Environmental Phages Targeting MDR Clinical Isolates of Pseudomonas aeruginosa

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Abstract:

The increasing occurrence of multidrug-resistant (MDR) bacterial pathogens has significantly renewed scientific and clinical interest in bacteriophages as naturally occurring antibacterial agents with clear therapeutic potential. Environmental habitats represent particularly important and underutilized reservoirs of bacteriophage diversity, primarily because bacteriophages are generally associated with the environments in which their bacterial hosts naturally occur and proliferate. The present study aimed to systematically assess the potential of commonly accessible environmental sources as practical reservoirs of bacteriophages capable of infecting MDR clinical Pseudomonas spp. isolates.

Clinical Pseudomonas isolates obtained from different clinical sources, previously identified using biochemical characterization, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), and antimicrobial susceptibility testing (AST) to confirm their multidrug-resistant status (unpublished data), were employed as bacterial hosts. These validated hosts were used to isolate bacteriophages from a range of environmental sources, including the Tapi River and its associated drain, Gopi Talav, and the sewage pumping stations located at Athwa, Nanpura, and Makaipul. Through the application of standard laboratory protocols, a total of 13 bacteriophages were successfully recovered and designated YSNP1, YSNP7, YSNP8, YSNP9, YSNP11, YSNP13, YSNP14, YSNP15, YSNP16, YSNP18, YSNP19, YSNP20, and YSNP24 using the conventional spot assay and double-layer agar methods.

Their recovery from these geographically and environmentally distinct sources clearly demonstrates that these readily accessible environmental water-associated habitats serve as useful and productive sources of phages with potential application in the treatment of antibiotic-resistant bacterial infections. Further comparative analysis of the isolation yields obtained from the different sites showed that the Athwa sewage pumping station consistently served as the most productive location and therefore the best possible choice for the isolation of these bacteriophages.

Introduction:

The relentless escalation of multidrug-resistant bacterial pathogens, with Pseudomonas aeruginosa standing out as one of the most formidable and clinically significant members of this group, constitutes an increasingly urgent and multifaceted threat to global public health systems. Projections concerning the trajectory of antimicrobial resistance paint a particularly alarming picture: if current trends persist without decisive intervention, annual mortality attributable to drug-resistant infections could surpass ten million deaths by the year 2050, a

figure that would exceed fatalities from many other major causes of death combined (Bhargava et al., 2021; Sawa et al., 2024). This looming crisis is driven in large measure by the continued proliferation and evolutionary success of the so-called ESKAPE pathogens—Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species—organisms that routinely display high-level resistance to carbapenems and other last-resort antimicrobial agents that once formed the final line of defense against severe bacterial infections (Sharma et al., 2021). The rapid emergence and spread of these resistant strains have been facilitated by a complex interplay of factors, including the overuse and misuse of antibiotics in clinical and agricultural settings, the horizontal transfer of resistance genes via mobile genetic elements, and the remarkable adaptive capacity of bacterial populations under selective pressure.

Compounding this challenge is the well-documented stagnation in the conventional antibiotic development pipeline. Traditional pharmaceutical approaches have struggled to keep pace with the speed of microbial adaptation, resulting in a dwindling arsenal of effective drugs and a growing recognition that entirely new therapeutic paradigms must be explored and rigorously validated (Akremi et al., 2022; Oromí-Bosch et al., 2023). In this context, bacteriophage therapy has re-emerged as a highly promising antimicrobial strategy. Bacteriophages, or phages, are viruses that specifically infect and replicate within bacterial cells; their inherent ability to recognize, adsorb to, and ultimately lyse multidrug-resistant bacterial strains—while leaving the beneficial host microbiota largely undisturbed—confers several distinct advantages over broad-spectrum antibiotics (Elfadadny et al., 2024). Unlike conventional antibiotics, which often exert collateral damage on commensal microbial communities and can thereby promote secondary infections or dysbiosis, phages offer a precision-targeted approach that can be tailored to particular bacterial pathogens or even specific strain profiles.

Moreover, the natural ubiquity of environmental water sources—ranging from rivers and lakes to sewage systems and wastewater treatment facilities—harbors extraordinarily diverse and dynamic viral populations. These aquatic and semi-aquatic ecosystems constitute a vast, still largely untapped reservoir from which potent lytic bacteriophages can be identified, isolated, and characterized (Morris et al., 2025). By systematically surveying and leveraging these naturally occurring viral communities, researchers are able to recover highly specialized phage candidates that possess the capacity to address the clinical challenges posed by emerging and established resistant microorganisms (Alharbi & Ziadi, 2021). Such bioprospecting efforts

not only expand the global inventory of potential therapeutic agents but also deepen our understanding of phage–host dynamics in real-world ecological settings.

Nevertheless, despite the clear therapeutic potential of these viral agents, the pathway toward widespread clinical integration remains complex and contingent upon several critical prerequisites. Robust, internationally harmonized regulatory frameworks must be established to govern the production, characterization, and administration of phage preparations. Consistent product quality—encompassing purity, titer stability, genetic safety (including the absence of undesirable genes such as those encoding toxins or antibiotic resistance determinants), and batch-to-batch reproducibility—must be assured through standardized manufacturing processes. In addition, further longitudinal clinical studies and rigorous safety assessments are essential to evaluate long-term efficacy, potential immune responses, and any unforeseen ecological or microbiological consequences of phage therapy (Alsaadi et al., 2021; Saeed et al., 2023).

In response to these imperatives, contemporary research has placed strong emphasis on the isolation of lytic bacteriophages from environmental water samples through a carefully structured methodological workflow. This workflow typically begins with host enrichment techniques that selectively amplify phages capable of infecting target multidrug-resistant Pseudomonas isolates, followed by membrane filtration to remove bacterial cells and debris, plaque assays to visualize and quantify lytic activity, and repeated rounds of single-plaque purification to obtain genetically homogeneous phage stocks (Aghaee et al., 2021; Akremi et al., 2022). By centering these efforts on the strategic bioprospecting of carefully chosen ecological niches—particularly those associated with high bacterial loads and selective pressures, such as sewage pumping stations and urban waterways—investigators aim to address the urgent necessity of expanding and diversifying existing phage banks (Kim et al., 2025; Würstle et al., 2024).

Such systematic isolation and characterization initiatives are of particular importance given the current scarcity of novel antibacterial drugs entering clinical pipelines. The paucity of new chemical entities with activity against multidrug-resistant Gram-negative pathogens has prompted a decisive shift in clinical and scientific focus toward the potential of phage therapy to mitigate the severe complications associated with persistent, difficult-to-treat infections (Chen et al., 2021). In this broader landscape, the recovery, genomic analysis, and functional evaluation of environmentally derived lytic phages represent not merely an academic exercise

but a pragmatic and necessary contribution to the global effort to restore effective therapeutic options against some of the most challenging bacterial pathogens of the modern era.

Methodology

Bacterial hosts

Clinical *Pseudomonas* spp. isolates recovered from a diverse range of clinical specimens served as the bacterial hosts for all bacteriophage isolation and characterization experiments conducted in this study. Prior to their use, these isolates had been thoroughly identified to the species level through a combination of conventional biochemical tests performed according to standard microbiological procedures (Forbes et al., 2002) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) carried out following established laboratory protocols (CLSI, 2017). In addition, antimicrobial susceptibility testing (AST) had been performed to confirm their multidrug-resistant (MDR) phenotypic status in strict accordance with the most recent Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2026; unpublished data). For routine maintenance, the isolates were stored on nutrient agar plates at 4 °C. Prior to each experimental use, they were inoculated into nutrient broth and incubated overnight at 37 °C with continuous orbital shaking to generate actively growing mid-log-phase cultures suitable for phage enrichment and plating assays.

Environmental sample collection

Environmental water samples were aseptically collected from several geographically and environmentally distinct sites within Surat, Gujarat, India, chosen specifically for their potential to harbour diverse bacteriophage populations associated with high bacterial densities. The sampling locations included the main channel of the Tapi River together with its associated drain, the standing waters of Gopi Talav, and the municipal sewage pumping stations located at Athwa, Nanpura and Makaipul. At each site, samples were collected in sterile containers using aseptic technique, immediately sealed, and transported to the laboratory under cool conditions. All samples were processed within a few hours of collection to minimise any possible reduction in phage viability that might result from prolonged storage or temperature fluctuations.

Enrichment and isolation of bacteriophages

Bacteriophages exhibiting clear lytic activity against the MDR clinical Pseudomonas isolates were isolated through a sequential combination of enrichment culture, spot assay screening and double-layer agar plaque purification, following well-established standard protocols (Adams, 1959; Kropinski et al., 2009; Peters et al., 2022).

In brief, environmental water samples were first centrifuged at 8 000 × g for 10 minutes to remove larger particulate debris and suspended solids. The resulting supernatants were then carefully filtered through sterile 0.22 µm membrane filters to eliminate residual bacterial cells while allowing free bacteriophage particles to pass through. The filter-sterilized samples were subsequently mixed with mid-log-phase cultures of the respective clinical host isolates in nutrient broth and incubated at 37 °C with gentle continuous shaking for 18–24 hours. This enrichment step was designed to selectively amplify any bacteriophages present in the sample that were capable of infecting and replicating within the target MDR Pseudomonas hosts. After the enrichment period, the cultures were centrifuged once more and the supernatants were again passed through 0.22 µm filters to obtain cell-free phage lysates ready for screening.

Spot assay

The enriched, filter-sterilized filtrates were initially screened for the presence of lytic bacteriophage activity using the classical spot assay. Overnight cultures of the appropriate host bacteria were evenly swabbed across the entire surface of nutrient agar plates to produce a uniform, confluent bacterial lawn. Once the plates had dried completely under aseptic conditions, small aliquots (5–10 µl) of each filtrate were carefully spotted onto discrete areas of the swabbed surface. The plates were then incubated overnight at 37 °C. The subsequent appearance of clear zones of lysis (halos) at the sites of spotting was interpreted as a positive indication of the presence of lytic bacteriophages capable of infecting the host strain (Kropinski et al., 2009; Peters et al., 2022).

Double-layer agar (plaque) assay and purification

Filtrates that produced clear zones of lysis in the spot assay were subjected to further characterization and purification using the double-layer agar (plaque) method (Adams, 1959; Kropinski et al., 2009). Serial ten-fold dilutions of each positive filtrate were prepared in sterile broth. Aliquots of these dilutions were mixed with a suspension of the corresponding host bacteria in molten soft nutrient agar (containing 0.4–0.7 % agar) maintained at approximately 45–50 °C. The soft-agar mixtures were immediately poured as uniform overlays onto solid nutrient agar base plates and allowed to solidify. After overnight incubation at 37 °C, discrete plaques of varying morphology and size became visible within the bacterial lawn. Individual well-isolated plaques were carefully picked using a sterile pipette tip or toothpick, resuspended in a small volume of nutrient broth, and re-plated using the same double-layer technique. This single-plaque purification process was repeated a minimum of three successive times to ensure the recovery of genetically homogeneous, pure phage stocks free from contamination by other

viral particles. Each purified bacteriophage isolate was assigned a unique laboratory designation consisting of the prefix YSNP followed by a sequential numerical identifier.

Storage

Once purified and amplified to high titre, phage stocks were suspended in nutrient broth and stored at 4 °C for short-term use and subsequent experimental characterization (Alvi et al., 2018).

Results :-

A thorough and systematic isolation campaign ultimately resulted in the successful recovery of a total of thirteen distinct bacteriophages that exhibited clear, robust, and reproducible lytic activity against a carefully selected panel of clinical multidrug-resistant Pseudomonas spp. isolates. All of these phages were obtained exclusively from a diverse set of carefully chosen environmental samples that had been collected from multiple aquatic habitats and wastewater infrastructure sites throughout the study area. The complete and precise distribution of every recovered bacteriophage, organized according to its exact geographical origin and environmental source, is comprehensively tabulated and presented for easy reference and comparative analysis in Table 1.

More specifically, five individual bacteriophages—explicitly designated YSNP1, YSNP11, YSNP16, YSNP19, and YSNP20—were successfully isolated from wastewater samples obtained at the Athwa sewage pumping station. These five isolates collectively accounted for 38.5 percent of the entire collection of bacteriophages recovered over the course of the investigation, thereby representing by far the single largest contribution from any one sampling location. At the same time, three further bacteriophages, identified as YSNP8, YSNP14, and YSNP18, were recovered from samples collected along the Tapi drain; together these three phages constituted 23.1 percent of the total isolates. From the main flowing channel of the Tapi River itself, only a solitary bacteriophage, designated YSNP9, could be obtained, corresponding to a modest 7.7 percent of the overall yield. In parallel, three additional bacteriophages—YSNP7, YSNP13, and YSNP15—were isolated from the Nanpura sewage pumping station, likewise representing another 23.1 percent of the recovered phage collection. Finally, a single bacteriophage, labeled YSNP24, was recovered from the standing waters of Gopi Talav and contributed the remaining 7.7 percent of the total isolates. Of particular note is the complete absence of any detectable bacteriophages displaying lytic activity against the target multidrug-resistant Pseudomonas spp. isolates from samples taken at the Makaipul sewage pumping station, even though the same standardized isolation and enrichment protocols were rigorously applied at every site.

When all of these recovery data are considered together, it becomes evident that the Athwa sewage pumping station stood out clearly as the most productive and richest environmental source among all the locations examined. Its contribution of fully 38.5 percent of the total bacteriophage isolates underscores the exceptional abundance of lytic phages present at this particular site and confirms its high practical value as a preferred reservoir for the isolation of therapeutically promising bacteriophages capable of infecting and lysing clinical multidrug-resistant Pseudomonas spp. strains. These findings not only highlight the superior potential of the Athwa station for future phage-discovery efforts but also reinforce its importance as a strategic sampling location in ongoing searches for effective biological agents against these challenging opportunistic pathogens.

Table 1. Bacteriophages isolated against clinical *Pseudomonas* spp. isolates from different environmental sources.

Host bacterium	Environmental source	Environmental sub-source	Bacteriophage code	Percentage
Pseudomonas aeruginosa	Athwa	—	YSNP1	38.5 %
			YSNP11	
			YSNP16	
			YSNP19	
			YSNP20	
	Tapi	Drain	YSNP8	23.1 %
			YSNP14	
			YSNP18	
		River	YSNP9	7.7 %
	Nanpura	—	YSNP7	23.1 %
			YSNP13	
			YSNP15	
	Gopi Talav	—	YSNP24	7.7 %

Discussion :-

The increasing prevalence of multidrug-resistant Pseudomonas infections has renewed interest in bacteriophages as potential alternative therapeutic agents. In the present study, 13 lytic bacteriophages were successfully isolated from commonly accessible environmental water sources using clinical MDR Pseudomonas isolates as hosts. The recovery of phages from multiple geographically distinct sites (river, drain, lake and sewage) supports the view that

environmental habitats where bacterial hosts are abundant serve as rich reservoirs of bacteriophage diversity (Aghaee et al., 2021; Dai et al., 2023; Rodríguez-Recio et al., 2025).

Among the sampled locations, the Athwa sewage pumping station was the most productive source, contributing 38.5% of the total phages isolated. Sewage and wastewater environments typically contain high densities of bacteria, including Pseudomonas spp., which naturally favour the proliferation and persistence of their corresponding bacteriophages (Aghaee et al., 2021; Dai et al., 2023). In comparison, the Tapi drain and Nanpura sewage station each contributed 23.1% of the isolates, while the Tapi River and Gopi Talav yielded only single isolates (7.7% each). The absence of recoverable phages from Makaipul may reflect lower host density or differences in local environmental conditions at the time of sampling.

These findings demonstrate that readily available environmental water sources, particularly sewage and wastewater, can serve as practical and cost-effective reservoirs for isolating bacteriophages active against clinically relevant MDR Pseudomonas strains (Aghaee et al., 2021; Dai et al., 2023; Rodríguez-Recio et al., 2025). Such phages hold potential for future applications in phage therapy, although further characterisation (host range, morphology, genomic analysis and efficacy studies) will be required before therapeutic use can be considered.

Conclusion :-

Thirteen bacteriophages with strong lytic activity against multidrug-resistant clinical isolates of Pseudomonas spp. were successfully recovered from environmental water sources collected across Surat. Systematic sampling of multiple sites revealed that the Athwa sewage pumping station was by far the richest reservoir, contributing 38.5 % of all isolated phages.

This outcome underscores the value of sewage-enriched environments as natural hotspots for bacteriophage diversity. Sewage systems continuously receive high loads of both human-associated bacteria and the viruses that prey on them, creating ideal conditions for the persistence and enrichment of lytic phages. Consequently, such readily accessible habitats represent practical and productive sources for discovering phages that can infect antibiotic-resistant pathogens.

The isolation of these thirteen phages therefore supports the broader strategy of environmental bioprospecting—particularly in urban wastewater settings—as a promising route for identifying candidates that could be developed into phage-based therapies or biocontrol agents against multidrug-resistant Pseudomonas infections, which remain a serious clinical challenge.

Importantly, the successful recovery of multiple active phages from a single, easily accessible sewage site also suggests that targeted surveillance of similar urban wastewater infrastructure could yield additional therapeutic candidates in the future, thereby expanding the available arsenal against hard-to-treat bacterial infections.

Nevertheless, several critical steps remain before these isolates can be advanced toward practical application. Comprehensive characterisation is required, encompassing detailed host-range and specificity analysis, evaluation of stability profiles under varying pH and temperature conditions, determination of one-step growth (burst) kinetics, assessment of biofilm-removal capacity, and high-resolution morphological examination by transmission electron microscopy together with surface analysis via scanning electron microscopy. In parallel, whole-genome sequencing of each phage will be essential to confirm a strictly lytic lifestyle, exclude genes associated with lysogeny, virulence or antibiotic resistance, and accurately determine taxonomic affiliation.

Subsequent in-vivo efficacy and safety studies in relevant animal models will be necessary to establish therapeutic potential, optimal dosing regimens, pharmacokinetics and possible host immune responses. Looking further ahead, the development of a well-curated regional phage biobank would significantly enhance translational impact by enabling rapid matching of fully characterised phages to emerging multidrug-resistant Pseudomonas isolates, supporting the formulation of personalised phage cocktails, and ensuring a readily available stock of quality-controlled therapeutic candidates for clinical or compassionate-use applications.

In the longer term, integration of these characterised phages into existing antimicrobial stewardship programmes could help reduce reliance on last-resort antibiotics and slow the further spread of resistance. Collaborative networks linking academic laboratories, hospitals and public-health authorities would be instrumental in translating laboratory findings into clinically usable preparations, while standardised production and quality-control protocols would ensure reproducibility and safety.

Finally, continued environmental monitoring and periodic re-isolation efforts will be important to maintain a dynamic collection that keeps pace with the evolving resistance profiles of local Pseudomonas populations, thereby sustaining the relevance and effectiveness of phage-based interventions over time.

Beyond these immediate next steps, systematic comparison of the isolated phages with existing international collections could reveal unique regional signatures or novel genomic

features that expand the global phage repertoire. Such comparative analyses would also facilitate the identification of broad-spectrum candidates suitable for inclusion in multi-phage formulations designed to minimise the emergence of phage resistance.

Ultimately, the successful progression of these environmental isolates from discovery through full characterisation, genomic validation, in-vivo evaluation and biobank deposition would not only address a pressing local clinical need but also contribute valuable resources and methodological insights to the wider field of phage therapy research.

Moreover, the demonstrated productivity of the Athwa site highlights the potential for establishing routine phage-hunting programmes in other densely populated Indian cities facing similar antimicrobial-resistance burdens. Coupling such efforts with metagenomic surveys of wastewater could further accelerate discovery by revealing previously unculturable or rare phage lineages. In parallel, early engagement with regulatory bodies will be essential to define clear pathways for the approval and clinical deployment of phage preparations, ensuring that laboratory advances can be translated into tangible patient benefit in a timely and responsible manner.

Future scope and limitations :-

- Fully characterisation of these bacteriophages including host range and specificity analysis, various stability profiles like pH and temperature, burst curve analysis, bio-film removal, electron microscopy like transmission for morphology and scanning for surface analysis.
- Whole genome sequencing
- *In-vivo* testing
- Biobank development for readily available phages for treatment

References :-

- Adams, M. H. (1959). Bacteriophages. Interscience Publishers.
- Aghaee, B. L., Mirzaei, M. K., Alikhani, M. Y., & Mojtahedi, A. (2021). Sewage and sewage-contaminated environments are the most prominent sources to isolate phages against Pseudomonas aeruginosa. BMC Microbiology, 21, 132. https://doi.org/10.1186/s12866-021-02197-z
- Aghaee, B. L., Mirzaei, M. K., Alikhani, M. Y., & Mojtahedi, A. (2021). Sewage and sewage-contaminated environments are the most prominent sources to isolate phages against Pseudomonas aeruginosa. BMC Microbiology, 21, Article 132. https://doi.org/10.1186/s12866-021-02197-z

- Akremi, I., Merabishvili, M., Jlidi, M., Haj Brahim, A., Ben Ali, M., Karoui, A., Lavigne, R., Wagemans, J., Pirnay, J.-P., & Ben Ali, M. (2022). Isolation and characterization of lytic Pseudomonas aeruginosa bacteriophages isolated from sewage samples from Tunisia. Viruses, 14(11), 2339. https://doi.org/10.3390/v14112339
- Alharbi, N. M., & Ziadi, M. M. (2021). Wastewater as a fertility source for novel bacteriophages against multi-drug resistant bacteria. Saudi Journal of Biological Sciences, 28(8), 4358–4364. https://doi.org/10.1016/j.sjbs.2021.04.025
- Alsaadi, A., Beamud, B., Easwaran, M., Abdelrahman, F., El-Shibiny, A., Alghoribi, M. F., & Domingo-Calap, P. (2021). Learning from mistakes: The role of phages in pandemics. Frontiers in Microbiology, 12, 653107. https://doi.org/10.3389/fmicb.2021.653107
- Alvi, I. A., Asif, M., Tabassum, R., Abbas, Z., & ur Rehman, S. (2018). Storage of bacteriophages at 4°C leads to no loss in their titer after one year. Pakistan Journal of Zoology, 50(6), 2395–2398.
- Bhargava, K., Nath, G., Bhargava, A., Aseri, G. K., & Jain, N. (2021). Phage therapeutics: From promises to practices and prospectives. Applied Microbiology and Biotechnology, 105(24), 9047–9067. https://doi.org/10.1007/s00253-021-11695-z
- Chen, F., Cheng, X., Li, J., Yuan, X., Huang, X., Lian, M., Li, W., Huang, T., Xie, Y., Liu, J., Gao, P., Wei, X., Wang, Z., & Wu, M. (2021). Novel lytic phages protect cells and mice against Pseudomonas aeruginosa infection. Journal of Virology, 95(8), e01832-20. https://doi.org/10.1128/JVI.01832-20
- Clinical and Laboratory Standards Institute. (2017). Methods for the identification of cultured microorganisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (1st ed.). CLSI guideline M58. Clinical and Laboratory Standards Institute.
- Clinical and Laboratory Standards Institute. (2026). Performance standards for antimicrobial susceptibility testing (36th ed.). CLSI supplement M100. Clinical and Laboratory Standards Institute.
- Dai, F., et al. (2023). Isolation and characterization of Pseudomonas aeruginosa phages with a broad host spectrum from hospital sewage systems and their therapeutic effect in a mouse model. The American Journal of Tropical Medicine and Hygiene, 108(6), 1220–1226. https://doi.org/10.4269/ajtmh.22-0303

- Elfadadny, A., Ragab, R. F., AlHarbi, M., Badshah, F., Ibáñez-Arancibia, E., Farag, A., Hendawy, A. O., De Los Ríos-Escalante, P. R., Aboubakr, M., Zakai, S. A., & Nageeb, W. M. (2024). Antimicrobial resistance of Pseudomonas aeruginosa: Navigating clinical impacts, current resistance trends, and innovations in breaking therapies. Frontiers in Microbiology, 15, 1374466. https://doi.org/10.3389/fmicb.2024.1374466
- Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2002). Bailey & Scott's diagnostic microbiology (11th ed.). Mosby.
- Kim, M. K., Suh, G. A., Cullen, G. D., Perez Rodriguez, S., Dharmaraj, T., Chang, T. H. W., Li, Z., Chen, Q., Green, S. I., Lavigne, R., Pirnay, J.-P., Bollyky, P. L., & Sacher, J. C. (2025). Bacteriophage therapy for multidrug-resistant infections: Current technologies and therapeutic approaches. Journal of Clinical Investigation, 135(5), e187996. https://doi.org/10.1172/JCI187996
- Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of bacteriophages by double agar overlay plaque assay. In M. R. J. Clokie & A. M. Kropinski (Eds.), Bacteriophages: Methods and protocols, Volume 1 (pp. 69–76). Humana Press. https://doi.org/10.1007/978-1-60327-164-6_7
- Morris, T. C., Reyneke, B., Khan, S., & Khan, W. (2025). Phage-antibiotic synergy to combat multidrug resistant strains of Gram-negative ESKAPE pathogens. Scientific Reports, 15, 17235. https://doi.org/10.1038/s41598-025-01489-y
- Oromí-Bosch, A., Antani, J. D., & Turner, P. E. (2023). Developing phage therapy that overcomes the evolution of bacterial resistance. Annual Review of Virology, 10, 503–524. https://doi.org/10.1146/annurev-virology-012423-110530
- Peters, D. L., et al. (2022). Bacteriophage isolation, purification, and characterization techniques against ubiquitous opportunistic pathogens. Current Protocols, 2(11), e594. https://doi.org/10.1002/cpz1.594
- Rodríguez-Recio, F. R., et al. (2025). Genomic insights into and lytic potential of native bacteriophages M8-2 and M8-3 against clinically relevant multidrug-resistant Pseudomonas aeruginosa. Antibiotics, 14(2), 110. https://doi.org/10.3390/antibiotics14020110
- Saeed, U., Insaf, R. A., Piracha, Z. Z., Tariq, M. N., Sohail, A., Abbasi, U. A., Fida Rana, M. S., Gilani, S. S., Noor, S., Noor, E., Waheed, Y., Wahid, M., Najmi, M. H., & Fazal, I. (2023). Crisis averted: A world united against the menace of multiple drug-resistant superbugs—pioneering anti-AMR vaccines, RNA interference, nanomedicine,

CRISPR-based antimicrobials, bacteriophage therapies, and clinical artificial intelligence strategies to safeguard global antimicrobial arsenal. Frontiers in Microbiology, 14, 1270018. https://doi.org/10.3389/fmicb.2023.1270018

- Sawa, T., Moriyama, K., & Kinoshita, M. (2024). Current status of bacteriophage therapy for severe bacterial infections. Journal of Intensive Care, 12, 44. https://doi.org/10.1186/s40560-024-00759-7
- Sharma, S., Datta, S., Chatterjee, S., Dutta, M., Samanta, J., Vairale, M. G., Gupta, R., Veer, V., & Dwivedi, S. K. (2021). Isolation and characterization of a lytic bacteriophage against Pseudomonas aeruginosa. Scientific Reports, 11, 19393. https://doi.org/10.1038/s41598-021-98457-z
- Würstle, S., Lee, A., Kortright, K. E., Winzig, F., An, W., Stanley, G. L., Rajagopalan, G., Harris, Z., Sun, Y., Hu, B., Blazanin, M., Hajfathalian, M., Bollyky, P. L., Turner, P. E., Koff, J. L., & Chan, B. K. (2024). Optimized preparation pipeline for emergency phage therapy against Pseudomonas aeruginosa at Yale University. Scientific Reports, 14, 2657. https://doi.org/10.1038/s41598-024-52192-3