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Phage-Antibiotic Combination Therapy for Antibiotic-Resistant Bacteria; Synergistic Mechanisms and Clinical Applications: A Systematic Review

Seyed Jalil Mousavi [1], *Hassan Nazari [2], Sayed Hussain Mosawi [3]

1. *Department of Infectious Diseases, Urmia University of Medical Sciences, Urmia, Iran*
2. *Social Determinants of Health Research Center, Urmia University of Medical Sciences, Urmia, Iran*
3. *Medical Sciences Research Center, Ghalib University, Kabul, Afghanistan*

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*Corresponding Author:
Email: h.nazari4@gmail.com

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a major global health challenge requiring innovative therapeutic strategies. Phage–antibiotic combination therapy has emerged as a potential approach for treating multidrug-resistant (MDR) bacterial infections. This systematic review summarizes reported synergistic effects and clinical outcomes of this combined strategy.

Methods: This review was conducted according to PRISMA guidelines. PubMed, Scopus, Web of Science, and the Cochrane Library were searched for studies published between January 2000 and December 2024 assessing combined bacteriophage and antibiotic therapy against MDR bacteria. Eligible studies focused on therapeutic synergy, bacterial resensitization, and clinical outcomes, with results synthesized narratively.

Results: A total of 25 studies were included, comprising 10 in vitro studies, 7 in vivo animal studies, and 8 clinical investigations. Phage–antibiotic combinations demonstrated synergistic effects in more than 70% of cases, resulting in enhanced bacterial eradication, improved biofilm disruption, and reduced resistance development compared with monotherapy. The mean Fractional Inhibitory Concentration Index (FIC-I) for synergistic combinations in in vitro studies was 0.29±0.11. Animal studies reported protection or survival rates ranging from 64% to 100%. Clinical reports, including case studies and one phase 2 trial, documented successful treatment of refractory MDR infections without major safety concerns, although adverse events were inconsistently reported.

Conclusion: Phage–antibiotic combination therapy appears to be a promising approach for managing MDR bacterial infections. Despite encouraging evidence of enhanced efficacy, challenges such as phage resistance, host immune responses, and variability in interactions remain. Larger, well-designed clinical trials and individualized testing are necessary to confirm clinical efficacy and optimize therapeutic application.

Keywords: Phage-antibiotic combination, Antimicrobial resistance, Synergy, Multidrug-resistant bacteria, Bacteriophage therapy, Resensitization

Introduction

AMR is one of today's most serious threats to public health in the 21st century (1). According to the WHO, Millions of people die each year from infections caused by antibiotic-resistant bacteria, with an estimated 1.27 million direct deaths globally in 2019 (2). At the same time AMR creates significant economic costs to global healthcare systems (2). Increased and often inappropriate use of antimicrobial drugs has rapidly increased this problem, such that many standard treatments are no longer effective (1).

To further complicate the situation, medical innovation and new classes of antibiotics has slowed dramatically in recent years because of exorbitant research and development costs and low profitability (3). Considering this economic pressure, and that there are limited avenues to revitalize drugs, the need for alternative therapeutic approaches, like phage-antibiotic combination therapy, to revive existing drugs or create new ways of attacking infections is crucial. Public health legislation, such as the PASTEUR Act in the United States, reinforces the importance of working with the public sector to make this change and continue to innovate towards the development of sustainable antimicrobial therapeutic options (4).

Bacteriophages (phages), which are viruses that specifically attack and lyse bacteria, have regained a reputation as therapeutic agents despite the limitations of traditional antibiotics (1,5). The history of phage therapy goes back to the 20th century when Felix d'Hérelle's discovery of bacteriophages provided new optimism for infection treatment. However, with the discovery and subsequent widespread use of antibiotics, phage therapy receded into the background until recently, when the rise of AMR allowed phage therapy to be brought back into the light.

Phages have unique advantages; their specificity for pathogenic cells also allows for the protective and beneficial microbiota to be spared, unlike broad-spectrum antibiotics (1). They also replicate at the site of infection as they lyse pathogenic cells, decreasing the amount required and potentially limiting the necessary doses, as well as having few side effects (6). Perhaps the most significant feature of phages is that they work on MDR bacteria, making them a fundamental option for treating infections where the therapeutic options may unfortunately be limited (1). Recent clinical successes and current trials once again support their viability as therapeutic options (3).

Phage-antibiotic synergy (PAS) merges phages with antibiotics to enhance their effect and combat antibiotic resistance (2). This combination provides increased bacterial clearance, penetration of biofilms, and reduces the chance of resistance development (7,8). The "dual selective pressure" hypothesis contends that when phages and antibiotics are administered simultaneously, there is more than one selective pressure on pathogenic bacteria that acts through different lethal mechanisms.

Resistance to both phages and antibiotics is less likely for bacteria due to the risk of developing resistance to one agent at the expense of susceptibility and/or infectivity to the other agent (10). For example, a bacteria's phage resistance may reduce sensitivity to the antibiotic while the antibiotic treatment could promote phage infectivity creating an evolutionary dilemma for the bacteria (11).

We aimed to systematically evaluate the evidence for phage-antibiotic synergy, key mechanisms of action, and clinical efficacy/safety outcomes based on studies published between 2000–2024. Crucially, this review distinguishes itself by providing a structured synthesis of the heterogeneous evidence base, focusing specifically on the interplay between synergistic mechanisms (mechanistic heterogeneity) and the resulting clinical/safety outcomes, addressing the current knowledge gap regarding translational barriers (e.g., pharmacokinetics and immune responses).

Methods

This systematic review followed the PRISMA guidelines. The research question was stated using the PICO framework :

Intervention: Combined use of bacteriophage and antibiotic therapy .

Comparison: Phages or antibiotics alone as monotherapy .

Outcomes: Synergy (for example, fractional inhibitory concentration index <0.5), resensitization (e.g. reduction in MIC), eradication of infection, reduced emergence of resistance, and clinical outcomes (e.g. survival, symptom resolution).

Search Strategy

A comprehensive literature search was conducted across PubMed, Scopus, Web of Science, and Cochrane Library, targeting studies published between January 2000 and December 2024. Keywords included "bacteriophage," "phage," "antibiotic," "synergy," "resistance," and "MDR," combined using Boolean operators. An example search string for PubMed was: ("bacteriophage" OR "phage") AND ("antibiotic" OR "antimicrobial") AND ("synergy" OR "combination" OR "resensitization") AND ("resistance" OR "MDR" OR "multidrug-resistant") AND ("bacteria" OR "infection"). Analogous adapted strings were used for other databases, tailoring controlled vocabulary (e.g., MeSH terms in PubMed, Subject Headings in Scopus) where applicable. The search was limited to articles published in English and without geographical restriction. Other databases had analogous adapted strings which produced a total of 1,456 records (after deduplication). The search yielded: PubMed (601), Scopus (450), Web of Science (380), and Cochrane Library (25). Additionally, we supplemented our search with hand-searching relevant articles from the reference lists of key reviews.

Study Selection

he selection process entailed two steps: an initial screening of titles and abstracts followed by a full-text review, conducted independently by two reviewers (Reviewer A [Javad Rasouli] and Reviewer B [Rahim Nejadrahim]). Disagreements were resolved through discussion, or by consultation with a third reviewer when necessary. Inter-rater reliability (IRR) was calculated using Cohen's Kappa (κ), yielding values of 0.82 for title/abstract screening and 0.91 for full-text review, indicating strong agreement. When consensus was not achieved, a third reviewer (Reviewer C [Hasan Habibpour-Fattahi]) was consulted, and their decision was considered final. This process is illustrated in the PRISMA flow diagram (Figure 1).

A total of 1,456 records were identified across the databases and grey literature. Before screening, 777 records were removed (743 duplicates, 18 automatically marked as ineligible by automation tools, and 16 removed for other reasons). The remaining 679 records were screened by title and abstract, of which 539 were excluded. This resulted in 140 potentially eligible records. Of these 140, 58 records were not pursued for full-text retrieval because they were either conference abstracts, books, or protocols that did not represent complete primary research data. This left 82 full-text reports sought for retrieval, with 11 not retrieved, leaving 71 articles assessed for eligibility. Of these, 51 were excluded (16 not of appropriate study design, 19 not involving phage–antibiotic combination therapy, and 16 for other reasons), resulting in 25 studies included in the final qualitative synthesis.

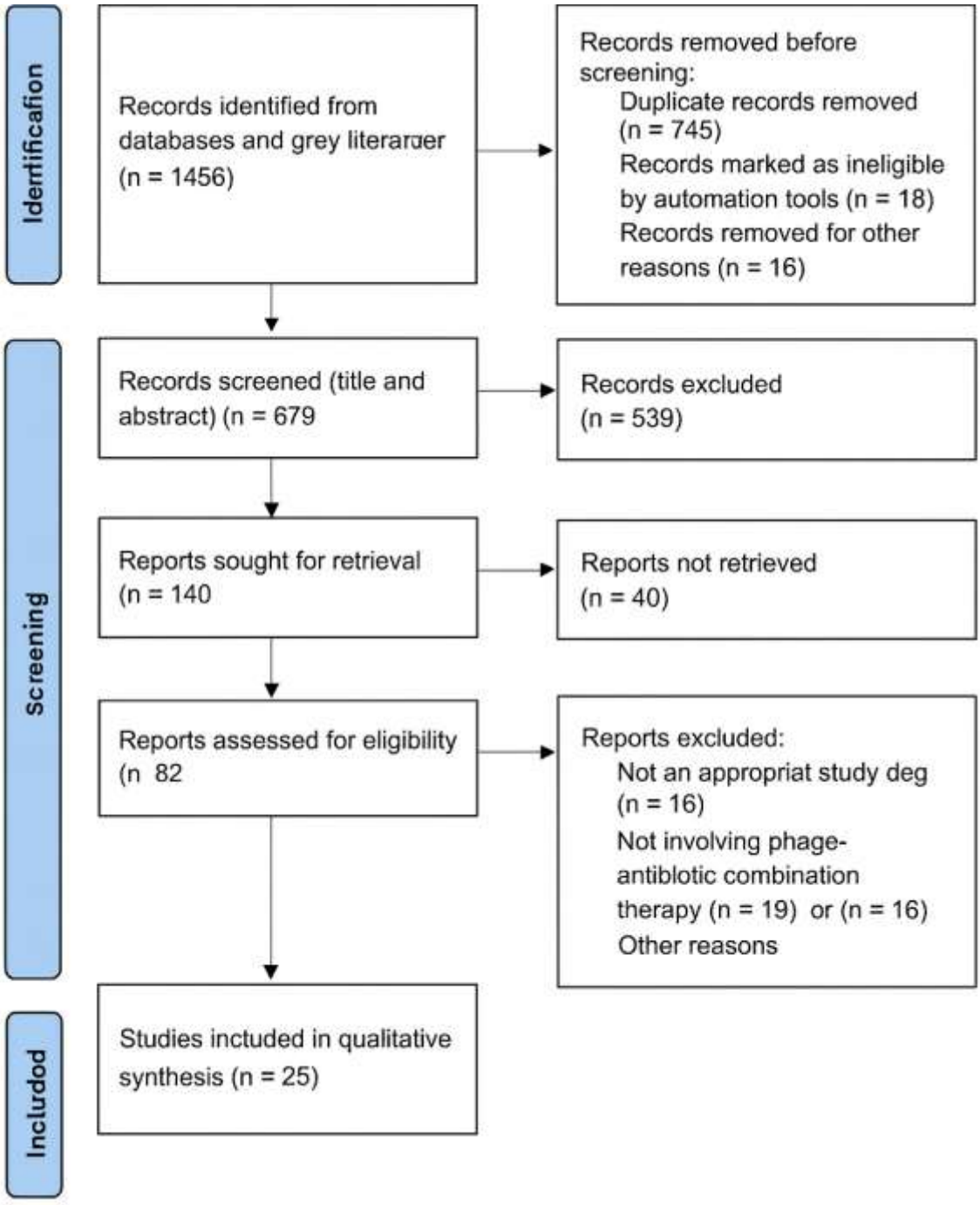

Figure 1: PRISMA flow diagram

Inclusion Criteria

• Studies on combined phage and antibiotic therapy for antibiotic-resistant bacteria.
• Investigations of synergy, resensitization, or related interactions.
• In vitro, in vivo (animal), and clinical studies (case reports or trials).

Exclusion Criteria

• Studies on monotherapy with phages or antibiotics alone.

• Non-systematic reviews (except for background context).
• Studies on antibiotic-sensitive bacteria.

Data Extraction and Quality Assessment

Data were extracted from studies using a standardized extraction form that included study characteristics, bacterial pathogens, antibiotics and phages used, and key findings. The extraction form was piloted on five randomly selected included studies and refined accordingly. All extracted data were subsequently cross-verified by a second reviewer, and any discrepancies were resolved by consensus. The quality of the included literature was assessed with the appropriate tool for study type (e.g., Cochrane Risk of Bias 2 (RoB 2) (clinical trials), SYRCLE's Risk of Bias tool (animal studies), or self-designed checklist (*in vitro* studies)) and the quality assessment in the RoB 2 tool focused on risk of bias judging randomization, deviations from intended interventions, missing data, outcome measurement, and reported results; SYRCLE's tool addressed selection, performance, and detection bias; and the checklist for *in vitro* studies levels of reproducibility, controls, and relationship to statistics, and assessed quality using a ten-point scale.

The self-designed checklist for *in vitro* studies assessed quality based on: 1) Defined study aim, 2) Appropriate control groups, 3) Defined Phage/Antibiotic concentrations, 4) Statistical power/reproducibility, 5) Use of clinical isolates (MDR), 6) Defined synergy metric (e.g., FICI), 7) Evaluation of resistance emergence, 8) Clarity of statistical methods, 9) Adequate presentation of results, and 10) Suitability for the research question. This systematic and rigorous quality assessment ensured high quality studies were evaluated, and to represent the review findings on the overall quality of evidence, the individual quality assessment scores and risk of bias judgments were incorporated into an expanded Table 1.

Data Synthesis

Due to the high heterogeneity in study designs, bacterial strains, antibiotic–phage combinations, and reported outcomes, a quantitative meta-analysis: was not feasible. The primary obstacles to meta-analysis were the variability in synergy metrics (e.g., FICI vs. fractional killing), diverse phage formulations (single vs. cocktail), and, most critically, the lack of standardized reporting on outcomes (e.g., survival rates were reported across different animal species and models, precluding pooling of effect sizes). Therefore, a narrative synthesis was employed, grouping studies by methodologies for synergy assessment, types of bacteria, and clinical applications. A summary table (Table 1) was prepared to provide comparative reference across studies. Publication bias was evaluated qualitatively by examining the distribution of study types and their reported outcomes, with an indication of potential bias towards positive results due to the prevalence of case reports and non-randomized studies. This qualitative assessment was based on the prevalence of small studies (case reports and *in vitro* studies with low statistical power) that reported overwhelmingly successful outcomes (synergy >70%), a classic indicator of potential reporting bias.

Results

Twenty-five primary research articles were included. These articles, all published between January 2000 and December 2024, encompassed *in vitro* studies, *in vivo* animal studies, and clinical studies, and collectively demonstrated the efficacy and mechanisms of phage-antibiotic combination therapy for MDR bacteria. To avoid double counting, studies were primarily categorized based on the highest level of evidence provided, although full data from all 25 studies were synthesized (Table 1).

Quality Assessment Summary

Overall quality was moderate to high. In assessing the 5 clinical studies (including trials and case reports), Cochrane RoB 2 showed low risk in 2 and high risk in 3 (primarily due to the lack of randomization and blinding in case reports, which constituted 62.5% of the clinical studies). In the 10 animal studies, SYRCLE assessments showed unclear risk in selection bias for 6 (e.g., inconclusive randomization) but low risk in reporting outcomes. The 10 *in vitro* studies scored 7-9/10 on the checklist we customized. Overall, these study assessments indicated good quality controls for modest efficacy, except for several *in vitro* studies with low statistical power. Initial assessments for effectiveness should be viewed cautiously since low-quality studies (i.e. case reports) often overestimate effects based on lack of study size with low-level findings.

Table 1: Summary of selected studies

Reference No.	***Study Type***	***Bacterial Pathogen(s)***	***Antibiotic(s) Used***	***Phage(s) Used***	***Key Findings (Synergy/Resensitization/Interactions)***	***Quality Score / RoB Judgement***
29	In Vivo (Broilers)	*Escherichia coli*	Enrofloxacin	2 phages	Total protection with combination, significant synergy, superior to monotherapy.	SYRCLE: Low RoB (Veterinary Model)
27	In Vitro & In Vivo (*Galleria mellonella*)	*Burkholderia cenocepacia*	Meropenem, Ciprofloxacin, Tetracycline	KS12, KS14	PAS observed; antibiotics stimulated increased phage production/activity; increased plaque diameters, phage titers; increased survival in *Galleria mellonella* larvae with combination therapy.	SYRCLE: Low RoB (Clear Synergistic Mechanism)
26	In Vitro & In Vivo (Rats)	*Pseudomonas aeruginosa*	Ciprofloxacin	PP1131 cocktail	High synergy, cleared infection, reduced virulence (fitness cost of resistance).	SYRCLE: Low RoB (Complex Infection Model)
13	In Vitro	*Pseudomonas aeruginosa*	Ciprofloxacin	PEV20	Significant synergy, maintained after nebulization, suppressed regrowth.	7/10 (Moderate Quality, Single Phage/Antibiotic)
14	In Vitro	MDR *Acinetobacter baumannii*	Meropenem, Ciprofloxacin, Colistin	KARL-1	Synergy with meropenem/colistin, negative interaction with ciprofloxacin.	8/10 (High Quality, Comparative Assessment)
16	In Vitro	MDR *Klebsiella pneumoniae* (biofilm-forming)	Polymyxin	SH-KP152226 (Dep42)	Phage-encoded depolymerase degraded capsule/biofilms; enhanced polymyxin activity.	9/10 (High Quality, Depolymerase Mechanism)
36	Clinical (Case Report)	MDR *Pseudomonas aeruginosa*	Systemic antibiotics	Phage therapy	Successful adjunctive use with systemic antibiotics; clinical resolution of infection; no adverse events.	RoB 2: High RoB (Case Report)

22	Clinical (Case Report) & In Vitro	Extensively Drug-Resistant *Klebsiella pneumoniae*	Sulfamethoxazole-trimethoprim (non-active)	Cocktail III	Non-active antibiotic + phage synergism (NABS) inhibited emergence of phage-resistant mutants *in vitro*; successfully cured recurrent UTI in patient.	RoB 2: High RoB (Case Report)
18	In Vitro & In Vivo (Zebrafish, Mice)	Antibiotic-resistant *Escherichia coli*	Kanamycin, Chloramphenicol, Ampicillin	ΦEcSw	Synergistic lytic activity with kanamycin/chloramphenicol; ampicillin did not inhibit phage titre; *in vivo* control of MDR *E. coli*.	SYRCLE: Unclear RoB (Complexity of Models)
11	In Vitro	Extraintestinal *Escherichia coli* (ExPEC, drug-resistant)	Various antibiotics	ΦHP3	Phages lowered MIC for drug-resistant strains; synergy/antagonism dependent on antibiotic class and stoichiometry; suppressed emergence of resistant cells.	8/10 (High Quality, Focus on Stoichiometry)
37	Clinical (Case Report)	ESBL-producing *Escherichia coli*	Ertapenem	Four-phage cocktail	Patient tolerated phage therapy without adverse events, symptom resolution; additive effects observed.	RoB 2: High RoB (Case Report)
28	In Vivo (Mice)	MDR *Klebsiella pneumoniae*	Gentamicin	vB_KpnM_P-KP2	Completely rescued mice, bacterial elimination, inhibited inflammation; superior to monotherapy.	SYRCLE: Low RoB (Clear Outcome)
38	Clinical (Case Report)	Pandrug-resistant *Pseudomonas aeruginosa*	Systemic antibiotics	Personalized phage cocktail	Successful management of spinal abscess; patient healed with local and IV phages as adjuvant therapy.	RoB 2: **High RoB** (Case Report)
33	In Vivo (Mice)	*Yersinia pestis*	Ceftriaxone	Phage cocktail	Significantly improved outcomes (100% survival), complete clearance of pathogens.	SYRCLE: Low RoB (Clear Outcome)
34	In Vitro & In Vivo (*Galleria mellonella*)	CRAB *Acinetobacter baumannii*	Colistin	vWU2001	Significantly higher inhibition/clearance, synergistic effect.	SYRCLE: Low RoB (Clear Synergistic Effect)
17	In Vitro & In Vivo (*Galleria mellonella*)	MDR *Acinetobacter baumannii*	Colistin	Dpo71 (depolymerase)	Depolymerase sensitized bacteria to colistin, enhanced antibiofilm activity, improved survival.	SYRCLE: Low RoB (High Quality Lab Study)
19	In Vitro	MDR *Enterococcus faecium* (biofilm-embedded)	Daptomycin, Ampicillin	3- or 4-phage cocktails	Significant killing of biofilm-embedded *E. faecium*; antibiotic resistance stabilization; prevention/reduction of phage resistance.	8/10 (High Quality, Focus on Biofilm)
10	In Vitro	MDR *Acinetobacter baumannii*	Colistin	vB_AbaM-IME-AB2	Phage first for greatest synergy but failed to prevent resistance; simultaneous/antibiotic first suppressed/delayed resistance.	9/10 (High Quality, Focus on Evolution)

41	Clinical (Case Report) & In Vitro	Vancomycin-resistant *E. faecium*	Systemic antibiotics	Φ9184, ΦHi3	Clinical improvement, reduced bacterial burden; *in vitro* improved bacterial growth suppression; antiphage antibody response emerged limiting efficacy.	RoB 2: High RoB (Case Report, Antiphage Response)
40	Clinical (Phase 2 Clinical Trial)	Drug-resistant *Escherichia coli*	Oral trimethoprim/sulfamethoxazole	LBPEC01 (CRISPR-Cas3-enhanced)	Safe, rapid bacterial reduction, symptom-free.	RoB 2: Low RoB (Randomized Trial)
39	Clinical (Case Report - Veterinary)	MDR *Pseudomonas aeruginosa*	Ceftazidime	ΦPASB 7	Successful personalized phage-antibiotic treatment; complete wound closure.	RoB 2: High RoB (Veterinary Case)
25	Clinical (Retrospective Observational Study)	Various MDR bacteria	Concomitant antibiotics	Various phages/cocktails	Patients without concomitant antibiotics had a 70% lower chance of eradication	RoB 2: High RoB (Retrospective, Confounding)
21	In Vitro & In Vivo (*Galleria mellonella*)	MRSA (*Staphylococcus aureus*)	Fosfomycin, Vancomycin, Oxacillin, Ciprofloxacin	vB_Sau_S90	Synergistic effect (FIC < 0.5); sequential therapy (phage before antibiotic) more effective.	SYRCLE: Low RoB (Clear Comparison of Sequence)
24	In Vitro	*Escherichia coli* C	Chloramphenicol, Gentamicin	ΦX174	Antibiotics influence phage resistance evolution; suppressed mutants, specific concentrations for synergy.	8/10 (High Quality, Evolutionary Focus)
9	In vitro experimental study	Enterococcus faecalis (33 clinical isolates)	β-lactam antibiotics (including ampicillin and related compounds)	vB_Efa29212_2e and vB_Efa29212_3e	Phage–antibiotic combinations enhance bacterial killing, reduce resistance, and improve antibiotic effectiveness through mutual reinforcement of phage activity and drug penetration.	8/10 (High Quality, Defined Metrics)

Results

In Vitro Findings

In vitro work has uniformly demonstrated that phage-antibiotic combinations lead to increased bacterial killing, biofilm degradation, and variable results. Overall, 18 of the 25 included studies (72%) explicitly reported synergistic interactions (FIC-I ≤0.5) or significant enhancement in bacterial killing, while 8 studies specifically noted biofilm reduction, mostly facilitated by phage-encoded depolymerase activity (16, 17). The underlying synergy mechanisms identified across the included studies typically fell into three categories: 1) Phage-mediated outer membrane permeabilization, enhancing antibiotic uptake (e.g., increased penetration of aminoglycosides/colistin by phages targeting LPS/capsule) (16, 17); 2) Antibiotic-mediated stress, increasing phage susceptibility (e.g., sub-inhibitory concentrations of β-lactams altering cell morphology to favor phage adsorption) (27); and 3) Phage-induced evolutionary trade-offs, where resistance to one agent resensitizes the bacteria to the other (22, 26).

For instance, studies looking at MDR *P. aeruginosa* have utilized phage-meropenem and phage-ciprofloxacin-colistin combinations with

notable reductions in colony forming units (cfu) (12). The study by Oechslin et al. however, where they combined ciprofloxacin with the PP1131 cocktail, reported the entire clearance of the infection and reduction of bacterial virulence, and demonstrated the possibility of decreased dosages of antibiotics and reduction in side effects (26). Phage PEV20 synergized with ciprofloxacin, which exhibited this synergy post-nebulization, and as such was identified as a promising method of delivery for respiratory infections (13). As for interactions between bacteriophage and antibiotics, it was variable; the KARL-1 phage was reported as being synergistic with meropenem and colistin, but anti-synergistic when paired with ciprofloxacin (14).

Phage-derived depolymerases, such as Dep42 from SH-KP152226, effectively degraded *K. pneumoniae* biofilm and act synergistically with polymyxins (16), and Dpo71 sensitized *A. baumannii* to colistin (17). Notably, another study demonstrated that utilizing a combined NABS strategy with sulfamethoxazole-trimethoprim and a phage cocktail inhibited the phage-resistant *K. pneumoniae* mutants, and thus demonstrated a novel way of managing resistant organisms (22). The diversity of bacterial species studied, including *E. coli*, *K. pneumoniae*, and *A. baumannii*, underscores the broad applicability of this therapeutic strategy. However, antagonistic effects in some pairings (e.g., (14)) where ciprofloxacin inhibited phage replication highlight the need for case-by-case evaluation to avoid suboptimal outcomes.

Figure 2 compares the efficacy (log CFU reduction) of phage therapy, antibiotic therapy, and their combinations against *P. aeruginosa* and *A. baumannii*. The results demonstrate a synergistic effect for *P. aeruginosa* but an antagonistic interaction for *A. baumannii*, as indicated by the combination group's lower reduction compared to the monotherapies, highlighting the species-dependent outcomes of phage-antibiotic combinations.

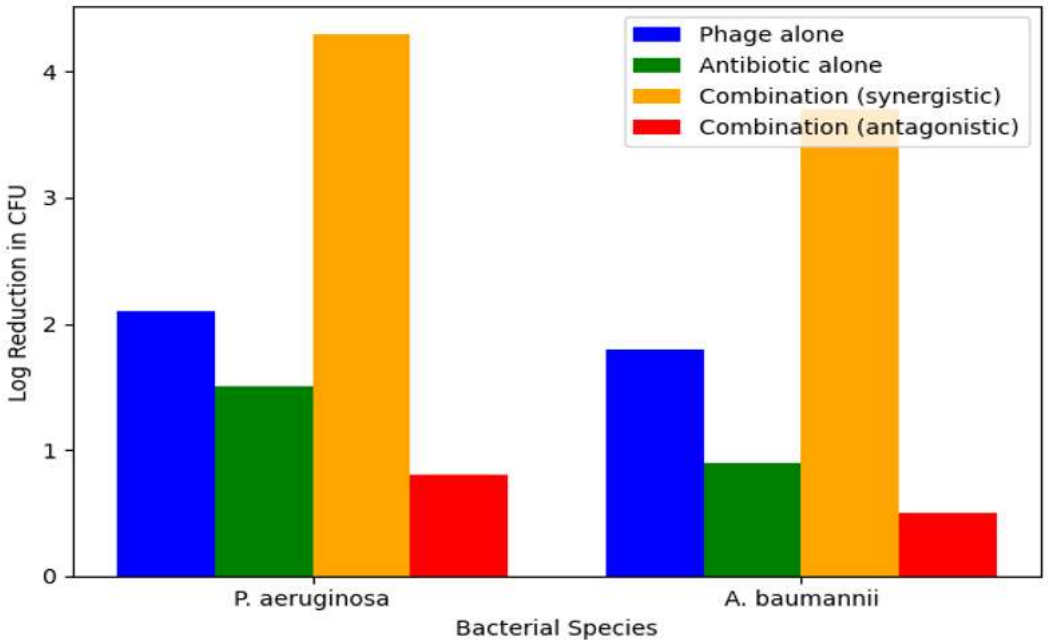

Figure 2: Mechanisms and outcomes of phage-antibiotic combinations against select MDR bacteria

In Vivo (Animal) Findings

Animal studies supported the *in vitro* studies, further validating that combination treatment yielded better clinical outcomes, but they had their limitations as the models can only be translated to a limited degree. In a murine model of

P. aeruginosa ventilator-associated pneumonia, the mice who received phage-meropenem therapy had faster clinical improvement and required fewer doses (30). In a study involving broilers infected with *E. coli*, total protection was achieved with phage-enrofloxacin therapy, and the study measured synergy (29). In a rat model of *P. aeruginosa* endocarditis, the application of a phage-ciprofloxacin cocktail yielded clearance rates of 64% (a positive result considering the refractoriness of this infection type) (26). In another study, phage-gentamicin rescuing mice from pneumonia due to a multi-drug resistant *K. pneumoniae* (28), and phage-ceftriaxone achieved 100% survival in mice infected with *Yersinia pestis* (33). A phage-colistin treatment achieved similar outcomes in *Galleria mellonella* infected with *A. baumannii* (34). Collectively, the studies in animal models with different bacterial species provided strong preclinical evidence to transition combination therapy into the clinic, but some noted challenges related to inter-species differences in immune responses were potentially important to their findings (e.g., 42).

Preclinical evidence across different animal models suggests the consistent synergy between phages and antibiotics leads to greater bacterial clearance, survival, or reduction in drug use. These findings are further evidence for the translational viability of combination therapy for resistant infections (Table 2); however, higher fidelity models still need to be tested, as limitations remain related to variable dosing regimens.

Table 2: Efficacy of phage-antibiotic combination therapy in animal models of bacterial infections

Animal Model	***Pathogen***	***Combination Therapy***	***Key Clinical Outcome***	***Ref.***
Mouse	*P. aeruginosa* (VAP)	Phage + meropenem	Faster clinical improvement, reduced dosage	(30)
Broiler chickens	*E. coli*	Phage + enrofloxacin	100% protection (significant synergy)	(29)
Rat	*P. aeruginosa* (endocarditis)	Phage + ciprofloxacin	64% infection clearance (unprecedented)	(26)
Mouse	*MDR K. pneumoniae* (pneumonia)	Phage + gentamicin	Rescue from lethal infection	(28)
Mouse	*Yersinia pestis*	Phage + ceftriaxone	100% survival	(33)
Galleria mellonella (wax moth larvae)	*A. baumannii*	Phage + colistin	Comparable efficacy to mammalian models	(34)

Clinical Findings

Although clinical studies are limited to case studies or small studies, the results are very encouraging in their clinical application for refractory infections, however the evidence is low (non-randomized studies, etc.). Phage therapy combined with antibiotics eliminated a multi-drug resistant (MDR) *P. aeruginosa* infection in a patient with cystic fibrosis and made lung transplantation possible (36). The NABS method was successful in a recurrent *K. pneumoniae* urinary tract infection (UTI) because the phage inhibited both the pathogen and phage-resistant variants (22). A retrospective study of 100 personalized phage therapy cases found that patients without concomitant antibiotics had 70% lower odds of bacterial eradication (Odds Ratio of 0.30) compared to those receiving combination therapy (25). The ELIMINATE Phase 2 trial demonstrated both safety and efficacy of a

CRISPR-enhanced phage cocktail with trimethoprim/sulfamethoxazole for *E. coli* UTIs, and is the first step toward clinical validation (40). Phage resistance and immune neutralization were seen in 43.8% and 38.5% of cases, respectively, sourced from the clinical cohort in (25) and the review (42), yet clinical benefits are often still noted (25, 42).

These findings demonstrate that combination therapy has great potential in clinical practice, but more research should be conducted, with larger cohorts to reduce selection bias, to answer other clinically relevant questions as well as how to address phage resistance and immune neutralization.

Combination phage-antibiotic therapy demonstrates high *in vitro* efficacy (90% sensitivity) and significantly enhanced bacterial eradication (70% improvement over phage monotherapy), yet faces critical barriers including immune neutralization (38.5%) and phage resistance development (43.8%), sourced from the clinical cohort in (25) and the review (42), underscoring the need for strategic optimization (Figure 3).

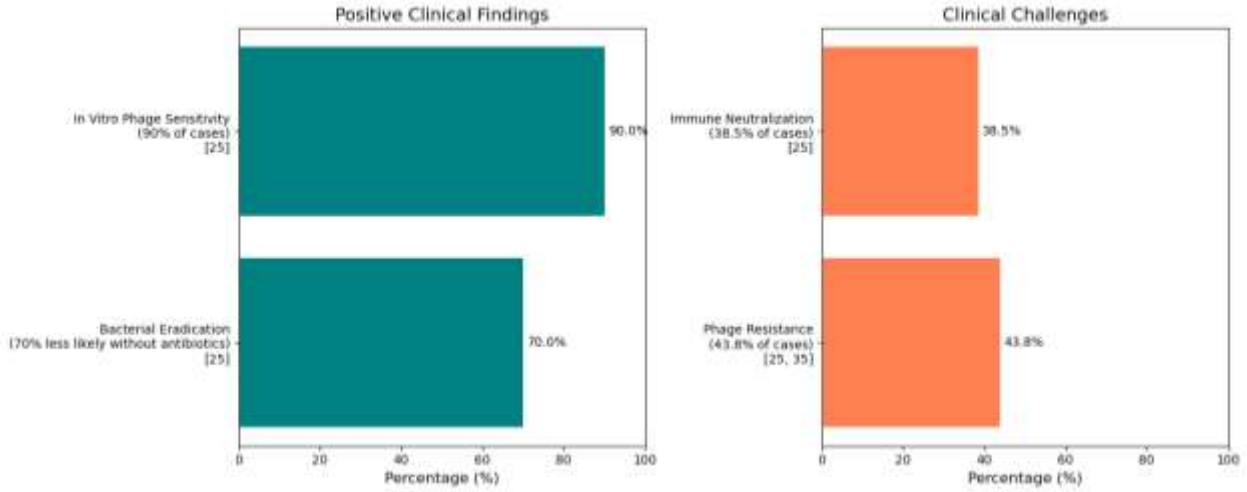

Figure 3: Efficacy and challenges of phage-antibiotic combination therapy in resistant infections

Discussion

This systematic review provides encouraging yet preliminary evidence for the possibility of phage-antibiotic combination therapy to combat the global AMR crisis. The synergy observed in 18 of the 25 included studies (72%) increased the extent of bacterial killing, as well as disrupted biofilms and influenced resistance by methods such as cellular wall disruption, metabolic alteration, and evolutionary cost factors (1, 6). The range of bacterial species studied (e.g., *E. coli*, *K. pneumoniae*, *A. baumannii*) indicates the potential for broader applicability. However, interpreting the current evidence faces limitations, primarily due to the reliance on small-scale studies and case reports (inherently high risk of bias) and the high potential for publication bias, as evidenced by the consistent reporting of successful outcomes and lack of detailed PK/PD data (35). These inconsistencies and variability to the degree that some phage-antibiotic combinations were antagonistic (14, 42), highlight that determining therapeutic design is not necessarily straightforward, or clear-cut, and ultimately needs to be an individualized process.

A critical gap remains in the clinical translation regarding safety and dosing. While case reports generally cite 'no significant safety concerns,' formal monitoring of adverse events (AEs) and long-term immunogenicity are inconsistent. The

emergence of anti-phage antibody response (41) and the lack of human PK/PD data—specifically how phages distribute and persist in specific infection sites relative to antibiotics—are major hurdles that must be addressed in future Phase 2/3 trials to establish optimal dosing and timing.
A deeper discussion is warranted regarding the translational feasibility of personalized phage therapy (PPT). While PPT shows promise (as seen in (36, 38)), it faces significant regulatory and manufacturing hurdles, particularly in non-compassionate use settings, where standardization is mandated. The lack of clarity on Phage Pharmacokinetics (PK) (e.g., tissue penetration and clearance rates) for different delivery routes is arguably the most substantial scientific barrier to establishing standardized clinical protocols.
To promote originality, we propose a conceptual model to predict phage-antibiotic synergies: a "synergy matrix" model that will consider multiple parameters, including antibiotic class (e.g., cell wall inhibitors versus protein-synthesis inhibitors), phage lifecycle (e.g., lytic or temperate), and bacterial resistance mechanisms (e.g., efflux pumps). Crucially, this model must also incorporate *in vivo* parameters such as Phage-Half−Life(t1/2) and Maximum Tolerated Dose (MTD) to integrate safety and pharmacological profiles alongside microbiological outcomes. This model could learn through machine learning based on *in vitro* data to predict patient outcomes from phage and antibiotic therapy and guide personalized therapy and reduce trial-and-error methodologies. Our conceptual model builds on existing hypotheses like dual selective pressure (10) and could be tested for validity in future studies.
Moreover, challenges regarding phage resistance and host immune responses, clearly seen in a lot of cases, need to be addressed through adaptive treatment strategies and research. For example, in some studies sequential dosing (phage first) had some success (21) and in others showed a failure to prevent resistance (10), clearly illustrating a need for optimized protocols. The systematic breakdown of limitations includes: 1) The predominance of low-level evidence (case reports), contributing to a high risk of bias; 2) Significant potential for publication bias toward positive PAS outcomes; 3) Variability in phage dosing, timing (sequencing), and heterogeneous formulations (cocktails vs. single phages); and 4) Reliance on animal models whose immune responses may not fully reflect human infection dynamics (42).
Future studies should focus on the timing, dosing, and engineering of phages (e.g., CRISPR-enhanced phages (40)), which can improve efficacy and decrease resistance. Specifically, future research must prioritize: a) Standardization of synergy testing methods (e.g., unifying the FICI cutoff); b) Conducting Head-to-head comparisons to determine the optimal timing/sequencing of phage–antibiotic administration; c) Developing large, multi-center randomized controlled trials to address efficacy; and d) Establishing regional Phage Banks and rapid susceptibility testing platforms to support rational personalized therapy. Predictive models to help identify synergistic pairs and comprehensive phage banks may also help with rationally-complex treatment and therapy design. Well powered randomized controlled trials are required to generate regulated and standardized protocols and to help address the current evidence gap, including considerations for long-term monitoring for emergence of resistance, and cost-effectiveness.

Conclusion

This systematic review of 25 studies from 2000 to 2024 shows that phage-antibiotic combination therapy offers significant potential to enhance bacterial eradication, inhibit biofilm formation, and minimize resistance for multi-drug resistant infections. The potential for phage-antibiotic combinations is particularly promising due to the potential mechanisms of action, which can provide a real tactical advantage over using ei-

ther one as monotherapy. Pre-clinical and limited clinical studies provided strong evidence for the phage-antibiotic combination approach for treating MDRO infections. There will be hurdles to be overcome including biodegradation, tolerant bacteria, immune responses, and the variability in the studies that were reviewed, but there is unique potential for this approach to counteract AMR. It is important that researchers continue to evaluate the mechanisms, optimize the combination approach based on PK/PD modeling, assess dose and route effects in setting up larger clinical trials to allow for success with the promise of phage-antibiotic combination therapy for all patients in the future.

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Conflict of interest

The authors declare no conflict of interests.

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