Attribution note: Background claims below are grounded in four peer-reviewed sources retrieved via PubMed (Rotman 2024, DOI; Li 2023, DOI; Bao 2020, DOI; Yu 2025, DOI).

Trapping Carbapenem-Resistant *Klebsiella pneumoniae*: A Receptor-Targeted Phage Cocktail that Drives Virulence Loss and Antibiotic Resensitization

Project Summary / Abstract

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a WHO and CDC critical-priority pathogen that causes hospital-acquired pneumonia, bloodstream infection, complicated urinary tract infection, and liver abscess, with invasive-disease mortality frequently reported at 40–50% because carbapenemases (KPC, NDM, OXA-48) eliminate last-line therapy. Lytic bacteriophages offer a mechanistically orthogonal option: they self-amplify at the infection site and kill independently of antibiotic-resistance pathways. Critically, *K. pneumoniae* phages recognize surface polysaccharides — the capsule (K-antigen) and lipopolysaccharide (LPS) — that also mediate virulence, so escape from phages can force the bacterium to alter these receptors. In contemporary CRKP studies this trade-off has been observed to attenuate virulence (Rotman 2024; Li 2023; Yu 2025) and, in at least one CRKP system, to restore susceptibility to clinically important antibiotics (Yu 2025), while phage–antibiotic synergy can suppress resistant mutants even with otherwise inactive drugs (Bao 2020). However, the same body of work issues a warning we treat as central: under phage pressure, phage populations themselves can co-evolve toward **higher virulence and broader host range**, and some bacterial escape routes preserve fitness (Rotman 2024). This proposal turns that double-edged biology into a designed therapeutic "trap." We will (1) assemble and genomically characterize a receptor-diverse, capsule- and LPS-spanning anti-CRKP phage cocktail against a contemporary U.S. isolate panel, selecting compositions that minimize *both* bacterial escape *and* gain-of-host-range phage evolution; (2) map the receptor-mutation → virulence-loss → antibiotic-resensitization trade-off and phage–antibiotic synergy for cocktail-selected escapers, explicitly enumerating fitness-preserving escape; and (3) test cocktail efficacy and gut decolonization, with paired monitoring of phage and bacterial evolution, in murine CRKP models. The work serves NIAID's antimicrobial-resistance (AMR) priorities and is designed to de-risk a future investigational phage product for partner-driven

(BARDA, CARB-X) development. [ILLUSTRATIVE]

Specific Aims

CRKP causes invasive infections with mortality frequently reported near 40–50%, and carbapenemase production leaves clinicians with few reliable drugs. Lytic phages kill independently of antibiotic resistance and bind capsule and LPS, the same structures that drive *K. pneumoniae* virulence. A recurring trade-off makes this strategically powerful: phage escape can cost CRKP its surface polysaccharides, attenuating virulence (Rotman 2024; Li 2023; Yu 2025) and, in a CRKP system, restoring antibiotic susceptibility (Yu 2025), while phage–antibiotic synergy can suppress survivors even with a non-active antibiotic (Bao 2020). The same literature also defines the principal risk: phages can co-evolve to **higher virulence and broader host range**, and not every escape route is costly (Rotman 2024). A credible program must therefore engineer *for* the trade-off while engineering *against* adverse co-evolution. We will:

Aim 1. Assemble and characterize a receptor-diverse, capsule- and LPS-spanning anti-CRKP phage cocktail. Using a PhageBank-style approach (Rotman 2024), we will curate lytic phages against a contemporary U.S. CRKP isolate panel spanning major capsular (K) types, O-antigen types, and high-risk clones (e.g., ST258, ST11). We will determine host range and cross-resistance (efficiency-of-plating matrix), quantify capsule-depolymerase and biofilm-degradation activity, confirm a lytic, toxin-/AMR-gene-free genome for each phage, and down-select a defined cocktail that maximizes coverage while combining distinct receptors to suppress single-step resistance.

Aim 2. Map the receptor-mutation → virulence-loss → antibiotic-resensitization trade-off and phage–antibiotic synergy, including fitness-preserving escape. We will isolate cocktail and single-phage escape mutants, whole-genome sequence them, and map receptor mutations — anchored on validated determinants (LPS-associated *fabF*, after Li 2023; capsule/LPS-synthesis loci, after Yu 2025) and reporting novel loci agnostically. We will phenotype capsule, serum resistance, and virulence, and compare antibiotic susceptibility (carbapenems, aminoglycosides, colistin, trimethoprim–sulfamethoxazole) between parental and escape strains. We will quantify phage–antibiotic synergy (checkerboard, time-kill), including non-active agents (after Bao 2020), and explicitly enumerate "fit" escapers that retain capsule/virulence so the cocktail and partner antibiotics can be designed to close them.

Aim 3. Test efficacy, gut decolonization, and paired phage/bacterial evolution in murine CRKP models. Using established murine models (Rotman 2024), we will evaluate the cocktail alone and with a synergistic antibiotic (from Aim 2) in (a) a gut colonization/decolonization model and (b) a systemic-infection model, measuring bacterial burden, survival, and virulence/resistance changes in recovered isolates. We will longitudinally sequence *both* bacterial escapers *and* the phages themselves

to detect host-range expansion or virulence-associated phage evolution, providing a go/no-go safety readout for cocktail design.

Impact. This project will deliver a defined, mechanistically de-risked CRKP phage cocktail plus the trade-off, resensitization, and co-evolution-safety data needed to justify a future investigational phage product against one of the deadliest gaps in the AMR pipeline.

Significance

CRKP is among the most lethal antibiotic-resistant threats in U.S. hospitals. Invasive CRKP disease — pneumonia, bacteremia, and complicated UTI — carries mortality frequently reported at 40–50%, driven by carbapenemases (KPC, NDM, OXA-48) that abolish last-line carbapenem activity and often co-occur with aminoglycoside and colistin resistance. The convergence of carbapenem resistance with hypervirulence in clones such as ST11 and ST258 threatens strains that are simultaneously near-untreatable and highly invasive, a scenario for which no dependable small-molecule pipeline exists. This is why NIAID lists carbapenem-resistant *Enterobacterales* among its highest AMR priorities.

Phage therapy addresses this gap through a fundamentally different kill mechanism. Because *K. pneumoniae* phages recognize the capsule or LPS as receptor, phage pressure attacks the same surface structures that mediate serum resistance, phagocyte evasion, and biofilm protection. The therapeutically decisive consequence is an evolutionary trade-off documented across independent CRKP and MDR-Kp systems. Yu et al. (2025) showed, in carbapenem-resistant strain Kp2092, that escape arose through mutation of a phage receptor and LPS-synthesis genes and that evolved clones gained sensitivity to clinically important antibiotics while losing virulence in an infection model. Li et al. (2023), in a personalized phage-therapy case for an MDR *K. pneumoniae* pulmonary infection, identified LPS as the therapeutic-phage receptor and showed that a *fabF* deletion altered LPS and reduced virulence in mouse and zebrafish models. Bao et al. (2020) cured a recurrent extensively drug-resistant *K. pneumoniae* UTI using synergy between a phage cocktail and an otherwise non-active antibiotic (trimethoprim–sulfamethoxazole), which suppressed the emergence of phage-resistant mutants. Rotman et al. (2024) moved this from anecdote toward design, building a *Klebsiella* PhageBank, identifying host factors via transposon screening, and formulating cocktails that selectively suppressed gut-resident CRKP in mice and drove loss of virulence factors that serve as phage receptors.

Two honest caveats shape our design and distinguish this proposal from optimistic framings. First, the resensitization benefit is not universal: it is best documented in a single CRKP system (Yu 2025), whereas other reports establish virulence attenuation without claiming restored susceptibility (Li 2023). Second, and most importantly, Rotman et al. (2024) found that phage-driven diversification can select **phage variants with higher virulence and broader host range**, and that some bacterial

escapers remain fit. A program that ignores this risks selecting for a worse phage or a still-virulent survivor. By characterizing the trade-off *and* its failure modes, and by treating the gut reservoir that seeds invasive disease and nosocomial transmission as a primary target, this work would move CRKP phage therapy from bespoke compassionate use toward a generalizable, safety-aware precision adjunct.

Innovation

This proposal is innovative in three respects. **First, it operationalizes "resistance-as-therapy" without naïveté:** rather than treating phage escape as failure, we design the cocktail so that the most accessible escape routes force capsule/LPS alteration toward a disarmed, frequently resensitized state — while explicitly enumerating and countering the fitness-preserving escapers and the adverse phage co-evolution that the literature shows can otherwise undermine this logic (Rotman 2024; Yu 2025). **Second, it pairs rational, PhageBank-style cocktail design** (host-range- and depolymerase-guided selection across capsular and O-antigen types, after Rotman 2024) **with systematic phage–antibiotic synergy mapping that includes otherwise inactive agents** (after Bao 2020), rather than relying on single phages or empirically chosen pairs. **Third, it makes paired phage-and-bacterium evolutionary monitoring a primary endpoint,** so that host-range expansion or virulence-associated phage mutations become a designed-in go/no-go safety criterion rather than an afterthought — directly addressing the central caution raised by the foundational study in this space. Together these features generate the defined product and mechanistic/safety dossier needed to de-risk later-stage, partner-driven development (BARDA, CARB-X).

Approach

Aim 1 — Assemble and characterize a receptor-diverse, capsule- and LPS-spanning anti-CRKP phage cocktail

Rationale. *K. pneumoniae* phages are strain-specific and bind capsule (K-type) or LPS; broad coverage therefore requires a curated multi-phage cocktail spanning receptor classes, as established by the PhageBank approach (Rotman 2024).

Experimental design. We will assemble a contemporary U.S. CRKP isolate panel (carbapenemase-genotyped: KPC/NDM/OXA-48; spanning K-types, O-antigen types, and high-risk clones including ST258 and ST11) from clinical-microbiology collaborators and public repositories, with provenance and resistance genotype recorded. Lytic phages will be isolated/curated and screened by a quantitative host-range matrix (efficiency-of-plating). For each phage we will: (i) sequence and annotate the genome to confirm a strictly lytic lifestyle and absence of toxin, AMR, and integrase genes; (ii)

quantify capsule-depolymerase activity (halo assays, capsule staining) and biofilm-matrix degradation on abiotic and catheter-mimetic surfaces; and (iii) measure single-step resistance frequency. A defined cocktail (a small number of complementary phages) will be down-selected to maximize panel coverage while combining *distinct receptors* (capsule + LPS) to minimize shared-receptor cross-resistance. Cocktail composition will be scored not only on coverage but on its tendency to select fit vs. disarmed escapers (feeding Aim 2).

Expected outcomes. A defined, sequence-characterized cocktail with documented host range across multiple K-/O-types, measured depolymerase/biofilm activity, and lower in vitro resistance emergence than any constituent phage alone.

Rigor & reproducibility. Phages and isolates authenticated by sequencing; assays run in biological and technical triplicate with defined acceptance criteria; titers standardized; reagents and isolate metadata catalogued for reuse.

Potential pitfalls & alternatives. Coverage of rare K-/O-types may be incomplete; we will expand the phage pool, prioritize broad-reach depolymerase-bearing phages, and note that host-range engineering and CRISPR-/computational design are emerging routes to broaden coverage. If one phage drives rapid resistance, composition will be rebalanced toward distinct receptors.

Aim 2 — Map the trade-off and phage–antibiotic synergy, including fitness-preserving escape

Rationale. Across CRKP/MDR-Kp systems, phage escape via capsule/LPS receptor mutation can cause virulence attenuation (Li 2023; Yu 2025) and, in at least one CRKP system, antibiotic resensitization (Yu 2025); phages can synergize even with otherwise inactive antibiotics (Bao 2020). Quantifying *and bounding* this for our cocktail is essential to its therapeutic logic.

Experimental design. We will select escape mutants against the Aim 1 cocktail and individual phages and whole-genome sequence them. Variant mapping will be anchored on validated determinants — LPS-associated *fabF* (Li 2023) and capsule/LPS-synthesis loci (Yu 2025) — while reporting novel loci agnostically rather than presupposing specific capsule genes. Mutants will be phenotyped for capsule production, serum resistance, and virulence-associated traits, with virulence first triaged in an invertebrate/zebrafish-style model and confirmed in mice (Aim 3). Antibiotic susceptibility (carbapenems, aminoglycosides, colistin, trimethoprim–sulfamethoxazole) will be compared between parental and escape strains by CLSI-standard MIC. Phage–antibiotic synergy will be quantified by checkerboard and time-kill assays for phage + each antibiotic class, including non-active agents. We will explicitly enumerate and rank **fitness-preserving escapers** (capsule/virulence retained) and design cocktail/antibiotic combinations to close these routes.

Expected outcomes. A mapped relationship between defined receptor mutations and the magnitude of capsule loss, virulence attenuation, and resensitization; a catalogue of fit escape routes; and a synergy profile identifying antibiotic partners that suppress resistant mutants.

Rigor & reproducibility. Independent mutant lineages sequenced; MICs read by two blinded operators; synergy scored by predefined FIC/log-kill thresholds; parental controls in every run.

Potential pitfalls & alternatives. Some escape routes will preserve capsule/virulence (expected per Rotman 2024); we prioritize cocktail/antibiotic combinations that suppress them, and lean on phage–antibiotic synergy (including non-active agents) as an orthogonal route. If resensitization proves partial or strain-specific (as the literature suggests it may), the value proposition shifts toward virulence attenuation plus synergy-driven burden reduction, which we will quantify rather than assume.

Aim 3 — Test efficacy, gut decolonization, and paired phage/bacterial evolution in murine CRKP models

Rationale. The gut is a CRKP reservoir seeding invasive disease and transmission; rationally designed cocktails have suppressed gut-resident CRKP and driven virulence-factor loss in mice (Rotman 2024). That same study shows phages can co-evolve undesirably, so in vivo evolutionary surveillance is required.

Experimental design. Using established murine CRKP models, we will test the cocktail alone and with a synergistic antibiotic (selected in Aim 2) in: (a) a gut colonization/decolonization model, measuring fecal CRKP burden over time and characterizing residual isolates for capsule/virulence/resistance changes; and (b) a systemic-infection model assessing bacterial burden and survival. **Both sexes will be included** and analyzed as a biological variable; group sizes will be set by power analysis to the minimum required [ILLUSTRATIVE]. Endpoints include bacterial burden, survival, virulence-factor loss in recovered isolates, and — as a co-primary readout — **longitudinal sequencing of recovered phages** to detect host-range expansion or virulence-associated phage mutations. Adverse phage evolution will trigger predefined cocktail-redesign criteria.

Expected outcomes. Reduced CRKP burden and/or improved survival with cocktail ± antibiotic versus controls; evidence that survivors trend toward reduced virulence and (where applicable) restored susceptibility; and a defined safety readout on phage evolution.

Rigor & reproducibility. Randomized, blinded scoring; both sexes; predefined humane and statistical endpoints; isolates and phages archived and sequenced.

Potential pitfalls & alternatives. Phage pharmacokinetics, gut clearance, or neutralization may limit

effect; we will optimize dose/route/timing, consider repeated dosing, and use synergy to lower the burden the host must clear. If phages co-evolve broader host range or higher virulence (per Rotman 2024), we will down-select to compositions that constrain phage evolution (e.g., receptor diversity, controlled dosing) and, if needed, fix cocktail composition to limit adaptive trajectories. Incomplete decolonization will be interpreted alongside virulence/resensitization shifts that may still reduce invasive risk.

Timeline

[ILLUSTRATIVE] 5-year R01.

- **Year 1 [ILLUSTRATIVE]:** Isolate-panel assembly; phage curation, sequencing, host-range and depolymerase screening (Aim 1).
- **Year 2 [ILLUSTRATIVE]:** Cocktail down-selection and in vitro resistance characterization (Aim 1); begin escape-mutant generation and mapping (Aim 2).
- **Year 3 [ILLUSTRATIVE]:** Complete trade-off mapping, fit-escaper cataloguing, and synergy profiling (Aim 2); initiate murine models (Aim 3).
- **Years 4–5 [ILLUSTRATIVE]:** Murine efficacy and gut-decolonization studies; paired in vivo phage/bacterial evolution monitoring; integration and translational-dossier assembly (Aim 3).

Budget Justification

Modular R01-style sketch; all figures [ILLUSTRATIVE].

- **Personnel [ILLUSTRATIVE]:** PI (microbiology/phage biology) effort; Co-I in infectious disease/animal models; 1–2 postdoctoral scientists; research technician; bioinformatics support for phage genomics and escape-mutant mapping.
- **Direct costs [ILLUSTRATIVE]:** Requested at the standard $250,000/year modular direct-cost cap (NIH module size, [ILLUSTRATIVE]), totaling roughly $1.25M direct over 5 years [ILLUSTRATIVE], plus applicable F&A.
- **Major categories [ILLUSTRATIVE]:** Whole-genome sequencing for phages and escape mutants (Aims 1–2); depolymerase/biofilm and antibiotic-susceptibility/synergy reagents (Aims 1–2); vertebrate-animal per-diems and study costs including paired phage/bacterial sequencing (Aim 3); biosafety/containment for CRKP (BSL-2 practices); publication and dissemination.
- **Justification:** Costs scale with the breadth of the isolate/phage panel, sequencing depth for resistance and phage-evolution mapping, and the two murine model arms, which constitute

the principal expense in Years 4–5 [ILLUSTRATIVE].

Vertebrate Animals

Animal work is proposed (Aim 3; supportive invertebrate/zebrafish-style virulence assays in Aim 2). Murine CRKP gut-colonization/decolonization and systemic-infection models will assess bacterial burden, virulence-factor loss, survival, and paired phage/bacterial evolution, consistent with published murine CRKP phage studies (Rotman 2024). Both sexes will be included and sex analyzed as a biological variable. All procedures will follow an IACUC-approved protocol with humane endpoints, the minimum number of animals required for statistical power [ILLUSTRATIVE], appropriate anesthesia/analgesia, and euthanasia per AVMA guidelines. Justification for species and numbers and reduction/refinement strategies will be detailed in the full Vertebrate Animal Section.

Human Subjects / Clinical Trial

Not applicable to the proposed aims. This R01 is preclinical (phage isolation/characterization, mechanism, and murine efficacy); no human subjects or clinical trial are included. We note the translational pathway: investigational phage products for individual CRKP patients have to date proceeded via FDA single-patient emergency/expanded-access INDs (eIND) with IRB oversight, the route used in personalized cases such as those informing Li et al. (2023) and Bao et al. (2020). Any future clinical evaluation arising from this work would require a separate IND and IRB approval, pursued through appropriate mechanisms and partners.

Investigators & Environment

Template roles to be completed with named personnel/institutions [TO FILL]; the assembled team is designed to cover every required competency below.

- **Principal Investigator [TO FILL]:** Bacteriophage biology/microbial genetics; leads cocktail design, escape-mutant mapping, and phage-evolution analysis. Expected qualifications: prior phage isolation/characterization and CRKP or Enterobacterales experience.
- **Co-Investigator, Infectious Disease / In Vivo Models [TO FILL]:** Directs murine CRKP efficacy, decolonization, and survival studies; expected track record in Gram-negative infection models.
- **Co-Investigator/Collaborator, Clinical Microbiology [TO FILL]:** Provides genotyped contemporary U.S. CRKP isolates, carbapenemase typing, and clinical context.
- **Bioinformatics Lead [TO FILL]:** Phage genome annotation, bacterial variant calling, and

longitudinal phage/host evolutionary analysis.

- **Consultants/Partners [TO FILL]:** Phage-therapy translational expertise (academic phage centers; NIAID Antibacterial Resistance Leadership Group) to guide eventual product development with BARDA/CARB-X.
- **Environment [TO FILL]:** BSL-2 microbiology and phage facilities, high-throughput genomics core, AAALAC-accredited animal facility, and institutional IACUC/biosafety oversight — together providing the infrastructure required for all three aims.

References

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