



## Review Article

# Phage Therapy for Hospital-Acquired Respiratory Bacterial Infections: A Review



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## ABSTRACT

Antibiotic-resistant respiratory infections necessitate alternative or adjunctive therapeutic strategies to reduce the bacterial burden in patients, particularly in the context of hospital-acquired infections. Bacteriophage therapy has emerged as a promising tool, with a resurgence of research in Western countries for various infectious diseases. The application of phage therapy against pulmonary infections has been primarily investigated for pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* in in vivo experiments and for *Mycobacterium abscessus* and *P. aeruginosa* in compassionate use cases. This review summarizes recent work on phage therapy targeting clinically relevant drug-resistant bacteria that cause nosocomial pulmonary infections, encompassing animal research, clinical cases, and clinical trials.

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## Fagoterapia para infecciones respiratorias bacterianas de adquisición nosocomial: revisión de la literatura

## RESUMEN

Las infecciones respiratorias resistentes a los antibióticos requieren opciones de tratamiento alternativas o aditivas para disminuir la carga bacteriana de los pacientes, particularmente en las infecciones adquiridas en el hospital. La terapia con bacteriófagos es una herramienta prometedora cuyas investigaciones han resurgido en los países occidentales contra diferentes tipos de infección. La terapia con fagos contra infecciones pulmonares se ha investigado principalmente para los patógenos *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* y *Acinetobacter baumannii* en experimentos *in vivo* y para *Mycobacterium abscessus* y *Pseudomonas aeruginosa* en casos de uso compasivo. Esta revisión resume el trabajo reciente de la terapia con fagos contra bacterias relevantes resistentes a los medicamentos que causan infecciones pulmonares nosocomiales, incluida la investigación con animales, casos clínicos y ensayos clínicos.

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## Introduction

Multidrug resistance (MDR) represents a significant challenge in modern medicine due to the limitations it imposes on patient management within hospital settings. Moreover, recent reports have

highlighted the impact of multidrug resistance on mortality across diverse patient populations, a problem that continues to escalate globally.<sup>1</sup>

Bacterial respiratory tract infections, especially those acquired in hospitals, are a major clinical concern affecting both immunocompromised and immunocompetent patients and causing acute and chronic conditions. Pathogens commonly involved in nosocomial lung infections include Gram-positive cocci, particularly methicillin-sensitive and methicillin-resistant *Staphylococcus*

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*aureus* (MRSA), and Gram-negative bacteria such as *Acinetobacter*, *Enterobacter*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus*, *Pseudomonas aeruginosa*, and *Serratia marcescens*. Despite often originating from aspiration, anaerobic bacteria do not play a significant role in the development of hospital-acquired pneumonia. *S. aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* are the most frequently implicated microorganisms when pneumonia develops within 4–7 days of hospitalization, whereas *P. aeruginosa*, MRSA, and Enterobacterales become more prevalent with longer hospital stays.<sup>2</sup>

Research into phage therapy has re-emerged in Western countries as a promising alternative for treating infections caused by MDR bacteria. Concurrently, its use under compassionate protocols became common in Eastern countries during the last century. The Center for Innovative Phage Applications and Therapeutics (IPATH, University of California) reported that from its inception in 2018–2020, the three main pathogens of interest for phage therapy requests were *P. aeruginosa*, *S. aureus*, and *Mycobacterium abscessus*.<sup>3</sup> Similarly, the PHAGEinLYON Clinic program, established in 2022, identified bone and joint infections (61%) and pulmonary infections (10%) as the most common targets, with *S. aureus* and *P. aeruginosa* being the most prevalent bacteria. Monoinfections constituted 73% of all cases.<sup>4</sup>

In 2024, the World Health Organization (WHO) updated its Bacterial Priority Pathogens list to guide research, development, and antimicrobial resistance strategies.<sup>5</sup> Pathogens are categorized as critical, high, and medium priority based on their resistance profiles. The critical group includes carbapenem-resistant *Acinetobacter baumannii* and third-generation cephalosporin- and carbapenem-resistant Enterobacterales. The top three prioritized pathogens are carbapenem-resistant *K. pneumoniae*, cephalosporin-resistant *E. coli*, and carbapenem-resistant *A. baumannii*. Notably, carbapenem-resistant *P. aeruginosa* has moved from the second priority in the 2017 list to the tenth in the current one.

This review focuses on recent phage therapy research (from 2020 to the present) against nosocomial respiratory bacteria of clinical relevance, reporting on investigations in vertebrate animal models, compassionate use cases, and clinical trials.

## Summary of key in vivo findings with clinical relevance

Extensive preclinical research across various vertebrate animal models—including mice, rats, pigs, and zebrafish—provides compelling evidence for its efficacy and delineates key parameters crucial for clinical translation. Results of in vivo experiments against *A. baumannii*, *E. coli*, *K. pneumoniae*, *M. abscessus*, *P. aeruginosa*, and *S. aureus* are summarized in Table 1.

For pulmonary infections, direct administration to the lungs via inhalation or intratracheal instillation was consistently more effective than systemic (intraperitoneal/intravenous) routes. For instance, a single intratracheal dose of a phage cocktail protected 100% of mice from a lethal MDR *P. aeruginosa* infection, even when treatment was delayed by up to 6 h, whereas intraperitoneal administration alone failed.<sup>6</sup> In a porcine model of ventilator-associated pneumonia (VAP), nebulized phages achieved a significant 1.5-log reduction in pulmonary bacterial load, demonstrating the feasibility of this delivery method in a large animal model that closely mimics human clinical settings.<sup>7</sup>

Interestingly, a mathematical model was created coupling in vitro and in vivo results for pneumonia induced by *E. coli* to characterize the interaction between phage and bacteria during the infection.<sup>8</sup> This approach was inspired in the strategy used for developing new drugs considering pharmacokinetic and pharmacodynamic (PK/PD) data. After intravenous administration of

phages in non-infected mice, phages reached the peak concentration in lungs 4 h post-phage administration meanwhile, at the same time, the concentration of phages from via intratracheal was almost 100-fold higher. In addition, during the 48 h post-phage administration, the pulmonary phage elimination rate was slower after the intratracheal route than in the intravenous route with estimated half-life values of 12 h and 3 h, respectively. In turn, concentrations of phages were 100–1000-fold higher in infected mice than in uninfected mice, demonstrating in vivo replication. Successful phage therapy is not solely dependent on phage lytic activity but requires a synergistic interaction with the host's innate immune system, particularly neutrophils and macrophages. In neutropenic or macrophage-depleted models, phage therapy often failed to clear the infection. Phages can also enhance the bactericidal activity of neutrophils.<sup>9</sup> The mathematical model quantified this synergy, identifying an immunity threshold below which phages are essential to reduce bacterial load to a level manageable by the host's defenses.<sup>8</sup>

The combination of phages with some antibiotics often produces a synergistic or additive effect, proving more effective than either treatment alone. For instance, an inhalable co-spray-dried powder of phage PEV20 and ciprofloxacin achieved a 5.9-log<sub>10</sub> reduction in pulmonary *P. aeruginosa* load in mice, whereas monotherapy with either agent was ineffective.<sup>10</sup> Similarly, a triple combination of phage, the adjuvant CaEDTA, and ceftazidime/avibactam completely cleared chronic *P. aeruginosa* lung infections in mice, achieving 100% survival.<sup>11</sup>

A primary clinical concern is the potential for an inflammatory “cytokine storm” from the rapid lysis of Gram-negative bacteria. However, multiple studies confirm that phage therapy does not cause overstimulation of the inflammatory response compared to antibiotic treatment. Phage therapy demonstrated a dose-dependent effect on suppressing pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ), with higher doses being more effective.<sup>12</sup> Highly purified, low-endotoxin phage preparations were well-tolerated with no significant adverse effects.

The development of phage-resistant bacteria in vivo is a recognized challenge, with the proportion of resistant isolates increasing with higher phage treatment doses. However, the acquisition of phage resistance often comes at a fitness cost to the bacterium. This “evolutionary trade-off” can result in re-sensitization to antibiotics to which the pathogen was previously resistant. For instance, phage-resistant *P. aeruginosa* mutants regained susceptibility to ciprofloxacin, amikacin, and tobramycin.<sup>13</sup> Resistant mutants may also exhibit reduced virulence, including impaired motility and lower production of virulence factors. This phenomenon makes phage therapy a powerful tool not just for direct killing but also for steering bacterial evolution toward a less virulent and more treatable state.

Curiously, a 4D X-ray imaging was proposed to monitor the progression of phage therapy in a cystic fibrosis (CF) mouse model.<sup>14</sup>

In conclusion, preclinical evidence strongly supports phage therapy as a safe and effective treatment for MDR respiratory infections. Its success relies on local delivery, synergy with the host immune system, and strategic combination with antibiotics. The evolutionary pressure exerted by phages can re-sensitize bacteria to conventional drugs, offering a dual therapeutic benefit. These findings provide a robust rationale for advancing phage therapy into well-designed clinical trials for patients with refractory lung infections.

## Compassionate use of phage therapy

Case reports describing phage therapy in compassionate use have been described since the last century against different

**Table 1**

Summary of in vivo phage therapy studies for bacterial lung infections.

| Bacteria                                                  | Infection model                                                                                                                | Phage therapy                                                                                                                                                                                                               | Phage or more                                              | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                            | Ref. |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <i>A. baumannii</i> (RUH 2037; MDR)                       | Acute pneumonia. C57BL/6N mice (female, 8–10 weeks old). <b>Infection:</b> $5 \times 10^8$ CFU, i.n.                           | <b>Single phage:</b> vB.AbaM.Acibel004. <b>Dose:</b> $1.25 \times 10^5$ PFU. <b>Route:</b> i.t. aerosolization. <b>Timing:</b> Single dose, 12 h post-infection.                                                            | Phage                                                      | Reduced pulmonary bacterial burden, lung permeability, and cytokine release. Faster recovery from hypothermia. Less inflammation.                                                                                                                                                                                                                                                                                                                  | 47   |
| <i>A. baumannii</i> (CGMCC 1.90331; MDR clinical isolate) | Acute pneumonia. Neutropenic Wistar rats (male, 6 weeks old). <b>Infection:</b> $2 \times 10^7$ CFU, airway drip injection     | <b>Phage cocktail:</b> vB.AbaM.P1 + vB.AbaM.DP45. <b>Dose:</b> $10^{12}$ PFU. <b>Route:</b> i.p. <b>Timing:</b> Prophylactic, 1 day before bacteria                                                                         | Phage                                                      | Prophylactic phage treatment protected 80% of rats, reduced bacterial load in lungs, decreased oxidative stress (MDA levels), and lowered serum cytokine levels (TNF- $\alpha$ , IL-1 $\beta$ , IL-6).                                                                                                                                                                                                                                             | 48   |
| <i>A. baumannii</i> (AB-5; MDR clinical isolate)          | Acute pneumonia in Wistar rats (male, 6 weeks old). <b>Infection:</b> $2 \times 10^9$ CFU, non-invasive i.t. drops.            | <b>Phage cocktail:</b> vB.AbaM.P1 + vB.AbaM.DP45. <b>Dose:</b> $2 \times 10^{10}$ PFU. <b>Route:</b> i.t. <b>Timing:</b> Single dose, 1 h post-infection.                                                                   | Phage                                                      | Improved survival rates to 90%. Bacterial load in lungs decreased by 4.59, 6.43, and 8.48 log <sub>10</sub> at 12 h, 24 h, and 48 h, respectively.                                                                                                                                                                                                                                                                                                 | 49   |
| <i>E. coli</i> (536; wild type)                           | Acute pneumonia. BALB/cJrj mice (male, 8 weeks old). <b>Infection:</b> $10^7$ CFU, i.n.                                        | <b>Single phage:</b> 536.P1. <b>Dose:</b> $\sim 10^9$ PFU. <b>Route:</b> i.n. <b>Timing:</b> Single dose, 4 h post-infection.                                                                                               | Phage (compared to ceftriaxone; i.p.)                      | Decreased bacterial load faster than antibiotics, prevented bacteremia, limited pulmonary edema, faster correction of blood cell count abnormalities.                                                                                                                                                                                                                                                                                              | 50   |
| <i>E. coli</i> (LM33; ESBL, MDR)                          | Acute pneumonia. BALB/cJrj mice (male, 8 weeks old). <b>Infection:</b> $5 \times 10^7$ CFU, i.n.                               | <b>Single phage:</b> LM33.P1. <b>Dose:</b> $\sim 5 \times 10^9$ PFU. <b>Route:</b> i.n. <b>Timing:</b> Single dose, 4 h post-infection.                                                                                     | Phage (compared to ceftioxin or imipenem-cilastatin; i.p.) | Phage therapy significantly reduced bacterial load more effectively than either antibiotic and reduced lung edema.                                                                                                                                                                                                                                                                                                                                 | 50   |
| <i>E. coli</i> (536; wild type)                           | Acute pneumonia. BALB/cJrj mice (male, 8 weeks old). <b>Infection:</b> from 7.6 to 8.2 log <sub>10</sub> CFU, i.t.             | <b>Single phage:</b> 536.P1. <b>Dose:</b> Low (6.6 log <sub>10</sub> PFU), Medium (7.6 log <sub>10</sub> PFU), High (8.6 log <sub>10</sub> PFU). <b>Route:</b> i.t. or i.v. <b>Timing:</b> Single dose, 2 h post-infection. | Phage                                                      | Phage Biodistribution: In uninfected mice, i.t. administration showed higher and more sustained phage concentrations in the lungs than i.v. via. Phage half-life in lungs was 12 h after i.t. route and 3 h after i.v. route. Phage-Bacteria Dynamics: In infected mice, phage titers in lungs reached levels 2–3 log <sub>10</sub> higher than in uninfected mice. The i.t. route faster time to peak viral load in lungs compared to i.v. route. | 8    |
| <i>K. pneumoniae</i> (ATCC 10031)                         | Lobar pneumonia. BALB/C mice (male, 6–8 weeks old). <b>Infection:</b> $1-2 \times 10^6$ CFU, i.n.                              | <b>Single phage:</b> vB.KpnM-Teh.1. <b>Dose:</b> $10^8$ or $10^9$ PFU. <b>Route:</b> i.p. <b>Timing:</b> Single dose, simultaneously with or 24 h post-infection.                                                           | Phage                                                      | Significant reduction in lung bacterial load (5–7 logs) up to 3 days post-treatment.                                                                                                                                                                                                                                                                                                                                                               | 51   |
| <i>K. pneumoniae</i> (MTCC109)                            | Pneumonia. BALB/c mice (6–8 weeks old). <b>Infection:</b> $10^9$ CFU, i.n.                                                     | <b>Single phage:</b> VTCCBPA43. <b>Dose:</b> $10^9$ PFU. <b>Route:</b> i.n. <b>Timing:</b> Single dose, 2 h post-infection.                                                                                                 | Phage                                                      | Significant reduction in lung bacterial load and lesion severity. Active phage was detected in lungs for up to 6 days.                                                                                                                                                                                                                                                                                                                             | 52   |
| <i>K. pneumoniae</i> (W-KP2; K47 serotype, MDR)           | Acute pneumonia. C57BL/6J mice (female, 18–20 g). <b>Infection:</b> $10^9$ CFU ( $2 \times$ MLD), i.n.                         | <b>Single phage:</b> vB.KpnM.P-KP2. <b>Dose:</b> $10^7$ , $10^8$ , or $10^9$ PFU. <b>Route:</b> i.n. <b>Timing:</b> Single dose, 1 h post-infection.                                                                        | Phage or gentamicin                                        | Phage therapy at $10^9$ PFU was comparable to gentamicin, increasing survival to 70%.                                                                                                                                                                                                                                                                                                                                                              | 53   |
|                                                           |                                                                                                                                | <b>Phage-antibiotic combination:</b> $10^9$ PFU + gentamicin (1.5 mg/kg), both i.n. <b>Timing:</b> Single dose, phage 1 h and gentamicin 1.5 h post-infection                                                               | Phage with gentamicin                                      | The combination rescued 100% of mice, almost eliminated bacteria in lungs after 6 days, and maintained cytokine levels comparable to healthy mice.                                                                                                                                                                                                                                                                                                 | 53   |
| <i>K. pneumoniae</i> (MTCC 432; MDR)                      | Zebrafish ( <i>Danio rerio</i> ). <b>Infection:</b> $10^3$ CFU, i.m.                                                           | <b>Single phage:</b> KpG. <b>Dose:</b> $10^6$ PFU. <b>Route:</b> i.m. <b>Timing:</b> Single dose, 2 h post-infection.                                                                                                       | Phage or streptomycin                                      | Phage treatment alone reduced bacterial load by 77.7%, and streptomycin alone by 62.8%. Phages showed no toxicity.                                                                                                                                                                                                                                                                                                                                 | 54   |
|                                                           |                                                                                                                                |                                                                                                                                                                                                                             | Phage with streptomycin                                    | Combination with streptomycin reduced bacterial load by 97.2%.                                                                                                                                                                                                                                                                                                                                                                                     | 54   |
| <i>K. pneumoniae</i> (ATCC BAA-2146; MDR)                 | Systemic infection. Zebrafish larvae ( <i>Danio rerio</i> ). <b>Infection:</b> $3.3 \times 10^6$ CFU/mL, immersion for 30 min. | <b>Single phage:</b> UPM2146. <b>Dose:</b> $10^6$ PFU/mL. <b>Route:</b> Immersion. <b>Timing:</b> Single treatment after infection period.                                                                                  | Phage                                                      | Phage treatment completely lysed the bacteria by 10 h post-treatment, preventing mortality. Phage was safe for the larvae.                                                                                                                                                                                                                                                                                                                         | 55   |
| <i>K. pneumoniae</i> (S-2007; K1-ST23, MDR)               | Acute pneumonia. BALB/c mice (female, 3–5 weeks old). <b>Infection:</b> $2 \times 10^6$ CFU, i.n.                              | <b>Single phage:</b> BUCT541. <b>Dose:</b> $2 \times 10^5$ , $2 \times 10^6$ , $2 \times 10^7$ , or $2 \times 10^8$ PFU. <b>Route:</b> i.n. <b>Timing:</b> Single dose, 6 h post-infection.                                 | Phage                                                      | A high dose ( $2 \times 10^7$ PFU, MOI=10) rescued 100% of mice, reducing lung bacterial load to $<10^4$ CFU/mL (vs. $10^9$ CFU/mL in controls) and alleviating lesions after 30 h.                                                                                                                                                                                                                                                                | 56   |

**Table 1**  
(Continued)

| Bacteria                                                                         | Infection model                                                                                                                                     | Phage therapy                                                                                                                                                                                                              | Phage or more                      | Outcome                                                                                                                                                                                                                                                                                                                                                                                  | Ref.     |
|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| <i>K. pneumoniae</i> (C6, C10 and SY1; ST383, ST11, and ST23, respectively;MDR)  | Pneumonia. C57/6J mice (male, 7 weeks old). <b>Infection:</b> $10^{11}$ CFU ( $2 \times$ MLD), i.n.                                                 | <b>Single phages or Phage cocktail:</b> pKp11, pKp383. <b>Dose:</b> $10^9$ PFU. <b>Route:</b> i.n. <b>Timing:</b> Single dose, 2 h post-infection                                                                          | Phage (single or cocktail)         | Increased survival rates to 80–100%. Reduction of bacterial loads, inflammation (cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$ ), and pathological injuries in lungs. The phage cocktail reduced more bacterial loads, inflammation, and pathogenic injuries than individual phages. No severe side effects or histopathological changes were observed in treated mice (liver or kidney). | 57       |
| <i>K. pneumoniae</i> (SCNJ1; K54 serotype, CR-hvKP).                             | Pneumonia. Immunodeficient BALB/c mice (female, 4–5 weeks old). <b>Infection:</b> $1 \times 10^6$ CFU, i.n.                                         | <b>Single phages or Phage cocktail:</b> vB.KpnA.SCNJ1-Z, vB.KpnS.SCNJ1-C, vB.KpnM.SCNJ1-Y. <b>Dose:</b> $10^8$ PFU. <b>Route:</b> i.n. <b>Timing:</b> Single dose, 2 h post-infection.                                     | Phage (single or cocktail).        | Both single phages and the cocktail rescued 70–80% of mice and significantly reduced bacterial loads in lung, liver, and spleen.                                                                                                                                                                                                                                                         | 58       |
| <i>K. pneumoniae</i> (Kp20 and Kp9H; both pandrug-resistant).                    | Respiratory infection model. Wistar rats (male, 120–150 g). <b>Infection:</b> $2 \times 10^6$ CFU, i.n.                                             | <b>Single phage:</b> NK20. <b>Dose:</b> $2.5 \times 10^6$ PFU (MOI = 1). <b>Route:</b> i.n. <b>Timing:</b> 6, 24, and 48 h post-infection.                                                                                 | Phage.                             | Despite the in vitro development of resistant mutants, i.n. phage rescued 100% of infected rats. Reduced the bacterial load in both lungs (by 6.3 logs) and blood. Histological and immunochemical analysis of lung treated-animals tissue revealed less inflammation and lower TNF- $\alpha$ and caspase-3 expression.                                                                  | 59       |
| <i>K. pneumoniae</i> (Kp20; K62 serotype, CRKP, blaOXA-48)                       | Systemic infection in Zebrafish ( <i>Danio rerio</i> ). <b>Infection:</b> $1.8\text{--}3.5 \times 10^7$ CFU (LD <sub>50</sub> ), cloacal injection. | <b>Single phage:</b> vB.kpnP.KPYAP-1. <b>Dose:</b> MOI 1 or 10. <b>Route:</b> Cloacal injection. <b>Timing:</b> Single dose, 30 min post-infection.                                                                        | Phage.                             | Phage treatment significantly improved survival rate to 91.6% at MOI = 10 and 70.6% at MOI = 1. The phage was safe administered alone.                                                                                                                                                                                                                                                   | 60       |
| <i>K. pneumoniae</i> (THR60 and THR60r (phage resistant derivative); ST11, CRKP) | Pulmonary infection in C57 mice (6–8 weeks old). <b>Infection:</b> $10^9$ CFU; i.n.                                                                 | <b>Single phages or Phage cocktail:</b> GZ7 infecting THR60, GZ9 infecting THR60r. <b>Dose:</b> $10^{10}$ (MOI = 10). <b>Route:</b> i.n. <b>Timing:</b> Single dose, 1 h post-infection.                                   | Phage (GZ7 or GZ7 + GZ9).          | GZ7 and GZ7 + GZ9 showed similar effect. Reduced bacterial burdens in lungs. Less body weight loss, reduced levels of inflammatory cytokines (TNF- $\alpha$ and IL-6), and significantly improved lung lesion conditions. Increased survival from 0% to 37.5% in treated mice with GZ7 or the cocktail.                                                                                  | 61       |
| <i>M. abscessus</i> (subsp. massiliense GD01; MDR)                               | Systemic infection in CFTR-depleted Zebrafish embryos ( <i>Danio rerio</i> ). <b>Infection:</b> 250–300 CFU, caudal vein injection.                 | <b>Single phage:</b> Muddy. <b>Dose:</b> MOI 50. <b>Route:</b> Caudal vein injection. <b>Timing:</b> After 24 h post-infection, daily injections for 5 days.                                                               | Phage.<br><br>Phage with rifabutin | Phage therapy increased survival and significantly reduced bacterial burden, cording, and abscess formation.<br>The combined treatment further increased the survival rate to 70% at 12 dpi (vs. ~40% with phage alone) and markedly reduced bacterial loads and pathological signs.                                                                                                     | 62<br>62 |
| <i>P. aeruginosa</i> (FADDI-PA001; MDR clinical isolate)                         | Neutropenic murine model. Immunosuppressed BALB/c mice (female, 6–8 weeks). <b>Infection:</b> $10^6$ CFU; i.t.                                      | <b>Phage:</b> PEV31. <b>Dose:</b> $10^9$ PFU. <b>Route:</b> I.t. <b>Timing:</b> Single dose, 2 h post-infection                                                                                                            | Phage                              | Suppressed bacterial growth, resulting in a >4-log difference in bacterial load at 26 h post-infection. Phage titer increased by almost 2-log <sub>10</sub> in the presence of bacteria.                                                                                                                                                                                                 | 13       |
| <i>P. aeruginosa</i> (FADDI-PA001; MDR)                                          | Acute pneumonia in neutropenic BALB/c mice (female, 8–10 weeks old). <b>Infection:</b> ~ $10^6$ CFU, i.t. spray.                                    | <b>Phage-antibiotic combination:</b> PEV20 + ciprofloxacin co-spray-dried powder. <b>Dose:</b> 1 mg powder ( $10^6$ PFU phage + 0.33 mg ciprofloxacin). <b>Route:</b> I.t. <b>Timing:</b> Single dose, 2 h post-infection. | Phage with ciprofloxacin.          | The combination powder synergistically reduced lung bacterial load by 5.9 log <sub>10</sub> , whereas single treatments did not. It also reduced inflammation (Mo/M $\phi$ and CD8+ T cells).                                                                                                                                                                                            | 10       |
| <i>P. aeruginosa</i> (PAO1 and W19; standard and clinical, respectively; MDR)    | Acute pneumonia in C57BL/6 mice (6–8 weeks old). <b>Infection:</b> $1\text{--}5 \times 10^6$ CFU, i.t.                                              | <b>Phages:</b> HX1 and MYY9 individually. <b>Dose:</b> Not specified. <b>Route:</b> I.t. or i.v. <b>Timing:</b> Single dose, 2 h post-infection.                                                                           | Phage                              | I.t. and i.v. routes reduced bacterial loads, although i.t. delivery was superior to i.v. in bacterial reduction.                                                                                                                                                                                                                                                                        | 63       |

**Table 1**  
(Continued)

| Bacteria                                                               | Infection model                                                                                                             | Phage therapy                                                                                                                                                                                                                              | Phage or more                                      | Outcome                                                                                                                                                                                                                                                                                                                    | Ref. |
|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <i>P. aeruginosa</i> (FRD1; mucoid clinical isolate)                   | Chronic pneumonia. C57BL/6 mice (6–8 weeks old). <b>Infection:</b> FRD1-laden agar beads ( $10^7$ CFU), i.t.                | <b>Phage:</b> MY9. <b>Dose:</b> $1 \times 10^7$ PFU (MOI 1). <b>Route:</b> Intratracheal. <b>Timing:</b> Single dose, 3 days post-infection.                                                                                               | Phage.                                             | Phage treatment significantly reduced bacterial load ( $\sim 4.7$ log units at 4 dpi) and inflammatory cytokines (TNF- $\alpha$ , IL-6). No bacteria were detected at 7 dpi (limit of detection $10^2$ CFU).                                                                                                               | 63   |
| <i>P. aeruginosa</i> (D4)                                              | Pneumonia in ICR mice (male, 6 weeks old). <b>Infection:</b> $5 \times 10^7$ CFU, i.n.                                      | <b>Single phage:</b> KPP10. <b>Dose:</b> $4 \times 10^9$ PFU (MOI = 80). <b>Route:</b> i.n. <b>Timing:</b> Single dose, 2 h or 8 h post-infection.                                                                                         | Phage.                                             | Phage therapy significantly improved survival (87% vs 13%), reduced bacterial loads in lungs and serum, and decreased serum inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$ ).                                                                                                                        | 64   |
| <i>P. aeruginosa</i> (PA01; MDR)                                       | Pneumonia. BALB/c mice (female, 8 weeks old). <b>Infection:</b> $10^6$ CFU, i.t.                                            | <b>Single phage:</b> vB.PaeP.PA01EW. <b>Dose:</b> $10^9$ PFU. <b>Route:</b> I.t. <b>Timing:</b> Single dose, 1 h post-infection.                                                                                                           | Phage.                                             | Phage therapy significantly reduced bacterial burden and alleviated lung histopathological changes (edema, inflammatory infiltration).                                                                                                                                                                                     | 65   |
| <i>P. aeruginosa</i> (UNC-D; MDR)                                      | Acute pneumonia. Immunocompromised BALB/c mice (female, 8 weeks old). <b>Infection:</b> $10^{5.5}$ CFU, i.m., i.t.          | <b>Phage cocktail:</b> PaAH2 $\Phi$ P, PsBAP5 $\Phi$ 2, Pa $\Phi$ 134. <b>Dose:</b> $2.5 \times 10^9$ PFU (IMIT) or $10^9$ PFU (i.p.). <b>Route:</b> IMIT, i.p., or combined. <b>Timing:</b> 3 h post-infection, i.p. every 8 h for 120 h. | Phage or with meropenem (if i.p. phage route).     | A single IMIT dose of phage cocktail was 100% effective, even when delayed by 6 h. IP cocktail alone was ineffective but showed an additive effect combined with meropenem or with IMIT delivery.                                                                                                                          | 6    |
| <i>P. aeruginosa</i> (PAK-Lux; Beta-lactam and tetracycline resistant) | BALB/c mouse. <b>Infection:</b> $10^7$ CFU; Orotracheal instillation                                                        | <b>Phage cocktail:</b> Mix A: PP1450, PP1777, PP1902, and mix B: PP1450, PP1777, PP1902, PP1792, PP1797. <b>Dose:</b> MOI 10 or 100. <b>Route:</b> Orotracheal instillation. <b>Timing:</b> Single dose, 2 h post-infection.               | Phage (mix A or mix B cocktails).                  | Mix A and Mix B showed similar in vitro efficacy. However, in vivo mix A rescued 83% to 100% of mice infected. In vivo mix B was nearly ineffective.                                                                                                                                                                       | 7    |
| <i>P. aeruginosa</i> (PAK-Lux; Beta-lactam and tetracycline resistant) | VAP, piglets (Large White, 8–9 weeks old). <b>Infection:</b> $1.5 \times 10^8$ CFU, bronchoscopic instillation.             | <b>Phage cocktail:</b> Mix A: PP1450, PP1777, PP1902. <b>Dose:</b> MOI 10–100. <b>Route:</b> Inhalation via nebulizer. <b>Timing:</b> Two doses, 2 and 11 h post-infection.                                                                | Phage (mix A cocktail).                            | Reduction of bacterial load in the pneumonic foci by 1.5-log. Phages were well-distributed in the lungs but not detected in serum or urine.                                                                                                                                                                                | 7    |
| <i>P. aeruginosa</i> (FADDI-PA001; MDR)                                | Acute pneumonia. Neutropenic BALB/c mice (female). <b>Infection:</b> $2 \times 10^4$ CFU, i.t.                              | <b>Single phage:</b> PEV31. <b>Dose:</b> $7.5 \times 10^4$ , $5 \times 10^6$ , or $5 \times 10^8$ PFU. <b>Route:</b> I.t. <b>Timing:</b> Single dose, 2 h post-infection.                                                                  | Phage.                                             | All doses significantly reduced pulmonary bacterial load by 1.3–1.9 log <sub>10</sub> . Higher doses suppressed inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) more effectively. Phage resistance was dose-dependent, but mutants showed increased sensitivity to ciprofloxacin.                             | 12   |
| <i>P. aeruginosa</i> (P20; host-adapted-derivative from D4)            | Chronic lung infection in immunocompromised C57BL/6 mice (female, 8 weeks old). <b>Infection:</b> $2 \times 10^7$ CFU; i.n. | <b>Triple combination:</b> Phage KPP10 + CaEDTA + CZA. <b>Dose:</b> 0.128 mg/mL CaEDTA + 0.5 $\mu$ g/mL CZA + $2 \times 10^5$ PFU/mL phage. <b>Route:</b> I.n. <b>Timing:</b> Single dose, 48- and 60-h post infection.                    | Phage with CaEDTA and CZA                          | Triple therapy completely cleared the infection and ensured 100% survival. Dual combinations were less effective. CaEDTA reduced virulence gene expression.                                                                                                                                                                | 11   |
| <i>P. aeruginosa</i> (DSM 107574)                                      | Naïve, uninfected murine model in C57BL/6J (female mice, 8–10 weeks old)                                                    | <b>Phage cocktail:</b> DSM 19872, DSM 22045. <b>Dose:</b> $5 \times 10^7$ PFU per phage. <b>Route:</b> I.p. <b>Timing:</b> Daily for 7 days                                                                                                | Phage (cocktail)                                   | Phages were detected in lungs, spleen, blood, and peritoneal cavity 6 h post-injection. Repetitive exposure to phages did not provoke obvious adverse effects or significant activation of innate/adaptive immune cells and did elicit a minimal humoral response with detectable phage-specific IgG antibodies by day 21. | 66   |
| <i>P. aeruginosa</i> (PA103; ExoU+)                                    | Acute lung pneumonia. ICR mice (male, 8–12 weeks old). <b>Infection:</b> $1.0 \times 10^6$ CFU, i.t.                        | <b>Single phage:</b> $\Phi$ R18. <b>Dose:</b> $4.0 \times 10^7$ PFU. <b>Route:</b> I.t. <b>Timing:</b> Single dose, 5 min post-infection.                                                                                                  | Phage alone or with anti-PcrV antibody (1 $\mu$ g) | <b>Survival:</b> 7.1% in the saline group, 26.7% in the anti-PcrV group, 41.2% in the phage group, and 66.7% in the anti-PcrV + phage group. Reduced lung edema, MPO activity, and inflammatory cytokines.                                                                                                                 | 67   |
| <i>P. aeruginosa</i> (PACL; clinical isolate)                          | Pneumonia in C57BL/6 mice (male, 12 weeks old). <b>Infection:</b> $2 \times 10^7$ CFU, i.t.                                 | <b>Single phage:</b> vB.PaeM-AL. <b>Dose:</b> $2 \times 10^8$ PFU. <b>Route:</b> I.t. or i.v. <b>Timing:</b> Single dose, 2 h post-infection.                                                                                              | Phage                                              | Both i.t. and i.v. administration attenuated pneumonia, improving survival and reducing bacterial loads and inflammation. I.v. and i.t. routes showed similar phage abundance in BALF.                                                                                                                                     | 9    |

**Table 1**  
(Continued)

| Bacteria                                        | Infection model                                                                                         | Phage therapy                                                                                                                                                                                                           | Phage or more                          | Outcome                                                                                                                                                                                                                                                                                                            | Ref. |
|-------------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <i>S. aureus</i> (A MRSA clinical isolate AW7). | Ventilator-associated pneumonia. Wistar rats. <b>Infection:</b> $\sim 8 \times 10^9$ CFU, endotracheal. | <b>Phage cocktail:</b> K, 3A, 2002, and 2003. <b>Dose:</b> $3 \times 10^{10}$ PFU or $3 \times 10^{11}$ PFU. <b>Route:</b> nebulized/aerosolized. <b>Timing:</b> Single dose, 6 h before infection).                    | Phage (prophylaxis)                    | Prophylactic aerophages improved survival from 0% in controls to 60–70%. Surviving animals had significantly fewer bacteria in their lungs ( $1.6 \times 10^6$ CFU/g) compared to untreated controls that succumbed to pneumonia ( $8.0 \times 10^8$ CFU/g).                                                       | 68   |
| <i>S. aureus</i> . (MSSA strain Xen29).         | Acute pneumonia. BALB/c mice. <b>Infection:</b> $3.0 \times 10^8$ CFU, i.n.                             | <b>Phage cocktail AB-SA01</b> (Sa83, Sa87, J-Sa36). <b>Dose:</b> $5 \times 10^8$ PFU per phage. <b>Route:</b> i.n. <b>Timing:</b> 2 and 6 h post-infection.                                                             | Phage                                  | AB-SA01 was as effective as vancomycin in reducing lung bacterial burdens. The mean reduction in bacterial load was $1.64 \log_{10}$ CFU for AB-SA01 and $1.80 \log_{10}$ CFU for vancomycin, compared to untreated controls. No phage-resistant colonies were observed                                            | 69   |
| <i>S. aureus</i> (MRSA UNT144-3).               | Acute pneumonia. Neutropenic: CD-1 mice. <b>Infection:</b> $9.5 \times 10^6$ CFU, i.n.                  | <b>Phage cocktail AB-SA01</b> (Sa83, Sa87, J-Sa36, J-Sa37). <b>Dose:</b> $10^9$ , $10^8$ , or $10^7$ PFU. <b>Route:</b> i.n. <b>Timing:</b> 2 and 6 h post-infection.                                                   | Phage                                  | The two higher phage doses significantly reduced lung bacterial load, similar to vancomycin. The highest dose ( $4 \times 10^9$ total PFU) reduced the bacterial load by 3.09 $\log_{10}$ CFU relative to untreated mice.                                                                                          | 69   |
| <i>S. aureus</i> (MRSA clinical isolate AW7).   | VAP. Wistar rats. <b>Infection:</b> $\sim 10^{10}$ CFU, endotracheal)                                   | <b>Phage cocktail</b> (2003, 2002, 3A, and K). <b>Dose:</b> $1.5 \times 10^{10}$ PFU/mL [0.3 mL i.v. or 2 mL aerosol]; <b>Route:</b> aerosolized, i.v., or both. <b>Timing:</b> 2, 12, 24, 48, and 72 h post-infection. | Phage or combination with linezolid    | Combination of aerophages and i.v. phages was most effective, rescuing 91% of animals, better than either monotherapy (50% survival each). Combination therapy with i.v. linezolid did not improve survival (55%) compared to linezolid alone (38%), possibly because the antibiotic impaired phage amplification. | 70   |
| <i>S. aureus</i> (MDRSA from sewage, MDR).      | Haematogenous pneumonia. BALB/c mice. <b>Dose:</b> $10^8$ CFU/mL; <b>route:</b> intravenous)            | <b>Single phage. Dose:</b> $10^8$ PFU/mL. <b>Route:</b> i.v. <b>Timing:</b> Single dose 72 h post-infection.                                                                                                            | Phage or combination with clindamycin. | Phage therapy lowered the viable MDRSA count in lung homogenates compared to clindamycin, combination therapy, and untreated controls.                                                                                                                                                                             | 71   |
| <i>S. aureus</i> (MRSA clinical isolate).       | VAP. Rats.                                                                                              | <b>Phage cocktail</b> (2003, 2002, 3A, and K). <b>Dose:</b> $2-3 \times 10^9$ PFU/mL. <b>Route:</b> i.v. <b>Timing:</b> 2, 12, 24 h post-infection, then once daily for 4 days.                                         | Phage or combination with teicoplanin. | Phage therapy increased survival from 0% to 58%, comparable to teicoplanin (50% survival). Combining phages with teicoplanin did not improve survival (45%). Survival correlated with reduced bacterial loads in the lung.                                                                                         | 72   |

Abbreviations: MDR, Multidrug-resistant; ESBL, Extended-spectrum  $\beta$ -lactamase; CRKP, Carbapenem-resistant *Klebsiella pneumoniae*; CR-hvKP, Carbapenem-resistant hyper-virulent *K. pneumoniae*; CZA, Ceftazidime/avibactam; MLD, Minimum lethal dose; dpf, days post-fertilization; IMIT, intubation-mediated intratracheal; i.p., intraperitoneal; i.v., intravenous; Ref, reference; MRSA, Methicillin-resistant *S. aureus*; SSA, Methicillin-sensitive *S. aureus*; VAP, Ventilator-associated pneumonia.

infections. Phage therapy against compassionate respiratory infections are frequent against *P. aeruginosa* and *M. abscessus* infections with no recent cases against *E. coli*. Table 2 summarizes case reports of lung infections from 2020 to date.

#### A. baumannii

*A. baumannii* is a nosocomial pathogen, frequently causing pneumonia associated with mechanical ventilation and mortality rates ranging from 35% to 70%.<sup>15</sup> It poses a significant threat in intensive care units (ICUs) and, like other environmental bacteria, can develop resistance to numerous antimicrobials, sometimes leading to pandrug resistance. Tigecycline and colistin are often the last-resort treatments, but resistance to these agents has also been reported.<sup>16,17</sup> Although phages targeting *A. baumannii* have been isolated and characterized since 2010, the first compassionate use case was not reported until 2017.

Recent case reports of phage therapy for respiratory *A. baumannii* infections are scarce. Tan et al. described the case of a man with chronic obstructive pulmonary disease (COPD) and

type-2 diabetes who had recurrent lung infections.<sup>18</sup> In 2020, a carbapenem-resistant *A. baumannii* strain, resistant to all tested antibiotics except tigecycline and polymyxin E, was isolated. Due to concerns about poor lung penetration of tigecycline and the nephrotoxicity of polymyxin E in a patient with renal failure, phage therapy was proposed. The de novo isolated phage Ab.SZ3 was administered via nebulization in escalating doses (from  $5 \times 10^6$  to  $5 \times 10^{10}$  PFU) over 16 days, alongside short courses of tigecycline and polymyxin E. Phages were detected in bronchoalveolar lavage fluid (BALF) 1 h after administration, with concentrations increasing to  $10^7$  PFU/mL, suggesting in situ replication. From day seven of phage therapy onward, BALF/sputum cultures were negative for the carbapenem-resistant strain. The patient's lung function gradually improved, and no significant side effects were observed.

Wu et al. reported on four critically ill COVID-19 patients (aged 62–81) in the ICU with secondary pulmonary infections caused by carbapenem-resistant *A. baumannii* who received phage therapy after antibiotic failure. All patients were SARS-CoV-2-free at the time of treatment.<sup>19</sup> Patient 1 initially received phage  $\phi$ Ab124 via nebulization, which led to the isolation of a phage-resistant strain

**Table 2**

Summary of phage therapy cases for lung bacterial infections.

| Target bacterium     | Patient, infection                                                                                                                       | Phage therapy                                                                                                                                                                                                                                                                                                                            | Concurrent antibiotics                                                                                                                                     | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Ref.  |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| <i>A. baumannii</i>  | Male, 88 years old. COPD with hospital-acquired pneumonia.                                                                               | <b>Phage:</b> Ab.SZ3. <b>Dose:</b> Started at $5 \times 10^6$ PFU, increased to $2.5 \times 10^7$ PFU, $10^8$ PFU, $10^9$ PFU, and $5 \times 10^{10}$ PFU. <b>Via:</b> Nebulized every 12 h (except for the first 2 doses, once daily). <b>Duration:</b> 16 days.                                                                        | Tigecycline: 50 (twice daily) for 6 days before and the first 5 days of phage therapy. Polymyxin E: (twice daily) for 5 days (days 6–10 of phage therapy). | <b>Bacterial isolation:</b> Phage-resistant bacteria isolated on days 2 and 3 of therapy. CRAB was not detected in BALF culture on day 7 for the first time. All subsequent sputum/BALF cultures were negative for CRAB, except for one positive culture on day 15, which remained susceptible to the phage. <b>Clinical:</b> Clearance of the pathogen and clinical improvement in lung function. No significant side effects were noted. <b>Survival:</b> The patient was alive and stable at the time of publication.                                                                                                                                                                                | 18    |
| <i>A. baumannii</i>  | Four male patients, ages 62–81 years. Critical COVID-19 with secondary pulmonary infections.                                             | <b>Phage:</b> Optimized phage cocktail ( $\phi$ Ab124 and $\phi$ Ab121). <b>Dose:</b> $10^9$ PFU total ( $10^8$ PFU/mL each phage in 10 mL saline/dose). <b>Via:</b> 2 doses with a 1-h interval. Via nebulization for lung infections and via wet compress for a topical wound infection (Patient 2). <b>Duration:</b> One day for all. | All patients were on antibiotic treatment: Cefoperazone-sulbactam, tigecycline, levofloxacin, meropenem, and imipenem were used, depending on the patient. | <b>Bacterial isolation:</b> Reduced CRAB burden in all patients. Phage resistance emerged in 4 out of 6 patients. In Patient 3, CRAB was eliminated, but a subsequent <i>K. pneumoniae</i> infection occurred. For Patient 2, the wound infection resolved with no CRAB detection. <b>Adverse Events:</b> Patient 1 had fever and cytokine storm (high IL-6 and IL-8) 4 h after phage delivery, resolved the next day. <b>Clinical/Survival:</b> Patients 1 and 2 were discharged from the hospital. Patient 4 was discharged from the ICU but died a month later from respiratory failure. Patient 3 died 10 days after phage therapy due to respiratory failure from CRKP infection.                  | 19    |
| <i>K. pneumoniae</i> | Male, 40 years old. Lung infection after heart transplantation (immune-suppression).                                                     | <b>Phage:</b> Cocktail (KPV811 and KPV15). <b>Dose:</b> $1 \times 10^8$ PFU/mL. <b>Via:</b> 2 mL via inhalation and 18 mL via nasogastric tube, once daily for 2 days, then twice daily for 2 days. <b>Duration:</b> 4 days.                                                                                                             | Ceftazidime, linezolid, avibactam (twice/day), colistin, meropenem (3 times/day), cotrimoxazole (once/day), tobramycin.                                    | <b>Bacterial isolation:</b> <i>K. pneumoniae</i> was not detected in bronchial lavage after therapy. However, an antibiotic-susceptible strain of <i>K. pneumoniae</i> was later found in stool samples, suggesting phage-induced resensitization to antibiotics. <b>Clinical:</b> No side effects were observed. <b>Survival:</b> The patient was alive at the time of publication.                                                                                                                                                                                                                                                                                                                    | 23    |
| <i>K. pneumoniae</i> | Male, 54 years old. Hospital-acquired pulmonary infection following a car accident.                                                      | <b>Phage:</b> First FKp.GWPB35; then cocktail FKp.GWPB35 + FKp.GWPA139. <b>Dose:</b> $>1 \times 10^9$ PFU/mL, 5 mL/dose. <b>Via:</b> Nebulized, 3 doses/course. <b>Duration:</b> Two therapies, 14 days apart.                                                                                                                           | Antibiotic treatment continued throughout phage therapy.                                                                                                   | <b>Bacterial isolation:</b> Phage-resistant <i>K. pneumoniae</i> emerged after the second phage treatment with significantly reduced virulence in mouse and zebrafish infection models. <b>Clinical:</b> Symptoms of pulmonary infection improved significantly after both courses of therapy.                                                                                                                                                                                                                                                                                                                                                                                                          | 24    |
| <i>M. abscessus</i>  | Male, 81 years old. Non-CF bronchiectasis and refractory pulmonary infection with <i>M. abscessus</i> subsp. <i>massiliense</i> (rough). | <b>Phage:</b> Cocktail (Muddy, BPs $\Delta$ , ZoeJ $\Delta$ ). <b>Dose:</b> $1 \times 10^9$ PFU/phage. <b>Via:</b> i.v., twice daily for 6 months, then switched to nebulized, twice daily for 12 months. <b>Duration:</b> 18 months total.                                                                                              | Multidrug: Imipenem, omadacycline, clofazimine, amikacin, and bedaquiline at various times.                                                                | <b>Bacterial isolation:</b> Bacterial load decreased 10-fold after 1 month of i.v. therapy but rebounded. During nebulized therapy, a transient reduction in bacterial load was also observed. The bacterium remained sensitive to phages Muddy and BPs $\Delta$ throughout; intermittent resistance to ZoeJ $\Delta$ was noted. <b>Anti-phage ab:</b> A potent neutralizing IgG and IgM antibody response developed after 2 months of i.v. therapy, correlating with treatment failure. During nebulized therapy, a weak sputum IgA response was noted, with only mild neutralization. <b>Clinical:</b> Transient improvements were seen with both i.v. and nebulized therapy, but were not sustained. | 38,39 |

**Table 2**  
(Continued)

| Target bacterium                      | Patient, infection                                                                                                                                                                                                                                                                                                                    | Phage therapy                                                                                                                                                                                                                                                                                                                                                                                                                               | Concurrent antibiotics                                                                                                                                                                                      | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Ref. |
|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <i>M. abscessus</i>                   | Male, 26 years old. CF with advanced bronchiectasis and treatment-refractory pulmonary infection by <i>M. abscessus</i> subsp. <i>abscessus</i> (rough).                                                                                                                                                                              | <b>Phage:</b> Cocktail of two engineered lytic phages (BPsΔ33HTH.HRM10 and D29.HRMGD40). <b>Dose:</b> ~10 <sup>9</sup> PFU of BPsΔ and 10 <sup>8</sup> PFU of D29.HRMGD40. <b>Via:</b> i.v., twice daily. <b>Duration:</b> Ongoing for over 500 days.                                                                                                                                                                                       | Continuous 4–5 drug antibiotic regimen. Concurrently treated for <i>P. aeruginosa</i> and MRSA.                                                                                                             | <b>Bacterial isolation:</b> After 118 days of phage therapy, sputum cultures became predominantly negative for <i>M. abscessus</i> . Explanted lung tissue post-transplant was culture-negative for <i>M. abscessus</i> . Post-phage isolates were susceptible to both phages. <b>Anti-phage ab:</b> The patient had pre-existing IgG to both phages but without neutralizing activity. Significant neutralizing antibodies against BPsΔ developed after 242 days, but only mild neutralization against D29. <b>Clinical:</b> Marked improvement ended in lung transplant on day 379. Urine LAM levels (marker of mycobacterial lysis) peaked at day 47 and declined, consistent with bacterial clearance. Overall: Favorable or partial responses were observed in 9 of the 17 pulmonary infection patients.                                                                                                                                                                                                                                                                                                                                                                                                                                 | 41   |
| <i>M. abscessus</i> / <i>M. avium</i> | 17 patients with pulmonary infections: Ages were pediatric to adult. CF (14 patients), scleroderma (1 patient), and chronic bronchiectasis (2 patients). Pathogens: 16 patients had <i>M. abscessus</i> (12 subsp. <i>abscessus</i> , 4 subsp. <i>massiliense</i> ); 1 had <i>M. avium</i> . Some mixed rough and smooth morphotypes. | <b>Phage:</b> Single phages (8 patients) or cocktails of 2–4 phages (9 patients). <b>Dose:</b> Most 10 <sup>9</sup> PFU/dose. One increased to 10 <sup>10</sup> PFU/dose. <b>Via:</b> i.v., nebulized, or both. Some patients switched from i.v. to aerosolized delivery due to lack of efficacy or neutralizing antibodies. One also received bronchoscopic administration. <b>Duration:</b> Varied widely from 10 days to over 3.5 years. | All patients received concomitant antimycobacterial treatment with at least 2 drugs based on prior drug susceptibility testing and tolerability. Antibiotic regimens were adjusted by clinicians as needed. | <b>Clinical/Microbiological:</b> Outcomes for the 9 responders included: culture conversion to negative; improved FEV1; resolution of disseminated infection aspects; improved clinical signs/symptoms; and successful bridging to lung transplant. In 4 patients, the response was partial or difficult to assess due to complications from other infections. Inconclusive outcomes were noted in 4 patients, and 4 patients had no evident clinical improvement. Safety: Phage administration was well-tolerated by all patients via all routes, with no serious adverse reactions attributed to the phage itself. Phage Resistance: No phage resistance was observed in any of the 6 patients treated with a single phage from whom isolates were recovered post-treatment. Resistance was only seen against 1 of 3 phages in a cocktail used for two patients. <b>Anti-phage ab:</b> Serum neutralizing antibodies were detected in 8 patients after i.v., administration. This correlated with a lack of response in 3 patients but was not consistently associated with unfavorable outcomes in others. Nebulized delivery was sometimes used to overcome serum neutralization, but a weak sputum IgA response could still be detected. | 40   |
| <i>M. abscessus</i>                   | Male, 60 s. Bilateral lung transplant recipient with sternal osteomyelitis and soft tissue infection (rough and smooth).                                                                                                                                                                                                              | <b>Phage:</b> Single phage Muddy, then cocktail (Muddy+ BPsΔ33HTH.HRM10pMC09). <b>Dose:</b> 10 <sup>9</sup> PFU. <b>Via:</b> i.v., twice daily. <b>Duration:</b> Muddy ~340 days; cocktail ~50 days. Total duration ~400 days.                                                                                                                                                                                                              | After phage therapy failed, dual beta-lactam therapy (meropenem and ceftazidime-avibactam).                                                                                                                 | <b>Bacterial isolation:</b> ~4 months after phage therapy, only the smooth colony morphotype (phage-resistant) was isolated. <b>Anti-phage ab:</b> Little to no serum neutralization of phage Muddy was detected after 255 days, likely due to immunosuppression. <b>Clinical:</b> Phage therapy eliminated the rough strain but not the infection. Subsequent dual beta-lactam therapy led to clinical and radiographic improvement. In vitro, phages showed synergy with meropenem against the smooth strain.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 42   |

**Table 2**  
(Continued)

| Target bacterium                                                 | Patient, infection                                                                                        | Phage therapy                                                                                                                                                                                                                                                                                               | Concurrent antibiotics                                                                                                         | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Ref. |
|------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Mix: <i>E. faecium</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> | Male, 52 years old. Prosthetic infection complicated by pleural empyema and purulent bronchial infection. | <b>Phage:</b> Cocktail of <i>S.</i> phage CH1, <i>E.</i> phage Enf1, and <i>P.</i> PA5 and PA10. <b>Dose:</b> $1 \times 10^8$ PFU/mL. <b>Via:</b> 25 mL local and 50 mL oral dose on day 1. Then, 25 mL local on day 3. <b>Duration:</b> 3 days.                                                            | Cefepime (once daily), daptomycin (once daily), linezolid (once daily), and tobramycin.                                        | <b>Bacterial isolation:</b> <i>S. aureus</i> , <i>E. faecium</i> , and <i>P. aeruginosa</i> were not detected for 16 days after the second phage application. <b>Clinical:</b> No severe adverse side effects observed. <b>Survival:</b> The patient died 2 months after phage therapy due to a new infection caused by <i>E. coli</i> and a different strain of <i>P. aeruginosa</i> .                                                                                                                                                                                                                                                                                                                                                                                         | 23   |
| Mix: <i>S. aureus</i> / <i>P. aeruginosa</i>                     | Male, 17 years old. CF with chronic lung infection.                                                       | <b>Phage:</b> Two sequential monophage treatments. First, <i>S. aureus</i> phage Mallokai. Second, <i>P. aeruginosa</i> phage LPS-5. <b>Dose:</b> Mallokai: $2 \times 10^9$ PFU/dose. LPS-5: $10^{10}$ PFU/dose. <b>Administration:</b> Nebulized daily. <b>Duration:</b> 10 days for each treatment.       | <i>S. aureus</i> treatment: Cotrimoxazole then linezolid. <i>P. aeruginosa</i> treatment: Alternating colistin and tobramycin. | <b>Bacterial isolation:</b> <i>S. aureus</i> treatment: >5 log reduction in <i>S. aureus</i> with no growth detected 2 days post-therapy, but it returned to pre-treatment levels by day 51. <i>P. aeruginosa</i> load increased during this time. No phage resistance observed. <i>P. aeruginosa</i> treatment: No significant reduction in bacterial load was observed. No phage resistance observed. <b>Anti-phage ab:</b> Mallokai: Neutralizing antibodies detected in serum from day 10, with >3 log reduction in phage titer by day 51. LPS-5: Low-level neutralizing activity detected from day 17. <b>Clinical:</b> No improvement was reported for either treatment. Phages were detected in sputum up to 3 days (Mallokai) and 8 days (LPS-5) after treatment ended. | 36   |
| <i>P. aeruginosa</i>                                             | Male, 43 years old. CF with chronic lung infection.                                                       | <b>Phage:</b> Pyo and Intesti preparations. Later 2 custom phages sequentially. <b>Dose and via:</b> Pyo/Intesti: 8 mL orally, 2 mL nebulized, daily. Custom phages: ( $\sim 1 \times 10^7$ PFU/mL) nebulized daily. <b>Duration:</b> 2017–2021.                                                            | Antibiotics only once in 2019 during phage shortage.                                                                           | <b>Bacterial isolation:</b> <i>P. aeruginosa</i> persisted in all sputum samples, but bacterial load was reduced 10 to 100-fold after custom phage therapy. Phage resistance to Pyo/Intesti and the first custom phage emerged but sensitivity to Pyo/Intesti later returned. PFGE analysis showed population diversification, with different genetic clusters dominating over time. <b>Clinical:</b> Patient was able to completely replace antibiotics with phages.                                                                                                                                                                                                                                                                                                           | 26   |
| <i>P. aeruginosa</i>                                             | Female, 64 years old. Primary ciliary dyskinesia and bronchiectasis with lung infection.                  | <b>Phage:</b> 5 sequential custom phages. <b>Dose:</b> $4-6 \times 10^6$ PFU/mL. <b>Via:</b> Orally, twice daily for 20-day courses. <b>Duration:</b> 2018 – 2021.                                                                                                                                          | Staphylococcal bacteriophage for co-infection. No other antibiotics.                                                           | <b>Bacterial isolation:</b> <i>P. aeruginosa</i> persisted through most of the therapy, with resistance to custom phages developing and sometimes disappearing. PFGE showed the bacterial population remained genetically uniform. At recent analysis, <i>P. aeruginosa</i> was not detected. <b>Clinical:</b> The patient successfully replaced antibiotics with phages.                                                                                                                                                                                                                                                                                                                                                                                                       | 26   |
| <i>P. aeruginosa</i>                                             | Female, lung transplant recipient.                                                                        | <b>Phage:</b> Cocktail AB-PA01. Then, modified cocktail AB-PA01-m1 and a Navy cocktail. <b>Dose:</b> $4-5 \times 10^5$ PFU/mL of phage spiked into serum for neutralization assays. <b>Via:</b> First i.v. 14 days, inhaled days 7–28. Then: i.v. and nebulized days 53–92. <b>Duration:</b> 92 days total. | Patient was on maintenance immunosuppression (sirolimus, prednisone) and received monthly IVIG.                                | <b>Bacterial isolation:</b> Microbiological cure in the second episode. <b>Anti-phage ab:</b> Phage-specific CD4+ T cells, IgG, and neutralizing antibodies developed. Neutralizing antibodies for AB-PA01 were first observed on day 21, with titers peaking around day 63. <b>Clinical:</b> Patient weaned off ventilator after first course. Despite the immune response, treatment was successful.                                                                                                                                                                                                                                                                                                                                                                          | 29   |
| <i>P. aeruginosa</i>                                             | Male, 40 years old. Interstitial lung disease with severe, chronic pulmonary infection.                   | <b>Phage:</b> dsRNA phage phiYY. <b>Dose:</b> $10^8$ PFU/mL in 10 mL saline. <b>Via:</b> Nebulized. First two courses: twice daily with a 4-h interval for 1 day each. Third course: twice daily for 3 days. <b>Duration:</b> Three courses over several weeks.                                             | Antibiotics were stopped after the second phage course.                                                                        | <b>Bacterial isolation:</b> Transient elimination of <i>P. aeruginosa</i> after each of the first two 1-day courses, with recurrence after 1–3 days. The recurrent isolates remained susceptible to the phage but showed different antibiotic susceptibility profiles. Phage titer in sputum decreased by $\sim 10,000$ -fold within 24 h and was undetectable after 48 h. <b>Adverse Events:</b> Transient fever ( $38.7^\circ\text{C}$ ) occurred after each of the first two treatments. <b>Clinical:</b> Significant improvement in infection symptoms (cough, expectoration). Lung transplant and recovered well.                                                                                                                                                          | 28   |

**Table 2**  
(Continued)

| Target bacterium     | Patient, infection                                                                     | Phage therapy                                                                                                                                                                                                                                           | Concurrent antibiotics                                                                                                           | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Ref. |
|----------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <i>P. aeruginosa</i> | Male, 68 years old. BPF-associated empyema and pneumonia after right upper lobectomy.  | <b>Phage:</b> Cocktail (PA3 and PA18). <b>Dose:</b> First: $1.25 \times 10^{10}$ PFU/mL per phage. Then: $3 \times 10^{10}$ (PA3) and $1.5 \times 10^{11}$ (PA18) PFU/mL. <b>Via:</b> Nebulized 2/day and intrapleural 1/day. <b>Duration:</b> 24 days. | Amikacin, ceftazidime-avibactam, fosfomycin, and polymyxin concomitantly.                                                        | <b>Bacterial isolation:</b> CRPA was isolated from pleural effusion on days 0, 1, 4, and 5 of therapy. After day 7, PE cultures did not yield CRPA. Carbapenem-sensitive <i>P. aeruginosa</i> detected later was considered colonization. <b>Clinical:</b> Treatment was well-tolerated. Inflammatory markers decreased, and consolidations on chest X-rays improved. The patient was discharged with no signs of infection.                                                                                                                                                                                            | 27   |
| <i>P. aeruginosa</i> | Male, 41 years old. Kartagener syndrome with chronic, life-threatening lung infection. | <b>Phage:</b> vFB297. <b>Dose:</b> $5 \times 10^9$ PFU daily. <b>Via:</b> Aerosolized. <b>Duration:</b> Iterative treatments: an initial 7-day course, followed by a 5-day course ~15 months later, and three further doses ~1 month after that.        | i.v. antibiotics administered the first 24 days of the initial phage therapy, then switched to intermittent suppressive therapy. | <b>Bacterial isolation:</b> Phage replication was observed in vivo, with phage DNA and PFU counts increasing in sputum until day 3 of the first course. The bacterial population was clonal but phenotypically diverse (hypermutator strains), with some isolates resistant to the phage at baseline. All isolates after phage treatment remained phage-susceptible. <b>Clinical:</b> Significant clinical improvement, progressive clearance of lung consolidations on CT scans, and eventual cessation of all systemic antibiotics. No adverse events after an initial transient drop in oxygen saturation and fever. | 31   |
| <i>P. aeruginosa</i> | Female, 6 years old. CF lung infection requiring non-invasive ventilation.             | <b>Phage:</b> INF. <b>Dose:</b> $1 \times 10^{10}$ PFU in 3 mL PBS. <b>Via:</b> Inhaled twice daily. <b>Duration:</b> 7 days.                                                                                                                           | Concurrently with meropenem and ciprofloxacin.                                                                                   | <b>Bacterial isolation:</b> Sputum culture on day 4 was negative for <i>P. aeruginosa</i> . However, by day 7, it was again detected. <b>Clinical:</b> Symptomatic improvement: weaned from continuous BiPAP to high-flow nasal cannula, decreased sputum production, and improved energy level. Laboratory findings remained stable. <b>Survival:</b> Transferred for lung transplant evaluation but passed away 6 months later from respiratory failure.                                                                                                                                                              | 32   |
| <i>P. aeruginosa</i> | Female, 26 years old. CF and advanced lung disease.                                    | <b>Phage:</b> Sequential single-phage therapy: INF and pB. <b>Dose:</b> $1 \times 10^{10}$ PFU in 3 mL PBS. <b>Via:</b> INF inhaled twice/day for 2 days, then once/day for 5 days. pB inhaled/day for 7 days. <b>Duration:</b> 14 days total.          | Concurrently with i.v. meropenem.                                                                                                | <b>Bacterial isolation:</b> <i>P. aeruginosa</i> persisted. On day 8, a mucoid strain susceptible to meropenem was isolated. By day 11, both mucoid and rough strains were pan-resistant again. <b>Clinical:</b> Initial symptomatic improvement (less dyspnea, decreased cough, oxygen returned to baseline) in the first week, but symptoms returned in the second week. Experienced a pneumothorax 1 week after completing therapy, likely attributable to underlying disease. <b>Survival:</b> Received a lung transplant 5 months after phage therapy.                                                             | 32   |
| <i>P. aeruginosa</i> | Female, 12 years old. CF with chronic lung infection.                                  | <b>Phage:</b> PBPA103. <b>Via:</b> First: instilled via bronchoscopy into lung lobes. Then, 2/day nebulization. <b>Duration:</b> 7 days.                                                                                                                | Concurrently Piperacillin/Tazobactam and Tobramycin.                                                                             | <b>Bacterial isolation:</b> No <i>P. aeruginosa</i> isolated from sputum samples at days 3, 7, 14, and at 3, 6, and 9 months post-treatment. <b>Clinical:</b> FEV1% increased by 4% from her best in the past 3 years and by 12% from baseline. No adverse events were observed. A temporary rise in WBC and IL-6 was noted. <b>Survival:</b> Outcome not specified beyond 9 months.                                                                                                                                                                                                                                    | 33   |
| <i>P. aeruginosa</i> | Male, 17 years old. CF with chronic lung infection.                                    | <b>Phage:</b> PBPA103. <b>Dose:</b> Not specified. <b>Via:</b> Initial dose instilled via bronchoscopy into all lung lobes, followed by twice-daily nebulization. <b>Duration:</b> 7 days.                                                              | Concurrently Piperacillin/Tazobactam and Tobramycin.                                                                             | <b>Bacterial isolation:</b> Continued to isolate non-mucoid <i>P. aeruginosa</i> post-treatment, but the isolate demonstrated increased antibiotic sensitivity. The strain remained susceptible to the phage. <b>Clinical:</b> FEV1% increased by 5% from his best in the past 3 years and by 8% from baseline. No adverse events observed. A rise in TNF- $\alpha$ and IL-6 was noted. <b>Survival:</b> Outcome not specified.                                                                                                                                                                                         | 33   |

**Table 2**  
(Continued)

| Target bacterium     | Patient, infection                                                                               | Phage therapy                                                                                                                                                                                                                                           | Concurrent antibiotics                                                        | Outcome                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Ref. |
|----------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <i>P. aeruginosa</i> | Nine adult patients (8 female, 1 male), median age 32. CF with chronic lung infection.           | <b>Phage:</b> Single phages (n = 3) or cocktails of 2–3 phages (n = 6). <b>Dose:</b> $1 \times 10^{10}$ PFU. <b>Administration:</b> Nebulized, once or twice daily. <b>Duration:</b> 7–10 days.                                                         | All patients were concurrently on or had recently completed antibiotics.      | <b>Bacterial isolation:</b> Sputum <i>P. aeruginosa</i> decreased by a median of $10^4$ CFU/mL 5–18 days after therapy. Phage resistance emerged in post-therapy isolates. The 2 patients receiving phage OMKO1, post-therapy isolates with increased antibiotic susceptibility. For patients receiving TIVP-H6, some isolates showed reduced pyocyanin production and decreased adherence to epithelial cells. <b>Clinical:</b> Median FEV1% improved by 6% (mean 8%) at 21–35 days post-therapy. No adverse events noted; some transient low-grade fever and fatigue. No significant change in sputum microbiome diversity was observed. | 34   |
| <i>P. aeruginosa</i> | Male, 57 years old. CVID and bilateral lung transplant, with chronic bronchopulmonary infection. | <b>Phage:</b> Cocktail (PP1450, PP1792, PP1797). <b>Dose:</b> 4 mL/phage at $10^{10}$ PFU/mL in 48 mL saline. <b>Via:</b> First: inhaled (8 mL) and oral (6 mL), twice/day. Then: bronchoscopy and nasogastric tube. <b>Duration:</b> Two 7-day cycles. | Cefiderocol and colistin i.v. Also received IgM/IgA-enriched immunoglobulins. | <b>Bacterial isolation:</b> XDR <i>P. aeruginosa</i> still present after the first cycle but susceptible to phages. After the second cycle, all samples (rectal, bronchial) were sterile from day 5 until death. <b>Clinical:</b> Respiratory condition improved during the first cycle. However, the patient developed acute respiratory failure before the second cycle and ultimately died of multi-organ failure 24 months post-transplant.                                                                                                                                                                                            | 30   |
| <i>S. aureus</i>     | Male, 62 years old. Fulminant pleural empyema after LVAD implantation.                           | <b>Phage:</b> CH1. <b>Dose:</b> 20 mL of $1 \times 10^9$ PFU/mL. <b>Via:</b> Local drainage every 12 h. <b>Duration:</b> 7 days (14 doses).                                                                                                             | Daptomycin: once per day.                                                     | <b>Bacterial isolation:</b> No bacteria were detected from wound swabs after therapy. <b>Clinical:</b> The LVAD remained uninfected, confirmed by PET-CT scan 2 months later. No side effects were observed. <b>Survival:</b> Died 20 months after heart transplantation due to transplant failure.                                                                                                                                                                                                                                                                                                                                        | 23   |

i.v., intravenous.

and a cytokine storm. A subsequent cocktail of  $\phi$ Ab124 and  $\phi$ Ab121 resulted in a reduced bacterial burden. Patients 2, 3, and 4 received this cocktail, with minimal radiographic improvement in Patient 2 and no change in the others. Patients 1 and 2 were discharged from the hospital, while Patient 3 cleared the *A. baumannii* infection but succumbed to a carbapenem-resistant *K. pneumoniae* infection 10 days later. Patient 4 was discharged from the ICU but died of respiratory failure one month later.

Given that phage therapy research for *A. baumannii* is relatively nascent, further studies are needed to identify and characterize new bacteriophages. Preclinical studies are crucial for determining optimal administration routes and pharmacokinetics, while clinical trials are necessary to establish effective doses and assess the true impact on survival and bacterial clearance.

## *E. coli*

*E. coli*, a Gram-negative bacillus of the family Enterobacteriaceae, is a common inhabitant of the healthy human intestine. However, certain strains can cause nosocomial lung infections in ventilated patients, posing a high mortality risk, particularly when resistant to antibiotics such as quinolones or cotrimoxazole. Its ability to carry extended-spectrum beta-lactamase (ESBL) enzymes is a notable characteristic.<sup>20</sup> Despite there are animal models describing phage therapy against respiratory *E. coli* infections, there is no recent literature on the compassionate use of phages about this bacterium.

## *K. pneumoniae*

*K. pneumoniae*, a Gram-negative bacillus belonging to the Enterobacteriaceae family, is responsible for numerous respiratory tract infections. The intestines of hospitalized patients often serve as a reservoir for this pathogen. The increased use of carbapenems against MDR strains has driven the emergence of carbapenem resistance, limiting treatment options to colistin and tigecycline, although resistance to these last-resort antibiotics has also been described.<sup>21,22</sup>

Two recent case reports have described the use of phage therapy under compassionate use protocols for critically ill patients with respiratory infections caused by *K. pneumoniae*. The first case involved a 40-year-old patient who developed a lung infection with a pan-resistant strain following drug-induced immunosuppression after a heart transplantation in 2016. The patient received a combination of phages KPV811 and PKV15 ( $10^8$  PFU/mL) administered via inhalation (2 mL) and nasogastric tube (18 mL), alongside a regimen of ceftazidime, linezolid/avibactam, colistin, meropenem, cotrimoxazole, and tobramycin. One month later, *K. pneumoniae* was undetectable in bronchial lavage samples, and the patient survived the entire three-year follow-up period without severe adverse side effects.<sup>23</sup>

The second case involved a 54-year-old patient with a hospital-acquired pulmonary infection caused by a MDR *K. pneumoniae* strain. Phage Kp7450 was administered via nebulization for 14 days, followed by a cocktail containing phages Fkp.GWPB35 and

FKp.GWPA139. Despite concurrent antibiotic administration and two courses of phage therapy, the patient's sputum remained positive for a phage-resistant strain. In vivo analysis of this resistant isolate revealed a reduction in virulence compared to the parental strain.<sup>24</sup>

## *P. aeruginosa*

*P. aeruginosa* is a Gram-negative bacillus responsible for acute and chronic pulmonary infections, especially in immunocompromised patients. It is a frequent colonizer in individuals with CF, affecting 30% of children and 80% of adults over 25, and is a major cause of morbidity and mortality in this population.<sup>25</sup>

Two patients with lower respiratory tract infections treated with personalized phages at the Eliava Phage Therapy Center in Georgia experienced no adverse effects but did not achieve bacterial clearance.<sup>26</sup> In contrast, a 68-year-old patient with an empyema-associated broncho-pleural fistula and pneumonia achieved clearance of a carbapenem-resistant *P. aeruginosa* strain and clinical improvement after 24 days of treatment with phages PA3 and PA18 alongside conventional antibiotics.<sup>27</sup> The first-ever administration of a double-stranded RNA phage (phiYY) was reported in a 40-year-old patient with interstitial lung disease.<sup>28</sup> Nebulized phage treatment was associated with transient fever, reduced bacterial burden, and symptom improvement, allowing for the cessation of antibiotics. Although the infection recurred, a subsequent course of phage therapy relieved symptoms, and the patient successfully underwent a lung transplant six months later.

Other authors described for the first time the development of phage-specific CD4<sup>+</sup> T cells, with consequent IgG and neutralizing antibodies against phages used in the successful treatment of pneumonia caused by a MDR *P. aeruginosa* strain.<sup>29</sup> The treatment consisted in AB-PA01 phage intravenously (14 days) and inhaled (21 days) obtaining an improvement, but worsening was experienced and, consequently, the patient received AB-PA01-m1, a modified version of AB-PA01, and a cocktail of phages from the United States Navy via nebulization and intravenously. The patient also received photopheresis, monthly IVIG, and maintenance sirolimus and prednisone. The authors defined success in the first treatment episode as progress from sedation and ventilation to an ambulatory condition (without microbiological cure), and success in the subsequent steps as the absence of pneumonia and microbiological cure.

A curious report describes a 57-year-old patient with lung transplantation who developed a chronic bronchopulmonary infection caused by a XDR *P. aeruginosa* strain.<sup>30</sup> The treatment consisted of IgM/IgA-enriched immunoglobulins and bacteriophage therapy. Phages were provided by the Frech company Pherecydes Pharma and were administered via inhalation (2 doses daily) and oral ingestion (2 doses daily) for 7 days. Sterilization of the infected sites was obtained, however, the patient presented a severe obstructive pattern on the ventilator, ending to multi-organ failure and passed through 24 months post-lung transplant.

A chronic lung infection due to MDR *P. aeruginosa* was also treated in a 41-year-old patient with aerosolized phages.<sup>31</sup> No complete eradication was achieved, and sequencing of remaining bacteria showed a diverse population with spontaneous mutations, but susceptible to phages.

Phage therapy has been particularly explored in CF patients. Two patients (aged 6 and 26) with pandrug-resistant *P. aeruginosa* infections received inhaled phage therapy, which was well-tolerated and produced symptomatic improvement, but neither cleared the bacterium.<sup>32</sup> The 6-year-old patient passed away while awaiting a lung transplant, while the 26-year-old received a transplant five months later. However, a 12-year-old and 17-year-old patients treated with bronchoscopic instillation and nebulization of phages

alongside intravenous antibiotics experienced a clinical improvement. No *P. aeruginosa* isolated from sputum samples were found in the 12-year-old patient.<sup>33</sup>

A recent publication involving nine CF patients with MDR *P. aeruginosa* infections reported that nebulized, tailored phage therapy led to a decrease in sputum bacterial counts, reduced antibiotic resistance in remaining isolates, and improved lung function without adverse effects.<sup>34</sup>

These cases, while highly variable, consistently show at least some degree of clinical improvement, positioning CF as a promising indication for phage therapy against *P. aeruginosa* infections.

## *S. aureus*

*S. aureus*, a Gram-positive pathogen, is the etiological agent in 1.7% of community-acquired pneumonia cases, of which 0.7% involve methicillin-resistant strains (MRSA). However, the risk of *S. aureus* infection is significantly higher in hospital settings, partly because approximately 30% of individuals are colonized with the bacterium in their anterior nares, and nasal colonization with MRSA is on the rise.<sup>35</sup>

A case involved a 62-year-old male patient who developed fulminant pleural empyema caused by *S. aureus* following LVAD implantation. The infection did not respond to intravenous daptomycin. A single-phage preparation, *Staphylococcus* phage CH1 ( $1 \times 10^9$  PFU/mL), was used. The treatment consisted of a 20 mL local application via a drainage tube every 12 h for seven consecutive days. This regimen led to the successful eradication of *S. aureus*, with no bacteria detected in subsequent wound swabs. The patient showed no further signs of bacterial infection and died 20 months later from transplant failure, considered unrelated to the prior infection or phage therapy.<sup>23</sup>

A 17-year-old male patient with CF suffered from a chronic lung infection with a predominant *S. aureus* presence and a secondary *P. aeruginosa* infection. He received two separate, sequential phage treatments. The first course, targeting *S. aureus*, involved the daily nebulization of phage alongside courses of cotrimoxazole and linezolid. This resulted in a significant short-term reduction of the *S. aureus* bacterial load by more than 5 log CFU/mL. However, a concurrent increase in the *P. aeruginosa* count was observed during this period. Consequently, a second 10-day course of nebulized phage therapy was administered, this time targeting *P. aeruginosa* with phage LPS-5. This second treatment did not produce a significant change in the *P. aeruginosa* bacterial load, which remained relatively low (between  $10^3$  and  $10^5$  CFU/mL). Following both treatments, neutralizing anti-phage antibodies were detected in the patient's serum: 10 days after the *S. aureus* phage administration and 17 days after the *P. aeruginosa* phage administration. While the patient's overall clinical status remained stable without adverse events, the development of antibodies may have limited the long-term efficacy of the therapies.<sup>36</sup>

## *M. abscessus*

*M. abscessus* is a rapidly growing nontuberculous mycobacterium (NTM) associated with infections in immunocompromised individuals, such as those with CF. The species is divided into three subspecies (*abscessus*, *bolletii*, and *massiliense*), with the first two often being macrolide-resistant due to the *erm*(41) gene. Intrinsic and acquired resistance makes treatment exceptionally challenging, often requiring months or years of multi-antibiotic regimens that are frequently associated with side effects and high failure rates.<sup>37</sup>

Several patients have received phage therapy for *M. abscessus* infections in the last 5 years. An 81-year-old patient with bronchiectasis received an intravenous three-phage cocktail for

a *M. abscessus* subsp. *massiliense* infection. The treatment was safe and initially reduced the bacterial burden, but counts later returned to baseline levels, a failure attributed to the development of robust neutralizing IgM and IgG antibodies.<sup>38</sup> Switching to nebulized administration to bypass systemic neutralization resulted in a decrease in bacterial burden and C-reactive protein levels.<sup>39</sup>

In a larger study, lytic phages were identified for 55 out of 200 mycobacterial isolates from symptomatic patients, and 20 of these patients received phage therapy.<sup>40</sup> No adverse effects were reported, and microbiological improvement was observed in 11 patients, including complete resolution in some cases and one successful lung transplant. The presence of neutralizing antibodies was potentially linked to treatment failure in four patients. Another case involved a 26-year-old CF patient with declining lung function due to a chronic *M. abscessus* subsp. *abscessus* infection.<sup>41</sup> Intravenous administration of two phages led to predominantly negative airway cultures by day 118, and the patient successfully underwent a lung transplant on day 379 with no subsequent detection of *M. abscessus*.

Finally, has been recently reported the case of a clinical improvement of a sternal wound infection against *M. abscessus* caused by one smooth and one rough *M. abscessus* strain. Phage therapy succeeds eliminating the rough strain, but not the smooth morphotype. Interestingly, the combination of phage therapy and meropenem and ceftazidime-avibactam showed clinical improvement.<sup>42</sup>

These cases underscore the need for animal models to better understand optimal administration routes (aerosolized, intravenous, or combined) and to devise strategies for minimizing the host humoral response against phages.

### Clinical trials of phage therapy against respiratory infections

Several clinical trials investigating phage therapy for pulmonary infections are registered, primarily targeting *P. aeruginosa* in CF patients.

An early trial (NCT04636554) aimed to evaluate intravenous phage therapy for bacterial co-infections in COVID-19 patients, but no results were posted. A trial for NTM infections (NCT06262282) began recruiting in 2024, planning to test a one-year course of phage therapy plus antibiotics in about 10 CF patients.

Several trials focus on *P. aeruginosa*. A phase 1b/2a study (NCT05010577) by BiomX is testing a nebulized three-phage cocktail (BX004) in CF patients, with initial reports indicating favorable safety and efficacy. A completed phase 2 study at Yale (NCT04684641) testing the phage product YPT-01 did not meet its primary microbiological efficacy endpoint. Armata Pharmaceuticals completed a phase 1b/2a trial (NCT04596319) of an inhaled five-phage cocktail (AP-PA02) in CF patients, concluding it was well-tolerated with minimal systemic exposure. A subsequent phase 2 trial (NCT05616221) was conducted in non-CF bronchiectasis patients to define a safe and effective dose for a future Phase 3 trial. Finally, Adaptive Phage Therapeutics is conducting a phase 1b/2 trial (NCT05453578) with 72 CF patients to assess the safety and microbiological activity of an intravenous four-phage product (WRAIR-PAM-CF1).

Previous failures of clinical trials include poor trial design, reflecting a lack of standardization common in early research. Trials frequently used phage cocktails with inadequate lytic activity against the target bacterial strain, or the phages used were poorly characterized. Another critical failing was related to PK/PD: early studies often did not ensure the phage could reach the site of infection (e.g., deep tissues or bone) in sufficient concentrations to maintain a high MOI and rapidly kill the bacteria. Furthermore, antagonism with bacteriostatic antibiotics was not always stud-

ied. Fortunately, many of these considerations have been overcome with the increasing preclinical data and Phase I/II assays.

### Clinical perspectives and current limitations

Phage therapy has demonstrated a strong safety profile in animal models, case reports, and clinical trials. However, the absence of Phase III clinical trial data prevents its routine implementation in clinical practice. A promising development is the 2025 Horizon Europe “Health” call, which will fund clinical trials to test the efficacy of phage therapy for treatment-resistant infections, likely targeting chronically infected patients first. This initiative should provide the rigorous statistical analysis needed to validate phage therapy alongside other antimicrobials.

Key knowledge gaps remain, including limited evidence for polymicrobial infections and the need for standardized manufacturing protocols and clinical guidelines on dosing and administration routes. A deeper understanding of phage PK/PD to ensure the phage can reach the site of infection, and of interactions with the host immune system is essential for developing these guidelines. The impact of temperate phages within target bacteria also remains an underexplored area.

### Discussion

Notably, no phage therapy research in animal models or patients has been published for other priority lung pathogens on the 2024 WHO list, such as *Enterobacter* spp., *H. influenzae*, *Proteus* spp., or *S. pneumoniae*. In the case of *S. marcescens*, there is only a case report published analysing safety of a pulmonary infection with good results.<sup>43</sup> For *S. pneumoniae*, the prevalence of temperate phages in clinical isolates has shifted research focus toward phage-derived endolysins. Similarly, phage therapy research on other problematic CF pathogens like *Stenotrophomonas maltophilia* and *Burkholderia cepacia* is lacking.

Murine models of lung infection have most frequently targeted *P. aeruginosa* and *K. pneumoniae*, typically in acute settings with single phage doses. A major difference between these models and clinical reality is the controlled timing and dosage in experiments, whereas the bacterial burden in patients is unknown and variable. Although higher phage doses generally correlate with better outcomes, they may also drive the emergence of phage-resistant bacteria—a concern mitigated if resistance confers reduced virulence or re-sensitization to antibiotics.

Phage therapy against polymicrobial infections has been poorly investigated. Compassionate use cases have predominantly focused on monomicrobial infections caused by *M. abscessus* and *P. aeruginosa*, often using phages in combination with antibiotics. Almost the half therapies were cocktails of two phages. The development of neutralizing antibodies was not universally linked to treatment failure, and nebulized and intravenous routes were the primary modes of administration. A key factor for success in these cases was the careful selection of phages with potent in vitro lytic activity, often resulting in personalized or “tailored” therapy. In some instances, phage therapy served as a bridge to a definitive medical procedure, such as a lung transplant. For particularly challenging infections like those caused by *M. abscessus*, genetically modified phages have been used to enhance lytic activity. While only two compassionate studies have investigated the replacement of antibiotics with phages, phage therapy is typically advocated as an adjunctive agent alongside antibiotics. Exceptions to this strategy include specific, uncommon scenarios, such as documented antibiotic allergy.

In contrast to the tailored approach of compassionate use, recent clinical trials have shifted toward fixed phage cocktails. This strategy is driven by regulatory demands from agencies

like the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), which require a standardized, stable product manufactured under Good Manufacturing Practices (GMP). GMP production of phages is challenging since they are alive products. Belgium has pioneered a regulatory framework that accommodates individualized phage preparations, classifying them as “magistral preparations” made in a hospital pharmacy. Regardless of the approach, the inherent need for personalization in dose, timing, and antibiotic combinations makes it challenging to evaluate outcomes in clinical trials with high patient variability. Approximately 90 clinical trials involving bacteriophages are ongoing worldwide (including respiratory infections), with 41 studies in the United States reflecting the growing commitment to this therapeutic area.<sup>44</sup>

Given the significant role of the host immune system, future clinical trials should consider stratifying immunocompetent and immunocompromised patients to better interpret results. Treatment failures are often linked to bacterial resistance, neutralizing immune responses, or inefficient phage delivery to damaged lung tissue. For CF patients, pre-treatment with mucolytic agents has been proposed to improve outcomes by degrading mucins that can induce phage resistance.<sup>45</sup> Finally, optimizing phage-antibiotic combinations to ensure synergy and avoid antagonism is critical. Pre-screening for such interactions, which is not always performed, could prevent therapeutic failures. For instance, bacteriostatic antibiotics that inhibit protein synthesis can antagonize lytic phage replication.<sup>46</sup>

Patients with underlying structural lung diseases like CF, bronchiectasis, or COPD could be the prime candidates for phage therapy, especially when these infections complicate their clinical course, lead to frequent exacerbations, and contraindicate procedures like lung transplantation.

In conclusion, phage therapy is a promising, evolving strategy for difficult-to-treat respiratory infections. It can currently be considered for compassionate use with caution, in short- to medium-term respiratory practice mainly against *P. aeruginosa*, while awaiting the results of ongoing and future clinical trials to establish its place in modern medicine.

## Artificial intelligence involvement

The authors confirm that the following generative AI and AI-assisted technologies were used in the preparation of this manuscript, in line with the journal's guidelines: Gemini (Google) and NotebookLM (Google). These tools were specifically used for: (1) review and improvement of English language and readability, and (2) assistance with the formatting and structural layout of the tables. The authors take full responsibility for the content, integrity, and accuracy of all scientific insights, data, and conclusions presented in the tables and the manuscript, which were verified, corrected, and approved by the authors.

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## Authors' contributions

Conceptualization, J.E., M.G.-Q.; writing—original draft preparation, M.C.M.-E. A.R., J.E., M.G.-Q.; writing—review and editing M.C.M.-E. A.R., J.E., M.G.-Q.; funding acquisition, J.E., M.G.-Q. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

The authors declare no conflicts of interest.

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