Bacteriophage Therapy: a review and the Portuguese landscape

Terapia fágica: da evidência científica global à implementação em Portugal

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ABSTRACT

This review provides a comprehensive overview of bacteriophage biology and classification, phage–bacteria interactions, and the mechanisms underlying phage-therapy efficacy, including its activity against biofilms and multidrug-resistant pathogens.

It further examines phage–antibiotic synergy, resistance dynamics, safety considerations, pharmacokinetic and pharmacodynamic aspects, and the role of phage banks.

Finally, the article discusses clinical evidence supporting phage therapy, emerging applications, and the Portuguese experience, highlighting regulatory, logistical, and clinical pathways that support the integration of phage therapy into modern infectious disease management.

Keywords: Bacteriophages; Phage Therapy; Antimicrobial Resistance; Chronic bacterial infections; Bacterial viruses.

RESUMO

Esta revisão oferece uma visão abrangente da biologia e classificação dos bacteriófagos, das interações fago-bactéria e dos mecanismos subjacentes à eficácia da terapia fágica, incluindo a atividade contra biofilmes e infeções por microorganismos multirresistentes.
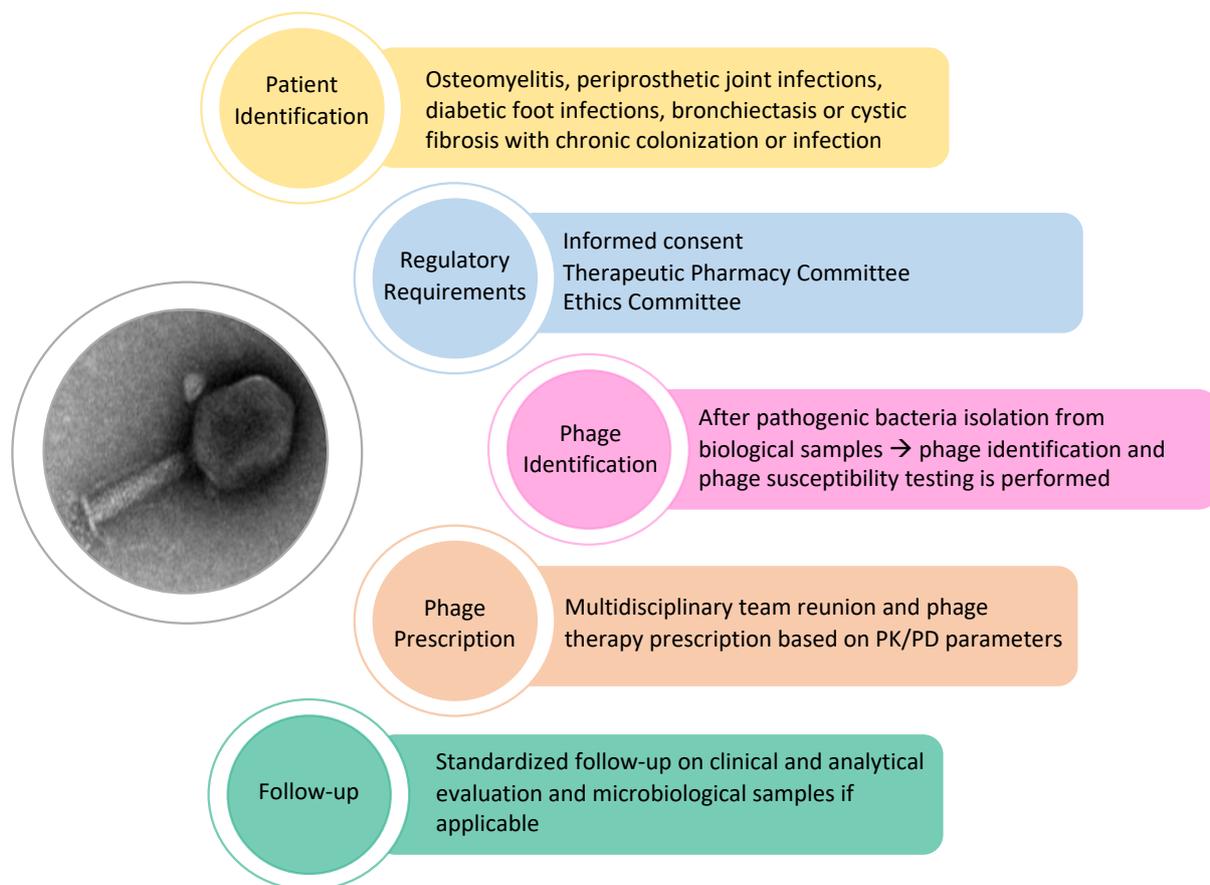
Descreve a sinergia fago-antibiótico, a dinâmica da emergência de resistências, as considerações de segurança, características farmacocinéticas e farmacodinâmicas e o papel dos bancos de fagos.

Por fim, o artigo discute as evidências clínicas que apoiam a eficácia da terapia com fagos, as aplicações emergentes e a experiência portuguesa, destacando os fluxos de trabalho regulatórios, logísticos e clínicos que apoiam a integração da terapia fágica na prática clínica.

Palavras-Chave: Bacteriófagos; Terapia fágica; Resistência antimicrobiana; Infeções bacterianas crónicas; Vírus bacterianos.

GRAPHICAL ABSTRACT

Phage therapy workflow developed by CEB in collaboration with the Queen Astrid Military Hospital and with Portuguese hospitals

Patient Identification — Osteomyelitis, periprosthetic joint infections, diabetic foot infections, bronchiectasis or cystic fibrosis with chronic colonization or infection

Regulatory Requirements — Informed consent, Therapeutic Pharmacy Committee, Ethics Committee

Phage Identification — After pathogenic bacteria isolation from biological samples → phage identification and phage susceptibility testing is performed

Phage Prescription — Multidisciplinary team reunion and phage therapy prescription based on PK/PD parameters

Follow-up — Standardized follow-up on clinical and analytical evaluation and microbiological samples if applicable

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Introduction

By the time of bacteriophages' discovery, in 1915 by Frederick Twort and in 1917 by Félix d' Hérelle[1,2], the research was focused on the treatment of bacterial infections, but the discovery and introduction of highly effective antibiotics in the following years largely displaced bacteriophage therapy from clinical practice. However, decades of extensive antibiotic use have selected for resistant microorganisms, and together with the limited development of new effective agents, this has become a major global health threat, with rising morbidity and mortality attributable to bacterial infections. In recent years, from 2018 to 2023, the incidence of resistant bacteria increased by more than 40.0%[3]. This scenario has led to a renewed interest in bacteriophages (phages) and their use in treating bacterial infections - phage therapy[4].

Phage therapy represents a personalized therapeutic approach, with a growing number of successful clinical applications reported in recent years. Nevertheless, access to phage therapy remains limited by economic and regulatory barriers, as well as by geographic distance from specialized academic phage research centers.

Phage therapy has recently been approved in Portugal under a regulatory framework (INFARMED Deliberation nº 112/CD/2024) aligned with the Belgian model based on magistral preparations, representing a significant milestone for the clinical implementation of this personalized antimicrobial strategy. This development opens new opportunities to expand phage therapy as a complementary or alternative approach to antibiotics and to address the growing challenge of antimicrobial resistance, which is prevalent in Portugal[5].

This review provides a comprehensive overview of phage biology and classification, phage–bacteria interactions, and the mechanisms underlying phage therapy efficacy, including its activity against biofilms and multidrug-resistant (MDR) pathogens. It further explores phage–antibiotic synergy, resistance dynamics, safety considerations, pharmacokinetic and pharmacodynamic aspects, and the role of phage banks. Finally, we address the clinical evidence supporting phage therapy, its emerging applications, and the recent Portuguese experience, highlighting regulatory, logistical, and clinical workflows that support the integration of phage therapy into a modern infectious disease approach to infection.

1. Bacteriophage Principles

Bacteriophages, or phages, are viruses that infect bacteria and are considered the most abundant biological entities on Earth[6−8]. They are also a natural and significant component of the human microbiome. Most known phages belong to the class *Caudoviricetes*, which comprises tailed viruses (figure 1) characterized by icosahedral capsids and double-stranded deoxyribonucleic acid (DNA) genomes[9].

Previously, phages were traditionally classified based on their tail morphology into three major families: *Myoviridae*, characterized by long contractile tails; *Podoviridae*, possessing short non-contractile tails; and *Siphoviridae*, exhibiting long non-contractile tails[10]. However, the 2022 taxonomic update by the ICTV Bacterial Viruses Subcommittee has abolished these morphology-based families, which were replaced by genome-based taxonomy. Morphological descriptors such as "myovirus", "siphovirus", and "podovirus" are still used for descriptive purposes but no longer have formal taxonomic significance[9].

This taxonomic revision resulted in extensive reorganization, including the establishment of one new order (*Crassvirales*), 22 new families, 30 subfamilies, 321 genera, and 862 species, reflecting a shift toward a genome-based classification system[9].

Phages exhibit two primary life cycles, namely the lytic and lysogenic cycles. In both cases, phages start by attaching to bacterial surfaces via specialized tail proteins, enabling the injection of their genetic material[11,12]. In the lytic cycle, phage DNA is transcribed and replicated within the host cell, leading to the assembly of new viral particles and, ultimately, bacterial lysis. The release
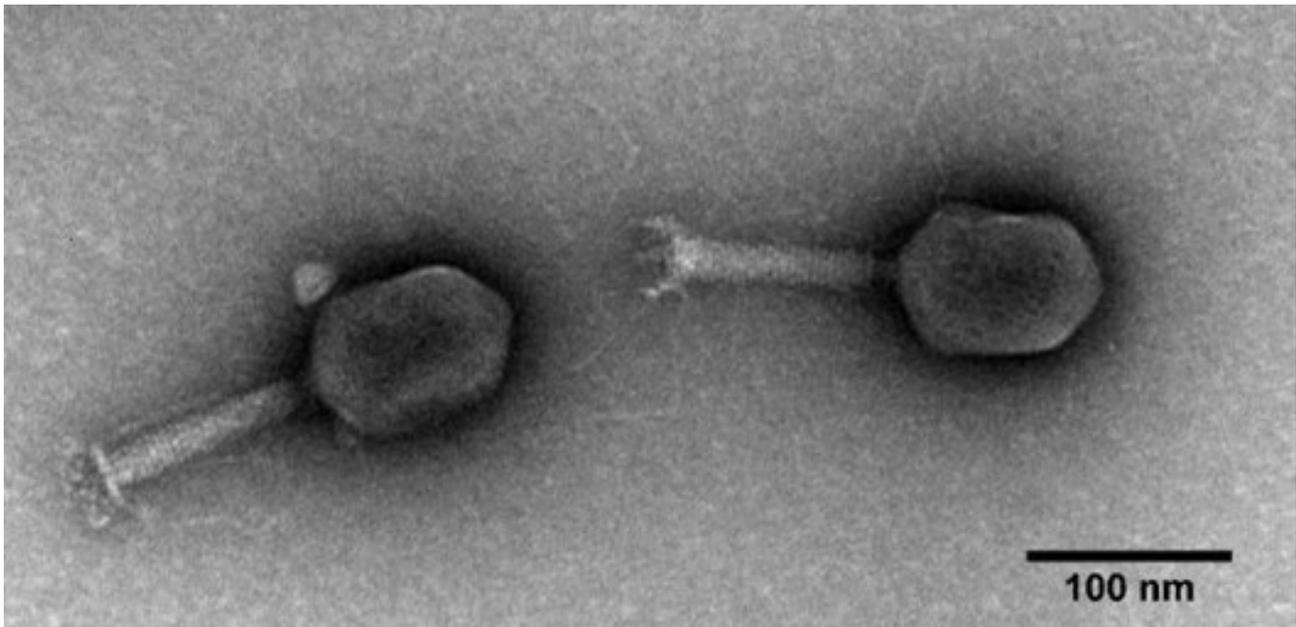
Figure 1. Transmission electron micrograph of an *Escherichia coli*–infecting bacteriophage with myovirus-like morphology from CEB's phage collection.

of progeny phages enables subsequent rounds of bacterial infection[11,12]. In contrast, in the lysogenic cycle, the injected phage genome integrates into the bacterial chromosome as a prophage and remains dormant, being passively replicated alongside the host DNA during normal bacterial cell division[13].

Due to their capacity to specifically and exclusively infect and lyse bacterial cells, phages that exhibit a strictly lytic cycle (often called virulent phages) that do not transduce have attracted increasing attention across several fields, particularly regarding their potential application in phage therapy[14]. Phages that can alternate between a lytic and lysogenic cycle are called temperate phages and are avoided in phage therapy due to risks of bacterial persistence and gene transduction[13].

Phages are known for their high degree of host specificity, targeting only a particular range of bacterial strains and replicating without affecting non-target cells. Consequently, phage-based approaches enable the elimination of pathogenic bacteria while preserving healthy commensal microbiota[15,16].

Bacterial resistance to phages frequently arises through the emergence of bacteriophage-insensitive mutants (BIMs). Although this resistance differs mechanistically from antibiotic resistance, bacteria can employ a wide range of antiphage defense systems, including receptor modification, superinfection exclusion, restriction–modification, and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) mediated genome degradation, abortive infection systems, and antiphage signaling[17−19]. Interestingly, while phage-resistant bacteria often arise rapidly under experimental conditions, the acquisition of resistance is commonly associated with significant fitness costs, frequently manifested as reduced growth rates, reduced virulence, and restored antibiotic sensitivity[20,21].

Moreover, phages have also evolved multiple counter-defense strategies to circumvent bacterial antiviral systems, including anti-CRISPR proteins, nucleotide modifications evading restriction-modification systems, and tail fiber mutations recognizing altered bacterial receptors[19,22]. Consequently, co-evolutionary dynamics between phages and bacteria enable phage adaptation to BIMs, thereby restoring bacterial susceptibility and lytic activity despite initial resistance.

Phages can also act on complex environments, such as bacterial biofilms. Biofilms are clusters of bacteria encased in a self-produced matrix of extracellular polymeric substances (EPS). This protective mode of bacterial growth confers increased tolerance to antibiotics and host defenses, making biofilms more resistant than free-floating (planktonic) bacteria[23]. Several phages have demonstrated the ability to inhibit biofilm formation and reduce pre-established biofilms[24]. However, the biofilm EPS matrix itself can still provide substantial protection to bacterial cells, highlighting the need for combined or adjunctive treatment strategies[25−27].

2. Phage and antibiotic interactions

Phage therapy can be used alone or in combination with antibiotics, depending on which strategy offers the greatest therapeutic efficacy and the lowest likelihood of resistance development in each case[28−30].

It has been demonstrated that, in some cases, phages can be particularly effective against bacteria when combined with antibiotics. This phenomenon, known as phage–antibiotic synergy (PAS), is commonly observed in vitro as an increase in phage plaque size upon exposure of phage-infected bacteria to sublethal concentrations of antibiotics. Enlarged plaque size has been linked to enhanced phage adsorption rates, shortened latent periods, and increased burst sizes[28].

One of the mechanisms underlying PAS involves antibiotic-induced bacterial filamentation, a protective response of the bacteria when exposed to antibiotics and associated with inhibition of cell division. Antibiotics such as fluoroquinolones, β-lactams, and trimethoprim promote the formation of elongated, multinucleated bacterial cells, and these filamentous bacteria exhibit an increased surface area, which may enhance phage adsorption and subsequent infection[31].

Filamentous bacteria may further enhance phage production either by delaying cell lysis—allowing greater accumulation of phage particles before cell rupture—or by accelerating lysis, thereby increasing the rate of infection of neighboring cells[31].

Although multiple PAS mechanisms have been described, no single mechanism fully explains the antibacterial efficacy observed in phage–antibiotic combinations. In addition, several studies have shown that phage–antibiotic combinations can potentiate antibiotic activity, reduce the emergence of resistance, or even restore the efficacy of antibiotics against resistant bacterial strains[32].

By simultaneously targeting distinct bacterial structures, phages and antibiotics impose multiple selective pressures, which may explain the reduced likelihood of emergence of resistance. This principle is analogous to that underlying phage cocktails, where multiple phages are selected and used simultaneously to target different bacterial receptors, reducing the chance of resistance emergence[33,34].

Additional synergistic mechanisms include enhanced antibiotic penetration into biofilms, as some phages encode enzymes that can degrade the biofilm matrix, thereby facilitating antibiotic access to embedded bacterial cells[35].

Despite these synergistic effects, antagonistic interactions between phages and antibiotics have also been reported. Because virulent phages depend on host cellular machinery involved in transcription, translation, and DNA replication, antibiotics that inhibit these processes may impair phage replication, resulting in phage–antibiotic antagonism[36−38].

Furthermore, certain antibiotics may indirectly reduce phage efficacy by enhancing bacterial defense systems[31,39]. For example, some antibiotics modulate CRISPR–Cas immunity, which provides adaptive protection against phage infection through spacer acquisition and targeted cleavage of phage DNA[31,40].

Bacteriostatic antibiotics have been shown to enhance CRISPR-mediated immunity by prolonging the eclipse period, reducing phage production, and extending the temporal window for spacer acquisition[31,39].

Given the complexity and variability of phage–antibiotic interactions, in vitro evaluation is essential before clinical application. Commonly employed assays include time–kill curves and phage–antibiotic interaction analyses, such as synograms. As demonstrated by Liu et al., PAS is strongly influenced by multiple factors, including the antibiotic mechanism of action, stoichiometry, resistance determinants, phage-specific parameters (e.g., burst size), and environmental conditions[41].

3. Tackling the challenges of antibiotic resistance

Antimicrobial resistance is a global public health issue and threatens the effective treatment of bacterial infections[3].

A key concern is the rising number of infections caused by invasive Gram-negative bacilli, which are linked to increased mortality rates[42]. The World Health Organization has updated its list of bacterial priority pathogens, highlighting the urgent need for investment, especially in treating infections caused by *Acinetobacter baumannii* resistant to carbapenems, *Enterobacterales* resistant to third-generation cephalosporins, and other carbapenem-resistant strains[43].

Phage therapy narrows the gap between MDR infections and available treatment options. Various centers offer patients phage therapy for multiple bacterial infections, including those caused by *Acinetobacter baumannii*, *Burkholderia cepacia*, *Citrobacter spp.*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella spp.*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella spp.*, *Serratia marcescens*, *Shigella spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Streptococcus salivarius*, *Streptococcus sanguis*, and *Stenotrophomonas maltophilia*, among others[44].

In various environments like oceans, soil, freshwater, the human body, fermented foods, and waste,

there are countless phages, each capable of infecting specific bacterial strains. Compared to antibiotics, phages are widely distributed throughout the biosphere and exhibit high diversity. However, each phage typically infects only a narrow range of bacterial strains. Because bacterial populations show substantial variation even within the same species, effective phage therapy usually requires a combination of different phages in a so-called phage cocktail. This highlights the inherently personalized nature of phage therapy, in which efficacy depends on the specific interaction between the phage receptor-binding machinery and the surface receptors expressed by the patient's bacterial isolate. In many clinically important pathogens, these receptors vary substantially between strains (even within the same species), so a phage (or a cocktail) that is effective in one patient may fail in another, despite infection by the same bacterial species[45,46]. This issue is particularly pronounced in capsulated bacteria, where the capsular polysaccharide (CPS) often acts as a dominant adsorption determinant and shows extensive strain-to-strain diversity. In *Klebsiella pneumoniae*, capsule (serotype/K-locus) differences can strongly affect phage susceptibility[47]. Similarly, in *Acinetobacter baumannii*, a critical-priority MDR pathogen, the capsule is frequently a primary receptor for phage attachment, and CPS structures are highly diverse (with large numbers of distinct capsule loci/types described), meaning that most phages infect only a limited subset of clinical isolates unless cocktails are tailored to the infecting strain's capsule type[48].

4. Phage Banks

The renewed interest in phage therapy has led to increased attention toward historical phage banks, alongside the emergence of new laboratories dedicated to phage research. Consequently, there has been a rapid expansion in the number of phage collections, ranging from small laboratory-based repositories to large institutional banks. However, this growth has occurred with relatively limited coordination and a lack of standardized catalogu-

ing practices, and to date, no comprehensive international registry of phage collections exists[49]. Enhanced coordination and data sharing among phage researchers are therefore critical to expanding access and increase the likelihood of successful therapeutic matching in phage therapy. Many existing collections operate as service-providing culture collections or Biological Resource Centers (BRCs), acting as custodians of phage diversity, supplying phages for a fee, and supporting both internal and external research activities[49].

Among the most well-known phage banks are those originating from the former Soviet Union, particularly the Eliava Institute of Bacteriophages, Microbiology and Virology in Georgia, dating back to 1923, which maintains over 1,000 phages, and the Hirszfeld Institute of Immunology and Experimental Therapy in Poland, established in 1952, which holds more than 850 phages[50,51].

Additional phage banks have been established over time primarily to support laboratory research, including the Félix d' Hérelle Reference Center for Bacterial Viruses in Canada (>400 phages), the American Type Culture Collection (ATCC) in the United States (approximately 350 phages), the German Collection of Microorganisms and Cell Cultures (DSMZ; approximately 450 phages), the National Collection of Type Cultures (NCTC) in the United Kingdom (>100 phages), and the Korean Phage Bank (>1,000 phages)[50].

Although efforts have been made to centralize phage collections, the establishment of national or international infrastructures remains challenging. As phage therapy has not yet been fully integrated into routine clinical practice, securing sustained public or private investment for centralized repositories is difficult[49].

Also, a critical requirement for the long-term viability of phage biobanks is the availability of reliable cryopreservation strategies. Despite their importance, standardized and universally accepted protocols for phage cryopreservation are still lacking[52,53].

5. Phage Therapy Efficacy

Phage therapy has a wide range of applications alone or in combination with antibiotics, in different anatomic sites: urinary tract infections; genital tract infections; postsurgical infections; respiratory tract infections; soft and skin tissue infections; bone and joint infections; cardiovascular and intravascular infections[51]. Phages can also penetrate complex bacterial communities, such as biofilms, and infect persistent or hard-to-reach cells (e.g., areas with poor blood circulation)[24]. In addition, phages can respond effectively to the emergence of resistant bacterial mutants, since phages replicate in situ and, under selective pressure, spontaneous mutations within the phage population can give rise to variants with advantageous traits, such as expanded host range or enhanced infectivity[4,54,55].

The inappropriate clinical use of phages between 1920 and 1930 raised important concerns about safety and efficacy[29], contradicted by recent research[56,57], where the efficacy of phage therapy against MDR bacteria has been demonstrated by several in vitro studies, showing significant reduction in bacterial loads and biofilms after phage treatment.

There is also evidence of the safety and efficacy of phages in reducing bacterial loads in in vivo models of infections, such as lung infections and wound infections[62−66]. Besides the promising results from in vitro and in vivo studies, there is an increasing number of case studies reporting encouraging results of patients with life-threatening antibiotic-resistant bacterial infections that have been successfully treated with phage therapy[63−65].

Table I (see supplementary material at the end of the article) summarizes reported clinical cases and case series describing the use of phage therapy across multiple infection sites to date.

Osteoarticular infections represent the largest body of published clinical experience with phage therapy. In this setting, phage therapy is predominantly administered locally, as summarized

in Table I, enabling high local phage concentrations despite the absence of standardized pharmacokinetic data. The heterogeneous phage dosing parameters in clinical literature reflect the lack of standardized guidelines and the individualized nature of its use.

Osteoarticular infections are difficult to treat, as microorganisms adhere to devitalized bone and prosthetic material, forming mature biofilms[108,122−124] with limited antibiotic penetration and high bacterial persistence. Phage therapy may target bacteria embedded within biofilm partly through the production of depolymerases that degrade the biofilm matrix and through local phage amplification at the site of infection[24].

Cardiovascular and intravascular infections are rare but severe, including endocarditis, prosthetic valve infections, and vascular graft infections. In this context Phage therapy has been almost exclusively used as salvage adjunctive therapy (commonly intravenous), typically combined with prolonged systemic antibiotics and, when feasible, surgery. The observed tolerability of intravenous phage administration, even in critically ill patients, is a clinically relevant finding[89−98].

Clinical experience with phage therapy in respiratory infections remains limited but has increased in recent years. Phage therapy is predominantly delivered by nebulization, and doses are rarely reported, as summarized in Table I. In this setting, even when phage therapy could not achieve complete microbiological eradication, the majority of patients showed consistently clinical benefit[99,101,102,105−107].

The lung represents a dynamic compartment with rapid clearance mechanisms[125], making pharmacokinetics particularly relevant yet poorly characterized as reflected by inconsistent dosing and limited pharmacokinetic data reported in published clinical cases.

In urinary tract infections, different administration routes are reported, such as oral, rectal, and intravesical instillations. Again, data on specific

dosing schedules is rarely reported. Of notice, in a notable proportion of cases, phages were administered without concomitant antibiotics[112−115,121].

In summary, Table I illustrates an important wide range of concomitant antimicrobial agents spanning from narrow- to broad-spectrum antibiotics. No evidence of phage-antibiotic antagonism was reported.

Across the reported cases, phage selection was consistently guided by phagogram testing, and systemic antibiotics are almost always prescribed concomitantly with phage therapy. There was marked heterogeneity in phage dosing, routes of administration, and treatment duration. These findings reflect a prescription experience-based and highlight the need of standardized treatment protocols. As discussed by Abedon et al.[126], phages are self-amplifying biological agents whose replication depends on the local bacterial burden, suggesting that precise dosing may be less critical than for conventional antimicrobials and that repeated administration at the site of infection may not always be necessary.

Several case reports document the use of cocktails comprising two or more phages[72,74,75,77,82,84,87,95,96,106,107] targeting the same bacterial strain and administered either simultaneously or sequentially to avoid the likelihood of resistance emergence during phage therapy[127]. Importantly, even when phage resistance is documented, it does not necessarily correlate with clinical failure, as demonstrated by Yang et al. and Li et al.[99,105].

Furthermore, across reported clinical cases, phage therapy was associated with consistently low rates of phage-related adverse events, and no dose-dependent toxicity has been described to date[127,128].

Personalized phage therapy, while offering high specificity and the potential for targeted therapy, is inherently time-consuming. The process typically requires bacterial isolation, phage susceptibility testing, regulatory approval, and often customized phage preparation. These steps may

take several days to weeks, limiting its feasibility in acutely septic patients in need of immediate therapy[130−132]. This limitation is particularly relevant in cardiovascular and pulmonary infections, where patients frequently present with acute or rapidly progressive disease. Consistent with this constraint, the summarized cases in Table I predominantly describe phage therapy as adjunctive therapy, initiated after prolonged antibiotic exposure, infection recurrences, or when surgical options were limited.

Interestingly, phage therapy use is not restricted to infection treatment, and it can play an important role as a prophylactic agent or microbiome regulator in colonized patients, as well as in the construction of antivenoms and biocontrol in food manufacturing, among other applications[44,133−135].

6. Phage Pharmacokinetics and Pharmacodynamics

Therapeutic optimization of phages is essential to achieve the desired therapeutic effect while minimizing the occurrence of toxicity and resistance[136]. In this context, a thorough understanding of their pharmacokinetic (PK) and pharmacodynamic (PD) properties is required. Pharmacokinetics refers to the mechanisms involved in determining phage concentrations within the organism, whereas pharmacodynamics describes the effects that phages may exert on the human body[137].

The wide diversity of phages, formulations, and treatment regimens hinders the establishment of general conclusions regarding their in vivo pharmacokinetic/pharmacodynamic (PK/PD) characteristics and bioavailability[136]. Despite the extensive use of phages in several countries, the lack of PK/PD information remains a persistent challenge for phage therapy[136,138,139]. As biological entities capable of rapid self-replication at the site of infection and characterized by single-hit bacterial killing kinetics, phages exhibit highly complex PK/PD behavior[136,139,140].

The main pharmacokinetic and pharmacodynamic elements to be considered in phage therapy include

their pharmacological characteristics, the route of administration, the site of bacterial infection, the relative concentrations of phages and bacteria at different anatomical locations (including the site of infection, when feasible), and the interaction with the host immune system[137].

6.1 Phage Pharmacokinetics

In comparison with antibiotics, phages are living biological entities that replicate in the presence of susceptible bacteria, exhibiting PK profiles that are considerably more complex than those of conventional antibacterial agents[139]. Therefore, sensitive and validated quantification methods are required for appropriate PK/PD monitoring of phages[136].

To describe PK in phage therapy, it is necessary to consider how phages interact with the host organism, including the evaluation of absorption, distribution, metabolism, and elimination processes, as well as phage dosing strategies[137].

The phage dose is the number of particles of each administered phage solution and forms the basis for PK determination[136].

Several techniques have been employed to investigate the in vivo PK properties of phages, including the measurement of phage titers in body fluids, as well as phage labeling as an alternative approach to assess their distribution[136].

Intravenous administration of phages has proven to be effective, providing good tissue penetration, particularly in tissues affected by inflammatory processes, with a consequent increase in endothelial barrier permeability[137].

Bacteriophages can circulate and accumulate in different organs and tissues[136]. They have been detected in blood, bronchoalveolar lavage fluid, feces, lungs, heart, liver, kidneys, spleen, and brain tissue, indicating that they are capable of crossing the blood–brain barrier[136]. Although phages appear to access multiple tissues with relative ease, it remains unclear whether therapeutic con-

centrations at sites of infection can be achieved regardless of the administration route[136].

Phages accumulate in the liver and spleen, which are the organs responsible for their inactivation and elimination[137]. The elimination of phages through urine is low[137].

It is important to note that the innate immune system also plays a fundamental role in phage elimination, mainly through phagocytosis[140].

6.2 Phage Pharmacodynamics

PD focuses on defining the relationship between phage concentration and bacterial elimination and on evaluating potential adverse effects associated with phage therapy. Primary PD studies aim to determine the effective phage concentration capable of reducing or eradicating bacterial loads to restore health, commonly quantified using the multiplicity of infection, which represents the ratio of phages to bacterial cells[136]. Because not all administered phages successfully interact with bacteria, PD modelling must account for adsorption rates and bacterial density, reflecting the dynamic predator–prey relationship unique to phage therapy. Two main therapeutic strategies are described: passive therapy, which relies on administering phages in excess of the bacterial burden, and active therapy, which leverages phage replication at the infection site once bacterial and phage threshold concentrations are exceeded[137]. Secondary PD studies address safety considerations, including toxicity linked to endotoxin contamination and broader effects on the microbiota, immune system, and eukaryotic cells, underscoring the need for rigorous purification and evaluation[137]. Together, these PD principles highlight that, unlike antibiotics, phage exposure and response are interdependent, making careful optimization of phage dosing and bacterial targeting essential for effective and safe clinical application[137].

7. Safety profile and Adverse Effects

Phage therapy has demonstrated safety, tolerability, and efficacy in multiple clinical trials target-ing different bacterial infections, resulting from its specific targeting of bacteria without affecting mammalian cells[127]. Even at high concentrations, phage therapy has demonstrated excellent biosafety profiles[128].

Although clinical trials suggest that phage therapy is generally safe and well tolerated across a range of diseases and patient profiles, it is essential to recognize that patient-specific characteristics—such as immune status and comorbidities—as well as the phages used, the dosing regimen, and the route and/or method of administration, may influence the occurrence and severity of adverse reactions[127].

In cases where adverse effects were reported—such as pulmonary exacerbations in cystic fibrosis, bruising, dizziness, fatigue, headaches, weight gain, cough, nasal congestion, postoperative wound complications, and gastrointestinal disturbances — they were often nonspecific and their direct relation to phage treatment remains unproven[127]. The adverse effects may have been attributable to other factors, such as the underlying clinical condition and/or concomitant treatments, rather than to phage therapy[71−140].

One of the main safety concerns regarding phages involves the presence of residual endotoxins in preparations[128]. Endotoxins are lipopolysaccharides found in the outer membrane of Gram-negative bacteria and are released during bacterial lysis or death[128]. These endotoxins are highly immunogenic and can raise body temperature, reduce white blood cell counts, and even cause shock[128]. Therefore, effective endotoxin removal processes are necessary to meet stringent safety standards and facilitate the standardized use of phage therapies[128].

Another challenge lies in the production of anti-phage antibodies, which can limit the therapeutic efficacy of phage preparations[128]. In mammalian hosts, serum immunoglobulin M and immunoglobulin G antibodies can reduce or inhibit phage activity, respectively[128]. The host immune response to bacterial infection may lead to rapid clearance

of phages from the system, thereby compromising their therapeutic efficacy[128].

Although phages have been reported to induce only mild immune responses, further studies are still required[128].

Finally, phages are composed of proteins and nucleic acids, which can interact with the human immune system and elicit immunogenicity and potentially trigger allergic reactions[128].

Pharmacovigilance efforts, including post-marketing surveillance and adverse event reporting systems, are essential for monitoring the safety of phage-based products in real-world settings and for identifying any rare or unexpected adverse effects that may arise over time[127].

8. Local Experience - Phage therapy in Portugal

Phage therapy is already a commonly used practice in the treatment of antibiotic-resistant infections in Australia, Belgium, France, Georgia, Germany, Poland, Russia, and the United States of America in dedicated phage therapy centers[141−142].

A framework allowing the use of phage therapy in clinical practice was recently approved in Portugal (INFARMED Deliberation nº 112/CD/2024). According to this resolution, phage therapy may be used as a magistral preparation for individual patients, in accordance with Article 3(1) of Directive 2001/83/EC (magistral formula) and Article 5 (direct responsibility of a healthcare professional). Only non-genetically modified bacteriophages are covered under this framework[143].

The magistral preparation must be carried out exclusively in a hospital pharmacy, based on an individualized medical prescription for a specific patient, and in compliance with Good Practices for the Preparation of Manipulated Medicines. The entity responsible for producing the active agents (bacteriophages) must provide well-characterized bacteriophages, maintain a master phage bank, and perform quality assessments at two levels: ge-netic control and quality parameter control of the different production batches, ensuring the maintenance of appropriate batch records and certificates of analysis[143].

Once the clinical team decides to propose phage therapy, its use requires validation by the Ethics Committee, the Pharmacy and Therapeutics Committee, and the local Program for the Prevention and Control of Infections and Antimicrobial Resistance unit. Following each treatment, the outcome must be reported to INFARMED[143].

In Portugal, several laboratories and companies are dedicated to phage research and production. The Centre of Biological Engineering (CEB) at the University of Minho is a national reference in phage research with a substantial body of published literature, including review articles and pre-clinical studies addressing both the efficacy and the challenges of phage therapy[25,26,145,146]. CEB is also involved in personalized phage production for Portuguese patients, in collaboration with the Queen Astrid Military Hospital - QAMH (Belgium) and, in 2025, formally announced the creation of a laboratory dedicated to the production of therapeutic phages[144].

Technophage, a Lisbon-based biotechnology company, has nearly two decades of experience in the development and manufacture of phage cocktails. The company has already supported compassionate-use treatments in countries such as France and Israel[147].

LxBio is another Portuguese biopharmaceutical company engaged in phage innovation and integrated into international networks such as PhageEU, although it does not yet have a publicly disclosed clinical pipeline[148].

In addition, numerous national academic laboratories generate knowledge, tools, and biomaterials that may support phage therapy programs. The PhaBRIC laboratory at the Institute for Medicines Research (iMed), University of Lisbon, focuses on phage biology and lytic proteins, studying mechanisms of bacterial lysis and phage–host interactions, and developing phage-derived agents target-

ing *Enterococcus spp.*, *Mycobacterium tuberculosis* and *Staphylococcus spp.*[149].

The BeSurf group at i3S, University of Porto, specializes in BioEngineered Surfaces, developing biomaterials and surfaces functionalized with phages or phage-derived elements to prevent device-associated infections and osteomyelitis, exploring targeted and antibiotic-free approaches[150].

Finally, the International Iberian Nanotechnology Laboratory (INL), located in Braga, develops projects such as "Phages on Chip", which integrate bacteriophages into microfluidic devices for diagnostic applications and for addressing the antimicrobial resistance crisis[151].

To date, CEB has contributed to more than 20 phage therapy treatments in Portugal targeting strains of *Mycobacterium abscessus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

In the remainder of this section, we describe the workflow implemented at CEB that goes from the identification of a suitable patient to the follow-up of those receiving phage therapy.

Box 1: Phage therapy workflow developed by CEB in collaboration with the Queen Astrid Military Hospital and with Portuguese hospitals.

Identification of Suitable Patient

Patient identification is conducted in both inpatient and outpatient units during routine medical appointments. Following initial selection, an Infectious Diseases specialist evaluates the patient's clinical condition and discusses the case within a multidisciplinary team, according to the patient's condition and the source of infection.

Eligible candidates are then referred to the CEB via email. The physician responsible for the case must complete the patient's clinical file and submit it to CEB. This file is subsequently reviewed by a multidisciplinary evaluation team, composed of members from both CEB and the QAMH. The team determines whether the clinical condition justifies the use of phage therapy, considering patient characteristics, infection site, microbiological data, and other relevant factors.

In general, phage therapy is considered for patients with chronic osteomyelitis, periprosthetic joint infections without curative surgical options, diabetic foot infections caused by difficult-to-treat bacteria, and patients with structural lung diseases (such as bronchiectasis or cystic fibrosis) presenting chronic colonization or infection with no clinical improvement under standard-of-care treatments.

Regulatory Requirements

If treatment is approved and active phages against the patient's strain are available, the patient is provided with an informed consent form. The treating physician must then submit the required documentation for review by the hospital's Therapeutic Pharmacy Committee and the local Ethics Committee.

Clinical Procedures

Following approval by the Therapeutic Pharmacy Committee and the Ethics Committee, collection of bacteriological samples from the infection site is scheduled. Pathogenic bacteria are isolated from these samples and forwarded to CEB, where phage susceptibility testing is performed on all recovered bacterial strains.

When multiple phages demonstrate activity against the same strain or strains, those with the highest efficiency of plating (EOP) are prioritized. If uncertainty remains regarding optimal phage selection, additional pharmacokinetic and efficacy assays are conducted. These assays evaluate both individual phages and phage combinations to identify the formulation that most effectively reduces bacterial burden.

The selected phages are produced and supplied by the QAMH. Upon receipt, phage stocks are stored at 2–8 °C to preserve viability. Final preparation is performed according to the medical prescription and maintained at 2–8 °C until administration, for a maximum period of one week.

Medical Prescription

Phage therapy is prescribed by the treating physician, with the dose, administration frequency, and treatment duration being previously discussed within a multidisciplinary team involving an Infectious Diseases Specialist and agreed upon with the QAMH team.

Follow-up

After completion of treatment, the physician responsible for the case completes a standardized follow-up form including information from the pre-treatment, treatment, and post-treatment periods, considering microbiological analyses, clinical symptoms, concomitant medications, and any potential adverse events related to therapy. The physician also reports whether, following phage therapy, the patient eradicated the target strain, experienced clinical improvement, or showed no noticeable changes during treatment.

Following treatment, additional experimental assays may be conducted at CEB using serum or sputum samples collected from the patient before, during, and after therapy. Serum-based assays are used to determine whether anti-phage antibodies were generated during treatment and, when present, to quantify antibody titers.

In patients with respiratory tract infections, sputum-based assays are performed to evaluate the reduction or eradication of the target bacterial strain and to assess potential changes in the strain's antibiotic susceptibility over the course of therapy.

In both cases, phage susceptibility testing is repeated using the phages administered during treatment to determine whether any alterations in phage sensitivity occurred during therapy.

Conclusion

This review provides a comprehensive overview of phage therapy, spanning fundamental phage biology, pharmacokinetic and pharmacodynamic considerations, and available clinical evidence regarding efficacy and safety.

There is marked heterogeneity in phage dosing, routes of administration, and treatment duration, reflecting the current reliance on experience-based prescribing and highlighting the need for standardized treatment protocols.

Overall, the majority of reported cases described clinical resolution, with consistently low rates of phage-related adverse events, supporting the favorable safety profile of phage therapy.

Finally, in Portugal, several laboratories and companies are actively engaged in phage research and production, and a growing body of clinical experience is emerging. Together, these developments support the progressive integration of phage therapy into modern infectious disease management frameworks.

Author Contributions

Funding / Sponsorship

Ethics statement

This study is a review of previously published literature and did not involve human participants or animals. Therefore, ethical approval and informed consent were not required.

Conflicts of Interest

The authors declare no conflict of interest.

Table I. Clinical use of bacteriophage therapy: reported cases and case series

Author, year	Infection	n	Microbiology	Phage preparation[1]	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Osteoarticular infections									
Eschweiler et al., 2025	Prosthetic joint infection	3	MDR Gram - *S. epidermidis*, *S. capitis*, *Cutibacterium acnes*	Commercial phage cocktail	Oral and intralesional 10–30 mL per dose (variable) Once or twice daily 1–8 days (short patient-dependent courses)	Ampicillin/ sulbactam Teicoplanin Amoxicillin/ clavulanate	Resolution	Not reported	[66]
Cammuso et al., 2025	Prosthetic joint infection	1	*S. epidermidis*	Personalized phage therapy	Intra-articularly and intravenously 7×10^9 PFU/dose, IA, twice daily, 7 days and IV, daily 14 days	Daptomycin Rifampin Linezolid	Resolution under suppressive antibiotic therapy	Hypotension, hypertension, low-grade fever, chest pain, rigors, and wheezing; increased liver enzymes	[67]
Wahl et al., 2025	Prosthetic joint infection	1	MRSA	Personalized phage therapy	Intra-articularly and intravenously 1×10^7 PFU/mL, 10 days	Daptomycin Trimethoprim/ sulfamethoxazole	Resolution; death at 6 months unrelated to infection	Not reported	[68]
Doub et al., 2023	Prosthetic joint infection	1	*Enterococcus faecalis*	Personalized phage therapy	Intra-articularly then intravenously 1×10^{10} PFU/mL (10 mL, IA), 2 days 1×10^{10} PFU/mL (50 mL, IV), 4 days	Daptomycin	Resolution	Not reported	[69]
Doub et al., 2022	Chronic PJI	1	MRSA	Personalized phage therapy	Intra-articularly then intravenously 1×10^9 PFU/mL (10 mL, IA), single dose 2×10^8 PFU/mL, 3 doses, IV	Daptomycin Ceftaroline	Resolution	Increased liver enzymes	[70]

Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Doub et al., 2022	Chronic PJI	1	*Klebsiella pneumoniae* ESBL +	Personalized phage therapy	Intra-articularly then intravenously 1×10^{10} PFU/mL (20 mL, IA), 2 days 1×10^{10} PFU/mL (50 mL, IV), 2 days	Ertapenem	Resolution	Not reported	[71]
Ferry et al., 2022	Spondylodiscitis with spinal abscess	1	*Pseudomonas aeruginosa*	Personalized phage therapy	Intraoperative and intravenously 1×10^{6} PFU/mL (7 mL) IO, single dose 1×10^{6} PFU/mL (30 mL) IV, 21 days	Cefiderocol Colistin	Resolution	No adverse events reported	[72]
Schoeffel et al., 2022	Prosthetic joint infection	1	MRSA	Personalized phage therapy	Intra-articularly or intravenously 1.2×10^{9} PFU/mL (10 mL), IA, single dose 1.2×10^{9} PFU/mL, IV, 3 days	Daptomycin Trimethoprim/ sulfamethoxazole	Resolution	Increased liver enzymes	[73]
Eskenazi et al., 2022	Osteoarticular infection related to left femur fracture	1	*Klebsiella pneumoniae* MSSA	Personalized phage therapy	Intraoperative and catheter drainage 1×10^{8} PFU/mL (100 mL) IO 1×10^{8} PFU/mL (20 mL) CD, q8h, 5 days	Meropenem Colistin Ceftazidime/ avibactam	Resolution	Not reported	[74]
Racenis et al., 2022	Periprosthetic joint infection and chronic osteomyelitis	1	MDR *Pseudomonas aeruginosa*	Personalized phage therapy	Intraoperative and catheter drainage 1×10^{9} PFU/mL (100 mL) IO 1×10^{8} PFU/mL (20 mL) CD, q8h, 14 days	Colistin Meropenem Ceftazidime	Resolution	Not reported	[75]
Neuts et al., 2021	Osteoarticular/ recurrent chronic infection Prosthetic Hip Infection	1	*Enterococcus faecalis*	Commercial phage cocktail	Oral (dose not reported), twice daily Two 19-day cycles separated by a 2-week break (no surgery)	Amoxicillin Doxycycline	Resolution	Not reported	[76]
Ferry et al., 2021	Osteoarticular Prosthetic Knee Infection	1	*Pseudomonas aeruginosa*	Personalized phage therapy	Intra-articularly 1×10^{9} PFU/mL (30 mL) Single dose	Ceftazidime Ciprofloxacin	Resolution (death not related to infection)	Not reported	[77]

Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Doub et al., 2021	Osteoarticular Prosthetic Knee Infection	1	MRSE	Personalized phage therapy	Intra-articularly 2×10^{10} PFU (10 mL) Single dose	Daptomycin	Resolution	Increased liver enzymes	[78]
Cano EJ et al., 2021	Osteoarticular PJI	1	*Klebsiella pneumoniae*	Personalized phage therapy	Intravenously 6.3×10^{10} PFU (50 mL) Once daily, 8 weeks	Minocycline	Resolution	Not reported	[79]
Ramirez-Sanchez et al., 2021	Prosthetic Joint Infection	1	MSSA	Personalized phage therapy	Intra-articularly and intravenously 1×10^{10} PFU/mL (50 mL) IA, single dose 1×10^{10} PFU/mL (30 mL) IV, BID, 6 weeks	Cefazoline	Resolution	Serum neutralization	[80]
Van Nieuwen-huyse et al., 2021	Chronic polymicrobial infection of pelvic allografts	1	*Clostridium hathewayi, Finegoldia magna, Proteus mirabilis, S. aureus*	Personalized phage therapy	Intraoperative and catheter drainage 1×10^{7} PFU/mL (50 mL) IO, single dose 1×10^{7} PFU/mL (40 mL) CD, TID, 7 days 1×10^{7} PFU/mL (30 mL) CD, BID, 7 days	Clindamycin Rifampicin Ciprofloxacin	Resolution	Not reported	[81]
Ferry et al., 2020	Prosthetic Knee Infection	3	MSSA	Personalized phage therapy	Intra-articularly 1×10^{9} PFU/mL, IA, single dose	Daptomycin-based combination, followed by oral therapy and SAT	Resolution	Not reported	[82]
Doub et al., 2020	Chronic periprosthetic infection	1	MRSA	Personalized phage therapy	Intra-articularly and intravenously 5.4×10^{9} PFU/mL (10 mL) IA, single dose 2.7×10^{9} PFU/mL (50 mL) IV, 3 days	Daptomycin	Resolution	Increased liver enzymes (IV phage therapy discontinued)	[83]

Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Onsea et al., 2019	Chronic osteomyelitis	4	*S. epidermidis* *Pseudomonas aeruginosa* *S. aureus* *Enterococcus faecalis*	Personalized phage therapy (one patient with *P. aeruginosa* was non-susceptible to phage); Commercial phage cocktail (for *E. faecalis*)	Intraoperative and catheter drainage 1×10^7 (10–40 mL) IO, single dose 1×10^7 (10–40 mL) CD, TID, 7–10 days	Concomitant systemic antibiotics (including glycopeptides $\pm$ rifampicin), adjusted to pathogen susceptibility	Resolution (one patient had recurrence of infection, although with a different agent)	One patient with local pain and redness (patient with the commercial phage cocktail)	[84]
Nir-Paz et al., 2019	Bone traumatic left tibial infection	1	*Acinetobacter baumannii*, *Klebsiella pneumoniae*	Personalized phage therapy	Intravenously 5×10^7 PFU/mL (1 mL), TID, 5 days	Colistin Meropenem	Resolution	Not reported	[85]
Ferry et al., 2018	Chronic periprosthetic infection	1	*S. aureus*	Personalized phage therapy	Intra-articularly 1×10^{10} PFU/mL (10 mL), single dose	Daptomycin Amoxicillin Clindamycin	Resolution	Not reported	[86]
Ferry et al., 2018	Periprosthetic infection	1	XDR *Pseudomonas aeruginosa*	Personalized phage therapy	Intra-articularly $\approx 10^8$ PFU/mL (10 mL), every 3 days, 4 administrations	Ceftolozane–tazobactam (local: colistin)	Resolution	Not reported	[87]
Fish et al., 2018	Diabetic foot ulcer with osteomyelitis	1	MRSA	Commercial phage cocktail	Local perilesional soft-tissue and intraosseous injection 0.7 mL (PFU not reported), once weekly, 7 weeks	Levofloxacin (without any improvement and suspended)	Resolution	Not reported	[88]
Cardiovascular and intravascular infections									
Eifferman et al., 2025	Recurrent prosthetic valve endocarditis with infected Bentall (aortic graft infection)	1	*P. aeruginosa*	Personalized phage therapy	Intravenously 1×10^{10} PFU (10 mL), BID, 7 days	Ceftazidime Ciprofloxacin	Resolution	Not reported	[89]

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Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Hameed et al., 2024	Post-cardiac surgery mediastinitis	1	MDR *Klebsiella pneumoniae*	Personalized phage therapy	Local administration via mediastinal drains	Systemic antibiotics maintained (not fully detailed)	Resolution	Not reported	[90]
Racenis et al., 2023	LVAD driveline infection	1	MDR *Pseudomonas aeruginosa*	Personalized phage therapy	IV + local; IV concentrations 10^7–10^{11} PFU/mL	Colistin Ceftazidime/ avibactam Amikacin	Resolution	Not reported	[91]
Rojas et al., 2022	Intrapericardial LVAD outflow graft infection	1	*Staphylococcus aureus*	Personalized phage therapy	Local application at wound closure	Systemic antibiotics maintained (not fully detailed)	Significant reduction of infection	Not reported	[92]
Grambow et al., 2022	Infected thoracic aortic stent graft	1	MSSA	Personalized phage therapy	Local extravascular + endovascular application, repeated instillation over 3 days	Flucloxacillin Cefuroxime	Resolution	Not reported	[93]
Puschel et al., 2022	LVAD driveline infection	1	*Proteus mirabilis* *S. aureus*	Personalized phage therapy	Local application after debridement	Piperacillin tazobactam	Resolution	Not reported	[94]
Rubalskii et al., 2020	Implant- and transplant- associated cardiothoracic infections	8	*S. aureus*, *E. faecium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli*	Personalized phage therapy	Variable: local ± oral ± inhaled $\sim 1 \times 10^8$ PFU/mL Duration not standardized	Systemic antibiotics maintained (not fully detailed)	Eradication in 7/8 patients	Not reported	[95]
Aslam et al., 2019	LVAD infection with sternal osteomyelitis and recurrent bacteremia	1	MSSA	Personalized phage therapy	Intravenously 3×10^9 PFU BID 28 days	Cefazolin Minocycline	Resolution	Not reported	[96]

Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Gilbey et al., 2019	Prosthetic valve endocarditis	1	MSSA	Personalized phage therapy	Intravenously 1×10^9 PFU BID 14 days	Flucloxacillin + ciprofloxacin + rifampicin	Resolution	No adverse event attributable to phage therapy	[97]
Chan et al., 2018	Aortic graft infection with aorto-cutaneous fistula	1	*Pseudomonas aeruginosa*	Personalized phage therapy	Single local application directly to graft	Ceftazidime	Resolution	Not reported	[98]
Respiratory tract infections									
Yang et al., 2025	Lung infection	1	Carbapenem-resistant *Pseudomonas aeruginosa*	Personalized phage therapy	Nebulized 9×10^9 PFU/mL 14 days	Colistin	Clinical improvement; persistent *Pseudomonas aeruginosa* colonization (no eradication). Phage resistance detected	Not reported	[99]
Tenney et al., 2024	VAP (burned patient)	1	XDR *Pseudomonas aeruginosa*	Personalized phage therapy	Nebulized and intravenous 2×10^9 PFU/mL, 3 inhaled doses, 5 mL 2×10^8 PFU/mL, daily IV, 50 mL, 7 days	Imipenem–relebactam Colistin (inhaled)	Resolution (pneumonia recurrence after 1 month; repeated phage course with favorable outcome)	No adverse event attributable to phage therapy	[100]

Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Kohler et al., 2023	Lung infection	1	MDR *Pseudomonas aeruginosa*	Personalized phage therapy	Nebulized daily dose of 5×10^9 PFU, 7 days	Concomitant suppressive antibiotics (not specified)	Clinical improvement without eradication; recurrence requiring repeated phage course	Transient hypoxia, single febrile episode	[101]
Li et al., 2023	Chronic lung infection (interstitial lung disease)	1	*Pseudomonas aeruginosa*	Personalized phage therapy	Nebulized 1×10^8 PFU/mL (10 mL) BID, 3 days	Multiple systemic antibiotics prior to phage therapy, discontinued during repeated nebulized phage courses	Transient elimination of *Pseudomonas aeruginosa* in sputum; significant improvement of infection; recurrence, not eradicated	Not reported	[102]
Haidar et al., 2023	Lung infection (lung transplant)	1	MDR *Burkholderia multivorans*	Not specified	Nebulized 3.33×10^9 PFU per dose 3 mL per dose 7 days	Concomitant suppressive antibiotics (not specified)	Clinical failure and death	No adverse event attributable to phage therapy	[103]
Levêque et al., 2023	Lung infection (lung transplant)	1	XDR *Pseudomonas aeruginosa*	Personalized phage therapy	Multiple nebulized doses (not specified)	Continuous inhaled colistin and multiple systemic antibiotics (including cefiderocol and aminoglycosides, adjusted over time)	Microbiological response without clinical success; death	Not reported	[104]

Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Li et al., 2023	Chronic pulmonary infection	1	MDR *Klebsiella pneumoniae*	Personalized phage therapy	Nebulized $> 1 \times 10^9$ PFU/mL 5 mL, 2 single-day courses, each with 3 nebulized doses	Concomitant systemic antibiotics (regimens not specified)	Clinical improvement despite incomplete eradication. Phage resistance detected	Not reported	[105]
Chen et al., 2022	Chronic pleuropulmonary infection after lobectomy	1	MDR *Pseudomonas aeruginosa*	Personalized phage therapy	Nebulized and intrapleural Dosage not consistently reported Nebulized BID and intrapleural QD, 11 days and then intensified nebulized regimen (duration not specified)	Amikacin Ceftazidime–avibactam Fosfomycin	Persistent colonization; increased susceptibility to antibiotics after phage therapy	Not reported	[106]
Wu et al., 2021	Secondary bacterial pneumonia	4	Carbapenem-resistant *Acinetobacter baumannii*	Personalized phage therapy	Nebulized 2×10^9 PFU per administration Repeated doses (exact duration varied per patient)	Concomitant suppressive antibiotics (not specified)	Reduced bacterial load with clinical improvement	Not reported	[107]
Maddocks et al., 2019	VAP	1	MDR *Pseudomonas aeruginosa*	Personalized phage therapy	Nebulized and intravenously 4 mL undiluted, BID, Neb 100 mL, BID, IV	Ciprofloxacin Gentamicin	Resolution	Not reported	[108]
Aslam et al., 2019	Lung transplant	3	*Pseudomonas aeruginosa* (n=2), *Burkholderia dolosa* (n=1)	Personalized phage therapy	Nebulized and intravenously (exact PFU and duration not specified)	Concomitant suppressive antibiotics (not specified)	Clinical improvement in 2/3 patients; relapse and death in 1 patient	Not reported	[109]
Law et al., 2019	Lung infection (waiting lung transplant)	1	MDR *Pseudomonas aeruginosa*	Personalized phage therapy	Intravenously 1×10^9 PFU/mL (IV dosing, repeated)	Colistin Azithromycin Piperacillin–tazobactam Carbapenem	Resolution	Not reported	[110]

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Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Genito-Urinary Tract Infections									
Cook et al., 2025	Recurrent UTI	1	MDR *E. coli*	Personalized phage therapy	Oral, intravesical instillation and then topical formulation 4.3×10^{11} PFU in 100 mL, single dose, oral 4.3×10^{11} PFU in 100 mL, single dose 2.6×10^{9} PFU (topical), 3 days	Not specified	Clinical improvement with reduction in UTI episodes	Not reported	[111]
Cesta et al., 2025	Chronic bacterial prostatitis	1	*E. coli*	Personalized phage therapy	Rectal and oral administration for several weeks (exact PFU and duration not specified)	No combined antibiotics	Resolution	Not reported	[112]
Johri et al., 2023	Chronic bacterial prostatitis	1	*E. coli*	Personalized phage therapy	Rectal and oral administration for several weeks (exact PFU and duration not specified)	No combined antibiotics	Resolution	Not reported	[113]
Le et al., 2023	Recurrent UTI (transplant)	1	*Klebsiella pneumoniae* ESBL +	Personalized phage therapy	Intravenously, 4 weeks (exact PFU not specified)	No combined antibiotics	Resolution, no recurrence for 6 months	Not reported	[114]
Johri et al., 2021	Chronic prostatitis	1	*E. coli*	Personalized phage therapy	Oral and rectal Several weeks (exact PFU and duration not specified)	No combined antibiotics	Resolution	Not reported	[115]
Rostkowska et al., 2021	Recurrent UTI	1	*Klebsiella pneumoniae* ESBL +	Phage cocktail with confirmed lytic activity	Intrarectal, repeated courses (exact PFU and duration not specified)	Meropenem Colistin	Resolution only after nephrectomy	Not reported	[116]
Terwilliger et al., 2021	Recurrent UTI and prostatitis	1	*E. coli* ESBL +	Personalized phage therapy	Intravenous, 2 weeks (exact PFU not specified)	Ertapenem	Resolution	Not reported	[117]
Bao et al., 2020	UTI	1	*Klebsiella pneumoniae*	Personalized phage therapy	Intravesical, repeated instillations (exact PFU and duration not specified)	Not clearly specified	Reduction in bacterial burden and symptom improvement	Not reported	[118]

Table I. Clinical use of bacteriophage therapy (continued)

Author, year	Infection	n	Microbiology	Phage preparation	Route of administration, dosage, frequency and duration	Combined antibiotics	Reported outcome	Adverse events	Ref.
Kuipers et al., 2019	UTI (renal transplant recipient)	1	*Klebsiella pneumoniae* ESBL +	Phage cocktail with confirmed lytic activity	Oral and intravesical, 12 weeks total (exact PFU and duration not specified)	Meropenem	Resolution	Not reported	[119]
Khawaldeh et al., 2011	Recurrent UTI	1	*Pseudomonas aeruginosa*	Personalized phage therapy	Intravesical, 10 days (exact PFU and duration not specified)	Meropenem Colistin	Resolution	Not reported	[120]
Letkiewicz et al., 2009	Chronic bacterial prostatitis	3	*Enterococcus faecalis*	Personalized phage therapy	Rectal; 2.8×10^8 / 7.5×10^7 / 4.5×10^7 PFU/mL (10 mL) BID 28–33 days	No combined antibiotics	Resolution	Not reported	[121]

Notes: (1) Phage preparation terminology: **Personalized phage therapy** indicates bacteriophages specifically selected, adapted, or produced for an individual patient following susceptibility testing. **Commercial phage cocktail** denotes standardized phage products administered without documented patient-specific selection. **Phage cocktail with confirmed lytic activity** describes phage preparations obtained from established phage banks or pre-existing cocktails, for which in vitro lytic activity against the clinical isolate was confirmed before administration.

Abbreviations. BID, twice daily; CD, catheter drainage; ESBL, extended-spectrum beta-lactamase; IA, intra-articular; IO, intraoperative; IV, intravenous; LVAD, left ventricular assist device; MDR, multidrug-resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MSSA, methicillin-susceptible *Staphylococcus aureus*; Neb, nebulized; PFU, plaque-forming units; PJI, prosthetic joint infection; QD, once daily; SAT, suppressive antibiotic therapy; TID, three times daily; UTI, urinary tract infection; VAP, ventilator-associated pneumonia; XDR, extensively drug-resistant.

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