www.brn.cat

PERMANYER

BRN Rev. 2025;11(4):287-301

BRN Reviews

REVIEW ARTICLE

Phage therapy in mycobacteria

María C. Cumplí-Gargallo[1], Patrick J. Hurley[1], Jose G. González-García[1], and Marisol Domínguez-Álvarez[1,2,3]*

[1]Department of pneumology, Hospital del Mar; [2]Department of Medicine and Life Sciences, Universitat Pompeu Fabra; [3]Hospital del Mar Research Institute. Barcelona, Spain

ABSTRACT

This review explores the potential of bacteriophage therapy as an emerging strategy against mycobacterial infections, including *Mycobacterium tuberculosis* and non-tuberculous mycobacteria. Conventional treatments for these pathogens are prolonged, poorly tolerated, and increasingly ineffective due to multidrug- and extensively drug-resistant strains, highlighting the urgent need for innovative alternatives. Bacteriophages, viruses that specifically infect and lyse bacteria, offer a targeted and adaptable alternative or adjunct to antibiotics. The review summarizes the biology of mycobacteriophages, methods for their isolation and formulation, and mechanisms of bacterial lysis. It also compiles preclinical and clinical evidence supporting their efficacy in reducing bacterial burden, enhancing antibiotic susceptibility and improving outcomes, particularly in refractory *Mycobacterium abscessus* infections. Despite promising advances, challenges such as phage resistance, host immune responses, and regulatory inconsistencies remain. Well-designed clinical trials and standardized production protocols are essential to translate this therapy into clinical practice.

Keywords: Bacteriophage therapy. Mycobacteriophages. *Mycobacterium tuberculosis*. Non-tuberculous mycobacteria. Phage.

***Correspondence to:**
Marisol Domínguez-Álvarez
E-mail: mdominguez@hmar.cat

Received: 10-10-2025
Accepted: 30-10-2025
DOI: 10.23866/BRNRev:2025-M0142
www.brnreviews.com

INTRODUCTION

Mycobacteria are aerobic bacilli with lipid -rich cell walls (mycolic acids, glycolipids, and glycopeptidolipids), which confer resistance to multiple antibiotics, the ability to form biofilms, and the characteristic acid–alcohol–resistant staining[1-3]. Within this genus are both *Mycobacterium tuberculosis*, the cause of tuberculosis (TB), and non-tuberculous mycobacteria (NTM), responsible for opportunistic infections in immunocompromised patients or those with chronic pulmonary diseases[4].

NTM encompasses all species distinct from *M. tuberculosis* and *Mycobacterium leprae*[5]. They are ubiquitous environmental microorganisms, able to survive in water and soil, adapt to different conditions, and resist both disinfectants and antimicrobials[6]. Among the species of greatest clinical relevance are the *Mycobacterium avium complex*, *Mycobacterium kansasii*, *Mycobacterium xenopi*, *Mycobacterium abscessus*, *Mycobacterium Chelonae,* and *Mycobacterium malmoense*. Their prevalence has increased in recent decades due to advances in diagnostic techniques, improved survival of patients with chronic respiratory diseases, widespread use of immunosuppressants, and recognition of genetic susceptibility factors[6-8]. However, the finding of an NTM in respiratory samples does not always indicate active infection, which necessitates applying clinical, radiological, and microbiological criteria from guidelines such as ATS, BTS, or SEPAR[9-11].

Treatment of infections caused by NTM is complex: it requires combinations of antibiotics for prolonged periods, with high toxicity, poor adherence, and limited cure rates. Moreover, after microbiological conversion, relapses and reinfections are frequent[9-12].

TB remains one of the leading causes of infectious mortality. In 2023, the World Health Organization estimated 10.8 million new cases and 1.25 million deaths[13]. Its transmission occurs through the airborne route, with predominant pulmonary involvement and possible extrapulmonary dissemination. The high global burden is related to social determinants such as poverty, overcrowding, HIV coinfection, malnutrition, the presence of chronic diseases, and immunosuppressive treatments[14].

The diagnosis combines clinical and radiological assessments with microbiological and molecular tests. Bacilloscopy and culture remain the reference methods, whereas molecular techniques such as Xpert MTB/RIF have significantly accelerated detection and the assessment of drug resistance[15,16]. The standard treatment for sensitive TB consists of 6 months of isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by isoniazid and rifampicin. However, the emergence of mutations under antibiotic pressure has favored the rise of multidrug-resistant TB (MDR) and extensively drug-resistant TB (XDR), which require prolonged regimens that are poorly tolerated and have lower success rates[16,17]. The incorporation of new drugs such as bedaquiline has improved outcomes, with cure rates of 82% in MDR/XDR-TB[18], although the WHO warns that without innovative strategies, resistant strains could cause more than 75 million deaths in the coming decades.

Both in TB and in NTM, the diagnostic challenges, intrinsic or acquired resistance, the

need for prolonged treatments, and the high frequency of relapses highlight the urgency for new therapeutic approaches. In this context, bacteriophages emerge as a promising option.

BACTERIOPHAGES: GENERAL FUNDAMENTALS

A history of phage therapy

Phage therapy, or phagotherapy, is based on the use of bacteriophages (phages), viruses that specifically infect bacteria[19,20]. Its discovery represents one of the most significant advances in microbiology of the 20th century, with contributions from Twort and Félix d'Hérelle.

In 1915, Twort, from St. Thomas Hospital in London, observed in cultures of *Micrococcus* the appearance of transmissible glassy spots that destroyed the colonies during his study of the *vaccinia* virus. Although he suggested the existence of an ultramicroscopic virus, he could not confirm this due to technical and financial limitations during World War I, which constituted the first scientific description of the phenomenon[21,22].

In 1917, d'Hérelle, from the Pasteur Institute, investigated an outbreak of dysentery in French troops and detected clear areas in cultures of *Shigella*. He concluded that they were caused by a bactericidal virus, which he named "bacteriophage," and presented his findings the same year[21,23]. In 1919, he successfully treated a child with dysentery in Paris using phage therapy, and shortly thereafter, its use was reported against staphylococcal infections. D'Hérelle also promoted campaigns in Asia and Africa against cholera and bubonic plague[21,24].

In the 1920s and 1930s, phages were produced commercially in France, Brazil, and the United States, although the lack of standardization, variable results, and the emergence of antibiotics led to their decline in the West[21]. In contrast, phage therapy thrived in the Soviet Union thanks to George Eliava, who founded the Eliava Institute in Tbilisi in 1923 alongside d'Hérelle. Despite Eliava's execution during the Stalinist purges, the institute remained active and, favored by Soviet isolation during the Cold War, established itself as an international reference center, maintaining phage therapy uninterruptedly.

In recent decades, the rise of antibiotic resistance has rekindled interest in phages as a therapeutic alternative.

Structure of phages

Phages are the most abundant microorganisms on the planet, outnumbering bacteria by 10 times, and can be found in various habitats including water, soil, saliva, skin, and faeces. They bind to specific receptors on the bacterial surface and inject their genetic material, which is then replicated[19].

Structurally, most phages have a protein capsid that encapsulates nucleic acid, either DNA or RNA, in single or double strands. Morphologically, they are divided into two major groups: tailed phages (96%), belonging to the order *Caudovirales*, and non-tailed phages (4%), which display cubic, filamentous, or

pleomorphic shapes. Among the former, the families *Myoviridae* (contractile tails), *Siphoviridae* (long, non-contractile tails), and *Podoviridae* (short tails) stand out. Although they share a general architecture, they exhibit notable structural variability (in the capsid and tail) that reflects specific functional adaptations. It is tailed phages that represent most of the phages employed in therapy[25,26].

At the genomic level, phages exhibit extraordinary diversity, with sizes ranging from ~41 to ~165 kbp. To classify this variability, they are grouped into clusters and subclusters based on sequence similarity and gene content; additionally, there are *singletons* that do not group with any others, further highlighting their genetic diversity[27]. The complete and updated list of sequenced phages and their status is available in the databases PhagesDB (https://phagesdb.org) and Phamerator (https://phamerator.org)[28].

Bacteriophages present three different life cycles when infecting a bacterial cell: the lytic, lysogenic, and pseudolysogenic cycles. In the lytic cycle, following infection, there is active replication of the phage and subsequent lysis of the bacterial cell, releasing new viral particles. In the lysogenic cycle, on the other hand, the phage genome integrates into the bacterial chromosome, remaining in a latent state as a prophage without causing immediate cellular damage, replicating along with the bacterial DNA. In the pseudolysogenic cycle, the phage remains within the bacterium without integrating into the genome or initiating lytic replication. It remains inactive, and the host cell survives without immediate phenotypic changes. Phages that reproduce through the lytic cycle are called virulent or lytic phages, while those that can replicate through both the lytic and lysogenic cycles are referred to as temperate phages[20,29].

The infectious process begins with the adsorption of the phage to specific receptors located on the outer membrane of the bacterium, such as porins, glycolipids, or membrane proteins. This interaction determines host specificity. Next, the phage tail acts as a channel to inject the viral DNA into the bacterial cytoplasm, marking the start of the replicative cycle[20,30].

In obligate lytic phages, once the DNA is injected, the expression of early genes involved in viral genome replication and the inhibition of bacterial defense systems is activated. Subsequently, structural proteins are synthesized, capsids and tails are assembled, and the DNA is packaged inside the viral particles. Finally, lysis genes are expressed that code for proteins such as endolysins and holins, responsible for degrading the cell wall and facilitating the release of new virions[20,31,32].

In temperate phages, viral DNA integrates into the bacterial genome through a specific integrase, forming a lysogenic strain in which the virus remains in a latent state. During this phase, the phage's genetic material replicates passively along with that of the bacterium, and its lytic expression is suppressed by regulatory genes. However, this latency is not necessarily permanent: under certain conditions, such as environmental stress, exposure to ultraviolet radiation, or damage to bacterial DNA, the prophage can excise from the chromosome and initiate the lytic cycle, leading to the production of new viral particles and the lysis of the host cell[29,31].

Mechanism of action against hosts

Bacteriophages produce specialized proteins that mediate their adsorption, multiplication, and lysis in susceptible hosts. In mycobacteria, the cell envelope formed by mycolyl–arabinogalactan–peptidoglycan constitutes a key barrier to persistence, virulence, and antibiotic resistance[33]. To overcome this, holins are involved, which are small hydrophobic polypeptides that form pores and facilitate or regulate the action of endolysins on peptidoglycan, ensuring effective lysis[34,35]. Among the endolysins, bacteriophages encode Lysin A and Lysin B, enzymes with modular organization: a C-terminal binding domain and two catalytic domains that break peptidoglycan bonds[35,36]. It is noteworthy that Lysin B is a stearase that hydrolyzes the ester bonds that attach mycolic acids to arabinogalactan and degrades trehalose dimycolate (a glycolipid), thereby weakening the wall and reducing the virulence of mycobacteria[37,38]. In addition to lysis, secondary mechanisms have been described, such as the generation of superoxide radicals, the induction of bacterial apoptosis[39], and the opsonization of bacterial cells that favors phagocytosis by macrophages and innate immune cells[40].

Preparation of phages for therapeutic use

Bacteriophages are found in environments such as freshwater, seawater, wastewater treatment plants, soils, or in inanimate material. For therapeutic use, phages must be isolated, purified, and transformed into safe products, with lytic phages being the most suitable. The process begins with infective characterization through propagation, double-layer agar titration, *spot test*, host range analysis, and plaque efficiency. At this stage, they are quantified as plaque-forming units (PFU), selecting concentrations ≥ 10^8 PFU/mL to ensure their clinical potential. Assays such as adsorption curves and kinetics in liquid medium provide information on the dynamics of infection[41,42].

The next step is genomic characterization, where sequencing and bioinformatic analysis confirm identity and rule out lysogenic, resistance, or virulence genes. Subsequently, phages can be adapted to the target strain using the Appelmans method and combined into cocktails to broaden their spectrum[27,43].

Next, purification is carried out using techniques such as ultrafiltration, chromatography, or cross-flow filtration, removing contaminants and endotoxins and obtaining titers of 10^8-10^{12} PFU/mL suitable for clinical use[44,45]. Finally, the preparations, considered active pharmaceutical ingredients (APIs), undergo quality controls (identity, infective potency, biological load, endotoxins, pH, and impurities) and are stored in neutral buffers at 4°C or through lyophilization, remaining ready as safe, specific, and effective tools in therapy[42].

Advantages and limitations of phage therapy

This type of therapy has been used for almost 100 years. Unlike antibiotics, which affect both pathogenic bacteria and beneficial bacteria in the microbiome, bacteriophages act selectively, exclusively targeting the specific

bacterial species for which they have been designed[20,32,42]. In addition, they have low toxicity and are often better tolerated by patients with allergies to antibiotics[46,47]. They can be administered as a single dose or in combination, in the form of phage cocktails[48]. The main routes of administration include intravenous, topical, intraoperative, and nebulized[47,49]. The oral route is used less frequently due to the instability of phages in gastric pH or their inactivation by bile acids[42]. Among the additional benefits are the optimization of the antibiotic susceptibility pattern[50,51] and the increase in their efficacy in infections associated with biofilm formation[52]. Their synergistic action with antibiotics[53,54] and their ability to adapt to bacterial resistance mechanisms[31,55] is particularly relevant.

Despite its promising therapeutic potential, the use of phages still faces significant challenges that limit its large-scale clinical implementation. One of the main obstacles is the limited availability of specialized laboratories[32] with the necessary technical capacity to carry out all stages of the process up to the safe formulation for clinical use[32]. This difficulty is exacerbated by the need for these phages to have a broad spectrum within the same bacterial species (including different strains), to tailor the treatment to specific infections[56]. This specificity, while representing an advantage by minimizing the impact on the commensal microbiota, also complicates the empirical treatment of infections, especially in comparison to broad-spectrum antibiotics[32].

Another relevant challenge is that bacteria can develop resistance mechanisms against phages. These include, for example, the degradation of phage DNA (through systems such as CRISPR-Cas, restriction modification, and other strategies), blocking the transcription and replication of phage DNA or the synthesis of its proteins (through anti-phage signaling systems and infection abort mechanisms), as well as the modification of bacterial receptors, which prevent phage adhesion to host cells[32,55,57].

These factors are compounded by other limitations, such as the possible generation of neutralizing antibodies by the patient's immune system, which can reduce the effectiveness of treatment after multiple administrations[58]. The lack of a clear and uniform regulatory framework also constitutes a significant obstacle: in Eastern Europe (Georgia, Russia, and Poland), phages have been used for decades as commercial preparations; in the United States, their use is restricted to compassionate cases under the *emergency* or *expanded access IND* pathways, complying with Good Manufacturing Practices (GMP); Belgium has implemented a unique model based on magistral formulation in accredited pharmacies[59]; and in Spain, the AEMPS considers them drugs subject to GMP, with their current use limited to clinical trials. These differences reflect the urgent need for specific regulatory frameworks that facilitate their transition to routine clinical practice. Finally, uncertainties regarding their long-term efficacy and safety persist[20,31,32].

MYCOBACTERIOPHAGES

Mycobacteriophages are viruses that infect bacteria of the genus *Mycobacterium*. All of

those sequenced so far show a tailed morphology and double-stranded DNA genomes, primarily belonging to the families *Siphoviridae* (predominant) and *Myoviridae*, with no members of *Podoviridae* described[60]. Genomically, mycobacteriophages exhibit remarkable diversity. Initiatives such as the SEA-PHAGES program[61] have allowed the classification of 34 clusters from A to Z as well as AA, AB, AC, AD, AE, AF, AG, and AH, with cluster A being the most extensive. In addition, multiple subclusters have been identified (A1, A2, A3...). Furthermore, there are six distinct *singletons* that are genetically unrelated to the other clusters[60,62]. A central element in these advances has been the use of *M. smegmatis* mc²155, which is fast-growing and non-virulent, making it a safe model for phage propagation and manipulation. It is also used as a delivery system capable of transporting phages to intracellular pathogenic mycobacteria, acting as a host for their multiplication and enhancing their activity in macrophages and monocytes[62-64].

Numerous mycobacteriophages have been isolated from clinical and environmental sources, showing therapeutic potential against NTM and *M. tuberculosis* (Table 1). Their diversity, both in life cycles and in classification by clusters, translates into differentiated applications against NTM and against *M. tuberculosis*. In the field of NTM, phage D29 (cluster A2, lytic) stands out, which is active against *M. avium, M. scrofulaceum, M. ulcerans, M. fortuitum*, and *M. chelonae*, as well as phage TM4 (cluster K2, lytic), which has demonstrated efficacy against *M. avium*[35,65-68]. Muddy (cluster AB, lytic) is also notable, used in the treatment of *M. abscessus*. In addition, some initially temperate phages, such as ZoeJ (cluster K2) or BPs (cluster G1), have given rise to lytic derivatives (e.g., ZoeJD45 and BPs-D33HTH) with the ability to infect *M. abscessus* and *M. avium*[35,65,69].

In relation to *M. tuberculosis*, possible therapeutic effects of strictly lytic phages Bo4 (cluster G), Muddy (cluster AB) D29 (cluster A2), and TM4 (cluster K2) have been described[35,70,71]. Temperate phages from cluster K, such as Adephagia and Fionnbharth itself, have also been modified to generate lytic derivatives with activity against *M. tuberculosis*[65,72]. Similarly, DS6A (singleton and temperate) shows specific tropism for the *M. tuberculosis* complex and has demonstrated efficacy in cellular and murine models[35,73,74].

PRECLINICAL EVIDENCE OF TREATMENT WITH MYCOBACTERIOPHAGES

The study of therapy with mycobacteriophages *in vivo* animal models remains limited (Table 2). In *M. avium*, a study in mice employed the phage TM4 carried by *M. smegmatis* MC²155 as a strategy to reach intracellular bacteria. Although neither the phage nor *M. smegmatis* alone was able to reduce the bacterial load, their combination produced a significant decrease in the number of intracellular bacteria in the spleen compared with the untreated control, an effect explained by the fusion of vacuoles of the infected macrophages that allowed the intracellular release of the phage. However, 23% of the isolates developed resistance to TM4, supporting the need to use phage cocktails to prevent the emergence of resistant subpopulations[66]. In *M. ulcerans*, the mouse plantar pad model

Table 1. Mycobacteriophages with reported therapeutic potential

Phage	Cluster	Lytic or temperate	Mycobacterial targets
D29	A2	Lytic	*Mycobacterium avium, Mycobacterium scrofulaceum, Mycobacterium ulcerans, Mycobacterium fortuitum, Mycobacterium chelonae,* and *Mycobacterium tuberculosis*
TM4	K2	Lytic	*Mycobacterium avium,* and *Mycobacterium tuberculosis*
Muddy	AB	Lytic	*Mycobacterium abscessus,* and *Mycobacterium tuberculosis*
ZoeJ Δ45	K2	Engineered lytic derivative	*Mycobacterium abscessus* and *Mycobacterium avium*
BPs Δ33HTH	G1	Engineered lytic derivative	*Mycobacterium abscessus* and *Mycobacterium avium*
DS6A	Singleton	Temperate	*Mycobacterium tuberculosis*
Bo4	G	Lytic	*Mycobacterium tuberculosis*
Adephagia, Fionnbharth	K	Modified lytic derivatives	*Mycobacterium tuberculosis*

showed that a single dose of phage D29 significantly reduced the bacterial load in tissue and lymph nodes, in addition to modulating the immune response with an increase in TNF, IFN-γ, and IL-10 and a reduction in IL-6. Histological studies have confirmed less tissue destruction in treated animals, while in the same model, the endolysin D29 Lysin B also decreased bacterial proliferation, stimulated the production of protective cytokines, and showed synergy with rifampicin against *M. smegmatis*[67]. Another area of interest has been inhaled administration. Liu et al. demonstrated that inhaled aerosolization of Mycobacteriophage D29 in mice is safe, well tolerated, and an efficient delivery method, supporting its potential use for the treatment of respiratory mycobacterial infections[68]. In addition, non-mammalian models such as zebrafish (*Danio rerio*), *Galleria mellonella* larvae, and *Drosophila melanogaster* have been employed, offering advantages in terms of low cost, experimental speed, and greater ethical acceptability. In particular, zebrafish have allowed the study of complex immunological mechanisms and provided insights into the pathogenesis of *M. abscessus*[75], highlighting the formation of granulomas and the phenomenon of *cording* as a means of immune evasion[76].

In the case of TB, the first attempts at phage therapy date back to 1981, when Sula and collaborators treated guinea pigs infected with *M. tuberculosis* using the phages DS6A, GR-21/T, and My-327. The phage DS6A showed an effect comparable to that of isoniazid, with a significant reduction in bacterial load in the spleen, as reflected by a lower splenic index[73]. Subsequently, in another study with guinea pigs infected with sensitive strains, the intranasal administration of phage D29 reduced the bacterial load and pulmonary pathology with an efficacy similar to rifampicin, with no adverse effects observed[77]. More recently, prophylactic studies with aerosolized phage D29 have shown promising results in murine models, opening the possibility for its preventive use against *M. tuberculosis*[78]. In humanized NSG-SGM3 mice, the intravenous administration of phage DS6A achieved a reduction in pulmonary

and splenic bacterial load, accompanied by low levels of IgG and IgA antibodies against the phage, suggesting a limited but sufficient immune response to indicate antigen presentation[74].

CLINICAL EVIDENCE OF TREATMENT WITH MYCOBACTERIOPHAGES

Phage therapy has shown promising results in the management of chronic resistant and difficult-to-treat infections, including urinary tract infections, skin infections, osteomyelitis, infections associated with aortic grafts, and notably, respiratory infections[32,47,79-86]. Most of the available evidence comes from case series or isolated clinical cases involving MDR pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus, Acinetobacter baumannii, or Achromobacter xylosidans*[79,85,87-90]. In most patients, the administration of phage therapy was carried out in the context of compassionate use. A retrospective series conducted in Belgium described 100 consecutive cases of phage therapy for complex infections localized in the lung, bone, skin, and soft tissues[91]. In this study, 77% of patients showed clinical improvement, and 61% achieved eradication of the implicated pathogen, with a low incidence of adverse effects reported. In the realm of respiratory infections, this treatment has been primarily used in patients with cystic fibrosis (CF)[69,79,85,92,93], ventilator-associated pneumonia[85,94], or lung transplant recipients[51,69,85]. The predominant pathogens have been *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and species of *Achromobacter*[85,95,96]. The described routes of administration include nebulization, intravenous administration, or a combination of both; bronchoscopy has also been employed[97], and even instillation through a drainage tube in a patient with chronic empyema[98], with a favorable clinical response in most cases[85]. Most patients (75%) were treated with a combination of two or more phages. Notably, in two patients with a MDR *Pseudomonas aeruginosa* infection, an improvement in the antibiotic susceptibility profile was observed after phage therapy[51,85]. The duration of phage therapy varies between 2 days and 12 months[85].

The evidence regarding the use of phage therapy in infections caused by mycobacteria is still limited[49,58,69,99-101] (Table 2). One of the first reported cases involved a 15-year-old adolescent with CF who developed a disseminated infection due to *M. abscessus* subsp. *massiliense* following a lung transplant. The treatment, based on a cocktail of three phages (Muddy, BPsΔ33HTH_HRM10, and ZoeJΔ45) combined with antibiotics, was associated with a significant improvement in skin lesions and stabilization of lung function, with no development of resistance to the phages observed[69].

Subsequently, the case of an 81-year-old male, immunocompetent and with bronchiectasis not associated with CF, was documented, who had a refractory pulmonary infection caused by *M. abscessus* subsp. *massiliense* resistant to macrolides. Initially, he received 6 months of intravenous therapy with the same three-phage cocktail (Muddy, BPsΔ33HTH_HRM10, and ZoeJΔ45), which resulted in a transient reduction of the bacterial load that was lost after the appearance of neutralizing antibodies[58]. Given this limitation, it was decided to modify the route of administration to inhalation. The nebulized therapy,

Table 2. Preclinical and clinical studies on mycobacteriophage therapy

2.1. Preclinical efficacy and safety studies				
Study	**Animal model**	**Pathogen**	**Mycobacteriophage**	**Outcome**
Danelishvili et al.[66]	Mice	*M. avium*	TM4 phage delivered by M. smegmatis	↓ Bacterial load in spleen; resistance emerged
Fraga et al.[67]	Mice	*M. ulcerans*	D29	↓ Bacterial load, ↑ TNF/IFN-γ
Liu et al.[68]	Mice	Healthy	D29	Effective pulmonary delivery by inhalation
Sula et al.[73]	Guinea pigs	*M. tuberculosis*	DS6A, GR-21/T, My-327	DS6A efficacy comparable to isoniazid (↓ splenic bacterial load)
Li et al.[77]	Guinea pigs	*M. tuberculosis*	D29	D29 efficacy comparable to rifampicin (↓ Bacterial load and gross organ lesions)
Carrigy et al.[78]	Mice	*M. tuberculosis*	D29	Prophylactic protection

2.2. Clinical studies					
Study	**Condition**	**Pathogen**	**Mycobacteriophage**	**Route/Duration**	**Outcome**
Dedrick et al.[69]	Pediatric, CF lung transplant patient	*M. abscessus* subsp. *massiliense*	Muddy, ZoeJΔ45, BPsΔ33HTH	IV + topical 8 months	Clinical improvement, no resistance
Dedrick et al.[58] Dedrick et al.[99]	Bronchiectasis patient	M. abscessus subsp. *massiliense*	Muddy, ZoeJΔ45, BPsΔ33HTH	6 months IV→ 9 months aerosol	Initial IV response; loss of efficacy due to antibody development. Subsequent nebulized phage led to clinical improvement and reduced sputum bacterial load
Nick et al.[100]	CF patient	*M. abscessus* subsp. *abscessus*	Engineered phages: BPsΔ33HTH_HRM10 D29_HRMGD40	IV 13 months	Culture conversion, radiological improvement
Little et al.[101]	Adult, arthritis with cutaneous infection	*M.chelonae*	Muddy	IV 9 months	Infection resolved
Dedrick et al.[49]	Cohort of 20 patients (mostly with CF)	Predominantly *M. abscessus*	Single phage or phage cocktail	IV, aerosol or both 6 months	11 patients showed favorable clinical or microbiological responses

IV: intravenous; CF: cystic fibrosis. ↓: decrease, ↑: increase.

maintained for 9 months alongside antibiotics, showed a favorable safety profile and was associated with initial clinical benefits (including weight gain, decreased C-reactive protein, and relative reduction of bacterial load in sputum), while pulmonary function remained stable. No resistance to phage treatment was recorded. Unlike what was observed with intravenous administration, local neutralization by antibodies in sputum was weak, suggesting that nebulization could partially mitigate this immunological obstacle[99].

In another patient with advanced CF and refractory pulmonary infection due to *M. abscessus* subsp. *abscessus*, a cocktail of two genetically engineered phages (BPsΔ33HTH_HRM10 and D29_HRMGD40) was administered intravenously in combination with antibiotics. The treatment induced early

bacterial lysis, accompanied by progressive radiological improvement and conversion of respiratory cultures to negative in the following months. Genomic analysis of the isolates showed genetic stability, reduced bacterial diversity, and absence of resistance to phages or antibiotics. Although neutralizing antibodies against one of the phages were detected, the clinical response remained favorable until lung transplantation, with the explanted organ testing negative for *M. abscessus*[100].

In addition, a case of disseminated skin infection due to *M. chelonae* refractory in a patient with seronegative arthritis treated with tofacitinib has been reported. The combination of antimicrobials, surgery, and a single phage (Muddy) resulted in an excellent clinical response, with negative biopsies and no development of bacterial resistance, despite the appearance of neutralizing antibodies[101].

More recently, the experience of a cohort of 20 patients with drug-resistant mycobacterial disease treated with adjunct phage therapy under compassionate use was reported. Of these, 17 had an infection with *M. abscessus* (five subsp. *massiliense* and 12 subsp. *abscessus*), while the remaining patients had a disseminated BCG infection, a disseminated skin infection by *M. chelonae*, and one case of *M. avium* in a patient with CF. Most patients received phage therapy intravenously, administered twice weekly for 6 months, in combination with at least two antibiotics; in some cases, nebulized phage administration was also added. The treatment duration was individualized according to clinical and microbiological responses. In this cohort, approximately 55% showed a favorable or partially favorable clinical or microbiological response, five had inconclusive results or transient improvements, and four showed no response[49].

So far, all the published clinical evidence is limited to NTM infections. In the case of TB, the documented clinical experience is still limited. A case of favorable response in a disseminated BCG infection has been described, which opens the possibility for future applications[49]. In addition, a cocktail of candidate phages is already prepared for evaluation in clinical trials[72].

CONCLUSION

Phage therapy is emerging as a therapeutic strategy against mycobacterial infections, the incidence of which is on the rise, especially in patients with chronic respiratory diseases or immunocompromised conditions. The emergence of MDR and XDR strains of *M. tuberculosis* along with the limited efficacy and toxicity of the prolonged regimens used in NTM highlights the urgent need for innovative alternatives. In this context, bacteriophages stand out for their biological diversity, their ability to synergize with antimicrobials and the host's immune response, and their potential to reduce bacterial load with a favorable safety profile. However, to advance toward their routine clinical application, it is essential to develop extensive and well-characterized phage libraries, as well as to define regulatory frameworks that ensure their quality, safety, and efficacy.

Likewise, the optimization of critical parameters (including pharmacokinetic and pharmacodynamic factors, such as the route of administration, dosage, duration of treatment,

and modulation of the immune response) is an essential requirement to maximize the efficacy of therapy.

Similarly, challenges such as the emergence of phage resistance, the generation of neutralizing antibodies, and the complex interaction with host immunity must be systematically addressed.

While the current evidence, based on preclinical studies and compassionate use experiences, is promising, the absence of controlled clinical trials still limits its application. The design and execution of rigorous studies aimed at personalized medicine will be crucial to consolidate this strategy. If its efficacy is confirmed, phage therapy could establish itself not only as a complementary tool in the management of mycobacterial infections but also as a key resource in the global challenge of antimicrobial resistance.

FUNDING

The authors did not receive funds, grants, or support from any organization for the submitted work.

CONFLICTS OF INTEREST

None declared.

ETHICAL CONSIDERATIONS

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The study does not involve patient personal data nor requires ethical approval. The SAGER guidelines do not apply.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

REFERENCES

1. Jackson M. The mycobacterial cell envelope-lipids. Cold Spring Harb Perspect Med. 2014;4:a021105.
2. Pfysffer GE. *Mycobacterium*: general characteristics, laboratory detection and staining procedures. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. Manual of Clinical Microbiology. Washington, DC: ASM Press; 2007. p. 543-72.
3. Jacobo-Delgado YM, Rodríguez-Carlos A, Serrano CJ, Rivas-Santiago B. *Mycobacterium tuberculosis* cell-wall and antimicrobial peptides: a mission impossible? Front Immunol. 2023;14:1194923.
4. Fernández-Caso B, Vázquez R, Alarcón T, Girón R, López-Giménez MR, Domingo D. Prevalence and importance of non-tuberculous mycobacteria in adult patients with cystic fibrosis in a hospital in Madrid. Enferm Infecc Microbiol Clin (Engl Ed). 2020;38:323-6.
5. Runyon EH. Bacterial diseases. "Anonymous mycobacteria in pulmonary disease". Med Clin North Am. 1959;43:273-90.
6. Honda JR, Knight V, Chan ED. Pathogenesis and risk factors for nontuberculous mycobacterial lung disease. Clin Chest Med. 2015;36:1-11.
7. Bents SJ, Mercaldo RA, Powell C, Henkle E, Marras TK, Prevots DR Nontuberculous mycobacterial pulmonary disease (NTM PD) incidence trends in the United States, 2010-2019. BMC Infect Dis. 2024;24:1094.
8. Lake MA, Ambrose LR, Lipman MC, Lowe DM. "Why me, why now?" Using clinical immunology and epidemiology to explain who gets nontuberculous mycobacterial infection. BMC Med. 2016;14:54.
9. Griffith DE, Aksamit T, Brown-Elliot BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175:367-416.
10. Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF, et al. British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax. 2017;72:ii1-64.
11. García García JM, Palacios Gutierrez JJ, Sánchez Antuña AA. Infecciones respiratorias por micobacterias ambientales. Arch Bronconeumol. 2005;41: 206-19.
12. Cowman S, van Ingen J, Griffith DE, Loebinger MR. Non-tuberculous mycobacterial pulmonary disease. Eur Respir J. 2019;54:1900250.
13. World Health Organization. Global Tuberculosis Report, 2023. Geneva, Switzerland: WHO; 2023.
14. Jarde A, Romano E, Afaq S, Elsony A, Lin Y, Huque R, et al. Prevalence and risks of tuberculosis multimorbidity in low-income and middle-income countries: a meta-review. BMJ Open. 2022;12:e060906.
15. Caminero JA, García-García JM, Caylà JA, García-Pérez FJ, Palacios JJ, Ruiz-Manzano J. Actualización de la normativa SEPAR diagnóstico y tratamiento de la tuberculosis con resistencia a fármacos. Arch Bronconeumol. 2020;56:514-21.

16. Sanchez-Montalva A, Caminero JA, Guna MR, Sanz TR, Rabuñal R, Millet JP, et al. Executive summary: clinical practice guidelines on the management of resistant tuberculosis of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) and the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC). Arch Bronconeumol. 2024;60:759-67.
17. Saukkonen JJ, Duarte R, Munsiff SS, Winston CA, Mammen MJ, Abubakar I, et al. Updates on the treatment of drug-susceptible and drug-resistant tuberculosis: an official ATS/CDC/ERS/IDSA clinical practice guideline. *Am J Respir Crit Care Med*. 2025;211:15-33.
18. Starshinova A, Dovgalyk I, Belyaeva E, Glushkova A, Osipov N, Kudlay D. Efficacy of tuberculosis treatment in patients with drug-resistant tuberculosis with the use of bedaquiline: the experience of the Russian Federation. Antibiotics (Basel). 2022;11:1622.
19. Hyman P, Abedon ST. Smaller fleas: viruses of microorganisms. Scientifica (Cairo). 2012;2012:734023.
20. Elois MA, Silva RD, Pilati GV, Rodríguez-Lázaro D, Fongaro G. Bacteriophages as biotechnological tools. Viruses. 2023;15:349.
21. Chanishvili N. Phage therapy--history from Twort and d´Herelle through soviet experience to current approaches. Adv Virus Res. 2012;83:3-40.
22. Twort F. An investigation on the nature of ultramicroscopic viruses. Lancet. 1915;11:1241-3.
23. D'Herelle F. Sur un microbe invisible antagonistse des bacilles dysentériques. C R Acad Sci. 1917;165:373-5.
24. Kazhal N, Iftimovich R. From the History of Fight against Bacteria and Viruses. Bucharest: Nauchnoe Izdatelstvo; 1968.
25. Bradley DE. Ultrastructure of bacteriophage and bacteriocins. Bacteriol Rev. 1990;31:230-314.
26. Ackerman HW. Phage classification and characterization. Methods Mol Biol. 2009;501:127-40.
27. Dion MB, Oechlisin F, Moineau S. Phage diversity, genomics and phylogeny. Nat Rev Microbiol. 2020;18:125-38.
28. Russell DA, Hatfull GF. PhagesDB: the actinobacteriophage database. Bioinformatics. 2017;33:784-6.
29. Hobbs Z, Abedon ST. Diveristy of phage infection types and associated terminology: the problem with "lytic or lysogenic". FEMS Microbiol Lett. 2016;363:fnw047.
30. Abedon ST. Bacteriophage adsorption: likelihood of virion encounter with bacteria and other factors affecting rates. Antibiotics (Basel). 2023; 12:723.
31. Dicks LM, Vermeulen W. Bacteriophage-host interactions and the therapeutic potential of bacteriophages. Viruses. 2024;16:478.
32. Petrovic Fabijan A, Iredell J, Danis-Wlodarczyk K, Kebriaei R, Abedon ST. Translating phage therapy into the clinic: Recent accomplishments but continuing challenges. PLoS Biol. 2023;21:e3002119.
33. Dulberger CL, Rubin EJ, Boutte CC. The mycobacterial cell envelope - a moving target. Nat Rev Microbiol. 2020;18:47-59.
34. Catalão MJ, Gil F, Moniz-Pereira J, São-José C, Pimentel M. Diversity in bacterial lysis systems: bacteriophages show the way. FEMS Microbiol Rev. 2013;37:554-71.
35. Kumar Raman S, Reddy DV, Jain V, Bajpai U, Misra A, Singh AK. Mycobacteriophages: therapeutic approach for mycobacterial infections. Drug Discov Today. 2024;29:104049.
36. Loessner MJ. Bacteriophage endolysins--current state of research and applications. Curr Opin Microbiol. 2005;8:480-7.
37. Payne K, Sun Q, Sacchettini J, Hatfull GF. Mycobacteriophage lysin B is a novel mycolylarabinogalactan esterase. Mol Microbiol. 2009;73:367-81.
38. Gil F, Grzegorzewicz AE, Catalão MJ, Vital J, McNeil MR, Pimentel M. Mycobacteriophage Ms6 LysB specifically targets the outer membrane of *Mycobacterium smegmatis*. Microbiology (Reading). 2010;156:1497-504.
39. Samaddar S, Grewal RK, Sinha S, Ghosh S, Roy S, Das Gupta SK. Dynamics of mycobacteriophage-mycobacterial host interaction: evidence for secondary mechanisms for host lethality. Appl Environ Microbiol. 2016;82:124-33.
40. Van Belleghem JD, Dabrowska K, Vaneechoutte M, Barr JJ, Bollyky PL. Interactions between bacteriophage, bacteria, and the mammalian immune system. Viruses. 2019;11:10.
41. Luong T, Salabarria AC, Edwards RA, Roach DR. Standardized bacteriophage purification for personalized phage therapy. Nat Protoc. 2020;15:2867-90.
42. Zalewska-Piatek B. Phage therapy-challenges, opportunities and future aspects. Pharmaceuticals. 2023;16:163.
43. Brown-Jaque M, Calero-Cáceres W, Espinal P, Rodríguez-Navarro J, Miró E, González-López JJ, et al. Antibiotic resistance genes in phage particles isolated from human faeces and induced from clinical bacterial isolates. Int J Antimicrob Agents. 2018;51:434-42.
44. Abdelsattar A, Dawoud A, Makky S, Nofal R, Aziz R, El-Shibiny A. Bacteriophages: from isolation to application. Curr Pharm Biotechnol. 2022;23:337-60.
45. Van Belleghem JD, Merabishvili M, Vergauwen B, Lavigne R, Vaneechoutte M. A comparative study of different strategies for removal of endotoxins from bacteriophage preparations. J Microbiol Methods 2017;132:153-9.
46. Salmon GP, Fineran PC. A century of the phage: past, present and future. Nat Rev Genet. 2015;13:777-86.
47. Aslam S, Lampley E, Wooten D, Karris M, Benson C, Strathdee S, et al. Lessons learned from the first 10 consecutive cases of intravenous bacteriophage therapy to treat multidrug-resistant bacterial infections at a single center in the United States. Open Forum Infect Dis. 2020;7:ofaa389.
48. Tanji Y, Shimada T, Yoichi M, Miyanga K, Hori K, Unno H. Toward rational control of *Escherichia coli* O157: H7 by a phage cocktail. Appl Microbiol Biotechnol. 2004;64:270-4.
49. Dedrick RM, Smith BE, Cristinziano M, Freeman KG, Jacobs-Sera D, Belessis Y, et al. Phage therapy of *Mycobacterium* infections: compassionate use of phages in 20 patients with drug-resistant mycobacterial disease. Clin Infect Dis. 2023;76:103-12.
50. Chan BK, Sistrom M, Wertz JE, Kortright KE, Narayan D, Turner PE. Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*. Sci Rep. 2016;6:26717.
51. Aslam S, Courtwright AM, Koval C, Lehman SM, Morales S, Furr CL, et al. Early clinical experience of bacteriophage therapy in three lung transplant recipients. Am J Transplant. 2019;19:2631-9.
52. Gordon M, Ramirez P. Efficacy and experience of bacteriophages in biofilm-related infections. Antibiotics (Basel). 2024;13:125.
53. Oechslin F, Piccardi P, Mancini S, Gabard J, Moreillon P, Entenza JM, et al. Synergisitc interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. J Infect Dis. 2017;215:703-12.
54. Tagliaferri TL, Jansen M, Horz HP. Fighting pathogenic bacteria on two fronts: phages and antibiotics as combined strategy. Front Cell Infect Microbiol. 2019;9:22.
55. Hampton HG, Watson BN, Fineran PC. The arms race between bacteria and their phage foes. Nature. 2020;577:327-36.
56. Hyman P, Abedon ST. Bacteriophage host range and bacterial resistance. Adv Appl Microbiol. 2010;70:217-48.
57. Ambroa A, Blasco L, López M, Pacios O, Bleriot I, Fernández-García L, et al. Genomic analysis of molecular bacterial mechanisms of resistance to phage infection. Front Microbiol. 2022;12:784949.
58. Dedrick RM, Freeman KG, Nguyen J, Bahadirli-Talbott A, Smith BE, Wu AE, et al. Potent antibody-mediated neutralization limits bacteriophage treatment of a pulmonary *Mycobacterium abscessus* infection. Nat Med. 2021;27:1357-61.
59. Pirnay JP, Verbeken G, Ceyssens PJ, Huys I, De Vos D, Ameloot C, et al. The magistral phage. Viruses. 2018;10:64.
60. Hatfull GF. Mycobacteriophages: genes and genomes. Annu Rev Microbiol. 2010;64:331-56.
61. Jordan TC, Burnett SH, Carson S, Caruso SM, Clase K, DeJong RJ, et al. A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. mBio. 2014;5:e01051-1113.
62. Hatfull GF. Acinobacteriophages: genomics, dynamics and applications. Ann Rev Virol. 2020;7:37-61.

63. Grover N, Passkaleva EE, Mehta KK, Dordick JS, Kane RS. Growth inhibition of *Mycobacterium smegtatis* by mycobacteriophage-derived enzymes. Enzyme Microb Technol. 2014;63:1-6.
64. Li Q, Zhou M, Fan X, Yan J, Li W, Xie J. Mycobacteriophage SWU1 gp39 can potentiate multiple antibiotics against *Mycobacterium* via altering the cell permebility. Sci Rep. 2016;6:28701.
65. Opperman CJ, Wojno J, Goosen W, Warren R. Phages for the treatment of *Mycobacterium* species. Prog Mol Biol Transl Sci. 2023;201:41-92.
66. Danelishvili L, Young LS, Bermudez LE. *In vivo* efficacy of phage therapy for *Mycobacterium avium* infection as delivered by a non-virulent *Mycobacterium*. Microb Drug Resist. 2006;12:1-6.
67. Fraga AG, Trigo G, Murthy RK, Akhtar S, Hebbur M, Pacheco AR, et al. Antimicrobial activity of mycobacteriophage D29 Lysin B during *Mycobacterium ulcerans* infection. PLoS Negl Trop Dis. 2019;13:e0007113.
68. Liu KY, Yang WH, Dong XK, Cong LM, Li N, Li Y, et al. Inhalation study of mycobacteriophage D29 aerosol for mice by endotracheal route and nose-only exposure. J Aerosol Med Pulm Drug Deliv. 2016;29:393-405.
69. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K, et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nat Med. 2019;25:730-3.
70. Jeyasankar S, Kalapala YC, Sharma PR, Agarwal R. Antibacterial efficacy of mycobacteriophages against virulent *Mycobacterium tuberculosis*. BMC Microbiol. 2024;24:320.
71. Bin Yahia NM, Shan M, Zhu Y, Yang Y, Zhang S, Yang Y. From crisis to cure: harnessing the potential of mycobacteriophages in the battle against tuberculosis. J Appl Microbiol. 2024;135:lxae208.
72. Guerrero-Bustamante CA, Dedrick RM, Garlena RA, Russell DA, Hatfull GF. Toward a phage cocktail for tuberculosis: susceptibility and tuberculocidal action of mycobacteriophages against diverse *Mycobacterium tuberculosis* strains. mBio. 2021;12:e00973-21.
73. Sula L, Sulová J, Stolcpartová M. Therapy of experimental tuberculosis in guinea pigs with mycobacterial phages DS-6A, GR-21 T, My-327. Czech Med. 1981;4:209-14.
74. Yang F, Labani-Motlagh A, Moreira JD, Ansari D, Bohorquez JA, Patel S, et al. Bacteriophage therapy for the treatment of *Mycobacterium tuberculosis* infections in humanized mice. Commun Biol. 2024 Mar 9;7(1):294.
75. Davis JM, Clay H, Lewis JL, Ghori N, Herbomel P, Ramakrishnan L. Real-time visualization of *Mycobacterium*-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. Immunity. 2002;17:693-702.
76. Bernut A, Herrmann JL, Ordway D, Kremer L. The diverse cellular and animal models to decipher the physiopathological traits of *Mycobacterium abscessus* infection. Front Cell Infect Microbiol. 2017;7:100.
77. Li P. Therapeutic effect of bacteriophage D29 in the treatment for guinea pigs infected with sensitive strain of *Mycobacterium tuberculosis*. Chinese J Zoonoses. 2009;25:733-6.
78. Carrigy NB, Larsen SE, Reese V, Pecor T, Harrison M, Kuehl PJ, et al. Prophylaxis of *Mycobacterium tuberculosis* H37Rv infection in a preclinical mouse model via inhalation of nebulized bacteriophage D29. Antimicrob Agents Chemother. 2019;63:e00871-19.
79. Uyttebroek S, Chen B, Onsea J, Ruythooren F, Debaveye Y, Devolder D, et al. Safety and efficacy of phage therapy in difficult-to-treat infections: a systematic review. *Lancet Infect Dis*. 2022;22:e208-20.
80. Corbellino M, Kieffer N, Kutateladze M, Balarjishvili N, Leshkasheli L, Askilashvili L, et al. Eradication of a multidrug-resistant, carbapenemase-producing *Klebsiella pneumoniae* isolate following oral and intra-rectal therapy with a custom-made, lytic bacteriophage preparation. Clin Infect Dis. 2020;70:1998-2001.
81. Rostkowska OM, Międzybrodzki R, Miszewska-Szyszkowska D, Górski A, Durlik M. Treatment of recurrent urinary tract infections in a 60-year-old kidney transplant recipient: the use of phage therapy. Transpl Infect Dis. 2021;23:e13391.
82. Jault P, Leclere T, Jennes S, Pirnay JP, Que YA, Resch G, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase ½ trial. Lancet Infect Dis. 2018;19:35-45.
83. Patel DR, Bhartiya SK, Kumar R, Shukla VK, Nath G. Use of customized bacteriophages in the treatment of chronic nonhealing wounds: a prospective study. Int J Low Extrem Wounds. 2021;20:37-46.
84. Markoishvili K, Tsitlanadze G, Katsarava R, Morris JG Jr., Sulakvelidze A. A novel sustained-release matrix based on biodegradable poly (ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. Int J Dermatol. 2002;41:453-8.
85. Mitropolou G, Koutsokera A, Csajka C, Blanchon S, Sauty A, Brunet JF, et al. Phage therapy for pulmonary infections: lessons from clinical experiences and key considerations. Eur Respir Rev. 2022;31:220121.
86. Khosravi A, Chen Q, Echterhof A, Koff JL, Bollyky PL. Phage therapy for respiratory infections: opportunities and challenges. Lung. 2024;202:223-32.
87. Fish R, Kutter E, Bryan D, Wheat G, Kuhl S. Resolving digital Staphylococcal osteomyelitis using bacteriophage-a case report. Antibiotics (Basel). 2018;7:87.
88. Fish R, Kutter E, Wheat G, Blasdel B, Kutateladze M, Kuhl S. Bacteriophage treatment of intransigent diabetic toe ulcers: a case series. J Wound Care. 2016;25:S27-33.
89. Chan BK, Turner PE, Kim S, Mojibian HR, Elefteriades JA, Narayan D. Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*. Evol Med Public Health. 2018;2018:60-6.
90. Petrovic Fabijan A, Lin RC, Ho J, Maddocks S, Zakour NL, Iredell JR, et al. Safety of bacteriophage therapy in severe *Staphylococcus aureus* infection. Nat Microbiol. 2020;5:465-72.
91. Pirnay JP, Djebara S, Steurs G, Griselain J, Cochez C, De Soir S, et al. Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study. Nat Microbiol. 2024;9:1434-53.
92. Law N, Logan C, Yung G, Furr CL, Lehman SM, Morales S, et al. Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant *Pseudomonas aeruginosa* infection in a cystic fibrosis patient. Infection. 2019;47:665-6.
93. Gainey AB, Burch AK, Brownstein MJ, Brown DE, Fackler J, Horne BA, et al. Combining bacteriophages with cefiderocol and meropenem/vaborbactam to treat a pan-drug resistant *Achromobacter* species infection in a pediatric cystic fibrosis patient. Pediatr Pulmonol. 2020;55:2990-4.
94. Maddocks S, Fabijan AP, Ho J, Lin RC, Zakour NL, Dugan C, et al. Bacteriophage therapy of ventilator-associated pneumonia and empyema caused by *Pseudomonas aeruginosa*. Am J Respir Crit Care Med. 2019;200:1179-81.
95. Wu N, Dai J, Guo M, Li J, Zhou X, Li F, et al. Pre-optimized phage therapy on secondary *Acinetobacter baumannii* infection in four critical COVID-19 patients. Emerg Microbes Infect. 2021;10:612-8.
96. Rao S, Betancourt-Garcia M, Kare-Opaneye YO, Swierczewski BE, Bennett JW, Horne B, et al. Critically ill patient with multidrug-resistant *Acinetobacter baumannii* respiratory infection successfully treated with intravenous and nebulized bacteriophage therapy. Antimicrob Agents Chemother. 2021;66:e0082421.
97. Lebeaux D, Merabishvili M, Caudron E, Lannoy D, Simaey LV, Duyvejonck H, et al. A case of phage therapy against pandrug-resistant *Achromobacter xylosoxidans* in a 12-year-old lung-transplanted cystic fibrosis patient. Viruses. 2021;13:60.
98. Rubalskii E, Ruemke S, Salmoukas C, Boyle EC, Warnecke G, Tudorache I, et al. Bacteriophage therapy for critical infections related to cardiothoracic surgery. Antibiotics (Basel). 2020;9:232.
99. Dedrick RM, Freeman KG, Nguyen JA, Bahadirli-Talbott A, Cardin ME, Cristinziano M, et al. Nebulized bacteriophage in a patient with refractory *Mycobacterium abscessus* lung disease. Open Forum Infect Dis. 2022;9:ofac194.
100. Nick JA, Dedrick RM, Gray AL, Vladar EK, Smith BE, Freeman KG, et al. Host and pathogen response to bacteriophage engineered against *Mycobacterium abscessus* lung infection. Cell. 2022;185:1860-74.e12.
101. Little JS, Dedrick RM, Freeman KG, Cristinziano M, Smith BE, Benson CA, et al. Bacteriophage treatment of disseminated cutaneous *Mycobacterium chelonae* infection. Nat Commun. 2022;13:2313.