Phage-Antibiotic Synergy Against Recurrent Biliary-Stent Infection: A Strain-Matched, Depolymerase-Armed Lytic Cocktail Targeting *Enterococcus* and *Enterobacteriaceae* in the Obstructed Biliary Tree

Project Summary / Abstract

Plastic and metal biliary stents fail because enteric bacteria colonize the device, build occluding biofilm "sludge," and trigger recurrent cholangitis. In the largest sonication series of explanted endoprostheses (343 stents), *Enterococcus* species were the single most prevalent colonizer (~22%) and *Enterobacteriaceae* such as *Klebsiella* spp. accounted for ~10%; 20% of stents occluded, and 35% of occlusions caused cholangitis or cholestasis—directly linking these taxa and their biofilm to device failure (Schneider 2014). These organisms are increasingly multidrug-resistant (vancomycin-resistant *Enterococcus* [VRE]; ESBL/carbapenem-resistant *Klebsiella*), and antibiotics penetrate biofilm and obstructed bile poorly, so infection recurs until the device is exchanged. Lytic bacteriophages are mechanistically suited to this niche: they self-amplify at the infection site, kill antibiotic-resistant strains, and degrade biofilm matrix via depolymerases. Hepatobiliary precedent now exists—an oral/IV anti-*Klebsiella pneumoniae* lytic phage cocktail suppressed the pathogen and attenuated biliary inflammation in a sclerosing-cholangitis model without off-target dysbiosis (Ichikawa 2023); phages targeting cytolytic *Enterococcus faecalis* abolished ethanol-induced liver injury in humanized mice (Duan 2019); and an IV two-phage magistral preparation cleared a VRE *E. faecium* abdominal infection in a one-year-old liver-transplant recipient, with vancomycin-susceptible isolates emerging from resistant progenitors (Paul 2021). This exploratory R21 will (1) build a strain-matched, depolymerase-bearing *Enterococcus*/*Enterobacteriaceae* phage cocktail against bile-stent isolates and quantify biofilm killing on stent material, and (2) test phage-antibiotic synergy (PAS) and antibiotic re-sensitization of MDR isolates on device biofilm in vitro. A contingent, go/no-go-gated proof-of-concept Aim 3 tests intrabiliary/systemic delivery in a catheter-based biliary-infection model. The work is deliberately preclinical, matched to the current evidence base, and designed to de-risk a future expanded-access/IND-enabled trial of phage-augmented stent management.

Specific Aims

Recurrent biliary-stent infection is a high-burden NIDDK hepatobiliary problem with no durable solution short of repeated device exchange. The dominant colonizers—*Enterococcus* and *Enterobacteriaceae*—are increasingly drug-resistant, and antibiotics fail because they cannot penetrate biofilm or obstructed bile (Schneider 2014). We hypothesize that a personalized, depolymerase-armed lytic phage cocktail, delivered into the biliary tree and paired with antibiotics, can clear device biofilm and break the recurrence cycle. This R21 is scaled to generate the missing biofilm and synergy data and a delivery rationale—not to leapfrog them.

Aim 1. Assemble and characterize a strain-matched lytic phage cocktail against bile-stent *Enterococcus* and *Enterobacteriaceae* isolates, with activity on device biofilm. Host-range phages against a panel of clinical *E. faecium/faecalis* and *Klebsiella/E. coli* isolates; prioritize depolymerase-bearing phages; quantify biofilm reduction on plastic and metal stent coupons. *Milestone/go-no-go:* a defined ≤4-phage-per-arm cocktail covering ≥70% of the isolate panel by efficiency-of-plating and achieving ≥2-log CFU biofilm reduction on coupons.

Aim 2. Determine phage-antibiotic synergy and antibiotic re-sensitization against MDR isolates on device biofilm in vitro. Test cocktail-plus-antibiotic combinations (e.g., daptomycin/ampicillin for *Enterococcus*; a clinically relevant β-lactam/carbapenem for *Klebsiella*) for synergy and for restoration of susceptibility in phage-resistant escape mutants. *Milestone/go-no-go:* quantified synergy (≥2-log improvement vs. best single agent) for ≥1 antibiotic per arm, and documented escape-mutant fitness/resistance cost (e.g., VRE re-sensitized toward vancomycin, as observed clinically in Paul 2021).

Aim 3 (contingent; gated on Aims 1–2 milestones). Establish proof-of-concept efficacy and safety of intrabiliary/systemic phage delivery in a catheter-based biliary-infection model. Compare cocktail (intrabiliary and/or IV) ± antibiotic vs. antibiotic alone vs. vehicle for biliary/hepatic bacterial burden, device biofilm, hepatobiliary inflammation, phage pharmacokinetics, and 16S-based microbiota disruption.

Impact: Success yields a defined, reproducible phage cocktail and a delivery/dosing rationale for a future eIND-enabled trial of phage-augmented biliary-stent management—converting a last-resort device infection toward a curable one.

Significance

Biliary stents are placed in hundreds of thousands of patients annually for malignant and benign obstruction, and stent occlusion is a defining complication. The mechanism is microbiological: bile

stasis allows enteric bacteria to adhere to the device, secrete extracellular matrix, and form "sludge" that occludes the lumen and seeds ascending cholangitis or cholestasis. In the largest sonication series of explanted endoprostheses, *Enterococcus* species were the most prevalent colonizer (~22%) and *Klebsiella* species ~10%; sludge formation was associated with longer indwelling time and sideholes, 20% of stents occluded, and 35% of occlusions produced cholangitis or cholestasis—directly linking these taxa and their biofilm to stent failure (Schneider 2014). Because these are precisely the organisms now most affected by acquired resistance—VRE, ESBL- and carbapenem-resistant *Klebsiella*—each recurrence is harder to treat, and systemic antibiotics are doubly handicapped by poor penetration into biofilm and into obstructed, poorly perfused bile. The current standard, repeated endoscopic stent exchange, is costly, procedurally risky, and does not address the underlying colonization.

Phage therapy targets this gap with a fundamentally different mechanism, and the hepatobiliary relevance is no longer hypothetical. Ichikawa and colleagues detected abundant pathological *K. pneumoniae* (and *Enterococcus gallinarum*) in primary sclerosing cholangitis patients and showed that a lytic *K. pneumoniae* phage cocktail—given orally and intravenously—suppressed the pathogen and attenuated liver inflammation in hepatobiliary-injury-prone mice without off-target dysbiosis (Ichikawa 2023). On the *Enterococcus* side, phages targeting cytolysin-positive *E. faecalis* reduced hepatic cytolysin and abolished ethanol-induced liver injury in humanized mice (Duan 2019). Closest to the abdominal/transplant setting, an IV two-phage magistral preparation cleared a VRE *E. faecium* abdominal infection in a one-year-old liver-transplant recipient (with underlying biliary atresia), and vancomycin-susceptible isolates emerged from resistant progenitors during therapy (Paul 2021). Together these establish biological plausibility for the two pathogen arms that dominate stent biofilm —yet no registered controlled trial for cholangitis or biliary-stent infection has reported results, leaving a clear, fundable exploratory gap that this R21 addresses. The work aligns squarely with NIDDK's mission in digestive and liver diseases and with the exploratory intent of the R21 mechanism.

Innovation

This proposal is innovative in target, mechanism, and translational framing. (1) *Indication:* to our knowledge the first dedicated preclinical program aimed specifically at biliary-stent biofilm rather than parenchymal liver disease, mapping the phage approach onto the precise organisms (*Enterococcus*, *Enterobacteriaceae*) shown to colonize explanted devices (Schneider 2014). (2) *Dual-pathogen, depolymerase-armed cocktail:* rather than a single-organism preparation, we co-target the two dominant colonizers and prioritize depolymerase-bearing phages to strip the exopolysaccharide shield on device biofilm, exposing persister cells to phage and antibiotic. (3) *Synergy and re-sensitization as design goals:* we exploit phage-antibiotic synergy and the evolutionary trade-off

whereby phage pressure can drive VRE back toward vancomycin susceptibility—using resistance evolution as a therapeutic lever, not merely a liability; this VRE-to-susceptible reversion was observed clinically in a liver-transplant recipient (Paul 2021). (4) *Microbiome sparing:* strain-specific receptor binding lets a matched cocktail lower the pathogen without broad dysbiosis, an advantage demonstrated for an oral anti-*Klebsiella* cocktail (Ichikawa 2023). (5) *Delivery framing toward the device:* by testing intrabiliary-relevant and systemic routes against stent material, the work points toward the long-term goal of a phage-flushed or phage-eluting stent matched to the patient's isolate at ERCP. We are explicit that current human evidence is preclinical plus compassionate-use case reports; this R21 is scaled to generate the missing biofilm and in vivo data, not to leapfrog them.

Approach

Aim 1 — Strain-matched lytic cocktail with anti-biofilm activity on stent material

Rationale. Phages bind strain-specific surface receptors (capsular polysaccharide, LPS, pili), so efficacy requires matching phages to the patient's isolates; depolymerase activity is what makes phages credible against the exopolysaccharide matrix of device sludge. *Enterococcus* (~22%) and *Klebsiella* (~10%) dominate explanted-stent colonizers (Schneider 2014), defining our target panel.

Experimental design. We will assemble a biobank of *E. faecium/faecalis* and *K. pneumoniae/E. coli* isolates from biliary stents/bile, characterized for species, capsular type, and antibiotic-resistance profile (including VRE and ESBL/carbapenem-resistant strains). Candidate lytic phages from collaborating banks and environmental isolation will be screened by host-range (spot and efficiency-of-plating) assays; depolymerase activity will be scored by halo formation. We will build [ILLUSTRATIVE] 2–4-phage cocktails per pathogen arm selected for complementary receptors and minimal cross-resistance, then quantify biofilm killing on plastic and metal stent coupons (CFU, confocal/biomass), including regrowth and resistance-frequency assays.

Expected outcomes & success criteria. A defined, reproducible dual-pathogen cocktail with documented depolymerase activity that covers ≥70% of the isolate panel and achieves ≥2-log CFU biofilm reduction on coupons with suppressed regrowth.

Potential pitfalls & alternative approaches. Narrow host range is the central risk; we mitigate with a personalized/banked design and periodic cocktail updating. If depolymerase-bearing phages are scarce for a given capsular type, we will prioritize receptor diversity and combine with sub-inhibitory matrix-disrupting conditions, and note purified endolysins/engineered phages as a longer-term angle (not a deliverable here).

Aim 2 — Phage-antibiotic synergy and antibiotic re-sensitization in MDR isolates

Rationale. Antibiotics fail in biofilm and obstructed bile, but combining lytic phage with antibiotics is hypothesized to both improve killing and restore drug activity. This rationale is grounded in the broader PAS literature and, directly, in the clinical observation that phage pressure drove a VRE *E. faecium* population back toward vancomycin susceptibility during therapy (Paul 2021). We test, rather than assume, synergy on device biofilm.

Experimental design. Using the Aim 1 cocktail, we will test phage-plus-antibiotic combinations (e.g., daptomycin and ampicillin for *Enterococcus*; clinically relevant β-lactam/carbapenem regimens for *Klebsiella*) in planktonic and stent-biofilm models, quantifying synergy versus either agent alone (checkerboard/time-kill on biofilm; Bliss/FIC framing). Phage-resistant escape mutants will be isolated and re-tested for antibiotic MICs to detect re-sensitization and for changes in capsule/virulence-associated phenotypes. [ILLUSTRATIVE] dosing sequences and ratios will be compared to define an optimal PAS schedule.

Expected outcomes & success criteria. Quantified synergy (≥2-log improvement vs. best single agent) for at least one antibiotic per pathogen arm, plus evidence that phage escape carries a resistance- or virulence-cost (e.g., VRE re-sensitized toward vancomycin), yielding a PAS dosing rationale for Aim 3.

Potential pitfalls & alternative approaches. Synergy may be antibiotic- and strain-dependent; we will screen several drug classes and report antagonism where it occurs. If re-sensitization is inconsistent, the program still advances on the synergy/biofilm-penetration benefit alone.

Aim 3 (contingent; gated on Aims 1–2 milestones) — Proof-of-concept efficacy and safety of intrabiliary/systemic delivery in vivo

Rationale. Oral and IV phage dosing suppressed hepatobiliary *K. pneumoniae* and reduced inflammation in a cholangitis model (Ichikawa 2023); phages against cytolytic *E. faecalis* abolished ethanol-induced liver injury in humanized mice (Duan 2019); and IV phage cleared a VRE abdominal infection clinically (Paul 2021). These support testing delivery against a stent-associated biliary infection. *Aim 3 proceeds only if Aim 1 and Aim 2 milestones are met*, ensuring R21 resources are committed to a validated cocktail.

Experimental design. In a catheter-based biliary-infection model colonized with a target isolate, we will compare cocktail (intrabiliary-relevant and/or IV) versus antibiotic alone versus vehicle, with PAS arms informed by Aim 2. Primary readout: biliary/hepatic bacterial burden and device biofilm; secondary: hepatobiliary inflammation/histology, phage pharmacokinetics, and 16S-based microbiota

disruption to test microbiome sparing. Group sizes [ILLUSTRATIVE] will be powered for the primary burden endpoint with biostatistical input.

Expected outcomes & success criteria. Demonstration that phage (especially with PAS) lowers biliary bacterial burden, biofilm, and inflammation more than antibiotic alone, without major off-target microbiota disruption—directly de-risking a future clinical study.

Potential pitfalls & alternative approaches. Biliary anatomy and phage clearance may limit residence time; we will compare routes and dosing intervals and consider repeat instillation. A faithful fully-stented rodent model may be infeasible within R21 scope; we therefore pre-specify the catheter-based biliary-infection surrogate as the primary in vivo system and clearly bound the translational claim.

Timeline

[ILLUSTRATIVE] Total period 2 years. **Months 1–9:** isolate biobanking, phage host-range/depolymerase screening, cocktail assembly (Aim 1). **Months 6–15:** stent-coupon biofilm and PAS/re-sensitization studies (Aims 1–2); Aim 1/2 go-no-go review at ~Month 12. **Months 12–24:** contingent in vivo efficacy/safety and microbiota analysis (Aim 3), data integration, and clinical-trial planning. Aims overlap intentionally so biofilm/PAS results feed the animal dosing design.

Budget Justification

[ILLUSTRATIVE] We request up to $275,000 in total direct costs across the 2-year project period (with no more than $200,000 in any single year), consistent with standard R21 limits. **Personnel:** PI/MPI effort to direct phage and hepatobiliary components; a microbiologist/phage scientist for isolation, host-range, and cocktail work; a research technician for biofilm/coupon assays; statistical and animal-core support. **Supplies:** clinical isolates and phage banking, media and molecular reagents, stent/coupon materials, antibiotics for PAS, confocal/sequencing consumables. **Animal costs:** per-diem and procedural costs for the contingent Aim 3 [ILLUSTRATIVE]. **Other:** sequencing/microbiota services, quality consultation, and limited travel. No major equipment is requested; existing institutional cores will be used. All figures are [ILLUSTRATIVE] and will be finalized with institutional budgeting.

Vertebrate Animals

Animal work is proposed only in the contingent Aim 3 and only if Aims 1–2 milestones are met. Procedures will use the minimum number of animals [ILLUSTRATIVE] necessary for statistical rigor

in an established catheter-based biliary/device-associated infection model, following ARRIVE principles and IACUC approval. Justification: in vivo testing of intrabiliary/systemic phage delivery cannot be replicated in vitro and is the key translational step, with direct precedent for oral and IV phage dosing in cholangitis/liver-injury models (Ichikawa 2023; Duan 2019). Endpoints include humane criteria, appropriate anesthesia/analgesia, and euthanasia consistent with AVMA guidelines. Microbiota and burden sampling will be designed to minimize distress and animal numbers.

Human Subjects / Clinical Trial

Not applicable as proposed; this R21 is preclinical and involves no prospective enrollment or interventional dosing of human participants. De-identified clinical bacterial isolates from biliary stents/bile will be obtained under IRB review or exemption with appropriate biosafety approval. We note the translational pathway for the future trial this work enables: investigational phage for individual patients in the US currently proceeds via FDA emergency/expanded-access IND (eIND) and magistral/compassionate-use routes (e.g., IPATH-type programs), under IRB oversight and institutional biosafety review—consistent with the published compassionate-use case in a liver-transplant recipient (Paul 2021). No human therapeutic administration is part of this application.

Team & Environment

[Fill with real names/institutions.] **Contact PI / MPI** — hepatobiliary/translational lead (gastroenterology–hepatology or infectious disease) directing indication strategy and Aim 3. **Co-Investigator, Phage Biology** — phage isolation, host-range, depolymerase, and cocktail design (collaboration with an established phage bank/center). **Co-Investigator, Interventional Endoscopy** — ERCP/biliary-stent expertise to ground delivery and modeling. **Microbiologist / Biofilm Scientist**, **Research Technician**, **Biostatistician**, and **Animal-Model Core** support complete the team. **Environment:** an academic medical center with endoscopy and explanted-stent access, a phage therapeutics partner or core, BSL-2 microbiology, confocal imaging, sequencing, and an IACUC-approved animal facility. Advisory input from compassionate-use phage programs (e.g., IPATH-type centers) will guide eventual eIND planning.

References

1. Schneider J, Hapfelmeier A, Fremd J, et al. Biliary endoprosthesis: a prospective analysis of bacterial colonization and risk factors for sludge formation. *PLoS One.* 2014;9(10):e110112. https://pubmed.ncbi.nlm.nih.gov/25314593/
2. Ichikawa M, Nakamoto N, Kredo-Russo S, et al. Bacteriophage therapy against pathological

Klebsiella pneumoniae ameliorates the course of primary sclerosing cholangitis. *Nat Commun.* 2023;14(1):3261. https://doi.org/10.1038/s41467-023-39029-9

3. Duan Y, Llorente C, Lang S, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature.* 2019;575(7783):505-511. https://doi.org/10.1038/s41586-019-1742-x
4. Paul K, Merabishvili M, Hazan R, et al. Bacteriophage Rescue Therapy of a Vancomycin-Resistant *Enterococcus faecium* Infection in a One-Year-Old Child following a Third Liver Transplantation. *Viruses.* 2021;13(9):1785. https://doi.org/10.3390/v13091785