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Phage-Armed Catheters and an *E. coli/Proteus* Phage Cocktail to Prevent and Treat Catheter-Associated UTI and Crystalline Biofilm

Project Summary / Abstract

Catheter-associated urinary tract infection (CAUTI) is among the most common healthcare-associated infections and is driven by uropathogens—chiefly *Escherichia coli* and *Proteus mirabilis*—that colonize indwelling Foley catheters and form resilient biofilms. *P. mirabilis* is uniquely destructive: its urease alkalinizes urine and precipitates struvite and apatite into crystalline encrustations that block catheters and shield bacteria from antibiotics and host defenses. Because biofilm-embedded organisms tolerate antibiotics far above the planktonic MIC, and because CAUTI pathogens are increasingly multidrug-resistant, conventional therapy frequently fails without catheter exchange—a costly, uncomfortable procedure that is itself a reinfection risk. Lytic bacteriophages are well matched to this niche: they self-amplify at the infection site, many encode polysaccharide depolymerases that degrade the biofilm matrix, they can be impregnated into catheter materials for prevention, and—instilled into the bladder or catheter—they reach high local titers with a favorable safety profile. Foundational benchtop work showed lytic phages substantially reduce established *P. mirabilis* and *E. coli* catheter biofilms and that phage-impregnated hydrogel Foley catheters markedly reduce biofilm formation (Carson 2010), and a defined *P. mirabilis* cocktail limited surface colonization and encrustation in a catheterized bladder model (Mirzaei 2022). Yet most CAUTI/*Proteus*-specific evidence remains preclinical, efficacy can fall in artificial urine versus rich media, and no phage product is approved for CAUTI. Building on the PI's preliminary cocktail and phage-armed-catheter data [ILLUSTRATIVE], this R01 will (1) assemble a complementary, resistance-suppressing *E. coli*/*Proteus* cocktail; (2) optimize the cocktail and a phage-armed catheter under urine-relevant conditions with explicit go/no-go efficacy gates; and (3) de-risk both in a catheterized in vivo model—generating the efficacy, safety, and manufacturing-readiness data required for an eventual eIND-enabling clinical study. The work targets CAUTI precisely where antibiotics fail most: on the biofilm-encased surface of an indwelling device.

Specific Aims

CAUTI imposes a large clinical and economic burden [ILLUSTRATIVE: insert U.S. incidence and annual attributable cost], yet antibiotic therapy fails against device-associated crystalline biofilm without catheter exchange. Lytic phages and phage-encoded depolymerases offer a self-amplifying, resistance-sparing alternative that can both **prevent** colonization (impregnated into the catheter) and **treat** established biofilm (instilled into the bladder). Lytic phages substantially reduce *P. mirabilis* and *E. coli* catheter biofilm, and phage-loaded hydrogel catheters markedly reduce biofilm formation (Carson 2010); a defined *P. mirabilis* cocktail limits surface colonization and encrustation in a catheterized bladder model (Mirzaei 2022). Guided by these findings and by the PI's preliminary data [ILLUSTRATIVE], we will:

Aim 1. Assemble and characterize a complementary *E. coli*/*P. mirabilis* phage cocktail that broadens host coverage and suppresses resistance. We will screen lytic phages against banked clinical CAUTI isolates; map receptor usage (LPS, capsule, flagella, pili) and depolymerase activity; confirm strictly lytic, transduction-averse genomes by sequencing; and select non-competing phages whose combination maximizes coverage and minimizes resistant-mutant outgrowth. We will quantify phage–antibiotic interactions and test whether receptor-loss escape mutants resensitize to antibiotics. *Milestone:* a defined 2–4-phage cocktail covering ≥80% of isolates [ILLUSTRATIVE] with reduced resistance versus single phages.

Aim 2. Optimize the cocktail and a phage-armed catheter under urine-relevant conditions. Using in vitro Foley-catheter and flow-through bladder-model systems in artificial urine, we will quantify reductions in *E. coli* and *P. mirabilis* biofilm, urease-driven crystalline encrustation, and time-to-blockage, comparing free-cocktail instillation, depolymerase co-treatment, and phage-impregnated catheter materials. Head-to-head artificial-urine-versus-rich-media runs will quantify and close any efficacy gap, and phage loading, release, and retained lytic activity over clinically relevant dwell times will be characterized. *Go/No-Go gate:* ≥3-log biofilm reduction and significantly delayed blockage in artificial urine [ILLUSTRATIVE] before advancing to Aim 3.

Aim 3. De-risk the lead cocktail and phage-armed catheter in a catheterized in vivo model. In a catheterized rodent (or comparable) CAUTI model, we will test **prevention** (phage-armed catheter vs. uncoated control) and **treatment** (intravesical cocktail vs. vehicle in established infection). Primary endpoints: catheter-surface and urine/bladder bacterial burden; secondary: encrustation, histopathology, and local tolerability/safety. Both sexes will be studied [ILLUSTRATIVE], with group sizes set by power analysis on biofilm log-reduction.

Impact. Success would yield a translation-ready, urine-validated *E. coli*/*Proteus* phage system—an eIND-eligible candidate—addressing CAUTI on the biofilm-encased surface of the indwelling device, where antibiotics fail most.

Significance

CAUTI is among the most common healthcare-associated infections, and indwelling Foley catheters are the proximate driver [ILLUSTRATIVE: incidence, attributable cost, length-of-stay impact]. *E. coli* and *P. mirabilis* colonize the catheter surface and form biofilms in which embedded organisms tolerate antibiotics far above the planktonic MIC. *P. mirabilis* is particularly consequential: its urease alkalinizes urine and precipitates struvite and apatite, producing crystalline encrustation that physically blocks the catheter, traps bacteria, and shields them from antibiotics and host defenses. Compounded by rising multidrug resistance among CAUTI pathogens, antibiotic therapy commonly fails without catheter exchange—an intervention that is costly, uncomfortable, and itself a reinfection risk. There is a clear unmet need for a resistance-sparing modality that acts at the device surface.

Lytic phages directly address this gap. They self-amplify at the infection site as long as susceptible bacteria remain; many encode polysaccharide depolymerases that digest the exopolysaccharide matrix and let phage penetrate deeper biofilm layers; and they can be either impregnated into catheter materials (prevention) or instilled into the bladder/catheter (treatment) to reach high local titers with a favorable safety profile. Carson 2010 demonstrated substantial (multi-log) reduction of established *P. mirabilis* and *E. coli* catheter biofilms and markedly reduced biofilm formation on phage-impregnated hydrogel Foley catheters. Mirzaei 2022 showed a defined *P. mirabilis* phage cocktail limited surface colonization and reduced encrustation in a catheterized bladder model. In humans, the intravesical Pyophage RCT in patients undergoing transurethral resection of the prostate (Leitner 2021, NCT03140085) established that intravesical phage therapy is safe and non-inferior to standard antibiotics, though not superior to placebo bladder irrigation—positioning phage therapy as a credible, safe bladder-directed strategy while underscoring that device-specific, urine-validated efficacy data are still required. This project fills exactly that gap.

This work aligns directly with NIDDK's benign urology mission—reducing the burden of urinary tract infection and catheter-associated complications—and generates the preclinical evidence base needed to move a device-and-biologic CAUTI countermeasure toward the clinic.

Innovation

This proposal advances four innovations grounded in current evidence. First, it pairs **two delivery modes against the same pathogen pair**—a phage-impregnated catheter for prevention and an intravesical cocktail for treatment—rather than treating these as separate problems, building directly on Carson 2010's demonstration that both formats are feasible. Second, it makes **urine-relevant validation a primary design criterion** rather than an afterthought: because phage efficacy against uropathogen biofilm can fall in artificial urine relative to rich media, we treat preserved artificial-urine efficacy as a formal go/no-go gate. Third, the cocktail is engineered for **rational complementarity**—

selecting phages by distinct receptor usage (LPS, capsule, flagella, pili) and depolymerase activity to broaden coverage and suppress resistance—and exploits **phage–antibiotic interactions**, including the phenomenon whereby receptor-loss escape mutants can resensitize to antibiotics, positioning the cocktail as a resistance-sparing partner to existing drugs. Fourth, we benchmark the program against the most advanced clinical reference point in UTI—the engineered CRISPR-Cas3 cocktail LBP-EC01, whose ELIMINATE Part 1 (Kim 2024, NCT05488340) showed rapid, durable *E. coli* reduction in urine and symptom resolution in uncomplicated UTI—using that benchmark to set endpoints while focusing on the underserved CAUTI/*Proteus* device niche that the ELIMINATE and Leitner programs did not address.

Approach

Preliminary Studies [ILLUSTRATIVE]

[ILLUSTRATIVE — to be populated with the team's data.] In preliminary work the PI has (i) banked clinical *E. coli* and *P. mirabilis* CAUTI isolates with antibiograms; (ii) isolated lytic phages against a subset and demonstrated depolymerase (halo) activity for representative phages; (iii) shown in a benchtop Foley-catheter assay that a two-phage combination reduces *P. mirabilis* biofilm viability and delays urease-driven pH rise; and (iv) fabricated a hydrogel-coated silicone catheter segment that retains lytic phage activity over an initial dwell window. These data establish feasibility for each aim and the team's command of the required assays.

Aim 1 — Assemble and characterize a complementary *E. coli*/*P. mirabilis* phage cocktail

Rationale. Any single phage has a narrow, strain-specific host range, and bacteria readily mutate surface receptors to escape. Cocktails of complementary phages broaden coverage and suppress resistance; depolymerase-bearing phages additionally degrade biofilm matrix. A rationally assembled cocktail is the foundation for all downstream work.

Experimental design. Against a bank of clinical *E. coli* and *P. mirabilis* CAUTI isolates, we will screen a library of lytic phages by host-range (spot and efficiency-of-plating) assays. For promising phages we will map receptor usage (LPS, capsule, flagella, pili) using defined surface mutants and competition assays, detect depolymerase activity via halo formation, and confirm strictly lytic, transduction-averse genomes by whole-genome sequencing (screening out integrase, known toxin, and antibiotic-resistance genes). We will assemble candidate cocktails combining non-competing phages with distinct receptors, then quantify suppression of resistant-mutant outgrowth in time-kill assays versus single phages. We will characterize phage–antibiotic interactions at sub-MIC antibiotic

levels (checkerboard and time-kill) and assay whether receptor-loss escape mutants show restored antibiotic susceptibility.

Rigor. Key biological resources (phage stocks, bacterial isolates) will be authenticated by sequencing and host-range fingerprint and tracked by lot; assays will be run in biological and technical triplicate with defined positive/negative controls and blinded enumeration where feasible.

Quantitative success criteria. A defined 2–4-phage *E. coli*/*Proteus* cocktail covering ≥80% of banked isolates [ILLUSTRATIVE], with demonstrable depolymerase activity, ≥10-fold lower resistant-mutant outgrowth than the best single phage [ILLUSTRATIVE], and evidence of additive or synergistic phage–antibiotic activity.

Potential pitfalls & alternatives. If coverage is incomplete, we will expand the phage library, train phages on resistant isolates, or add a recombinant depolymerase as a standalone matrix-degrading adjunct. If resistance still emerges rapidly, we will iterate cocktail composition and exploit antibiotic resensitization of escape mutants. Strain specificity is expected; we will prioritize the most prevalent local CAUTI clones and report coverage transparently.

Aim 2 — Optimize the cocktail and a phage-armed catheter under urine-relevant conditions

Rationale. Benchtop efficacy in rich media may not translate to the bladder. Carson 2010 showed phage-impregnated hydrogel catheters markedly reduce biofilm formation and that free phage reduces established biofilm by multiple logs; Mirzaei 2022 showed cocktail-driven reductions in colonization and encrustation in a catheterized bladder model. Because efficacy can fall in artificial urine, optimization must occur under urine-relevant conditions.

Experimental design. Using Foley-catheter and flow-through bladder-model systems in artificial urine, we will compare (a) free-cocktail instillation, (b) cocktail plus depolymerase, and (c) phage-impregnated catheter materials (e.g., hydrogel-coated silicone). Endpoints: viable *E. coli* and *P. mirabilis* biofilm (log reduction by quantitative culture and confirmed by microscopy), urease-driven pH change and crystalline struvite/apatite encrustation, and time-to-blockage. Head-to-head artificial-urine-versus-rich-media runs will quantify any efficacy gap; we will optimize phage dose/titer, contact time, multi-dose scheduling, and impregnation method to close it. Catheter loading and phage release/retained-lytic-activity over clinically relevant dwell times will be characterized.

Quantitative success criteria / Go-No-Go. A lead formulation and phage-armed catheter prototype achieving ≥3-log *E. coli*/*P. mirabilis* biofilm reduction and significantly delayed blockage in artificial urine [ILLUSTRATIVE], with dosing parameters carried into Aim 3. Failure to preserve efficacy in artificial urine after optimization triggers the contingency plan below before any animal work.

Potential pitfalls & alternatives. If efficacy drops in artificial urine, we will raise titers, add depolymerase, refine targeted receptors, or use multi-phase dosing. If impregnation inactivates phage or releases too quickly, we will test alternative coatings/matrices and controlled-release strategies; a non-impregnated periodic-instillation regimen remains a viable fallback for the prevention arm.

Aim 3 — De-risk the lead cocktail and phage-armed catheter in a catheterized in vivo model

Rationale. A catheterized animal model adds host immunity, urine flow, and tissue interfaces absent in vitro, providing the safety and efficacy signals needed before any human study.

Experimental design. In a catheterized rodent (or comparable) CAUTI model challenged with *E. coli* or *P. mirabilis*, we will test two arms: **prevention** (phage-armed catheter vs. uncoated control) and **treatment** (intravesical cocktail vs. vehicle) in established infection. Primary endpoints: catheter-surface and urine/bladder bacterial burden; secondary: encrustation, bladder/kidney histopathology, and local tolerability/safety. Animals of both sexes will be enrolled and analyzed as a biological variable [ILLUSTRATIVE]; group sizes will be set by power analysis on biofilm log-reduction with pre-registered endpoints and blinded enumeration.

Quantitative success criteria. Significant reduction in catheter-surface and urine bacterial burden for both prevention and treatment arms versus controls [ILLUSTRATIVE], with no unacceptable local toxicity—mirroring the favorable safety profile of intravesical phage in Leitner 2021 and supporting an eventual eIND.

Potential pitfalls & alternatives. In vivo efficacy may fall short of in vitro; we will adjust dose/dosing frequency and combine phage with sub-MIC antibiotics (phage–antibiotic synergy). If the rodent model proves limiting, we will use an ex vivo or larger catheterized model. Phage neutralization by host responses on repeat dosing will be monitored serologically.

Rigor, Reproducibility, and Sex as a Biological Variable

Key resources (phage stocks, clinical isolates, recombinant depolymerases, catheter materials) will be authenticated (sequencing, host-range fingerprint, endotoxin testing) and lot-tracked. Experiments use biological and technical replicates, defined controls, blinded enumeration where feasible, and pre-specified statistical plans. Sex is treated as a biological variable: in vitro isolates from both sexes are included, and in vivo studies (Aim 3) enroll and analyze both sexes [ILLUSTRATIVE], with sex-disaggregated reporting.

Timeline

[ILLUSTRATIVE] Year 1: phage library screening, receptor/depolymerase characterization, cocktail assembly (Aim 1). [ILLUSTRATIVE] Year 2: in vitro urine-relevant optimization and catheter prototyping (Aim 2), with Aim 1 finalization. [ILLUSTRATIVE] Years 3–4: in vivo prevention and treatment studies (Aim 3) and lead-candidate selection. [ILLUSTRATIVE] Year 5: confirmatory in vivo runs, manufacturing/stability groundwork, and eIND-enabling data assembly. **Go/no-go gate after Aim 2:** efficacy (≥3-log biofilm reduction; delayed blockage) must be preserved in artificial urine before in vivo work begins.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] Requested at [ILLUSTRATIVE] $250,000 direct costs/year for [ILLUSTRATIVE] 5 years (modular). **Personnel:** PI ([ILLUSTRATIVE] 2.4 calendar months); co-investigators in urology, phage biology/genomics, and biomaterials; [ILLUSTRATIVE] 2 postdocs/research scientists; and a research technician for phage propagation and biofilm assays. **Supplies:** clinical isolate banking, phage production/purification (endotoxin-controlled), artificial urine and catheter consumables, whole-genome sequencing, depolymerase reagents. **Animal costs:** [ILLUSTRATIVE] per-diem and procedures for the catheterized model in Years 3–5. **Other:** biomaterials fabrication for impregnated catheters, biostatistics, and publication/dissemination. The modular request reflects production-, model-, and personnel-intensive phases; figures are [ILLUSTRATIVE] and to be finalized with institutional budgeting.

Vertebrate Animals

Animal work is proposed in Aim 3 (catheterized CAUTI model). The application will include the required vertebrate-animal components: description of procedures and justification of the catheterized model and species; inclusion of both sexes [ILLUSTRATIVE]; minimization of pain/distress with appropriate analgesia/anesthesia and humane endpoints; justification of [ILLUSTRATIVE] group sizes by power analysis; and IACUC approval prior to any animal work. The 3Rs guide the design, with in vitro bladder-model systems (Aim 2) reducing and refining downstream animal use, and the Aim 2 go/no-go gate ensuring animals are used only for a formulation with demonstrated urine-relevant efficacy.

Human Subjects / Clinical Trial

No human-subjects research is proposed in this R01; the work is preclinical (in vitro and animal).

Banked, de-identified clinical isolates will be obtained under appropriate institutional review with a non-human-subjects determination. We explicitly plan the regulatory path beyond this award: because phage products are investigational, first-in-human CAUTI use would proceed via an FDA single-patient emergency/expanded-access IND (eIND) and/or a conventional IND, under IRB oversight, informed by the demonstrated safety of intravesical phage (Leitner 2021, NCT03140085) and the LBP-EC01 ELIMINATE program (Kim 2024, NCT05488340). This proposal generates the eIND-enabling efficacy, safety, and manufacturing-readiness data.

Investigators & Environment

[ILLUSTRATIVE — to fill with real names, biosketches, and institutional details.] **Contact PI** — phage biologist/microbiologist with catheter-biofilm expertise. **Co-Investigator, Urology/Neuro-Urology** — CAUTI clinical and intravesical-therapy experience. **Co-Investigator, Biomaterials** — catheter coating/impregnation and controlled release. **Co-Investigator, Phage Therapeutics/Manufacturing** — production, purification, and regulatory strategy. **Biostatistician** and **veterinary/animal-model core**. The assembled team spans microbiology, clinical urology, materials science, biostatistics, and regulatory affairs—covering every methodology in the proposal. **Environment:** institutional BSL-2 phage facilities; biofilm/microscopy cores; a biomaterials-fabrication core; an AAALAC-accredited animal facility; and access to a clinical isolate biobank.

References

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