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Phage therapy could be key to conquering persistent bacterial lung infections in children

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Persistent bacterial lung infections in children lead to significant morbidity and mortality due to antibiotic resistance. In this paper, we describe how phage therapy has shown remarkable efficacy in preclinical and clinical studies, demonstrating significant therapeutic benefits through various administration routes. Ongoing trials are evaluating its safety and effectiveness against different pathogens. Advancing phage therapy through systematic studies and international collaboration could provide a viable alternative to traditional antibiotics for persistent infections.

Persistent bacterial lung infections, including pneumonia, are the primary cause for hospitalization, morbidity, and mortality globally, accounting for over 40 million cases reported annually, and over 650,000 fatalities in children[1]. Persistent bacterial lung infections in children are often associated with underlying diseases including cystic fibrosis (CF), immune deficiencies, congenital pulmonary or cardiac anomalies, primary ciliary dyskinesia, and secondary lung damage from severe pneumonia, food or foreign bodies aspiration and chronic obstructive pulmonary diseases, which can hinder pathogens removal from the airways[2]. The hallmarks of persistent lung infections are intervals of stability interspersed with acute exacerbations of lung infection caused by bacterial pathogens including *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Burkholderia cenocepacia*, *Achromobacter xylosoxidans*, *Staphylococcus aureus*, and *Mycobacterium abscessus complex* respectively[3–10]. Because of the increasing selection for antibiotic resistance after prolonged exposure to broad-spectrum antibiotics, these pathogens display an important clinical issue of bacterial persistence even under antimicrobial therapy[3]. Furthermore, the microbes causing persistent lung infections are reported to develop in airways as microcolonies that aggregate into biofilms, which makes them more tolerant to antibiotics[3]. Although systemic or localized inhaled antibiotic therapy can reduce the pulmonary bacterial burden, insufficient antibiotic penetration to deeper lung areas and the bacterial capabilities to grow slowly or remain dormant inside lung epithelial and immune cells additionally contribute to the establishment of persistent lung

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infections[3,11]. Meanwhile, children with compromised immunity frequently experience persistent lung infections due to limited immune-mediated bacterial clearance which allows bacteria to persist even in the presence of antibiotic[12]. The combination of these adaptive modifications exhibited by pulmonary pathogens makes antibiotic therapy unsuccessful in overcoming persistent lung infections in children, leading to progressive, irreversible lung damage with impaired pulmonary functions[13]. This emphasizes the importance of finding a viable alternative therapy that will maximize the effectiveness of infection management in these pediatric patients. Owing to the growing issue of antibiotic resistance and limited possibilities for discovering novel antibiotics, bacteriophage therapy is increasingly reported as a promising alternative therapy for conquering persistent bacterial lung infections in children[9,14].

Bacteriophage (phage)

Bacteriophages (phages) are viruses that specifically target bacteria by binding to their unique surface receptors, injecting their genomes to replicate within the bacteria, and inducing bacterial lysis to release new virions which infect neighboring bacteria, thereby initiating their new lytic cycles[15–20]. Because these lytic phages preferentially kill their hosts while replicating, they represent tremendous potential as effective and targeted alternative therapy to antibiotics in combating bacterial infections, especially those triggered by antibiotic-resistant bacteria involved in persistent bacterial lung infections[15,16]. Meanwhile, temperate phages enter lysogenic cycles by integrating as prophages into bacterial genomes, allowing a significant proportion of bacteria to withstand phage-induced lysis[15,16]. Temperate phages can be genetically modified by deleting their repressor genes, which prevents them from entering lysogenic cycles and allows them to be used therapeutically to induce target bacterial lysis while avoiding the risks of lysogeny or transfer of virulent and antibiotic-resistant genes[15,16]. Currently, preclinical studies have been conducted to assess the safety and effectiveness of phage therapy using the clinically relevant animal models of acute and chronic lung infections, with the goal of validating the clinical applications of phage therapy as targeted therapy in overcoming bacterial lung infections.

Preclinical studies of phage therapy against Gram-negative bacteria

Phages-PAK-P1 and PAK-P3 demonstrated significant potential for treating and preventing acute multidrug-resistant (MDR) *P. aeruginosa* lung infections in mice, according to previous studies[21,22] (Table 1). The same bioluminescent *P. aeruginosa* strain was subsequently utilized in another study to develop acute murine lung infection and assessed activities of 9 distinct phages in vitro and in vivo[23] (Table 1). Although seven phages (PAK_P1, PAK_P2, PAK_P3, PAK_P4, PAK_P5, LBL3, LUZ19) demonstrated an excellent correlation between in vitro and in vivo activities for improving survival with a decrease in bacterial load signal of luminescence in treated animals, two phages (PhiKZ, CHA_P1) were insufficiently active in vivo, emphasizing the importance of isolating phages directly on its targeted host to clinically apply phage as personalized therapy for achieving its therapeutic significance in pulmonary infections[23] (Table 1). Meanwhile, intranasal phage-YH6 treatment was demonstrated to successfully decrease the bacterial load in internal organs including lungs, and rescued mice from death while treating murine hemorrhagic pneumonia triggered by MDR *P. aeruginosa*. Moreover, there were no inflammation or other pathological alterations in lungs of the mice following YH6 treatment[24] (Table 1). When treating MDR *P. aeruginosa*-associated lung infections, either immediately or 12-h post- infection, an intranasal phage-MMI-Ps1 treatment resulted in significantly longer survival in treated animals with a lower bacterial burden in their lungs and blood[25] (Table 1). Intranasal Bϕ-R656 or Bϕ-R1836 treatments improved animal survival and bacterial clearance in extensively drug-resistant (XDR) *P. aeruginosa*-induced murine pneumonia, with minimal histologic damages and no adverse consequences[26]. The phage-treated group showed higher levels of TNF-α and IL-6 compared to the negative control group, but still lower than the untreated group[26] (Table 1).

According to an earlier study, intratracheal administration of PEV31 significantly reduced bacterial load and proinflammatory cytokines in the lungs of mice having *P. aeruginosa*-associated lung infection[27] (Table 1). When mice with acute pneumonia caused by *P. aeruginosa*-PA01 were given intratracheal aerosolized phage-vB_PaeP_PA01EW, their lung tissues showed significantly lower bacterial burden, fewer inflammatory cell infiltration, and minimal pulmonary congestion as compared the control group[28] (Table 1). In acute lung infection caused by *P. aeruginosa*-FADDI-PA001, intratracheal inhalation of phage-PEV31 at three distinct dosages (7.5×10^4, 5×10^6, and 5×10^8 PFU per mouse) effectively decreased the pulmonary bacterial burden, regardless of phage dosage and triggered a dose-dependent reduction of pulmonary inflammatory cytokines[29] (Table 1). The bactericidal activities of PEV20 in a dry-powder formulation were also significantly effective when sprayed directly into the trachea of mice with MDR *P. aeruginosa*-induced lung infection, implying that pulmonary delivery of dry-powder formulation is an additional feasible therapeutic option for acute *P. aeruginosa*-associated lung infections[30] (Table 1). When the efficacy of the phage-derived lysin was further tested in the treatment of acute murine *P. aeruginosa* pneumonia, PlyPa9 given both intratracheally and intranasally resulted in higher animal survival rates (70%) than its intranasal therapy (20%), emphasizing the therapeutic significance of phage's product-lysin and its administration route for improving effectiveness in acute bacterial lung infections[31] (Table 1). In mice with *P. aeruginosa*-associated acute and chronic pneumonia, both intravenous and intratracheal administration of phages-MYY9 or HX1, resulted in a significant reduction in bacterial burden, with fewer inflammatory responses and pathological damage to the lungs[32] (Table 1). In murine chronic lung infection caused by MDR *P. aeruginosa*-LESB65 wild type and its adapted strain-NP22_2, intranasal administration of phage-PELP20 up to 7 days post-infection was reported to exhibit significant therapeutic efficacy to reduce pulmonary bacterial burden[33] (Table 1). The findings of these studies revealed the significant efficacy and safety of phage therapy, irrespective of the administration route or formulation, in treating both acute and chronic *P. aeruginosa*-associated lung infections.

Importantly, *P. aeruginosa* in the murine lung was successfully eradicated by a combination of myovirus (φNH-4) and podovirus (φMR299-2) administered intranasally, indicating the therapeutic efficacy of intranasal phage cocktail treatment for *P. aeruginosa*-associated lung infections[34] (Table 1). Intranasal phage cocktail therapy delivered simultaneously, 24 h-post infection and prophylactically (48 h prior to infection), was also observed to be efficient in eradicating *P. aeruginosa*-PAO1 in acute murine lung infection, and lowering inflammation, resulting in preventing the systemic dissemination of pathogen irrespective of when it was administered[35] (Table 1). In addition to displaying in vitro lytic activities against *P. aeruginosa* in both planktonic and biofilms, the cocktail containing six phages (PYO2, DEV, E215, E217, PAK_P1, and PAK_P4) demonstrated dose-dependent curative effects for reducing the respiratory bacterial burden in acute pneumonia, suggesting a potent synergistic effect of the combined phage cocktail in pulmonary infections[36] (Table 1). Meanwhile, intraperitoneal phage cocktail treatment for acute pulmonary infection of *P. aeruginosa* failed to protect mice from dying, despite a notable reduction in the bacterial burden in the lungs and spleen[37]. However, when a phage cocktail (PaAH2ΦP, PaBAP5Φ2, and PaΦ134) was given both intraperitoneally and intratracheally at 3 h after bacterial challenge, 100% of the mice revealed no mortality along with a significant decrease in bacterial load and pulmonary damage[37]. Remarkably, intratracheal phage cocktail monotherapy effectively prevented all mice from dying, and reduced lung bacterial burden and pathologic abnormalities even it was provided 6 h after bacterial challenge[37] (Table 1). When a phage cocktail (PP1450, PP1777, and PP1902) was administered via nebulization during mechanical ventilation to treat porcine ventilator-associated pneumonia (VAP) caused by *P. aeruginosa*, the bacterial load was rapidly and significantly decreased, and the phages were distributed uniformly throughout the treated animals' lungs[38] (Table 1). These studies suggest that inhaled phage cocktail therapy offers therapeutic benefits for both non-ventilated and ventilated patients with *P. aeruginosa*-related lung infections.

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Table 1 | Preclinical studies of phage therapy against different respiratory pathogens

Pathogen	Infection	Animal and its immune status	Treatment (No of phage: Name of phage)	Route and time of administration	Outcome parameters	Results	Reference
Phage therapy against *P. aeruginosa*-associated lung infections							
P. aeruginosa bioluminescent strain-PAK-lumi	Acute lung infection	Immunocompetent mice	1: Phage-PAK-P1	Intranasal instillation of phage at 2h after and 24 h prior to infection	- Survial - Bacterial load	Phage treatment not only was effective in saving animals from lethal infection, but also was able to prevent lung infection when given 24 h before bacterial infection	21
P. aeruginosa mucoid strain from cystic fibrosis patient - CHA strain	Acute lung infection	Immunocompetent mice	1; Phage-PAK-P3	Intranasal instillation of phage at 2 h after and at 4 days prior to infection	- Survial - Bacterial load	- A curative treatment (one single dose) administrated 2 h after the onset of the infection allowed over 95% survival. - A 4-day preventive treatment (one single dose) resulted in a 100% survival.	22
P. aeruginosa PAK strain, *P.aeruginosa* bioluminescent strain-PAK-lumi and CHA strain	Acute lung infection	Immunocompetent mice	9; Phages - PAK_P1 - PAK_P2 - PAK_P3 - PAK_P4 - PAK_P5 - LBL3 - LUZ19 - PhiKZ - CHA_P1	Intranasal instillation of phage at 2 h following infection	- Survial - Bacterial load by luminescence measurements	- 7 phages (PAK_P1, PAK_P2, PAK_P3, PAK_P4, PAK_P5, LBL3 and LUZ19) demonstrated an excellent corrrelation between in vitro and in vivo activities for improving survival with a decrease in luminescence in treated animals - 2 phages (PhiKZ and CHA_P1) were insufficiently active in vivo	23
MDR *P. aeruginosa* strain D9	Acute hemorrhagic pneumonia	Immunocompetent mice	1; Phage-YH6	A single dose of phage was administered intranasally at 2 h after the bacterial challenge	- Survival - Bacterial load - Histological damage	- Phage treatment successfully decreased the bacterial load in the lungs, blood, and spleen and rescued the treated mice from death - There were no inflammation or other pathological alterations in the lungs of the mice receiving phage treatment	24
MDR *P. aeruginosa* -PA9	Acute lung infection	Immunocompetent mice	1; Phage - MMI-Ps1	Intranasal administration of phage either immediately or 12 h afrer infection	- Survival - Bacterial load	- An intranasal phage-MMI-Ps1 treatment resulted in significantly longer survival in treated animals with a lower bacterial burden in their blood and lungs - Phage therapy was discovered to improve the efficiency of complement-mediated pathogen elimination in vitro	25
XDR *P. aeruginosa* - R656, R1836	Acute pneumonia	Immunocompromised mice	1; Phages - Bϕ-R656 (or) - Bϕ-R1836	Intranasal administration of phage at 4 h post-infection	- Survival - Bacterial load - Proinflammatory cytokine (TNF-α and IL-6)	- Intranasal Bϕ-R656 or Bϕ-R1836 treatments improved animal survival, induced bacterial clearance, with minimal histologic damages and no adverse consequences in treated animals. - The phage-treated group showed higher levels of TNF-α and IL-6 compared to the negative control group, but still lower than the untreated group.	26
MDR *P. aeruginosa*-FADDI-001	Acute lung infection	Immunocompromised mice	1; Phage-PEV31	- Intratracheal administration of phage at 2 h post-infection	- Bacterial load - Inflammatory cytokine	- Phagae therapy significantly reduced bacterial load and proinflammatory cytokines in the lungs of mice having *P. aeruginosa*-assocaited lung infection. - Phage therapy was observed to modify the bacteria's antimicrobial susceptibility profiles in vitro, making them more susceptible to tobramycin, ciprofloxacin, and amikacin but less susceptible to colistin.	27

Table 1 (continued) | Preclinical studies of phage therapy against different respiratory pathogens

Pathogen	Infection	Animal and its immune status	Treatment (No of phage: Name of phage)	Route and time of administration	Outcome parameters	Results	Reference
P. aeruginosa -PA01	Acute pneumonia	Immunocompetent mice	1, Phage -vB_PaeP_PA01EW	- Intratracheal administration of phage at 1 h post-infection	- Bacterial load - Histological damage	- In phage-traeted mice, their lung tissues showed significantly lower bacterial burden, fewer inflammatory cell infiltration, and minimal pulmonary congestion as compared the control group. - Because epithelial proliferation in alveoli and immune cell infiltration in alveolar walls and bronchi were identified despite phage therapy, combining phages with antibiotics can be used to achieve an improved clinical outcome.	28
MDR *P. aeruginosa*-FADDI-001	Acute pulmonary infection	Immunocompromised mice	1; Phage-PEV31	- Three doses of phage-PEV31 (7.5×10^4, 5×10^6, and 5×10^8 PFU per mouse) were given intratracheally at 2 h post- infection	- Bacterial load - Inflammatory cytokine (TNF-α, IL-1ß, and IL-6)	- Phage-PEV31 at three distinct dosages effectively decreased the pulmonary bacterial burden, regardless of phage dosage - Expression of pulmonar inflammtory cytokines were reduced in a dose- dependent manner	29
MDR *P. aeruginosa*-FADDI-001	Acute pulmonary infection	Immunocompromised mice	1; Phage-PEV20	Intratracheal spraying of phage as dry-powder by using a Dry powder insufflator at 2 h after infection	- Bacterial load - Histopathologic damage	Phage treatment in a dry-powder formulation showed siginificant effiveness in lowering bacterial load and lung damage	30
P. aeruginosa-PA01	Acute pneumonia	Immunocompetent mice	Phage-derived lysin-PlyPa91	- One intranasal and one intratracheal instillation of Phage lysin-PlyPa91 at 3 and 6h post- infection (or) - Intranasal instillations of Phage lysin-PlyPa91 for two times	- Survival	PlyPa9 given intratracheally and intranasally resulted in higher animal survival rates (70%) than intranasal therapy (20%)	31
- MDR P. aeruginosa- PAO1 - *P. aeruginosa*-FRD1 from cystic fibrosis patient's sputum	- Acute pneumonia - Chronic pneumonia	Immunocompetent mice	2; Phages - MYY9 - HX1	- Intravenous (IV) and intratracheal administration of phages – MYY9 and HX1 at 2 h post-infection in acute pneumonia - Intratracheal administration of MYY9 at day 3 in chronic pneumonia	- Bacterial load - Histopathologic damage - Inflammatory cytokine	- Each phage therapy reduced bacterial load in lungs and pulmonary damage of mice with acute pneumonia - Siginificant reduction of bacterial burden and inflammation with no obvious histopathologic damage was observed in lungs of phage-treated mice with chronic pneumonia	32
P. aeruginosa-LESB65 wild type and its adapted strain-NP22_2	Chronic lung infection	Immunocompetent mice	1; Phage-PELP20	Intranasal phage administration at: - 24 h and 36 h post infection (treatment 1) - at 48h and 60 h post infection (treatment 2) - 144 h (6 days)and 156h (6.5 days) post infection (treatment 3)	- Bacterial load	- Intranasal administration of phage at 24, 36, 48 or 60h post-infection produced complete clearance of bacterial in lungs - Intranasal administration of phage up to 7 days post-infection was highly effective against the chronic lung infection by completely clearing bacteria from the lungs of 70% of mice, and significantly reducing bacterial load in remaining 30% when compared with controls	33
P. aeruginosa-lux-tagged NH57388A or MR299	Acute lung infection	Immunocompetent mice	2; Cocktail of 2 phages (Combination of ϕMR299-2 and ϕNH-4)	Intranasal instillation of phage cocktail at 2 h following infection	- Bacterial load	Phage cocktail showed siginificant therapeutic efficacy in clearing the pathogen from murine lungs	34
P. aeruginosa-PAO1	Acute lung infection	Immunocompetent mice	3; Cocktail of 3 phages (Combination of 3 phages-*P. aeruginosa* 24, *P. aeruginosa* 25, and *P. aeruginosa* 7)	Intranasal instillation of phage cocktail simultaneously, 24 h post-infection and prophylactically (48 h prior to infection)	- Bacterial load - Inflammatory cells and cytokine in brochoalveolar fluid (BALF)	Phage cocktail-treated mice showed clearance of *P. aeruginosa* infection and siginificantly reduced both inflammatory cells and BALF cytokines at all conditions	35
MDR *P. aeruginosa*-PAK-lumi	Acute lung infection	Immunocompetent mice	6; Cocktail of 6 phages (Combination of PYO2, DEV, E215, E217, PAK_P1, and PAK_P4)	Intranasal administration of phage cocktail at 2 h post-infection	- Bacterial load	The phage cocktail demonstrated dose-dependent curative effects for substantially reducing the respiratory bacterial burden in acute *P. aeruginosa*-induced pneumonia	36

Table 1 (continued) | Preclinical studies of phage therapy against different respiratory pathogens

Pathogen	Infection	Animal and its immune status	Treatment (No of phage: Name of phage)	Route and time of administration	Outcome parameters	Results	Reference
MDR *P. aeruginosa*-UNC-D	Acute pulmonary infection	Immunocompromisedmice	3; Cocktail of 3 phages (Combination of PaAH2ΦP, PaBAP5Φ2, PaΦ134) monotherapy (or) Phage cocktail-Meropenem combination	Assessed therapeutic efficacy pf phage cocktail using different administration routes (intraperitoneal versus intratracheal), with/without meropenem	- Survival - Bacterial load - Histological damage	- Intraperitoneal phage cocktail failed to protect mice from death. - When phage cocktail was given both intraperitoneally and intratracheally, 100% of the mice revealed no mortality along with a significant decrease in bacterial load and pulmonary damage. - Intratracheal phage cocktail monotherapy effectively prevented all mice from dying, and reduced lung bacterial burden and pathologic abnormalities even it was provided 6h after the bacterial challenge. - Administering an intraperitoneal phage cocktail in conjunction with meropenem considerably enhanced animal survival (>50%).	37
P. aeruginosa-PAK-Lux	Acute ventilator-associated pneumonia	Immunocompetent white piglets with mechanical ventilation	3; Cocktail of 3 phages (Combination of PP1450, PP1777 and PP1902)	Inhaled phage cocktail was given 2 and 11 h after bacterial challenge	- Bacterial load	- The bacterial load was rapidly and significantly decreased after administration of nebulized phage cocktail - The phages were distributed uniformly throughout the treated animals' lungs	38
MDR *P. aeruginosa*-PAK-lumi	Acute pneumonia	- Healthy immunocompetent mice - Immunocompromised mice including lymphocyte-deficient, MyD88-deficient, and neutrophil-depleted mice	1; Phage-PAK_P1	- Intranasal therapeutic administration of phage in both types of mice at 2 h postinfection - Intranasal prophylatic administration of phage in immunocompet-ent mice at 4 days prior infection	- Survival	- Phage therapy is effcient in improving survival of immunocompetent, lymphocyte-deficient, and MyD88-deficient mice. - Phage was ineffective in neutrophil-depleted mice. - Neutrophil-phage synergy was crucial for successful curative and prophylactic efficacies of intranasal phage therapy.	39
MDR *P. aeruginosa*-FADD1-PA001	Acute pneumonia	Immunocompromised mice	1; Phage PEV20 (or) Ciprofloxacin (or) PEV20-Ciprofloxacin combination	Intratracheal administration of phage-PEV20 monotherapy or ciprofloxacin monotherapy or PEV20-Ciprofloxacin combination in spray dried powders at 2 h post-infection	- Bacterial load - Leukocyte recruitment to lungs	- Intratracheal treatment with PEV20-Ciprofloxacin powder significantly reduced the bacterial load in lungs, whereas single treatments failed to reduce the burden. - PEV20-Ciprofloxacin combination did not result in altered neutrophils levels, indicating no increase in inflammation upon administration of treatment. - Reduction of CD8+ T cells, B cells and Mo/Mφ cells with PEV20-Ciprofloxacin combination treatment correlates with reduction in bacterial load with effective bacterial clearance and more rapid resolution of immune response in lung.	41
MDR *P. aeruginosa*-D4 or P20	Chronic lung infection	Immunocompetent mice	1, Phage - KPP10 (or) - Ceftazidime/ avibactam (or) - CaEDTA (or) - KPP10 + Ceftazidime/ avibactam (or) - KPP10 + CaEDTA (or) - KPP10 + Ceftazidime/ avibactam + CaEDTA	Single intranasal inhalation of phage-KPP10, Ceftazidime/ avibactam and CaEDTA as their monotherapy and dual or triple combination therapy at 48 and 60 h post-infection	- Survival - Bacterial load	- Treatment with dual KPP10 +CaEDTA combination demonstrated a bacterial reduction in infected lungs, resulting in an 80% murine survival rate as compared to KPP10 + ceftazidime/avibactam or ceftazidime/avibactam + CaEDTA dual combinations - Triple therapy of KPP10 + ceftazidime/avibactam + CaEDTA combination completely cleared pulmonary bacterial burden and ensured 100% survival in treated animals. - Combination with CaEDTA treatment reduced the expression of several genes related to pathogenicity and virulence.	19

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Table 1 (continued) | Preclinical studies of phage therapy against different respiratory pathogens

Pathogen	Infection	Animal and its immune status	Treatment (No of phage: Name of phage)	Route and time of administration	Outcome parameters	Results	Reference
Phage therapy against *S. aureus*-associated lung infections							
- Methicillin-resistant *S. aureus* (MRSA) strain - UNT144-3 - Methicillin-sensitive *S. aureus* (MSSA) strain-Xen29	Acute pneumonia	- Immunocompromised CD1 mice - Immunocompetent mice	1; Phage AB-SA01 and its prototype product	- Intranasal administration of phage prototype product and AB-SA0, - Subcutaneous administration of antibiotics (vancomycin), at 2 and 6 h post-infection	- Bacterial load	- Both AB-SA01 phage and its prototype product were equally effective as vancomycin in reducing lung bacterial burden in acute pneumonia for both immunocompetent and neutropenic mice, despite using animals with different immunological conditions, genetic backgrounds, and *S. aureus* strains with varying methicillin susceptibility profiles.	63
MRSA strain -AW7	Ventilator associated pnemonia	Immunocompetent rat	4; Cocktail of 4 phages (Combination of 2003, 2002, 3A, and K)	IV administration of phagae cocktail, or teicoplanin, and its combination at 2, 12,24,48 and 72 h post infection	- Survival - Bacterial load	- Phage cocktail was as effective as teicoplanin in improving animal survuival and redcuing bacterial burden in lungs with improved histopathological outcomes. - The phage cocktail-teicoplanin combination did not incraese survival in trated rats.	64
MRSA strain -AW7	Ventilator associated pneumonia	Immunocompetent rat	4; Cocktail of 4 phages (Combination of 2003, 2002, 3A, and K)	- IV daptomycin and Nebulized placebo, or - Nebulized phage cocktail and IV Daptomycin at 2, 12, 24, 48, and 72 h post-infection	- Survival - Bacterial load	Combination of nebulized phage cocktail and intravenous daptomycin did not showed superior efficacy than nebulized phgae cocktail monotherapy for improving animal survival or reducing bacterial burdens in the lungs or spleen.	65
MRSA strain- AW7	Ventilator associated pnemonia	Immunocompetent rat	4; Cocktail of 4 phages (2003, 2002, 3A, and K)	- Nebulized delivery of aerosolized phage cocktail, or IV administration of phage cocktail, or combination of both at 12, 24, 48 and 72 h post-infection - IV linezolid or IV linezolid in combination with nebulized delivery of aerosolized phage cocktail at 2, 12,24,48 and 72 h post-infection	- Survival	- The combination of nebulized and IV phage cocktail significantly improved survival as compared to giving phage cocktail via intravenous or nebulized route. - The combination of IV linezolid and nebulized phage cocktail did not showed synergisim in improving survival.	66
MDR *S. aureus*	Acute pneumonia	Immunocompetent mice	1, *S. aureus*-specific phage	IV administration of phage monotherapy or clinidamycin montherapy or phage-clindamycin combination at 72 h post-infection	- Bacterial load - Histopathology in lungs	Phage monotherapy significantly reduced bacterial load and caused less pulmonary histologic damage than clinidmycin monotherapy or phage-clindanmycin combination groups.	67
S. aureus-SA27	Lung-derived septicemia	Immunocompromised mice	1, Phage-S13′	Intraperitoneal administration of phage at 6h after the intranasal inoculation of *S. aureus*	- Survival - Bacterial load - Inflammatory cytokine	Phage therapy was associated with significantly higher rates of animal survival and lower levels of inflammatory cytokines and bacterial burden in phage-treated mice compared to control mice.	68
Phage therapy against *A. baumannii*-associated lung infections							
Carbapenem-resistant *A. baumannii* (CRAB)-YMC13/01/C62	Acute pulmonary infection	Immunocompromised mice	1; Phage-Bϕ-C62	Intranasal administraion at 30 min after infection	- Survival - Bacterial load - Histological damage - Proinflammatory cytokines (TNF-α, IL-6)	Phage-treated mouse demonstrated a 100% survival, complete bacterial clearance, a decrease in pulmonary proinflammatory cytokines (TNF-α, IL-6) and improved histological damage without causing adverse effects	43
Carbapenem-resistant *A. baumannii* (CRAB)-YMC13/03/R2096	Acute pneumonia	Immunocompromised mice	1; Phage- Bϕ-R2096	Intranasal administraion at 30 min after infection	- Survival - Bacterial load - Histological damage	Phage-treated mouse demonstrated a 100% survival, complete bacterial clearance, and improved histological damage without causing adverse effects	44

Table 1 (continued) | Preclinical studies of phage therapy against different respiratory pathogens

Pathogen	Infection	Animal and its immune status	Treatment (No of phage: Name of phage)	Route and time of administration	Outcome parameters	Results	Reference
Carbapenem-resistant *A. baumannii* (CRAB)-15519	Acute pneumonia	Immunocompromised mice	1; Phage- SH-Ab15519	- Intranasal administraion at 1 or 2 h after infection	- Survival - Bacterial load - Histological damage - Proinflammatory cytokine	- Phage-treated mouse demonstrated a 100% survival, complete bacterial clearance and improved histological damage. - Mice that received early phage therapy shortly after infection survived longer than mice that underwent delayed treatment, indicating that phage therapy requires prompt intervention to be successful.	45
MDR *A. baumannii* strain- MDR-AB2	Acute pneumonia	Immunocompromised mice	1; Phage- vB_AbaM-IME-AB2	- Intranasal administraion at 1, 4 and 24 h after infection	- Survival - Bacterial load - Histological damage - Pathologic lesion under microcomputed tomography	- Intranasal delivery of phage demonstrated dose- and time-dependent therapeutic effectiveness, with the highest beneficial effects at MOI-10 given 1h after infection in preventing 100% of mice against fatal pneumonia. - Mice treated with phage revealed no inflammatory cell infiltration or pathologic abnormalities in their lungs during histology or microcomputed tomography studies.	46
MDR *A. baumannii*-RUH 2037	Acute pneumonia	Immunocompetent mice	1; Phage-vB_AbaM_Acibel004	- Intratracheal aerosolization of phage at 12 h post-infection	- Clinical signs of pneumonia - Bacterial load - Histopathologic lesion - Inflammatory cytokine	- The animals treated with phage showed a lower lung bacterial burden as a result of phage replication at the infection site, as well as milder signs of pneumonia. - The severity of pulmonary histopathologic lesions in phage-treated mice was dramatically diminished, with decreased inflammation and cytokine production, and no phage-related adverse effects.	47
MDR *A. baumannii*	Acute pulmonary infection	Immunocompromised mice	5; Cocktail of 5 phages (Combination of PBAB08, PBAB25, PBAB68, PBAB80, and PBAB93)	- Intranasal administraion at 4 h post infection and daily for 7 days - Intraperitoneal, intranasal and oral administraion of phage cocktail dialy for 7 days to investigate immune reaction against phage cocktail	- Survival - Bacterial load - Inflammatory cytokine, IgE and histamine	- Phage cocktail-treated mice showed a better survival with significant reduction of bacterial load in their lungs. - Minimal inflammatory responses against phage cocktail via different administration routes due to the absence of significant increase in serum IgE, inflammatory cytokines and histamine level in phage-treated mice.	48
Phage therapy against *K. pneumoniae*-associated lung infections							
K. pneumoniae-B5055	Acute lobar pneumonia	Immunocompetent mice	1; Phage-SS	A single intraperitoneal injection phage administered immediately after, 3 h prior to and 6 h delay of infection	- Bacterial load	Administration of phage immediately resulted in complete clearance of bacteria from lungs. - Phage given 3 h prior to infection provided significant protection of mice from infection. - Delayed treatment with phage 6h after induction of bacterial infection was ineffective for treating lobar pneumonia.	49
K. pneumoniae-KP1513	Acute pneumonia	Immunocompetent mice	1; Phage-1513	Intranasal administration at 2 h post-infection	- Survival - Bacterial load - Inflammatory cytokines	Phage therapy was efficient significantly in improving animals survival and lowering bacterial load and inflammatory cytokines with improvement of lung lesions	50
K. pneumoniae-MTCC109	Acute pneumonia	Immunocompetent mice	1; Phage-VTCCBPA43	Intranasal administration at 2 h post-infection	- Bacterial load - Histopathologic lesion in lungs - Inflammatory cytokine	Significant reduction of bacterial load and and an improvement in the severity of the pathologic lesion in the murine pneumonic lung following phage therapy	51

Table 1 (continued) | Preclinical studies of phage therapy against different respiratory pathogens

Pathogen	Infection	Animal and its immune status	Treatment (No of phage: Name of phage)	Route and time of administration	Outcome parameters	Results	Reference
K. pneumoniae-B5055	Acute lobar pneumonia	Immunocompetent mice	1; Phage- KPO1K2 as liposome-entrapped and nonliposomal phages	Therpaeutic and prophylatic intraperitoneal administration of: - Liposome-entraped phage at 24, 48, and 72 h after infection; and at 6, 24, 48, 72 h prior to infection - Nonliposomal phage at 6, 24, and 48h after infection; and 3, 6, 24 h prior to infection	- Survival - Bacterial load - Inflammatory cytokines - Antiinflammatory cytokines	- Liposome-entrapped phages showed an improved effectiveness of IP phage therapy even though the therapy was administered 72 h after and 48h before the mice developed pneumonia. - Nonliposmal phage resulted in a lower bacterial burden when given 24 h after and 6 h before infection. - It highlighted the superiority of liposomal delivery in IP phage administration for both therapeutic and prophylatic treatment of pnemonia.	52
MDR *K. pneumoniae* of ST11 and ST383	Acute pneumonia	Immunocompetent mice	2; Cocktail of 2 phages (Combination of pKp11, and pKp383)	Intranasal administration of phages as monotherapy or cocktail at 2h post-infection	- Survival - Bacterial load - Histopathologic lesion in lungs - Inflammatory cytokine (IL-1β, IL-6, and TNF-α)	A phage cocktail outperformed its monotherapy in terms of animal survival, bacterial load, inflammatory cytokines, and lung damage in early stage of murine pneumonia	53
MDR *K. pneumoniae*-LPKP	Acute pneumonia	Immunocompetent mice	Phage-derived endolysins-LysCA and LysG24	Intranasal administration of phage-derived endolysins -LysCA/LysG24 at the onset of clinical symptoms	- Bacterial load - Histopathologic lesion in lungs	Phage-derived endolysin-LysCA and LysG24 exhibited significant antibacterial activity by drastically lowering the bacterial load while leaving no pathologic lesions in the lungs of treated mice	54
Phage therapy against *E. coli*-associated lung infection							
- *E. coli* luminescent strain-536-Lux - *E. coli*-PDP302, a virulent strain of VAP	Acute pneumonia	Immunocompetent mice	- 1; Phage 536_P1 (or) Ceftriaxone - 1; Phage-536_P7 (or) Adapted phage-536_P7_PDP302	Intranasal administration of phage or intraperitoneal administration of Ceftriaxone, at 2h post infection	- Survival - Bacterial load - Inflammatory cytokine - Complete blood count	- Intranasal phage-536_P1 therapy was equivalently successful as ceftriazone in enhancing animal survival and lowering bacterial load. - Phage-536_P7 did not significantly reduce mortality in mice infected with *E. coli*-PDP302 strain, a virulent strain of VAP. - Adapting phage-536_P7_PDP302 against *E. coli*-PDP302 in vitro improved its therapeutic efficacy against *E. coli*-associated pneumonia in clinical settings, as evidenced by a significant increase in survival.	55
E. coli strains- 536 and LM33	Acute pneumonia	Immunocompetent mice	- 2; Phage - 536_P1 - LM33_P1 (or) antibiotic	Intranasal phage therapy or intraperitoneal antibiotics therapy (ceftriaxone, cefoxitin, or imipenem-cilastatin), at 4h post infection	- Bacterial load - Inflammatory cytokine - Complete blood count	- intranasal treatment with specific phages (536_P1 and LM33_P1) resulted in a greater reduction of the bacterial load, a quicker correction of abnormal blood cell counts, and lower inflammatory responses than intraperitoneal antibiotic treatments.	56
Phage therapy against *B. cenocepacia*-associated lung infections							
B. cenocepacia strain-AU0728	Acute pulmonary infection	Immunocompetent mice	1; Phage-BcepIL02	Intraperitoneal and intransal administraion at 24h after infection	- Bacterial load - Inflammatory cytokines	Systemic administration of phage intraperitoneally was more effective than localized intranasal administration in reduction of pulmoary bacterial burden and inflammatory cytokines.	57
B. cenocepacia-K56-2 or C6433	Acute pulmonary infection	Immunocompromised mice	4; Phages - KS12 - KS4-M - KS5 - DC1	Intraperitoneal and NOID-delivered nebulization of phage at 1 day, 2 days and 3 days post infection	- Bacterial load	Aerosol nebulization of phage was more effective than intraperitoneal administration for lowering bacterial load.	58

In a prior study, phage therapy was discovered to improve the efficiency of complement-mediated pathogen elimination in vitro, underscoring the significance of a functioning complement system for complete eradication of *P. aeruginosa* during phage therapy[25] (Table 1). When the effects of host immunity on phage therapy in acute *P. aeruginosa*-induced pneumonia were investigated by comparing treatment responses in healthy immunocompetent, MyD88-deficient, lymphocyte-deficient, and neutrophil-depleted mice, the neutrophil-phage synergy was crucial for providing the curative and prophylactic efficacies of intranasal phage therapy in respiratory infection[39] (Table 1).

Furthermore, phage therapy was observed to modify the bacteria's antimicrobial susceptibility profiles in vitro, making them more susceptible to tobramycin, ciprofloxacin, and amikacin, but less susceptible to colistin, which may in part be related to the mechanism of action of colistin (outer membrane) and their interaction with the phage receptors[27] (Table 1). Because epithelial proliferation in alveoli and immune cell infiltration in alveolar walls and bronchi were identified despite phage therapy, the prior study proposed combining phages with antibiotics for improving clinical outcomes[28] (Table 1). Interestingly, delivering an intraperitoneal phage cocktail in conjunction with meropenem at sub-inhibitory dosage subcutaneously improved animal survival (>50%), suggesting the potential additive outcome of a phage-antibiotic combination in treating murine pulmonary infections[37] (Table 1). Following nebulization with air-jet or vibrating mesh nebulizers, aerosolization of the PEV20-ciprofloxacin combination was observed to retain synergistic antibacterial efficacy against MDR *P. aeruginosa* isolates in vitro[40]. While mice treated with phage or ciprofloxacin monotherapy showed no reduction in bacterial burden or inflammation, intratracheal aerosolization of a dry powder formulation containing PEV20-ciprofloxacin combination dramatically decreased pulmonary bacterial load and inflammatory responses in acute lung infection[41] (Table 1). Intranasal inhalation of a triple combination therapy containing phage-KKP10, ceftazidime/avibactam, and CaEDTA was recently discovered to be significantly effective in managing chronic *P. aeruginosa* lung infections, resulting in 100% survival with complete pulmonary bacterial clearance in treated animals, and reducing bacterial pathogenicity and virulence[19] (Table 1). These studies demonstrate the significant therapeutic potential of phage-antibiotic synergy in overcoming acute and chronic *P. aeruginosa*-associated lung infections.

A prior study revealed that higher phage doses were linked to a greater development of phage resistance in a *P. aeruginosa* subpopulation recovered from the lungs of neutropenic mice with acute pulmonary infection[29]. The phage-resistant *P. aeruginosa* displayed modified bacterial pathogenicity in vitro, such as increased ciprofloxacin susceptibility, decreased twitching motility, and reduced synthesis of blue-green pigment[29]. According to a prior study, *P. aeruginosa*-PAO1 developed phage resistance in vitro associated with an increase in pyomelanin synthesis, a greater susceptibility, and a faster rate of killing by peptides-LL-37 and colistin, after being exposed to phages-AM.P2, Mat, and Kat[42]. In human blood, serum, and a mouse pneumonia model, these phage-resistant mutants with genomic deletions showed significantly lower survival than its wild type *P. aeruginosa*-PAO1, suggesting the evolutionary trade-offs from phage resistance that reduce bacterial pathogenicity and increase susceptibility to immune-mediated clearance[42]. However, the emergence of phage-resistant variants in the presence of immune signaling deficiency was documented in a prior study, highlighting the importance of complementing phage therapy to maximize its effectiveness in immunodeficient individuals with *P. aeruginosa*-associated lung infections[39].

Previous studies on mice with *A. baumannii*-induced pneumonia found that administering phages-Bϕ-C62 or Bϕ-R2096 intranasally at 30 min after infection resulted in 100% animal survival, complete bacterial clearance, a decrease in pulmonary proinflammatory cytokines, and an improvement in histological damage without causing adverse effects, revealing the therapeutic potential of phage therapy for overcoming pneumonia caused by carbapenemase-producing *A. baumannii*[43,44] (Table 1). In a prior investigation, mice with lung infections were given time-dependent phage SH-Ab15519 treatment intranasally at 1 or 2 h after carbapenem-resistant *A. baumannii* (CRAB) challenge[45]. Intranasal phage therapy effectively lowered proinflammatory cytokines and bacteria in the lungs while also protecting mice from fatal infections[45]. The promising efficacy of intranasal phage SH-Ab15519 therapy in resolving CRAB-associated lung infection was further evidenced histopathologically by reduced pulmonary congestion and inflammatory cell invasion[45]. Notably, mice that received early phage therapy shortly after infection survived longer than mice that underwent delayed treatment, indicating that phage therapy requires prompt intervention to be successful[45] (Table 1). Intranasal delivery of phage-vB_AbaM-IME-AB2 demonstrated dose- and time-dependent therapeutic effectiveness, with the highest beneficial effects at MOI-10 given 1-h after infection, in preventing 100% of mice against fatal *A. baumannii*-associated pneumonia[46]. Mice treated with phage revealed no inflammatory cell infiltration or pathologic abnormalities in their lungs during histology or microcomputed tomography studies. Collectively, intranasal vB_AbaM-IME-AB2 therapy was revealed to be a highly efficacious therapeutic option for treating pneumonia caused by MDR *A. baumannii*[46] (Table 1). The effectiveness of phage-vB_AbaM_Acibel004 against MDR *A. baumannii*-induced pneumonia in mice was assessed using intratracheal aerosolization 12-h after the bacterial challenge[47]. The animals treated with phage showed a lower lung bacterial burden as a result of phage replication at the infection site, as well as milder signs of pneumonia[47]. The severity of pulmonary histopathologic lesions in phage-treated mice was dramatically diminished, with decreased inflammation and cytokine production, and no phage-related adverse effects[47] (Table 1). Learning from investigating into the effects of intranasal phage cocktail (PBAB08, PBAB25, PBAB68, PBAB80, and PBAB93) treatment in MDR *A. baumannii*-associated acute lung infections, this phage cocktail demonstrated remarkable therapeutic efficacy for enhancing animal survival and reducing pulmonary bacterial burden[48]. Interestingly, phage cocktail administration via intraperitoneal, intranasal, and oral routes was found to be safe, with minimal inflammatory responses, as phage-treated mice showed no significant increase in serum IgE, inflammatory cytokines, or histamine levels[48] (Table 1). The findings of different studies indicate the potential benefit of utilizing phage in clinical settings for managing MDR *A. baumannii* lung infections.

A prior study discovered that an intraperitoneal phage-SS therapy administered both immediately after and 3 h before an intranasal bacterial challenge was significantly efficient in rescuing mice from *K. pneumoniae*-mediated pneumonia[49]. Significantly, the phage treatment became ineffective when it was administered 6 h after the infection was induced[49] (Table 1). In murine acute pneumonia produced by MDR *K. pneumoniae*-KP1513, mice were protected from mortality by a single intranasal dosage of phage1513[50]. Furthermore, phage-treated mice showed a reduced burden of *K. pneumoniae* in their lungs with an improvement in lung damage and lower inflammatory cytokine in lungs as compared to the control group[50] (Table 1). In an earlier investigation, intranasal application of phage-VTCCBPA43 at 2-h post-infection with virulent *K. pneumoniae* resulted in a significant reduction in bacterial load and an improvement in the severity of the pathologic lesions in the murine pneumonic lung[51] (Table 1). Significantly, liposome-entrapped phages had been demonstrated to improve the effectiveness of intraperitoneal phage therapy, resulting in complete clearance of *K. pneumoniae*-induced lobar pneumonia even though the therapy was administered 72 h after and 48 h before the mice developed pneumonia[52]. Meanwhile, non-liposomal phage administration resulted in a lower bacterial burden when given 24 h after and 6 h before infection, highlighting the superiority of liposomal delivery in IP phage administration for both therapeutic and prophylactic treatment of *K. pneumoniae*-induced lobar pneumonia[52] (Table 1). A phage cocktail containing pKp11 and pKp383 outperformed its monotherapy in terms of animal survival, bacterial load, inflammatory cytokines, and lung damage in early stage of murine pneumonia caused by refractory ST11 and ST383 *K. pneumoniae*, suggesting the promising potential of phages in addressing difficult-to-treat *K. pneumoniae* lung infections[53] (Table 1). Furthermore, phage-derived endolysin-LysCA and LysG24 exhibited remarkable antibacterial activity

in vitro and therapeutic effects in vivo, drastically lowering the bacterial load while leaving no pathologic lesions in the lungs of treated mice with *K. pneumoniae*-associated pneumonia. These endolysins demonstrated excellent environmental adaptability and safety, suggesting the possibility of endolysin as an alternative therapy for the treatment of *K. pneumoniae*-associated lung infections[54] (Table 1).

When treating pneumonia caused by highly virulent *E. coli* 536-*Lux* strain, intranasal phage-536_P1 therapy was proven to be as successful as ceftriaxone therapy in animal survival and lowering bacterial load[55]. Phage-536_P7 treatment, meanwhile, did not significantly reduce mortality in VAP mice infected with a virulent strain of *E. coli*-PDP302[55]. Adapting phage-536_P7_PDP302 against *E. coli*-PDP302 in vitro improved its therapeutic efficacy against *E. coli*-associated pneumonia in clinical settings, as evidenced by a significant increase in animals' survival[55] (Table 1). Irrespective of the virulence and antimicrobial resistance profiles of the targeted *E. coli* strains-536 and LM33, intranasal treatment with specific phages (536_P1 and LM33_P1) resulted in a greater reduction of the bacterial load, a quicker correction of abnormal blood cell counts, and lower inflammatory responses than intraperitoneal antibiotic treatments in mice with acute *E. coli*-associated pneumonia[56] (Table 1). These findings highlight the potential significance of phage therapy in the treatment of *E. coli*-associated lung infections in clinical settings.

Interestingly, intraperitoneal phage therapy was found to be more efficient than intranasal inhalation for lowering bacterial load and inflammatory cytokines in acute murine *B. cepac*ia lung infection, implying that phage could be more readily accessible to these pulmonary pathogens through systemic administration in this study[57] (Table 1). In contrast, aerosol inhalation of *B. cenocepacia*-specific phages-KS12, KS5, KS14 and DC1 via NOID (nose-only inhalation device) reduced bacterial load more significantly than intraperitoneal delivery in a murine lung infection model, suggesting that aerosol phage delivery is an effective administration route for treating antibiotic-resistant *B. cepacia* complex-induced respiratory infections[58] (Table 1). The discrepancies in treatment responses via different administration routes observed between studies could be explained by differences in the phage species, immunological condition of the mice, or bacterial strains used in those studies.

In prior studies, lytic phages exhibiting potential antibacterial efficacy against MDR *A. xylosoxidans* were isolated and characterized, including a detailed genomic study[59–61]. These findings highlight the significance of utilizing phage therapy as a potent alternative therapy to address the growing issue of antibiotic resistance in *A. xylosoxidans*-associated infections[59–61].

Each of these preclinical studies involved isolating phages from the environment that displayed target-specific lytic activities against clinical pathogens that are highly resistant to conventional antibiotics. Importantly, different phage therapies have been investigated, primarily in preclinical scenarios, for the treatment of animal lung infections caused by a single pathogen. There is limited evidence on the efficacy of phage therapy against polymicrobial respiratory infections. Furthermore, these studies documented that prompt administration of phage therapy immediately after development of infection is necessary for successful therapeutic outcomes in animals. However, phage therapy is not expected to be administered to patients shortly after the initial onset of lung infection in clinical settings, which could frequently lead to the development of chronic pulmonary infections. Further preclinical research is needed to better understand the potential of phage therapy as an alternative treatment for acute and chronic lung infections involving polymicrobial scenarios. Despite promising preclinical results, there are still important issues to be addressed about the toxicity, safety, and effectiveness of phage therapy in clinical settings. Moreover, preclinical findings from mice should be extrapolated with caution to humans due to the significant immunological differences between humans and mice[62]. Therefore, clinical evaluations, including case studies and clinical trials, are required to validate the efficacy and safety of phage therapy in addressing bacterial lung infections in children.

Preclinical studies of phage therapy against Gram-positive bacteria

When given intranasally, both phage-AB-SA01 and its prototype products were equally effective as vancomycin in reducing lung bacterial burden in acute pneumonia, despite using animals with different immunological conditions, genetic backgrounds, and *S. aureus* strains with varying methicillin susceptibility profiles[63] (Table 1). In VAP with methicillin-resistant *S. aureus* (MRSA) in rats, intravenous therapy of a phage cocktail (2003, 2002, 3A, and K) was comparably efficacious as teicoplanin in improving animal survival and reducing bacterial burden in lungs with improved histopathological outcomes[64]. The phage cocktail-teicoplanin combination however did not increase survival in treated rats[64] (Table 1). The same phage cocktail also failed to outperform its monotherapy in improving animal survival or lowering bacterial burdens in the lungs or spleen when administered via nebulization in combination with IV daptomycin in a rat model of VAP caused by MRSA, implying the limited effectiveness of this phage cocktail-daptomycin combination for individuals with MRSA pneumonia[65] (Table 1). When the therapeutic outcomes of different delivery routes for the same phage cocktail were evaluated in rats with MRSA-induced VAP, the combination of intravenous and nebulized delivery of aerosolized phage cocktail demonstrated significant synergistic potential in rescuing animals from death when compared to giving phage cocktail via an intravenous or nebulized route. However, the synergistic effects failed to occur when linezolid was given in combination with nebulized phage cocktail[66] (Table 1). Meanwhile, when the phage isolated from sewage was administered intravenously in murine MDR *S. aureus*-induced pneumonia, phage monotherapy significantly reduced bacterial load and caused less pulmonary histologic damage than clindamycin monotherapy or phage- clindamycin combination treatment[67] (Table 1). In a previous study, intraperitoneal phage therapy was demonstrated to be therapeutically effective in saving mice from death and reducing the severity of infection in lung-derived septicemia triggered by *S. aureus*, as evidenced by significantly higher rates of animal survival and lower levels of inflammatory cytokines and bacterial burden in phage-treated mice compared to control mice[68] (Table 1).

Although the antimycobacterial phages isolated using *M. smegmatis* have been demonstrated to be effective against *Mycobacterium* species, limited preclinical study has been undertaken on their lytic activities against *M. abscessus*[69]. A recent study discovered that the combination of antimycobacterial phage-Muddy and the conventional antibiotic-rifabutin, was significantly improved *M. abscessus*-GD01 clearance and enhanced treated animals' survival in CF transmembrane conductance regulator-depleted zebrafish model, revealing the appealing potential of phage-antibiotic combination in resolving *M. abscessus*-associated infections[70]. Importantly, functional innate immunity was necessary for phage-Muddy to be efficacious, as evidenced by the lack of efficacy in macrophage-ablated larvae[70].

Clinical case reports of phage therapy in bacterial lung infection of children

An earlier study discovered that high and repeated dosages of phages (3–5 ml) instilled into the pleural cavity or around abscesses, experienced no adverse consequences during treatment of suppurative lung diseases in neonates and infants[71]. The author therefore hypothesized that applying phages directly into the site of infection by lung puncture would avoid the necessity for additional surgical operations[71] (Table 2). A previous case study used three rounds of nebulized phage therapy to treat pulmonary *S. aureus* and *P. aeruginosa* infections in a 5-year-old cystic fibrosis child, and the patient's overall well-being improved as a result of microbial eradication from the phage treatment[72] (Table 2). Both *P. aeruginosa* and *S. aureus* were found to have chronically colonized in the lungs of a 7-year-old cystic fibrosis girl, and the patient required broad-spectrum antibiotic treatment for several years despite the absence of inhibitory effects on bacterial colonization. In this patient, delivering the Pyophage cocktail and Sb-1 phage by nebulization, significantly reduced both pathogens during therapy and follow-

Table 2 | Case reports of phage therapy in bacterial lung infection of children

Clinical case	Pathogen	Treatment	Type of therapy	Outcomes	Adverse effects	Reference
Newborns and infants with suppurative pleuritis	*S. aureus*	High and repeated dosages of phages (3–5 ml) instilled into the pleural cavity or around abscess	Localized therapy	- Clinical improvement with no production of pus - Applying phages directly into the site of infection by lung puncture would avoid the necessity for additional surgical operations	No adverse consequences	71
5-year-old cystic fibrosis patient	*S. aureus* and *P. aeruginosa*	Nebulized phage therapy three times a day for 3 rounds	Localized therapy	- Improvement of general conditions - Eradication of pathogens after 3rd round of treatment	No adverse consequences	72
7-year-old cystic fibrosis patient	*S. aureus* and *P. aeruginosa*	Nebulized Pyophage cocktail and Sb-1 phage treatment was provided nine times (with approximately 4–6-week interval between phage therapies)	Localized therapy	- Improvement of general conditions - Eradication of pathogens after the end of phage treatment	No adverse consequences	73
6-year-old cystic fibrosis patient with end-stage lung diseases	*P. aeruginosa*	Inhalation of personalized phage cocktail containing 3 phages two time a day for 7 days	Localized therapy	Symptomatic improvement and patient was then transferred out for lung transplantation	No adverse consequences	74
13-year-old patient with sternal wound abscess following bilateral lung-transplant surgery	*P. aeruginosa*	Local application of *Pseudomonas*-specific phage-PA5 and PA10 (4 ml) intraoperatively	Localized therapy	Complete wound healing and microbiological eradication	No adverse consequences	75
12-year-old lung-transplanted patient with cystic fibrosis	*A. xylosoxidans*	- Nebulization of the first cocktail-APC 1.1 (JWDelta, JWT and 2-1), 3 times a day, but this treatment was stopped due to absence of clinical improvement and persistence of *A. xylosoxidans* - Instillation of a second cocktail-APC 2.1 (Jwalpha, JWDelta, JWT, and 2-1) to each pulmonary lobe through the fibroscope followed by continued phage nebulization at home (three times a day for 14 days)	Localized therapy	- Improvement of patient's respiratory functions - Although the low microbial density of *A. xylosoxidans* persisted in airways for months, this colonization eventually went negative and did not reoccur more than two years after phage therapy	No adverse consequences	78
17-year-old cystic fibrosis patient with persistent lung infection	*A. xylosoxidans*	Therapeutic *Achromobacter* phage cocktail was given via inhalation using a compression nebulizer once daily and orally twice daily, for 20 days. The treatment course was repeated a total of 4 times; at 1 month, 3 months, 6 months and 12 months after initial treatment.	Localized therapy	- Improvement of general conditions - Resolution of clinical symptoms - Improvement of lung functions - Reduced need for antibiotics and hospitalization	No adverse consequences	79
10-year-old cystic fibrosis patient with multiple acute pulmonary exacerbations	*Achromobacter* spp.	Concomitant administration of intravenous phage (Ax2CJ45ϕ2) in combination with antibiotics-cefiderocol and meropenem/vaborbactam for 14 days	Systemic therapy	- Significant improvement of clinical symptoms - Improvement of pulmonary functions with eradication of pathogens - This treatment regimen was observed to be safe, well-tolerated and efficacious for clearing infection from the lungs while improving patient's pulmonary functions	No adverse consequences	80
15-year-old cystic fibrosis patient with a disseminated *M. abscessus* infection following bilateral lung transplants	*M. abscessus*	Intravenous administration of phage cocktail containing 3 genetically engineered phages- Muddy, BPs33ΔHTH-HRM10, and ZoeJΔ45 every 12 h for at least 32 weeks	Systemic therapy	- Improvement of lung functions, liver functions and gradual healing of surgical wound and skin lesions - Eradication of pathogens	No adverse consequences	81

up, resulting in improved clinical conditions, pathogen eradication and less antibiotic needs with no phage-related adverse consequences[73] (Table 2). Using a personalized phage cocktail inhalation, a 6-year-old cystic fibrosis patient with end-stage lung diseases positive for MDR *P. aeruginosa* demonstrated symptomatic improvement and well-tolerance to phage treatment without inducing bronchospasm or compromising liver functions[74] (Table 2). When phage therapy was given to treat MDR *P. aeruginosa*-associated sternal wound abscess in a 13-year-old bilateral lung-transplanted patient, phage treatment led to complete wound healing and microbiological eradication, highlighting the potential benefits of phage therapy for antibiotic-resistant bacterial infections following cardiothoracic surgery[75] (Table 2).

A recent cohort study of patients with antibiotic-resistant mycobacterial infections, comprising 13 adult and 7 pediatric patients, revealed that 11 individuals—6 of whom were pediatric patients—showed favorable clinical and microbiological responses[76]. Administration of different phages via IV or aerosolized delivery was well-tolerated, and no patient experienced any serious phage-related side effects[76]. Although neutralizing antibodies against the phage were discovered in serum after the initial administration of intravenous phage therapy in eight patients, and may have contributed to treatment failure in four of these cases, they were not consistently associated with unsuccessful outcomes in the other cases[76]. Notably, none of the bacteria isolate from the 11 patients who received phage monotherapy

Table 3 | Clinical trials of phage therapy

Phase	Study	Trial No	Disease	Objective	Pathogen	Participant	Starting year	Completedyear	Status	Reference
Phase 2	A prospective, randomized, placebo-controlled, double-blinded, single-site study - CYstic Fibrosis bacterioPHage Study at Yale (CYPHY)	NCT04684641	Cystic fibrosis with chronic airway infection	To evaluate the efficacy and safety of nebulized phage therapy-YPT-01	*P. aeruginosa*	Adult (≥18 years)	2021	2023	Completed	82
Phase 1b/2a	A multi-center, double-blind, randomized, placebo-controlled, single and multiple ascending dose study (SWARM-Pa)	NCT04596319	Cystic fibrosis with chronic pulmonary infection	To evaluate the safety, tolerability and phage recovery profile inhaled phage therapy-AP-PA02	*P. aeruginosa*	Adult (≥18 years)	2020	2022	Completed	83
Phase 2	A multi-center, double-blind, randomized, placebo-controlled study (Tailwind)	NCT05616221	Non-cystic fibrosis with bronchiectasis and chronic pulmonary infection	To evaluate the safety, phage kinetics, and efficacy of inhaled phage therapy-AP-PA02	*P. aeruginosa*	Adult (≥18 years)	2023	2024	Recruiting	84
Phase 1b/2a	A randomized, double-blind, placebo-controlled, multicenter study	NCT05010577	Cystic fibrosis with chronic pulmonary infection	To evaluate safety and tolerability of nebulized phage-BX004-A	*P. aeruginosa*	Adult (≥18 years)	2022	2024	Recruiting	85
Phase 1b/ 2	A multicenter, randomized placebo-controlled double-blind study	NCT05453578	Cystic fibrosis	To assess the safety and microbiological activity of intravenous phage therapy-WRAIR-PAM-CF1	*P. aeruginosa*	Adult (≥18 years)	2022	2024	Recruiting	86

developed phage resistance[76]. The effectiveness of phages administered orally and locally via inhalation was studied in 93 patients between 1981 and 1986 who had lung abscess, bronchitis, and pneumonia caused by *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *S. pyogenes*, and *Salmonella*. Of these, 83 patients experienced successful therapeutic outcomes, 8 patients showed a temporary improvement, and only 2 had no therapeutic impact[77].

Due to persistent lung infection with pandrug-resistant (PDR) *A. xylosoxidans*, a 12-year-old lung-transplanted patient with cystic fibrosis underwent two rounds of 2 phage cocktails given via nebulization alone (first phage cocktail-APC 1.1) and direct instillation followed by nebulization (second phage cocktail-APC 2.1), resulting in clinical tolerance with improvement of patient's respiratory functions[78]. Although the low microbial density of *A. xylosoxidans* persisted in airways for months, this colonization eventually went negative and did not reoccur more than two years after phage therapy[78] (Table 2). Periodic phage cocktail therapy, given orally and by inhalation, effectively healed a 17-year-old cystic fibrosis patient's persistent lung infection caused by *A. xylosoxidans*, improving the patient's general well-being and lung functions while decreasing the need for antibiotics and hospitalization[79] (Table 2). According to a prior report, a 10-year-old cystic fibrosis patient with PDR *Achromobacter* spp. was successfully treated with intravenous phage-cefiderocol-meropenem/vaborbactam triple combination therapy[80]. This treatment regimen was observed to be safe, well-tolerated and efficacious for clearing infection from the lungs while improving patient's pulmonary functions[80] (Table 2).

Meanwhile, a 15-year-old cystic fibrosis patient with a disseminated *M. abscessus* infection following bilateral lung transplants received an engineered three-phage cocktail intravenously with good tolerance, resulting in microbial clearance, clinical improvement with no occurrences of adverse reactions[81] (Table 2).

Clinical trials of phage therapy in children

Among the clinical trials conducted on pulmonary infections, most of them assessed the safety and effectiveness of phage therapy in adult patients with cystic fibrosis who had chronic *P. aeruginosa*-induced pulmonary infections. The safety and efficacy of nebulized YPT-01 phage treatment for reducing the bacterial load in sputum of cystic fibrosis patients with *P. aeruginosa* was investigated in a phase II clinical trial (NCT04684641)[82] (Table 3). The trials-SWARM-Pa (NCT04596319) and Tailwind (NCT0561622) used the same inhaled phage therapy-AP-PA02 in patients who had different clinical presentations[83,84]. The SWARM-Pa trial, a phase 1b/2a study that assessed the safety, tolerability, and phage recovery profile in CF patients with *P. aeruginosa*-induced lung infections completed in 2022[83] (Table 3). Meanwhile, the Tailwind phase 2 trial continues to enroll non-CF subjects with *P. aeruginosa*-induced bronchiectasis and persistent lung infection to evaluate the safety, phage kinetics, and effectiveness of inhaled AP-PA02 phage therapy[84] (Table 3). Patients with CF and persistent *P. aeruginosa*-induced lung infection continue to recruit for a trial (NCT05010577) investigating the safety and tolerability of nebulized phage, BX004-A[85] (Table 3). Concurrently, a multicenter randomized double-blind study (NCT05453578) has begun enrolling CF patients to evaluate the safety

and antibacterial activity of intravenous phage therapy-WRAIR-PAM-CF1 against *P. aeruginosa*[86] (Table 3).

A clinical trial involving 6–24-month-old children with acute diarrhea who received oral phage therapy demonstrated the safety of phage therapy by achieving safe phage transit with no alterations to their intestinal microbiome, while there was no discernible improvement in intestinal phage replication or diarrhea outcomes[87]. A single-arm, non-randomized, open-labeled trial with the Clinical Trial Registry-ACTRN12622000767707 will begin recruiting children with CF who tested positive for *P. aeruginosa* even after eradication therapy, with the objective of investigating the safety and tolerability of intrabronchial and nebulized phage treatment. In this trial, the selected phage will be administered endo-bronchially under direct vision, followed by twice-daily nebulization for 7 days while receiving standard CF therapy. Following phage therapy, the patients' progress will be evaluated using clinical assessments which involve spirometry and follow-up sputum cultures at 3, 6, 9, and 12 months[88].

Clinical benefits of phage therapy

Phage therapy offers several clinical benefits as an alternative or adjunct to traditional antibiotic treatments. Its specificity to target pathogenic bacteria without affecting the beneficial microbiota, ability to disrupt biofilms, and potential to address antibiotic-resistant infections are among its key advantages. These benefits can significantly improve patient outcomes and provide new avenues for combating bacterial infections.

Ease of isolating target-specific potent phages from environment

With their approximate population of 10^{32}, phages are the most prevalent naturally existing lifeform which influence the microbial equilibrium in human and animal environments[89,90]. According to fecal metagenomic studies, phages comprise a vital component of the microbiome and are more abundant than eukaryotic viruses, having 10^{15} versus 10^{8}–10^{9}/g of feces. When compared to developing novel antibiotics, such ubiquitous characteristics make it easier and less expensive to isolate highly efficient target-specific phages from a variety of environments[89,90]. This simple and affordable procedure of isolating phages, particularly in low-resource regions of the world, is an appealing reason for investing in phage production to establish a phage bank for future use as an alternative antimicrobial therapy in persistent bacterial lung infections, and it additionally may render importing phages from abroad less necessary[89,90]. The discovery of therapeutic phages in the specific regions is also beneficial due to the co-evolution of phage and bacteria under the same selective pressures in a shared geographic region, leading to retaining phage-bacteria sensitivity, which is not typically observed in phages obtained from geographically distinct environments[89,90].

Reduced selection of antibiotic resistance

The widespread use of broad-spectrum antibiotics puts the pathogens at the site of infection and commensal microbes in the respiratory tract under selection pressure[89,91]. This eventually leads to the emergence of difficult-to-treat antibiotic-resistant organisms, generating substantial public health concerns and demanding immediate global action due to the extremely limited treatment options in the affected population[89,91]. In contrast, phage therapy does not pose the risk of triggering antibiotic selection pressure, making it a fascinating possibility for implementation in the global attempt to mitigate antibiotic resistance[89,91]. By employing phage as an alternative therapy, patients will be exposed to fewer antibiotics, preserving the effectiveness of currently available antibiotics while minimizing the incidence of antibiotic-induced adverse effects and establishment of antibiotic resistance[89,91]. Moreover, a synergistic impact can be achieved by using specific phages in combination with antibiotics to re-sensitize pathogens to antibiotics to which they were previously resistant[89,91].

Reversal of antibiotic resistance by phage-antibiotic synergy (PAS)

When phages were added to pre-existing antibiotic regimens, the combined effect exhibited stronger antibacterial effects than the individual effects, a phenomenon known as phage-antibiotic synergy (PAS)[92–94]. During PAS, target pathogens, even those resistant to the paired antibiotic, were eliminated because of the accelerated multiplication and larger plaque size of combined phages which were facilitated by antibiotic-enhanced phage production in the presence of subinhibitory antibiotic doses[92–94]. Bacterial filamentation caused by β-lactam-related PBPs inhibition, or SOS-response-facilitated survival mechanisms when exposed to sublethal concentrations of β-lactam or DNA-disrupting antibiotics and lethal concentrations of protein or RNA synthesis inhibitors, have also been reported to trigger the PAS response in several studies[92–94]. In addition to increasing bacterial size and surface area, filamentation enables bacterial genomic expression to continue without cell division, allowing phages' replication for the PAS-associated lysis achievable[92–94]. Furthermore, antibiotic-induced bacterial filamentation improved phage adsorption through enhancing the density of phage receptors on bacterial cell walls, consequently accelerating the phage-induced lysis for PAS[92–94]. However, filamentation mediated by protein or RNA synthesis inhibitors usually results in shorter bacterial cells than those seen with β-lactams or fluoroquinolones, while the specific mechanisms remain unclear[92–94]. The greater probability of producing PAS with β-lactams and fluoroquinolones compared to other antibiotics therefore could be attributed to these distinct variations in bacterial cell surface area modifications[92–94]. Importantly, if the antibiotic dose is insufficient to elicit a subinhibitory response to the bacteria, or if the bacteria are highly resistant to the prescribed drugs, filamentation is unlikely to initiate, and PAS may not occur[92–94]. Therefore, selecting the appropriate antibiotic class and dose for a given phage is crucial for achieving the PAS response[92–94]. Antibiotic-induced bacterial membrane destabilization could shorten the phages' latent time to invade and destroy bacteria, resulting in the production of PAS and accelerated bacterial lysis[92–94]. Because of a shorter latent interval and faster bacterial lysis, phages can infect nearby bacteria quickly for initiating new lytic cycles, potentially increasing PAS[92–94]. Not only has rapid lysis been linked to PAS, but delayed cell lysis also allows for a longer period for phage synthesis, maturation, and assembly, resulting in greater burst size and phage plaque enlargement, which contributes to PAS[92–94].

Antibiofilm effects

Biofilms are composed of bacterial microcolonies that aggregate inside a matrix predominantly containing exopolysaccharides, extracellular DNA, secreted proteins, and lipids and have been commonly reported in persistent lung infections[95]. During phage infection, lytic phages and host bacteria interact by phage adsorption, followed by the release of new progenies through bacteriolysis[95]. However, phage invasion may be physically impeded by the existence of biofilm matrix[95]. To overcome this barrier, phages use depolymerase enzymes associated with their tails, which cause the progressive cleavage of polymer bonds, culminating in the degradation of not only the exopolysaccharide matrix of biofilm, but also host bacteria's polysaccharide capsules[95]. Consequently, depolymerases facilitate the infiltration and distribution of phages through biofilms while simultaneously eradicating the target bacteria present in biofilm, hence eliminating the pre-existing biofilms and preventing new biofilms' development[95]. Reduced bacterial virulence resulting from bacterial capsules degradation by phage-encoded polymerase is an additional therapeutic benefit of phage therapy[95]. Concurrently, phages generate lysin or virion-associated lysins, which are the hydrolytic enzymes present in phage tails. They serve as receptor recognition proteins, allowing phage genomic material entry into host bacterial cells via bacterial cell wall penetration[95]. Additionally, they assist to release virions by cleaving the bacterial cell wall from within[95]. Lysins, can also degrade the extracellular matrix of biofilms and trigger the lysis of bacteria that reside within them[95]. These phage-related enzymes outperform broad-spectrum antibiotics in clinical settings with their abilities to selectively target pathogens without harming the normal flora, improve their

effectiveness through genetic engineering, produce strong synergistic effects when combined with other antibiotics, and rapidly lyse host bacteria with a lower risk of resistance development[95]. Phages exhibit excellent antibiofilm effects in the presence of these enzymes and phage-induced bacteriolysis, implying that phage or phage-related enzymes, either alone or in conjunction with antibiotics, could ultimately result in greater clinical cure and shorter therapeutic duration while combating biofilm-associated lung infections in children[95].

No cross-resistance with antibiotics

Previous studies have revealed that cross-resistance was frequently observed in phages that employed the same target receptors while producing phage-mediated bacteriolysis against their target bacterial infections[96,97]. However, antibiotic and phage use completely distinct mechanisms for achieving their antibacterial effects, whereas phages infect to replicate within specific bacteria for bacterial lysis and death[92,93,98], and antibiotics target cellular mechanisms to disrupt bacterial growth and metabolism[99,100]. Interestingly, there is no cross-resistance between antibiotics and phages, because the specific mechanisms generating antibiotic resistance such as enzymatic inactivation or modification of antibiotics, reduced permeability of antibiotics, target site protection or modification, target bypass, increased efflux and decreased influx of antibiotics[101], cannot be translated into phages. Additionally, while bacteria develop resistance to a specific phage under its selection pressure, this resistance fails to confer cross-resistance to antibiotics as these bacteria employ distinct anti-phage defense mechanisms to generate phage resistance, such as modifying host surface receptors to prevent phage adsorption, restricting phage's genome entry into the host, minimizing secondary phage infection, activating the restriction-modification and CRISPR-Cas systems, and initiating an abortive infection system, which cannot be translated into antibiotics[102–104]. Among the different phage-resistant mechanisms, the alteration in host surface receptors such as lipopolysaccharide, capsule polysaccharide, pili, outer membrane porins, and efflux pumps are the most significant mechanisms involved in bacterial resistance to phage[102–104]. The receptors alteration can potentially affect bacterial fitness because these surface structures are essential for various cellular functions, bacterial virulence, and antibiotic resistance[90,104–106]. Therefore, the presence of phage resistance frequently triggers several trade-offs in bacterial pathogens, including decreased pathogenicity, delayed bacterial growth, and increased antibiotic susceptibilities, which eventually cause phage-resistant bacteria regaining their susceptibility to antibiotics[105–107]. Phage-induced restoration of antibiotic sensitivity is reported in several studies to positively impact antibiotic resistance in clinical settings, providing an important clinical benefit for repurposing the conventional antibiotics in phage combination therapy to combat drug-resistant respiratory pathogens[90–93,104–107]. However, pleiotropic alterations in bacterial susceptibilities to antibiotics was observed when phage resistance evolved from a variety of mutations in distinct bacterial target structures that antibiotics employed to demonstrate their antibacterial effects[108–110]. It was reported in a prior study whereas resistance to both phages-T6, U115, and albicidin has observed in *E. coli* through mutations in Tsx, an outer membrane porin that functions as a receptor for these phages, or a porin for an uptake of albicidin, an antibiotic inhibiting DNA gyrase[109]. A recent study found that phage-U136B employed the antibiotic efflux pump-TolC and lipopolysaccharide (LPS) as its target receptors to induce bacteriolysis in *E. coli*. Mutations in the *tolC* or LPS synthesis gene of phage-resistant *E. coli* in their study resulted in lower resistance to tetracycline and colistin[110]. Interestingly, a subgroup of their LPS mutants developed a greater resistance to tetracycline while reducing colistin resistance, demonstrating that these LPS mutants displayed a pleiotropic impact on tetracycline susceptibilities[110]. These findings highlighted not only the development of trade-offs, but also the pleiotropic evolution of trade-ups could be generated by specific phage-resistant mutations, emphasizing the necessity of screening to identify phages capable of pleiotropically selecting against drug-resistance genes in target pathogens.

Self-replication at the site of infection

During the phage infection cycle, the first infection of host bacteria for phage-induced lysis is primary infection, and the subsequent replication in nearby bacteria by virions released from lysed cells is referred to secondary infection[90,111]. Phage therapy for bacterial lung infections frequently results in a secondary infection of phage replication in the lungs[90,111,112]. Phage replication additionally allows phages to multiply in deeper lung infection areas that are more difficult to access but have a high bacterial load[90,111,112]. Because they can only exist while their target pathogenic bacteria are present at the infection site, their replication is however self-limiting[90,111,112]. When treating persistent bacterial lung infections, this self-replicating ability offers the benefit of auto-dosing, whereas high-dosage of phage administrations will be less needed to achieve the optimal therapeutic outcomes[89,90]. Auto-dosing additionally contributes the benefit of safety with few to no side effects as its antibacterial impact is confined to its infection site at lower dosage[89,90].

Coevolution of phages and bacteria

Because phages and bacteria coexist in diverse environments, bacteria are constantly adapting to survive phage attacks by generating anti-phage defense mechanisms as described previously[102–104]. Meanwhile, phages also adopt a variety of coevolutionary strategies to keep attacking bacteria that resist its infection[106,113–115]. One of these is enhanced phage's adsorption and entry through the identification of new or altered host surface receptors and modification of receptor-binding regions to compensate for alterations in its bacterial surface receptors[106,113–115]. Other strategies also assist phages to overcome restriction modification-mediated phage resistance by modifying the restriction sites within genomes, methylating genomes, and eliminating the cofactors required for restriction-modification systems[106,113–115]. Phages effectively circumvent CRISPR-Cas-mediated resistance by generating anti-CRISPR proteins and introducing point mutations in the protospacer regions or CRISPR-targeted sequences[106,113–115]. Phages generate antitoxin proteins and mutations in their genomes to terminate abortive infection-mediated phage resistance[106,113–115]. Because of these coevolutionary interactions between phages and bacteria, there is a lower risk of phage and/or antibiotic-resistant bacterial escape, which is a significant therapeutic benefit of utilizing phages over antibiotics[106,113–115].

Minimal to no toxicity

Phages can be regarded as non-toxic because their main components are single or double-stranded nucleic acids wrapped in protein capsids[116,117]. Due to their receptor-mediated host specificity, phages pose no threat to mammalian eukaryotic cells as these cells lack the required surface receptors to allow for phage infection[116,117]. These characteristics provide phage therapy tolerable, resulting in minimal to no toxicity when compared to antibiotics[116,117].

Minimal risks of dysbiosis

While broad-spectrum antibiotics produce deleterious impacts on the airway microbiome due to their non-specific antibacterial effects, target-specific phage therapy offers a significant therapeutic benefit with minimal dysbiosis risks[118,119]. Phage therapy has been reported in human and animal studies to have no impact on the microbiome, preventing the eradication of potentially beneficial microbes, secondary pathogens proliferation, and the emergence of resistant microorganisms[118,119].

Clinical challenges of phage therapy

Bacteriophages, used to treat persistent bacterial lung infections, present several clinical challenges despite their potential as an alternative to antibiotics. These challenges span various aspects, including therapeutic efficacy, safety, and logistical considerations. The complexity of phage-bacteria interactions, the body's immune response to phages, and the stability of

phage preparations are key factors that influence the clinical success of this therapeutic approach. Understanding and addressing these challenges is crucial for the effective implementation of phage therapy in medical practice.

Therapeutic failure from strains variation and phage resistance

The most notable obstacle related to phage's host specificity is the failure of phage-induced bacteriolysis among bacterial isolates belonging to same species[120,121]. Because there is a significant diversity across bacterial strains within a species, this strain variation contributes to phage non-susceptibility[120,121]. The use of phage cocktails, which comprise various clinically efficacious phages belonging to different families with diverse mechanisms of action, and the administration of phages with broad-spectrum host ranges against diverse set of clinical strains belonging to the major lineages of target pathogens species, are promising approaches to address this issue[120,121].

Another unavoidable and frequent issue encountered during phage therapy is the emergence of phage-insensitive mutations, which ultimately leads to inadequate bacterial clearance and phage therapy failure[122,123]. Sequential or simultaneous administration of a phage cocktail comprising different phages with distinct modes of action, provided encouraging results in the fight against the development of bacterial phage resistance[122,123]. Meanwhile, the use of genetically engineered phages can bring the advantages of increasing the therapeutic efficacy and safety of phages and limiting the development of phage resistance in the clinical settings[122,123].

Antagonistic phage–phage interactions

Antagonistic phage-phage interaction occurs when phages hinder or oppose the growth, replication, or functionality of other phages while infecting the same host bacterium[124,125]. It occurs through different mechanisms, including superinfection and competitive exclusion by blocking host surface receptors and decreasing cellular resources as a result of phage-phage competition, phage-mediated defense triggered by restriction modification systems that identify and degrade competing phage's DNA, and the production of anti-phage proteins like bacteriocins or phage-encoded abortive infection systems that target and limit the growth or replication of other phages[124,125]. The effectiveness of phage therapy may therefore be adversely affected by antagonistic phage-phage interactions[124,125]. Undoubtedly, administering different phages sequentially or individually could lower the possibility of undesirable phage-phage interactions when compared to giving combined phage cocktails[124,125]. Because minimal research has been conducted on these detrimental consequences of antagonistic interaction during phage therapy, it can be challenging to predict how these phenomenon would impact different treatment modalities[124,125]. It is therefore imperative to continue researching the mechanisms underlying antagonistic phage-phage interactions to develop alternative strategies to increase the efficacy of phage-based therapies in combating persistent lung infections[124,125].

Safety concerns

Currently, the potential clinical risk linked with phage therapy is that phages, particularly temperate phages, could trigger safety challenges by dissemination of new genetic characteristics among bacterial populations[116,126]. During lysogenic cycles, temperate phages integrate into host bacterial genomes as stable prophages and do not induce host bacterial lysis[116,126]. However, they can facilitate lysogenic conversion of host bacteria, resulting in the emergence of pathogenic strains in their hosts via phage-mediated transduction of new genetic characteristics, such as genes involved in bacterial virulence, toxin production, and antibiotic resistance[116,126]. Risks assessment therefore needs to involve an evaluation of the complete phage genomes, along with transduction capacity and host range studies for a particular phage to address safety concerns related to phage therapy[116,126].

Immunity to phages

Due to their antigenic nature, prolonged exposure to phages and their byproducts can trigger the mammalian immune system to generate phage-specific T and B cells immune responses in vitro and in vivo[127,128]. Previous studies discovered that phage survival was significantly longer in immunocompromised animals and humans as compared to immunocompetent ones, highlighting the critical role of adaptive immunity in phage clearance[127,129,130]. The phage titer remained unchanged in B-cell-deficient mice, suggesting that anti-phage antibody production was significantly responsible for the phage clearance[131]. Although IgM accounts for the majority of anti-phage neutralizing antibodies, IgG and IgA are also produced[132]. It has been revealed that both environmental and genetically engineered phages can trigger the development of anti-phage antibodies[132]. Although it has not been further investigated in controlled trials, administering phages at the site of infection was reported to generate stronger antibody responses than oral administration in patients[133]. Individuals who have been given structurally comparable phages could possess phage-specific antibodies, which could mitigate the effectiveness of subsequent phage treatments[127,132]. Interestingly, enrolled human subjects were discovered to have preexisting anti-phage antibodies with significant reduction of phage's activity, even though none of them had previously received phage therapy[134,135]. Nevertheless, the impact of anti-phage antibodies on phage therapy outcomes remains uncorrelated since there was no discernible relationship between the patient's treatment responses and the development of phage-specific humoral immunity[133,136]. The absence of controlled trials using standardized phage products, administration protocols, and treatment durations makes it extremely difficult to effectively evaluate how patients' development of anti-phage antibodies influences therapeutic outcomes[127,132]. It will also be necessary to explore whether the existence of neutralizing antibodies to a specific phage limits the future therapeutic application of that particular phage or other phage families.

Stability and pharmacokinetic issues

Under diverse environmental conditions, phages containing nucleic acids encased in proteinaceous capsids have the same potential to denature as proteins[137–139]. Phage manufacturing, transportation, and storage are therefore challenging due to its limited stability[137–139]. Phage instability can be triggered by fluctuations in temperature, pH, and mechanical stresses during manufacturing, resulting in a reduction in phage therapeutic effectiveness[137–139]. Therefore, it is preferable to implement minimal steps to ensure the stability and potency of phages throughout manufacturing[137,138]. Moreover, the stability of phages in different formulations has been documented to be phage-specific, and variations in stabilities between phages may jeopardize the process of developing phage cocktails in diverse formulations[137–139].

Furthermore, phages possess different pharmacokinetic profiles from conventional antibiotics whereas their ability for self-replication is associated with eradication of pathogens[137–139]. The effectiveness of phage therapy is significantly influenced by the presence of target pathogens, the relative quantity of phages to bacteria, and the ideal timing for phage delivery[137–139]. The pharmacokinetics of phages, including their distribution, metabolism, and excretion in vivo, need to be investigated for optimizing treatment regimens[137–139]. However, the limited knowledge about phage pharmacokinetics renders manufacturing and developing a standardized protocol for phage therapy challenging.

Future research directions and conclusion

The use of phage therapy has demonstrated remarkable therapeutic potential in preclinical and clinical settings when convectional antibiotics failed to control persistent bacterial lung infections in children. Moving forward, research endeavors in phage therapy for bacterial lung infections in children should focus on several key areas to advance its clinical applicability and effectiveness. First and foremost, standardizing phage formulations is crucial to ensure consistent therapeutic outcomes and streamline the regulatory approval process. This involves refining phage production techniques, enhancing the stability of phage formulations for extended storage, and determining the optimal phage-to-bacteria ratios for maximizing its treatment efficacy. Understanding the pharmacokinetics of phages is pivotal

for optimizing treatment regimens. Investigating the distribution, metabolism, and excretion of phages in vivo will provide insights into their bioavailability, persistence, and overall therapeutic profile. Additionally, it's imperative to delve deeper into the mechanisms governing antagonistic interactions between phages. Uncovering these interactions will pave the way for developing strategies to mitigate their adverse effects, thereby boosting phage treatment's efficacy.

Long-term studies are also essential to ascertain the safety and efficacy of phage therapy across diverse patient populations and geographical locations. These studies should focus on monitoring potential adverse effects, tracking the emergence of phage and antibiotic resistance, and assessing long-term patients' outcomes. Exploring the synergistic potential of combining phage therapy with other therapeutic modalities, such as antibiotics or immunomodulatory agents, could offer a multifaceted approach to enhancing treatment outcomes. Lastly, the establishment of comprehensive regulatory frameworks and ethical guidelines is paramount to ensure patient's safety and foster broader acceptance of phage therapy. This includes the development of standardized protocols, rigorous safety assessments, and clear guidelines for informed consent and patients' monitoring.

In conclusion, advancing our understanding of phage therapy through systematic research and addressing these pivotal research directions will be instrumental in realizing its full therapeutic potential for combating persistent bacterial lung infections in children.

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Authors contributions

D.L.W., S.A, P.G.H. and A.Ki. conceived the idea and formatted the structure of the manuscript. A.M.S.S and P.H. wrote the main body, A.K., P.P., K.M., L.C., K.S., M.A., P.M, and T.C. reviewed and added to the manuscript. The authors read and approved the final manuscript.

Competing interests

A.M.S.S., P.H., A.K., P.P., K.M., L.C., K.S., M.A., P.M, A.Ki., T.C., P.G.H., and S.A. declare no financial or non-financial competing interests. D.L.W. serves as an editorial board member of this journal and had no role in the peer-review or decision to publish this manuscript. D.L.W. declares no financial competing interests.

Additional information

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