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REVIEW ARTICLE

Bacteriophage cocktails and bacterial coevolution: a systematic review of resistance prevention strategies against multidrug-resistant pathogens in sewage water

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ABSTRACT

Background and Objectives: This systematic review aimed to determine whether phage cocktails and co-evolutionarily trained phages prevent or delay the emergence of resistance in multidrug-resistant (MDR) bacteria more effectively than single-phage therapy in sewage and wastewater-derived systems, and to characterize the underlying mechanisms and the maturity of the current evidence base.

Materials and Methods: Following PRISMA guidelines, a systematic review was performed using the PubMed and Scopus databases, with screening conducted via Rayyan Premium.

Results: Among 4,795 records, 20 studies (≈0.4%) fulfilled the inclusion criteria; this low fraction reflects a deliberately broad search combined with strict eligibility criteria targeting original experimental studies of phage activity against MDR pathogens specifically isolated from sewage or wastewater. Phage cocktails showed a wider range of host coverage, a delay in resistance development, and improved penetration of biofilms, particularly when paired with depolymerases or antibiotics. Phage co-evolution training resulted in a more prolonged suppression of multidrug-resistant bacteria.

Conclusion: Phage cocktails, especially those that are co-evolved and enhanced with enzymes, show potential for applications in wastewater; however, additional long-term and large-scale research is necessary.

Keywords: Bacteriophages; Drug resistance; Wastewater; Sewage; Biofilms; *Klebsiella pneumoniae*; Anti-bacterial agents

INTRODUCTION

The global Antimicrobial Resistance (AMR) crisis is of immense concern for public health in the 21st Century. With each passing day, conventional antibiotics become weaker when it comes to multidrug-resistant (MDR) pathogens, and most experts are concerned that if AMR is not seriously mitigated, it could result in more than a million deaths every year (1-3). The "one-drug-one-target" paradigm collapse reaffirms that we need new, more creative ways of dealing with this problem (2).

Phage therapy has come into focus as one of the solutions to tackle resistant infections in chronic cases. Phages are precision viruses that infect bacteria in a highly targeted manner, and they are unique in

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that they split the bacteria and replicate at the wounded site autonomously without needing an external stimulant (4-6). For instance, custom-engineered phages were used on patients suffering from cystic fibrosis and infections caused by *Mycobacterium abscessus* that antibiotics could not heal alone (7). Clinical studies from 2011 to 2021 that included the use of phage therapy reported an 85% success rate in dealing with multidrug-resistant infections (8). Phages are unquestionably effective, but resistance is a noted impediment to therapy, as bacteria exhibit quick, effortless modifications against monophage therapy (9), and combination systems may help overcome the limitations posed by single phages and antibiotics (10, 11).

The use of single-phage therapy (SPT) in sewage or wastewater settings encounters numerous significant obstacles. Phages generally demonstrate a limited host range, frequently infecting only a few bacterial strains, and due to the extensive diversity of bacterial populations in sewage, it is improbable that a single phage can effectively reduce a wide range of targeted pathogens (12). Bacteria in sewers also swiftly develop resistance to specific phages via mutations in surface receptors, CRISPR-Cas immunity, and various other defense mechanisms, allowing resistant subpopulations to rapidly develop in dense and diverse microbial environments and constrain the long-term efficacy of SPT (4, 13, 14). In addition, sewage presents a very changeable and frequently inhospitable habitat for phages: variables including pH, temperature, organic matter concentration, and competing microbial enzymes can diminish phage stability and efficacy, rendering SPT unreliable for wastewater applications. A further constraint stems from the possible existence of temperate phages, as a solitary therapeutic phage that is lysogenic may integrate into bacterial genomes rather than lysing cells, potentially transferring antibiotic resistance or virulent genes — a risk that is particularly alarming in sewage, where horizontal gene transfer frequently transpires among bacterial populations (4). Finally, research indicates that different infection patterns manifest even within a single susceptible bacterial population, where certain bacteria are rapidly lysed while others endure or recuperate, indicating that dependence on a single phage seldom results in sustained population control among intricate wastewater ecosystems (15). Together, these issues underscore the superiority of multi-phage cocktails or integrated antibacterial methods in wastewater treatment applications.

Wastewater and sewage environments are acknowledged as significant reservoirs for MDR and extensively drug-resistant (XDR) bacteria, including clinically pertinent nosocomial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (16). Conventional wastewater treatment techniques, although proficient at diminishing organic and chemical burdens, do not entirely eradicate harmful bacteria, consequently heightening the risk of environmental spread of antibiotic resistance (17). In these diverse and dynamic systems, phage cocktails can simultaneously target multiple strains or species, thereby achieving a higher level of bacterial suppression, and have demonstrated effectiveness in biofilm destruction, especially when used in conjunction with antibiotics (16, 18). Beyond cocktails, coevolutionary phage training has emerged as an innovative strategy to improve phage efficacy: phages are pre-adapted against their bacterial hosts through repeated co-culturing, allowing them to bypass resistance mechanisms that arise in natural bacterial populations (19). In sewage water systems, where bacteria are constantly exposed to selective pressures, coevolutionary training enhances the robustness of phages to environmental challenges and resistance mutations, making trained phages a sustainable tool for maintaining long-term suppression of MDR pathogens (19, 20). Multiphage therapy and coevolutionary training therefore offer synergistic benefits — cocktails provide extensive coverage and prompt reduction of bacterial populations, whereas co-evolutionarily trained phages enhance resilience and postpone the emergence of resistant strains — and when implemented in wastewater and sewage systems, these solutions not only diminish the transmission of pathogenic bacteria but also aid in alleviating the environmental proliferation of antibiotic resistance (21-23).

Notwithstanding the increasing research underscoring the advantages of multiphage therapy, phage cocktails, and coevolutionary training in sewage and wastewater systems (19, 21-23), a significant deficiency persists in the form of systematic evidence synthesis. Current research is disjointed, concentrating on singular case studies, experimental frameworks, or theoretical analyses, lacking the synthesis of results across many contexts. There is currently no exhaus-

tive systematic study that aggregates evidence about the coevolutionary dynamics of phages in sewage systems and their function in alleviating bacterial resistance. Addressing this gap will yield essential information regarding the long-term sustainability, scalability, and efficacy of phage-based therapies against multidrug-resistant bacteria in wastewater ecosystems.

To address this gap, the present systematic review synthesizes original experimental evidence — laboratory in vitro studies, in vivo infection-model studies, and field/pilot studies — on bacteriophage applications against MDR pathogens isolated from sewage and wastewater. Specifically, it asks whether phage cocktails and co-evolutionarily trained phages prevent or delay the emergence of bacterial resistance more effectively than single-phage therapy, and through what mechanisms. The secondary objectives were to characterize host-range breadth, biofilm-disruption and antibiotic-synergy outcomes, and the maturity of the evidence base (study designs, scale, and reporting consistency) relevant to wastewater applications.

The studies synthesized here were original experimental investigations of bacteriophages active against MDR bacteria from sewage or wastewater. Most were laboratory/in vitro studies (phage isolation, characterization, host-range and biofilm assays, and genomics), alongside a smaller set of in vivo infection-model studies (*Galleria mellonella*, zebrafish, or mice) and one pilot-scale field study in hospital wastewater. Reviews, editorials, commentaries, and non-experimental reports were excluded (Table 1; see Materials and Methods).

MATERIALS AND METHODS

Search strategy. A detailed literature review was performed using PubMed and Scopus to find pertinent studies released from January 2015 to June 2025. A 2015 cutoff was applied to capture the contemporary evidence base on phage-mediated control of MDR pathogens, which expanded substantially following the renewed clinical and research interest in phage therapy and the integration of CRISPR-Cas-based resistance mechanisms into phage–bacteria co-evolution studies from the mid-2010s onward. This ensured that included studies reflected current isolation, characterisation, and resistance-reporting standards relevant to the review's focus on resistance prevention. Only publications in the English language were included. The search utilised a combination of controlled vocabulary terms (MeSH) and free-text keywords associated with bacteriophages and bacterial co-evolution. Core terms included bacteriophage, phage therapy, phage–host interactions, lytic phage, temperate phage, bacteriophage resistance, phage predation, phage diversity, bacterial resistance, host bacteria, *Escherichia coli*, *Pseudomonas*, *Vibrio*, microbial community, bacterial evolution, co-evolution, evolutionary arms race, adaptation, phage–bacteria dynamics, selective pressure, and mutual adaptation. Environmental and regional terms include wastewater, sewage, waterborne pathogens, microbiome, antimicrobial resistance, biocontrol, infection treatment, and multidrug resistance. Boolean operators were applied to combine terms, and the strategy was adapted for each database. No grey literature was searched.

A deliberately broad search strategy was used to

Table 1. PICO Framework for Eligibility

Element	Definition in This Review	Examples Applied
Population (P)	Bacteria and bacteriophages are isolated from environmental and clinical water-related sources.	*E. coli*, *Pseudomonas*, *Vibrio*, other Gram-negative bacteria; wastewater, sewage, coastal/marine/estuarine water
Intervention/Exposure (I)	Interaction between bacteriophages and bacterial hosts; studies involving isolation, characterization, or experimental analysis.	Phage therapy trials, plaque assays, co-evolution experiments, genomic studies of phage–host dynamics
Comparator (C)	Not always applicable; it could be baseline or control bacterial strains without phage exposure, or different phage types/conditions	Untreated bacteria vs. phage-exposed; lytic vs. temperate phages; ancestral vs. evolved strains
Outcomes (O)	Evidence of co-evolutionary dynamics, bacterial resistance, phage adaptation, diversity, or therapeutic/biocontrol potential	Resistance patterns, plaque morphology changes, host-range shifts, AMR reduction, microbiome/community impact

maximize sensitivity; the review's focus on MDR pathogens in sewage and wastewater was then applied through the eligibility criteria during screening, accounting for the clinical, wastewater-derived character of the included studies.

Eligibility criteria. Studies were included if they met the following criteria (Table 1):

Population/setting: Experimental or observational studies of bacteriophage–bacteria interactions in environmental or clinical contexts, particularly wastewater or sewage.

Intervention/exposure: Isolation, characterization, or experimental analysis of bacteriophages and bacterial hosts.

Outcomes: Data on host resistance, co-evolutionary dynamics, bacterial adaptation, phage diversity, or therapeutic/biocontrol potential.

Study design: Original research articles (laboratory-based, field studies, or clinical studies).

Exclusion criteria were reviews, editorials, commentaries, conference abstracts without full text, and studies not in English or published before 2015 (Table 2).

Screening and selection. All retrieved records were imported into Rayyan Premium (n = 4,795). After duplicate removal (n = 1,774), 3,021 unique records remained. Rayyan's machine-learning auto-screening function was applied to exclude records predicted to be ineligible with high confidence (n = 2,668). The remaining 353 records were independently screened by two reviewers based on titles and abstracts in a blind manner. Discrepancies were resolved by discussion. Of these, 190 articles were retrieved for full-text review. Following detailed eligibility assessment, 170 were excluded (reasons documented within Rayyan), leaving 20 studies eligible for inclusion (Fig. 1).

Our study is based on sewage or wastewater as the isolation sources (urban, clinical wastewater and mixed hospital ponds), mostly from hospitals, as reflected by the studies screened. They were observed to be yielding phages that were active against some of the clinically significant MDR pathogens. Thus, we found wastewater or sewage to be a diverse ecological source for phage discovery (Table 2).

Data extraction. Data extraction was conducted in Rayyan utilizing a predefined template. For every included study, the following information was gathered: study ID, authors, publication year, country/region, sample source, bacterial host species, type of phage (lytic/temperate), methodologies employed (like plaque assay, genomic analysis, or co-evolution experiments), duration of experiments, key findings (such as resistance patterns or host-phage interactions), and outcomes reported regarding antimicrobial resistance or biocontrol. Extraction was performed independently by two reviewers, and discrepancies were resolved through consensus.

Quality assessment. Given the heterogeneity of study designs (laboratory, environmental sampling, and experimental co-evolution), no single standard-

Table 2. Reasons for full-text exclusions in the systematic review

Reason for exclusion	Number (n)
Ineligible study design (not original experimental research)	40
Background/conceptual article with no experimental data	33
Not a wastewater source or habitat	21
Ineligible publication type (review, commentary, editorial, or abstract without full text)	13
Ineligible population (bacterial host or context not relevant to the review)	18
No water-related data reported	12
Other or multiple/overlapping reasons	33
Total excluded at full text	170

Exclusion categories reflect the review's eligibility criteria. "Ineligible population" denotes studies whose bacterial hosts or experimental context fell outside the review's focus on MDR pathogens in sewage/wastewater; and "ineligible publication type" denotes reviews, commentaries, editorials, and abstracts lacking a full text.

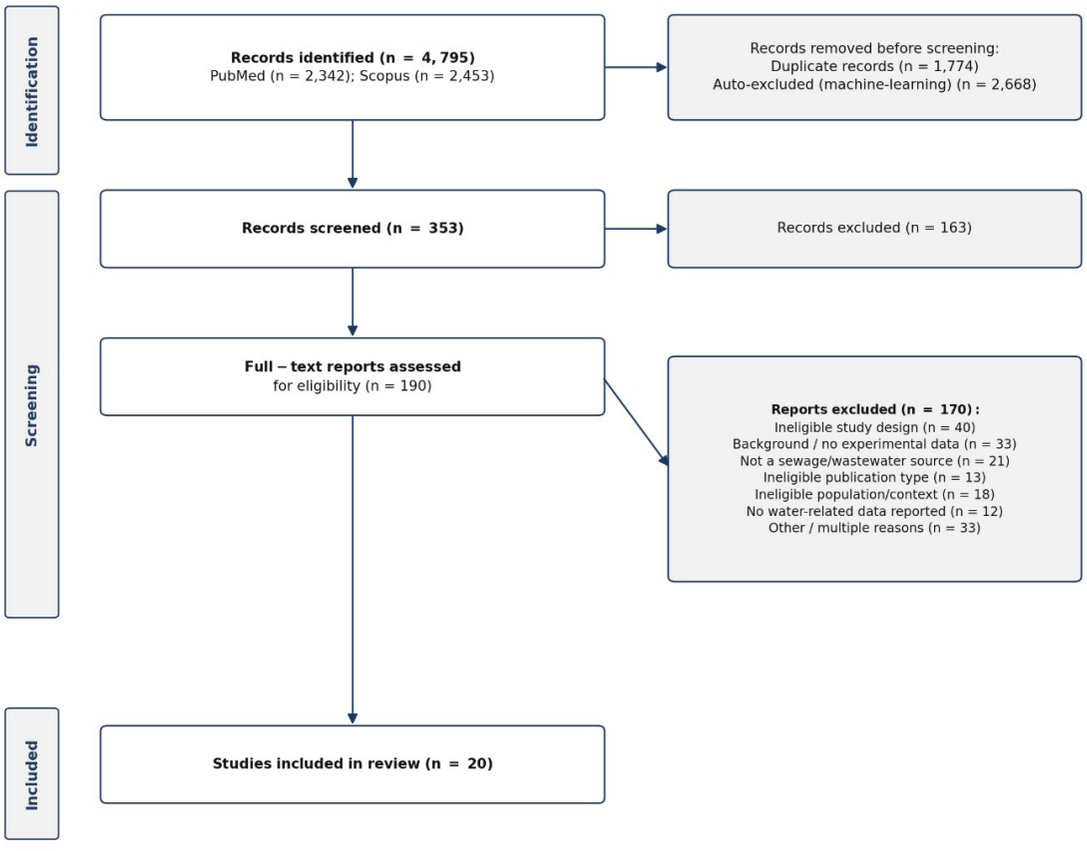

Fig. 1. PRISMA 2020 flow diagram illustrating the systematic search, screening, and selection process for studies included in the review on bacteriophage–bacteria co-evolution in wastewater environments

ized risk-of-bias tool was universally applicable. Instead, methodological strengths and limitations were narratively appraised, including sample source representativeness, clarity of phage–host identification, reproducibility of co-evolution assays, and completeness of reported outcomes.

RESULTS

Study selection and characteristics. We screened 4,795 records, out of which 190 full texts were reviewed. We selected 20 studies published between 2018 and 2025 that met our inclusion criteria, as shown in the PRISMA flow diagram (Fig. 1) (25).

Most of the studies (13 of 20) included laboratory-based and in vitro investigations. A smaller set of 7 studies used in vivo infection models such as *Galleria mellonella* larvae, zebrafish, and mice. Asian countries, such as China, India, Iran, and Taiwan, carried out most of the studies (11 of 20, with 7 from China alone), followed by African countries (5), European countries (3), and North America (1). Only one pilot-scaled field study was identified: a six-month trial carried out in Iran, which applied a cocktail of 42 phages to hospital wastewater. This study provides large-scale, real-world validation for the work performed in this area (Table 3).

Phage cocktails and co-evolutionary strategies. We observed that in 3 out of 20 studies, the phage cocktails consistently performed better than SPTs in both the variety of host range and durability (Tables 3 and 4). The mode of target of the multiphage cocktail targeted distinct bacterial receptors, such as capsular polysaccharides and lipopolysaccharides, and effectively delayed the emergence of resistance in the pathogens as compared to single phage therapies. This phenomenon was particularly evident against high-risk *Klebsiella pneumoniae* clones (ST11, ST147, ST307) and MDR *Escherichia coli* (Tables 3 and 4).

Several studies, on purpose, isolated phages from bacteria escape mutants and then incorporated these trained phages into cocktails to close the evolutionary

Table 3. Characteristics of Included Studies

Study (Author, Year)	Country/ Region	Sewage/WW Source	Type of Study	Experimental Setting & Conditions	Duration
Chen et al., 2024 (20)	China & Australia	Hospital sewage (Wenzhou Medical University)	Lab-based & animal model Laboratory & zebrafish infection model	Phages ΦK2044, ΦKR1, ΦKR8 tested against *K. pneumoniae* biofilms; catheter biofilm model; mice & larvae infection assays	Short-term (days–weeks)
Natarajan et al., 2024 (26)	Taiwan	Sewage		Novel N4-like phage vB_kpnP_KPYAP-1 against CRKP; genomic characterization; zebrafish survival model	Short-term (≤72h)
Fang & Zong, 2022 (27)	China	Hospital sewage (West China Hospital)	Lab-based	Phage P13 against ST11 K47 CRKP; stability & resistance emergence assays	Hours–days
Zaki et al., 2023 (28)	Egypt	Urban & medical sewage	Laboratory	Phage vB_Kpn_ZCKp20p against MDR K. pneumoniae; biofilm disruption; genome analysis	Lab assays only
Sun et al., 2022 (29)	China	Sewage	Laboratory	Novel *Proteus vulgaris* phage vB_PvuS_Pm34; stability & genome sequencing	Lab assays
Yang et al., 2025 (30)	China	Hospital sewage	Lab & in vivo (Galleria mellonella)	Phage vB_KpnP_IME1309 with depolymerase gene; biofilm disruption; synergy with kanamycin	Short-term (days)
Pu et al., 2022 (24)	China	Hospital sewage	Lab & in vivo (larvae)	Phage BUCT610; stability pH 3–12, 4–75°C; survival benefit in larvae	≤72h
Fikadu et al., 2024 (31)	Ethiopia	Hospital sewage (Jimma Medical Center)	Laboratory	Phages against MDR *E. coli*; coevolution step using resistant variants; cocktails tested	Short-term
Hanzada et al., 2025 (32)	Canada	Sewage samples (University of Ottawa Environmental Lab)	Laboratory & in vivo	Four lytic *P. aeruginosa* phages (*myovirus*, *podovirus*, *siphovirus*); broad host range; tested in vitro and *Galleria mellonella* larvae	Short-term (hours–months stability tests)
Peijun Lin et al., 2025 (4)	China (Guangdong)	Hospital sewage (Guangdong Provincial People's Hospital)	Lab & in vivo (mice)	Phage MSP15 isolated against MRSA; broad host range; systemic infection mouse model	Short-term survival study
Sara Sadeqi et al., 2022 (16)	Iran (22 hospitals, 8 cities)	Mixed hospital wastewater pooled into sedimentation pond	Pilot-scale field study	42 lytic phages isolated; cocktail applied to hospital pond; reduced multiple pathogens (*E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *S. aureus*, etc.)	6 months (March–Aug 2019)
Senhaji-Kacha et al., 2024 (33)	Spain	Clinical isolates	In vitro	Two novel phages vs. CR-Kp	24–48 h
Wang et al., 2024 (34)	China	Sewage	In vitro	Lytic phage vB_KpnP_23	24 h
Yazdi et al., 2018 (35)	Iran	UTI isolates	In vitro	Genomic analysis of VB_EcoS-Golestan	24 h
Badawy et al., 2022 (36)	Egypt	Clinical isolates	In vitro	Characterization of fEg-Eco19	24 h
Akremi et al., 2022 (37)	Tunisia	Sewage	In vitro	Lytic *P. aeruginosa* phages	24 h
Mirza et al., 2025 (38)	Germany	Hospital sewage	In vitro	vbKpUKJ_2, genomic analysis	24 h
Ponsecchi et al., 2024 (39)	Italy	Hospital sewage	In vitro	Four phages vs. MDR-Kp	24 h -48 h
Menon et al., 2022 (40)	India	Wastewater	In vitro	N4-like phage vs. MDR *P. aeruginosa*	24 h
Ochieng' Oduor et al., 2019 (41)	Kenya	Clinical isolates	In vitro	Lytic *S. aureus* phage	7-14 days

Table 4. Target Bacteria and Phage Therapy Approaches

Study (Author, Year)	Bacteria Targeted	Resistance Profile	Phage Source	Phage Type (Single/ Cocktail/Evolved)	Cocktail Composition	Evolution and strategy
Senhaji-Kacha et al., 2024	*K. pneumoniae*	Carbapenem-resistant	Clinical isolates	Single	N/A	N/A
Wang et al., 2024	*K. pneumoniae* (ST11-K64) *E. coli*	CRKP	Sewage	Single	N/A	N/A
Yazdi et al., 2018		MDR (UTI isolates)	UTI clinical samples	Single	N/A	N/A
Badawy et al., 2022	*E. coli*	Antibiotic-resistant	Clinical UTI isolate	Single	N/A	N/A
Akremi et al., 2022	*P. aeruginosa*	MDR	Sewage (Tunisia)	Single (multiple isolated) Single	N/A	N/A
Mirza et al., 2025	*K. pneumoniae*	MDR (hospital-associated) MDR, high-risk clones	Hospital sewage		N/A	N/A
Ponsecchi et al., 2024	*K. pneumoniae* (ST147, ST307)		Hospital sewage	Multiple phages (single isolates)	N/A	N/A
Menon et al., 2022	*P. aeruginosa*	MDR clinical strains	Wastewater (India)	Single (N4-like)	N/A	N/A
Ochieng'Oduor et al.,	*S. aureus*	2019MDR (MRSA)	Clinical isolates	Single	N/A	N/A
Chen et al., 2024	*Klebsiella pneumoniae* (incl. hypervirulent KL1; multiple capsular serotypes)	Hypervirulent, highly drug resistant strains; biofilm-associated tolerance	Hospital sewage (initial phage ΦK2044; additional phages from resistant derivatives)	Cocktail (ΦK2044+ ΦKR1+ΦKR8); evolved selection via resistant hosts	ΦK2044 (KL1-specific) + ΦKR1 + ΦKR8 (broader serotype coverage)	Use resistant mutants to isolate broadened-host-range phages; cocktail delays resistance; effective in biofilms and improves survival in *Galleria* & mouse models
Natarajan et al., 2024	*Klebsiella pneumoniae*, capsular type K62 (CRKP, *bla*OXA 48)	Carbapenem resistant; K62 capsule linked to higher resistance vs other K types	Sewage	Single lytic N4 like phage (Schitoviridae, Enquatroviri-nae, Kaypoctavirus)	N/A	Short latent period, large burst; in vivo efficacy in zebrafish; genome lacks AMR/virulence; host recognition via tail fiber variation
Fang & Zong, 2022	*Klebsiella pneumoniae* ST11 K47 (CRKP)	Carbapenem resistant; rapid emergence of phage resistance	Hospital sewage	Single lytic phage (Autographiviridae, Przondovirus)	N/A	Resistance via IS903B insertion disrupting wbaP (capsule synthesis); recommendation to assemble cocktails targeting different receptors
Zaki et al., 2023	Multidrug resistant *Klebsiella pneumoniae* (clinical isolates; biofilm forming)	MDR; device associated infections; biofilm tolerance	Urban and medical sewage	Single strictly lytic siphovirus	N/A	Anti biofilm activity; thermal/pH stability; proposed as safe therapeutic; could be combined in future cocktails
Yang et al., 2025	*Klebsiella pneumoniae* ST11 K64 (CRKP)	Carbapenem resistant; robust capsule (K64) and biofilm forming	Hospital sewage (phage vB_KpnP_IME1309; depolymerase ORF37)	Phage derived enzyme (depolymerase); adjunct to antibiotics	Dep37 + antibiotics (e.g., kanamycin) for enhanced biofilm clearance	Capsule degradation increases serum susceptibility; improves *Galleria* survival; synergy with antibiotics > either alone Excellent
Pu et al., 2022	*Klebsiella pneumoniae* (highly virulent strain K1119)	MDR context; evaluated for stability and in vivo efficacy	Hospital sewage	Single lytic phage	N/A	thermal/pH stability; increased *Galleria* survival from 13% to 83%
Fikadu et al., 2024	*Escherichia coli* (MDR clinical isolates); phage resistant variant *E. coli* 4307R	Multidrug resistant; rapid phage resistance on single phage exposure	Hospital sewage	Multiple single lytic phages; cocktails assessed for host infectivity	Cocktails of the three isolated coliphages (titers up to ~9.17×10^12 PFU/mL for VBW)	Isolated a new phage targeting the resistant variant; demonstrates iterative isolation and cocktail use to manage resistance
Sun et al., 2022	*Proteus vulgaris*	Not specified in source tables	Sewage	Single	N/A	N/A
Hanzada et al., 2025	*P. aeruginosa*	MDR	Sewage	Cocktail	Four lytic phages	Broad host range; tested in vitro and in *Galleria mellonella*
Peijun Lin et al., 2025	*S. aureus* (MRSA)	MDR (MRSA)	Hospital Sewage	Single	N/A	MSP15 broad host range; systemic infection mouse model
Sara Sadeqi et al., 2022	Multiple MDR pathogens	MDR	Mixed hospital wastewater	Cocktail	42 lytic phages	Field-scale cocktail applied to hospital wastewater pond over 6 months

escape routes effectively. This strategy was observed in 2 studies by (17) that demonstrate effective implementation of bacterial–phage co-evolution for resistance prevention (Tables 3 and 4).

Evidence from in vivo and environmental models. We also found results from the in vivo studies, though limited but promising. Across 7 studies using *Galleria mellonella*, zebrafish, or murine models, cocktail or enzyme-augmented therapy improved survival and reduced bacterial loads (Tables 3 and 5). The clearest example was (24), where larval (Galleria) survival increased from 13% to 83% following phage treatment. In the single Iranian pilot study (16), sustained suppression of multiple MDR pathogens (*E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *S. aureus*) was observed over six months, providing proof of their environmental application (Table 3).

Mechanistic insights into resistance prevention. From the mechanistic data that we collected, we observed in 3 studies that bacteria become resistant mostly from modifications in the capsule or losing some of its receptors (e.g., wbaP disruption in *K. pneumoniae*; K62 and K47 capsule-mediated escape (Table 6).

In our findings, the phage cocktails that targeted multiple bacterial receptors reduced the likelihood of simultaneous escape mutations. An extra layer of efficacy was noted in 1 study where a phage-derived depolymerase was integrated (30). The depolymerase exposed new receptors by degrading the capsule, thus enhancing antibacterial action when additional phages or antibiotics are used. This strategy was particularly useful against biofilm-associated infections, where enhanced biofilm clearance was observed when the depolymerase (Dep37) was combined with the antibiotic kanamycin (Table 6).

DISCUSSION

In this systematic review, we observe that phage cocktails consistently performed better than SPTs in terms of both variety of host range and efficiency against resistance. In 4 studies, out of the 20 in total, cocktail therapies delayed or, in some cases, prevented the emergence of bacterial resistance more effectively than the single phages, especially against *Klebsiella pneumoniae* and MDR *Escherichia coli* (38, 40, 42). These observations also align with the previous reports that indicated that when multiple phages are combined in a therapy that targets specific bacterial receptors, they reduce the possibility of simultaneous escape mutations (43). Furthermore, the geographically varied sites utilized for the investigations indicate that sewage and wastewater serve as possible reservoirs for phage isolation. This environmental prevalence highlights the practicality of creating region-specific, scalable phage-based solutions for wastewater-associated multidrug-resistant bacteria.

There is mechanistic evidence available from the studies we screened that demonstrates that resistance in bacteria usually results from the modification of the capsule or because of the loss of receptors. For instance, the disruption of wbaP occurs in *K. pneumoniae*. However, it is to be noted that such resistance comes with a fitness cost, reducing bacterial competitiveness (27). Addition of depolymerases enhanced the antibacterial mode. This is because it breaks down the bacterial capsule, which opens new receptors that antibiotics or phages can attack. One of the examples is that of Dep37 depolymerase, which significantly decreased biofilm biomass in combination with kanamycin (30). On the other hand, vB_Kpn_ZCKp20p showed strong lytic and anti-biofilm activity against MDR *K. pneumoniae* (28).

Despite promising outcomes, there remain gaps to be addressed. Most of the studies were short-term and were less than 72-hour in vitro experiments; long-term co-evolutionary dynamics in sewage or wastewater remain poorly explored. Inconsistent outcomes were also noted: metrics, such as time-to-resistance, biofilm biomass reduction, or clearance rates, were inconsistently reported, limiting comparability across studies. In studies, there was also a limitation of genomic mapping of resistance mutations and receptor usage, restricting predictive cocktail design. We also could not find any other field-scale replication except for one pilot study in Iran.

In summary, we found that multiphage therapy or cocktails broaden host range and effectively delay the emergence of resistance compared to single phage therapy. Overall, the evidence indicates that bacteriophage cocktails, especially those informed by bacterial co-evolution and enhanced by the incorporation of depolymerases, represent promising tools to suppress or delay resistance in MDR pathogens derived from sewage ecosystems. However, we urgently need long-term co-evolutionary studies and

Table 5. Outcomes of Phage Applications in Sewage Systems

Study (Author, Year)	Bacterial Reduction Outcomes	Resistance Emergence	Coevolution Dynamics	Phage Stability	Synergy with Antibiotics/Other Treatments
Chen et al., 2024	Strong killing of hypervirulent and MDR *K. pneumoniae*; effective in biofilm eradication	Delayed resistance compared to single phage	Use of resistant strains to isolate broader phages; adaptive trade-offs noted	Cocktail remained stable and active in biofilm & animal models	None tested with antibiotics
Natarajan et al., 2024	Efficient killing of CRKP ST45 K62	Not strongly reported (focus on efficacy)	No coevolution study	Stable genome; no resistance/virulence genes	Not tested
Fang & Zong, 2022	Rapid lysis of ST11 K47 CRKP Broad	Resistant mutants appeared within 4 h (via IS903B insertion in capsule gene)	Capsule gene mutation showed evolutionary arms race	Broad pH and thermal stability	None
Zaki et al., 2023	lysis of MDR *K. pneumoniae*; antibiofilm	Not highlighted	No explicit coevolution	Stable up to 70°C, pH 2–12	Not tested
Sun et al., 2022	Effective lysis of *P. vulgaris*	Not emphasized	Not studied	Stable pH 4–8; <40°C	Not tested
Yang et al., 2025	Strong anti-biofilm, serum bactericidal enhancement	Reduced capsule-mediated resistance	Capsule depolymerase directly targets virulence structure Not studied	Not specifically tested	Synergistic with kanamycin against CRKP biofilms
Pu et al., 2022	Kills MDR *K. pneumoniae* K1119; increased larval survival	Not highlighted		Stable across 4–75°C and wide pH	Not tested
Senhaji-Kacha et al., 2024	Effective lysis of carbapenem-resistant *K. pneumoniae* clinical isolates	Not observed in short-term assays	Not tested	Stable across typical storage and pH range	No synergy data reported
Wang et al., 2024	Strong lytic activity against CRKP ST11-K64	Resistance emerged in some hosts after 24–48 h	Not tested	Stable at 4–50°C; active pH 4–10	No antibiotic synergy tested
Yazdi et al., 2018	Clear reduction in MDR *E. coli* UTI isolates	No resistance observed in 24 h tests	Not tested	Stable pH 5–9; thermotolerant to 50°C	Not tested
Badawy et al., 2022	Marked lysis of UTI *E. coli* isolate; reduced CFU counts	No resistance within 24 h; lysogeny genes absent	Not tested	Stable under heat and pH stress	Not tested
Akremi et al., 2022	Effective clearance of MDR *P. aeruginosa* in vitro	Some resistance after serial passage	Not tested	Stable at various pH/temperature	Not tested
Mirza et al., 2025	Reduced MDR *K. pneumoniae* growth in vitro	Resistance not reported	Not tested	Strong pH and thermal stability (broad tolerance)	Not tested
Ponsecchi et al., 2024	Effective lysis of ST147 and ST307 CRKP Significant lysis of MDR	Resistance emerged in some strains; diversity of phages helped coverage	Not tested	Phages stable during genomic characterization	Not tested
Menon et al., 2022	*P. aeruginosa* clinical isolates	Resistance suppressed longer compared to standard phages	Coevolution dynamics partly tested; showed host–phage adaptation	Stable at different pH and temperatures	Synergy with antibiotics (enhanced killing, reduced resistance) No direct synergy tested
Ochieng'Oduor et al., 2019	Reduced *S. aureus* bacterial load in mice; improved survival	No resistance noted during in vivo trial	Not tested	Stable for animal experiments	
Fikadu et al., 2024	Effective lysis of MDR *E. coli*; cocktail active against phage-resistant variant (*E. coli* 4307R)	Single-phage exposure selected a resistant variant; addressed by isolating a new phage	Practical phage–host co-evolution: new phage isolated against the resistant variant	Not reported	Not tested
Hanzada et al., 2025	Broad-host-range lysis of MDR *P. aeruginosa* in vitro and in *Galleria mellonella*	Not reported	Not studied	Stable across hours–months stability tests	Not tested
Peijun Lin et al., 2025	Phage MSP15 reduced MRSA burden; improved survival in systemic infection mouse model	No resistance noted in vivo	Not studied	Stable for animal experiments	Not tested
Sara Sadeqi et al., 2022	Sustained reduction of multiple MDR pathogens	Not reported	Not studied	Maintained activity over 6-month field application	Not tested

Table 6. Mechanistic Insights, Limitations, and Knowledge Gaps

Study	Mechanistic Insights (Resistance Mechanisms, Phage Adaptations, Ecological Impact)	Limitations	Knowledge Gaps	Quality/Risk of Bias
Chen et al., 2024	Resistance in *K. pneumoniae* linked to capsule-type specificity; resistant mutants used to isolate broader-host phages (ΦKR1/ΦKR8); cocktail delays resistance and is active against biofilms.	Focused on one species; preclinical only (*Galleria*, mouse, catheter model).	Long-term coevolution in wastewater/sewage not assessed; clinical translation data lacking.	Moderate (robust models; no blinding/clinical randomization).
Natarajan et al., 2024	Encodes virion RNA polymerase (N4-like); lacks lysogeny/AMR/virulence genes; unique tail fiber suggests specific host recognition of K62 capsule.	Single capsular type focus; limited strain diversity.	Cross-capsular host range and sewage ecology impact untested.	Moderate–High (genomics + zebrafish model).
Fang & Zong, 2022	Rapid resistance via IS903B insertion interrupting wbaP (capsule synthesis); narrow host ST11 K47; highlights capsule-based escape routes.	Narrow host range; resistance emerged within 4 h.	Need multi-receptor/cocktail strategies for ST11 K47; environmental persistence unstudied.	Moderate (clear mechanism; short-term assays).
Zaki et al., 2023	Strictly lytic; antibiofilm activity demonstrated; genome indicates safety (no AMR/virulence).	Single phage; no resistance evolution experiments.	Mechanism of antibiofilm action; ecological impact in sewage networks.	Moderate.
Sun et al., 2022	First P. vulgaris–specific lytic phage reported; tolerates moderate pH/temperature.	No resistance/co-evolution analysis.	Therapeutic scope and sewage ecosystem interactions not defined.	Low–Moderate (exploratory characterization). High (strong mechanistic evidence; functional assays).
Yang et al., 2025	Dep37 cleaves K64 capsule, reduces virulence, enhances serum killing and antibiotic (kanamycin) action; mechanistic protein characterization.	Only in *Galleria* model; no mammalian validation yet.	Mechanistic synergy mapping with multiple antibiotics; durability of depolymerase in wastewater.	
Pu et al., 2022	Genomically safe (no AMR/virulence); wide stability range (4–75°C; pH 3–12).	No explicit resistance dynamics tested.	Coevolutionary trajectories and environmental persistence not assessed.	Moderate.
Fikadu et al., 2024	Single-phage exposure selected resistant variant; subsequent phage (VBA) lysed the resistant host—demonstrates practical phage–host coevolution strategy.	Geographically limited isolates; lab-scale validation only.	Validation in larger wastewater systems and diverse MDR strains.	Moderate.
Senhaji-Kacha et al., 2024	Isolated phages showed lysis of CRKP; mechanisms of resistance not fully explored.	Short-term in vitro only; no animal validation.	Resistance development over time; phage-antibiotic synergy not assessed.	Moderate risk of bias — no randomization, limited strain set.
Wang et al., 2024	vB_KpnP_23 lysed CRKP (ST11-K64); some resistance observed after 24–48 h.	No in vivo validation; focus on one phage.	Evolutionary dynamics with host strains; biofilm impact.	Moderate — basic characterization, no long-term follow-up.
Yazdi et al., 2018	VB_EcoS-Golestan lysed MDR *E. coli*; no lysogeny or AMR genes detected.	Tested only on UTI isolates; no cocktail strategy.	Broader host range assessment; in vivo efficacy unknown.	Low-moderate — good genomics, but limited scope.
Badawy et al., 2022	fEg-Eco19 stable and lytic vs UTI *E. coli*; no lysogeny or AMR genes.	Single isolate focus; in vitro only.	Long-term resistance risk; combination therapy.	Moderate — solid molecular data, but small sample base.
Akremi et al., 2022	Sewage-derived phages lysed MDR *P. aeruginosa*; demonstrated broad host killing.	Limited to Tunisian isolates; no genomic deep dive.	Stability in UAE sewage; in vivo testing.	Moderate — multiple phages, but uneven reporting.
Mirza et al., 2025	vbKpUKJ_2 lytic vs MD*R K. pneumoniae*; strong pH and temperature tolerance.	In vitro only; tested on small isolate panel.	Resistance suppression over time; clinical synergy.	Low-moderate — detailed genomics, but no in vivo.
Ponsecchi et al., 2024	Four phages targeted CRKP ST147/ST307; effective but resistance emerged in some strains.	Focused on two high-risk clones; no clinical trial.	Broader applicability to UAE CRKP diversity; biofilm testing.	Moderate — genomic work solid, but experimental design narrow.
Menon et al., 2022	N4-like phage suppressed resistance, stable across conditions; synergistic with antibiotics.	In vitro only; limited regional strains.	Long-term coevolution; UAE isolate testing.	Low-moderate — robust lab data, but limited ecological scope.
Ochieng'Oduor et al., 2019	*S. aureus* phage reduced infection burden in mice; no resistance observed in vivo.	Single phage; small animal model study.	Combination therapy in staphylococcal sepsis; durability.	Moderate — in vivo validation strong, but sample small.
Hanzada et al., 2025	Four lytic *P. aeruginosa* phages (myovirus, podovirus, siphovirus) with broad host range; combined as a cocktail	In vitro and Galleria only; no mammalian mode	Resistance dynamics and sewage-scale application not assessed	Moderate (in vitro + invertebrate model)
Peijun Lin et al., 2025	Phage MSP15 active against MRSA; broad host range; efficacy in systemic infection mouse model	Single phage; mouse model only	Cocktail strategy and resistance durability not assessed	Moderate (in vivo validation, single phage)
Sara Sadeqi et al., 2022	42-phage cocktail reduced multiple MDR pathogens in hospital wastewater over 6 months; only field-scale evidence	No mechanistic/genomic resistance analysis; pilot scale	Mechanism of suppression and long-term ecological impact unstudied	Moderate (real-world field data; limited controls)

large-scale environmental trials to confirm durability, scalability, and clinical relevance.

The studies indicate the potential of phage cocktails as a viable and sustainable method for wastewater treatment. They have achieved more than a 99% reduction in bacterial load in hospital sewage; moreover, phage-antibiotic combinations have revived the antibiotic efficacy in otherwise resistant bacterial strains (16). The global applicability of multiphage therapy can be obtained from the studies coming from diverse regions, such as Tunisia (37), India (40), and Iran (16). Phage therapy has also broadened its public health relevance by going beyond gram-negative bacteria to gram-positive MDR pathogens, such as MRSA (4).

CONCLUSION

The future work should focus on long-term co-evolution studies targeting sewage microcosms to determine phage efficacy (16). Comprehensive, genomic investigations of bacterial escape mutants and phage adaptations are also required to enable the practical design of phage cocktail therapy (38). The wastewater trials also need to be scaled up in multiple regions to establish reproducibility and ecological stability (16). More research is needed with Gram-positive MDR pathogens and needs to go beyond *Enterobacteriaceae* (4). Lastly, more integration of phages is required with existing wastewater technologies and antibiotics for hybrid AMR control.

While this review provides a focused synthesis of phage strategies against MDR pathogens in wastewater, a few limitations should be noted. The search was confined to two major databases (PubMed and Scopus) and to peer-reviewed, English-language studies from 2015 onward, ensuring high-quality evidence base while acknowledging that some relevant work may lie beyond this scope. The included studies, though methodologically diverse, were largely laboratory or in vitro investigations supported by one promising field study; this diversity enriched the qualitative synthesis but meant that a quantitative meta-analysis was not appropriate. The concentration of evidence on key pathogens such as *Klebsiella pneumoniae* and *Escherichia coli* reflects current research priorities and offers a strong foundation that future work can extend to a broader range of MDR species. Collectively, these observations point to clear and encouraging directions for larger, standardized, and field-scale studies to confirm the long-term potential of phage cocktails and co-evolutionary training in wastewater applications.

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