

PHAGE THERAPY AS AN ALTERNATIVE TO ANTIBIOTICS: HARNESSING PHAGES IN ORAL MEDICINE

Filip Pospiszyl^{1*} , Monika Brzychczy-Włoch² , Anna Morys¹ , Tomasz Kaczmarzyk³ 

¹Student Scientific Association at the Department of Oral Surgery, Jagiellonian University Medical College, 31-155 Krakow, Poland

²Chair of Microbiology, Department of Molecular Medical Microbiology, Jagiellonian University Medical College, 31-121 Krakow, Poland

³Chair of Oral Surgery, Jagiellonian University Medical College, 31-155 Krakow, Poland

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Abstract: This narrative review aims to discuss the potential of phage therapy as an alternative to antibiotic therapy, with a particular emphasis on its potential applications in dentistry.

Despite historical fluctuations, phage therapy is gaining increasing acceptance and becoming more widespread in the treatment of multidrug-resistant (MDR) infections. Contemporarily, phage therapy has become a prevalent treatment modality for bacterial infections of the digestive system in countries such as Georgia. In clinical trials, it has been used to treat wounds, bone and blood infections. In theory, it can be used in any instance of bacterial infection, including within the oral cavity. There are numerous methods of administration, and the therapy itself is regarded as both safe and effective, particularly in cases where antibiotics are ineffective, such as in the development of biofilms or infections by MDR bacteria.

Despite the lack of ample clinical studies evaluating the effectiveness of this therapy in dentistry, a growing body of evidence suggests its potential use in restorative dentistry, endodontics, periodontology, as well as oral and maxillofacial surgery.

To determine whether phage therapy can be considered a viable alternative or to assess its effectiveness when used in combination with antibiotic therapy, further regulations and research are warranted.

1. Introduction. 2. Discovery and development of phage therapy. 3. The characterization of phages. 4. Methods of phage administration. 4.1. Phage clearance. 5. Safety of phage therapy. 5.1. Safety comparison of phages and antibiotics. 6. The legal regulation of phage therapy. 7. Biofilm penetration by phages. 8. Examples of phage prophylaxis and concept of phage prophylaxis in dental surgery. 9. Phage therapy in clinical trials. 10. Use of phage therapy in dentistry and oral surgery. 10.1. Phage therapy in restorative dentistry and endodontics. 10.2. Phage therapy in periodontics. 10.3. Phage therapy in oral and maxillofacial surgery. 11. Bacterial phage resistance? - the arms race between phage and bacteria. 12. Combined antibiotic and phage therapy. 13. Conclusions

Keywords: antibiotic resistance; bacteriophage; biofilm; dentistry; phage therapy

1. Introduction

Bacteriophages are a group of viruses that can adhere to bacteria, inject their genome into the bacterial cell, and eventually induce bacterial lysis (Naureen et al. 2020). Their discovery dates to the beginning of the 20th century, and their clinical implementation in the treatment of bacterial infections was tested shortly after. However, due to the introduction and development of antibiotic therapy, phage therapy was greatly abandoned by Western science (Marongiu et al. 2022).

Nowadays, humanity stands on the verge of the post-antibiotic era (Kwon and Powderly 2021), with a desperate need to find alternatives to treat infection of multidrug-resistant (MDR) bacteria, mostly belonging to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* sp.). Contemporary scientific data indicate that the emergence of antibiotic resistance in bacterial pathogens is a growing concern for global health (Ventola 2015; Frieri et al. 2017; Hofstee et al. 2020; Man-

* Corresponding authors: Monika Brzychczy-Włoch, Jagiellonian University Medical College, Faculty of Medicine, Chair of Microbiology, Department of Molecular Medical Microbiology, Czysa 18, 31-121 Krakow, Poland e-mail: m.brzychczy-wloch@uj.edu.pl. Filip Pospiszyl, Student Scientific Association at the Department of Oral Surgery, Jagiellonian University Medical College, ul. Montel-pich 4, 31-155 Krakow, Poland, e-mail: filip.pospiszyl@student.uj.edu.pl;

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cusso et al. 2021). There is a risk that bacteria causing infections that have been treatable with antibiotics will become resistant to these drugs within the next few decades. This imminent threat has prompted a response from the scientific community in diverse fields, with researchers undertaking efforts to identify novel, more efficacious antibiotics (Miethke et al. 2021).

There is a need to find alternative solutions that could mitigate the impact of this looming crisis. One of these is bacteriophage therapy, also known as phage therapy (PT). This approach traces its origins to the

early 20th century, yet it has only recently garnered significant attention (Figure 1). The concept involves the use of carefully selected bacteriophages to combat pathogenic bacteria (Sawa et al. 2024).

This narrative review examines the potential of phage therapy, which can be utilized to both prevent and treat bacterial infections, particularly in dental settings. It revises forms of its administration, analyses the results of published clinical trials, and discusses its safety and potential risks.

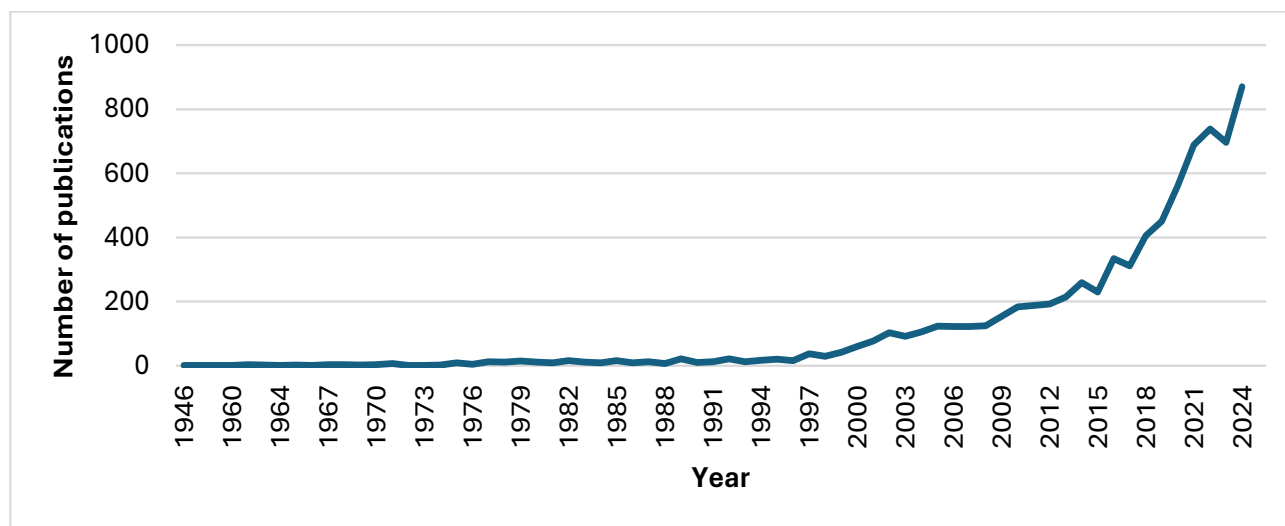


Figure 1. The number of papers retrieved from PubMed using the keyword ‘phage therapy’ that were published between 1946 and 2024 shows a significant increase in research in recent years.

2. Discovery and development of PT

Many scientists have observed bacteriophage activity since the late 19th century. However, the first to understand that they were dealing with a new organism, rather than, for example, an enzyme, was the French Canadian microbiologist Félix d’Hérelle (Marongiu et al. 2022). In 1915, during World War I, he investigated the outbreak of severe haemorrhagic dysentery among French troops. He made filtrates of faeces that were free from bacteria. Then, he spread them on the agar cultures of *Shigella* spp strains collected from sick soldiers. He observed blank spots that he later named *plaques*. Two years later, in 1917, he presented his findings during a meeting of the Academy of Sciences, and he proposed the name “bacteriophage” from bacteria and the Greek word *phage*, which translates as “to eat” (Chanishvili 2012). He realized the importance of his discovery, so after conducting small animal trials and testing phages for safety on himself, he proceeded to

clinical trials on humans, initiating what is now known as PT (Marongiu et al. 2022).

In 1919, three young brothers with symptoms of dysentery were admitted to the hospital where Félix d’Hérelle worked. Their sister had died of this disease a day before. One day after the introduction of phage therapy, they fully recovered. He later treated in a similar way cholera with positive results, which encouraged other researchers to test the therapy. For example, British Lieutenant Colonel John Morison used phage therapy as a prophylaxis for cholera in the Indian region of Naogaon (now Bangladesh). He allegedly administered phages to 530,000 people, which resulted in zero cases of cholera in that region between 1925 and 1935. In contrast, during the same period in the neighbouring region, 1,500 people died of the disease (Summers 1993).

Despite many clinical successes, there were voices in the scientific community that d’Hérelle and his colleagues conducted experiments that did not meet

scientific standards and, therefore, did not provide a factual basis for using this form of therapy. Notably, he was accused of lacking controlled conditions and statistical significance in his results. In connection with the above, the scientific community at the time did not accept PT as an effective treatment method and continued to rely on preventive measures, such as improved hygiene and vaccinations (Summers 1993).

In 1930, two American physicians, E. D. Crutchfield and B. F. Stout, conducted a study on 57 patients with staphylococcal wound infections using phage therapy, achieving a success rate of over 90% (Crutchfield and Stout 1930). This result encouraged d'Hérelle to bring his discovery to the market and start mass production. However, over time, it became apparent that the effectiveness of these phage preparations was significantly lower than initially reported in preliminary studies, which discouraged pharmaceutical companies, patients, and scientists alike. The decrease in efficacy was caused by several factors, including poor storage conditions of the preparations, their short shelf life, the use of mercury as a preservative, the failure to inactivate them by gastrointestinal substances, and the implementation of phage therapy to treat viral infections (Ho 2001; Marongiu et al. 2022).

The subsequent introduction of the first antibiotics (penicillin and sulfanilamide), which was then considered a breakthrough in contrast to bacteriophages where each phage affected only one type of bacterium, led to a further decline in the popularity of PT in the West (Marongiu et al. 2022).

During d'Hérelle work at the Institute Pasteur in Paris, he met Georgian bacteriologist Georgiy Eliava. Eliava was fascinated by d'Hérelle's discoveries, and in 1923, he founded the Institute of Bacteriology in Tbilisi, the capital of Georgia. It received massive support from the authorities of the Soviet Union, who wanted to catch up with the West, also in the field of bacteriology. Unfortunately, in 1937, during the Great Purge, Eliava was declared an enemy of the people and then shot. This fact led the USSR to lose a scientist recognized in the West (Marongiu et al. 2022). Despite this, the Soviets continued to develop and use phage therapy (Summers 2001). The Iron Curtain after World War II hindered the flow of scientific knowledge and strengthened the belief of phages as a communist medicine among Western scientists (Summers 2012).

Further controversy was also caused by d'Herell's views, which some scientists even described as heresy.

He claimed that phages, which naturally exist in the human microbiota, are responsible for the body's immunity and that phages can spread just like pathogens they are supposed to kill, so one should not pay too much attention to personal hygiene. He argued that contamination with faeces can paradoxically contribute to the improvement of the patient's condition because, in this way, one receives new portions of phages (Fruciano and Bourne 2007).

In 1946, at St. Mary's Hospital in London, the same hospital where penicillin was discovered, F. Himmelweit presented the concept of therapy based on the joint administration of phages and penicillin. The assumption was that this would lead to a reduction in the emergence of penicillin-resistant strains. The preliminary results of the research were auspicious, which was probably due to the different effects of penicillin and phages on bacteria. Unfortunately, this principle was not widely accepted in the scientific community (MacNeal et al. 1946; Fruciano and Bourne 2007).

It is estimated that in the process of finding new antibiotics, only five of the 5,000 to 10,000 molecules tested make it through to the first stage of testing, but only one of these is approved for use in humans. Given the enormous costs and complexity of developing new antibiotics, as well as the increasing multi-drug resistance of bacteria, the scientific world is seeking alternatives. Today, more than 100 years after d'Herell's discovery, PT is being rediscovered and increasingly tested; however, scientists agree that it needs to be standardized, and more clinical studies are warranted (Gordillo Altamirano and Barr 2019; Suh et al. 2022).

3. The characterization of phages

Phages, as representatives of viruses, are unable to function and replicate outside the host organism. They are the most abundant organisms on Earth, and in addition to bacteria, it has been proven that they can occur inside archaea and even eukaryotes (Lehti et al. 2017; Naureen et al. 2020). The reproductive cycles of bacteria and phages are closely linked; however, each cycle produces between 100 and 200 new phages but only two daughter bacterial cells. This phenomenon suggests that there would be a significant disproportion in the number of bacteria or even their disappearance, but this does not occur. To understand this, it is essential to have a comprehensive understanding of

phage biology and its impact on bacteria (Naureen et al. 2020).

Bacteriophages have a complex structure, particularly when compared to simpler viruses, such as those with only a capsid and genetic material. It consists of DNA or RNA, single or double-stranded, enclosed in a protein capsid that occurs in three forms: head with tail, head without tail, and filamentous form (Naureen et al. 2020). Tailed phages (collected in the *Caudovir-*

icetes class) are the most common group, previously represented as the families *Myoviridae*, *Siphoviridae*, and *Podoviridae* (Figure 2). However, the predominant feature they exhibited was morphology; presently, the emphasis is on classification based on the genome. Despite this, attention is drawn to the importance of morphological similarities. Hence, the terms myovirus, siphovirus and podovirus remain in use (Turner et al. 2023).

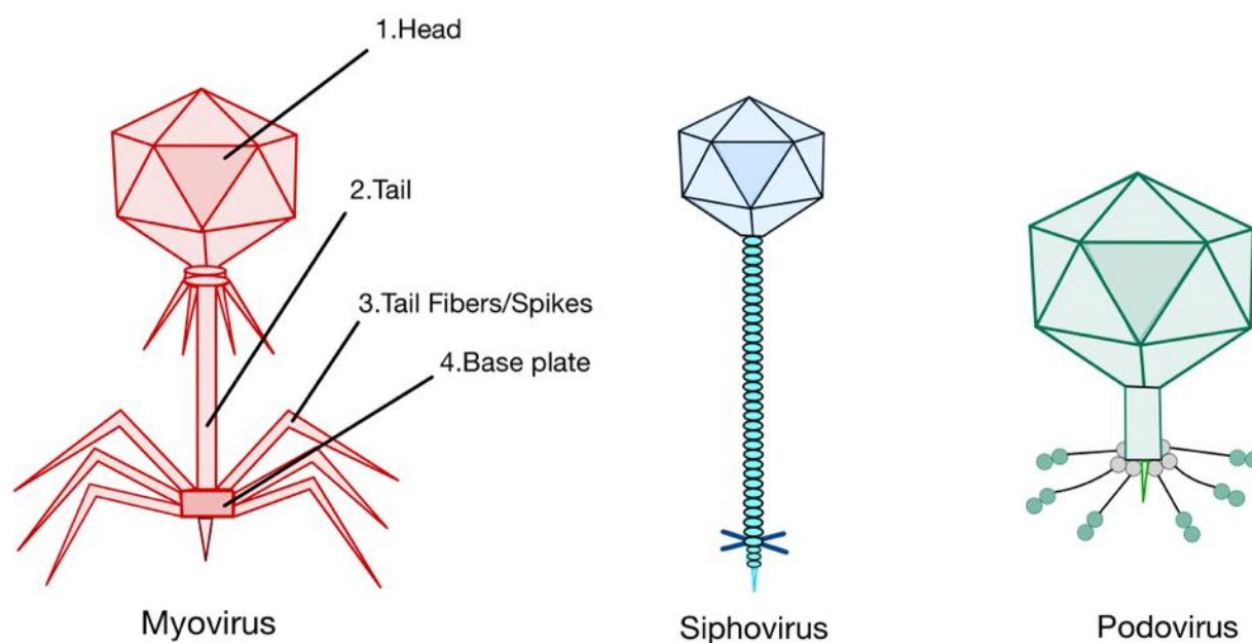


Figure 2. The diagram illustrates a simplified representation of the structural composition of different types of tailed phages class (*Caudoviricetes*): 1. Head - a protein structure, often icosahedral in shape, containing the phage's genetic material (single or double strand of DNA or RNA); 2. Tail - a tubular structure used by the phage to inject its genetic material into the host cell; 3. Tail fibres or spikes - enable the phage to recognize and attach to the appropriate receptors on the surface of the bacterial cell and determine the specificity of the bacteriophage to a particular bacterium; 4. Base plate - anchors the tail fibres and binds them to the surface of the bacterium, enabling the infection to begin. Graphic design was based on Nobrega et al. (2018).

There are four different phage life cycles: lytic, lysogenic, pseudo-lysogenic and chronic. Only lytic phages are currently considered the most suitable for treating humans (Figure 3) (Lin et al. 2017). First, the phage must attach to the bacterial cell wall by recognizing its specific receptor on the bacterial surface. Some phages possess the ability to produce specific enzymes, such as hydrolases, which can degrade the polysaccharide envelope of a bacterium, thereby exposing the appropriate receptors (Wittebole et al. 2014). After attaching to the cell wall, the phage creates a hole in

it through which it injects its genetic material into a bacterium while the protein capsid remains outside. In the case of lytic phages, the bacterial ability to reproduce viral proteins and genetic material is exploited downstream. Phage assembly is followed by lysis of the bacterial cell and the release of new viral particles. The amount of viruses released depends on several factors, including the type of virus, the type of bacterium, and environmental conditions (Wittebole et al. 2014; Naureen et al. 2020).

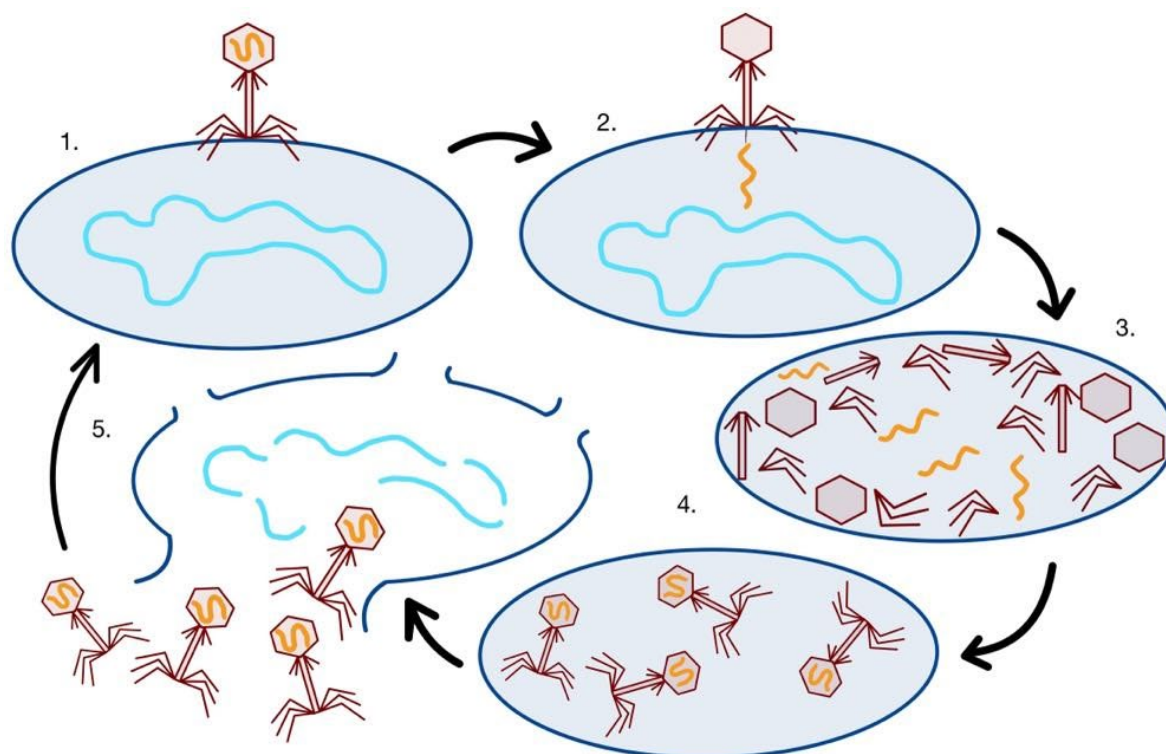


Figure 3. Diagram illustrating the lytic cycle of a bacteriophage, highlighting its mechanism of bacterial cell destruction – a key process in phage therapy: 1. Attachment; 2. Penetration; 3. Biosynthesis; 4. Replication and maturation; 5. Lysis. Graphic design based on Adesanya et al. (2020).

4. Methods of phage administration

The specificity of phage therapy predicated on the necessity to administer a particular phage to combat a specific bacterium and the brief duration of phage activity within the body necessitate the development of novel methodologies for phage administration to patients. In addition to oral or intravenous administration, there are also inhalation, implantation of biomaterials, intraperitoneal, intramuscular, subcutaneous, intranasal, endotracheal, rectal, intrauterine, vaginal or transdermal routes of administration (Dąbrowska 2019; Rotman et al. 2020). It had been suspected that phages might be rendered ineffective by the presence of low gastric acid pH, which required their administration in conjunction with acid-neutralizing medications. However, subsequent research has demonstrated that the co-administration of these drugs with phages results in no alteration to their efficacy (Dąbrowska 2019). The oral route of administration is efficacious in treating gastrointestinal diseases (Qadir et al. 2018; Dąbrowska 2019). For instance, a study was conducted on the treatment of *Clostridioides difficile* infection using phage therapy in an animal model, resulting in

a reduction in *C. difficile* colonization and a delay in the onset of symptoms (Nale et al. 2016). However, the oral route of administration is the least effective for systemic penetration of phages. It is essential to note that increasing the administered dose of phages results in enhanced systemic penetration. A systematic analysis has demonstrated the superiority of parenteral administration, whether intravenous (IV), intramuscular (IM), or intraperitoneal (IP) over oral dosing (Dąbrowska 2019).

Regarding IV administration, Speck and Smithyman, in their work on the safety of intravenous phage therapy, have described the use of highly specific phage cocktails in treating rhinosinusitis caused by *Staphylococcus aureus*. Furthermore, they discussed a potential application of phage therapy in the management of acute infective endocarditis (Speck and Smithyman 2016). This condition may be caused by bacteraemia following oral surgery (e.g. tooth extraction) in a high-risk patient.

Recent studies on the intramuscular (IM) administration of phages are lacking; however, a significant number of such studies have been documented in the Soviet scientific literature. For instance, in the treat-

ment of typhoid fever, patients were divided into three groups: intramuscular (IM), oral, and combined intramuscular and oral administration. The most unfavourable outcomes were observed for oral administration, while significantly superior outcomes were demonstrated in other groups (Chanishvili 2012).

To date, no studies on IP administration have been conducted in humans. However, animal research demonstrated the efficacy of this route of administration, surpassing the effectiveness of phage inhalation, for instance, in the treatment of pneumonia caused by *Burkholderia cenocepacia* (Carmody et al. 2010).

Following systemic administration, phages can reach virtually all tissues and organs of the body, including, but not limited to, skeletal muscle, heart, bone marrow, salivary glands, kidneys, and even the brain and bones (Dąbrowska 2019). However, oral administration may be ineffective due to the poor penetration of phages into the circulation, and intravenous administration appears to be significantly more efficacious (Dąbrowska 2019; Vila et al. 2024).

Regarding oral diseases, the most efficacious treatment may be rinsing the affected area with a phage solution or the local application of a slow-releasing hydrogel, which enables the appropriate concentration of phages within the oral cavity.

4.1 Phage clearance

The primary organs responsible for the uptake of bacteriophages from the bloodstream are the liver and spleen, with the macrophages present in these organs playing a specific role in this process. Animal research has demonstrated that phages are filtered and reach their highest concentrations in the spleen; however, the fastest inactivation occurs in the liver via Kupffer cells (Inchley 1969). The renal excretion of phages in the urine is minimal, likely attributable to their morphology, as they are too large to be filtered by the kidneys. The dosage of phages, as well as their type (size) and route of administration, seem to be all significant factors. No renal clearance was observed with subcutaneous administration as opposed to intraperitoneal injection (Dąbrowska 2019).

There are several ways to delay phage clearance, such as increasing the dose or inactivating the complement system; however, the most promising approach appears to be encapsulation of the phage particles, as seen in the case of phages against *Klebsiella pneumoniae*, which have been enclosed in a liposomal shell. It has been demonstrated that this procedure results in a prolonged retention of phages within the body, even in the absence of the targeted bacteria (Singla et al. 2015).

Numerous technological methodologies have been developed to extend the shelf life of phages and delay their removal from the body through stabilization and encapsulation techniques. The most widely employed techniques are those involving freeze drying, spray freeze drying and spray drying. Freeze-drying, also known as lyophilization, is a process that can be categorized into two distinct stages. The liquid containing the phages is subjected to a freezing process at a very low temperature, after which it is dried and ground to yield a powder which may be used for inhalation. Spray freeze drying is based on a similar principle. The process involves exposing a spray containing phages to liquid nitrogen, resulting in the production of a porous phage powder. This method has been shown to induce a lower degree of thermal stress in the phages compared to the spray drying process, which involves spraying a suspension containing phages into a chamber filled with hot, dry gas. The water evaporates rapidly, leaving the phages in a powdered state. It should be noted, however, that numerous alternative methods are available (Malik et al. 2017). In terms of phage storage, maintaining a temperature of 4°C has been demonstrated to be an efficient method for prolonging shelf life (Xu et al. 2023).

5. Safety of phage therapy

It is widely accepted that PT is safe and has no severe side effects (Uyttebroek et al. 2022; Kim et al. 2024; Palma and Qi 2024). The safety of phage therapy stems from the specificity of the phage, which can target a particular bacterial cell exclusively. This selectivity distinguishes it from antibiotics, which can affect the host microbiota. The specificity of phage action also means that pathological bacteria acquire phage resistance much less frequently than with antibiotics. While it is acknowledged that phage administration can elicit an immune response, to date, there is no evidence that phages are capable of directly attacking human cells. In the presence of compatible bacterial cells, phages can replicate; otherwise, they tend to self-regulate (Uyttebroek et al. 2022).

A substantial body of scientific research (Uyttebroek et al. 2022; Kim et al. 2024; Palma and Qi 2024) has demonstrated the safety of phage therapy in treating patients. No severe adverse reactions have been reported; however, some authors have noted inadequate standardization of safety studies (Chung et al. 2023). However, it is acknowledged that there exists a risk of toxic shock precipitated by the release of bacterial endotoxins following phage-induced bacterial lysis (de Tejada et al. 2015; Aslam et al. 2019). A 2017

study provides a notable example. It documented the case of a 2-year-old child infected with multidrug-resistant *Pseudomonas aeruginosa*, complicated by sepsis. The patient exhibited a chronic medical condition characterized by DiGeorge syndrome, a complex congenital heart defect involving an interrupted type B aortic arch, and an allergy to antibiotics, among other co-morbidities. The presence of both allergies and infection with MDR *P. aeruginosa* led researchers to consider bacteriophage therapy as a potential treatment. The child was administered two bacteriophages, resulting in the resolution of the sepsis. However, this intervention led to an exacerbation of the underlying heart failure, most likely attributable to the release of endotoxins following bacterial degradation (Duplessis et al. 2018).

Despite extensive research conducted over several decades on phage administration and its potential adverse effects, no documented cases of anaphylactic shock have been reported in patients receiving phage therapy (Speck and Smithyman 2016; Doub et al. 2020; Chung et al. 2023).

The safety of PT in children has been well-documented (Howard-Jones et al. 2022). To the best of our knowledge, there are no reports on the safety of phage therapy in pregnancy.

5.1 Safety comparison of phages and antibiotics

It is now widely acknowledged that the gut microbiota plays a pivotal role in the optimal functioning of the human body (Khalil et al. 2024). The use of antibiotics can result in a substantial reduction of beneficial microflora within the gastrointestinal tract, thereby promoting the proliferation of multidrug-resistant (MDR) *Clostridioides difficile* or the yeast *Candida albicans*. Recent studies have shown that phages have a minimal impact on the intestinal microflora, mainly due to their specificity (Chung et al. 2023).

In addition to the adverse effects of antibiotics on the human microbiota, their use can cause many, often severe, complications like pseudomembranous colitis. Other serious complications may also arise, including ototoxic effects of macrolides and aminoglycosides, liver damage, encephalopathy, or anaphylactic shock, among numerous others (Cunha 2001). In contrast, most studies on PT report no adverse effects. However, some authors do note the occurrence of some minor side effects, including redness, inflammation, fever and hypotension. To provide an accurate assessment of possible side effects, further research is warranted, along with the systematization and regulation of PT in its entirety.

6. The legal regulation of phage therapy

The issue of the absence of appropriate legal regulations is raised in this work and numerous other studies on phages. As stated in the historical introduction, PT was utilized on a broader scale in the Union of Soviet Socialist Republics (USSR) following World War II, with a particular emphasis on Georgia Republic. It was also used in Poland. Following Poland's accession to the European Union in 2004, this therapy has become more comprehensive and systematic. Nowadays, it is considered an experimental treatment based on the Act on the Professions of Physician and Dentist, the Constitution of the Republic of Poland, as well as EU regulations, such as Directive 2005/28/EC, and the European Medicines Agency. PT is exclusively administered by the Phage Therapy Unit, a constituent of the Hirsfeld Institute of Immunology and Experimental Therapy (Yang et al. 2023).

In contrast, in countries such as Georgia and Russia, the regulatory landscape is significantly different, with phage preparations, including 'Intestiphage' and 'Pyophage', available for purchase without a prescription. Regarding Georgia, the prescription-only provision for personalized phage medicines is permitted in designated pharmacies. However, these products have not been recognized by Western regulatory authorities (Yang et al. 2023).

7. Biofilm penetration by phages

Many species of bacteria form a biofilm, a complex environment composed of polysaccharides, proteins, and lipids that are designed to protect the bacteria from external factors. In practical terms, biofilms can effectively block antibiotic access to bacterial cells, making the treatment of infections significantly more challenging (Yuan et al. 2019).

There are numerous reports of bacteriophages' effectiveness in penetrating the bacterial biofilms of bacteria such as *P. aeruginosa* or *Streptococcus suis*. However, these studies indicate the need for further trials and the potential possibility of antibiotic-phage combination therapy (Hanlon et al. 2001; Meng et al. 2011; Yuan et al. 2019).

Bacterial biofilms, which pose a serious clinical challenge due to their ability to block access to antibiotics, are present in many parts of the body, such as the oral cavity (Rath et al. 2021), intestines (Jandl et al. 2024), and the female reproductive tract (Obuobi and Škalko-Basnet, 2024). A mixture of aerobic and anaerobic bacterial strains generally constitutes it. As previously outlined, the capacity of phages to penetrate

deeply into biofilms offers a promising prospect for the effective management of the bacteria that form these structures, potentially in conjunction with or as an alternative to antibiotics (Figure 4).

Although phages isolated from the oral cavity have not yet been used clinically, they have shown prom-

ising results *in vitro*. The SMHBZ8 phage is a prime example, as it has been demonstrated to be efficacious in combating both planktonic and biofilm cultures of *Streptococcus mutans*. This phage has the potential for practical use in future clinical trials (Zhu et al. 2025).

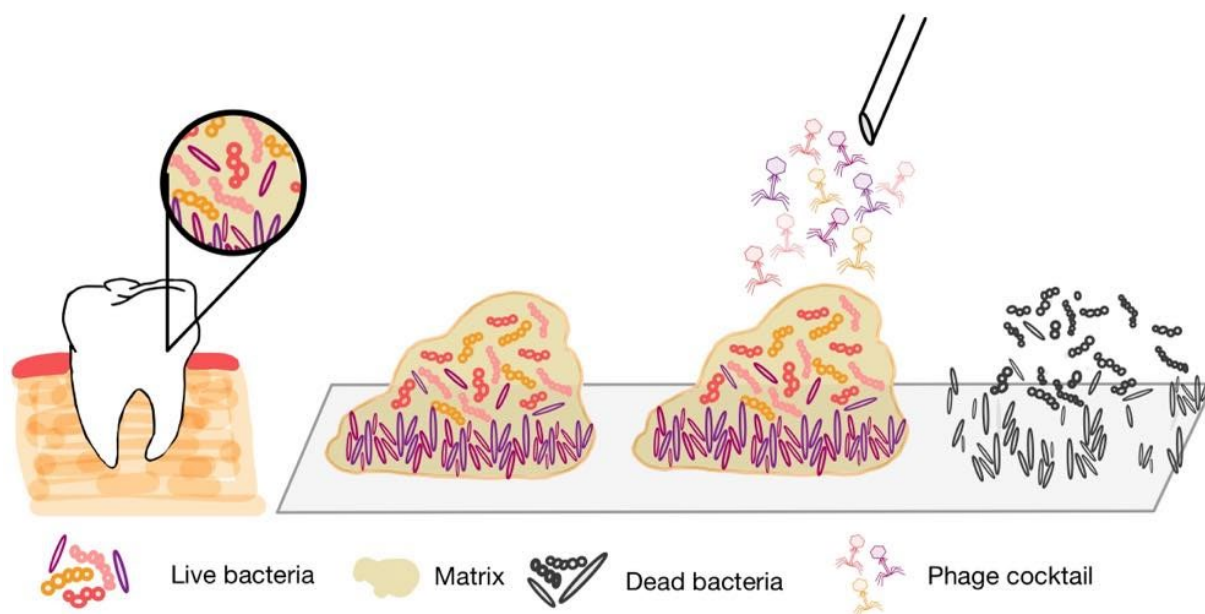


Figure 4. The biofilm in the oral cavity can lead to oral infections such as dental caries, gingivitis, or periodontal disease, and a carefully selected phage cocktail may have a potential effect on biofilm degradation.

8. Examples of phage prophylaxis and the concept of phage prophylaxis in oral surgery

For PT to be considered a complete alternative to antibiotic therapy, it would also need to be used as part of the prevention of bacterial infections. Broad-spectrum antibiotics, such as amoxicillin, are often administered to patients to prevent infection. Preventive action is challenging with phages due to their specificity; however, it is possible to create specific cocktails consisting of multiple different phages that are suitable for the groups of bacteria most likely to cause infections in a given area. The finding that encapsulated phages exhibit prolonged persistence within the human body (Singla et al. 2015; Malik et al. 2017) may provide a rationale for exploring the potential of phages in preventing bacterial infections. However, further research is necessary to test this hypothesis. A promising example of the prophylactic use of phage therapy is the study by Yu-Huai Ho et al., in which phage against *A. baumannii* carbapenem-resistant (CRAB) was aerosolized in intensive care units. This study demonstrated a reduction in the incidence of CRAB from 8.57 per 1,000 patient days in the hospital to 5.11 per 1,000 patient days (Ho et al. 2016). Another similar study was

conducted by Chun-Chieh Tseng et al. in a unit with patients undergoing extracorporeal membrane oxygenation (ECMO). As before, an aerosol was used, this time containing a cocktail of four phages against the bacteria most commonly causing nosocomial infections in ECMO patients: *A. baumannii*, *K. pneumoniae*, *P. aeruginosa* and *Stenotrophomonas maltophilia*. As a result, no ECMO patient whose environment was decontaminated with phage aerosol became infected with any of the four target bacteria (Tseng et al. 2025).

The utilization of phages for prophylactic purposes in the domains of oral and general surgery is a matter of considerable complexity. The term “antibiotic prophylaxis” may refer to the administration of a broad-spectrum antibiotic to a patient at high risk of infective endocarditis prior to a dental procedure, such as tooth extraction. However, it is worth noting that phages require the presence of bacteria to be active and to carry out their life cycles. Consequently, these measures should be administered during or even after the procedure, in which case there is no question of prophylaxis. Advancements in phage encapsulation methodologies have the potential to extend the duration of phage presence in the body and delay clearance, thereby enabling the prophylactic use of phages prior to scheduled surgical interventions (Singla et al. 2015).

9. PT in clinical trials

Currently, one of the most popular methods of phage therapy (PT) is what can be described as profiled or even personalized PT. It is based on sampling the causative strain and selecting the appropriate phages for clinical administration. This method is most suitable for patients with intractable or recurrent infections caused by antibiotic-resistant bacteria. The following are examples of such use.

A patient with a spinal abscess caused by multi-drug-resistant *P. aeruginosa* underwent surgery during which the abscess was drained and a personalized cocktail of three bacteriophages, specifically prepared for the procedure, was administered into the abscess cavity along with a bicarbonate solution. At the same time, an antibiotic (cefiderocol) was administered intravenously. The pre-existing back pain disappeared after the operation, but there was diarrhoea associated with *C. difficile*. For the second surgery, related to the screw-in osteosynthesis implants, phages and antibiotics were again administered. In the months that followed, the patient experienced no pain or adverse effects (Ferry et al. 2022).

Another promising example of the use of personalized phage therapy is that of a 30-year-old female victim of the 2016 Brussels airport terrorist attack.

The patient suffered severe injuries, including to her left thigh. The left thigh wound was found to be infected with pan-drug resistant (PDR) *K. pneumoniae*. Antibiotic treatment was implemented, which led to numerous side effects without eradicating the infection. After exhausting other treatment options, PT was administered 702 days after the accident. Phages were administered locally through a catheter left over from a previous procedure. They were given for 6 days along with a general course of antibiotics. There was a dramatic improvement in the patient’s condition, with the permanent eradication of the *K. pneumoniae* infection (Eskenazi et al. 2022).

A retrospective observational analysis of 100 cases of personalized PT for difficult-to-treat infections showed that this therapy is most effective when combined with antibiotics. Without co-administration of antibiotics, bacterial eradication by phages alone was 70% less likely to occur. These examples imply that, in some cases, antibiotics and phage therapy can be synergistic (Pirnay et al. 2024).

The potential of PT to treat bacterial infections in any organ or tissue in the body is a promising avenue for further research (Figure 5).

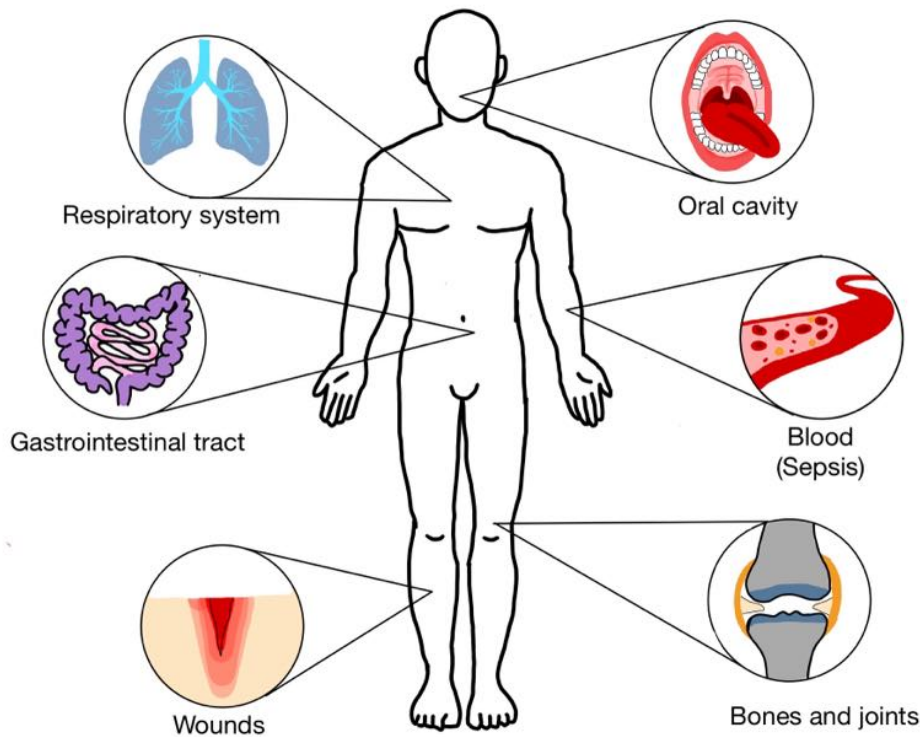


Figure 5. The diagram indicating the possible areas of PT use: oral cavity (Khalifa et al. 2016; Guo et al. 2024), blood (Górski et al. 2017), bones and joints (Peng et al. 2024), wounds (Jault et al. 2019), gastrointestinal tract (Nale et al. 2016) and respiratory system (Abedon 2015).

10. The use of phage therapy in dentistry and oral surgery

In the oral cavity, bacteria pose a significant challenge, given their role in the development of various conditions, including dental caries, pulp inflamma-

tion, periodontal disease, abscesses, peri-implantitis, and bone necrosis. This comprehensive list illustrates the diverse array of oral issues that can arise from bacterial infection (Figure 6). Antibiotics are frequently employed in the treatment of these; however, PT has the potential to either complement or replace them.

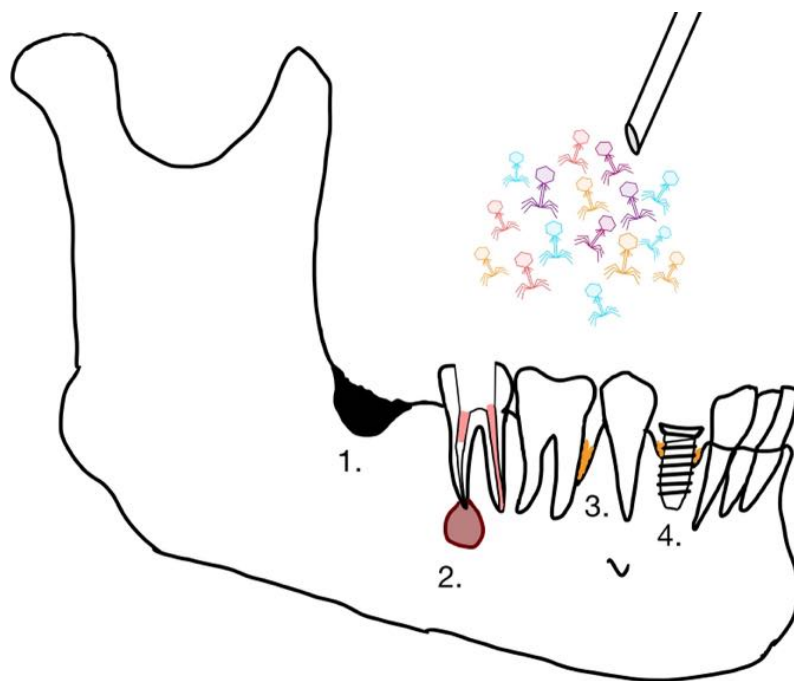


Figure 6. Examples of potential applications of PT in dentistry: 1. Osteonecrosis; 2. Necessity of endodontic re-treatment; 3. Periodontal treatment; 4. Periimplantitis.

10.1 Phage therapy in restorative dentistry and endodontics

The potential of phages to penetrate biofilms and tissues, their specificity of action, and the increasing antibiotic resistance of bacteria have led to the emergence of phage therapy (PT) as a promising tool in dentistry (Zhu et al. 2025).

Regarding restorative dentistry and caries treatment, the objective is to prevent or inhibit tooth decay by limiting the growth of pathogens responsible, such as *S. mutans*. *In vitro* and *in vivo* animal studies have demonstrated the efficacy of SMHBZ8 phage against the specified bacterium. This finding suggests that the phage could be utilized in future human treatments (Wolfoviz-Zilberman et al. 2021)). Concerning the endodontic treatment, particularly in cases of re-treatment, a frequently detected and profoundly problematic *Enterococcus faecalis* has been identified. *In vitro* studies on extracted human teeth have demonstrated

the efficacy of phages in eradicating *E. faecalis* from root canals, particularly when combined with 0.5% sodium hypochlorite (NaOCl) rinsing, resulting in an 84% reduction in biofilm mass. This result is comparable to that obtained with the use of NaOCl at a concentration of 5.25%, which is potentially toxic to periapical and surrounding tissues. That result suggests that a lower concentration of NaOCl could be equally effective. Further research, including human trials, is necessary (Khalifa et al. 2016; Tinoco et al. 2017; Basak Erol et al. 2024).

10.2 Phage therapy in periodontics

Socransky's seminal work (Socransky et al. 1998) described the bacteria present in the oral cavity in the form of complexes, among them a red complex containing the bacteria most virulent in the periodontium, namely *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*. In contrast to the latter

two, to date, no bacteriophage with lysogenic activity against *P. gingivalis* has been identified. The identification and description of phages active against other bacteria responsible for periodontitis has been successfully achieved. Among these are *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* (the discovered phage is specific only against one serotype of this strain), *Actinomyces naeslundii* and *E. faecalis* (often present in deep periodontal pockets). Further research is needed to determine the potential application of phages in the treatment of periodontitis in humans (Pazhouhnia et al. 2022; Guo et al. 2024).

Although no lytic phage has yet been identified against highly problematic *P. gingivalis*, it has been demonstrated that there is a relationship between *P. gingivalis* and *Streptococcus gordonii*, where the latter, as the pioneer colonizer, must create the appropriate conditions for *P. gingivalis* to attach to by creating specific binding sites and later to form its biofilm. One study implementing the phage ΦSG005 targeted against *S. gordonii* revealed a promising significant reduction in *P. gingivalis* abundance of over 99% (Wu et al. 2024). Another in vitro study revealed phage peptides capable of stimulating non-neoplastic proliferation of mucosal epithelial cells. This approach has the potential to be developed into a future treatment for oral mucosal healing, similar to the use of phages to heal skin wounds (Li et al. 2013).

However, it is important to note that phages may not necessarily have an exclusively positive effect on the periodontium. In patients diagnosed with periodontitis, the Siphoviridae_29632 phage was isolated, with a significantly higher prevalence observed in patients with periodontal disease compared to healthy individuals. This observation raises the suspicion that the virus may create favourable conditions for the development of periodontal disease; however, there is a lack of compelling evidence to substantiate this hypothesis. Further research is warranted to ascertain the potential correlation between this phage and the development of periodontal disease (Zhang et al. 2019).

10.3 Phage therapy in oral and maxillofacial surgery

There is great potential for the use of PT in oral and maxillofacial surgery. Phages have been demonstrated to be a safe and effective treatment option for bone and joint infections. Their ability to penetrate bacterial biofilms makes them an ideal choice for treating chronic infections. The effectiveness of treating bone infections using phage therapy (PT) has been demonstrated through both the administration of phage cocktails and individualized therapy. There are high hopes for their development (Clarke et al. 2020).

To date, no research has been conducted on the use of phage therapy in the treatment of periimplantitis. Recent systematic reviews and meta-analyses have demonstrated that *Staphylococcus epidermidis*, *F. nucleatum*, *T. denticola*, *T. forsythia*, *P. intermedia* and *P. gingivalis* are predominantly implicated in the development of peri-implantitis (Săndulescu et al. 2023). The efficacy of phage therapies has been demonstrated against *F. nucleatum*, *T. denticola*, *T. forsythia* and *S. epidermidis* (Štrancar et al. 2023; Guo et al. 2024). Regarding *P. gingivalis*, a reduction in titre has been observed (Wu et al. 2024), but no effective phage against this bacterium has yet been identified. Moreover, there are reports of the capacity of phages to adhere to zirconia, which engenders considerable optimism for the prospective treatment of periimplantitis with these microbes (Hashimoto et al. 2011). Consequently, the prospect of formulating a phage cocktail that is efficacious in treating periimplantitis is conceivable; however, conducting clinical trials is imperative to validate this hypothesis.

It has been demonstrated that bacteria such as *P. gingivalis*, *F. nucleatum*, and *T. denticola* can influence the development of oral squamous cell carcinoma (OSCC) by stimulating epithelial cell proliferation, inhibiting apoptosis, and modulating the inflammatory microenvironment. This dysbiosis is considered to represent a specific link between periodontal disease and OSCC. This may necessitate the implementation of PT against the aforementioned bacteria (Guo et al. 2024).

Animal studies also demonstrated that oral immunization with phage MS2-L2 VLPs can protect against infection with highly oncogenic types of HPV associated with head and neck cancers (mostly against HPV-16, -35, -39 and -58). This may offer the possibility of creating phage vaccines against oral malignancy (Zhai et al. 2019).

It is also evident that phages have the potential to be utilized in cancer therapy in various ways. It has been demonstrated that suitably modified phages can function as specific transporters of imaging agents or even drugs directly into cancer cells, thereby facilitating rapid diagnosis and treatment (Easwaran et al. 2024; Cao et al. 2025).

PT has been extensively employed in the treatment of osteomyelitis (Kishor et al. 2016; Onsea et al. 2019; Cobb et al. 2020; Simner et al. 2022). This condition is typically caused by a *Staphylococcus aureus* infection, which can take place via three primary routes. Haematogenous (most common in children), through infected adjacent tissues, but also trauma (including surgical) as well as vascular or neurological insufficiency (e.g. in the course of diabetes) (Birt et al. 2016;

Hofstee et al. 2020). A particular instance that poses a unique challenge in oral surgery is medication-related osteonecrosis of the jaw. Antiresorptive drugs, such as bisphosphonates or denosumab, used in the treatment of osteoporosis, have been shown to inhibit osteoclast activity and, consequently, bone remodelling. In cases involving surgical oral intervention in patients treated with antiresorptive drugs, a potential risk of osteonecrosis exists, which is often further complicated by the occurrence of bacterial infections such as with *Actinomyces* spp. (Ibrahim et al. 2022). Consequently, these conditions could be treated with phage therapy. However, to date, there have been no documented cases of phage therapy utilization in the field of oral and maxillofacial surgery for the management of this infectious inflammation. *Actinomyces* spp. bacteria occur naturally in the oral cavity but can become pathogenic bacteria under favourable conditions. Bacteria of this genus are found in oral abscesses, often infect necrotic bone tissue and are responsible for actinomycosis (mainly *A. israeli*) (Ibrahim et al. 2022). To date, phages have been developed that mainly target *A. naeslundii*. Thus, further research is needed (Szafrński et al. 2017).

To date, no studies have been conducted which describe the use of phages in the treatment of acute osteomyelitis. The process of identifying bacteria and finding a specific phage is often time-consuming, and bacterial biofilms in acute osteomyelitis are less developed than in chronic disease. This supports the greater likelihood of success with antibiotic treatment. However, PT may also be considered, although this method is typically used to treat chronic inflammations that demonstrate resistance to conventional treatment methods (Suh et al., 2023). This observation does not imply the inefficiency of PT in addressing acute inflammations; instead, it highlights the absence of studies specifically designed to investigate its effectiveness in such contexts. Consequently, there is a need for further research on this topic in the future.

Tuberculosis and actinomycosis are among the bacterial diseases that pose clinical challenges to oral surgeons. In the case of tuberculosis, treatment with phage therapy is complicated by the fact that the pathogen lives intracellularly in macrophages, which significantly reduces the availability of phages to the bacteria. In the later stages of the disease, significant amounts of bacteria are present in the extracellular environment, allowing phages to be effective, as demonstrated *in vitro*. Interestingly, studies have shown that some phages attack a wide range of *Mycobacterium tuberculosis* isolates, an unusual observation among phages (Guerreiro-Bustamante et al. 2021).

11. Bacterial phage resistance? - the arms race between phage and bacteria

Bacteria and phages have coexisted for centuries, and we know that bacteria developed mechanisms that allow them to avoid phages. Bacterial defence against phages occurs at several stages of the phage reproductive cycle. These include modification of the bacterial surface to prevent phage adhesion, prevention of phage DNA entry and replication, cleavage of the phage genome, CRISPR-Cas systems, or abortive infection (Abi system), which involves the suicidal death of a bacterium attacked by a phage before its duplication occurs. Comprehension of these mechanisms may play a crucial role in counteracting the acquisition of phage resistance by bacteria (Hampton et al. 2020; Safari et al. 2020; Teklemariam et al. 2023).

However, unlike antibiotics, phages do not remain passive in the face of increasing bacterial resistance; instead, they produce systems that allow them to continue attacking bacteria. They adapt to new receptors on the bacterial surface, create anti-restriction modification systems, produce mutations that bypass CRISPR-Cas, or even create genes that counter the CRISPR-Cas system (Hampton et al. 2020; Safari et al. 2020; Teklemariam et al. 2023).

From a clinical perspective, it is noteworthy that phage administration in the form of a phage cocktail, rather than phage monotherapy, appears to be an effective method for preventing the development of bacterial resistance (Chan et al. 2013).

12. Combined antibiotic and phage therapy

As demonstrated by the following case report, the combination of antibiotic and phage therapy has been successfully implemented in several instances. The report details the treatment of a patient who, following a motorcycle accident, developed an infection of the tibia caused by multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*. In the absence of effective antibiotic treatment, the patient was at risk of losing his limb. However, combined phage and antibiotic therapy resulted in rapid healing and complete eradication of the bacteria from the infected site. No adverse effects of the therapy were observed (Nir-Paz et al. 2019).

Combined antibiotic and phage therapy has been demonstrated to yield significant and interesting results. The available research suggests that combining both forms of therapy increases and/or prolongs the effective action of the antibiotic. It has been hypothesized that phage infection exerts selective pressure

on bacteria, causing them to mutate and reduce the expression of factors related to toxicity, antibiotic resistance, and growth inhibition. Consequently, even if a bacterial strain develops resistance to phages, it becomes less toxic and more susceptible to antibiotics (Li et al. 2021; Diallo and Dublanchet 2022).

Further research is needed into this phenomenon. In addition, assigning specific phages to specific antibiotics and developing a sequence for administering them to patients must be undertaken, so that treatment is as effective and safe as possible (Diallo and Dublanchet 2022).

13. Conclusions

According to the World Health Organization, by 2050, approximately 10 million people worldwide may die annually due to the multidrug-resistant bacterial infections (de Kraker et al. 2016). This predicament underscores the need for the development of novel antibiotics or alternative therapeutic modalities.

Considering the studies presented in this review, it seems probable that PT may become a viable alternative to antibiotic therapy in the future, or at least a support for it.

In principle, its application is feasible in any medical discipline where bacterial infections are a problem, including dentistry, where it can potentially be used in caries prevention, re-endodontic treatment, peri-implantitis, periodontitis or even osteomyelitis.

PT has been demonstrated to have considerable potential; however, numerous challenges must be overcome before it can be implemented on a large scale clinically. These include the necessity for additional clinical trials, long-term observational studies on efficacy and safety, and the establishment of appropriate and uniform regulations, including phage administration regimens similar to those employed for other drugs. It is also necessary to reduce production costs and extend their shelf life.

The renaissance of phage therapy, or its rediscovery, evokes optimism for the future and the ongoing effective combat against bacterial diseases.



Filip Pospiszyl <https://orcid.org/0000-0002-1305-103X>
 Monika Brzychczy-Włoch <https://orcid.org/0000-0002-7415-0154>
 Anna Morys <https://orcid.org/0009-0008-4733-4776>
 Tomasz Kaczmarzyk <https://orcid.org/0000-0003-3024-9926>

Conflict of interest

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