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REVIEW

Examining the Efficacy and Safety of Bacteriophage Therapy on ESKAPE Pathogens and its Potential for Mitigating Disease Outbreaks

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Abstract

Purpose of the Review Antimicrobial resistance (AMR) poses a global health crisis, with ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.) driving severe, multidrug-resistant infections. Bacteriophage therapy (PT) offers a targeted alternative; however, its clinical efficacy, safety, and potential outbreak mitigation remain underexplored. This narrative review synthesized evidence from 30 clinical studies to evaluate PT for ESKAPE infections.

Recent Findings Complete bacterial clearance was achieved in 10 studies, primarily for *P. aeruginosa* and *K. pneumoniae*, with clinical improvement in 24 studies, including complex cases like osteomyelitis and cystic fibrosis-related pneumonia. PT was safe, with no serious adverse effects across 25 studies; mild, transient events (e.g., fever) were rare. Mortality, reported in nine studies, was unrelated to PT. One study demonstrated a reduction in nosocomial transmission of *A. baumannii* using environmental phages, suggesting a potential for outbreak control.

Summary PT shows promise as a safe, effective adjunct for MDR infections, but larger trials and standardized protocols are needed to address resistance, optimize dosing, and explore public health applications.

Keywords Bacteriophage therapy · ESKAPE pathogens · Antimicrobial resistance · Phage therapy efficacy

Introduction

Antimicrobial resistance (AMR) threatens global health, undermining decades of progress in infectious disease management. The World Health Organization (WHO) designates AMR as a critical priority, driven by the proliferation of multidrug-resistant (MDR) bacteria—those resistant to three or more antibiotic classes [1, 2]. This crisis is compounded by a decline in antibiotic research and development, with few novel agents entering clinical pipelines over the past three decades [3]. Global surveillance of AMR remains fragmented, limiting comprehensive data on its impact [1]. Nevertheless, regional estimates reveal a dire situation: in the United States, AMR causes approximately 29,000 deaths, over 2 million infections, and $4.7 billion in healthcare costs annually [4]. In Europe, it claims 33,000 lives, accounts for 874,000 disability-adjusted life years (DALYs) lost, and incurs $1.5 billion in direct and indirect costs [5, 6]. Developing countries face even graver challenges, where infectious diseases remain the leading causes

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of mortality, exacerbated by limited access to diagnostics, second-line antibiotics, and robust healthcare infrastructure [7, 8]. While difficult to quantify globally, the economic burden is substantial, with ripple effects on productivity and healthcare systems [9]. This escalating crisis shows the urgent need for innovative solutions to combat resistant pathogens and mitigate their societal toll.

Among MDR bacteria, ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.—are particularly concerning due to their ability to evade multiple antibiotics and cause severe, often nosocomial, infections [4]. These pathogens are implicated in various conditions, from bloodstream infections to ventilator-associated pneumonia, significantly increasing morbidity, mortality, and treatment costs [10]. For instance, methicillin-resistant *S. aureus* (MRSA) alone accounts for thousands of deaths annually, while carbapenem-resistant *K. pneumoniae* and *A. baumannii* pose growing threats in intensive care settings [11]. ESKAPE pathogens exploit diverse resistance mechanisms, including efflux pumps, enzymatic degradation of antibiotics, and target site alterations, rendering conventional therapies increasingly obsolete [12]. Biofilm formation further complicates treatment, as these structured communities shield bacteria from antibiotics, environmental culprits, and immune responses, promoting persistent infections [13]. The limited therapeutic options for ESKAPE infections highlight the need for a coordinated global response, including enhanced surveillance, stewardship programs, and alternative treatment modalities.

The growing inefficacy of antibiotics has spurred renewed interest in bacteriophage therapy (PT), a century-old approach now gaining traction as a viable alternative [14]. PT employs lytic bacteriophages—viruses that infect and lyse specific bacteria—to target pathogens, such as ESKAPE organisms [15]. Historically, phage therapy was pioneered in the early 20th century, notably in the Soviet Union and Eastern Europe, where it was used to treat bacterial infections before antibiotics became widespread [16]. The advent of antibiotics, however, relegated PT to the sidelines in Western medicine, despite its continued use in regions like Georgia and Russia [17]. Today, PT is experiencing a renaissance, driven by AMR's rise and advances in genomics, which enable precise phage selection and engineering [18]. Phages offer distinct advantages over antibiotics: they are highly specific, precisely targeting only the intended bacterial species, thus preserving the host's microbiota and reducing dysbiosis [19].

Despite its promise, PT faces significant challenges. Though less frequent than antibiotic resistance, bacterial resistance to phages can emerge via mutations in phage receptors, necessitating cocktail therapies or engineered phages [19]. Given these challenges, systematic evaluation of PT against ESKAPE pathogens is essential to unlock its potential and integrate it into clinical practice. This study aims to assess the efficacy and safety of bacteriophage therapy.

Methods

Search Strategy

This narrative review synthesized evidence on the efficacy, safety, and outbreak mitigation potential of PT for infections caused by ESKAPE pathogens. A literature search was conducted across PubMed, Scopus, Web of Science, DOAJ, Cochrane Library, and Google Scholar, covering studies published from the databases' inception to March 2025. Search terms combined Medical Subject Headings (MeSH) and keywords, including "bacteriophage," "phage therapy," "ESKAPE pathogens," "antimicrobial resistance," "infection control," "clinical trials," "cocktail therapy," "outbreak management," and specific pathogens (e.g., "MRSA," "carbapenem-resistant Acinetobacter"). Boolean operators (AND, OR, NOT) refined queries to enhance precision. For example, searches used combinations like ("bacteriophage" OR "phage therapy") AND ("ESKAPE" OR "Klebsiella pneumoniae") AND ("efficacy" OR "safety"). To capture additional relevant studies, reference lists of included articles were hand-searched, and grey literature, including clinical trial registries (e.g., ClinicalTrials.gov) and conference abstracts, was reviewed. Two independent reviewers searched, with discrepancies resolved through discussion to ensure consistency.

Inclusion and Exclusion Criteria

Eligible studies included peer-reviewed, English-language publications evaluating PT for ESKAPE pathogen infections in humans, such as randomized controlled trials (RCTs), cohort studies, case-control studies, and clinical case reports. Studies were included if they assessed PT as a standalone or adjunctive therapy, reporting outcomes like clinical cure rates, microbiological clearance, adverse events, or outbreak containment. Comparative studies (e.g., PT vs. antibiotics) and those exploring PT's role in infection control during outbreaks were also considered. To balance mechanistic insights with clinical relevance, select in vitro and animal studies were included only if they directly informed human PT applications. Exclusions encompassed studies on non-ESKAPE infections, non-peer-reviewed sources (e.g., editorials, commentaries), and non-English publications. Systematic reviews were excluded as primary

Table 1 Details of 30 phage therapy studies

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Jault et al. (2019)	RCT, double-blind	France, Belgium	27	*P. aeruginosa*	Phage cocktail PP1131 (12 lytic *Pseudomonas aeruginosa* phages) applied topically once daily for 7 days Standard care: 1% sulfadiazine silver emulsion cream applied daily for 7 days	Topical	Cocktail	Allowed	Slower vs. standard care	No significant difference	23% phage vs. 54% standard; none serious	Terminated early
Wright et al. (2009)	RCT, double-blind	London, UK	24	*P. aeruginosa*	Single dose of 200 µL Biophage-PA (each containing 100,000 PFU of six bacteriophages)	Topical (ear)	Cocktail	Without antibiotics	Significant reduction	3/12 complete resolution	Mild, transient	Mixed: resolution or recurrence
Tolkunovna et al. (2024)	RCT	Tashkent, Uzbekistan	212	*S. aureus* (others)	Inhaled bacteriophage therapy using liquid polyvalent pyobacteriophage (LPPB) via nebulizer; 5 mL once daily for 5 days	Inhalation	Cocktail	With amoxicillin	30-fold reduction	1.4x faster recovery	None reported	Faster recovery

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
McCallin et al. (2018)	Phase 1 safety trial	Switzerland, Belgium, Bangladesh	21	*S. aureus*	*Metagenome analysis*: Two commercial Pyophage cocktails from Georgia and Russia analyzed *Clinical trial*: Participants received either a single *Staphylococcus aureus* monophage, a phage cocktail (Pyophage), or placebo Oral administration: 10 mL of the phage or placebo three times daily for two days Nasal administration: Phage solution applied in the nasal cavity	Oral, nasal	Monophage, cocktail	Without antibiotics	Not assessed	Not applicable	9% unrelated abnormalities	Not assessed
Dobretsov et al. (2021)	RCT, double-blind	Krasnoyarsk, Russia	40	*S. aureus*, *P. aeruginosa*, *Klebsiella*	Intranasal application of bacteriophage gel ("Otophag") twice daily for ten weeks	Intranasal	Cocktail	Without antibiotics	Significant reduction	Improved symptoms	None reported	Reduced inflammation
Ho et al. (2016)	Prospective intervention	Hualien, Taiwan	264 CRAB	*A. baumannii*	Aerosolized bacteriophage solution applied via ultrasonic humidifier, 500 mL of 10^7 PFU/mL phage stock used per session	Environmental (aerosol)	Lytic/Mono-phage	Without antibiotics	Reduced acquisition rate	Not applicable	None reported	Reduced antibiotic use

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Nir-Paz et al. (2022)	Phase I/II trial	Israel	26	*S. aureus*, *P. aeruginosa*, *A. baumannii*	TP-102 bacteriophage cocktail applied topically at 10^9 PFU/mL/cm³ of the target ulcer Cohort 1 (non-infected DFU): Treatment for 1 week Cohort 2 (infected DFU, grade 2 or 3 PEDIS classification): Treatment for 28 days, three times a week	Topical	Cocktail	With standard care	Not reported	Not reported	No severe events	Not reported
Beschastnov et al. (2023)	Prospective cohort	Nizhny Novgorod, Russia	60	*P. aeruginosa*, *K. pneumoniae*, *S. aureus*	Topical application of bacteriophage-infused hydrogel (Polypran polymer film with bacteriophage suspension)	Topical	Lytic/Mono-phage	Without antibiotics	Complete in 5 (*P. aeruginosa*)	Improved healing	None reported	Enhanced graft engraftment
Jennes et al. (2017)	Case report	Brussels, Belgium	1	*P. aeruginosa*	Intravenous infusion of 50 µL of bacteriophage cocktail (BFC1) over 6 h daily for 10 days Wound irrigation with 50 mL of BFC1 every 8 h for 10 days	Intravenous, topical	Cocktail	Without antibiotics	Immediate clearance	Improved septic symptoms	None reported	Temporary resolution

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Bao et al. (2020)	Case report	Shanghai, China	1	*K. pneumoniae*	**First round**: Bladder irrigation with 50 mL of phage cocktail I (5×10^8 PFU/mL for each phage) for 5 days **Second round**: Bladder irrigation with phage cocktail II for 5 days **Third round**: Bladder irrigation with phage cocktail III combined with oral trimethoprim-sulfamethoxazole (800 mg–160 mg) twice daily for 5 days	Bladder irrigation, oral	Cocktail	With non-active antibiotics	Complete eradication	Full UTI resolution	None reported	No recurrence (6 months)
Chen et al. (2022)	Case report	Shenzhen, China	1	*P. aeruginosa*	Intrapleural injection of bacteriophage cocktail (PA3 and PA18) once daily Nebulized bacteriophage administration twice or three times daily Phage dose increased after 11 days due to persistent infection	Intrapleural, nebulization	Cocktail	With antibiotics	Eliminated from effusion	Improved pneumonia	Mild (polymyxin-related)	No infection at discharge
Law et al. (2019)	Case report	San Diego, USA	1	*P. aeruginosa*	Intravenous administration of AB-PA01 (a four-phage cocktail). 4×10^9 PFU in 5 mL IV syringe every 6 h for 8 weeks. Antibiotics (ciprofloxacin, piperacillin-tazobactam, and later doripenem) given concomitantly	Intravenous	Cocktail	With antibiotics	Infection resolved	Full resolution	None reported	Lung transplantation (9 months)

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Petrovic et al. (2020)	Single-arm trial	Sydney, Australia	13	*S. aureus*	Intravenous infusion of AB-SA01 (a three-phage Myoviridae cocktail) 50–100 mL of AB-SA01 in 0.9% NaCl infused over 10–30 min twice daily for 14 days	Intravenous	Cocktail	With antibiotics	Cleared in days	62% improved	None reported	No recurrence (90 days)
Schooley et al. (2017)	Case report	San Diego, USA	1	*A. baumannii*	Intravenous (IV) administration of phage cocktail every 2 h. Intracavitary (directly into abscess cavities) administration of phage cocktail every 6–12 h	Intravenous, intracavitary	Cocktail	With antibiotics	Infection cleared	Significant improvement	None reported	No recurrence; returned to work
Li et al. (2023)	Case report	Shanghai, China	1	*K. pneumoniae*	First course: Single nebulized phage therapy (FKp_GWPB35) for 14 days Second course: Phage cocktail (FKp_GWPB35 + FKp_GWPA139) for 14 days	Nebulization	Cocktail	With antibiotics	Not eradicated, less virulent	Improved symptoms	None reported	Surgery post-therapy
Köhler et al. (2023)	Case report	Geneva, Switzerland	1	*P. aeruginosa*	Initial phage therapy: Daily nebulization of 5×10^9 PFU of phage vFB297 for 5 days Additional two doses given after a 2-day break Subsequent phage courses: Phage vFB297 aerosolized over several days as needed	Nebulization	Mono-phage	With antibiotics	Significant reduction	Respiratory obstruction resolved	Transient fever, desaturation	Lung improvement

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Singh et al. (2024)	Case report	Sydney, Australia	2	*P. aeruginosa*	Bronchoscopic instillation of bacteriophage PBPA103 (Lyse N Tech, South Korea) into all lobes Subsequent nebulization of PBPA103 twice daily for 7 days Concurrent intravenous administration of piperacillin/tazobactam and tobramycin	Bronchoscopy, nebulization	Mono-phage	With antibiotics	1/2 eradicated	Improved lung function	None reported	FEV1% increased
Teney et al. (2024)	Case report	Lyon, France	1	*P. aeruginosa*	Inhaled phage therapy: Three doses (one every 2–3 days) using a vibrating mesh nebulizer Intravenous (IV) phage therapy: Daily injections for 7 days Phages used: PP1792 and PP1797, each at 2×10^9 PFU/mL (inhaled) and 2×10^8 PFU/mL (IV)	Nebulization, intravenous	Cocktail	With antibiotics, interferon-γ	Reduced, not eradicated	Improved pneumonia	None reported	Reduced ventilation

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Gupta et al. (2019)	Prospective observational	Varanasi, India	20	*S. aureus*, *P. aeruginosa*	Customized bacteriophage cocktail (targeting *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) Topical application of 0.1 mL/cm² (10^9 PFU/mL) on alternate days Treatment continued until the wound was microbiologically sterile	Topical	Cocktail	Without antibiotics	60% *S. aureus*, 55.5% *P. aeruginosa* sterile	7/20 healed	None reported	Improved wound margins
Racenis et al. (2023)	Case report	Riga, Latvia	1	*P. aeruginosa*	Intravenous infusion of phages PNM and PT07 (10^7 PFU/mL each) daily for 8 days Local application of 50 mL of phage solution to the wound daily for 3 days Intravenous antibiotics (ceftazidime/avibactam and amikacin) for 6 weeks	Intravenous, local	Cocktail	With antibiotics	Complete eradication	Full resolution	None reported	No recurrence (21 months)
Li & Zhong et al. (2023)	Case report	Shanghai, China	1	*P. aeruginosa*	Three courses of nebulized dsRNA phage phiYY therapy Each course consisted of phage administration via vibrating-mesh nebulizer Phage solution diluted in 10 mL saline (10^8 PFU/mL) Two doses per treatment course with a 4-hour interval	Nebulization	Lytic/Mono-phage	Without antibiotics	Transient reduction	Temporary improvement	Mild fever	No infection post-transplantation

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Liu et al. (2022)	Case report	USA	1	*A. baumannii*	Phage therapy with two cocktails (ΦPC and ΦIV), each containing four bacteriophages Intracavitary administration of ΦPC via percutaneous drains Intravenous administration of ΦIV A ninth phage (AbTP3phi1) was added later to target emerging phage-resistant *A. baumannii* strains	Intravenous, intracavitary	Cocktail	With antibiotics	Initial reduction, resistance	Significant improvement	None reported	Patient recovery
Ramirez-Sanchez et al. (2021)	Case report	San Diego, USA	1	*S. aureus*	First course: Intra-articular phage injection followed by intravenous (IV) infusions of AB-SA01 every 12 h for 2 weeks Second course: A single intraoperative phage dose (SaGR51ø1) plus IV phage infusions every 12 h for 6 weeks Concomitant cefazolin (2 g IV every 8 h) for 6 weeks in both courses	Intravenous, intra-articular	Cocktail, mono-phage	With antibiotics	Eradicated after second cycle	Full resolution	None reported	No recurrence (20 months)

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Zaldastanishvili et al. (2021)	Case series	Tbilisi, Georgia	3	*P. aeruginosa*, *K. pneumoniae*	Patient #1 (Pseudomonas aeruginosa lung infection): Pyo Bacteriophage & Intesti Bacteriophage (8 mL orally, 2 mL via nebulizer daily for 20 days) Custom phage therapy added later Patient #2 (Pseudomonas aeruginosa lung infection): Custom phage therapy administered orally (10 mL twice daily for 20 days) Staphylococcal Bacteriophage for co-infections Patient #3 (Klebsiella pneumoniae urinary tract infection): Custom phage therapy orally (twice daily for 20 days) Custom phage vaginal suppositories for 10 day	Oral, nebulization, vaginal	Cocktail, mono-phage	Mixed	No complete eradication	2/3 improved	None reported	Antibiotic replacement (1/3)
Khatami et al. (2021)	Case report	Sydney, Australia	1	*P. aeruginosa*	Intravenous (IV) administration of phage PASA16 (Pa14NPΦPASA16) 0.9 mL of 10^{11} PFU/mL once daily (days 1, 2, 4–7) or twice daily (days 3 and 8–14) Given as adjunctive therapy with colistin and aztreonam	Intravenous	Mono-phage	With antibiotics	Significant reduction	Pain-free weight-bearing	Transient fever, pain	Radiological improvement

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Corbellino et al. (2020)	Case report	Milan, Italy	1	*K. pneumoniae*	Oral phage therapy: 10 mL of bacteriophage solution (10^6 PFU/mL) twice daily for 3 weeks Intra-rectal administration: 1 million phages daily for 2 weeks No concurrent antibiotic therapy during phage treatment	Oral, intra-rectal	Mono-phage	Without antibiotics	Complete eradication	Resolution	None reported	No recurrence (12 months)
Nir-Paz & Gelman et al. (2019)	Case report	Jerusalem, Israel	1	*A. baumannii*, *K. pneumoniae*	Intravenous (IV) administration of phages ϕAbKT21phi3 and ϕKpKT21phi1 targeting *Acinetobacter baumannii* and *Klebsiella pneumoniae* 1 mL of each phage (5×10^7 PFU/mL) administered intravenously three times daily for 5 days A second course of IV phages was given for an additional 6 days Phage therapy combined with meropenem (2 g TID) and colistin (4.5×10^6 units BID)	Intravenous	Cocktail	With antibiotics	Complete eradication	Full wound healing	None reported	No recurrence (8 months)
Wu et al. (2021)	Case series	Shanghai, China	4	*A. baumannii*	Two successive doses of a 2-phage cocktail (10^9 PFU) administered via inhalation or topical application Phages used: *ϕAb124* (Podoviridae) and *ϕAb121* (Myoviridae)	Nebulization, topical	Cocktail	With antibiotics	Significant reduction	2/4 discharged	1/4 cytokine storm	No recurrence in survivors

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Doub et al. (2022)	Case report	Baltimore, USA	1	*K. pneumoniae*	Intra-articular (IA) bacteriophage therapy (KP1 and KP2) at 10^{10} PFU/mL daily Intravenous (IV) bacteriophage therapy (KP1 and KP2) at 10^{10} PFU/mL daily for 2 days Concurrent 6-week intravenous ertapenem therapy Chronic suppressive therapy with amoxicillin-clavulanate	Intra-articular, intravenous	Cocktail	With antibiotics	Detectable, attenuated	No recurrence	None reported	Improved joint function (14 months)

Table 1 (continued)

Author & Year	Study Design	Study Location	Sample Size	Pathogen (ESKAPE)	Intervention	Route of Administration	Phage Type/Specificity	Combination Therapy	Bacterial Clearance	Clinical Cure	Adverse Effects	Long-term Outcomes
Van Nieuwenhuyse et al. (2021)	Case report	Brussels, Belgium	1	*S. aureus*	In situ phage therapy using BacterioFaag Cocktail 1 (BFC1), a cocktail containing one *Staphylococcus aureus* phage (ISP) and two *Pseudomonas aeruginosa* phages (PNM and 14/1) First dose: 50 mL of BFC1 (10^7 PFU/mL) instilled directly into the surgical site intraoperatively Subsequent treatment: 40 mL of BFC1 instilled through a catheter 3 times daily for 7 days 30 mL of BFC1 instilled 2 times daily for an additional 7 days Site alkalization using sodium bicarbonate solution before each phage instillation Concurrent IV antibiotic therapy (clindamycin, rifampin, ciprofloxacin, later switched to piperacillin-tazobactam)	In situ (catheter)	Cocktail/Mono-phage	With antibiotics	Eradicated, reappeared	Initial improvement	None reported	Infection-free > 2 years

RCT Randomised Controlled Trial, *CRAB* Carbapenem-resistant *Acinetobacter*

sources but used to identify additional references. Studies lacking clear patient outcomes or focusing solely on laboratory models without clinical relevance were excluded.

Data Extraction and Quality Assessment

Data were extracted using a standardized template in Microsoft Excel. Two reviewers independently extracted data, cross-verifying entries for accuracy and completeness.

Data Synthesis

A narrative synthesis was conducted to integrate findings on PT's efficacy, safety, and potential for mitigating outbreaks, structured around key themes: clinical outcomes, adverse events, phage resistance, and infection control applications.

Results

A total of 30 studies were included, comprising randomized controlled trials (n=5), prospective cohort or intervention studies (n=3), single-arm clinical trials (n=2), case series (n=2), and case reports (n=18). Sample sizes ranged from single patients to 264 new acquisitions of carbapenem-resistant *A. baumannii* (CRAB), aged 7 to 81 years. Infections targeted ESKAPE pathogens, primarily *P. aeruginosa* (n=15), *S. aureus* (n=8), *K. pneumoniae* (n=6), *A. baumannii* (n=5), *E. coli* (n=3), with fewer studies on *Enterobacter* spp. (n=1) and none on *E. faecium*. PT was administered via intravenous (n=12), topical (n=7), nebulization/inhalation (n=7), intracavitary/intra-articular (n=4), oral/intra-rectal (n=2), or bronchoscopic routes (n=1), using mono-phage (n=7), cocktail (n=22), or both (n=1). Most studies combined phages with antibiotics (n=21), while nine used phage monotherapies. Outcomes included bacterial clearance, clinical cure, time to eradication, adverse effects, mortality, and long-term effects, with one study addressing outbreak mitigation Table 1.

Phage Types (Monophages & Cocktails)

A variety of phage combinations were used to treat ESKAPE infections across the studies we reviewed. Some studies used monophage therapy (n=7), others employed phage cocktails ranging from 32 phages to 2 phages or less (n=23), and three studies used both, with two studies alternating between monophage and cocktail therapies. Broad-spectrum cocktails containing 12 natural lytic bacteriophages, 32 different phages, polyvalent pyobacteriophages, and multiple lytic phages were separately used in 4 different studies to treat *P. aeruginosa* infections in burn wounds, rhinosinusitis, pediatric tonsillitis, and *S. aureus* infections, respectively [20–23]. Strain specific cocktails containing 6 bacteriophages, 5 different bacteriophages, 4 different lytic bacteriophages, 3 lytic bacteriophages, and AB-SA01 (3 lytic phages), were respectively used to treat *P. aeruginosa* infections in chronic otitis, diabetic foot infections, *P. aeruginosa* mediated cystic fibrosis, *S. aureus* infections, and prosthetic joint infection [24–28]. Non-healing wound infections, ventilator-associated pneumonia from *P. aeruginosa* infection, K. pneumoniae infection, P. aeruginosa infection, and bone infection management were respectively targeted in five different studies with 3 lytic bacteriophages, 2 bacteriophages, 2 lytic bacteriophages, PNM and PT07 (both lytic bacteriophages), and 2 lytic bacteriophages [25, 29–32]. A lytic cocktail of PA3 and PA18 used to treat *P.aeruginosa* associated empyema, KP1 and KP2 (both lytic phages) were employed in the treatment of K.pneumoniae, and two lytic bacteriophages were used to treat *A.baumannii* in COVID-19 patients. Colistin-only-sensitive *P. aeruginosa* strains, *K. pneumoniae*-associated recurrent UTI, multidrug-resistant *A. baumannii*, *A. baumannii* infection, bone allograft infection, chronic lung infections (*P. aeruginosa*) & recurrent urinary tract infection (*K. pneumoniae*), were respectively targeted in 6 different studies with two bacteriophages, multiple lytic phages, custom-designed lytic phages, T4-like myophages and a podophage, *S. aureus* phage ISP and two *P. aeruginosa phages* (PNM & 14/1), and a combination of commercially available monophages with a custom-designed monophage [33–38].

Bacterial Clearance

Bacterial clearance varied by pathogen and study design. Complete eradication was reported in 10 studies, primarily for *P. aeruginosa* [29, 33] and *K. pneumoniae* [34, 39]. A study reported sterilization rates of 60% for S. aureus, 83.3% for *E. coli*, and 55.5% for *P. aeruginosa* by day 13 in chronic wounds [30]. Significant reductions without full clearance occurred in eight studies, including two recent studies [40, 41] for *P. aeruginosa* and *K. pneumoniae*, respectively, often with reduced bacterial virulence. Three studies noted persistent infections despite therapy, particularly in cases involving *P. aeruginosa* and *K. pneumoniae* [32, 33, 35, 38, 42, 43]. In spite of clinical resolution, Chen et al. detected the presence of *P. aeruginosa* in pleural fluid samples collected on days 1, 2, and 5 post-phage therapy; Jennes et al. reported the loss of a patient, 4-months after PT, due to sepsis caused by *K. pneumoniae*; and Zaldastanishvili et al. noted the presence of *K. pneumoniae* in the

urine samples of a patient after multiple 20-day courses of phage therapy. Serum levels of these pathogens remained detectable, even in the presence of clinical resolution of symptoms. In 2016, a study reported a decrease in the CRAB acquisition rate (8.57 to 5.11 per 1,000 patient-days), following environmental phage application [44]. Due to low phage concentrations, a study found slower bacterial reduction with phages than standard care for *P. aeruginosa* burn wounds [20]. Data were unavailable for one ongoing trial [25].

Clinical Cure Rates

Clinical cure or significant improvement was observed in 24 studies. Full resolution occurred in nine cases, including burn wounds [45], osteomyelitis [25], and cystic fibrosis-related pneumonia [32]. One study reported a 62% improvement rate for *S. aureus* infections [27]. Another study noted a 1.4 times faster recovery in children with tonsillitis using PT compared to antibiotics [21]. Results from a single study showed complete wound healing in 7 of 20 patients by day 21 [30]. Partial improvement, characterized by reduced symptoms but incomplete bacterial clearance of *K. pneumoniae*, *P. aeruginosa*, *and A. baumannii*, was reported in six studies [31, 40, 41]; pulmonary symptoms, resolution of respiratory obstruction, improvement in pneumonia and wound healing with successful extubation, reduced cough and expectoration, and no recurrence of symptoms of prosthetic joint infection were reportedly improved. Three studies [35] demonstrated symptom relief without a cure, and one trial found no significant difference in healing compared to standard care [20]. As at the time of this publication, clinical outcomes are pending for one ongoing Phase I/II clinical trial [25].

Time To Bacterial Eradication

Time to eradication ranged from hours to weeks. Rapid clearance occurred in five studies, with negative cultures within days for *P. aeruginosa* and *A. baumannii*/*K. pneumoniae* [25, 29]. In one case report, *K. pneumoniae* clearance was achieved in 5 days with a combination of phage and antibiotic therapy [34]. Also, in a prospective cohort study, phage therapy demonstrated sterilization of various pathogens between 9 and 13 days [30]. Furthermore, a randomized controlled trial observed a median time to bacterial eradication of 144 h for phages versus 47 h for standard care [20]. Partial reductions took longer, often 6–14 days [40, 46]. Seven studies reported no complete eradication, and the timing was unspecified in others due to the study's focus [22].

Adverse Effects

Adverse effects were minimal across studies. No serious phage-related events were reported in 25 studies. Mild effects included transient fever [32, 47], oxygen desaturation [40], and localized pain [47], resolving quickly. One study noted adverse events in 23% of phage patients vs. 54% in standard care, none phage-specific [20]. One patient had a transient cytokine storm that was clinically suspected as a result of elevated body temperature and confirmed by elevated serum levels of IL-6 and IL-8 which resolved within 24 h; this reaction was deemed to be due to immune dysregulation following a previous infection with COVID-19 and a coexisting *C. albicans* infection [48]. Abnormal observations of up to 9% were reported in one study; however, these findings were unrelated to PT [22]. Four studies lacked adverse effect data [30, 35, 39, 44].

Mortality

Mortality was low and unrelated to PT. Nine studies reported deaths: one study noted two deaths from underlying conditions, with another reporting five deaths (38%) due to disease severity, with one death from unrelated sepsis [27, 33, 48]. Most studies ($n=21$) reported no deaths, with patients surviving after treatment [36, 39].

Long-Term Outcomes

Long-term outcomes, assessed from 6 weeks to 3 years, were favorable in 18 studies. No infection recurrence was reported for up to 21 months [29], 20 months [28], and 12 months [39]. Successful lung transplantation 9 months post-therapy was possible in one study [26]. In another study, improved lung function was reported in two patients, 9 months post-PT; FEV_1 (Forced Expiratory Volume in 1 s) improved by 4% and 5% when compared to previously recorded values over the preceding three years, with an overall improvement of 12% and 8% from baseline [46]. Despite incomplete bacterial clearance, sustained symptom control was reported in two patients [35]. Similarly, a study noted infection-free status for over two years post-surgery, despite *S. aureus* reappearance in one patient. Seven studies lacked long-term data, and one was ongoing [25].

Outbreak Mitigation Potential

One study directly addressed the mitigation of outbreaks [44]. Applied aerosolized phages in an ICU, reduced CRAB acquisition rates ($p=0.0029$) and antibiotic use, with CRAB resistance dropping from 87.76 to 46.07% ($p=0.001$). Other studies indirectly support infection control, with

rapid clearance in hospital settings [29, 33], suggesting the potential to limit nosocomial spread, although this was not explicitly measured [29, 43].

Discussion

This narrative review synthesizes findings from 30 studies to evaluate the efficacy, safety, and potential for outbreak mitigation of PT for infections caused by ESKAPE pathogens. The evidence presents a compelling picture of PT as a targeted and safe alternative to antibiotics amid the growing crisis of AMR, while also highlighting the hurdles that must be overcome to realize its clinical and public health potential fully. PT reduces bacterial loads in infections caused by ESKAPE pathogens, with 10 studies reporting complete eradication, particularly for *P. aeruginosa* and *K. pneumoniae* [29, 33, 34, 39]. These successes are important, given the resistance of these pathogens to multiple antibiotics, which often leaves clinicians with few options. Even when total bacterial clearance was not achieved, PT consistently reduced the severity of infections and improved patient outcomes, as seen in complex cases such as chronic wounds and cystic fibrosis-related pneumonia [26, 30, 40, 41]. A standout feature of PT is its synergy with antibiotics, as several studies have demonstrated that phage-antibiotic combinations not only enhance bacterial clearance but also restore susceptibility in previously resistant strains [34, 36, 42]. For instance, one case report documented the clearance of *K. pneumoniae* in just five days using this combination, demonstrating the potential to bypass resistance barriers [34]. Phage monotherapy also proved effective, particularly for localized infections like burn wounds, suggesting that PT can be tailored to diverse clinical scenarios [33, 45].

However, the efficacy of PT is not uniform. Outcomes varied depending on the pathogen, delivery method, and type of infection. *P. aeruginosa* and *K. pneumoniae* responded more reliably than *S. aureus* or *Acinetobacter baumannii*, possibly due to differences in phage specificity or the complexity of bacterial biofilms [30]. Delivery methods, such as intravenous or nebulized administration, often resulted in rapid clearance, whereas topical applications, although effective for chronic wounds, sometimes required longer treatment times [20, 29]. These differences highlight the need for customized phage selection and optimized delivery protocols. Most studies have employed phage cocktails to reduce the risk of resistance, but challenges such as phage stability and precise dosing have persisted [20]. One study, for example, attributed slower bacterial reduction to unexpectedly low phage concentrations (10–100 PFU/mL [Plaque-Forming Units/mililiter]), emphasizing the critical role of quality control in phage preparations [20] Figs. 1 and 2.

Safety is a clear strength of PT. Across 25 studies, no serious adverse events were linked to phage therapy, a stark contrast to antibiotics, which can disrupt the body's microbiota or cause toxicity [20, 22, 32, 40, 47]. Mild, short-lived effects, such as fever or localized pain, were rare and typically resolved quickly, even in vulnerable groups, including children and immunocompromised patients [21, 46]. One study reported adverse events in only 23% of PT patients, compared to 54% in those receiving standard care, with none of the events directly tied to phages [20]. Mortality, reported in nine studies, was consistently unrelated to PT, with deaths attributed to underlying conditions or unrelated complications like sepsis [27, 33, 48]. Despite this reassuring safety profile, gaps remain. Four studies did not report adverse effects data, and the lack of large-scale clinical trials limits a comprehensive understanding of PT's safety across diverse populations and long-term use.

One of the most important findings is PT's potential to curb nosocomial outbreaks. A pivotal study demonstrated that aerosolized phages in an intensive care unit significantly reduced the acquisition rate of carbapenem-resistant *A.r baumannii*, dropping from 8.57 to 5.11 per 1,000 patient days [42]. This intervention also lowered antibiotic use and resistance rates, suggesting that PT could play a dual role in infection control and AMR mitigation [42]. Other studies indirectly supported this potential by showing rapid bacterial clearance in hospital settings, which could limit pathogen spread [29, 43]. These findings are particularly relevant for ESKAPE pathogens, which are major drivers of hospital-acquired infections. Yet, with only one study directly addressing outbreak control, more research is needed to explore how environmental phage applications can be scaled up for broader public health impact.

Despite its promise, PT faces challenges. Bacterial resistance to phages, although less common than antibiotic resistance, has been observed in some studies, often due to mutations in phage receptors [35, 38]. In one study, despite the resolution of clinical symptoms, K. pneumonia remained detectable in blood samples for up to six months post-treatment [43]. Resistance was also observed in the treatment of a patient with carbapenem-resistant *A.baumanii* co-infection with COVID-19 [48]. Furthermore, strain diversification and altered phage susceptibility led to the incomplete eradication of *P. aeruginosa* infection in three patients [35]. This issue shows the need for dynamic phage cocktails or engineered phages to stay ahead of evolving bacteria [19]. Variability in phage stability and specificity also affected outcomes, with one study noting reduced efficacy due to suboptimal phage concentrations [20]. The personalized nature of PT, while a strength for targeting

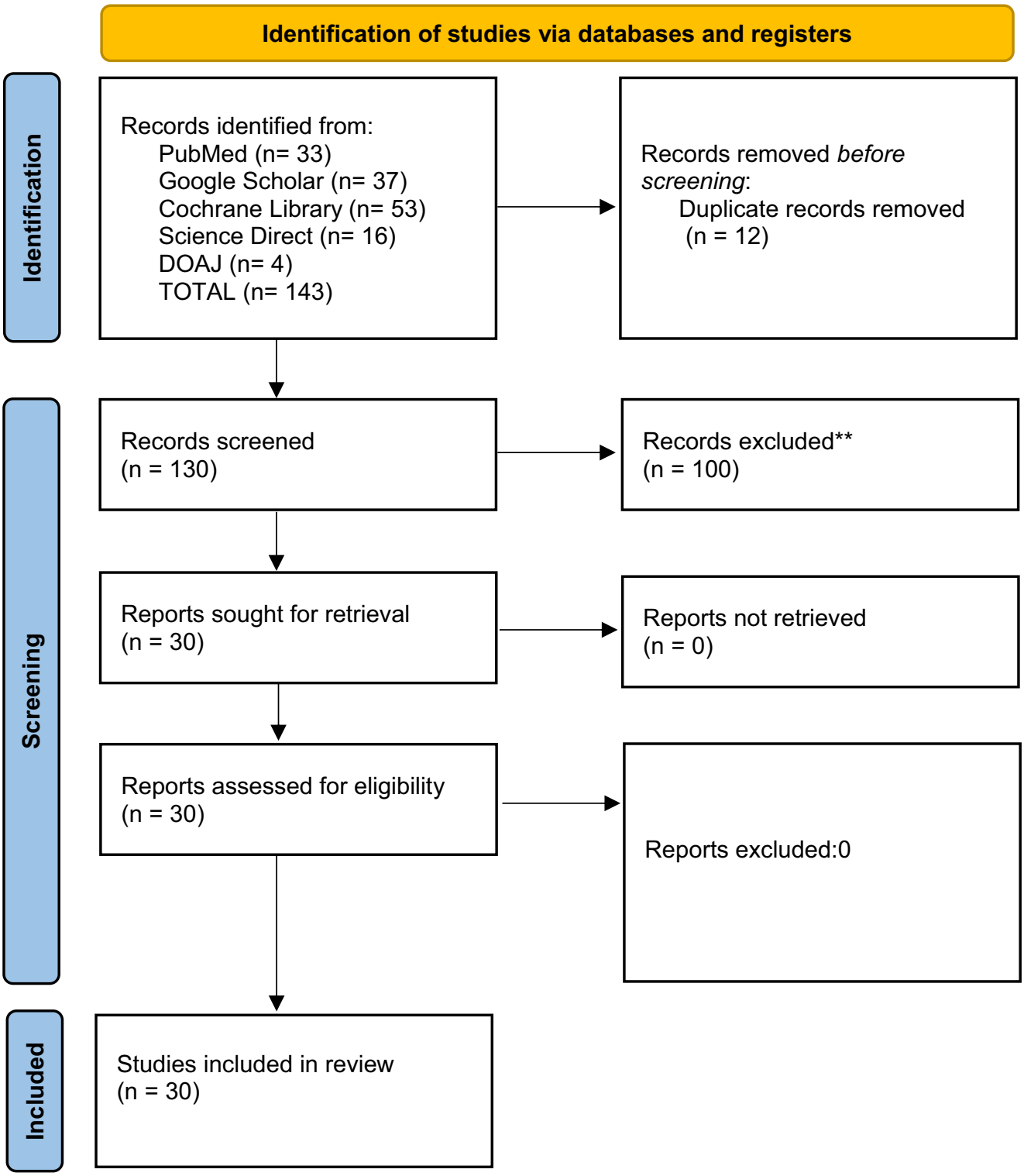

Fig. 1 PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

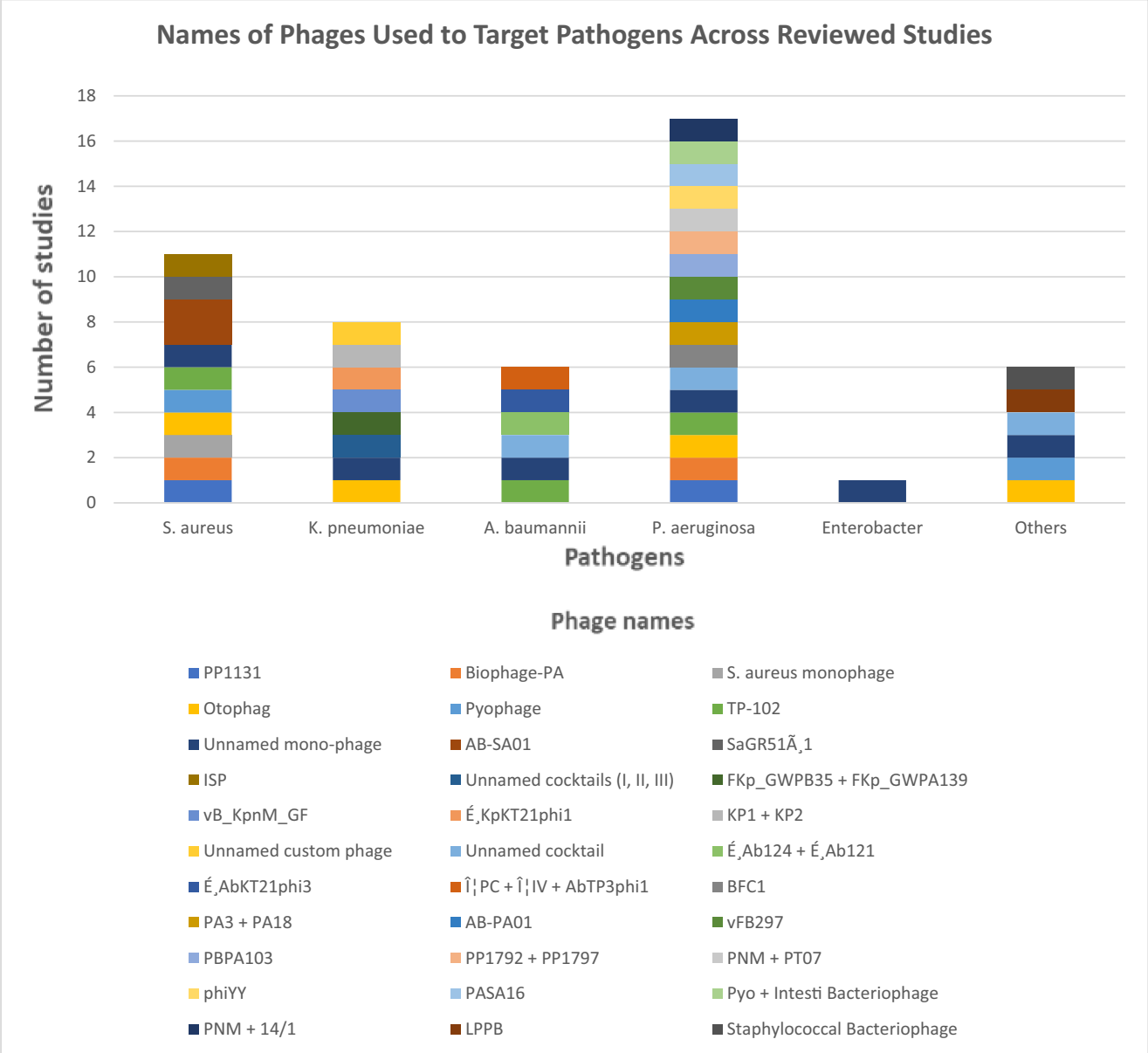

Fig. 2 Names of phages used to target pathogens across reviewed studies

specific bacterial strains, creates logistical hurdles, such as the need for rapid phage matching and production, which is particularly challenging in acute infections. The absence of standardized protocols for phage preparation, dosing, and administration further complicates the reproducibility and scalability of these approaches. Additionally, long-term data on phage resistance and the body's immunological response to repeated PT are scarce, limiting insights into its sustained effectiveness [35].

A potential challenge that may arise stems from the localization of microbiota into specific body compartments. This poses a challenge for bacteriophage therapies with limited volumes of distribution, and constitutional designs that make certain body compartments impregnable. This may create pseudo-resistance where, though a bacteriophage is efficacious against a microorganism, it is unable to completely eradicate it as a result of limited body compartment distribution. This necessitates the development of phage preparations that are optimized to concentrate in specific body compartments where infections are localized, increasing their location specificity and potency in eliminating ESKAPE infections.

Methodologically, the evidence base has limitations. Many studies were small-scale, with 18 case reports and few randomized controlled trials, which restricts generalizability. The complete absence of studies on *E. faecium* and

limited data on *Enterobacter* spp. reveal gaps in addressing the full ESKAPE spectrum. While in vitro and animal studies offered valuable mechanistic insights, their applicability to human infections remains uncertain without larger clinical trials.

This review represents the first systematic compilation of clinical evidence on PT's role against ESKAPE pathogens, providing a comprehensive assessment of its efficacy, safety, and potential in outbreak control. Its inclusion of diverse study designs and administration routes provides a robust foundation for understanding the clinical applications of PT. The synergy of phage-antibiotic combinations and PT's ability to restore antibiotic susceptibility makes a strong case for its integration into strategies to combat AMR [34, 36, 42]. The evidence of PT's role in infection control, though preliminary, opens an exciting avenue for public health innovation [44].

PT's specificity, adaptability, and synergy with antibiotics position it as a powerful tool in the fight against AMR. By combining phages with antibiotics, clinicians can leverage complementary mechanisms, such as antibiotics promoting bacterial changes that enhance phage effectiveness [49]. This approach could prolong the utility of existing antibiotics, easing the pressure to develop new ones. PT's potential in outbreak mitigation aligns with global AMR strategies that prioritize infection prevention, but its integration into clinical practice requires overcoming regulatory and scientific barriers. Standardized production protocols and flexible regulatory frameworks, similar to those used for biologics like viral vector vaccines, are essential [50, 51].

To advance PT, policymakers and clinicians should prioritize several steps. First, regulatory bodies must establish clear guidelines for PT, drawing on existing frameworks for biologics to ensure safety and efficacy [50]. Second, large-scale randomized controlled trials are crucial for validating PT's effectiveness and safety across diverse populations and pathogens, particularly those that are underrepresented in current research. Third, pilot programs testing environmental phage applications in high-risk settings, such as intensive care units, could confirm PT's role in outbreak control, thereby shaping hospital infection control policies.

Future studies should focus on next-generation phage therapies, such as genetically engineered phages with enhanced specificity and resistance-proof designs [52, 53]. Personalized phage banks, continuously updated with new phages, could keep pace with evolving bacterial populations. Long-term studies are also needed to track bacterial resistance to phages and the immunological effects of repeated PT, providing insights into how to delay resistance [54–56]. In addition, scalable models for PT delivery, such as regional phage libraries or automated phage-matching platforms, could streamline its use in clinical settings, making it more accessible [57].

Conclusion

PT offers a promising, safe, and effective solution for combating ESKAPE-related infections and addressing the global crisis of antimicrobial resistance. Its demonstrated ability to clear bacterial loads, improve clinical outcomes, and potentially curb nosocomial outbreaks underscores its transformative potential in modern medicine. However, challenges such as phage resistance, variability in efficacy, and regulatory hurdles demand urgent attention through rigorous research and policy innovation. By prioritizing large-scale clinical trials, standardized protocols, and innovative phage technologies, such as engineered phages and personalized phage banks, bacteriophage therapy can be positioned as a cornerstone of strategies to mitigate multidrug-resistant infections, providing a critical lifeline in an era where antibiotic options are increasingly limited.

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  This article was chosen because it is the only study that explores the potential of bacteriophage therapy to mitigate disease outbreaks. In the study, aerosolised phages were used to limit the transmission of carbapenem-resistant *A. baumannii*, reducing infection rates from 8.57 to 5.11 per 1000 patient days ($p=0.0029$), also reducing carbapenem resistance from 87.76 to 46.07% ($p=0.0001$), with decreased antibiotic use.

- Jault P, Leclerc T, Jennes S, Pirnay JP, Que YA, Resch G, Rousseau AF, Ravat F, Carsin H, Le Floch R, Schaal JV. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. The Lancet Infectious Diseases. 2019 Jan 1;19(1):35–45. https://www.thelancet.c

om/journals/laninf/article/PIIS1473-3099(18)30482-1/abstract.

This study was selected because of its rigorous methodology. Being a double-blind, randomized controlled trial, its results have stronger evidence for generalizability compared to case reports. Also, the unexpected challenges of low phage concentrations reinforce the need for standardized dosing protocols as highlighted in our discussion.

- Petrovic Fabijan A, Lin RC, Ho J, Maddocks S, Ben Zakour NL, Iredell JR, Westmead Bacteriophage Therapy Team Khalid Ali 1 3 Venturini Carola 1 3 Chard Richard 3 7 Morales Sandra 8 Sandaradura Indy 2 3 Gilbey Tim 2. Safety of bacteriophage therapy in severe Staphylococcus aureus infection. Nature microbiology. 2020 Mar 2;5(3):465−72. https://www.nature.com/articles/s41564-019-0634-z.

  This study was chosen because of the generalizability potential of its findings. It is the only clinical trial among the studies we reviewed that focused on the safety profile of bacteriophage therapy medications. By establishing the safety profile of intravenous phage therapy without infusion-related adverse events or phage resistance, it is a key study in the corpus of evidence for the safety of phage therapy.

Abbreviations

WHO	World Health Organization
AMR	Antimicrobial Resistance
MDR	Multi–Drug Resistant bacteria
ESKAPE	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species*
PT	Phage Therapy
IND	Investigational New Drug
IST	Initial Safety Testing
CDE	Center for Drug Evaluation
NMPA	National Medical Products Administration

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Declarations

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Competing interests The authors declare no competing interests.

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