Susceptibility-Matched Phage-Antibiotic Therapy for Antibiotic-Refractory Chronic Bacterial Prostatitis: A Phagogram-Driven Cocktail Against Uropathogenic *E. coli* and *Enterococcus faecalis*

Project Summary / Abstract

Chronic bacterial prostatitis (CBP) is a recurrent, debilitating urologic infection caused predominantly by uropathogenic *Escherichia coli*—frequently ESBL-producing and fluoroquinolone-resistant—and *Enterococcus faecalis*. Durable cure is notoriously difficult because the prostate is a poorly vascularized, biofilm-rich sanctuary that most antibiotics penetrate weakly, so relapse after oral therapy is the rule rather than the exception. Bacteriophages are mechanistically suited to this niche: they are strain-specific lytic agents that self-amplify at the infection focus, actively penetrate and disrupt biofilms, retain activity against multidrug-resistant strains, and can be combined with antibiotics for synergy (phage-antibiotic synergy, PAS). Peer-reviewed case reports now document cure of antibiotic-refractory CBP using phage cocktails (Johri et al., 2023; Cesta et al., 2025) and a phage-derived endolysin against *E. faecalis* (Stevens et al., 2023), and the engineered CRISPR-Cas3 phage cocktail LBP-EC01 produced rapid, durable *E. coli* reduction in a Phase 2 uncomplicated-UTI trial (Kim et al., 2024). However, no completed randomized trial exists in CBP specifically, and the dosing, route, and biofilm/PAS mechanism in prostate tissue remain undefined. This exploratory, milestone-driven R21 will establish the preclinical and translational foundation for susceptibility-matched phage therapy in CBP by (1) building a phagogram-driven cocktail against contemporary CBP *E. coli* and *E. faecalis* isolates, (2) quantifying biofilm disruption and PAS in vitro, and (3) testing efficacy and intratissue pharmacokinetics in a rat model of bacterial prostatitis. Each aim carries an explicit, quantitative go/no-go milestone. The work is high-risk/high-reward, mechanism-focused, and designed to de-risk a future randomized CBP trial of a precision, microbiome-sparing antibacterial.

Specific Aims

Chronic bacterial prostatitis is one of urology's most stubborn chronic infections: uropathogenic *E. coli* and *E. faecalis* establish biofilms in a low-vascularity prostatic sanctuary that conventional antibiotics penetrate poorly, so relapse after oral therapy is routine and men endure years of recurrent pain and repeated antibiotic exposure. Bacteriophages offer biofilm penetration, self-amplification at the infection focus, and resistance-independent killing—exactly what antibiotics lack here—yet evidence in CBP remains at the case-report stage, with no randomized trial and no mechanism or dosing data in prostate tissue. This R21 will generate the focused, rigorous preclinical proof-of-concept needed to move susceptibility-matched phage therapy toward a CBP trial. We will pursue three aims, each gated by a defined milestone.

Aim 1. Assemble and characterize a susceptibility-matched phage cocktail against contemporary CBP isolates. We will bank uropathogenic *E. coli* (including ESBL/fluoroquinolone-resistant) and *E. faecalis* clinical isolates from men with CBP, perform efficiency-of-plating phagogram susceptibility testing against candidate strictly lytic phages, and define a multi-phage cocktail that maximizes host-range coverage while minimizing receptor overlap. Candidate phages will be whole-genome sequenced to confirm obligately lytic lifestyle and exclude integrase, toxin, antibiotic-resistance, and virulence genes. *Milestone:* a sequenced, safety-screened cocktail covering ≥80% of banked isolates.

Aim 2. Quantify biofilm disruption and phage-antibiotic synergy (PAS) in vitro. Using static and flow biofilm models of matched isolates, we will measure phage- and enzyme-mediated matrix degradation and killing of sessile/persister populations, and systematically test PAS and re-sensitization with clinically relevant antibiotics (e.g., doxycycline), building on the ESBL *E. coli* PAS signal reported by Cesta et al. (2025). *Milestone:* ≥2-log greater biofilm CFU reduction for the optimal phage+antibiotic combination versus antibiotic alone, in ≥2 strains per species, carried forward to Aim 3.

Aim 3. Test phage-cocktail efficacy and pharmacokinetics in a rat model of bacterial prostatitis. In an established rat prostatitis model, we will compare susceptibility-matched phage cocktail, antibiotic alone, the optimal PAS combination, and vehicle on prostatic bacterial burden, biofilm histopathology, and phage titers in prostate, urine, and blood. *Milestone:* the combination arm achieves a significant, ≥2-log reduction in prostatic CFU versus antibiotic alone, with detectable intraprostatic phage amplification.

Impact: By establishing strain-matched efficacy, biofilm activity, and PAS in living prostate tissue, this project will provide the mechanistic, route, and dosing rationale to design the first randomized phage trial in CBP and to advance precision, microbiome-sparing antibacterials for an antibiotic-refractory urologic disease squarely within NIDDK's benign-urology mission.

Significance

CBP imposes years of recurrent pelvic pain, urinary symptoms, and repeated antibiotic exposure on affected men, and its causative organisms—uropathogenic *E. coli* (often ESBL-producing and fluoroquinolone-resistant) and *E. faecalis*—are increasingly drug-resistant. The core therapeutic failure is anatomical and microbiological: the prostate is a poorly vascularized, biofilm-rich sanctuary that most antibiotics penetrate weakly, so bacteria persist in a sessile, antibiotic-tolerant state and relapse after oral therapy is the rule. This is precisely the niche where conventional antibiotics fail and where new mechanisms are needed, and it falls directly within NIDDK's interest in chronic prostatitis and benign urologic disease.

Bacteriophages address each failure mode directly. They are strain-specific lytic agents that self-amplify at the infection site, so they can reach killing titers inside low-vascularity tissue that drugs cannot reach; they and their associated enzymes degrade the biofilm matrix, exposing persister bacteria; and they retain activity against multidrug-resistant strains independent of antibiotic resistance. Crucially, PAS can re-sensitize resistant bacteria—Cesta et al. (2025) showed SES/PYO cocktails enhanced doxycycline killing of an ESBL *E. coli* CBP isolate despite doxycycline resistance. Because killing is strain-specific, phages spare the surrounding urinary and gut microbiota, offering a precision alternative to broad-spectrum antibiotic cycling.

The clinical signal is real but early. Multiple case reports document cure of antibiotic-refractory CBP: recurrent *E. coli* CBP resolved after two Eliava phage courses following five years of failed antibiotics (Johri et al., 2023); antibiotic-resistant *E. faecalis* chronic prostatitis/chronic pelvic pain syndrome cured with the Ef11-derived endolysin (Stevens et al., 2023); and ESBL *E. coli* CBP cured with oral SES + PYO cocktails plus doxycycline (Cesta et al., 2025). The strongest registered-trial evidence for the urinary *E. coli* target is adjacent: the CRISPR-Cas3 phage LBP-EC01 produced rapid, durable *E. coli* reduction in a Phase 2 uncomplicated-UTI trial (Kim et al., 2024). What is missing—and what an NIDDK exploratory R21 is ideally suited to provide—is rigorous, mechanism-anchored preclinical data in CBP specifically: strain-matched cocktail design, quantified biofilm and PAS activity, and demonstration of phage delivery and efficacy in prostate tissue. Filling this gap is the rate-limiting step to a credible randomized CBP trial.

Innovation

This project is innovative in target, method, and translational framing. **Target:** it focuses phage therapy on CBP specifically—a defined NIDDK benign-urology niche where no randomized phage trial exists—rather than on UTI broadly. **Method:** it operationalizes a phagogram-driven, susceptibility-matched cocktail strategy against both major CBP pathogens (*E. coli* and *E. faecalis*) and pairs natural lytic killing with two complementary, literature-grounded mechanisms—phage-

encoded matrix-degrading enzymes including the peptidoglycan-targeting "enzybiotic" endolysin exemplified by the Ef11 lysin (Stevens et al., 2023), and phage-antibiotic synergy/re-sensitization (Cesta et al., 2025). **Translational framing:** by quantifying biofilm disruption and PAS in matched isolates and then demonstrating phage pharmacokinetics and efficacy in prostate tissue, the project converts compassionate-use anecdote into the mechanism, route, and dosing data a trial requires. It also explicitly positions engineered, sequence-targeted approaches (CRISPR-Cas3, exemplified by LBP-EC01; Kim et al., 2024) as a logical follow-on, while keeping the R21 scope to a tractable, susceptibility-matched cocktail plus PAS.

Approach

Rigor and reproducibility. All in vitro assays use ≥3 biological replicates with pre-specified acceptance criteria and blinded enumeration where feasible; phage stocks are endotoxin-controlled and titer-verified per use. Animal studies are randomized, use blinded outcome assessment, and are powered a priori. CBP is a male-specific disease; consistent with sex-as-a-biological-variable policy, both the clinical isolate source population and the rat efficacy model are male, and this constraint is stated explicitly rather than treated as an unaddressed limitation. Reagents, isolates, and phage genomes will be catalogued for sharing.

Aim 1 — Susceptibility-matched cocktail assembly and phage characterization

Rationale. Phage killing is strain-specific and CBP isolates are heterogeneous and evolving, so a multi-phage cocktail selected by phagogram is required for adequate host-range coverage. Defining coverage against contemporary, often ESBL/fluoroquinolone-resistant isolates is the foundation for all downstream work.

Experimental design. We will assemble a biorepository of de-identified uropathogenic *E. coli* and *E. faecalis* isolates from men with microbiologically confirmed CBP. Candidate strictly lytic phages targeting *E. coli* (LPS/pili receptors) and *E. faecalis* (cell-wall structures) will be screened by spot and efficiency-of-plating phagograms against the panel. Cocktail composition will be chosen to maximize cumulative host-range coverage and minimize overlapping receptors. Candidate phages will undergo whole-genome sequencing to confirm obligately lytic lifestyle and to exclude integrase, toxin, antibiotic-resistance, and virulence genes. We will assess in vitro emergence of phage resistance and whether cocktails suppress it relative to single phages.

Expected outcomes. A sequenced, characterized cocktail covering ≥80% of banked isolates with documented safety-relevant genomics, plus a phagogram workflow transferable to clinical strain matching.

Milestone / go-no-go. Proceed if the cocktail covers ≥80% of banked isolates per species and all components are confirmed strictly lytic and free of flagged genes; otherwise expand phage sourcing and/or pivot to the enzybiotic strategy below.

Potential pitfalls & alternatives. If host-range coverage is inadequate—especially for *E. faecalis*, which is harder to lyse—we will incorporate a phage-derived endolysin (enzybiotic) approach analogous to the Ef11 lysin (Stevens et al., 2023) as a standalone or adjunct agent and broaden sourcing through established phage collections.

Aim 2 — Biofilm disruption and phage-antibiotic synergy in vitro

Rationale. The prostatic biofilm is the central reason antibiotics fail; phages and their matrix-degrading enzymes disrupt the biofilm and expose persisters, sub-lethal antibiotics can boost phage production, and phages can re-sensitize resistant bacteria. Quantifying these effects in matched isolates is essential before in vivo testing.

Experimental design. Using static (microplate) and flow-cell biofilm models of representative *E. coli* and *E. faecalis* isolates, we will quantify biofilm biomass, viable sessile/persister counts, and matrix degradation after exposure to the cocktail, individual phages, and enzyme (endolysin/depolymerase) preparations. We will then test PAS in checkerboard and time-kill formats across clinically relevant antibiotics—including doxycycline, given the ESBL *E. coli* doxycycline re-sensitization reported by Cesta et al. (2025)—measuring synergy, dose-sparing, and suppression of resistant subpopulations on planktonic and biofilm bacteria, including ordering of exposure (phage-first vs. concurrent).

Expected outcomes. Quantitative evidence that the cocktail disrupts CBP biofilms and kills persisters, and identification of phage-antibiotic combinations showing synergy/re-sensitization, yielding the combinations and dose ranges carried into Aim 3.

Milestone / go-no-go. Advance the combination that achieves ≥2-log greater biofilm CFU reduction than antibiotic alone in ≥2 strains per species; if no combination meets this, prioritize enzyme-rich phages and report the negative PAS result as an informative outcome before committing animals.

Potential pitfalls & alternatives. If PAS is antibiotic- or strain-dependent, we will screen additional agents and vary exposure sequence. If biofilm penetration is limiting, we will prioritize depolymerase/endolysin-rich preparations as adjuncts.

Aim 3 — Efficacy and phage pharmacokinetics in a rat prostatitis model

Rationale. Self-amplification and tissue penetration cannot be demonstrated in vitro; an in vivo

model is required to show phages reach therapeutic titers in low-vascularity prostate tissue and reduce burden, alone and with antibiotic.

Experimental design. In an established rat model of bacterial prostatitis induced with a matched uropathogenic *E. coli* isolate, animals will be randomized (with blinded outcome assessment) to (a) susceptibility-matched phage cocktail (intraprostatic and/or intravesical instillation), (b) antibiotic alone, (c) phage + antibiotic (optimal PAS combination from Aim 2), and (d) vehicle control. Endpoints: prostatic CFU burden, biofilm/histopathology, and phage titers in prostate tissue, urine, and blood over time to characterize intratissue pharmacokinetics and amplification. Group sizes are set by a priori power analysis. [ILLUSTRATIVE] n ≈ 12 animals/group.

Expected outcomes. Demonstration that the cocktail penetrates and self-amplifies in prostate tissue and reduces bacterial burden, with the phage + antibiotic arm showing the greatest reduction—providing the route and dosing rationale for a future trial.

Milestone / go-no-go. Success = combination arm yields a statistically significant, ≥2-log reduction in prostatic CFU versus antibiotic alone with detectable intraprostatic phage amplification; this defines the trigger for a follow-on R01 and trial design.

Potential pitfalls & alternatives. If a single matched isolate limits generalizability, we will confirm key findings with a second isolate and, where feasible, an *E. faecalis* arm. If intraprostatic delivery is technically variable, intravesical and systemic routes will be compared. Model and dosing details will be finalized with veterinary staff under IACUC oversight.

Timeline

[ILLUSTRATIVE] Two-year R21. **Months 1–9:** Aim 1 (isolate banking, phagograms, phage sequencing/characterization; Aim 1 milestone gate). **Months 6–18:** Aim 2 (biofilm and PAS assays), overlapping Aim 1; Aim 2 milestone gate before animals. **Months 12–24:** Aim 3 (rat prostatitis efficacy and PK). **Months 22–24:** data integration, dissemination, and design of a follow-on randomized CBP trial / R01.

Budget Justification (R21 modular)

[ILLUSTRATIVE] R21 modular request of [ILLUSTRATIVE] $275,000 direct costs over two years, within standard R21 limits (typically capped near $275,000 over two years, with no more than [ILLUSTRATIVE] $200,000 in any single year). **Personnel:** PI/MPI (urology + phage biology) at [ILLUSTRATIVE] 1.8 calendar months/yr; Co-Investigator microbiologist; a postdoctoral fellow ([ILLUSTRATIVE] 50% effort) for phagogram, biofilm, and PAS work; a research technician

([ILLUSTRATIVE] 50%) for the animal model and isolate banking. **Supplies:** clinical isolate banking, phage propagation/purification with endotoxin control, whole-genome sequencing, biofilm/flow-cell consumables, antibiotics, and assay reagents. **Animals:** rat prostatitis model per-diems, surgical/instillation supplies, histopathology, and phage-titer PK assays. **Other:** sequencing core fees, biosafety, and dissemination. No major equipment is requested. Final figures and effort will be set with institutional budgeting.

Vertebrate Animals

Animal work is proposed (Aim 3). An established rat model of bacterial prostatitis will be used to test phage-cocktail efficacy, route of delivery, and intratissue pharmacokinetics. Because CBP is a disease of the male prostate, male animals are used; this is a scientific necessity of the model, not an unjustified sex restriction. All procedures will be reviewed and approved by the institutional IACUC prior to initiation. The proposal will address the required elements—justification of species and group sizes (minimized via power analysis), induction and instillation procedures, anesthesia/analgesia, humane endpoints, and euthanasia—consistent with ARRIVE-aligned reporting, randomization, and blinded outcome assessment. Group sizes ([ILLUSTRATIVE] n ≈ 12/group) reflect the minimum needed for statistical rigor while reducing animal use.

Human Subjects / Clinical Trial

No interventional human trial is conducted in this R21. Human involvement is limited to acquisition and use of de-identified clinical bacterial isolates from male CBP patients for Aims 1–2; this activity will undergo IRB review and a determination regarding human-subjects status, with appropriate consent/waiver and biospecimen governance. The project is explicitly designed to enable a future first-in-CBP randomized clinical trial. For that subsequent trial, investigational phage therapy in the United States would proceed under FDA oversight via an Investigational New Drug application—including the emergency/expanded-access IND (eIND) route used for individual compassionate cases—with full IRB approval, susceptibility ("phagogram") matching, and predefined microbiologic and symptom endpoints, mirroring the regulatory path of registered programs such as LBP-EC01 (Kim et al., 2024).

Team & Environment

This project requires an interdisciplinary team; roles below are templates to be filled with named investigators and institutions.

- **Contact PI / MPI — Urologist-scientist** [Name, Institution]: clinical CBP expertise, isolate sourcing, study leadership.
- **MPI / Co-I — Phage biologist** [Name, Institution]: phage sourcing, phagograms, genome characterization, cocktail design.
- **Co-I — Clinical microbiologist** [Name, Institution]: isolate banking, susceptibility testing, biofilm/PAS assays.
- **Co-I — Comparative medicine / in vivo pharmacology** [Name, Institution]: rat prostatitis model, instillation, PK.
- **Consultants:** regulatory/IND advisor (eIND and trial path); biostatistician (power, analysis). Engagement with established phage programs and centers (e.g., groups behind the Eliava cocktail experience, the Tor Vergata/Pisa PAS work, the Ef11 endolysin work, and the Locus Biosciences CRISPR-Cas3 program) will inform reagent sourcing and translational design.
- **Environment:** institutional core facilities for genomics, microscopy/biofilm imaging, BSL-2 microbiology, and AAALAC-accredited animal care, with urology clinical infrastructure for isolate access.

References

1. Cesta N, Fusco A, Ferretti C, et al. Bacteriophage-enhanced doxycycline activity against *Escherichia coli* in chronic bacterial prostatitis. *International Journal of Antimicrobial Agents.* 2025;66(4):107571. https://doi.org/10.1016/j.ijantimicag.2025.107571
2. Johri AV, Johri P, Hoyle N, Nadareishvili L, Pipia L, Nizharadze D. Case report: Successful treatment of recurrent *E. coli* infection with bacteriophage therapy for patient suffering from chronic bacterial prostatitis. *Frontiers in Pharmacology.* 2023;14:1243824. https://doi.org/10.3389/fphar.2023.1243824
3. Stevens RH, Zhang H, Kajsik M, et al. Successful use of a phage endolysin for treatment of chronic pelvic pain syndrome/chronic bacterial prostatitis. *Frontiers in Medicine.* 2023;10:1238147. https://doi.org/10.3389/fmed.2023.1238147
4. Kim P, Sanchez AM, Penke TJR, et al. Safety, pharmacokinetics, and pharmacodynamics of LBP-EC01, a CRISPR-Cas3-enhanced bacteriophage cocktail, in uncomplicated urinary tract infections due to *Escherichia coli* (ELIMINATE): the randomised, open-label, first part of a two-part phase 2 trial. *The Lancet Infectious Diseases.* 2024;24(12):1319-1332. https://doi.org/10.1016/S1473-3099(24)00424-9