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Bacteriophages Across Seven Application Fields: A One Health Integrative Review from Medicine to Aquaculture

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Abstract

Antimicrobial resistance has intensified the global search for alternatives to conventional antibiotics. Bacteriophages offer one of the few mechanistically distinct options, yet the literature remains fragmented across separate clinical, veterinary, food, agricultural, environmental, and aquaculture fields. Most reviews focus on a single application area and rarely compare multiple fields under a unified analytical framework. The cumulative evidence is therefore stronger than any single literature suggests, and recurring barriers remain invisible from within any single field. This integrative review examines bacteriophage applications across seven fields through a One Health perspective. It evaluates seven recurring translational barriers within a comparative cross-domain framework and assesses how each barrier manifests across the different application areas. Three findings stand out. First, regulatory fragmentation, not technical failure, is the dominant barrier in every field examined, with at least four distinct regulatory paradigms operating in parallel. Second, formulation and environmental persistence, rather than phage isolation, now drive most active commercial development; the scalability of personalised cocktails remains incompatible with conventional drug-approval pathways. Third, many of the operational tools needed to address these barriers are already in routine use within at least one field, so the practical task is cross-domain transfer rather than new development. Bacteriophage applications can no longer be treated as an isolated niche within any single field. The evidence positions them as an integrated, mechanistically distinct One Health intervention that complements the classical antibiotic, vaccine, and biosecurity-based control of bacterial infection.

Keywords:
AMR; One Health; phage therapy; sustainable disease management.

1. Introduction

Antimicrobial resistance (AMR) is one of the most pressing problems in modern medicine and public health. The Antimicrobial Resistance Collaborators estimated 4.95 million deaths associated with bacterial AMR in 2019, of which 1.27 million were directly attributable to resistance (Murray et al. 2022). More recent modelling forecasts a cumulative 39.1 million deaths directly attributable to bacterial AMR between 2025 and 2050, and a peak of 1.91 million annual attributable deaths by 2050 if current trends continue (Naghavi et al. 2024). The 2024 World Health Organization Bacterial Priority Pathogens List confirms that resistance to last-line antibiotics is now established in twenty-four pathogen-drug combinations of greatest concern, including carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant Enterobacteriaceae, third-generation cephalosporin-resistant

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Escherichia coli and methicillin-resistant *Staphylococcus aureus* (Sati et al. 2025). The classical approaches to bacterial infection control, including antibiotics, vaccination, and hygiene measures, remain essential across medical, veterinary, and food-production systems. The continued spread of multidrug-resistant pathogens, the slow pace of new antibiotic discovery, and the limited coverage of bacterial vaccines together indicate that additional, mechanistically distinct interventions are needed alongside the classical approaches, particularly for pathogens where vaccine development remains difficult and resistance is already established.

Among the alternative strategies under active investigation, bacteriophages (viruses that specifically infect and lyse bacteria) have re-entered mainstream scientific and clinical research after almost a century of intermittent use. First described by Twort and d'Hérelle, they were used therapeutically in Eastern Europe and the former Soviet Union throughout the twentieth century, but were largely abandoned in the West with the rise of broad-spectrum antibiotics. Their reappearance in modern medicine has been driven by several landmark clinical and regulatory developments. Compassionate-use applications against multidrug-resistant infections, genetically engineered phage therapies, and expanding personalised treatment programs have collectively demonstrated renewed clinical feasibility (Schooley et al. 2017; Dedrick et al. 2019). Belgium's 2018 magistral preparation framework provided the first formal legal route for their clinical use within the European Union, and parallel developments in regulatory science, genome-level safety screening, and good manufacturing practice are now reshaping the field (Pirnay et al. 2024).

However, the renewed clinical interest is only one aspect of a much broader phage research landscape. Phages and their derived products are now being developed and deployed across multiple interconnected application domains: human medicine, veterinary medicine, food safety and pathogen detection, agriculture and phytopathology, environmental biotechnology, biofilm control, and aquaculture. Each of these faces its own version of the AMR problem and its own translational constraints, but they share the same biological tools and the same regulatory uncertainties. The One Health framework, which treats human, animal, plant, and environmental health as a single interconnected system, is particularly relevant here because resistance genes, pathogens, and the viruses that act on them all move between these compartments. Despite this conceptual unity, the literature on bacteriophage research has remained largely compartmentalised. Clinical reviews seldom engage with aquaculture or agricultural evidence, food-safety reviews rarely discuss environmental release, and environmental and aquaculture studies often remain disconnected from the regulatory discussions that dominate the human therapeutic field. As a result, potentially transferable knowledge and shared translational barriers often remain isolated within separate literatures, making an integrative perspective both timely and necessary.

This review was prepared as an integrative narrative synthesis designed to evaluate evidence across heterogeneous study designs and application fields within a One Health perspective. Articles published between 2000 and 2026 and indexed in Web of Science and Google Scholar were screened for relevance to bacteriophage applications across the fields examined here. Around 250 articles were assessed, and the 173 most relevant to the scope of the review were retained and cited. Literature retrieved from these databases, together with relevant institutional and policy documents, was evaluated thematically across the application fields, with particular emphasis on recurring translational barriers, regulatory fragmentation, formulation and delivery, environmental persistence, and resistance dynamics.

The aim of this review is to provide an integrated assessment of the current state of bacteriophage applications across these interconnected fields while identifying the recurring scientific, technical, and regulatory constraints that extend across application boundaries. Most existing reviews focus on a field, or at most pair two related ones, so the cumulative evidence and the shared constraints remain scattered across separate literatures. The novelty of this review lies in bringing seven application fields together within a unified analytical framework through three integrative components that remain limited in the published literature: a comparative cross-domain table of translational constraints, a structured synthesis examining how these constraints manifest differently across each field, and a perspective informed by aquaculture and aquatic microbiology, where the open-water, food-chain, and One Health dimensions of phage applications are especially visible. It revisits the biological foundations and historical trajectory of phage research, examines each of the seven fields in turn, and brings the field-specific findings together in a cross-domain synthesis. Finally, the review evaluates the regulatory, manufacturing, and scientific interfaces most likely to shape the future development and implementation of bacteriophage applications. An integrative overview of phage applications across the seven major fields covered in this review, including pathogen targets, representative products, application routes, reported efficacy, and regulatory status, is presented in Table 1.

Table 1. Overview of bacteriophage applications across major fields with pathogen-specific phage products, application routes, reported efficacy and regulatory status.

HUMAN MEDICINE					
Pathogen	**Product**	**Application Route**	**Outcome**	**Regulatory Status**	**Reference**
S. aureus (MRSA, MSSA, endocarditis)	AB-SA01/AP-SA02 (3-phage cocktail)	Intravenous (twice d, 14 d);intranasal rhinosinusitis	Phase 1b/2a trial positive; case series 54% improvement	FDA QIDP (2026); TGA Special Access (AU)	Petrovic et al. 2020
Pseudomonas aeruginosa (cystic fibrosis)	BX004 (BiomX); Pa-WRAIR / AP-PA02 (Armata)	Inhaled/nebulized (BX004); intravenous (AP-PA02)	BX004 Phase 1b/2a well tolerated, bacterial reduced	Multiple Phase 1b/2a trials (US, EU, Australia); no authorization	Ramirez-Sanchez et al. 2021
Mycobacterium abscessus (Pulmonary)	Engineered phages: Muddy, ZoeJΔ´3, BPsД13HTH_HRM10	Intravenous, twice daily, prolonged	First personalized phage teenager; infection cleared	Compassionate-use only; expanded-access framework	Dedrick et al. 2019
Escherichia coli (recurrent UTI)	LBP-EC01 (Locus Biosciences, CRISPR-Cas3 engineered)	Intravesical, intravenous, oral	Eliminate Phas 2: significant bacterial reduc.; well tolerated	FDA IND; Phase 2 complete; CRISPR-enhanced program	Suh et al. 2023
Klebsiella pneumoniae (CR-Kp, MDR)	Eliava Institute cocktails; personalized use	Topical, oral, intravenous (compassionate use)	Multiple case reports recovery in ICU patients	Magistral preparations (Belgium); compassionate use	Pirnay et al. 2024
VETERINARY MEDICINE					
Salmonella enterica in poultry	SalmoFREE® (SciPhage, Colombia); Bafasal® (Proteon Pharma, Poland)	Oral in drinking water; feed-grade additive	Significant reduction in at slaughter; no effect growth	Commercial in Latin America, EU; EFSA opinions; FDA CVM	Clavijo et al. 2019
Campylobacter jejuni, *C. coli* in broilers	Experimental phage cocktails (CP8,CP30,NCTC12669derived)	Oral via feed (most effective) drinking water aerosol	cecal reduction; 13% resistant mutants	Experimental; EU emergency authorizations only	Sarrami et al. 2022
E. coli (in calves, piglets, lambs)	Smith-Huggins phage preparations; modern feed-additive cocktails	Oral (single dose 10^5 PFU therapeutic; 10^2 PFU prophylactic)	Single dose cured exp. diarrhoea; prophylaxis	Foundational work; descendants in feed-additive market	Smith et al. 1987
P. aeruginosa (wounds, otitis, implants)	Personalized cocktails; case-specific preparations	Topical; intra-articular for implant infections	Implant infection in cat; vet. case	EMA guideline framework	Bianchessi et al. 2024
S. aureus (mastitis)	Experimental phage cocktails	Intramammary infusion; topical	Mastitis models; biofilm strains challenging	EMA (CVMP/NTWP/ 32862/2022)	Kwiatek et al. 2020
FOOD SAFETY AND PATHOGEN DETECTION					
Listeria monocytogenes meat, cheese, smoked fish	ListShield™ (Intralytix); LISTEX P100 PhageGuard Listex™ (Micreos)	Surface spray; immersion dip; food-packaging incorporation	Reduction in fresh cheese; below detection in ham at 0.5%	FDA GRAS (2006, first ever); USDA FSIS; EFSA favorable; EU processing-aid	Grigore-Gurgu et al. 2024
S. enterica on poultry, fish, fresh produce	SalmoFresh™ (Intralytix); Salmonelex™ PhageGuard S	Surface spray on carcasses; produce wash dip	1–2 $\log_{10}$ CFU reduction on poultry; across matrices	FDA GRAS; USDA FSIS; Canada; commercial in EU, US, Israel	Atterbury et al. 2007
E. coli O157:H7 on beef, lettuce, sprouts	EcoShield™ (Intralytix) FINALYSE® (ElancoPhageGuard)	Surface spray on beef carcasses; hide spray pre-slaughter	Not provided	FDA GRAS; USDA FSIS approved for direct application	Moye et al. 2018
Shigella spp. on RTE foods	ShigaShield™ (Intralytix)	Surface spray on RTE meat, fish, dairy, produce	Effective against multiple *Shigella* species in food matrices	FDA GRAS Notice (GRN 672)	Soffer et al. 2017
Cronobacter sakazakii in infant formula, powdered milk	Experimental phages (CR3, CR8, CR9)	Spray on reconstituted formula; processing-line application	Significant reduction infant formula colonization neonatal mouse	Experimental; no FDA-approved product for this matrix	Bai et al. 2016
AGRICULTURE AND PHYTOPATHOGENS					
Xanthomonas campestris /*P. syringae* pv. *X. citri* (tomato, pepper citrus)	AgriPhage Spot & Speck (OmniLytics; EPA Reg. 67986-1; 1.55×10^{13} PFU/gallon)	Foliar spray; fogging; preventive + curative	17 years' use Florida tomato, no detectable resistance 8% yield increase	EPA-registered (2005, first US ag phage); USDA NOP organic; MRL-exempt; 0-day PHI	Nakayinga et al. 2021
Erwinia amylovora (fire	AgriPhage-Fire Blight (EPA Reg. 67986-8);	Blossom spray; whole-tree foliar application during bloom	Reduceblossom infection flower greenhouse trials; first EU cocktails 2024	EPA-registered; NOP organic; no full EU authorization	Svircev et al. 2018; Biosca et al. 2024

blight apple, pear, quince)	European phage cocktails				
Clavibacter michiganensis (tomato bac.)	AgriPhage-Tomato Canker (CMM; EPA Reg. 67986-6)	Foliar spray; preventive and curative	Reduced bacterial symptoms tomato	EPA-registered (2011); USDA NOP organic	Balogh et al. 2010
Ralstonia solanacearum (bacterial wilt of solanaceae)	Experimental phages (φRSA1, φRSL1)	Soil drench; irrigation-based delivery	Lytic phage reduced tomato greenhouse assays	Experimental; under research evaluation in Asia	Fujiwara et al. 2011
Dickeya/Pectobacterium (potato soft rot, blackleg)	Experimental phage cocktails (BF25/12 and related)	Seed-tuber dip; in-furrow application	Reduced seed-tuber rot in storage; effect on planted tubers	Experimental; commercial development in EU and US	Buttimer et al. 2017
ENVIRONMENTAL APPLICATIONS AND BIOFILM					
Gordonia, *Microthrix*, *Nocardia*	Activated-sludge-derived phages; exper. lytic isolates	Direct dosing into activated sludge tanks	Pilot-scale control sludge bulking foaming; improved quality	No environ.phage-release framework pilot deployment only	Withey et al. 2005
Antibiotic-R *Enterobacteriaceae* in hospital wastewater	Polyvalent phage cocktails; experimental isolates from hospital effluent	Dosing into hospital wastewater stream before discharge	Phage cocktails reduced plasmid ARG (blaNDM-1), inhibited ARB growth in sludge	Pre-regulatory; ecological impact assessment under discussion (EU, WHO, FDA)	Cáceres& Muniesa 2016
Legionella pneumophila, *P. aeruginosa* in cooling towers	Experimental species-specific phage preparations	Pulsed dosing into water phase; biofilm-targeted	Labscale reduction biofouled surfaces	No commercial pathway; mass-transfer challenges in chlorinated water	Garvey 2022;
P. aeruginosa, *S. aureus* biofilms	Phage-derived depolymerases; engin. phage	Surface application; CIP cycle integration; encapsulated phage	Degradation; up to 99% *A. baumannii* biofilm reduction	Parent sector; no dedicated biofilm-phage category	Abedon 2023;
AQUACULTURE					
Aeromonas hydrophila (*Labeo rohita*)	Phage AhFM11 (168 kbp *Straboviridae*, AMR-gene-free)	Injection, immersion, oral via phage-coated feed	Injection 100%, immersion 95%, oral 93% survival	Research stage; EMA 2023 veterinary guideline applies	Tu et al. 2020
A. hydrophila in (*P.hypophthalmus*)	Phage PVN02; phage cocktail φAHBHU12/16/19	Spray feeding intramuscular water immersion	Survival 75.6%- 87.8%; cocktail effective 24 h	Research stage; commercial Vietnam, India	Le et al. 2018;
A. salmonicida in salmonids	Experimental phages sewage farm water	Injection in trial settings; oral feed in farm trials	Limited success vs *A. hydrophila*; encapsulated phenotype	Research stage; comm.pathway unclear	Choi et al. 2022
V. parahaemolyticus in shrimp	Anti-AHPND phage cocktails commer. Asian products	Pond water dosing; bath immersion; feed incorporation	60-90% morta.reduction in *L. vannamei*, *P. monodon*	Several Asian national approvals; no centralised EU/US	Chen et al. 2019
Yersinia ruckeri in rainbow trout	Novel *Y. ruckeri* phages isolated Turkish farm	Bath immersion; oral feed; injection in trials	Broad host range vs biotype 1 and 2;	National interest (biotype 2 vaccine failures)	Welch 2020
F.psychrophilum /F. columnare	Experimental phages hatchery	Bath immersion; egg-surface treatment	Mortality reduction in salmonid fry	Research stage	Castillo et al. 2019

2. Bacteriophage Biology and the Foundations of Phage Therapy

Bacteriophage activity rests on a small set of biological principles and phage therapy has a long and uneven history. Phage structure, replication, host specificity, and the early therapeutic record together provide the basis for interpreting the seven application fields that follow. The word bacteriophage, often shortened to phage, comes from the Greek for "to eat or devour" and refers to viruses that infect bacteria as obligate intracellular parasites (Sulakvelidze et al. 2001). Most phages are between 20 and 200 nm in size. They are also the most abundant biological entities on Earth, with around 10^{31} particles globally; roughly ten times the number of bacteria. Host specificity is one of their defining features: each phage lyses only the bacteria it targets while leaving the surrounding microbiota and the host organism unaffected. They have recovered from almost every environment where bacteria occur, including river and seawater, sediments, soil, sewage, food and human and animal feces and urine. Phage structures vary considerably. A typical particle has a protein capsid that encloses the viral genome, together with a tail of variable length and tail fibers or spikes that serve as receptor-binding proteins. Phages display considerable structural and genomic diversity, including differences in capsid morphology, tail architecture, and genome composition, all of which influence host recognition and infection dynamics (Turner et al. 2023).

Phage taxonomy was reorganized in 2022. Until then, the International Committee on Taxonomy of Viruses (ICTV) placed tailed phages within the order *Caudovirales*, with three morphology-based families: *Myoviridae* (long contractile tails), *Siphoviridae* (long non-contractile tails), and *Podoviridae* (short tails). Tailless phages were grouped in several additional families. Later phylogenomic analyses revealed that the classical families were polyphyletic and did not reflect shared evolutionary histories. ICTV therefore dissolved the order *Caudovirales* together with these three families and replaced them with the class *Caudoviricetes*, which now covers all tailed bacterial and archaeal viruses with icosahedral capsids and dsDNA genomes. A binomial nomenclature system was adopted and the tailed phages were reclassified into numerous new families, genera and species (Turner et al. 2023). Despite this change, the older morphological groupings are still used in much of the applied phage therapy and aquaculture literature.

Replication depends entirely on a bacterial host. After phages attach to the cell and inject their genome, they follow one of two main routes: the lytic (virulent) cycle or the lysogenic (temperate) cycle (Borysowski & Gorski 2008). In the lytic cycle, the phage quickly replicates its genome, builds new structural proteins inside the host, and then lyses the cell to release the new particles. One cycle usually lasts 20 minutes to 2 hours, and a single lysed cell may release 50-200 progeny phages depending on the phage and the host. Lytic phages are also called virulent phages, since they consistently kill the host. Lysogenic phages behave differently. They integrate their genome into the host chromosome as a prophage and stay in a dormant state, with most phage genes silenced by a phage-encoded repressor protein (Borysowski & Gorski 2008). For therapeutic use this is a problem. Temperate phages can move virulence genes and antimicrobial resistance (AMR) determinants between bacteria through horizontal gene transfer and may turn previously harmless strains into pathogens. The major stages of bacteriophage attachment, genome replication and bacterial cell lysis during the lytic cycle are illustrated in Figure 1.

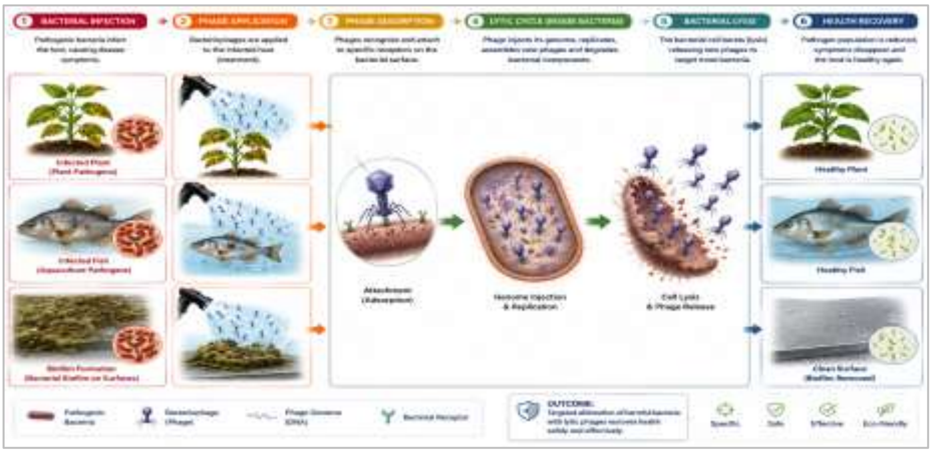

Figure 1. Schematic overview of bacteriophage attachment, genome replication, and bacterial cell lysis during the lytic cycle.

Beyond intact phage particles, two phage-derived molecules feature throughout the applied literature and are introduced here once. Endolysins are bacteriophage enzymes that hydrolyze the bacterial cell wall during the lytic cycle; recombinant endolysins can kill target bacteria from outside the cell without productive infection and are particularly active against Gram-positive pathogens.

The history of phage research extends from Hankin's 1896 observation of antibacterial activity in the Ganges, through the discoveries of Twort and d'Hérelle, to the establishment of the Eliava Institute in Tbilisi in 1923 and the continued therapeutic use of phages in Eastern Europe throughout the twentieth century; detailed historical accounts are available elsewhere (Sulakvelidze et al. 2001). The major milestones shaping the historical development of bacteriophage research and therapy are summarised in Figure 2. Bacteriophages are now used in a wide range of fields, including human medicine, veterinary practice, food safety, agriculture, environmental biotechnology, and aquaculture. Their host specificity and targeted antibacterial activity make them suitable for diverse biological and industrial applications. An overview of application fields of phages discussed here is given in Figure 3.

Figure 2. Key milestones in the historical development of bacteriophage research and therapy.

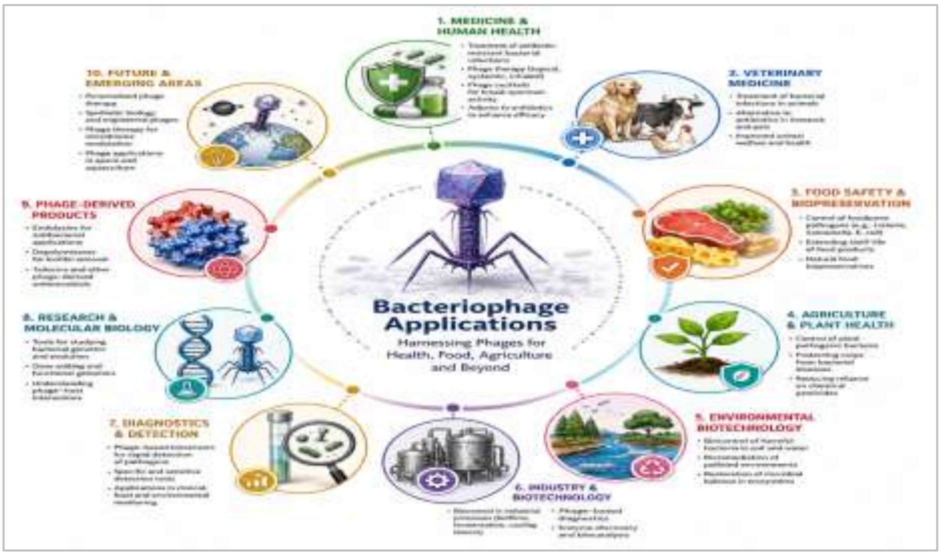

Figure 3. Overview of bacteriophage applications across human medicine, veterinary practice, food safety, agriculture, aquaculture, environmental biotechnology and biofilm control.

3. Human Medicine Applications

Antimicrobial resistance (AMR) is now among the most serious public health problems of the present century. The development pipeline for new antibiotics has stalled at the same time, and few candidates with novel mechanisms of action have reached the clinic in the past decade. The bacterial groups are most often involved in difficult-to-treat infections, the so-called ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), increasingly carry combinations of resistance determinants that leave very few therapeutic options. Against this background, phages have re-entered modern medicine as a serious alternative or adjunct to antibiotics.

Modern interest is driven largely by the rapid spread of multidrug-resistant (MDR) bacterial infections and the need for alternative therapeutic and prophylactic options. Phages can target antibiotic-resistant bacteria while preserving commensal microbiota and self-amplifying at infection sites. Patient-specific phage therapy has shown promising results against *S. aureus*, *A. baumannii* and *M. abscessus* in cases where standard treatment failed (Strathdee et al. 2023). The 2016 compassionate-use treatment of Tom Patterson at UC San Diego recovered a patient from a disseminated multidrug-resistant *A. baumannii* infection after intravenous personalized phage cocktail therapy under emergency FDA approval (Schooley et al. 2017). In 2019, Dedrick and colleagues used three genetically engineered mycobacteriophages to treat a cystic fibrosis patient with disseminated *M. abscessus* infection after lung transplantation (Dedrick et al. 2019).

Clinical activity in phage therapy has expanded considerably during the past five years. By mid-2024, approximately ninety active or completed clinical trials had been registered worldwide, reflecting growing scientific and commercial investment. Most studies remain early-phase trials, although several phase II programs have begun reporting clinical data.

Clinical applications now span a broad range of infection types. Frequently targeted pathogens include *S. aureus* (including methicillin-resistant strains), *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, *E. faecalis*, *E. coli*, and *M. abscessus*. Clinical indications cover chronic urinary tract infections, rhinosinusitis, skin and soft tissue infections, diabetic foot ulcers, prosthetic joint and bone infections, cardiac device infections, and chronic respiratory infections in cystic fibrosis patients. Biofilm- and device-associated infections are particularly common indications, because antibiotics often fail in these settings and surgical revision is technically difficult. In cystic fibrosis, nebulized phage therapy against *P. aeruginosa* has produced measurable reductions in sputum bacterial load without disrupting the lung microbiome (Wang & Yu 2025).

Phages can be administered orally, topically, by inhalation, or intravenously, providing flexibility across different clinical settings (König 2025). Intravenous delivery remains the standard for systemic infections, whereas inhaled formulations are mainly used for chronic respiratory disease and topical or intra-articular applications for wound and prosthetic infections. However, important delivery challenges remain. Neutralizing antibodies may reduce efficacy during prolonged intravenous therapy, while oral administration is limited by phage instability in the gastrointestinal tract (Kim et al. 2025). Enteric-coated capsules and liposomal formulations have therefore been developed to improve phage stability and delivery efficiency.

The clinical evidence base is now large enough to allow a first quantitative picture of outcomes. The largest observational study to date is the multinational case series by Pirnay et al. (2024), which reported on 100 consecutive patients treated with personalized phage therapy across several centers; clinical improvement was observed in 77.2% of patients, and bacterial eradication or marked reduction was documented in a substantial proportion of cases. A recent systematic review and meta-analysis of clinical phage therapy reported overall clinical improvement rates around 71% (95% CI 60-80) and bacterial eradication rates around 51% (95% CI 30-72), with low to serious risk of bias depending on study design. Smaller series, such as the twelve-case expanded-access cohort from the TAILΦR program at Baylor College of Medicine and the twenty-case mycobacterial series reported by the Hatfull group at the University of Pittsburgh, have given more detailed information on individual outcomes and have shown success in roughly half to two-thirds of treated patients. Safety has been consistently good across these studies: serious adverse events directly attributable to phages have been very rare, even with prolonged intravenous administration.

Lytic killing is not the only therapeutic mechanism. Synergy between phages and antibiotics, in which sublethal antibiotic doses enhance phage replication and bacterial killing, is one of the more important recent developments (Gordillo Altamirano & Barr 2021). Endolysins offer another option, since they lyse bacteria quickly and are less likely to drive resistance. Engineering and synthetic phage platforms have also opened new ground. The ELIMINATE Phase II trials, for example, tested engineered phage cocktails against drug-resistant urinary tract infections, with the aim of disrupting bacterial resistance mechanisms directly. The number of phage trials registered on ClinicalTrials.gov has grown steadily, which reflects both the scientific momentum behind the field and a more open regulatory stance (Pirnay et al. 2024).

Despite the volume of clinical activity, no phage therapeutic has yet received full marketing authorization in either the United States or the European Union. Treatment in these regions is mostly conducted under compassionate use or expanded access provisions, or within approved clinical trials. As noted in the Introduction, Belgium is the only Western jurisdiction with a dedicated national framework that allows hospital pharmacists to prepare personalized phage products on prescription (Pirnay et al. 2024). The European Medicines Agency published its first guideline on veterinary phage products in 2023 and is now preparing an equivalent guideline for human therapeutics (König 2025). Phage therapy has remained part of routine health care in Georgia, Russia, and Poland throughout the past century, with the Eliava Institute in Tbilisi and the Hirszfeld Institute in Wrocław producing cocktails for clinical use and accepting patients from Europe and North America for treatment of chronic resistant infections. India, Australia, and Brazil are at earlier stages of developing dedicated regulatory pathways (Yang et al. 2023). These barriers are not primarily scientific but systemic.

First, the personalization that drives clinical success is incompatible with conventional drug-approval pathways: bespoke phage cocktails matched to individual patient isolates cannot be filed as a single regulatable product, and the Belgian pathway introduced above remains the only jurisdiction that has resolved this contradiction at law. Second, the pharmacokinetic and pharmacodynamics standards that regulators apply to small-molecule antibiotics, such as minimum inhibitory concentration, area under the curve, and dose-response linearity, do not transfer to self-replicating biological agents, and no internationally agreed framework has yet replaced them. Third, GMP manufacturing biological preparations with deliberately flexible composition is expensive, and the per-treatment cost cannot be amortized across a defined product line when each cocktail is unique to a patient. These three barriers, namely personalization versus scalability, missing PK/PD frameworks, and batch-flexible GMP, are not unique to human medicine, but they take their most acute form here. The veterinary, food-safety, and aquaculture application areas face structurally similar problems on different scales and the cross-domain analysis later in this review returns to them as a translational signature shared across areas.

Clinical advances in human phage therapy also cannot be considered independently from veterinary, food, agricultural, environmental, and aquatic systems, where the same pathogens, resistance genes, and selection pressures continue to circulate within the broader One Health network.

4. Veterinary Medicine Applications

Just as in human medicine, veterinary phage therapy has long historical roots that predate modern clinical use, and the same AMR pressures now drive its return. The rising global burden of AMR has made the search for alternatives to conventional antibiotics a priority in veterinary medicine, where phages are now used as therapeutic and prophylactic agents in livestock, poultry, and companion animals. The main reason for adopting phages in veterinary settings is not animal welfare on its own, but the need to slow the spread of AMR linked to agricultural antibiotic use. Routine prophylactic and metaphylactic antibiotic use in intensive animal husbandry have been directly linked to the accumulation and spread of resistance genes, which eventually re-enter human populations through food chains and environmental microbiomes (Gigante & Atterbury 2019). The veterinary application area accounts for a major share of total global antibiotic consumption, and pressure to reduce this use, both for animal health and human public health reasons, has accelerated the move toward biological alternatives such as phages (Ferriol-González & Domingo-Calap 2021).

Veterinary phage research has continued through two parallel tracks: in vitro screening of phage-bacterial pairings and in vivo testing in challenge models. In vitro work focuses on host range characterization, plaque assays, efficiency of plating (EOP) measurements, and biofilm disruption assays. These approaches allow researchers to select the most active phages from environmental sources such as sewage, slurry, and animal faeces and to assemble cocktails that cover the local strain diversity of a target pathogen (Bianchessi et al. 2024). In vivo studies use experimentally infected animals or, less commonly, naturally infected production flocks, and measure outcomes such as reduction in faecal shedding, tissue bacterial load, mortality, weight gain, and clinical signs. A 2022 systematic review and meta-analysis of preclinical phage therapy across 124 animal studies, mostly in rodents but including pigs and poultry, reported significant reductions in mortality and tissue bacterial burdens in systemic infections, skin and burn infections, and pneumonia, although the risk of bias in individual studies was often high (Onsea et al. 2022).

Phage-based treatments have worked well against a wide range of zoonotic and economically important pathogens. Controlled studies have reported successful suppression of *E. coli* (Kim et al. 2025), *Salmonella* spp. (Atterbury et al. 2007), *Campylobacter* spp., *S. aureus* (Kwiatek et al. 2020) and difficult Gram-negative species including *P. aeruginosa* and *A. baumannii* (Ferriol-González & Domingo-Calap 2021). More than a decade of experimental and field studies now supports the use of phage therapy in production animal settings (Gigante & Atterbury 2019). Cocktails directed at *S. enterica* serovars *Enteritidis*, *Typhimurium* and *Gallinarum* have reduced caecal colonization in broilers, while preparations against *C. jejuni* and *C. coli* have produced reductions of approximately 2 log10 CFU/g in faecal counts when administered through feed or oral gavage.

Field and experimental outcomes have been generally positive, although the quality of evidence remains uneven. A commercial-scale broiler trial using the SalmoFREE® phage cocktail in Colombia reported significant reductions in *Salmonella* prevalence at processing without negative effects on growth performance (Clavijo et al. 2019). The systematic review by Sarrami et al. (2022) found significant overall reductions in challenge bacteria across twelve poultry trials, with strongest effects when phages were administered through feed and in older birds. A Lancet Microbe systematic review covering 124 preclinical animal studies reported reductions in mortality in 73 of 78 relevant studies and reductions in tissue bacterial burden in 83 of 91, although nearly all studies carried at least some risk of bias (Onsea et al. 2022). Resistant bacterial mutants have been recovered from animals treated at frequencies of around 13% in *Campylobacter* feed trials, but these variants often show reduced fitness or virulence and do not always replace the susceptible parent strain.

From a regulatory standpoint, the growing evidence supports phage feed additives as a replacement for the growth-promoting antibiotic regimens banned under European Union Regulation (EU) 2019/6. The European Medicines Agency issued its first dedicated guideline on veterinary medicinal products designed for phage therapy in January 2023, with legal effect from October 2023 (EMA/CVMP/NTWP/32862/2022). Importantly, this framework allows products with flexible composition, reflecting the biological variability of phage preparations. The United States Food and Drug Administration regulate veterinary phages through the Center for Veterinary Medicine and have granted GRAS status to several phage products used in animal food. By 2024, around nine veterinary phage products had received FDA approval and three had received EFSA approval, mostly from companies such as Intralytix, PhageGuard, PhagePharm, and Fixed-Phage (Wu & Zhu 2021). Brazil has authorized at least two phage-based products for *Salmonella* and *E. coli* control in poultry, while China and South Korea are expanding their commercial offerings. Despite this progress, no phage product yet holds a centralized EMA marketing authorization for veterinary use, and most companion-animal applications still proceed through compassionate use or research protocols (Fauconnier et al. 2025).

Veterinary phage therapy occupies a position quite different from its human counterpart, and the translational picture reflects that difference. The economic logic favours scalable, low-cost interventions for production animals rather than the personalized regimens that dominate the human clinical pipeline, while the regulatory environment has moved more rapidly: the 2023 EMA guideline explicitly accepts flexible composition, a concession that human regulators have not yet made. The barriers in this area are therefore less about scalability and more about evidence quality and field validity. Many published trials still carry serious risk of bias, control groups are inconsistent, and field outcomes are often reported without independent strain typing of the challenge organism, making it difficult to distinguish true phage efficacy from background variation. Companion-animal applications, where personalized cocktails may be justified by case value, remain largely anecdotal and confined to compassionate use. Phage-host mismatch is also more visible at herd level than in individual human cases because farm bacterial populations are genetically heterogeneous and shift between cohorts. The translational challenge in veterinary medicine is therefore not to obtain a first regulatory framework, but to convert a permissive framework into standardized, reproducible products with documented efficacy under field conditions. Similar evidence-quality and heterogeneity problems reappear in food safety and aquaculture, where herd- or batch-level variability shapes the same translational barriers.

Despite these limitations, veterinary phage therapy offers several practical advantages. Phages are highly specific, leave the commensal microbiota largely intact, can be produced relatively cheaply at scale, and leave no chemical residues in animal products, which represents an important operational advantage over conventional antibiotics. They can also be combined with antibiotics in synergistic regimens and can target biofilm-associated infections that are otherwise difficult to eradicate (Ferriol-González & Domingo-Calap 2021). The growing commercial activity in veterinary phage development, the recent EMA guideline, and the steady accumulation of in vivo evidence together suggest that phage products will likely see expanded use in veterinary practice over the next decade, particularly as One Health-oriented AMR policies continue to push livestock production toward antibiotic reduction (Bianchessi et al. 2024). The continuity with human medicine remains direct, and food safety is the next logical extension of the same One Health problem.

5. Food Safety and Pathogen Detection Applications

Foodborne illness remains a major global public health burden. The World Health Organization estimates around 600 million cases and 420,000 deaths from contaminated food each year, with diarrhoeal pathogens including non-typhoidal *Salmonella*, Shiga toxin-producing *E. coli*, *Campylobacter* spp., *L. monocytogenes*, and *Vibrio* spp. responsible for most of this burden (Alomari et al. 2021). AMR has reduced the effectiveness of conventional sanitizers and antibiotics used along the food chain, and consumer preference is steadily shifting toward natural, residue-free preservation methods. High target specificity, self-amplification at the contamination site, and a strong safety profile make phages well suited to this role (Moye et al. 2018). They can be applied at almost every step of the food production chain without altering taste, appearance, or nutritional value, and several products have already been authorized by regulators in multiple jurisdictions.As biocontrol agents, phages are now used throughout the food production chain, from pre-harvest agriculture to post-harvest processing and retail environments (Aziz et al. 2025). Applications include surface spraying, immersion, packaging coatings, and sanitation systems designed to reduce contamination by pathogens such as *E. coli*, *Salmonella* and *Listeria* without affecting food quality (Alomari et al. 2021; Grigore-Gurgu et al. 2024). Biocontrol applications now cover most major food categories. Ready to eat meat, poultry, and dairy products were among the earliest targets because *L. monocytogenes* contamination in these matrices has caused some of the most severe foodborne outbreaks in recent decades, with reported case fatality rates of approximately 20-30% (Endersen & Coffey 2020; Grigore-Gurgu et al. 2024). Fresh produce, seafood, dairy products, and eggs have since become additional targets for phage-based control of pathogens including *E. coli O157, Salmonella, Listeria* and *Vibrio* spp. (Clavijo et al. 2019).

The pathogens most often targeted by food-related phage products are those that combine high disease burden, environmental persistence, and resistance to conventional control. *L. monocytogenes* remains the leading target because of its ability to grow at refrigeration temperatures, its psychrotrophic nature, and its tolerance to salt, low pH, and many sanitizers (Grigore-Gurgu et al. 2024). *S. enterica* serovars Enteritidis and Typhimurium are next in importance, particularly in poultry, eggs, and produce. Shiga toxin-producing *E. coli*, including O157:H7, is a major target in beef and leafy greens. *C. jejuni* and *C. coli* are addressed at the poultry pre-harvest stage rather than on the finished product, since these organisms are difficult to control once carcasses enter processing (Goodridge & Bisha 2011). Other targets include *S. aureus* in dairy products and *Cronobacter sakazakii* in infant formula and powdered milk (Endersen & Coffey 2020).

Several phage products are already commercially approved. ListShield and LISTEX P100 target *L. monocytogenes* in ready-to-eat meat and poultry products, and SalmoFresh is used against *Salmonella* on poultry, fish, and fresh produce. These products were among the first to receive regulatory approval and made way for broader phage-based food safety applications (Moye et al. 2018).

Food-related phage applications are generally divided into pre-harvest and post-harvest stages. Pre-harvest interventions target live animals or crops before slaughter or harvest, whereas post-harvest applications focus on food products, packaging materials, and processing environments (Clavijo et al. 2019). Surface sprays, dips, and packaging coatings are the most widely used delivery formats, and biofilm removal on food-contact surfaces has become an increasingly active area of research. Under realistic storage conditions, post-harvest phage applications commonly produce reductions of approximately 1-3 log10 CFU/g or CFU/cm² in target pathogens on poultry, fresh produce, and dairy surfaces (Endersen & Coffey 2020; Grigore-Gurgu et al. 2024).

Phage-based biosensors and detection systems have also improved the rapid identification of viable foodborne pathogens (Li et al. 2026). Because phages bind only to viable host cells, these systems can distinguish live bacteria from dead cells more effectively than many PCR- or antibody-based approaches. Reported technologies include surface plasmon resonance sensors, fluorescent reporter phages, magnetoelastic biosensors, and impedance-based platforms. Detection limits as low as 10-100 CFU/mL have been reported for E. coli, Salmonella, and Listeria in food and water samples within a few hours, considerably faster than conventional culture-based methods requiring 24-72 hours.

In parallel with whole-phage products, phage-derived proteins are now being developed as a new class of food-safety agents. Endolysins, introduced in the previous section, retain activity in food matrices where phage particles can be inactivated and are particularly useful against Gram-positive pathogens such as *L. monocytogenes*, *S. aureus*, and *Bacillus cereus*. They also face fewer regulatory hurdles than whole phages because they are well-defined proteins. Recent reviews describe a steady increase in the number of endolysins entering food-safety pipelines, with broad spectrum and high specificity available depending on the cell-wall binding domain selected.

Across published literature, phage-based food safety interventions consistently produce measurable reductions in target pathogens, although the magnitude varies by matrix and pathogen. Reported reductions of *L. monocytogenes* on RTE meat surfaces typically fall between 1.0 and 3.5 $\log^{10}$ CFU/g, with stronger effects in liquid or surface applications than in solid matrices (Grigore-Gurgu et al. 2024). On fresh produce, *Salmonella* and *E. coli* O157:H7 reductions of 1 to 2 $\log^{10}$ CFU/g have been documented in lettuce, sprouts, and melons (Endersen & Coffey 2020). Industrial-scale broiler trials with the SalmoFREE® cocktail showed significant reductions in *Salmonella* prevalence at slaughter without affecting growth performance (Clavijo et al. 2019). Effects are usually higher when phage cocktails rather than single phages are used, when contamination levels are moderate, and when phages are applied early in the post-harvest chain.

The regulatory framework for food-safety phages is more developed than that for therapeutic phages. The United States Food and Drug Administration first granted Generally Recognized as Safe (GRAS) status to a phage product, ListShield (formerly LMP-102), in 2006, opening the way for direct phage use in food systems. Several additional approvals followed for products including LISTEX P100, EcoShield, SalmoFresh, ShigaShield, and Salmonelex (Moye et al. 2018). The US Department of Agriculture has also approved phage products for use on meat and poultry. In Europe, the European Food Safety Authority has issued favourable scientific opinions on several phage-based products, although direct authorization for broad food applications remains limited. Similar approvals or commercial acceptance now extend to Australia, New Zealand, Canada, Switzerland, Israel, and China. Nevertheless, regulatory pathways remain fragmented, and the absence of an internationally harmonized framework continues to limit global commercialization.

Food-safety phages occupy the most regulatorily mature corner of the field, and this maturity defines what counts as a translational barrier here. Since the 2006 GRAS status of ListShield, half a dozen products have entered the United States market, and parallel approvals exist in Australia, Canada, Israel, and several European jurisdictions. The translational question is therefore not whether phages can reach commercial use, they have, but why the uptake by the global food industry remains uneven. Three factors keep adoption below its technical potential. The matrix effect is the first: log reductions reported in liquid or surface applications shrink in dense, low-moisture, or chilled food matrices, and processors must absorb this efficacy loss without a clear performance specification from regulators. The second is consumer perception, particularly in European markets where the label “virus-treated food” creates resistance that no scientific argument has fully overcome. The third is the absence of an internationally harmonized framework: a product cleared by the FDA may face a separate dossier in the EU, a third in Mercosur countries, and yet another in Asia, which fragments the commercial case for global manufacturers. Food-safety phages thus illustrate a different kind of translational gap from human medicine. Here technical and regulatory feasibility is established, but market and harmonization barriers limit deployment.

Phage-based food safety offers several practical advantages, including high host specificity, absence of chemical residues in the final product, preservation of the natural food microbiota, and minimal impact on sensory or nutritional properties when used at recommended doses (Goodridge & Bisha 2011; Endersen & Coffey 2020). Phage activity is also maintained under cold-chain conditions where many chemical preservatives become less effective, and phages can be integrated with other biopreservation strategies such as bacteriocins, modified-atmosphere packaging, and lactic acid bacteria. Important limitations nevertheless remain. Broad pathogen coverage generally requires phage cocktails, resistant bacterial mutants may emerge rapidly, and phage viability can decline in dry, low-moisture, or extreme pH environments. Consumer perception also continues to limit adoption in some markets. Despite these constraints, the growing number of approved products and ongoing commercial scale-up strongly suggest that phage-based food safety will continue expanding within broader One Health-oriented AMR reduction strategies (Alomari et al. 2021; Grigore-Gurgu et al. 2024). The same logic extends upstream to agricultural systems, where phages increasingly function as direct biocontrol agents against plant pathogens.

6. Agriculture and Phytopathogen Applications

Bacterial plant diseases cause large economic losses every year and threaten the production of staple and high-value crops worldwide. Conventional control relies on copper-based bactericides and a small number of agricultural antibiotics such as streptomycin and oxytetracycline, but both groups are losing effectiveness and face increasing regulatory pressure. Copper accumulates in soil and surface water and is toxic to non-target organisms, while antibiotic use on crops has been linked to the spread of resistance genes that can later move into human and animal pathogens (Svircev et al. 2018). An alternative class of agents is phages, which combine narrow host specificity, self-amplification in the presence of the target pathogen, and an absence of chemical residue on the harvested crop. These features have made phage-based products one of the most active areas of biological plant protection over the past two decades.

In agriculture and plant pathology, phages are used as biocontrol agents against phytopathogenic bacteria that cause major crop losses worldwide. Reported targets include *E. amylovora* , *X. oryzae* and *Dickeya solani*, *P. syringae*, *Ralstonia solanacearum* (Holtappels et al. 2021) as well as *Xylella* and *Pectobacterium*. Both greenhouse and open-field studies have reported measurable reductions in disease incidence, although outcomes still vary according to the crop type, pathogen pressure and application method (Nakayinga et al. 2021). Current agricultural phage research spans most major crop systems. Fire blight caused by E. amylovora remains a major focus in pome fruits, while tomato and pepper are extensively studied for bacterial spot and bacterial speck caused by *Xanthomonas* and *P. syringae* pathovars (Svircev et al. 2018). Rice production systems are targeted for bacterial leaf blight caused by *X. oryzae* pv. *oryzae*, whereas potato and related Solanaceae crops are investigated for *Dickeya*-, *Pectobacterium*- and Ralstonia-associated soft rot and wilt diseases (Buttimer et al. 2017). Additional applications include citrus canker and *Xylella*-associated diseases in olive production systems.

In practice, phages are delivered through foliar sprays, soil treatments, seed coatings, or irrigation-based systems. Their primary mechanism is host-specific lysis, although some applications also interfere with biofilm formation on plant surfaces, particularly within the phyllosphere. Environmental exposure remains a major limitation: UV radiation, temperature fluctuations, rain wash-off, and desiccation can rapidly reduce phage stability under open-field conditions. Encapsulation strategies and polymer-based delivery systems are therefore being developed to improve persistence and efficacy in agricultural environments (Fernández et al. 2018; Nazir et al. 2023).

Research on agricultural phage applications is divided between laboratory characterization, controlled greenhouse trials, and open-field experiments. Laboratory studies focus on host range determination, plaque morphology, lytic spectrum against pathogen collections, genome sequencing, stability under different temperatures and UV exposures. Greenhouse trials test phage efficacy on inoculated plants under controlled conditions, while field trials assess performance under realistic environmental pressure. The first European phage cocktails effective against *E. amylovora* on pear blossoms were reported in 2024 and produced significant reductions in fire blight symptoms in greenhouse and detached-blossom assays (Biosca et al. 2024). Cocktails against *X. euvesicatoria* pv. *perforans* have reduced bacterial spot severity on tomato by 44 to 78% in greenhouse and field tests (Nakayinga et al. 2021). Application method depends on the pathogen, plant tissue and disease cycle. Foliar sprays remain the dominant approach for bacterial pathogens colonizing leaves, blossoms and fruit surfaces whereas soil drenching and irrigation-based systems are used for soilborne pathogens such as *R. solanacearum*. Preventive application or treatment at the earliest visible stage of disease produces the strongest results, and repeated weekly applications are common during high-risk periods. Seed treatments, drone-assisted delivery, and commercial ground-spray systems are also being explored to improve field-scale implementation (Sharma et al. 2022).

Several agricultural phage products have already reached the commercial market. The most established line is AgriPhage, developed by OmniLytics in the United States, which holds EPA registration for five products: AgriPhage Spot & Speck for *X. campestris* pv. *vesicatoria* and *P. syringae* pv. tomato on tomato and pepper, AgriPhage-Tomato for *Clavibacter michiganensis*, AgriPhage-Fire Blight for *E. amylovora*, AgriPhage-Citrus Canker for *X. citri*, and AgriPhage-Nut & Stone for stone fruit and walnut pathogens. All products are approved for organic production under the USDA National Organic Program, are exempt from maximum residue limits, and have a zero-day pre-harvest interval (Nakayinga et al. 2021). Additional commercial activity includes phage-based products from European manufacturers and from research institutes in Brazil, Korea, and China, where the regulatory environment for biological plant protection has grown more accommodating in recent years.

Field outcomes remain variable between trials, but performance is generally consistent when phages are well matched to local pathogen populations and applied at appropriate stages of disease development. Long-term field data from Florida tomato production indicate that weekly AgriPhage applications over more than a decade have not generated detectable phage resistance under commercial conditions, partly because the formulation contains multiple genetically distinct phages (Nakayinga et al. 2021). Disease severity reductions of approximately 20-50% relative to untreated controls are commonly reported, together with moderate yield improvements. Multi-phage cocktails against fire blight have significantly reduced blossom infection in detached-flower assays, while combinations of phages with biological control bacteria such as *Pantoea agglomerans* have shown additive effects in field conditions (Biosca et al. 2024). Integrated management approaches combining phages with resistant cultivars, copper treatments, or biological control agents are also increasingly common in stone fruit and kiwifruit systems (Buttimer et al. 2017).

The regulatory framework for agricultural phages is more developed in some regions than others. In the United States, phages used as plant bactericides are registered with the Environmental Protection Agency under FIFRA, and several products have been registered since AgriPhage first received approval in 2005. Products approved for use on USDA-certified organic crops carry the National Organic Program seal. In the European Union, the regulatory pathway is less clear; phages can in principle be registered as plant protection products under Regulation (EC) No 1107/2009, but no product has yet received full EU-wide authorization, and some member states have used emergency or temporary authorisations to allow phage trials on specific crops (Holtappels et al. 2021). The 2023 EMA veterinary phage guideline has prompted parallel discussions for plant protection products, although a dedicated agricultural guideline is not yet in place. Other countries with active phage product approvals or pilot use include Brazil, Canada, South Korea, China, and several African states where *Xanthomonas* wilt and cassava bacterial blight create major pressure.

Several factors still limit broader adoption of agricultural phage products. UV exposure remains the dominant environmental constraint, with phage titres on plant surfaces often decreasing by 1–3 log10 PFU within only a few hours of midday sunlight. Temperature extremes, rain wash-off, and surface desiccation further reduce field persistence, while slow movement across dry leaf surfaces can leave untreated areas susceptible to infection. Although phage resistance develops more slowly than antibiotic resistance, resistant field strains of *E. amylovora, Xanthomonas* and *Pseudomonas* have all been reported under selective pressure. The narrow host range of individual phages also makes multi-phage cocktails essential for most agricultural applications, and batch-to-batch stability remains an important formulation challenge. Encapsulation within alginate, chitosan, and other biopolymers, together with UV-protective formulations, is therefore being developed to improve persistence under field conditions (Fernández et al. 2018; Nazir et al. 2023).

Agricultural phages have reached the field in a way that human therapeutics have not, but the translational picture is shaped by physics more than by policy. AgriPhage products have been registered since 2005 and applied across multiple crops without producing detectable field resistance over more than a decade of weekly use, an outcome that human and aquaculture application areas have not been able to match. The barrier here is environmental persistence: UV light, temperature swings, rain wash-off, and desiccation reduce phage titres on leaf surfaces by one to three logs within hours, so any field formulation must combine biological activity with physical protection. This pushes the development pipeline toward formulation science, encapsulation, UV-protective adjuvants, polymer-based delivery, rather than toward new phage isolates, and shifts the cost structure away from manufacturing and toward applied chemistry. The regulatory side is also uneven: the EPA pathway in the United States is established, but the European Union still lacks a dedicated framework, which constrains commercial expansion in one of the largest agricultural markets in the world. Agricultural phage applications therefore illustrate that the dominant translational barrier is not always scientific or regulatory; in open-field settings it is physical persistence, a problem that recurs in environmental and aquaculture applications where phages must remain active in open systems for clinically meaningful periods.

Despite these limits, the advantages of phage-based plant protection are substantial. Such products leave no chemical residue on the harvested crop, meet the requirements of organic production, are compatible with most existing spray equipment, and can be combined with biological control bacteria, fungicides, and resistant cultivars in integrated disease management programmes. They offer a credible alternative to copper and to remaining agricultural antibiotics, particularly in jurisdictions where copper use is being restricted on environmental grounds. The commercial success of AgriPhage, the steady expansion of new product registrations, and the strong research pipeline on encapsulation and engineered phages all suggest that agricultural phage products will see expanded use in plant disease management over the next decade, particularly in tree fruit, vegetables, and rice production (Nakayinga et al. 2021; Holtappels et al. 2021). Agricultural phage applications also feed directly into the wider environmental compartment: surface runoff, irrigation return water, and animal manure from treated farms all carry phages, target bacteria, and resistance genes downstream, so the next section examines how phages are deployed in those receiving environments.

7. Environmental Applications and Biofilm Control

Bacterial contamination and biofilm formation create persistent problems in environmental and industrial systems, including municipal wastewater plants, drinking water networks, cooling towers, ship hulls and food processing surfaces. Rising antimicrobial resistance, restrictions on chemical biocides and tighter discharge limits have pushed researchers to look for biological control options that act with high specificity and leave no toxic residue. Among the options, phages fit this profile well. Their ability to self-amplification at the target, to selectively infect bacterial hosts and avoids interaction with eukaryotic components of treatment systems or a downstream ecosystem makes them particularly suitable for environmental applications (Calero-Cáceres & Muniesa 2016; Garvey et al. 2022).

Although these applications overlap partly with the food-safety and agricultural uses discussed earlier, the scale, open-system nature, and regulatory expectations are substantially different.

Environmental applications extend well beyond clinical and agricultural settings. In wastewater treatment systems, phages are used to selectively reduce pathogenic and antibiotic-resistant bacterial populations, thereby improving water quality and limiting environmental contamination (Garvey et al. 2022; Pires et al. 2023). Recent studies also suggest that phages may help reduce the spread of antimicrobial resistance genes between environmental reservoirs, which has direct implications for sustainable environmental management (Fernández et al. 2018). Industrial applications focus mainly on biofilm control and biofouling reduction in pipelines and membrane filtration systems, where conventional chemical treatments are increasingly constrained by both efficacy limitations and environmental regulations (Tian et al. 2021).

Wastewater treatment plants accumulate large quantities of organic matter, human and animal microbiota, and pharmaceutical residues, including sub-inhibitory concentrations of antibiotics. These conditions favour the selection and horizontal transfer of resistance genes, and wastewater facilities are therefore increasingly recognised as important reservoirs and dissemination points for environmental AMR. Several stages of the treatment chain have been explored for phage intervention. In activated sludge systems, lytic phages have been used at laboratory and pilot scale to control filamentous bacteria responsible for sludge bulking and foaming, particularly *Gordonia, Microthrix* and *Nocardia* spp. During disinfection, phage cocktails targeting E. coli, Salmonella, and antibiotic-resistant *Enterobacteriaceae* have been applied to reduce bacterial loads before discharge or water reuse. In hospital wastewater, phages targeting carbapenem-resistant *K. pneumoniae* and *A. baumannii* have also been investigated as a strategy to limit the release of clinically important resistant strains into municipal systems (Garvey et al. 2022).

Drinking water distribution systems and industrial water networks face a different set of challenges, dominated by biofilm formation on pipe interiors, cooling towers, heat exchangers, and reverse-osmosis membranes. These biofilms frequently harbour opportunistic pathogens such as *L. pneumophila*, *P. aeruginosa* and *Mycobacterium* spp., while also contributing to biofouling, corrosion, and loss of system efficiency. In these settings, phage applications target specific biofilm-forming species rather than total bacterial loads and are commonly delivered through water-phase dosing or controlled pulse treatments (Garvey et al. 2022). Laboratory column and flow-cell studies have reported reductions of approximately 2-3 log10 CFU/cm² on biofouled surfaces, although translation to full-scale industrial systems remains limited by mass-transfer constraints and the difficulty of maintaining phage viability in chlorinated water.

Soil and sediment applications are less developed than aquatic ones but are receiving growing attention. In agricultural and contaminated soils, phages are used to target plant pathogens and to limit the persistence of antibiotic-resistant bacteria introduced through manure and biosolids application. They have also been tested as part of bioremediation strategies aimed at removing antibiotic-resistant indicator organisms from sites where wastewater sludge or animal slurry has been applied to land. Soil application is more challenging than aquatic application because phage diffusion through the soil matrix is slow, and phage particles can be adsorbed and inactivated by clay minerals, organic matter, and pH extremes. Encapsulation in biopolymers and the use of carrier bacteria are being tested to address these constraints (Calero-Cáceres & Muniesa 2016).

Biofilms are surface-associated bacterial communities embedded within a self-produced EPS matrix composed mainly of polysaccharides, proteins, lipids, and extracellular DNA. Bacteria within biofilms are estimated to be 10-1000 times more tolerant to antibiotics and disinfectants than planktonic cells, and approximately 80% of chronic or recurrent bacterial infections in humans are considered biofilm-associated (Samson et al. 2023). Although this protective architecture creates barriers for phage penetration, several mechanisms still allow phages to disrupt biofilm structure. The most important involves depolymerases that cleave EPS and capsular polysaccharides, thereby opening channels for phage particles and lytic products to spread through the matrix (Abedon 2023). Additional mechanisms include lysis from within and lysis from without, both of which contribute to biofilm reduction even when productive infection remains incomplete.

When phages are released into open environmental systems, evaluation cannot be restricted to efficacy against target bacteria alone. Their potential contribution to horizontal gene transfer and their impact on resident microbial diversity must also be considered. At present, no major regulatory framework, including those of the European Union, the World Health Organization, or the United States Food and Drug Administration, has fully incorporated ecological oversight into environmental phage assessment, although the need for such regulation is increasingly recognised (Calero-Cáceres & Muniesa 2016; Pires et al. 2023). Biofilm barriers can nevertheless be disrupted through direct bacterial lysis and enzymatic degradation of the EPS matrix, both of which destabilise biofilm architecture and improve phage penetration (Tian et al. 2021).

Healthcare-associated biofilms represent an important overlap between environmental and clinical phage applications. Biofilms readily develop on catheters, prosthetic joints, contact lenses, endotracheal tubes, and other indwelling medical devices, while also colonising hospital sink drains, taps, and high-touch surfaces. Phage-based interventions have therefore been investigated for hospital sink decontamination, catheter-associated urinary tract infections, and removal of *S. aureus* biofilms from orthopaedic implant materials. In one ICU study, fogging with a phage suspension targeting carbapenem-resistant *A. baumannii* reduced environmental contamination that conventional alcohol- and hypochlorite-based disinfectants could not fully eliminate and lowered the proportion of drug-resistant isolates from 87.76% to 46.07% (Garvey et al. 2022).

Food-processing environments represent another major area where biofilms contribute to persistent pathogen contamination. *L. monocytogenes* can survive for years in dairy and meat-processing facilities by colonising pipe joints, conveyor belts, and floor drains, while *Salmonella* and *E. coli* O157 biofilms create similar persistence problems in slaughterhouses and fresh-produce facilities. Current applications include surface spraying, dipping treatments, foam formulations, and incorporation into cleaning-in-place (CIP) systems. Combining phages with conventional sanitizers often produces synergistic effects because chemical treatment disrupts the outer EPS layer and improves phage access to viable cells in deeper biofilm regions. Endolysins and recombinant depolymerases are also increasingly incorporated into these approaches, particularly against Gram-positive biofilms (Abedon 2023).

Environmental phage applications carry a translational burden that no other application area faces to the same degree: the deliberate release of replicating biological agents into open systems, with potential consequences for resident microbial communities and for the horizontal movement of resistance or virulence genes. Wastewater and biofilm studies have already produced strong efficacy data, including reductions of two to three log orders on befouled surfaces and measurable suppression of carbapenem-resistant pathogens in ICU environments. However, very little of this evidence has been translated into routine practice, largely because of regulatory caution rather than technical failure. No current framework, including EMA veterinary guidance, EPA agricultural regulation, or food-safety approval pathways, has fully incorporated ecological oversight into approval requirements, even though the genomic safety screening now routinely applied to human and veterinary phages could also address many environmental concerns. The translational challenge in this field is therefore not simply to generate additional efficacy data, but to integrate genomic safety screening into formal environmental risk assessment systems acceptable to regulators and water-management authorities. Until such frameworks exist, most applications will likely remain at pilot or research scale, and horizontal gene transfer will continue to be treated as a secondary concern rather than a primary endpoint. The integrative cross-domain evidence presented in this review suggests that this position is becoming increasingly difficult to justify.

Environmental phage applications face limitations that are partly shared with other application areas and partly unique to open systems. Phage particles may be inactivated by UV exposure, oxidising disinfectants, extreme pH, or high salinity, all of which are common in environmental matrices. Diffusion through dense biofilms, soil, and sediment is also slow, and effective contact between phages and target bacteria often depends on careful timing and flow conditions. Bacterial resistance may emerge during long-term applications, although the natural diversity of environmental phage populations and the use of cocktails partly compensate for this risk. Regulatory concern remains centred on horizontal gene transfer because lysogenic and transducing phages can transfer antibiotic resistance and virulence genes between bacterial hosts (Calero-Cáceres & Muniesa 2016). Many of these same constraints, including open-water delivery, biofilm penetration, resistance dynamics, and ecological release, reappear in aquaculture, which combines characteristics of veterinary medicine, food production, and open environmental systems. Aquaculture therefore functions as the integrative endpoint of the One Health phage applications discussed throughout this review.

8. Aquaculture Applications

Aquaculture is now the fastest-growing food production application area worldwide and accounts for approximately half of all aquatic animal protein consumed globally. The intensification of fish and shellfish farming has been followed by recurrent bacterial disease outbreaks, with motile aeromonad septicaemia, furunculosis, vibriosis, yersiniosis, columnaris disease, edwardsiellosis, and streptococcosis among the main causes of mortality and economic loss (Choi et al. 2022). Antibiotic use has been the default response to these outbreaks for decades, but the practice now faces strict regulatory limits in Europe under Regulation (EU) 2019/6, in the United States under FDA Veterinary Feed Directive rules, and in many Asian producer countries. The accumulation of antibiotic residues in water, sediment, and seafood, the selection of resistant strains in farm and downstream environments, and the documented horizontal transfer of resistance genes between aquatic and human-associated bacteria have made phage therapy a near-necessity for the area (Pereira et al. 2022).

Aquaculture phage applications have expanded considerably over the past four decades, beginning with the first documented therapeutic trial against *Aeromonas hydrophila* in 1981. Since then, phages have been characterized against major freshwater and marine pathogens, including *Aeromonas*, *Vibrio*, *Flavobacterium*, *Yersinia*, *Edwardsiella*, *Lactococcus*, *Pseudomonas* and *Streptococcus* spp. *Aeromonas* is the most economically important genus, with two pathogen groups dominating: the non-motile *A. salmonicida* (causing furunculosis in salmonids) and the motile aeromonads (*A. hydrophila*, *A. veronii*, *A. dhakensis* and related species, causing motile *Aeromonas* septicemia, MAS, in freshwater species). Therapeutic applications have been documented across a wide range of freshwater and marine species, including salmonids, tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), European sea bass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*) and whiteleg shrimp (*Litopenaeus vannamei*) (Jun et al. 2013).

In vitro characterization remains the first step of every aquaculture phage development programme. Isolation is performed from farm water, sediment, sewage, and the gut and tissues of diseased fish, followed by screening for host range, efficiency of plating (EOP), one-step growth curves, and stability across pH and temperature ranges relevant to fish production. Genome sequencing is now standard practice and is used to exclude integrase, antibiotic resistance, virulence, and toxin genes before any in vivo work (Pereira et al. 2022). Recent isolates are typically assigned to the *Caudoviricetes* class, with *Myoviridae*- and *Podoviridae*-like morphologies dominating *Aeromonas* and *Vibrio* collections. The lytic phage AhFM11 against hypervirulent *A. hydrophila* was characterized as a Straboviridae-like member free of antibiotic resistance genes before in vivo evaluation in rohu, while vB_AhaP_PZL-Ah8 against multidrug-resistant *A. hydrophila* showed rapid replication characteristics suitable for therapeutic use (Dien et al. 2022).

In vivo challenge trials are the most direct measure of therapeutic efficacy. For *A. hydrophila* in rohu, phage AhFM11 produced 100% survival by injection, 95% by immersion, and 93% by oral feeding through phage-coated feed. In rainbow trout, phage MJG raised post-challenge survival to 100% (injection), 66.7% (immersion), and 50% (oral). In striped catfish (*Pangasius hypophthalmus*), phage PVN02 increased survival from 75.6% to 87.8% via spray feeding (Le et al. 2018). Cocktail studies in *Pangasius buchanani* identified strong protection under prophylactic or early therapeutic application regimes (Dien et al. 2022). Relative percent survival (RPS) has reached 70-90% in several optimized trials.

The *Aeromonas* literature is the most developed within aquaculture phage research and illustrates the practical lessons that apply more widely. Single-phage applications routinely fail in field-like conditions because of the rapid emergence of phage-resistant bacterial mutants and the high genetic heterogeneity of farm isolates. Two-phage and multi-phage cocktails reduce the mutation frequency of *A. hydrophila* to phages by more than an order of magnitude compared with single-phage treatment (Dien et al. 2022). Application timing is just as important as dose and composition: prophylactic or simultaneous phage treatment consistently outperforms delayed therapeutic treatment, and the protective effect is lost when phage is given more than 24 hours after the bacterial challenge. For *A. salmonicida* infections in salmonids, in vivo work has been less successful than for *A. hydrophila*, partly because of the encapsulated phenotype of typical isolates and partly because of the cooler water temperatures that limit phage replication rate (Choi et al. 2022).

Beyond *Aeromonas*, three other genera now account for most aquaculture phage activity. Preparations against *V. parahaemolyticus*, *V. harveyi*, *V. anguillarum*, *V. alginolyticus* and *V. splendidus* have been developed in shrimp, sea bass, sea bream, oysters, and sea cucumber, with mortality reductions of 30 to 70% under experimental challenge (Choi et al. 2022). *Y. ruckeri* phages have been isolated from rainbow trout farms and municipal wastewater in Türkiye and elsewhere, where enteric red-mouth disease (ERM) continues to cause significant losses despite vaccination (Welch 2020). *F. psychrophilum* and *F. columnare* phages have also been characterized for bacterial cold-water disease and columnaris in salmonids and warm-water species (Castillo et al. 2019). Three administration routes are commonly used: intraperitoneal or intramuscular injection, bath immersion, and oral delivery through phage-supplemented feed. Each approach presents different trade-offs between therapeutic efficacy and operational feasibility at commercial scale.

Injection generally produces the strongest outcomes in challenge trials, while oral feed-based delivery is the most practical option for large-scale farm conditions. Stability under the acidic and proteolytic conditions of the fish gut remains the main obstacle for oral delivery, and biopolymer encapsulation and edible coatings are increasingly used to overcome it (Azhar et al. 2026). Prophylactic or simultaneous application of phages consistently outperforms delayed therapeutic treatment, but dose optimization is equally important. At excessively high doses, rapid bacterial lysis may release endotoxins and paradoxically increase host mortality, a phenomenon sometimes referred to as the "zone effect". Genomic safety screening for antibiotic resistance genes, virulence factors, and lysogeny-associated elements is now a routine prerequisite before any therapeutic application.

Shellfish and crustacean phage applications are technically distinct from finfish work because the hosts are immunologically simpler and the water-column delivery is direct. In shrimp aquaculture, *V. parahaemolyticus* carrying the *pirA*/*pirB* toxin genes is the cause of acute hepatopancreatic necrosis disease (AHPND), one of the most damaging shrimp diseases of the past decade. Cocktails against AHPND-causing *V. parahaemolyticus* have produced 60 to 90% reductions in mortality in challenge trials with whiteleg shrimp and giant tiger shrimp (*Penaeus monodon*) (Chen et al. 2019). In oysters and mussels, phages have been used at hatchery stage to reduce *V. harveyi* and *V. coralliilyticus* levels, and in juvenile sea cucumber (*Apostichopus japonicus*) preparations against *V. splendidus* have produced significant reductions in mortality. Crustacean phage applications also benefit from the relatively simple regulatory pathway, since the products are often classified as water-quality biocontrol agents rather than as therapeutic veterinary medicines.

Several features make phages an attractive non-antibiotic strategy for aquaculture. The narrow host range of individual phages makes cocktails essential for therapeutic coverage but limits collateral effects on non-target microbiota. Cocktails also reduce the emergence of resistant mutants compared with single-phage applications. Therapeutic options now extend beyond intact phage particles: recombinant endolysins can lyse Gram-negative aquaculture pathogens directly, even without outer membrane permeabilizers (Loc et al. 2021). Phage lysate vaccines provide an alternative to formalin-killed cell preparations, with protection rates higher than conventional inactivated vaccines. Phage-antibiotic synergy reduces antibiotic doses without sacrificing efficacy and therapeutic phages restore intestinal microbiota homeostasis following pathogen-induced dysbiosis, a clear advantage over broad-spectrum antibiotics. Commercial phage products targeting key aquaculture pathogens are reaching the market, and regulatory frameworks are progressively advancing (Rai et al. 2024; Azhar et al. 2026).

Some factors still limit broader adoption of phage products in aquaculture. Seawater conditions, including high salinity, dissolved organic matter, suspended solids, and UV exposure in surface waters can reduce phage titres substantially, and persistence on fish skin and gills is often a matter of hours rather than days. Cold water temperatures slow phage replication and limit therapeutic effect in salmonid farming, which is one of the largest commercial segments. Bacterial resistance, although slower to develop than in monoculture, remains a real risk in long-term applications. The narrow host range of single phages makes routine farm use dependent on strain-typing infrastructure that is rarely available outside research settings. Regulatory pathways for veterinary phage products in aquaculture are less developed than for terrestrial veterinary medicine; the 2023 European Medicines Agency guideline on veterinary phage products applies in principle, but no centrally authorised aquaculture phage product is yet on the EU market (Pereira et al. 2022; Choi et al. 2022).

Beyond these technical constraints, three issues shape the wider role of phages in aquaculture: sustainability, environmental dissemination and regulation. Within sustainable aquaculture, phages support the antibiotic-reduction goals now central to responsible production, because the accumulation of antibiotic residues in farmed fish and the associated import refusals and trade restrictions have made antibiotic alternatives a priority for the sector (Bondad-Reantaso et al. 2023). As a residue-free and species-specific option, phages fit alongside vaccination, probiotics, and quorum quenching within the integrated, antibiotic-sparing disease management that sustainable aquaculture requires (Albarella et al. 2025). Environmental dissemination is a distinct concern in open and flow-through systems, because applied phages and any mobilised resistance determinants can move from farm effluent into receiving waters and sediments, where antibiotic resistance genes are known to persist in both bacterial and bacteriophage fractions (Calero-Cáceres & Muniesa 2016). The same water exchange that disperses phages also prevents their retrieval, so the ecological consequences of large-scale release, including transduction-mediated gene transfer, must be assessed rather than assumed negligible (Pires et al. 2023). Regulatory concerns in aquatic systems therefore extend beyond product efficacy to formulation flexibility and environmental release. Centralised authorisation under Regulation (EU) 2019/6 remains costly and poorly suited to the low-cost, high-volume, frequently updated preparations that aquaculture requires, which has led some manufacturers toward feed-additive or water-biocontrol classifications that avoid full medicinal-product requirements (Fauconnier et al. 2025).

Aquaculture occupies a position that combines features of every other area and inherits the translational difficulties of each. Application happens in open water like agricultural and environmental uses; the host is a food-producing animal with the regulatory profile of veterinary medicine; the final product enters the human food chain with the safety expectations of food-safety phages; and intensive farming produces the same selection pressure on pathogens that drives the human AMR crisis. Aquaculture therefore serves as the practical test case for any cross-domain translational framework: a phage strategy that works here must address open-system delivery, regulatory fragmentation, environmental persistence, and food-chain safety in a single integrated solution.

The advantages of phage therapy in aquaculture justify the continued research and commercial activity. Applications leave no chemical residue in harvested products, do not select for cross-resistance to clinically important antibiotics, remain compatible with organic and antibiotic-free production systems, and can be produced at relatively low cost once scale-up methods are established. The overlap with One Health, the cumulative weight of in vivo evidence across major fish and shellfish species, and the growing list of national approvals all point to expanding use over the next decade. Practical priorities include broader-spectrum cocktail engineering, the routine integration of in-feed and in-water delivery, the standardization of genomic safety screening, and the negotiation of a clearer international regulatory pathway. Bacteriophages will not replace vaccines, biosecurity, and good husbandry in aquaculture, but they are positioned to play a defined role within integrated disease management (Pereira et al. 2022; Choi et al. 2022). Aquaculture thus serves as the integrative endpoint of the One Health phage applications reviewed here. Together, these links position phage-based interventions as a cross-domain AMR mitigation strategy rather than an area-specific tool.

9. Common Challenges across Application Fields

The six area-specific sections above show that bacteriophage applications have moved beyond proof of concept in every domain examined, yet none of these application areas has converted its scientific evidence into routine large-scale practice. The reasons differ by application area, but the underlying problems occur. This section steps back from application area-by-application area description and analyses the seven recurring constraints that define the translational profile of phage applications across human medicine, veterinary practice, food safety, agriculture, environmental biotechnology, and aquaculture: host specificity and cocktail design, phage resistance dynamics, formulation and delivery, manufacturing under good manufacturing practice (GMP), regulatory fragmentation, genomic safety screening, and environmental persistence with horizontal gene transfer risk. Each constraint is examined in turn, with attention to how its expression differs between application areas and what cross-domain lessons can be drawn. Table 2 summarizes the comparative picture and identifies the application areas where each constraint is most acute.

Table 2. Cross-areas comparison of translational constraints in bacteriophage applications.

Constraint	Human	Veterinary	Food	Agriculture	Environmental	Aquaculture
Host specificity / cocktail design	Personalized; per-patient	Herd-level; multi-strain	Multi-pathogen products	Up to 8 phages per formulation	Species-specific dosing	Farm-level; high heterogeneity
Phage resistance	Single-course emergence	Herd-scale selection	Matrix-dependent	Minimal under 10+ yr use	Long-term variable	High under monoculture
Formulation / delivery	IV, inhaled, topical	Oral / feed; encapsulation	Spray / dip / coating	Foliar; UV-protective	Water-phase dosing	In-feed, immersion, injection
GMP / cost	High; non-amortizable	Moderate; flexible composition	Food-grade; low	Biopesticide-grade; low	Undefined	Veterinary; moderate
Regulatory framework	Compassionate / magistral	EMA 2023; FDA CVM	FDA GRAS; EFSA	EPA FIFRA; no EU pathway	No dedicated framework	EMA in principle; no EU product

Genomic safety screening	Routine	Routine	Standard practice	Standard practice	Not formally required	Routine
Environmental persistence / HGT	Closed system; minimal	Mostly contained	Matrix decay	UV / weather loss	Open release; HGT key concern	Open water; HGT relevant

9.1. Host specificity and cocktail design

Host specificity is the defining therapeutic advantage of phages and the main source of their operational difficulty. A single phage typically lyses only a subset of strains within its target species, which preserves commensal microbiota but forces every area to develop cocktails that cover the local strain diversity of a target pathogen (Loc-Carrillo & Abedon 2011). The expression of this problem differs by scale. In human medicine, cocktails are matched to individual patient isolates and assembled on demand, as in the landmark case series treated under emergency-access provisions (Pirnay et al. 2024). In veterinary, food, and aquaculture settings, cocktails must cover the strain diversity of entire herds, batches, or farm populations, which is genetically far more heterogeneous (Goodridge & Bisha 2011; Choi et al. 2022). In agriculture, AgriPhage maintains efficacy partly by including up to eight genetically distinct phages in a single formulation, an approach that compensates for narrow individual host range through breadth of coverage (Holtappels et al. 2021; Nakayinga et al. 2021). The shared lesson across application areas is that cocktail composition is the variable that bridges efficacy and scalability, but the optimal design rules differ between personalized clinical use and population-level deployment.

9.2. *Resistance dynamics*

Bacterial resistance to phages can emerge within hours of exposure under selective pressure, but the rate and the fitness cost of resistance depend on the application context (Wang & Yu 2025). Long-term field data from agricultural AgriPhage use shows that no detectable resistance has appeared over more than a decade of weekly application on tomato crops, an outcome attributed to multi-phage formulations and to the absence of continuous high-density bacterial monoculture (Nakayinga et al. 2021). Aquaculture and intensive livestock production sit at the opposite end of this spectrum: continuous monoculture of fish or poultry pathogens creates exactly the selection pressure that drives resistance, and the genetic heterogeneity of farm isolates further accelerates the appearance of resistant subpopulations (Dien et al. 2022; Choi et al. 2022). Human clinical use occupies an intermediate position, where resistance often emerges within a single treatment course but is frequently accompanied by restored antibiotic susceptibility or reduced virulence (Gordillo Altamirano et al. 2021). Across application areas, the central conclusion is that resistance is not an intrinsic property of phage therapy but a consequence of how phages are deployed; cocktail diversity, intermittent application, and rotation regimens are the operational tools that contain it.

9.3. Formulation and delivery

Phages are biologically active particles whose viability is sensitive to pH, temperature, desiccation, UV exposure, salinity, and oxidative stress (Loc-Carrillo & Abedon 2011). Every application area has had to develop formulation chemistry to keep phages active under its specific application conditions, and the technical problems are very different. Intravenous and inhaled delivery in human medicine requires pyrogen-free, endotoxin-controlled preparations stable at clinical hold temperatures. Oral delivery in veterinary and aquaculture settings requires protection against gastric acidity and digestive proteases, which has driven the development of alginate, chitosan, and liposomal encapsulation. Surface and foliar applications in food and agriculture face UV-driven titre losses of one to three orders of magnitude within hours, which have pushed agricultural research toward UV-protective adjuvants and polymer-based encapsulation rather than toward new phage isolates (Fernández et al. 2018; Nazir et al. 2023). Environmental applications must contend with chlorine inactivation in drinking water systems and with adsorption by clay minerals in soil (Garvey et al. 2022; Calero-Cáceres & Muniesa 2016). Across application areas, formulation is now a more limiting constraint than phage isolation itself, and most active commercial development is now in delivery technology rather than in primary phage discovery.

9.4. Manufacturing and cost structure

Good manufacturing practice was developed for chemically defined small-molecule pharmaceuticals with stable, reproducible composition. Phage preparations sit awkwardly within this framework because they are biological products whose composition is intentionally variable, cocktails change with the target strain, and self-amplifying agents do not follow the dose–response assumptions of conventional pharmacology (Gordillo Altamirano & Barr 2021). Human medicine bears the full weight of this contradiction. Personalized phage products used under emergency or compassionate-use authorization cannot be cost-amortized across a defined product line, and per-treatment costs therefore remain high (König 2025). Veterinary and aquaculture application areas benefit from the 2023 European Medicines Agency guideline that explicitly accepts flexible composition, which lowers the regulatory cost barrier but does not eliminate the manufacturing variability problem (Fauconnier et al. 2025). Food and agricultural products escape this issue because their regulatory category does not require pharmaceutical GMP at all; they can be produced under food-grade or biopesticide-grade quality systems (Holtappels et al. 2021). The cross-domain lesson is that GMP cost is not a fixed property of phage therapy but a consequence of regulatory classification, and the variation between application areas is therefore informative about where regulatory reform would most reduce cost. Several practical strategies are now narrowing the gap between bespoke and scalable production. Standardised phage banks, in which well-characterised lytic isolates are pre-screened, sequenced, and stored under defined conditions, allow a defined-but-flexible cocktail to be assembled from a qualified library rather than developed from scratch for each case. Modular single-use bioreactor systems, already used in food-safety production at volumes up to 1500 litres, reduce cross-contamination risk and shorten changeover between batches, which suits the variable composition of phage products better than fixed stainless-steel lines. Validated upstream and downstream protocols covering host-strain control, endotoxin removal, and titre standardisation provide the reproducibility that regulators require without forcing a single fixed formulation. Together these approaches suggest that large-scale manufacturing feasibility depends less on new technology than on the adoption of qualified-library and single-use platforms across the application areas where production volume is highest (Moye et al. 2018; König 2025).

9.5. Regulatory fragmentation

No phage application sits within a single, harmonized international regulatory framework (König 2025). Human therapeutics relies on national magistral pathways in Belgium and on compassionate-use provisions elsewhere, with no centrally authorized product in the United States or the European Union. Veterinary products fall under the 2023 EMA guideline in Europe, under the FDA Center for Veterinary Medicine and GRAS pathways in the United States, and under separate Brazilian, Chinese, and South Korean approvals elsewhere (Fauconnier et al. 2025; Wu & Zhu 2021). Food-safety phages are governed by FDA GRAS status, USDA Food Safety and Inspection Service, EFSA opinions, and a patchwork of national approvals in Australia, Canada, Israel, and Switzerland (Moye et al. 2018). Agricultural phages fall under EPA FIFRA registration in the United States and lack a dedicated European framework (Holtappels et al. 2021; Nakayinga et al. 2021). Environmental applications have no purpose-built framework at all (Calero-Cáceres & Muniesa 2016; Pires et al. 2023). Aquaculture inherits the veterinary pathway but has not yet produced a centrally authorized European product (Pereira et al. 2022; Choi et al. 2022). The fragmentation has direct commercial consequences. A manufacturer seeking global market access must prepare separate dossiers under at least four distinct regulatory paradigms and the absence of a harmonized framework is now widely identified as the single most important policy barrier to large-scale phage deployment. Table 3 summarizes these regional frameworks and the strength or limitation of each.

Table 3. Comparison of bacteriophage regulatory frameworks across major world regions.

Region/Authority	**Regulatory approach**	**Phage handled**	**Pathway**	**Strength or limitation**	**Reference**
North America (USA, FDA)	No marketing authorization for therapeutic phages; food, agriculture handled through	Human therapy via Emergency IND /expanded access; food via GRAS; agriculture via EPA registration	eIND (Patterson case, 2016); ListShield GRAS (2006); AgriPhage EPA-registered	Flexible case by case clinical access, food and agriculture routes, no approve personalize therapeutics	Schooley et al. 2017; Moye et al. 2018
Europe- EU centralized (EMA)	Centralized marketing auth. mandatory; phages classed as novel therapies under Regulation (EU) 2019/6	Veterinary guideline adopted 2023 (EMA/CVMP/NTWP/32862/2022); human guideline in preparation	Centralized procedure; veterinary medicinal product framework	Harmonized and transparent but centralized route poorly suited to cocktails updates	European Medicines Agency 2023
Europe -Belgium	National magistral preparation framework since 2018, operating alongside EU	Hospital pharmacists prepare tailor-made phage medicines on prescription	Magistral preparation (FAMHP positive advice, January 2018)	Only Western framework that resolves personalproblem in law, but limited magistral	Pirnay et al. 2024

Region/Authority	Regulatory approach	Phage handled	Pathway	Strength or limitation	Reference
Europe -Eastern (Georgia, Russia, Poland)	Phage therapy never abandoned; embedded in routine clinical practice for over a century	Ready-to-use commercial personalized preparationsnot required GMPmanufactured	Eliava Institute (Tbilisi); Hirszfeld Institute (Wrocław)	Extensive clinical experience availability, but limited GMP standardization and few controlled trials	Yang et al. 2023
Oceania (Australia, TGA)	Phages regulated as medicines; no approved products; supply through unapproved-goods pathways	Special Access Scheme, Authorised Prescriber, and clinical-trial routes; -limited GMP exemption small-batch products	Special Access Scheme clinical access since December 2021	Supportive case-by-case access, but classification within the biological framework is not yet fully settled	Khatami et al. 2022
South America (Brazil, Colombia)	No dedicated central phage framework identified; commercial products operate mainly in agriculture and aquaculture	Commercial phage products marketed crop aquaculture use; approval mechanisms country-specific not harmonized	SalmoFREE (Colombia)	Active commercial uptake, but the regulatory basis is fragmented and not centrally defined	Clavijo et al. 2019
Asia (India, Vietnam, China, others)	No harmonized regional framework; several national-level approvals, mainly in aquaculture	Pond- and feed-based phage products approved shrimp fish; standards differ by country	Anti-AHPND phage products for shrimp aquaculture	Rapid uptake world's aquaculture region, fragmented standards limit use	Le et al. 2018; Chen et al. 2019

Abbreviations: AHPND, acute hepatopancreatic necrosis disease; CTN, Clinical Trial Notification; CTX, Clinical Trial Exemption; eIND, Emergency Investigational New Drug; EMA, European Medicines Agency; EPA, US Environmental Protection Agency; FAMHP, Belgian Federal Agency for Medicines and Health Products; FDA, US Food and Drug Administration; GMP, good manufacturing practice; GRAS, generally recognized as safe; IND, Investigational New Drug; TGA, Therapeutic Goods Administration.

9.6. Genomic safety and lysogeny screening

The transition from morphology-based to sequence-based phage characterization has changed what regulators expect before approval (Turner et al. 2023; Pereira et al. 2022). Whole-genome sequencing to exclude integrase genes, antibiotic resistance determinants, virulence factors, and toxin genes is now standard practice in human, veterinary, and aquaculture phage development. Lysogenic phages, which can integrate into the host chromosome and mediate horizontal gene transfer, are routinely excluded from therapeutic preparations. Food-safety and agricultural products apply similar screening, although their regulatory frameworks did not historically require it (Moye et al. 2018; Holtappels et al. 2021). Environmental applications represent the gap: large-scale release of phages into open systems creates the same transfer risk that genomic screening addresses in therapeutic applications, yet no environmental framework has yet incorporated genome safety as a formal approval requirement (Calero-Cáceres & Muniesa 2016). The cross-domain observation is that the technical tool to address environmental concerns already exists in routine use elsewhere; what is missing is the regulatory adoption of that tool outside therapeutic categories.

9.7. Environmental persistence and horizontal gene transfer

Open-system applications such as agriculture, environmental biotechnology, and aquaculture face two constraints that closed-system clinical and veterinary uses do not. Phage particles must remain active for clinically meaningful periods in environments that actively inactivate them, and the released phages cannot be retrieved. Persistence problems have been addressed primarily through formulation chemistry, as described in Section 9.3. The horizontal gene transfer risk is more fundamental, because even lytic phages can occasionally package and transfer bacterial DNA between hosts through transduction (Calero-Cáceres & Muniesa 2016). The probability of such events is low in any individual application but accumulates over large-scale deployment, and the ecological consequences of large-scale phage release on resident microbial communities remain poorly characterized (Pires et al. 2023; Samson et al. 2023). The integrated risk, persistence-driven exposure plus transfer-driven dissemination is highest in aquaculture, which combines open-water delivery with food-chain proximity, and lowest in closed clinical settings (Pereira et al. 2022). A cross-domain framework that calibrates genomic safety requirements to release scale and ecological exposure would address this gradient more rigorously than the current application area-by-application area approach.

Taken together, the seven constraints described above do not constitute independent technical problems but interconnected facets of a single translational challenge. Host specificity drives cocktail design, which in turn drives manufacturing variability and intersects with regulatory classification. Together, these factors determine whether GMP cost becomes a major barrier. Environmental persistence shapes formulation chemistry, which in turn limits available delivery routes and influences the regulatory category that applies. Resistance dynamics, genomic safety, and horizontal gene transfer risk cut across all application areas and connect the One Health compartments most directly. The integrative observation is that no single application area can solve these constraints independently. The cross-domain synthesis presented here instead suggests that the regulatory, manufacturing, and scientific infrastructure of phage therapy will mature fastest where boundaries between application areas are most permeable, particularly at the interfaces between veterinary medicine, food safety, environmental release, and aquaculture. Together, these sectors account for the largest production volumes and the most acute AMR-mitigation pressure. The same aquatic environments that receive agricultural runoff and treated effluent also supply aquaculture systems and return resistance determinants to the human food chain, so an intervention validated in one compartment cannot be evaluated in isolation from the others.

10. Conclusion and Future Perspectives

The evidence reviewed here shows that bacteriophage applications have moved beyond proof of concept in every area examined, from human compassionate-use therapy to commercial agricultural and food-safety products and to in-feed and water-phase deployment in aquaculture. Across the seven areas, phages and their derived products now offer a mechanistically distinct intervention against bacterial infections that complements the classical pillars of antibiotic, vaccine, and biosecurity-based control. The integrative picture across these areas is more advanced than any single sector-focused literature suggests.

The cross-domain synthesis indicates that the dominant barriers to translation are not technical but systemic. The technical tools needed to address these constraints already exist within at least one application area. The operational challenge is therefore to transfer them across sectors rather than to develop them anew.

Maturation of phage applications over the next decade is most likely to occur where policy and commercial conditions are already most favourable. The veterinary, aquaculture, and food-chain corridor represent the clearest example because it combines intense selection pressure for resistance, large production volumes, and some of the most permissive regulatory frameworks currently in place. Environmental applications and human medicine occupy opposite ends of this corridor, and the technical and administrative infrastructure developed within the middle sectors is likely to extend progressively toward both ends. Rather than replacing antibiotics, vaccines, or biosecurity, phages add a distinct mechanism within an increasingly integrated AMR-mitigation strategy. The priority for the coming decade is therefore not to demonstrate that phages work, but to harmonize the regulatory, manufacturing, and safety frameworks through which they reach the patient, the herd, the crop, the food product, the water system, and the fish farm.

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References

1. Abedon, S. T. (2023). Bacteriophage adsorption: Likelihood of virion encounter with bacteria and other factors affecting rates. Antibiotics, 12(4), 723. https://doi.org/10.3390/antibiotics12040723

2. Albarella, D., Dall'Ara, P., Rossi, L., & Turin, L. (2025). Bacteriophage therapy in freshwater and saltwater aquaculture species. Microorganisms, 13(4), 831. https://doi.org/10.3390/microorganisms13040831

3. Alomari, M. M. M., Dec, M., & Urban-Chmiel, R. (2021). Bacteriophages as an alternative method for control of zoonotic and foodborne pathogens. Viruses, 13(12), 2348. https://doi.org/10.3390/v13122348

4. Atterbury, R. J., Van Bergen, M. A. P., Ortiz, F., Lovell, M. A., Harris, J. A., De Boer, A., Wagenaar, J. A., Allen, V. M., & Barrow, P. A. (2007). Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. Applied and Environmental Microbiology, 73(14), 4543-4549. https://doi.org/10.1128/AEM.00049-07

5. Azhar, A. B., Muin, H., Tang, S. S., & Arshad, N. M. (2026). Expanding horizons in phage therapy: Addressing antibiotic resistance in aquaculture. Aquaculture, Fish and Fisheries, 6(1), e70171. https://doi.org/10.1002/aff2.70171

6. Aziz, N. S., Ibrahim, S., Zaharinie, T., & Tang, S. S. (2025). Bacteriophage encapsulation- trends and potential applications in aquaculture. Aquaculture, 594, 741398. https://doi.org/10.1016/j.aquaculture.2024.741398

7. Balogh, B., Jones, J. B., Iriarte, F. B., & Momol, M. T. (2010). Phage therapy for plant disease control. Current Pharmaceutical Biotechnology, 11(1), 48-57. https://doi.org/10.2174/138920110790725302

8. Bianchessi, L., De Bernardi, G., Vigorelli, M., Dall'Ara, P., & Turin, L. (2024). Bacteriophage therapy in companion and farm animals. Antibiotics, 13(4), 294. https://doi.org/10.3390/antibiotics13040294

9. Biosca, E. G., Delgado Santander, R., Morán, F., Figàs-Segura, À., Vázquez, R., Catalá-Senent, J. F., & Álvarez, B. (2024). First European *Erwinia amylovora* lytic bacteriophage cocktails effective in the host: Characterization and prospects for fire blight biocontrol. Biology, 13(3), 176. https://doi.org/10.3390/biology13030176

10. Bondad-Reantaso, M. G., MacKinnon, B., Karunasagar, I., Fridman, S., Alday-Sanz, V., Brun, E., Le Groumellec, M., Li, A., Surachetpong, W., Karunasagar, I., et al. (2023). Review of alternatives to antibiotic use in aquaculture. Reviews in Aquaculture, 15(4), 1421-1451. https://doi.org/10.1111/raq.12786

11. Borysowski, J., & Górski, A. (2008). Is phage therapy acceptable in the immunocompromised host? International Journal of Infectious Diseases, 12(5), 466-471. https://doi.org/10.1016/j.ijid.2008.01.006

12. Buttimer, C., McAuliffe, O., Ross, R. P., Hill, C., O'Mahony, J., & Coffey, A. (2017). Bacteriophages and bacterial plant diseases. Frontiers in Microbiology, 8, 34. https://doi.org/10.3389/fmicb.2017.00034

13. Calero-Cáceres, W., & Muniesa, M. (2016). Persistence of naturally occurring antibiotic resistance genes in the bacteria and bacteriophage fractions of wastewater. Water Research, 95, 11-18. https://doi.org/10.1016/j.watres.2016.03.006

14. Castillo, D., Christiansen, R. H., Espejo, R., & Middelboe, M. (2019). Bacteriophage resistance mechanisms in the fish pathogen *Flavobacterium psychrophil*um: Linking genomic mutations to changes in bacterial virulence factors. Applied and Environmental Microbiology, 85(9), e00027-19. https://doi.org/10.1128/AEM.00027-19

15. Chen, L., Fan, J., Yan, T., Liu, Q., Yuan, S., Zhang, H., Yang, J., Deng, D., Huang, S., & Ma, Y. (2019). Isolation and characterization of specific phages to prepare a cocktail preventing *Vibrio* sp. Va-F3 infections in shrimp (Litopenaeus vannamei). Frontiers in Microbiology, 10, 2337. https://doi.org/10.3389/fmicb.2019.02337

16. Choi, A. R., Patra, J. K., Kim, W. J., & Kang, S. S. (2022). Recent advances in the development of effective therapeutics against *Aeromonas salmonicida* infection in salmonid culture: A comprehensive review. Frontiers in Microbiology, 13, 964007. https://doi.org/10.3389/fmicb.2022.964007

17. Clavijo, V., Baquero, D., Hernandez, S., Farfan, J. C., Arias, J., Arévalo, A., Donado-Godoy, P., & Vives-Flores, M. (2019). Phage cocktail SalmoFREE® reduces Salmonella on a commercial broiler farm. Poultry Science, 98(10), 5054-5063. https://doi.org/10.3382/ps/pez251

18. Dedrick, R. M., Guerrero-Bustamante, C. A., Garlena, R. A., Russell, D. A., Ford, K., Harris, K., Gilmour, K. C., Soothill, J., Jacobs-Sera, D., Schooley, R. T., Hatfull, G. F., & Spencer, H. (2019). Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nature Medicine, 25(5), 730-733. https://doi.org/10.1038/s41591-019-0437-z
19. Dien, L. T., Ngo, T. P. H., Nguyen, T. V., Kayansamruaj, P., Salin, K. R., Mohan, C. V., Rodkhum, C., & Dong, H. T. (2022). Non-antibiotic approaches to combat motile *Aeromonas* infections in aquaculture: Current state of knowledge and future perspectives. Reviews in Aquaculture, 15(1), 333-366. https://doi.org/10.1111/raq.12721
20. Endersen, L. & Coffey, A. (2020). The use of bacteriophages for food safety. Current Opinion in Food Science, 36, 1–8. https://doi.org/10.1016/j.cofs.2020.10.006
21. Fauconnier, A., Pirnay, J. P., Verbeken, G., & Naranjo-Arcos, M. A. (2025). Bacteriophage-based veterinary products: Aligning regulatory framework and development challenges for market integration. Biologicals. https://doi.org/10.1016/j.biologicals.2025.101810
22. Fernández, L., Gutiérrez, D., Rodríguez, A., & García, P. (2018). Application of bacteriophages in the agro-food sector: A long way toward approval. Frontiers in Cellular and Infection Microbiology, 8, 296. https://doi.org/10.3389/fcimb.2018.00296
23. Ferriol-González, C., & Domingo-Calap, P. (2021). Phage therapy in livestock and companion animals. Antibiotics, 10(5), 559. https://doi.org/10.3390/antibiotics10050559
24. Garvey, M. (2022). Bacteriophages and food production: Biocontrol and bio-preservation options for food safety. Antibiotics, 11(10), 1324. https://doi.org/10.3390/antibiotics11101324
25. Gigante, A., & Atterbury, R. J. (2019). Veterinary use of bacteriophage therapy in intensively-reared livestock. Virology Journal, 16, 155. https://doi.org/10.1186/s12985-019-1260-3
26. Goodridge, R. & Bisha, B. (2011). Phage-based biocontrol strategies to reduce foodborne pathogens in foods. Bacteriophage, 1(3), 130-137. https://doi.org/10.4161/bact.1.3.17629
27. Gordillo Altamirano, F., Forsyth, J. H., Patwa, R., Kostoulias, X., Trim, M., Subedi, D., Archer, S. K., Morris, F. C., Oliveira, C., Kielty, L., Korneev, D., O'Bryan, M. K., Lithgow, T. J., Peleg, A. Y., & Barr, J. J. (2021). Bacteriophage-resistant *Acinetobacter baumannii* are resensitized to antimicrobials. Nature Microbiology, 6(2), 157-161. https://doi.org/10.1038/s41564-020-00830-7
28. Grigore-Gurgu, L., Bucur, F. I., Mihalache, O. A., & Nicolau, A. I. (2024). Comprehensive review on the biocontrol of *Listeria monocytogenes* in food products. Foods, 13(5), 734. https://doi.org/10.3390/foods13050734
29. Holtappels, D., Fortuna, K., Lavigne, R., & Wagemans, J. (2021). The future of phage biocontrol in integrated plant protection for sustainable crop production. Current Opinion in Biotechnology, 68, 60-71. https://doi.org/10.1016/j.copbio.2020.08.016
30. Khatami, A., Foley, D. A., Warner, M. S., Barnes, E. H., Peleg, A. Y., Li, J., Stick, S., Burke, N., Lin, R. C. Y., Warning, J., Snelling, T. L., Tong, S. Y. C., & Iredell, J. (2022). Standardised treatment and monitoring protocol to assess safety and tolerability of bacteriophage therapy for adult and paediatric patients (STAMP study): Protocol for an open-label, single-arm trial. BMJ Open, 12(12), e065401. https://doi.org/10.1136/bmjopen-2022-065401
31. 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., et al. (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
32. König, H. (2025). The prospect of approved and commercially available phage therapeutics. International Journal of Antimicrobial Agents, 66(4), 107612. https://doi.org/10.1016/j.ijantimicag.2025.107612
33. Kwiatek, M., Parasion, S., & Nakonieczna, A. (2020). Therapeutic bacteriophages as a rescue treatment for drug-resistant infections -an in vivo studies overview. Journal of Applied Microbiology, 128(4), 985-1002. https://doi.org/10.1111/jam.14535
34. Le, T. S., Nguyen, T. H., Vo, H. P., Doan, V. C., Nguyen, H. L., Tran, M. T., Tran, T. T., Southgate, P. C., & Kürtböke, D. İ. (2018). Protective effects of bacteriophages against *Aeromonas hydrophila* causing motile *Aeromonas septicemia* (MAS) in striped catfish. Antibiotics, 7(1), 16. https://doi.org/10.3390/antibiotics7010016

35. Li, S., Dong, J., Zhou, S., Cheng, B., & Ai, X. (2026). Antibiotic alternative strategies against *Aeromonas hydrophila* infections: New trends and advances. Reviews in Aquaculture, 18(1), e70117. https://doi.org/10.1111/raq.70117

36. Loc-Carrillo, C., & Abedon, S. T. (2011). Pros and cons of phage therapy. Bacteriophage, 1(2), 111-114. https://doi.org/10.4161/bact.1.2.14590

37. Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage applications for food production and processing. Viruses, 10(4), 205. https://doi.org/10.3390/v10040205

38. Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629-655. https://doi.org/10.1016/S0140-6736(21)02724-0

39. Naghavi, M., Vollset, S. E., Ikuta, K. S., Swetschinski, L. R., Gray, A. P., Wool, E. E., Robles Aguilar, G., Mestrovic, T., Smith, G., Han, C., Hsu, R. L., Chalek, J., et al. (2024). Global burden of bacterial antimicrobial resistance 1990-2021: A systematic analysis with forecasts to 2050. The Lancet, 404(10459), 1199-1226. https://doi.org/10.1016/S0140-6736(24)01867-1

40. Nakayinga, R., Makumi, A., Tumuhaise, V., & Tinzaara, W. (2021). *Xanthomonas* bacteriophages: A review of their biology and biocontrol applications in agriculture. BMC Microbiology, 21(1), 291. https://doi.org/10.1186/s12866-021-02351-7

41. Nazir, A., Qi, C., Shi, N., Gao, X., Feng, Q., Qing, H., Li, F., & Tong, Y. (2023). Bacteriophages as a potential treatment option for antibiotic-resistant bacteria. Frontiers in Cellular and Infection Microbiology, 13, 1209947. https://doi.org/10.3389/fcimb.2023.1209947

42. strOnsea, J., Uyttebroek, S., Chen, B., Wagemans, J., Lood, C., Van Gerven, L., Spriet, I., Devolder, D., Debaveye, Y., Depypere, M., Dupont, L., De Munter, P., Peetermans, W. E., Vanden Berghe, W., Rottiers, P., Lavigne, R., & Metsemakers, W.-J. (2022). Efficacy of phage therapy in preclinical models of bacterial infection: A systematic review and meta-analysis. The Lancet Microbe, 3(10), e756–e764. https://doi.org/10.1016/S2666-5247(22)00288-9

43. Pereira, C., Duarte, J., Costa, P., Braz, M., & Almeida, A. (2022). Bacteriophages in the control of *Aeromonas* sp. in aquaculture systems: An integrative view. Antibiotics, 11(2), 163. https://doi.org/10.3390/antibiotics11020163

44. Pires, J., Santos, R., & Monteiro, S. (2023). Antibiotic resistance genes in bacteriophages from wastewater treatment plant and hospital wastewaters. Science of The Total Environment, 892, 164708. https://doi.org/10.1016/j.scitotenv.2023.164708

45. Pirnay, J. P., Djebara, S., Steurs, G., Griselain, J., Cochez, C., De Soir, S., Glonti, T., Spiessens, A., Vanden Berghe, E., Green, S., Wagemans, J., Lood, C., Schroven, E. et al. (2024). Personalized bacteriophage therapy outcomes for 100 consecutive cases: A multicentre, multinational, retrospective observational study. Nature Microbiology, 9(6), 1434-1453. https://doi.org/10.1038/s41564-024-01705-x

46. Rai, S., Kaur, B., Singh, P., Benjakul, S., Vijay Kumar Reddy, S., Nagar, V., & Tyagi, A. (2024). Perspectives on phage therapy for health management in aquaculture. Aquaculture International, 32(2), 1349-1393. https://doi.org/10.1007/s10499-023-01220-6

47. Samson, R., Rajput, V., Yadav, R., Shah, M., Dastager, S., Khairnar, K., & Dharne, M. (2023). Spatio-temporal variation of the microbiome and resistome repertoire along an anthropogenically dynamic segment of the Ganges River, India. Science of the Total Environment, 872, 162125. https://doi.org/10.1016/j.scitotenv.2023.162125

48. Sarrami, Z., Sedghi, M., & Mohammadi, I. (2022). Efficacy of phage therapy in poultry: A systematic review and meta-analysis. Poultry Science, 101(2), 101559. https://doi.org/10.1016/j.psj.2021.101559

49. Sati, H., Carrara, E., Savoldi, A., Hansen, P., Garlasco, J., Campagnaro, E., Boccia, S., Castillo-Polo, J. A., Magrini, E., Garcia-Vello, P., Wool, E., Gigante, et al. (2025). The WHO Bacterial Priority Pathogens List 2024: A prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. The Lancet Infectious Diseases, 25(9), e479–e493. https://doi.org/10.1016/S1473-3099(25)00118-5

50. Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., Barr, J. J., Reed, S. L., Rohwer, F., Benler, S., Segall, A. M., Taplitz, R., et al. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated

resistant *Acinetobacter baumannii* infection. Antimicrobial Agents and Chemotherapy, 61(10), e00954-17. https://doi.org/10.1128/AAC.00954-17

51. Sharma, R. S., Karmakar, S., Kumar, P., & Mishra, V. (2022). Application of filamentous phages in environment: A tectonic shift in the science and practice of ecorestoration. Ecology and Evolution, 9(4), 2263-2304. https://doi.org/10.1002/ece3.4990
52. Smith, H. W., Huggins, M. B., & Shaw, K. M. (1987). The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. Journal of General Microbiology, 133(5), 1111-1126. https://doi.org/10.1099/00221287-133-5-1111
53. Suh, G. A., Ferry, T., Abdel, M. P., Berbari, E., Cunningham, S. A., Costerton, J. W., Heim, C. E., Hooten, S., Karau, M. J., Khalil, A. M., Patel, R., Stevens, R. H., et al. (2023). Retrospective, observational study of 12 cases of expanded-access customized phage therapy: Production, characteristics, and clinical outcomes. Clinical Infectious Diseases, 77(8), 1079-1091. https://doi.org/10.1093/cid/ciad335
54. Sulakvelidze, A., Alavidze, Z., & Morris, J. G., Jr. (2001). Bacteriophage therapy. Antimicrobial Agents and Chemotherapy, 45(3), 649-659. https://doi.org/10.1128/AAC.45.3.649-659.2001
55. Svircev, A., Roach, D., & Castle, A. (2018). Framing the future with bacteriophages in agriculture. Viruses, 10(5), 218. https://doi.org/10.3390/v10050218
56. Tian, F., Li, J., Nazir, A., & Tong, Y. (2021). Bacteriophage. A promising alternative measure for bacterial biofilm control. Infection and Drug Resistance, 14, 205-217. https://doi.org/10.2147/IDR.S290093
57. Turner, D., Shkoporov, A. N., Lood, C., Millard, A. D., Dutilh, B. E., Alfenas-Zerbini, P., van Zyl, L. J., Aziz, R. K., Oksanen, H. M., Poranen, M. M., Kropinski, A. M., Barylski, J., Brister, J. R., Chanisvili, N., Edwards, R. A., Enault, F., Gillis, A., Knezevic, P., Krupovic, M., Adriaenssens, E. M. (2023). Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. Archives of Virology, 168(2), 74. https://doi.org/10.1007/s00705-022-05516-5
58. Wang, Y., & Yu, Y. (2025). Phage therapy as a revitalized weapon for treating clinical diseases. Microbiome Research Reports, 4(3), 35. https://doi.org/10.20517/mrr.2025.31
59. Welch, T. J. (2020). Characterization of a novel *Yersinia ruckeri* serotype O1-specific bacteriophage with virulence-neutralizing activity. Journal of Fish Diseases, 43(2), 285-293. https://doi.org/10.1111/jfd.13124
60. Wu, N., & Zhu, T. (2021). Phage products for fighting antimicrobial resistance. Microorganisms, 9(7), 1324. https://doi.org/10.3390/microorganisms9071324
61. Yang, Q., Le, S., Zhu, T., & Wu, N. (2023). Regulations of phage therapy across the world. Frontiers in Microbiology, 14, 1250848. https://doi.org/10.3389/fmicb.2023.1250848