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EXPLORING BACTERIOPHAGES: NATURE'S OWN ANTIMICROBIAL AGENT

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ABSTRACT

Water is important for every facet of life. Surface water acts as a natural source of freshwater and is important for drinking, cooking as well as other household purposes. Access to safe and clean drinking water is a basic right to every human being for a healthy life. Globally freshwater is becoming a limited resource due to population expansion, anthropogenic contamination and climate change. It is reported that 80% of the diseases worldwide occurs due to contaminated water and water borne pathogens. According to the World Health Organization (WHO) 2022 report, more than two billion individuals rely on water sources contaminated with faecal matter, leading to approximately 485,000 deaths annually from diarrheal diseases associated with unsafe water, inadequate sanitation, and poor hygiene practices. Water pollution and antimicrobial resistance (AMR) are interconnected global crises that threaten public health, food security, and environmental sustainability. This global crisis extends beyond simple water scarcity and is increasingly defined by contamination from microbial pathogens and chemical pollutants. During 2021, United Nations International Children's Emergency Fund (UNICEF) & WHO jointly stated that drivers such as insufficient sanitation infrastructure, industrial effluents, agricultural runoff, and the accelerating impacts of climate change exacerbate the challenge, threatening both human health and environmental stability. Promising applications include wastewater decontamination, mitigation of bacterial outbreaks in aquaculture, and synergistic combinations with antibiotics through phage–antibiotic synergy (PAS). Moreover, phage-based strategies align with sustainability goals by minimizing ecological disruption compared to chemical antimicrobials. Bacteriophages provide a powerful, eco-friendly tool that, if developed and deployed strategically, could complement existing interventions and form a cornerstone of future efforts to combat AMR and reduce the burden of waterborne bacterial pathogens.

***Key words*:** Chemical pollutants, Climate change, Diarrheal diseases, Environmental stability, Pathogens

Introduction

The escalating global water crisis stems from the increasing prevalence of bacterial, viral, and chemical pollutants, which collectively undermine public health and environmental integrity. Contaminated water disproportionately affects low- and middle-income countries (LMICs), where inadequate infrastructure and limited resources heighten vulnerability. For instance, millions in Bangladesh are exposed to chronic arsenic poisoning via contaminated groundwater (Smith *et al.*, 2000), while recurrent cholera outbreaks in Sub-Saharan Africa highlight the consequences of poor water supply and sanitation systems (Mengel *et al.*, 2014). In Latin America, rivers and aquifers are heavily impacted by industrial and mining waste, with serious repercussions for local communities and ecosystems (Blaustein, 2020). Even high-income nations are not entirely immune, as aging infrastructure and localized contamination events can compromise water quality and safety.

Water pollution has emerged as a critical factor not only in the spread of infectious diseases but also in the proliferation of antimicrobial resistance (AMR). Antimicrobial resistance (AMR) is among the top ten global public health threats, according to the World Health Organization (WHO, 2022). Contaminants such as antibiotics, heavy metals, pesticides, microplastics, and pathogenic microorganisms create environments conducive to the selection and transmission of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Babuji *et al.*, 2023; Singh *et al.*, 2024; Chaturvedi *et al.*, 2021; Rzymski *et al.*, 2024). It compromises the effectiveness of antibiotics, antifungals, antivirals, and antiparasitic agents, leading to increased morbidity, mortality, and healthcare costs. Evidence indicates that water quality and accessibility are significant predictors of AMR-related morbidity and mortality. Key hotspots for resistance dissemination include wastewater treatment plants (WWTPs) (Mukherjee *et al.*, 2021), urban rivers (Kotwani *et al.*, 2021), and informal settlements, where incomplete removal of antibiotics and ARGs during water treatment, combined with anthropogenic inputs such as improper waste disposal, agricultural runoff, and pharmaceutical effluents, amplify the spread of resistant strains (Sengar & Vijayanandan, 2021; Zheng *et al.*, 2021; Eugenia *et al.*, 2020; Nadimpalli *et al.*, 2020).

Drivers of Antimicrobial Resistance in Water Systems

Multiple studies have confirmed that water bodies function as both reservoirs and transmission pathways for ARB and ARGs as represented in Fig. 1 (Sanganyado & Gwenzi, 2019; Eugenia *et al.*, 2020; Farrell *et al.*, 2021; Zhou *et al.*, 2018). The main drivers include:

1. Discharge of untreated or inadequately treated wastewater, particularly from hospitals, urban settlements, and pharmaceutical industries, which introduces high concentrations of antibiotics and resistant microorganisms into aquatic environments (Singh *et al.*, 2022; Huang *et al.*, 2019; Sengar & Vijayanandan, 2021; Rodríguez-Mozaz *et al.*, 2015; Eugenia *et al.*, 2020).
2. Agricultural runoff, which carries antibiotics, heavy metals, and animal waste from intensive farming practices, creating selective pressure for resistance development (Jones *et al.*, 2023; Kotwani *et al.*, 2021; Gupta *et al.*, 2022).
3. Rapid urbanization and informal settlements, where insufficient sanitation and water treatment infrastructure exacerbate contamination and facilitate the spread of resistant pathogens (Nadimpalli *et al.*, 2020; Bartley *et al.*, 2019).
4. Environmental co-selective agents, such as heavy metals and microplastics, which promote horizontal gene transfer and accelerate the dissemination of resistance traits within microbial communities (Komijani *et al.*, 2021; Rzymski *et al.*, 2024).

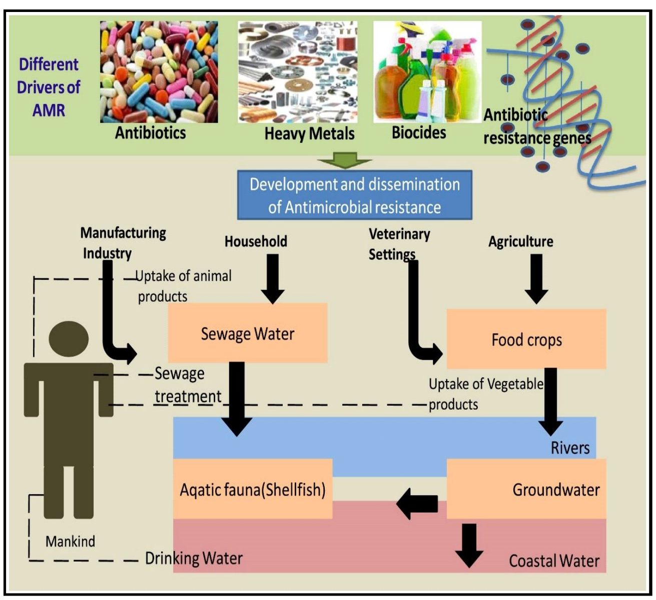

Fig. 1: Generalized scheme of different drivers of antimicrobial resistance (AMR) and their possible route of transmission to humans (Samreen *et al.*, 2021).

Integrative Approaches for Mitigation

Addressing the intertwined challenges of water pollution and antimicrobial resistance (AMR) requires a holistic, multi-sectoral strategy that combines technological innovations, effective governance, and community engagement. Strengthening water, sanitation, and hygiene (WASH) infrastructure has been shown to significantly reduce both waterborne disease burden and AMR dissemination (Nadimpalli *et al.*, 2020; Lewnard *et al.*, 2024). At the same time, the implementation of advanced wastewater treatment technologies capable of removing antibiotics and resistance genes is increasingly recognized as a key intervention for limiting environmental AMR hotspots (Zheng *et al.*, 2021; Sengar & Vijayanandan, 2021). Similarly, stringent regulation of industrial effluents and agricultural runoff plays a vital role in preventing the release of antibiotics, heavy metals, and other pollutants that co-select for resistance traits (Kotwani *et al.*, 2021; Gupta *et al.*, 2022). Continuous monitoring of environmental reservoirs such as urban rivers, hospital wastewater, and informal settlements is also necessary to track emerging threats (Mukherjee *et al.*, 2021; Monaco *et al.*, 2025).

Furthermore, integrating the One Health framework into policy and research agendas ensures that interventions account for the interconnectedness of human, animal, and environmental health, which is essential for sustainable AMR control (Bürgmann *et al.*, 2018; Singh *et al.*, 2022; La Rosa *et al.*, 2025). Such integrated approaches not only help reduce disease burden but also limit the environmental persistence of resistant pathogens and safeguard ecosystem resilience.

While these conventional measures are critical, innovative biological strategies are now emerging as complementary solutions. Among them, bacteriophages, viruses that specifically infect and kill bacteria offer a promising, eco-friendly, and highly targeted alternative for combating AMR pathogens in water systems (Abedon *et al.*, 2017). Their natural ability to control bacterial populations, adaptability, and safety profile position them as potential tools not only for therapeutic applications but also for environmental bioremediation, opening new possibilities in addressing the water-AMR nexus (Clokie, 2011).

Bacteriophages

Bacteriophages, or phages, are viruses that specifically infect and replicate within bacterial cells (Clokie, 2011). First discovered nearly a century ago, they are now recognized as the most abundant biological entities on Earth, playing pivotal roles in regulating bacterial populations across diverse environments, from oceans and soil to the human microbiome. Structurally, phages typically consist of a protein capsid encasing their genetic material—either DNA or RNA—and often possess a tail apparatus that facilitates attachment to their bacterial hosts. Once attached, phages inject their genetic material into the host, hijacking the bacterial machinery to produce progeny. Depending on their life cycle, they either lyse the host cell to release new virions (lytic cycle) or integrate their genome into the bacterial chromosome, replicating passively alongside the host (lysogenic cycle) (Kasman, 2022).

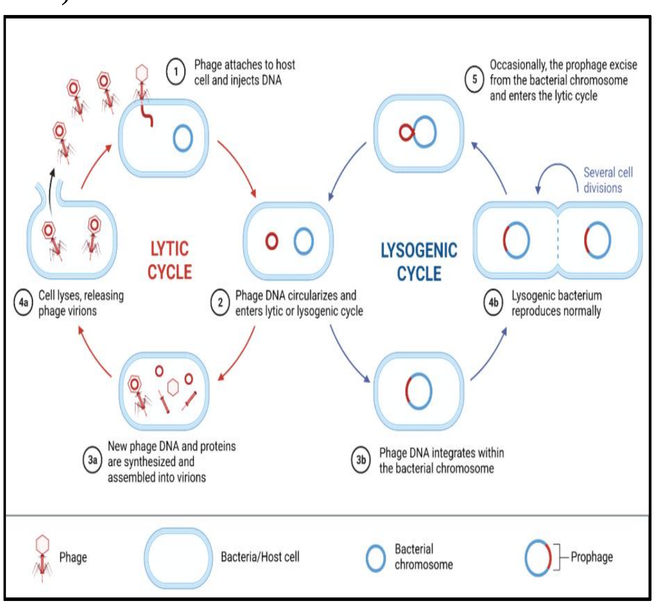

Fig. 2: Lytic and lysogenic bacteriophage replication cycles. (Source: Adapted from BioRender, 2025).

Phages primarily follows two life cycles: the lytic and lysogenic cycles (Fig. 2). In the lytic cycle, phages hijack the host cell machinery to rapidly replicate and lyse the host to release progeny phages. In the lysogenic cycle, temperate phages integrate their genome into the host genome, forming a prophage allowing the phage to persist passively as a prophage until certain conditions trigger a return to the lytic cycle.

Interest in phages has surged due to the global rise of antibiotic-resistant bacteria, positioning phage therapy as a promising alternative or complement to conventional antibiotics. Beyond their therapeutic potential, phages are integral to microbial ecology, shaping bacterial evolution through an ongoing predator–prey dynamic and influencing processes such as biogeochemical cycling and horizontal gene transfer. Their interactions with the mammalian immune system are complex, with evidence suggesting they can modulate immune responses and even impact human health directly. While challenges remain in standardizing phage therapy and understanding bacterial resistance mechanisms, ongoing research continues to expand their applications in medicine and biotechnology.

Ecologically, phages are crucial regulators of bacterial populations across multiple ecosystems, including soil, freshwater, marine environments, and the human microbiome. By lysing specific bacterial hosts, they influence microbial community structure, nutrient cycling, and genetic evolution via horizontal gene transfer. Their predatory activity also helps suppress pathogenic bacteria, maintaining microbial balance naturally. Beyond their ecological role, phages are increasingly applied in biotechnology for bacterial detection, genetic engineering, and as alternative antimicrobial strategies, underscoring their multifaceted importance to both natural ecosystems and human society (Clokie, 2011).

History of Bacteriophage Research

The history of bacteriophage discovery spans over a century, beginning with intriguing observations in natural environments. In 1896, British chemist Ernest Hanbury Hankin reported that waters from the Ganges and Jumna (Yamuna) rivers exhibited bactericidal activity against Vibrio cholerae. He noted that this antibacterial effect was retained only when the water was heated in airtight tubes, implying the presence of an unknown agent capable of killing bacteria. This early observation, though not fully understood at the time, foreshadowed the later identification of bacteriophages (Abedon *et al.*, 2011).

Nearly two decades later, in 1915, Frederick Twort observed viral agents that could destroy bacteria, laying the groundwork for the concept of bacteriophages. It

Table 1: Phage-Encoded Enzymes Relevant to Water Remediation.

S. No.	Enzyme Type	Function	Potential Role in Water Remediation	Example
1.	Endolysins (peptidoglycan hydrolases)	Hydrolyze bacterial cell walls from inside	Lyse pathogens such as *E. coli*, *Vibrio cholerae*, *Salmonella*, reducing microbial contamination	LysA, PlyV12
2.	Depolymerases	Degrade bacterial capsules, EPS, and biofilms	Remove protective biofilms from water system surfaces (pipes, membranes), making bacteria more susceptible to removal/disinfection	Tail spike depolymerases from *Klebsiella* phages
3.	Holins	Create pores in bacterial membranes to allow endolysin access	Work synergistically with lysins to rapidly kill bacteria in contaminated water	SppA-like holins
4.	Polysaccharide-degrading enzymes	Break down alginate, cellulose, chitin, etc.	Degrade extracellular polymeric substances in biofilms in wastewater plants or natural water systems	Alginate lyase from *Pseudomonas* phages
5.	Nucleases	Degrade bacterial DNA	Potentially reduce spread of antibiotic resistance genes (ARGs) in water bodies	DNase-like proteins
6.	Oxidoreductases	Catalyze oxidation/reduction of organic compounds	Could help break down phenolic pollutants, dyes, or aromatic hydrocarbons	Laccase-like oxidases in some cyanophages
7.	Hydrolases & esterases	Hydrolyze ester bonds in fats, oils, pesticides	Biodegrade agricultural runoff contaminants	Lipase/esterase genes in marine phages
8.	Phage-associated ARG suppressors	Inhibit bacterial defense or ARG transfer	Could limit survival of ARG-carrying bacteria in aquatic ecosystems	Anti-CRISPR proteins

was Felix d'Herelle in 1917, however, who formally described these entities, naming them "bacteriophages" and emphasizing their potential as therapeutic tools (Abedon *et al.*, 2011; Pasteur Institute, 2021). Early investigations primarily explored their ability to treat bacterial infections, offering hope at a time when antibiotics did not exist.

The discovery of antibiotics in the mid-20th century shifted the focus away from phage therapy, causing it to recede from mainstream medicine. Yet, the growing global challenge of antimicrobial resistance in recent decades has renewed interest in these bacterial viruses. Today, bacteriophages are being revisited both as a model system for understanding microbial interactions and as potential therapeutic agents, sparking a resurgence of research worldwide (Letarov, 2020).

Phage-host interaction mechanisms

Bacteriophage-host interactions are highly specific and follow a precise sequence of molecular events. Infection begins with the recognition of bacterial surface receptors by phage tail fibers or receptor-binding proteins (RBPs), which serve as the primary determinants of host specificity (Mourosi *et al.*, 2022). The efficiency of this attachment governs the phage's host range, which can vary from narrow, targeting a few strains, to broad, capable of infecting multiple bacterial species. After binding, phages inject their genetic material into the bacterial cytoplasm and hijack host cellular machinery for genome replication, protein synthesis, and assembly of new virions (Mourosi *et al.*, 2022). Host range is not static; it evolves through phage genetic mutations, recombination events, and selective pressures imposed by bacterial resistance mechanisms, leading to dynamic changes in infection potential over time. Environmental factors, such as nutrient availability, temperature fluctuations, and microbial competition, further influence the success of phage infection and host range adaptation.

Bacterial Defence Mechanisms and Phage Counter-Strategies

In response to phage predation, bacteria have developed an arsenal of defense mechanisms. These include restriction-modification systems that enzymatically degrade foreign DNA, CRISPR-Cas adaptive immunity that stores phage sequences for targeted defense, toxin-antitoxin systems, and abortive infection mechanisms that halt viral replication at the cost of host survival (Bleriot *et al.*, 2023; Mutalik *et al.*, 2020). Phages, in turn, counter these defences using strategies such as anti-CRISPR proteins, DNA base modifications, and regulatory proteins that evade host recognition. This molecular arms race drives co-evolution, resulting in diverse outcomes ranging from efficient lysis to partial resistance or complete bacterial immunity. The evolutionary trajectory of both phages and hosts is shaped not only by their genetic diversity but also by ecological pressures, including predation intensity, biofilm architecture, and interspecies interactions (Howard-Varona *et al.*, 2018; Mutalik *et al.*, 2020).

Ecological Complexity and Applied Implications

In complex microbial communities, such as biofilms, phage-host interactions extend beyond simple predator-prey dynamics. Phages can influence microbial diversity, mediate horizontal gene transfer, and, in certain contexts, enhance biofilm formation or stability, thus contributing to ecosystem resilience (Pires *et al.*, 2021). Environmental conditions, including pH, oxygen levels, and the presence of antimicrobial compounds, modulate these interactions and can select for phages with broader host ranges or enhanced infectivity. Understanding these dynamics is critical for applying phages in biotechnology, medicine, and agriculture. Phages are increasingly explored as therapeutic agents against multidrug-resistant bacteria, as biocontrol agents in crop protection, and as modulators of microbial consortia in industrial fermentations (Kauffman *et al.*, 2021). Additionally, insights into phage-host co-evolution and host range evolution provide predictive frameworks for microbial population dynamics, the spread of antimicrobial resistance, and the long-term stability of microbial ecosystems (Stone *et al.*, 2019).

Applications of Bacteriophages

Therapeutic applications

The therapeutic application of bacteriophages was first recognized in 1917 when Félix d'Hérelle described the phenomenon of bactériophage obligatoire (Abedon *et al.*, 2011). Shortly thereafter, Bruynoghe and Maisin (1921) pioneered phage therapy by successfully treating staphylococcal infections in patients. Since then, phage therapy has attracted attention as a promising alternative to conventional antibiotics, particularly in the era of rising antimicrobial resistance (Duckworth & Gulig, 2002; Sarker *et al.*, 2012). Extensive research has explored the therapeutic potential of bacteriophages against a wide range of bacterial pathogens (Matsuzaki *et al.*, 2003; Quintin *et al.*, 2005; Chatterjee *et al.*, 2015; Maszewska *et al.*, 2016).

The first commercial development of phage-based products was carried out in d'Hérelle's laboratory in Paris, where several preparations were produced to target different bacterial infections, including those caused by *Salmonella* spp., *Clostridium* difficile, and diarrheagenic Escherichia coli (Mai *et al.*, 2010; Frampton *et al.*, 2012). These formulations, later commercialized by the French company L'Oréal, included Bactécoliphage, Bactérhinophage, Bactéintestiphage, Bactépyophage, and Bactéstaphyphage (Summers, 1999; Sulakvelidze *et al.*, 2001).

During the 1940s, Eli Lilly and Company (Indianapolis, IN, USA) pioneered the large-scale production of seven bacteriophage-based therapeutics intended for human use. These preparations were formulated either as broth culture lysates (e.g., Colo-lysate, Ento-lysate, Neiso-lysate, and Staphylo-lysate) or as water-soluble jellies (e.g., Colo-jel, Ento-jel, and Staphylo-jel). They were specifically designed to target pathogenic bacteria such as *Staphylococcus*, *Streptococcus*, and *Escherichia coli*, and were clinically employed in the management of abscesses, septic wounds, mastoid infections, vaginitis, and respiratory tract infections (Sulakvelidze *et al.*, 2001).

Despite these advances, the rapid expansion of the antibiotic industry in the mid-20th century, driven by the dominance of large pharmaceutical companies, led to a significant decline in the use and commercial interest in phage-based therapeutics (Alisky *et al.*, 1998; Sulakvelidze & Kutter, 2005). Nevertheless, renewed interest in phage therapy has emerged in response to escalating antimicrobial resistance. Recent clinical investigations have demonstrated promising outcomes with phage cocktails applied to complex infections such as septic wounds and burn injuries. For instance, a phage formulation comprising 82 phages active against *Pseudomonas aeruginosa* and 8 targeting *Staphylococcus aureus* was successfully administered to eight patients, yielding favorable therapeutic effects (Merabishvili *et al.*, 2009; Nilsson, 2014).

Building on these encouraging results, modern biotechnology companies have advanced phage therapeutics into formal clinical trials. Notably, Pherecydes Pharma (France) has initiated a phase I/II single-blind multicenter clinical trial, termed *Phagoburn (http://www.pherecydes-pharma.com/phagoburn-clinical-study.html)*, to evaluate the efficacy of bacteriophage-based products in treating burn wound infections (Reardon, 2014; Ravat *et al.*, 2015; Servick, 2016).

Phage-based therapeutics are gaining renewed attention as alternatives to antibiotics, particularly for multidrug-resistant infections. The European Phagoburn project, coordinated by the French Ministry of Defense, represents one of the largest clinical evaluations of phage therapy, employing cocktails against Escherichia coli and Pseudomonas aeruginosa in burn patients across multiple centers in France, Switzerland, and Belgium. Such initiatives highlight the therapeutic potential of phages in areas where conventional antibiotics are increasingly ineffective, including Clostridium difficile infection (CDI), which is associated with dysbiosis, high recurrence, and poor antibiotic efficacy (Sangster *et al.*, 2014, 2015). Similarly, hospital-acquired P. aeruginosa infections remain a major clinical challenge due to their high morbidity, mortality, and resistance rates, reinforcing the importance of phage-based strategies (Viertel *et al.*, 2014; Chatterjee *et al.*, 2015).

Although controlled human trials remain limited, early clinical evidence is promising. Studies have reported successful outcomes using Shigella phages for dysentery (Babalova *et al.*, 1968) and phase I/II trials targeting chronic otitis, venous ulcers, and burn wounds with cocktails active against *S. aureus*, *P. aeruginosa*, and *E. coli* (Bruttin & Brüssow, 2005; Merabishvili *et al.*, 2009; Rhoads *et al.*, 2009). These studies indicate that relatively low phage titers (10^2–10^3 PFU) may suffice to control high bacterial loads (10v –10y CFU/mL), though higher doses are often applied in practice.

Beyond systemic use, innovative applications such as phage bioderm—a biodegradable polymer complex delivering phages—have been developed for treating wounds, osteomyelitis, and periodontal infections (Mathur *et al.*, 2003). While demonstrating high bacteriolytic activity (20–95% of isolates), challenges including phage neutralization by host immunity, induction of bacterial toxin genes, and endotoxin release during bacterial lysis remain barriers to widespread clinical adoption (Alisky *et al.*, 1998; Mathur *et al.*, 2003).

Biosensor development

According to the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is an analytical device that employs specific biochemical interactions mediated by isolated enzymes, antibodies, tissues, organelles, or whole cells to identify chemical compounds, generally through electrical, optical, or thermal signals (Nagel *et al.*, 1992; Hwang, 2014). Structurally, biosensors consist of a biological recognition element, a transducer, and an electronic system responsible for signal amplification, processing, and display. Their applications extend across multiple fields, including environmental monitoring, pharmaceutical analysis, and defense security. The advantages of biosensors include high sensitivity, specificity, rapid and accurate detection, minimal sample preparation, and cost-effectiveness (Singh *et al.*, 2013; Hwang, 2014). Notably, bacteriophages, due to their distinctive biological, structural, and mechanical features, can be harnessed as recognition elements in biosensors for applications such as bacterial identification, pathogen detection, and biocontrol (Hwang, 2014).

Developing biosensor surfaces using simple phage adsorption is comparatively simpler. However, it can yield variable results due to inconsistent phage immobilization densities (Balasubramanian *et al.*, 2007; Lakshmanan *et al.*, 2007). In contrast, chemically anchoring phages onto detection platforms—for example, using cysteamine-modified and glutaraldehyde-activated gold substrates—has been shown to provide more uniform phage coverage and improved detection efficiency (Cademartiri *et al.*, 2010; Singh *et al.*, 2013). When constructing chemically functionalized phage-based biosensors, the purity of the phage suspension, achieved via biopanning, is crucial; the preparation must be free from contaminants such as proteins, lipids, and carbohydrates (Naidoo *et al.*, 2012). Additionally, genetically engineered phages are generally more suitable as bio-probes than wild-type phages, as the latter can lyse host bacteria during detection, potentially reducing sensor signals (Singh *et al.*, 2010, 2013).

Recently, unique ability of phages called “phage display” has emerged as a powerful tool for screening diverse targets, including proteins, carbohydrates, or an entire cell by displaying peptides or proteins on their surface. Lambda, f1, M13, fd, T4, and T7 phages are commonly employed in phage display studies (Smith, 1985; Atias *et al.*, 2008; Singh *et al.*, 2013). This technology holds significant potential to advance diagnostics by generating molecules not readily accessible through conventional methods. Researchers have successfully displayed cellular proteins, peptides, antibody fragments, and antigens on phage surfaces for applications in pathogen detection, molecular imaging, and targeted gene delivery (Petrenko, 2008; Pande *et al.*, 2010; Singh *et al.*, 2013; Hwang, 2014; Chuang *et al.*, 2015).

Water decontamination

Conventional wastewater treatment generally relies on methods such as chemical precipitation, ion exchange, sedimentation, and coagulation–flocculation (Otte & Jacob, 2006). Although effective, these processes demand high energy input, costly infrastructure, and continuous maintenance. To overcome these limitations, researchers are increasingly exploring phage-based approaches for wastewater treatment, aiming to enhance effluent and sludge quality prior to discharge or reuse (Fu & Wang, 2011). Phage application in this context has the potential to control pathogenic bacteria, reduce foaming in activated sludge plants, improve sludge digestibility and dewaterability, and minimize competition between essential microbial communities and nuisance bacteria (Withey *et al*., 2005; Mulani *et al*., 2015). This approach is particularly relevant for developing nations like India, where sustainable, low-cost, and eco-friendly technologies are critically needed.

Wastewater harbours diverse microbial populations and naturally occurring bacteriophages, of which somatic coliphages are the most abundant. These phages, typically lytic in nature, belong to families such as *Myoviridae, Siphoviridae, Podoviridae,* and *Microviridae* and primarily target *Enterobacteriaceae,* especially *Escherichia coli* (Hayes, 1968). Their abundance in wastewater depends on host bacterial density and environmental conditions such as pH, temperature, concentrations of calcium and magnesium ions, and levels of organic matter (Grabow *et al*., 2000). Studies have demonstrated phage titers against *Salmonella typhi* reaching 10y –10^{1}p PFU/mL in sewage and culture media (Goyal *et al*., 1980). Effective phage replication requires at least 10t bacterial hosts per milliliter, however, under nutrient-limited conditions phages tend to associate with solid surfaces rather than remain in flowing water (Goyal *et al*., 1987).

Somatic Coliphages also inhabit the gastrointestinal tracts of humans and warm-blooded animals, where they replicate and are excreted with feces. Numerous investigations have reported coliphage densities in sewage ranging between 10v and 10x PFU/mL (Bell, 1976; Ignazitto *et al*., 1980; Havelaar & Hogeboom, 1984; Tartera *et al*., 1989). Interestingly, certain coliphages resemble human enteric viruses, such as enteroviruses (*Picornaviridae*) and F-RNA coliphages (*Leviviridae*), both of which possess icosahedral capsids (~25 nm) and single-stranded RNA genomes (Kamiko & Ohgaki, 1993). Due to these structural and genomic similarities, coliphages are widely considered suitable surrogates for enteric viruses (Grabow *et al*., 1995).

While coliphages are routinely released into sewage via fecal waste, human enteric viruses are typically shed during active infections. Notably, adenoviruses are frequently detected in raw sewage worldwide, often in higher abundance than enteroviruses. Approximately 80% of infectious adenoviruses found in sewage are considered enteric strains (Irving & Smith, 1981; Hurst *et al*., 1988; Krikelis *et al*., 1985a, 1985b). More recently, novel microbial source tracking methods have employed bacteriophages targeting *Bacteroides* strains (e.g., GA-17, GB-124, ARABA 84) and sorbitol-fermenting *Bifidobacteria* as indicators of human fecal contamination. Moreover, GI F-RNA coliphages have been proposed as effective indicators of virus reduction efficiency during wastewater treatment (Haramoto *et al*., 2015).

Agriculture

Bacteriophages, viruses that selectively infect and lyse bacteria, are increasingly recognized as promising biocontrol agents in agriculture. They offer a sustainable alternative to chemical pesticides and antibiotics, thereby supporting global strategies to mitigate antimicrobial resistance (AMR) and reduce reliance on synthetic agrochemicals (Czajkowski *et al*., 2025). Phages are applied through foliar sprays, seed coatings, soil drenches, and post-harvest treatments, effectively suppressing phytopathogens such as *Pseudomonas syringae, Xanthomonas campestris, Ralstonia solanacearum,* and *Erwinia amylovora*, which cause significant crop diseases like bacterial spot, black rot, and fire blight. Field trials with commercial products such as AgriPhage (EPA-registered in the US and Canada) and Erwiphage PLUS (authorized in Hungary) have shown reduced pathogen densities and delayed disease progression (Czajkowski *et al*., 2025). Similarly, in Africa, phage-based strategies are being explored against Banana *Xanthomonas Wilt* (BXW), where phages targeting *Xanthomonas campestris pv. musacearum* could protect staple crops vital to food security (Phage Guard, 2024).

The advantages of phage application extend beyond pathogen control. Their host specificity minimizes collateral effects on beneficial soil microbiota, while their ability to co-evolve with bacteria enhances long-term effectiveness. Moreover, phages can act synergistically with antimicrobials or beneficial microbes, reinforcing plant resilience. Globally, phage use is estimated to reduce crop losses by up to 41%, translating into billions in economic savings, without posing risks to humans, animals, or the environment (Czajkowski *et al*., 2025). Integration into livestock systems has also been successful, with products like BacWash™ and Finalyse™ shown to reduce Salmonella and E. coli O157:H7 contamination, thereby lowering zoonotic risks and limiting AMR transmission (Fernández *et al*., 2018).

Despite these benefits, challenges remain. Environmental factors such as UV radiation, temperature fluctuations, and soil pH often compromise phage stability, leading to inconsistent efficacy. Bacterial resistance is another concern, though the use of phage cocktails targeting multiple strains can mitigate this risk (Czajkowski *et al*., 2025). Regulatory inconsistencies further limit commercialization, particularly in the European Union under the Green Deal framework, where harmonized approval processes are still lacking. In Africa, technical and infrastructural barriers such as limited facilities for phage isolation, surveillance, and inadequate funding continue to restrict adoption (PhageGuard, 2024). Advanced approaches, including engineered phages and CRISPR-Cas–enhanced strategies, hold potential to overcome these limitations, though ethical and ecological implications of gene transfer remain under debate (Fernández *et al*., 2018).

Overall, phage-based interventions represent a climate-resilient and eco-friendly innovation for modern agriculture. Addressing challenges of stability, resistance, and regulatory frameworks will be crucial for scaling their application and realizing their full potential as sustainable alternatives to chemical inputs.

Aquaculture

Bacteriophages have emerged as a sustainable and targeted biocontrol strategy in aquaculture, providing an alternative to antibiotics at a time when antimicrobial resistance (AMR) poses increasing challenges. Aquaculture industries, which have grown rapidly over the past three decades as fish products become a vital and affordable source of protein worldwide, often face substantial economic losses due to uncontrolled microbial diseases that threaten their development and sustainability. Pathogenic bacteria such as *Flavobacterium psychrophilum, Photobacterium damselae, Vibrio anguillarum, Vibrio vulnificus, Aeromonas hydrophila,* and *Aeromonas salmonicida* have been reported to cause significant mortality in fish stocks and, in some cases, diseases in humans through consumption of contaminated seafood. Among these, vibriosis—caused by various *Vibrio* species (*V. vulnificus, V. anguillarum, V. parahaemolyticus*, *V. alginolyticus*, and *V. salmonicida*) remains one of the most common and destructive diseases in marine and estuarine fisheries, often resulting in mortality rates of up to 100% in larval stages.

Given the declining efficacy of antibiotics against multidrug-resistant strains, phage therapy has shown considerable promise. For example, phages have been effective against *V. anguillarum* infections in fish larvae. Their use extends across various applications, including immersion baths, feed incorporation, injections, and environmental decontamination in hatcheries and recirculating aquaculture systems (RAS). Phages such as AS-A have demonstrated control over *Aeromonas salmonicida* in Senegalese sole, significantly improving juvenile survival. Commercial formulations, such as BAFADOR® targeting *Aeromonas* and *Pseudomonas* species, and phage cocktails from Intralytix designed to combat *Vibrio* in shrimp larvae, exemplify the translational potential of this approach.

Studies showed up to 85% survival in shrimp larvae treated against *Vibrio harveyi*. Importantly, phage-based strategies align with sustainable aquaculture by supporting closed systems like RAS, reducing pathogen loads without releasing antibiotics into the environment. Approaches such as phage breeding, encapsulation for controlled release, and integration with endolysins have shown promise in enhancing stability and durability under aquaculture conditions. Nevertheless, the success of phage therapy ultimately depends on achieving sufficient phage proliferation following host lysis and minimizing the latent period for new infections, critical parameters for maintaining abundant phage populations in aquatic environments (Abedon *et al*., 2011)

Future Perspectives and Emerging Directions

The rapid advancement of phage research is opening new avenues for both fundamental and applied microbiology. Engineered and synthetic phages, designed through genome editing or modular assembly of receptor-binding proteins, offer the potential to expand host range, enhance infectivity, or overcome bacterial resistance mechanisms (Cafora *et al*., 2019). Computational and predictive modelling approaches are increasingly used to simulate phage-host co-evolution, providing insights into population dynamics, resistance emergence, and optimal phage therapy strategies (Fortier & Sekulovic, 2013). In addition, the integration of metagenomics, single-cell genomics, and high-throughput phenotypic screening is allowing researchers to explore phage diversity, host specificity, and ecological roles at unprecedented resolution. These approaches are particularly valuable in complex environments such as soil, marine systems, and human-associated microbiomes, where phage-host interactions influence nutrient cycling, pathogen suppression, and microbial community stability. Ultimately, combining molecular, ecological, and computational perspectives will enable rational design of phage-based interventions for combating antimicrobial resistance, managing microbial ecosystems, and developing next-generation biotechnologies, while also providing predictive frameworks for understanding microbial evolution in natural and engineered systems.

Conclusion

Water pollution and antimicrobial resistance (AMR) are interconnected global crises that threaten public health, food security, and environmental sustainability. Contaminated aquatic systems serve as hotspots for resistant bacteria and antibiotic resistance genes, accelerating their dissemination through ecosystems and into human and animal populations. Traditional reliance on antibiotics in aquaculture, wastewater management, and public health has proven increasingly inadequate, as the emergence of multidrug-resistant pathogens outpaces the development of new drugs. Against this backdrop, bacteriophages represent a compelling alternative. Their unique properties such as host specificity, self-replication at infection sites, and ability to evolve alongside bacterial hosts makes them highly suited for targeted biocontrol in aquatic environments. Promising applications include wastewater decontamination, mitigation of bacterial outbreaks in aquaculture, and synergistic combinations with antibiotics through phage–antibiotic synergy (PAS). Moreover, phage-based strategies align with sustainability goals by minimizing ecological disruption compared to chemical antimicrobials. However, widespread application still faces challenges such as phage resistance, narrow host ranges, environmental stability, and regulatory bottlenecks. Addressing these limitations through approaches like phage cocktails, encapsulation technologies, genome screening for safety, and integration with broader AMR action plans could accelerate their transition from experimental systems to mainstream practice. Ultimately, bacteriophages provide a powerful, eco-friendly tool that, if developed and deployed strategically, could complement existing interventions and form a cornerstone of future efforts to combat AMR and reduce the burden of waterborne bacterial pathogens.

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Author Contribution

CP: Manuscript writing, data collection, methodology, KS: Conceptualization, reviewing, editing and supervision.

Data availability

This paper includes all the relevant data or supplementary information.

Declarations

Competing interests

The authors declare that they have no competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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