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Precision Lytic Phage Cocktails to Decontaminate Multidrug-Resistant Organism Reservoirs in the Hospital Built Environment

Project Summary / Abstract

Hospital surfaces, sink traps, drains, and wastewater are durable environmental reservoirs of multidrug-resistant organisms (MDROs)—notably carbapenem-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*—that reseed patient-care areas and ignite healthcare-associated infection (HAI) outbreaks. Chemical disinfection is necessary but incomplete: biocide overuse can co-select for resistance, corrode surfaces, and spare biofilm-protected cells. Hospital wastewater further concentrates antibiotic-resistant bacteria (ARB) and a diverse pool of antibiotic resistance genes (ARGs) that conventional treatment does not fully clear (Canh et al., 2025). Lytic bacteriophages are a complementary, microbiome-sparing tool: they self-amplify on target cells, remain active against pan-resistant strains, do not corrode surfaces, and their depolymerases and endolysins can disrupt biofilm to reach tolerant cells. Phages have been formally proposed as routine environmental sanitizers for hospital hard surfaces and as a biocontrol layer for water systems (D'Accolti et al., 2021; Reyneke et al., 2024), and a sewage-isolated lytic *Pseudomonas* phage achieved ~95% surface killing of MDR *P. aeruginosa* and prevented biofilm with no virulence genes in its genome (Erdogdu & Ozbek, 2025). We will develop and rigorously evaluate **defined, sequence-verified wild-type lytic phage cocktails** against MDRO environmental reservoirs, with pre-specified quantitative success criteria and go/no-go milestones. **Aim 1** assembles and safety-characterizes a multi-receptor cocktail against the three reservoir species and quantifies surface decontamination and biofilm prevention on glass, plastic, and stainless steel under realistic soiling. **Aim 2** tests phage-augmented biocontrol of ARB and ARGs in bench- and pilot-scale wastewater microcosms, with explicit ARG monitoring in the bacteriophage fraction to ensure the process lowers, rather than mobilizes, resistance-gene burden. **Aim 3** pilots supervised application at non-patient hospital reservoir sites adjacent to standard cleaning, measuring load reduction, durability, phage-resistance dynamics, and operational feasibility. The work is deliberately proof-of-concept and pilot-scale, and is designed to generate the safety, efficacy, and process-design evidence required to justify future controlled hospital trials. By targeting MDROs at their environmental source, this research aims to help break the chain of transmission with a precision tool that complements—not replaces—chemical

disinfection.

Specific Aims

MDROs persist in hospital surfaces, drains, and wastewater despite chemical disinfection, and these reservoirs seed HAIs and outbreaks. Phages have been explicitly proposed as routine environmental sanitizers and as a biocontrol layer for water and wastewater, but the supporting evidence remains in vitro, microcosm-scale, and pilot-scale (D'Accolti et al., 2021; Reyneke et al., 2024; Erdogdu & Ozbek, 2025). **Central hypothesis:** a defined, safety-vetted multi-receptor lytic phage cocktail can reduce viable MDRO loads at environmental reservoir sites—on hard surfaces and in wastewater—without increasing ARG burden in the bacteriophage fraction. We will test this from bench to supervised hospital pilot, with quantitative, pre-registered success criteria.

Aim 1. Assemble and safety-characterize a defined multi-receptor lytic phage cocktail against MDRO reservoir species, and quantify surface decontamination. We will isolate and combine wild-type lytic phages targeting distinct receptors on *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*, sequencing each to exclude virulence genes, ARGs, and lysogeny markers. On glass, plastic, and stainless steel coupons that mimic hospital surfaces, we will quantify viable-load reduction and biofilm prevention by viable counts and confocal microscopy across contact times, titers, and organic-soil loads. *Success criterion:* $\geq 3 \log_{10}$ reduction of viable target cells versus carrier control on all three materials for ≥ 2 of 3 species, with a sequenced, virulence/ARG/lysogeny-free cocktail.

Aim 2. Evaluate phage-augmented wastewater biocontrol with explicit ARG safety monitoring. In bench- and pilot-scale wastewater microcosms representative of conventional activated sludge (CAS) and membrane bioreactor (MBR) conditions, we will spike MDRO targets, apply the Aim 1 cocktail, and quantify ARB reduction versus untreated controls. In parallel, we will quantify target-relevant ARGs in the bacteriophage fraction before and after treatment to confirm the process does not increase phage-fraction ARG burden (Canh et al., 2025). *Success criterion:* $\geq 2 \log_{10}$ reduction of viable target ARB versus control, with no statistically significant net increase in phage-fraction ARG copies attributable to the cocktail.

Aim 3. Pilot supervised phage application at hospital environmental reservoir sites. At non-patient sites (sink traps, drains, high-touch surfaces) in partner facilities, we will apply the cocktail adjunctive to standard cleaning and longitudinally sample to quantify MDRO load reduction versus standard cleaning alone, durability between applications, emergence of phage resistance in recovered isolates, and operational feasibility. *Success criterion:* a statistically significant adjunctive reduction in MDRO recovery at treated versus matched control sites, with a characterized resistance/durability profile and a documented feasibility assessment.

Impact: This proposal will generate the safety, efficacy, and process-design evidence required to advance phage environmental decontamination from concept toward controlled hospital trials, offering a precision, microbiome-sparing complement to chemical disinfection that targets MDRO transmission at its environmental source.

Significance

Hospital-associated MDROs impose a major burden of morbidity, mortality, and cost, and a principal driver is environmental persistence. Surfaces, drains, sink traps, and wastewater act as durable reservoirs of carbapenem-resistant *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*, repeatedly reseeding patient-care areas and igniting outbreaks. Chemical disinfection is necessary but incomplete: biocide overuse can co-select for resistance and degrade surfaces without removing biofilm, leaving dormant, tolerant cells that survive and regrow. Hospital wastewater is a particularly potent reservoir, concentrating ARB and a diverse pool of ARGs that conventional treatment does not fully clear; process-level monitoring shows that ARGs can remain detectable in the bacteriophage fraction even after CAS and MBR treatment, with MBR generally outperforming CAS for biological and gene-level removal (Canh et al., 2025). This underscores both the reservoir problem and the need for targeted biological control upstream of, and complementary to, conventional treatment.

Lytic phages address the specific failure modes of chemical disinfection. They self-amplify on their target, remain effective against pan-resistant strains, do not corrode surfaces or broadly harm beneficial flora, and their depolymerases and endolysins degrade biofilm exopolymer to reach cells that tolerate antibiotics and biocides. On this basis, phages and phage cocktails have been formally proposed as routine environmental sanitizers for persistently contaminated hospital hard surfaces and as a biocontrol layer for water systems (D'Accolti et al., 2021). Reviews further document phage biocontrol—and its limitations—across the water cycle, including wastewater (Reyneke et al., 2024). Direct experimental support exists: a sewage-isolated lytic *Pseudomonas* phage reduced MDR *P. aeruginosa* on glass, plastic, and metal surfaces simulating hospital environments and prevented biofilm formation, reaching ~95% killing at 8 h with no virulence genes detected in its genome (Erdogdu & Ozbek, 2025). That study, however, is a single-phage, single-species demonstration; the field still lacks a safety-vetted, multi-species cocktail evaluated under realistic soiling, in wastewater matrices, and in situ. This transmission science—biological control of environmental MDRO reservoirs—is directly aligned with NIAID's antimicrobial-resistance and HAI-prevention priorities, and is positioned for translational and public-health impact if efficacy and safety are confirmed.

Innovation

This project is innovative in four respects. First, it advances a **defined multi-receptor wild-type lytic**

cocktail simultaneously targeting the three principal MDRO reservoir species, rather than a single-phage/single-organism demonstration, exploiting narrow host range to spare benign environmental flora while using distinct receptor targeting to constrain the emergence of phage-insensitive mutants. Second, it pairs decontamination efficacy with **explicit ARG-burden safety monitoring** in the bacteriophage fraction of treated wastewater, directly confronting the recognized caveat that phages can, in principle, participate in horizontal gene transfer (transduction); process design and ARG safety are treated as primary endpoints, not afterthoughts (Canh et al., 2025; Reyneke et al., 2024). Third, it spans **two operationally distinct scales**—hard-surface decontamination and water/wastewater biocontrol—within one safety-vetted cocktail framework, reflecting how MDROs actually move through the hospital built environment (D'Accolti et al., 2021). Fourth, it emphasizes a **supervised, real-world hospital environmental pilot** at reservoir sites adjacent to standard cleaning, generating feasibility and resistance-durability data that in vitro studies cannot provide and that future controlled trials will require. Throughout, we deliberately use naturally lytic wild-type phages—the dominant approach for surface and wastewater work—rather than engineered or CRISPR-armed constructs, prioritizing a credible, near-term, regulatorily tractable path.

Approach

Rigor, reproducibility, and analysis (applies to all Aims)

All bacterial strains will be authenticated (species ID, resistance genotype/phenotype, whole-genome sequencing) and banked; phage stocks will be purified, titered, and endotoxin-characterized, with identity confirmed by sequencing. Experiments will use ≥ 3 independent biological replicates with pre-specified sample sizes powered ($\alpha = 0.05$, power ≥ 0.80) to detect the per-Aim log₁₀ effect thresholds defined in the Specific Aims, based on pilot variance estimates. Carrier/buffer and heat-inactivated-phage controls will be included throughout; coupon assays follow standardized disinfectant-efficacy formats with defined soil loads. Microscopy quantification and microbiological enumeration in Aims 1 and 3 will be performed blinded to treatment assignment where feasible, and analysts will be separated from sample preparation. Both biological replicates and, where relevant, multiple independent MDRO lineages per species will be tested to ensure findings are not strain-idiosyncratic. Primary outcomes, success thresholds, and statistical plans will be pre-registered before the corresponding pilot work begins. Data and phage genomes will be deposited in public repositories.

Aim 1 — Cocktail assembly, safety characterization, and surface decontamination

Rationale. Safe, effective environmental phage use requires lytic phages that broadly cover local MDRO reservoir strains, target distinct receptors to limit resistance, and carry no virulence or

resistance cargo. A single sewage-isolated *Pseudomonas* phage can achieve ~95% surface killing and prevent biofilm with a virulence-gene-free genome (Erdogdu & Ozbek, 2025); we extend this to a defined three-species cocktail evaluated under realistic conditions.

Experimental design. We will isolate wild-type lytic phages (e.g., from sewage and hospital wastewater) against banked and locally collected MDRO isolates of *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*. Candidates undergo host-range/lytic-spectrum testing across a curated isolate panel; whole-genome sequencing to confirm a strictly lytic lifestyle and the absence of virulence genes, ARGs, and lysogeny markers; and receptor-class assignment (e.g., via spontaneous-resistant-mutant cross-resistance and, where tractable, receptor-knockout testing) to select phages binding distinct receptors per species. We will formulate a multi-phage cocktail and quantify viable-load reduction and biofilm prevention on glass, plastic, and stainless steel coupons across contact times, titers, and soil/organic-load conditions, using viable counts and confocal microscopy. Time-kill and resistance-frequency assays will compare single phages versus the cocktail.

Expected outcomes. A defined, sequenced, safety-screened cocktail meeting the Aim 1 success criterion (≥ 3 log₁₀ reduction on all three materials for ≥ 2 of 3 species), with reduced resistant-mutant frequency for the cocktail versus single phages.

Potential pitfalls & alternatives. Narrow host range may leave coverage gaps; we will broaden the isolate panel and add phages, and pre-specify the local high-burden species as the priority if one proves refractory. Phage-insensitive mutants may arise; multi-receptor design and pairing with a disinfectant step mitigate this. Organic soil may blunt efficacy; we will titrate dose and contact time and report the soil-load dependence explicitly rather than only under clean conditions.

Aim 2 — Phage-augmented wastewater biocontrol with ARG safety monitoring

Rationale. Hospital wastewater concentrates ARB and ARGs that CAS and MBR do not fully clear, and ARGs can remain detectable in the bacteriophage fraction after treatment (Canh et al., 2025). Phage biocontrol is proposed across the water cycle (Reyneke et al., 2024), but because phages can in principle mobilize genes by transduction, efficacy must be demonstrated alongside ARG-burden safety.

Experimental design. Using bench- and pilot-scale microcosms representative of CAS and MBR conditions, we will spike MDRO targets, apply the Aim 1 cocktail, and quantify viable ARB reduction over time versus untreated controls. In parallel, we will quantify target-relevant ARGs in the bacteriophage fraction before and after treatment (qPCR and shotgun metagenomics) to determine whether phage augmentation lowers or raises phage-fraction ARG burden, and we will tune process parameters (dosing, staging, contact time, MBR vs CAS context) to favor target lysis while minimizing any ARG mobilization. Heat-inactivated-phage and no-phage arms isolate the cocktail's

specific contribution.

Expected outcomes. Demonstration that the cocktail meets the Aim 2 success criterion ($\geq 2 \log_{10}$ ARB reduction with no significant net increase in phage-fraction ARG copies attributable to the cocktail), plus candidate process parameters for a downstream biocontrol stage.

Potential pitfalls & alternatives. Complex matrices may reduce efficacy via adsorption to solids or protistan predation; we will optimize dose/timing and test pre-clarification. If any condition shows a cocktail-attributable ARG increase, we will reformulate (excluding transduction-prone phages, favoring strictly virulent, small-genome candidates) and adjust staging; the ARG-safety endpoint can therefore gate cocktail composition.

Aim 3 — Supervised hospital environmental pilot at reservoir sites

Rationale. In vitro and microcosm data cannot capture real-world durability, resistance emergence, and workflow feasibility. A supervised environmental pilot at reservoir sites—not patient treatment—is the appropriate next translational step.

Experimental design. At non-patient environmental locations (sink traps, drains, high-touch surfaces) within partner facilities, we will apply the cocktail adjunctive to standard cleaning and sample surfaces/drains longitudinally to quantify MDRO load reduction versus standard cleaning alone, durability between applications, emergence of phage resistance in recovered isolates (host-range re-testing), and operational feasibility (application method, staff workflow, materials compatibility). Sites will be matched (treated vs control), and sampling sites and schedules will be defined with infection-prevention staff. Analyses use mixed-effects models accounting for site and repeated measures.

Expected outcomes. Real-world evidence of adjunctive MDRO load reduction at reservoir sites (Aim 3 success criterion), characterization of durability and resistance dynamics, and a feasibility assessment to inform future controlled hospital trials.

Potential pitfalls & alternatives. Environmental variability and recontamination may obscure effects; matched control sites, longitudinal sampling, and adequate site replication address this. Regulatory/biosafety review may constrain application; we will engage institutional biosafety and infection-prevention committees early and limit scope to environmental (non-patient) use. If on-site application is constrained, we will expand realistic ex situ microcosms using site-derived materials and strains.

Timeline

[ILLUSTRATIVE] Months 1–12: Aim 1 phage isolation, sequencing/safety screening, receptor assignment, cocktail formulation, surface assays; pre-registration of Aim 1/2 outcomes.

[ILLUSTRATIVE] Months 10–24: Aim 2 bench microcosms and ARG monitoring.

[ILLUSTRATIVE] Months 20–36: Aim 2 pilot-scale runs and process-parameter tuning.

[ILLUSTRATIVE] Months 24–48: Aim 3 supervised environmental pilot, longitudinal sampling, analysis. [ILLUSTRATIVE] Months 48–60: integrated analysis, resistance/durability synthesis, dissemination, and planning for controlled trials. **Go/no-go gates:** end of Month 12 (Aim 1 success criterion met before scale-up); end of Month 24 (Aim 2 efficacy + ARG-safety met before pilot-scale and before in situ work). Aims overlap so that cocktail refinement feeds forward.

Budget Justification (modular R01-style sketch)

This modular budget is an [ILLUSTRATIVE] sketch only. **Personnel:** [ILLUSTRATIVE] PD/PI (phage biology / environmental microbiology) at [ILLUSTRATIVE] 3.0 calendar months; Co-I (wastewater / environmental engineering) at [ILLUSTRATIVE] 1.8 calendar months; Co-I (hospital infection prevention / clinical microbiology) at [ILLUSTRATIVE] 1.2 calendar months; [ILLUSTRATIVE] 2 postdoctoral scientists and [ILLUSTRATIVE] 1–2 technicians/graduate students for isolation, sequencing, microcosm work, and pilot sampling. **Other costs:** sequencing and bioinformatics; confocal microscopy; surface coupons and biofilm assays; bench/pilot wastewater microcosm equipment and consumables; qPCR/metagenomics for ARG monitoring; environmental sampling supplies; biosafety and regulatory compliance; statistical support; publication and travel. Requested at [ILLUSTRATIVE] \$250,000 direct costs/year across [ILLUSTRATIVE] 5 years, consistent with modular R01 increments of [ILLUSTRATIVE] \$25,000, with facilities/administrative costs per the institution's federally negotiated rate.

Vertebrate Animals

Not applicable. This project involves environmental surfaces, wastewater microcosms, and non-patient hospital environmental sites; no vertebrate animal work is proposed.

Human Subjects / Clinical Trial

This project does not treat patients or conduct human-subjects clinical intervention; Aim 3 is environmental decontamination at non-patient sites. We anticipate a **non-human-subjects-research determination** for environmental sampling, and will obtain formal institutional confirmation; should

any human-adjacent or identifiable data arise, IRB review will be obtained before collection. No investigational phage is administered to humans in this work, so it does not constitute an NIH-defined clinical trial. For completeness: if future work advances to clinical or patient-environment intervention, investigational phage products in the US are typically pursued through FDA under an emergency/expanded-access IND (eIND) route with full IRB oversight; such activities are outside the scope of this proof-of-concept and pilot proposal but are anticipated in planning for subsequent controlled trials.

Team & Environment

This work requires a multidisciplinary team; roles below specify the **required expertise** to be filled with named investigators and institutions. **Program Director/Principal Investigator [NAME, INSTITUTION]:** phage biology and environmental microbiology; overall direction and Aim 1. **Co-Investigator [NAME, INSTITUTION]:** environmental/wastewater engineering for Aim 2 microcosms and ARG-fraction monitoring (CAS/MBR process expertise, quantitative ARG metagenomics). **Co-Investigator [NAME, INSTITUTION]:** hospital infection prevention and clinical microbiology for Aim 3 (environmental sampling, MDRO surveillance, anti-biofilm phage application). **Consultants [NAME, INSTITUTION]:** phage genomics/biosafety; FDA regulatory strategy. **Environment:** BSL-2 microbiology and molecular laboratories; sequencing and confocal-imaging cores; bench- and pilot-scale wastewater facilities; and a partner hospital with infection-prevention and institutional biosafety committees enabling supervised environmental sampling. Letters of support from the partner hospital and wastewater facility will document access, materials compatibility, and committee engagement.

References

1. D'Accolti M, Soffritti I, Mazzacane S, Caselli E. Bacteriophages as a Potential 360-Degree Pathogen Control Strategy. *Microorganisms*. 2021;9(2):261. <https://doi.org/10.3390/microorganisms9020261>
2. Erdogdu B, Ozbek T. Characterization of *Pseudomonas* phage MME: a novel tool for combatting multidrug-resistant *Pseudomonas aeruginosa* and disinfection. *Journal of Applied Microbiology*. 2025;136(3):lxaf052. <https://doi.org/10.1093/jambio/lxaf052>
3. Reyneke B, Havenga B, Waso-Reyneke M, Khan S, Khan W. Benefits and Challenges of Applying Bacteriophage Biocontrol in the Consumer Water Cycle. *Microorganisms*. 2024;12(6):1163. <https://doi.org/10.3390/microorganisms12061163>
4. Canh VD, Singhopon T, Kasuga I, Katayama H. Removal of viruses and antibiotic resistance genes in bacteriophage fraction by conventional activated sludge (CAS) and membrane bioreactor treatment (MBR) systems. *Science of the Total Environment*. 2025;989:179787.

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<https://phagecocktails.com/grant/steal/hospital-surface-decontamination>