Phage-Cocktail Therapy with Re-Sensitizing Antibiotics for Pan-Resistant ESBL *Escherichia coli* Urosepsis

Project Summary / Abstract

Urosepsis caused by extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* is an escalating clinical emergency. ESBL strains hydrolyze most penicillins and cephalosporins and frequently co-carry fluoroquinolone and aminoglycoside resistance, forcing reliance on carbapenems and, on carbapenem failure, leaving few or no options ("pan-resistant"). Urinary tract infections (UTIs) are the most common source of Gram-negative bloodstream infection, and recurrent ESBL *E. coli* urosepsis in catheterized, immunosuppressed, and transplant patients carries high morbidity and mortality. Lytic bacteriophages are mechanistically suited to this crisis: their killing is independent of antibiotic-resistance machinery, so an ESBL or carbapenemase phenotype does not blunt them; they self-amplify at the infection site, penetrate biofilm on uroepithelium and catheters, and can be delivered intravenously, intravesically, or orally. Human evidence is real but early. A 2023 case report described a 17-year-old renal-transplant recipient with four episodes of recurrent ESBL *E. coli* urosepsis in four months who, after ertapenem and fecal transplant failed, received a 3-week IV course of a 2-phage isolate-specific cocktail and remained ESBL *E. coli* culture-negative for 4 years (Gainey et al., 2023). Part 1 of the randomized, open-label ELIMINATE Phase 2 trial showed that combined intraurethral plus IV dosing of the CRISPR-Cas3-enhanced cocktail LBP-EC01 with concurrent oral TMP-SMX was well tolerated, with favorable urine/blood pharmacokinetics and rapid *E. coli* reduction in *uncomplicated* UTI (Kim et al., 2024; NCT05488340). A 2023 systematic review confirms a small but consistent UTI safety signal while emphasizing the lack of controlled invasive-disease data (Al-Anany et al., 2023). The missing bridge is rigorous, mechanism-anchored preclinical evidence specific to *invasive* pan-resistant ESBL urosepsis. We will (1) assemble and mechanistically characterize a multi-phage anti-ESBL cocktail and test the hypothesis that phage-resistance mutations re-sensitize escape mutants to antibiotics; (2) test the cocktail plus a re-sensitizing antibiotic against biofilm and in a murine ascending-UTI-to-bacteremia model across IV, intravesical, and combined routes; and (3) define and prospectively validate a personalized isolate-to-phage matching and potency-release pipeline against quantitative milestones. The work converts a compassionate-use practice into controlled preclinical evidence positioned for FDA-regulated translation.

Specific Aims

Antimicrobial resistance threatens to make ESBL *E. coli* urosepsis untreatable. Because phage killing is wholly independent of beta-lactamase and carbapenemase activity, a lytic cocktail — paired with an antibiotic to which phage-resistant escape mutants may become re-sensitized — offers a rational, pathogen-agnostic strategy. Human evidence sits at the case-report (Gainey et al., 2023) and early-controlled-trial (ELIMINATE Part 1; Kim et al., 2024) stage: promising, not standard of care, and not yet tested in invasive disease. We propose:

Aim 1. Assemble and mechanistically characterize a multi-phage anti-ESBL *E. coli* cocktail. Against a curated bank of pan-resistant ESBL urosepsis isolates, we will select lytic phages with complementary receptor specificities (LPS O-antigen, outer-membrane proteins, type 1/F pili), map host range, quantify suppression of escape mutants by the cocktail versus single phages, and test the central hypothesis that phage-resistance mutations restore antibiotic susceptibility (phage-antibiotic synergy, PAS). *Success milestone:* a defined cocktail covering ≥80% [ILLUSTRATIVE] of banked isolates with measurable re-sensitization.

Aim 2. Establish efficacy against biofilm and in an *in vivo* ascending-UTI/bacteremia model. We will test the cocktail plus a re-sensitizing antibiotic on catheter/uroepithelial biofilm and in a murine ascending UTI progressing to bacteremia, comparing IV, intravesical, and combined dosing against bacterial burden, biofilm, phage PK, and survival. *Go/no-go:* ≥2-log burden reduction and survival benefit versus antibiotic alone [ILLUSTRATIVE].

Aim 3. Define and prospectively validate a personalized matching and potency-release pipeline. We will lock an overnight isolate-to-phage matching workflow with predefined potency, purity (endotoxin), and host-range release criteria, then prospectively test turnaround and reproducibility across the isolate bank, assembling an FDA emergency-IND (eIND) data package.

Impact. Success delivers the mechanistic, biofilm, *in vivo*, and pipeline evidence needed to move phage-cocktail therapy for pan-resistant ESBL urosepsis from heroic last resort toward a controlled, FDA-regulated precision option.

Significance

ESBL *E. coli* urosepsis sits at the intersection of two NIAID priorities — antimicrobial resistance and serious Gram-negative bloodstream infection. ESBL strains defeat most penicillins and cephalosporins and frequently co-carry fluoroquinolone and aminoglycoside resistance; carbapenem failure can leave patients effectively untreatable. Because UTIs are the leading source of Gram-negative bacteremia, and recurrence is common in catheterized, immunosuppressed, and transplant

patients, even incremental options have outsized value. Phages address the core problem directly: lytic activity does not depend on resistance machinery, so ESBL/carbapenemase status is irrelevant to killing; phages self-amplify at the infection site, penetrate catheter and mucosal biofilm, and can be delivered IV, intravesically, or orally to reach the gut ESBL reservoir that seeds recurrence. The clinical signal is real but thin: one durable cure in recurrent ESBL urosepsis after antibiotic and fecal-transplant failure (Gainey et al., 2023); favorable safety/PK and rapid bacterial reduction in *uncomplicated* UTI (Kim et al., 2024); and a broader UTI literature, including ESBL/MDR strains, summarized in a 2023 systematic review that explicitly calls for controlled studies (Al-Anany et al., 2023). Rigorous, invasive-disease-specific preclinical data and a reproducible matching pipeline are the missing translational bridge this proposal supplies.

Innovation

This project is innovative in four respects. First, it targets *invasive, pan-resistant* ESBL urosepsis — not uncomplicated cystitis — and treats PAS as a deliberate design principle: pairing the cocktail with an antibiotic to which phage-resistance mutations (altered LPS, porins, efflux) are hypothesized to re-sensitize the bacterium. Second, it engineers the cocktail to suppress escape mutants through complementary receptor specificities. Third, it compares route of delivery (IV, intravesical, combined) head-to-head in a model that progresses from ascending UTI to bacteremia, matching the clinical trajectory of urosepsis. Fourth, it prospectively validates an overnight matching and potency-release pipeline aligned to an FDA eIND, the pathway already used for compassionate phage therapy, so findings translate without a discovery gap. The engineered frontier — CRISPR-Cas3-enhanced cocktails such as LBP-EC01 (Kim et al., 2024) — informs our design while we keep the core program on near-term, deployable natural-phage cocktails.

Approach

Aim 1. Assemble and characterize a multi-phage anti-ESBL cocktail

Rationale. A cocktail with complementary receptors covers strain heterogeneity and suppresses escape mutants; resistance mutations frequently re-sensitize bacteria to antibiotics, the proposed basis of PAS.

Design. Against a bank of ~30 [ILLUSTRATIVE] pan-resistant ESBL *E. coli* urosepsis isolates, we will screen lytic phages, select those with distinct receptors, and confirm strictly lytic genomes lacking toxin/resistance genes. We will quantify host range, escape-mutant frequency (single phages vs. cocktail), and PAS with carbapenems and other partners by checkerboard and time-kill assays. Escape mutants will be tested for restored susceptibility and characterized for receptor/porin/efflux

changes.

Expected outcomes. A defined cocktail with broad coverage, suppressed escape, and documented re-sensitization.

Pitfalls & alternatives. If coverage is incomplete, we will expand the panel or add receptor specificities; if synergy is antibiotic-specific, we will map the optimal partner per resistance profile. Where natural phages underperform, CRISPR-Cas3-enhanced constructs (LBP-EC01 precedent) serve as a benchmark and contingency.

Aim 2. Biofilm and *in vivo* ascending-UTI/bacteremia efficacy

Rationale. Biofilm on catheters and uroepithelium shields bacteria; urosepsis begins as ascending UTI and progresses to bacteremia, so route and systemic control matter.

Design. We will quantify cocktail ± re-sensitizing antibiotic against static and catheter-associated ESBL biofilm (biomass, viable counts, matrix degradation). In a murine ascending UTI progressing to bacteremia [ILLUSTRATIVE n≈10–12/arm, set by formal power analysis], we will compare IV, intravesical, and combined dosing versus antibiotic alone and vehicle, with endpoints of bladder/kidney/blood burden, biofilm, phage PK in urine and blood, and survival.

Expected outcomes. Biofilm clearance and reduced burden/improved survival, with route-specific guidance (IV for bacteremia, intravesical for bladder/catheter source) mirroring the combined intraurethral/IV regimen used clinically (Kim et al., 2024).

Pitfalls & alternatives. Rapid phage clearance or neutralizing antibody may reduce exposure; we will adjust dosing/redosing and lean on combined-route delivery and PAS. If catheter biofilm is under-recapitulated, we will add an indwelling-catheter variant.

Aim 3. Personalized matching and potency-release pipeline

Rationale. Personalized, isolate-matched cocktails are how compassionate phage therapy is delivered today; a reproducible, release-tested pipeline is required for regulated use.

Design. We will lock an overnight workflow from urine/blood isolate to phage match, set potency, purity (endotoxin), and host-range release criteria, and prospectively validate turnaround and reproducibility across the isolate bank. We will assemble an eIND data package integrating manufacturing, characterization, and Aim 1–2 efficacy/safety data, with companion-diagnostic logic analogous to antibiogram-guided antibiotic selection.

Expected outcomes. A validated matching-and-release pipeline and an eIND-ready package.

Pitfalls & alternatives. If overnight matching is not consistently achievable, a pre-characterized banked cocktail provides a fallback while personalized matching is optimized.

Rigor & Reproducibility

Key resources will be authenticated: phage genomes sequenced to confirm strictly lytic lifecycle and absence of toxin/resistance genes; isolates confirmed by sequencing and antimicrobial-susceptibility testing. Assays use biological and technical replicates with blinded enumeration; *in vivo* group sizes are set by power analysis with prespecified endpoints, randomization, and humane go/no-go criteria.

Timeline

[ILLUSTRATIVE] **Years 1–2:** Aim 1 banking, cocktail assembly, host-range/PAS/re-sensitization. **Years 2–4:** Aim 2 biofilm and *in vivo* route comparison. **Years 3–5:** Aim 3 prospective pipeline validation and eIND package; pre-IND interactions.

Budget Justification (Modular R01 Sketch)

[ILLUSTRATIVE] **$250,000 direct costs/year for 5 years** (standard modular R01). Personnel: PI (phage biology), co-investigators (infectious disease, regulatory), a phage biologist, a molecular microbiologist, an *in vivo* technician. Other: isolate banking/sequencing, phage production/purification and endotoxin testing, biofilm/catheter assays, murine UTI/bacteremia studies, and eIND consulting. Modules/effort are illustrative and finalized per institutional rates.

Vertebrate Animals

Aim 2 uses a murine ascending-UTI-to-bacteremia model [ILLUSTRATIVE]. No *in vitro* system reproduces ascending infection, systemic dissemination, phage PK, and survival together. Procedures minimize pain/distress with appropriate analgesia/anesthesia and humane endpoints; group sizes set by power analysis to use the fewest animals consistent with rigor, under IACUC approval.

Human Subjects / Clinical Trial

No human-subjects research is conducted in this R01; the program is preclinical and translational. Banked clinical isolates will be obtained under institutional approvals and de-identification. Aim 3 prepares — but does not execute — a future clinical study; any subsequent personalized phage

administration would proceed under an FDA eIND and IRB oversight, consistent with current compassionate-use practice and the regulatory framework underlying ELIMINATE (NCT05488340).

Team & Environment

The project requires expertise in phage biology, ESBL/Gram-negative infectious disease, biofilm microbiology, *in vivo* infection modeling, and phage manufacturing/regulatory science, drawing on established academic phage-therapy programs and industry precedent (LBP-EC01/ELIMINATE; Kim et al., 2024). The host institution will provide BSL-2 microbiology, phage production/purification with endotoxin testing, a vivarium for UTI/bacteremia modeling, genomics, and regulatory-affairs support for eIND preparation.

References

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