siddiqui AR, Bernstein JM. Chronic wound infection: facts and controversies. Clin Dermatol. 2010 Sep-Oct;28(5):519-26. doi: 10.1016/j.clindermatol.2010.03.009. PMID: 20797512.

3. Williams M. Wound infections: an overview. Br J Community Nurs. 2021 Jun 1;26(Sup6):S22-S25. doi: 10.12968/bjcn.2021.26.Sup6.S22. PMID: 34106009.

4. Hurlow J, Couch K, Laforet K, Bolton L, Metcalf D, Bowler P. Clinical Biofilms: A Challenging Frontier in Wound Care. Adv Wound Care (New Rochelle). 2015 May 1;4(5):295-301. doi: 10.1089/wound.2014.0567. PMID: 26005595; PMCID: PMC4432968.

5. Lazarus GS, Cooper DM, Knighton DR, Percoraro RE, Rodeheaver G, Robson MC. Definitions and guidelines for assessment of wounds and evaluation of healing. Wound Repair Regen. 1994 Jul;2(3):165-70. doi: 10.1046/j.1524-475X.1994.20305.x. PMID: 17156107.

6. Hu MS, Borrelli MR, Lorenz HP, Longaker MT, Wan DC. Mesenchymal Stromal Cells and Cutaneous Wound Healing: A Comprehensive Review of the Background, Role, and Therapeutic Potential. Stem Cells Int. 2018 May 20;2018:6901983. doi: 10.1155/2018/6901983. PMID: 29887893; PMCID: PMC5985130.

7. Frykberg RG, Banks J. Challenges in the Treatment of Chronic Wounds. Adv Wound Care (New Rochelle). 2015 Sep 1;4(9):560-582. doi: 10.1089/wound.2015.0635. PMID: 26339534; PMCID: PMC4528992.

8. Martinengo L, Olsson M, Bajpai R, Soljak M, Upton Z, Schmidtchen A, Car J, Järbrink K. Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies. Ann Epidemiol. 2019 Jan;29:8-15. doi: 10.1016/j.annepidem.2018.10.005. Epub 2018 Nov 12. PMID: 30497932.

9. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010 Mar;89(3):219-29. doi: 10.1177/0022034509359125. Epub 2010 Feb 5. PMID: 20139336; PMCID: PMC2903966.

10. Falcone M, De Angelis B, Pea F, Scalise A, Stefani S, Tasinato R, Zanetti O, Dalla Paola L. Challenges in the management of chronic wound infections. J Glob Antimicrob Resist. 2021 Sep;26:140-147. doi: 10.1016/j.jgar.2021.05.010. Epub 2021 Jun 16. PMID: 34144200.

11. Rahim K, Saleha S, Zhu X, Huo L, Basit A, Franco OL. Bacterial Contribution in Chronicity of Wounds. Microb Ecol. 2017 Apr;73(3):710-721. doi: 10.1007/s00248-016-0867-9. Epub 2016 Oct 14. PMID: 27742997.

12. Tanno H, Kawakami K, Kanno E, Suzuki A, Takagi N, Yamamoto H, Ishii K, Imai Y, Maruyama R, Tachi M. Invariant NKT cells promote skin wound healing by preventing a prolonged neutrophilic inflammatory response. Wound Repair Regen. 2017 Sep;25(5):805-815. doi: 10.1111/wrr.12588. Epub 2017 Dec 8. PMID: 28940971.

13. Wilgus TA, Roy S, McDaniel JC. Neutrophils and Wound Repair: Positive Actions and Negative Reactions. Adv Wound Care (New Rochelle). 2013 Sep;2(7):379-388. doi: 10.1089/wound.2012.0383. PMID: 24527354; PMCID: PMC3763227.

14. Abdollahi M, Ng TS, Rezaeizadeh A, Aamidor S, Twigg SM, Min D, McLennan SV. Insulin treatment prevents wounding associated changes in tissue and circulating neutrophil MMP-9 and NGAL in diabetic rats. PLoS One. 2017 Feb 9;12(2):e0170951. doi: 10.1371/journal.pone.0170951. PMID: 28182694; PMCID: PMC5300126.

15. Mody L, Washer LL, Kaye KS, Gibson K, Saint S, Reyes K, Cassone M, Mantey J, Cao J, Altamimi S, Perri M, Sax H, Chopra V, Zervos M. Multidrug-resistant Organisms in Hospitals: What Is on Patient Hands and in Their Rooms? Clin Infect Dis. 2019 Nov 13;69(11):1837-1844. doi: 10.1093/cid/ciz092. PMID: 30980082; PMCID: PMC6853699.

16. Otter JA, Yezli S, Salkeld JA, French GL. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. Am J Infect Control. 2013 May;41(5 Suppl):S6-11. doi: 10.1016/j.ajic.2012.12.004. PMID: 23622751.

17. Chang C, Yu X, Guo W, Guo C, Guo X, Li Q, Zhu Y. Bacteriophage-Mediated Control of Biofilm: A Promising New Dawn for the Future. Front Microbiol. 2022 Apr 4;13:825828. doi: 10.3389/fmicb.2022.825828. PMID: 35495689; PMCID: PMC9048899.

18. Wolcott R, Costerton JW, Raoult D, Cutler SJ. The polymicrobial nature of biofilm infection. Clin Microbiol Infect. 2013 Feb;19(2):107-12. doi: 10.1111/j.1469-0691.2012.04001.x. Epub 2012 Aug 27. PMID: 22925473.

19. Rahim K, Saleha S, Zhu X, Huo L, Basit A, Franco OL. Bacterial Contribution in Chronicity of Wounds. Microb Ecol. 2017 Apr;73(3):710-721. doi: 10.1007/s00248-016-0867-9. Epub 2016 Oct 14. PMID: 27742997.

20. Wolcott R, Dowd S. The role of biofilms: are we hitting the right target? Plast Reconstr Surg. 2011 Jan;127 Suppl 1:28S-35S. doi: 10.1097/PRS.0b013e3181fca244. PMID: 21200270.

21. Stewart PS. Diffusion in biofilms. J Bacteriol. 2003 Mar;185(5):1485-91. doi: 10.1128/JB.185.5.1485-1491.2003. PMID: 12591863; PMCID: PMC148055.

22. Pires DP, Melo L, Vilas Boas D, Sillankorva S, Azeredo J. Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. Curr Opin Microbiol. 2017 Oct;39:48-56. doi: 10.1016/j.mib.2017.09.004. Epub 2017 Sep 28. PMID: 28964986.

23. Pinto AM, Cerqueira MA, Bañobre-Lópes M, Pastrana LM, Sillankorva S. Bacteriophages for Chronic Wound Treatment: from Traditional to Novel Delivery Systems. Viruses. 2020 Feb 20;12(2):235. doi: 10.3390/v12020235. PMID: 32093349; PMCID: PMC7077204.

24. Hausner M, Wuertz S. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. Appl Environ Microbiol. 1999 Aug;65(8):3710-3. doi: 10.1128/AEM.65.8.3710-3713.1999. PMID: 10427070; PMCID: PMC91555.

25. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002 Sep;8(9):881-90. doi: 10.3201/eid0809.020063. PMID: 12194761; PMCID: PMC2732559.

26. Rasamiravaka T, Labtani Q, Duez P, El Jaziri M. The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering

with control mechanisms. Biomed Res Int. 2015;2015:759348. doi: 10.1155/2015/759348. Epub 2015 Mar 19. PMID: 25866808; PMCID: PMC4383298.

27. Gaddy JA, Actis LA. Regulation of Acinetobacter baumannii biofilm formation. Future Microbiol. 2009 Apr;4(3):273-8. doi: 10.2217/fmb.09.5. PMID: 19327114; PMCID: PMC2724675.

28. Nadareishvili L, Hoyle N, Nakaidze N, Nizharadze D, Kutateladze M, Balarjishvili N, Kutter E, Pruidze N. Bacteriophage Therapy as a Potential Management Option for Surgical Wound Infections. Phage (New Rochelle). 2020 Sep 1;1(3):158-165. doi: 10.1089/phage.2020.0010. Epub 2020 Sep 16. PMID: 36147826; PMCID: PMC9041461.

29. Azeredo J, Sutherland IW. The use of phages for the removal of infectious biofilms. Curr Pharm Biotechnol. 2008 Aug;9(4):261-6. doi: 10.2174/138920108785161604. PMID: 18691087.

30. Knezevic P, Hoyle NS, Matsuzaki S, Gorski A. Editorial: Advances in Phage Therapy: Present Challenges and Future Perspectives. Front Microbiol. 2021 Jun 4;12:701898. doi: 10.3389/fmicb.2021.701898. PMID: 34220788; PMCID: PMC8248810.

31. Joerger RD. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poult Sci. 2003 Apr;82(4):640-7. doi: 10.1093/ps/82.4.640. PMID: 12710486.

32. Ma Y, Pacan JC, Wang Q, Xu Y, Huang X, Korenevsky A, Sabour PM. Microencapsulation of bacteriophage felix O1 into chitosan-alginate microspheres for oral delivery. Appl Environ Microbiol. 2008 Aug;74(15):4799-805. doi: 10.1128/AEM.00246-08. Epub 2008 May 30. PMID: 18515488; PMCID: PMC2519356.

33. Chang RYK, Morales S, Okamoto Y, Chan HK. Topical application of bacteriophages for treatment of wound infections. Transl Res. 2020 Jun;220:153-166. doi: 10.1016/j.trsl.2020.03.010. Epub 2020 Mar 19. PMID: 32268129; PMCID: PMC7293950.

34. Bourdin G, Schmitt B, Marvin Guy L, Germond JE, Zuber S, Michot L, Reuteler G, Brüssow H. Amplification and purification of T4-like escherichia coli phages for phage

therapy: from laboratory to pilot scale. Appl Environ Microbiol. 2014 Feb;80(4):1469-76. doi: 10.1128/AEM.03357-13. Epub 2013 Dec 20. PMID: 24362424; PMCID: PMC3911048.

35. Olofsson L, Ankarloo J, Andersson PO, Nicholls IA. Filamentous bacteriophage stability in non-aqueous media. Chem Biol. 2001 Jul;8(7):661-71. doi: 10.1016/s1074-5521(01)00041-2. PMID: 11451667.

36. Chhibber S, Kaur T, Sandeep Kaur. Co-therapy using lytic bacteriophage and linezolid: effective treatment in eliminating methicillin resistant Staphylococcus aureus (MRSA) from diabetic foot infections. PLoS One. 2013;8(2):e56022. doi: 10.1371/journal.pone.0056022. Epub 2013 Feb 13. PMID: 23418497; PMCID: PMC3572146.

37. Chhibber S, Shukla A, Kaur S. Transfersomal Phage Cocktail Is an Effective Treatment against Methicillin-Resistant Staphylococcus aureus-Mediated Skin and Soft Tissue Infections. Antimicrob Agents Chemother. 2017 Sep 22;61(10):e02146-16. doi: 10.1128/AAC.02146-16. PMID: 28739792; PMCID: PMC5610514.

38. Rai A, Khairnar K. Overview of the risks of Staphylococcus aureus infections and their control by bacteriophages and bacteriophage-encoded products. Braz J Microbiol. 2021 Dec;52(4):2031-2042. doi: 10.1007/s42770-021-00566-4. Epub 2021 Jul 12. PMID: 34251609; PMCID: PMC8578341.

39. Seth AK, Geringer MR, Nguyen KT, Agnew SP, Dumanian Z, Galiano RD, Leung KP, Mustoe TA, Hong SJ. Bacteriophage therapy for Staphylococcus aureus biofilm-infected wounds: a new approach to chronic wound care. Plast Reconstr Surg. 2013 Feb;131(2):225-234. doi: 10.1097/PRS.0b013e31827e47cd. PMID: 23357984.

40. Kifelew LG, Warner MS, Morales S, Vaughan L, Woodman R, Fitridge R, Mitchell JG, Speck P. Efficacy of phage cocktail AB-SA01 therapy in diabetic mouse wound infections caused by multidrug-resistant Staphylococcus aureus. BMC Microbiol. 2020 Jul 9;20(1):204. doi: 10.1186/s12866-020-01891-8. PMID: 32646376; PMCID: PMC7346408.

41. Gupta P, Singh HS, Shukla VK, Nath G, Bhartiya SK. Bacteriophage Therapy of Chronic Nonhealing Wound: Clinical Study. Int J Low Extrem Wounds. 2019

Jun;18(2):171-175. doi: 10.1177/1534734619835115. Epub 2019 May 13. PMID: 31081402.

42. Bagińska N, Pichlak A, Górski A, Jończyk-Matysiak E. Specific and Selective Bacteriophages in the Fight against Multidrug-resistant Acinetobacter baumannii. Virol Sin. 2019 Aug;34(4):347-357. doi: 10.1007/s12250-019-00125-0. Epub 2019 May 15. PMID: 31093881; PMCID: PMC6687786.

43. Kusradze I, Karumidze N, Rigvava S, Dvalidze T, Katsitadze M, Amiranashvili I, Goderdzishvili M. Characterization and Testing the Efficiency of *Acinetobacter baumannii* Phage *vB-GEC_Ab-M-G7* as an Antibacterial Agent. Front Microbiol. 2016 Oct 4;7:1590. doi: 10.3389/fmicb.2016.01590. PMID: 27757110; PMCID: PMC5047890.

44. Shivaswamy VC, Kalasuramath SB, Sadanand CK, Basavaraju AK, Ginnavaram V, Bille S, Ukken SS, Pushparaj UN. Ability of bacteriophage in resolving wound infection caused by multidrug-resistant Acinetobacter baumannii in uncontrolled diabetic rats. Microb Drug Resist. 2015 Apr;21(2):171-7. doi: 10.1089/mdr.2014.0120. Epub 2014 Nov 20. PMID: 25411824.

45. Mendes JJ, Leandro C, Corte-Real S, Barbosa R, Cavaco-Silva P, Melo-Cristino J, Górski A, Garcia M. Wound healing potential of topical bacteriophage therapy on diabetic cutaneous wounds. Wound Repair Regen. 2013 Jul-Aug;21(4):595-603. doi: 10.1111/wrr.12056. Epub 2013 Jun 11. PMID: 23755910.

46. Engeman E, Freyberger HR, Corey BW, Ward AM, He Y, Nikolich MP, Filippov AA, Tyner SD, Jacobs AC. Synergistic Killing and Re-Sensitization of *Pseudomonas aeruginosa* to Antibiotics by Phage-Antibiotic Combination Treatment. Pharmaceuticals (Basel). 2021 Feb 25;14(3):184. doi: 10.3390/ph14030184. PMID: 33668899; PMCID: PMC7996583.

47. McVay CS, Velásquez M, Fralick JA. Phage therapy of Pseudomonas aeruginosa infection in a mouse burn wound model. Antimicrob Agents Chemother. 2007 Jun;51(6):1934-8. doi: 10.1128/AAC.01028-06. Epub 2007 Mar 26. PMID: 17387151; PMCID: PMC1891379.

48. Marza JA, Soothill JS, Boydell P, Collyns TA. Multiplication of therapeutically administered bacteriophages in Pseudomonas aeruginosa infected patients. Burns. 2006

Aug;32(5):644-6. doi: 10.1016/j.burns.2006.02.012. Epub 2006 Jun 15. PMID: 16781080.

49. Piranaghl H, Golmohammadzadeh S, Soheili V, Noghabi ZS, Memar B, Jalali SM, Taherzadeh Z, Fazly Bazzaz BS. The potential therapeutic impact of a topical bacteriophage preparation in treating *Pseudomonas aeruginosa*-infected burn wounds in mice. Heliyon. 2023 Jul 13;9(7):e18246. doi: 10.1016/j.heliyon.2023.e18246. PMID: 37539104; PMCID: PMC10393627.

50. Dehari D, Chaudhuri A, Kumar DN, Patil R, Gangwar M, Rastogi S, Kumar D, Nath G, Agrawal AK. A Bacteriophage Microgel Effectively Treats the Multidrug-Resistant *Acinetobacter baumannii* Bacterial Infections in Burn Wounds. Pharmaceuticals (Basel). 2023 Jun 29;16(7):942. doi: 10.3390/ph16070942. PMID: 37513854; PMCID: PMC10385199.

51. Sevgi M, Toklu A, Vecchio D, Hamblin MR. Topical antimicrobials for burn infections - an update. Recent Pat Antiinfect Drug Discov. 2013 Dec;8(3):161-97. doi: 10.2174/1574891x08666131112143447. PMID: 24215506; PMCID: PMC4018441.

52. Wang B, Du L, Dong B, Kou E, Wang L, Zhu Y. Current Knowledge and Perspectives of Phage Therapy for Combating Refractory Wound Infections. Int J Mol Sci. 2024 May 17;25(10):5465. doi: 10.3390/ijms25105465. PMID: 38791502; PMCID: PMC11122179.

53. Gordillo Altamirano FL, Barr JJ. Phage Therapy in the Postantibiotic Era. Clin Microbiol Rev. 2019 Jan 16;32(2):e00066-18. doi: 10.1128/CMR.00066-18. PMID: 30651225; PMCID: PMC6431132.

54. Smith WPJ, Wucher BR, Nadell CD, Foster KR. Bacterial defences: mechanisms, evolution and antimicrobial resistance. Nat Rev Microbiol. 2023 Aug;21(8):519-534. doi: 10.1038/s41579-023-00877-3. Epub 2023 Apr 24. PMID: 37095190.

55. Akturk E, Melo LDR, Oliveira H, Crabbé A, Coenye T, Azeredo J. Combining phages and antibiotic to enhance antibiofilm efficacy against an *in vitro* dual species wound biofilm. Biofilm. 2023 Aug 2;6:100147. doi: 10.1016/j.bioflm.2023.100147. PMID: 37662851; PMCID: PMC10474582.

56. Macdonald KE, Stacey HJ, Harkin G, Hall LML, Young MJ, Jones JD. Patient perceptions of phage therapy for diabetic foot infection. PLoS One. 2020 Dec 14;15(12):e0243947. doi: 10.1371/journal.pone.0243947. PMID: 33315926; PMCID: PMC7735629.