Phage-Based Source Control of Antimicrobial-Resistant *Salmonella*, *Vibrio*, and *Aeromonas* Across Poultry and Aquaculture Production Systems

Project Summary / Abstract

Intensive poultry and warm-water aquaculture are among the largest on-farm reservoirs feeding the antimicrobial-resistance (AMR) crisis. Broiler flocks shed nontyphoidal *Salmonella* into the food chain, while shrimp and finfish operations rely on antibiotics to manage *Vibrio* (acute hepatopancreatic necrosis disease, luminous vibriosis) and *Aeromonas* (motile aeromonad septicemia, furunculosis). Because these are open, high-volume systems, prophylactic antibiotic use both selects for resistant strains and contaminates water and meat. This project develops a single, cross-commodity platform for **pre-harvest "source control"**: rationally composed lytic bacteriophages and cocktails delivered in feed, drinking water, and tank/pond water to drive pathogen loads below disease thresholds without expanding the resistome. Lytic phages adsorb to strain-specific surface receptors (LPS O-antigen, flagella, outer-membrane proteins) and lyse their host, so multi-phage cocktails are needed to cover relevant serovars and to suppress resistant mutants. The approach is regulatorily mature: an EU-authorized anti-*Salmonella* poultry feed additive and a U.S. FDA-GRAS *Salmonella* food cocktail are marketed, and the first commercial aquaculture phage product is dosed into salmon-farm water. We will (1) assemble and characterize host-range-matched, lysogeny/virulence/AMR-gene-free phages and cocktails against *Salmonella enterica*, *Vibrio* spp., and *Aeromonas hydrophila*; (2) optimize farm-relevant delivery and quantify efficacy in controlled in-vivo challenge models in broilers, shrimp postlarvae, and finfish; and (3) measure AMR-reservoir and antibiotic-sparing outcomes, build a refreshable regional phage-bank framework, and deliver producer-facing extension guidance. The work advances the USDA-NIFA AFRI One-Health priority of reducing the farm-to-fork AMR reservoir with a deployable, antibiotic-sparing biocontrol tool.

Specific Aims

Antimicrobial resistance arising from food-animal production is a defining One-Health threat, and

USDA-AFRI prioritizes interventions that reduce on-farm antimicrobial use and the AMR reservoir. Bacteriophages are uniquely suited to pre-harvest **source control**: self-amplifying, water- and feed-deliverable, commensal-sparing, and free of antibiotic-resistance genes when properly screened. Controlled in-vivo challenge studies in broilers, shrimp, and farmed fish report reduced pathogen burden and survival benefit, and authorized commercial products prove the regulatory and operational path. What is missing is a harmonized, cross-commodity U.S. platform pairing cocktail design with farm-relevant delivery, explicit AMR-sparing endpoints, and producer adoption pathways.

Aim 1 — Build and characterize host-range-matched, therapeutically curated phages and cocktails. Isolate/curate lytic phages; whole-genome screen to exclude lysogeny, virulence, and AMR genes; map host range across *S.* Enteritidis/Typhimurium/Kentucky, *V. harveyi/parahaemolyticus/diabolicus*, and *A. hydrophila*; compose preparations that suppress resistant mutants in vitro.

Aim 2 — Optimize farm-relevant delivery and demonstrate efficacy in controlled in-vivo challenge models. Formulate feed top-coats, acid/gut-stable encapsulation, and water dosing; quantify pathogen reduction, mortality, histopathology, and growth performance in broiler, shrimp-postlarval, and finfish challenge trials.

Aim 3 — Quantify AMR-reservoir and antibiotic-sparing impact, define a refreshable regional phage-bank framework, and transfer it to stakeholders. Measure shedding/water-column loads, resistant-subpopulation dynamics, and modeled antibiotic displacement; establish bank matching/refresh criteria; deliver extension-ready protocols.

Impact. A validated, antibiotic-sparing, cross-commodity phage platform that shrinks the AMR reservoir at its largest agricultural source, with a clear translation path to USDA/FDA-recognized on-farm use.

Significance

Livestock and aquaculture are open production systems in which blanket antibiotic prophylaxis simultaneously selects for resistant strains and contaminates water and meat. Source control — knocking pathogen loads down at the pre-harvest stage *without* adding selective pressure — is therefore the highest-leverage intervention. Phages meet that bar: self-amplifying, water- and feed-deliverable, commensal-sparing, and (when properly screened) carrying no antibiotic-resistance genes, so they reduce pathogen burden and antibiotic demand rather than adding to the resistome. The evidence base is now substantive. In broilers, a feed-delivered *Salmonella* phage cocktail significantly reduced colonization in challenged birds (Thanki et al., 2023), and a lyophilized cocktail reduced multidrug-resistant *Salmonella* burden in broilers (Nabil et al., 2024) — the latter directly targeting

resistant organisms. In aquaculture, a broad-host-range phage cocktail selectively and effectively eliminated *Vibrio* from the shrimp aquaculture environment (Lomelí-Ortega et al., 2022), and a novel lytic phage (AhFM11) was an effective therapy against hypervirulent *A. hydrophila* (Muliya Sankappa et al., 2024). Crucially, this indication has already crossed into authorized commercial use in both sectors, so a positive U.S. dataset has a credible adoption channel rather than an open-ended regulatory horizon. By unifying *Salmonella*, *Vibrio*, and *Aeromonas* under one design-and-delivery framework with explicit AMR-sparing endpoints and an extension pathway, this project produces evidence and tools that USDA, producers, and the feed industry can act on.

Innovation

- **Cross-commodity platform, not a single product.** Most prior work targets one pathogen in one species. We apply a shared pipeline — curate, screen out lysogeny/virulence/AMR genes, host-range match, suppress resistant mutants — to poultry *Salmonella*, shrimp/finfish *Vibrio*, and finfish *Aeromonas*.
- **Delivery-mode engineering for real farms.** We treat formulation (feed top-coat, acid/gut-stable encapsulation, direct tank/pond water dosing during transport, vaccination, and grading windows) as a primary experimental variable rather than an afterthought.
- **AMR-sparing as a measured endpoint.** Beyond log-reductions and survival, we quantify resistant-subpopulation dynamics and modeled antibiotic displacement — the outcomes a One-Health funder weighs.
- **Refreshable regional phage-bank framework with extension transfer.** Because phage activity is strain-restricted, we build and disseminate criteria for matching and refreshing banks against locally emerging strains, anticipating the standard-practice future the sector is moving toward.

Approach

Aim 1 — Host-range-matched, therapeutically curated phages and cocktails

Rationale. Lytic phages are strain/serovar-restricted because they recognize specific receptors (LPS O-antigen, flagella, outer-membrane proteins); cocktails cover relevant serovars and suppress resistant mutants, and therapeutic use demands phages free of lysogeny, virulence, and AMR genes.

Experimental design. Isolate and curate lytic phages against panels of *S.* Enteritidis/Typhimurium/Kentucky, *V. harveyi/parahaemolyticus/diabolicus*, and *A. hydrophila*. Perform whole-genome sequencing and bioinformatic screening to exclude integrases/repressors, known virulence factors, and AMR determinants. Quantify host range and efficiency-of-plating across

each panel; assemble candidate cocktails (e.g., a multi-phage *Salmonella* set; broad-host-range *Vibrio* pairs; an *Aeromonas* preparation built around a curated lytic phage such as AhFM11-type isolates) and test suppression of resistant-mutant outgrowth in vitro. Target composition: ~[ILLUSTRATIVE] 3–4 phages per pathogen.

Expected outcomes. ≥[ILLUSTRATIVE] one validated, fully sequenced, lysogeny/virulence/AMR-gene-free preparation per pathogen with documented panel coverage and resistant-mutant suppression.

Pitfalls & alternatives. Narrow host range or rapid resistance: broaden phage diversity, add receptor-diverse members, and evaluate phage-antibiotic synergy as a research-stage backstop (noting it remains largely preclinical in this sector).

Aim 2 — Farm-relevant delivery and in-vivo efficacy

Rationale. Documented effects in this sector — reduced cecal/tissue and water-column loads, lower mortality, reduced histopathology, improved growth — depend on getting viable phage to the colonization site via feed, water, or direct dosing during high-risk windows.

Experimental design. Develop feed top-coating, acid/gut-stable encapsulation (lyophilized formats included, per Nabil et al., 2024), and water dosing. Run controlled challenge trials: (a) broilers challenged with *Salmonella*, phage in feed/water (modeled on Thanki et al., 2023; Nabil et al., 2024); (b) shrimp postlarvae with *Vibrio*, phage dosed into rearing water (modeled on Lomelí-Ortega et al., 2022); (c) finfish (e.g., carp/tilapia/catfish) challenged with *A. hydrophila*, phage therapy (modeled on Muliya Sankappa et al., 2024). Trials use randomized allocation, blinded scoring of histopathology/mortality, and pre-specified biological variables. Endpoints: pathogen load (cecal/tissue/water column), mortality/survival, histopathology, growth. Group sizes ~[ILLUSTRATIVE] 20–40 animals/arm across [ILLUSTRATIVE] 3 arms (challenge-only, challenge+phage, untreated) with [ILLUSTRATIVE] 2 independent replicates per species; numbers set by a priori power analysis.

Expected outcomes. [ILLUSTRATIVE] ≥2–3 log reductions in pathogen burden and significant survival/performance benefit in at least one optimized delivery mode per species.

Pitfalls & alternatives. Gastric/environmental phage loss or titer decay: shift to encapsulation, increase dose/frequency, or re-time dosing to the high-risk window; if one species model underperforms, prioritize the two strongest for depth.

Aim 3 — AMR-reservoir impact, phage-bank framework, and extension transfer

Rationale. The funded goal is reservoir reduction and antibiotic sparing, so we measure these directly and move results to practitioners.

Experimental design. From Aim 2 cohorts, quantify shedding/water-column loads over time, track resistant subpopulations, and model antibiotic displacement under realistic prophylaxis scenarios. Define and pilot phage-bank matching/refresh criteria against newly collected regional isolates. Through an integrated extension component, co-develop dosing and bank-refresh protocols with poultry and aquaculture producers and Extension specialists, and disseminate via fact sheets and field-day demonstrations.

Expected outcomes. Quantified reservoir reduction, no phage-attributable expansion of resistant subpopulations, modeled antibiotic-use reduction, a documented bank-refresh standard operating procedure, and producer-ready guidance.

Pitfalls & alternatives. Phage resistance: rotate/refresh bank members and report kinetics; emphasize that, unlike antibiotics, properly screened phages add no AMR genes.

Timeline

[ILLUSTRATIVE] Total duration: 4 years. **Yr 1:** Aim 1 isolation/screening/cocktail assembly. **Yr 2:** Aim 2 formulation + first broiler and shrimp trials. **Yr 3:** Aim 2 finfish trials + replication; begin Aim 3 sampling. **Yr 4:** Aim 3 analysis, phage-bank framework, extension materials, and translation/regulatory dossier.

Budget Justification

[ILLUSTRATIVE] figures only; structured per the USDA-NIFA SF-424 R&R categories (not an NIH modular budget) and within the relevant AFRI program funding limit. Requested ~[ILLUSTRATIVE] $325,000 total costs/year × 4 years. **Senior/Key Personnel:** PI (2.4 cal-mo) plus co-PDs in poultry science and aquaculture/aquatic animal health, and an Extension specialist. **Other Personnel:** 2 postdocs (phage genomics; formulation), 1 technician, animal-facility staff, undergraduate trainees (workforce development). **Equipment/Supplies:** sequencing, phage-production and microbiology consumables, encapsulation materials, challenge-organism panels. **Animal costs:** broiler, shrimp-postlarval, and finfish challenge facilities and per-diem. **Other Direct Costs:** bioinformatics, biosafety, extension/outreach, travel, publication. Indirect costs per the institution's federally negotiated rate, subject to AFRI indirect-cost provisions.

Vertebrate Animals

Applicable. The project includes controlled challenge studies in broiler chickens and finfish (vertebrates); shrimp postlarvae are invertebrates and outside IACUC scope. All vertebrate work follows an IACUC-approved protocol covering justification of species and numbers, minimization of pain/distress, humane endpoints, and euthanasia consistent with AVMA guidelines. Group sizes [ILLUSTRATIVE] and replicate numbers [ILLUSTRATIVE] are statistically justified by power analysis and minimized accordingly. Biocontainment follows institutional biosafety requirements for challenge organisms.

Human Subjects / Clinical Trial

Not applicable. This is a pre-harvest agricultural biocontrol project with no human subjects and no clinical trial; no IRB review is required. The relevant oversight pathway is animal-feed/food and aquaculture biocontrol regulation (USDA-NIFA program scope; FDA CVM/GRAS precedent such as the existing GRAS *Salmonella* food cocktail; EU feed-additive precedent), addressed by the translation/dossier work in Aim 3.

Team & Environment

The team unites phage biology/genomics, poultry science, and aquatic animal health, with an Extension specialist and institutional cores for sequencing, bioinformatics, formulation, and approved animal-challenge/biocontainment facilities. The landscape includes directly relevant precedents the project will engage or build upon: an EU-authorized anti-*Salmonella* poultry feed additive, an FDA-GRAS *Salmonella* food/poultry cocktail, the first commercial aquaculture phage (anti-*Yersinia* in salmon), USDA-ARS aquatic-animal-health anti-*Aeromonas* catfish phage work, and academic in-feed broiler *Salmonella* phage programs. This positions the project for credible translation toward USDA/FDA-recognized on-farm use.

References

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