Isolate-Matched Oral Bacteriophage Cocktails for Microbiome-Sparing Gut Decolonization of Carbapenem-Resistant *Klebsiella pneumoniae* in High-Risk Patients

Project Summary / Abstract

Carbapenem-resistant *Klebsiella pneumoniae* (CR-*Kp*) is a CDC and WHO urgent threat, and the intestinal tract is its principal silent reservoir: gut carriage seeds bloodstream and surgical-site infection, drives ward-level transmission, and most endangers transplant, oncology, and ICU patients — precisely the hosts in whom no safe, microbiome-sparing decolonization tool exists. Antibiotic-based selective digestive decontamination is collateral-damaging and resistance-promoting and has never become standard for CRE. Lytic bacteriophages offer an alternative built on specificity: they kill the target strain while sparing commensals, and because they engage the *Klebsiella* capsule and O-antigen/lipopolysaccharide (LPS) structures that mediate virulence and transmissibility, the most common escape route — capsule loss — yields an attenuated bacterium that often remains sensitive to a co-formulated LPS-targeting phage.

The preclinical foundation is now reproducible across independent groups. Rationally designed cocktails drawn from a *Klebsiella* PhageBank suppress gut-resident CR-*Kp* and drive loss of capsule-associated virulence factors in gnotobiotic mice, and define a concrete pharmacodynamic target of ≥3×10⁸ PFU/g stool for effective killing (Rotman et al., 2024). Phages targeting two surface structures — capsule (φK64-1) and O1 LPS (φKO1-1) — achieved durable intestinal decolonization in 52.3% (15/28) of mice without altering the commensal microbiota, with capsule-negative escapes remaining LPS-phage-sensitive (Tan et al., 2022). A systematic review of 56 studies (51 animal, 5 clinical) found notable bacterial-load reduction in 80% (45/56) with no obvious adverse events, while only 5 achieved complete eradication and the authors stressed that optimization is required before clinical use (Fang et al., 2024). Notably, the only registered human gut-decolonization program for CRE/VRE to date uses a *defined live-microorganism consortium*, not phages (NCT07525089), leaving the oral-phage decolonization paradigm clinically unproven.

This R01 will (1) build and benchmark a genomically vetted isolate-matching PhageBank/cocktail-

design pipeline against a contemporary US CR-*Kp* biobank; (2) define oral pharmacology, decolonization efficacy, resistance dynamics, and microbiome-sparing of optimized cocktails in murine carriage models, using a replication-dependence (inactivated-cocktail) control to pin causality; and (3) execute a rigorously monitored proof-of-concept clinical decolonization study under an FDA eIND with IRB and DSMB oversight. The expected outcome is a validated, matchable, microbiome-sparing strategy plus the human pharmacokinetic and safety parameters needed to power a definitive efficacy trial — converting high-risk carriers to non-carriers before transplant, chemotherapy, or surgery without broad-spectrum collateral damage.

Specific Aims

Intestinal carriage of CR-*Kp* is the upstream driver of invasive *Klebsiella* infection and of ward transmission, yet decolonization remains an unmet need because antibiotic-based approaches damage protective colonization resistance and accelerate resistance. Lytic phages are compelling because they kill target strains with sub-species precision while sparing commensals, and because they bind the capsule and LPS receptors *Klebsiella* depends on for virulence — so escape frequently carries a fitness cost (Rotman et al., 2024; Tan et al., 2022). The evidence, however, is predominantly preclinical and incompletely optimized (Fang et al., 2024), and the sole registered human CRE/VRE gut-decolonization program is a non-phage live-biotherapeutic consortium (NCT07525089), leaving oral phage decolonization clinically untested. We will advance an isolate-matched oral cocktail from biobank to bedside, with explicit quantitative go/no-go gates between aims.

Aim 1 — Build and validate an isolate-matching PhageBank and cocktail-design pipeline against a contemporary US CR-*Kp* biobank. We will assemble lytic *Klebsiella* phages and clinical CR-*Kp* isolates spanning prevalent US capsular (K) types and carbapenemase genotypes; sequence each phage to confirm absence of integrase, known toxin, and antimicrobial-resistance genes; map host range and receptor usage (capsule, O-antigen/LPS) by efficiency-of-plating and capsule/LPS-mutant assays; and computationally compose cocktails that maximize panel coverage while minimizing shared escape routes (after Rotman et al., 2024). *Go/no-go (→Aim 2):* ≥2 lead cocktails each covering ≥80% of the isolate panel in vitro with no resistance regrowth over 24 h in killing assays, and a documented matching workflow.

Aim 2 — Define in vivo oral pharmacology, decolonization efficacy, resistance dynamics, and microbiome-sparing. In gnotobiotic and conventionally colonized mice carrying defined clinical CR-*Kp*, we will establish the oral dose/schedule that suppresses and clears CR-*Kp*; relate fecal phage exposure to burden reduction against the ≥3×10^8 PFU/g stool benchmark (Rotman et al., 2024); characterize escape frequency and phenotype (capsule status, re-sensitization to partner phage); and quantify commensal impact by 16S/shotgun sequencing. A **replication-incapable (heat/UV-inactivated) cocktail** arm will confirm that killing is replication-dependent. Both sexes will be

studied. *Go/no-go (→Aim 3):* ≥1.5–2 $\log_{10}$ fecal CR-*Kp* reduction versus vehicle at the defined dose, escapes attenuated/partner-sensitive, and no significant loss of commensal diversity versus vehicle.

Aim 3 — Conduct a monitored proof-of-concept clinical decolonization study under an eIND framework. In a small cohort of consenting adult CR-*Kp* gut carriers in high-risk settings, we will administer an isolate-matched oral cocktail (selected via the Aim 1 pipeline) under FDA expanded-access/emergency IND authorization with IRB oversight, pre-specified stopping rules, and an independent DSMB. **Primary objective:** safety/tolerability. **Secondary objectives:** change in intestinal CR-*Kp* burden, stool phage pharmacokinetics relatable to the Aim 2 exposure–response model, escape-variant emergence/phenotype, and microbiome preservation.

Impact. Success delivers a validated, matchable, microbiome-sparing decolonization strategy and the human pharmacokinetic and safety parameters required to design a definitive randomized efficacy trial — without the broad-spectrum collateral damage of antibiotic decontamination.

Significance

CR-*Kp* gut colonization is the reservoir from which invasive disease and hospital transmission arise, and carriage prevalence is highest among the most vulnerable: solid-organ and hematopoietic transplant recipients, ICU patients, and pre-chemotherapy oncology hosts. Decolonizing these patients before a high-risk window is a direct infection-control intervention, but available tools are inadequate — antibiotic-based selective digestive decontamination damages colonization resistance, selects for resistance, and has not become standard for CRE. This is squarely within NIAID's antimicrobial-resistance and AMR-transmission priorities.

Phage therapy addresses the core deficiency of antibiotic decolonization — specificity. Because lytic *Klebsiella* phages recognize capsule and LPS receptors, they kill the target strain while leaving the commensal community intact, and that same receptor dependence means bacteria escaping phage pressure frequently alter or lose the capsule, shedding a key virulence and transmissibility determinant (Rotman et al., 2024; Tan et al., 2022). The preclinical signal is reproducible across independent groups: optimized PhageBank cocktails suppress gut-resident CR-*Kp* and reduce capsule-associated virulence in gnotobiotic mice (Rotman et al., 2024), and a capsule-plus-LPS pair achieved durable decolonization in 52.3% of mice without disturbing the microbiota, with capsule-negative escapes remaining sensitive to the partner phage (Tan et al., 2022). A systematic review found load reduction in 80% of 56 studies with no obvious adverse events, but complete eradication in only 5 and an explicit call for optimization before clinical use (Fang et al., 2024).

The translational gap is therefore not *whether* phages can suppress gut *Klebsiella*, but whether a matchable, well-characterized cocktail can be delivered orally — safely, reproducibly, and with

quantified human pharmacokinetics. That gap is underscored by the clinical landscape: the only registered human CRE/VRE gut-decolonization program uses a defined consortium of intestinal microorganisms rather than phages (NCT07525089), so the oral-phage paradigm has no human efficacy or pharmacokinetic data of its own. This proposal supplies exactly those missing parameters.

Innovation

This proposal is innovative in four respects, each grounded in the cited evidence.

1. **Matchable, receptor-aware cocktail design from a curated PhageBank.** We operationalize rational, host-range-diverse cocktail composition (Rotman et al., 2024) as a benchmarked pipeline against a contemporary US clinical biobank, rather than relying on fixed, empirically assembled mixtures.
2. **The capsule as a therapeutic trap.** By combining phages against distinct surface receptors (capsule and O-antigen/LPS; Tan et al., 2022), escape requires simultaneous loss of multiple structures, and the most accessible route — capsule loss — yields an attenuated bacterium that remains vulnerable to the partner phage.
3. **Microbiome-sparing as a co-primary readout.** We treat preservation of colonization resistance (16S/shotgun) as an endpoint rather than an afterthought, the property that distinguishes phage from antibiotic decolonization (Tan et al., 2022; Fang et al., 2024).
4. **A regulatory and clinical bridge with the evidence prior efforts lacked.** We pre-specify the FDA expanded-access/eIND route for individualized phage products and generate the rigorous stool pharmacokinetic and safety data that compassionate-use experience has not produced (Fang et al., 2024) — establishing the oral-phage paradigm that the only registered CRE/VRE decolonization program does not address (NCT07525089).

Approach

Aim 1 — Isolate-matching PhageBank and cocktail-composition pipeline

Rationale. Reproducible decolonization begins with phages of defined host range, receptor usage, and genomic safety, plus a principled way to combine them. Rotman et al. (2024) showed that selecting host-range-diverse phages from a *Klebsiella* PhageBank and optimizing cocktails eliminates *K. pneumoniae* with minimal resistance; we reconstruct this logic against US clinical strains.

Design. We will (a) curate a biobank of clinical CR-*Kp* isolates spanning prevalent US K-types and carbapenemase genotypes; (b) isolate/propagate lytic *Klebsiella* phages, sequencing each to confirm absence of integrase, known toxin, and antimicrobial-resistance genes; (c) map host range and

receptor dependence (capsule, O-antigen/LPS) by spot and efficiency-of-plating assays plus capsule/LPS-mutant testing; and (d) computationally compose candidate cocktails maximizing isolate coverage and minimizing shared escape routes, validated in liquid-culture killing/resistance-emergence assays. A matching algorithm will nominate a cocktail for any input isolate.

Expected outcomes. A genomically vetted PhageBank; per-isolate coverage maps; ≥2 lead cocktails with broad in vitro coverage and durable suppression; a documented matching workflow. *Rigor & reproducibility:* phages/isolates authenticated by whole-genome sequencing and re-streaked stocks; assays run in biological triplicate with defined acceptance criteria.

Pitfalls & alternatives. Some isolates may be untargetable by the existing bank; we will expand phage sourcing and note host-range engineering as a field-consistent future direction, not a deliverable here. If escape arises rapidly in vitro, we will increase receptor diversity within cocktails, exploiting the capsule-loss/partner-sensitivity relationship (Tan et al., 2022).

Aim 2 — In vivo pharmacology, efficacy, resistance, and microbiome impact in murine gut-carriage models

Rationale. Oral decolonization depends on delivering and maintaining sufficient active phage at the carriage site. Rotman et al. (2024) reported that effective gut killing required maintaining ≥3×10^8 PFU/g stool the day after dosing, a concrete pharmacodynamic target; Tan et al. (2022) demonstrated durable intestinal decolonization via dual-receptor targeting.

Design. In gnotobiotic and conventionally colonized mice (both sexes) carrying defined clinical CR-*Kp*, we will administer lead oral cocktails across dose levels/schedules, quantifying fecal CR-*Kp* and phage titers longitudinally to relate stool exposure to burden reduction and benchmark against the ≥3×10^8 PFU/g target. A **replication-incapable (heat/UV-inactivated) cocktail** arm will confirm that suppression requires active phage replication rather than passive inoculum. Post-treatment CR-*Kp* will be characterized for escape frequency and phenotype (capsule status, virulence-factor loss, re-sensitization to the partner phage). Off-target effects will be assessed by 16S and shotgun metagenomics versus vehicle. Group sizes [ILLUSTRATIVE] will be set by formal power analysis with humane endpoints (see Vertebrate Animals).

Expected outcomes. A dose/schedule achieving ≥1.5–2 $\log_{10}$, ideally durable, fecal CR-*Kp* reduction; a defined stool exposure–response relationship; evidence that escape is infrequent and attenuated/partner-sensitive; and preserved commensal diversity versus comparator.

Pitfalls & alternatives. Gastric inactivation may lower delivered titer; we will test buffering/encapsulation and adjust dosing to meet the pharmacodynamic target. If suppression is incomplete — consistent with frequent reduction-without-eradication in the field (Fang et al., 2024)

— we will optimize composition/dosing intensity and define burden reduction, not sterilization, as the translational endpoint.

Aim 3 — Monitored proof-of-concept clinical decolonization under an eIND framework

Rationale. Human oral-phage decolonization is unproven; prior clinical phage use has been largely compassionate, with sparse pharmacokinetic and safety data (Fang et al., 2024), and the only registered CRE/VRE decolonization program uses a non-phage consortium (NCT07525089). A small, rigorously monitored study can establish safety/tolerability and generate the human exposure data needed to design an efficacy trial.

Design. Consenting adult CR-*Kp* gut carriers in high-risk settings will receive an isolate-matched oral cocktail (selected via the Aim 1 pipeline; lots characterized as in Aim 1) under FDA expanded-access/emergency IND authorization, with full IRB oversight, an independent DSMB, and pre-specified stopping rules. **Primary objective:** safety/tolerability. **Secondary objectives:** change in intestinal CR-*Kp* burden, stool phage pharmacokinetics, emergence/phenotype of escape isolates, and microbiome preservation by sequencing. Enrollment [ILLUSTRATIVE] and treatment duration [ILLUSTRATIVE] will be finalized with regulatory and statistical input. FDA pre-submission interaction and IRB protocol development (Years 2–4) precede first dosing to de-risk timing.

Expected outcomes. A documented safety/tolerability profile, human stool pharmacokinetics relatable to the Aim 2 exposure–response model, a preliminary decolonization signal, and confirmation of microbiome sparing — collectively de-risking a future randomized efficacy trial.

Pitfalls & alternatives. Per-patient matching may delay treatment; the Aim 1 pipeline and pre-vetted lead cocktails mitigate this, and uncovered isolates are not enrolled. If FDA interactions or manufacturing extend timelines, Aim 3 narrows to a first-patients safety/pharmacokinetic cohort while the efficacy design is deferred to the follow-on trial. If decolonization is partial, the study still delivers its primary safety and pharmacokinetic deliverables.

Timeline

[ILLUSTRATIVE] Years 1–2: Aim 1 PhageBank curation, genomic vetting, matching-pipeline validation; begin Aim 2 model setup. [ILLUSTRATIVE] Years 2–4: Aim 2 dose/schedule, resistance, and microbiome studies; parallel eIND-enabling lot characterization, FDA pre-submission interactions, and IRB protocol development. [ILLUSTRATIVE] Years 4–5: Aim 3 proof-of-concept clinical study, data analysis, and design of a follow-on efficacy trial. Go/no-go gates at the Aim 1 → 2 and Aim 2 → 3 transitions govern progression.

Budget Justification (modular R01-style sketch)

This is a modular R01 request of [ILLUSTRATIVE] $250,000 direct costs/year for [ILLUSTRATIVE] 5 years. **Personnel:** PI ([ILLUSTRATIVE] 2.4 calendar months); co-investigators in phage biology/microbial genomics and infectious diseases/clinical trials; a microbiome bioinformatician; [ILLUSTRATIVE] 2 postdoctoral scientists; a research technician; and a study coordinator for Aim 3. **Other than personnel:** sequencing (phage genomes, isolate typing, 16S/shotgun metagenomics); gnotobiotic and conventional murine studies (germ-free husbandry, per-diem); clinical-grade phage lot preparation and release testing for Aim 3; eIND regulatory and DSMB costs; clinical assays and patient-related costs; publication and data sharing. Equipment is assumed available through the host environment. Animal and clinical module sizes are [ILLUSTRATIVE] pending power analyses and regulatory input.

Rigor, Reproducibility, and Sex as a Biological Variable

Scientific premise rests on independently reproduced murine decolonization findings (Rotman et al., 2024; Tan et al., 2022) and a 56-study systematic review (Fang et al., 2024). Rigor is enforced by genomic authentication of all phages and isolates, pre-registered acceptance/go-no-go criteria, biological replication, blinded/randomized animal allocation, vehicle and **replication-incapable cocktail** controls, and ARRIVE-compliant reporting. Both sexes are included in Aim 2 and analyzed for sex-based differences; Aim 3 enrolls without sex restriction. Data and protocols will be shared per NIH policy.

Vertebrate Animals

Murine gut-carriage models (gnotobiotic and conventionally colonized) are required because intestinal colonization resistance and in vivo phage pharmacology cannot be reproduced in vitro. We will use the minimum number of animals justified by formal power analysis [ILLUSTRATIVE], with defined humane endpoints, oral (non-surgical) dosing, analgesia as indicated, and euthanasia per AVMA guidelines. Both sexes will be used. All procedures will be IACUC-approved before initiation; design and reporting follow ARRIVE guidelines, consistent with the murine decolonization models of Rotman et al. (2024) and Tan et al. (2022).

Human Subjects / Clinical Trial

Aim 3 is a small, prospective, single-arm proof-of-concept clinical decolonization study in consenting adult CR-*Kp* gut carriers. Because isolate-matched lytic phage products are investigational and

individualized, treatment will proceed under the FDA **expanded-access / emergency investigational new drug (eIND)** pathway — the established US route for personalized phage therapy — with formal FDA pre-submission interaction. The study operates under **IRB approval** with documented informed consent, an independent **DSMB**, and pre-specified stopping rules. The **primary endpoint is safety/tolerability**; secondary endpoints include intestinal CR-*Kp* burden change, stool phage pharmacokinetics, escape-variant emergence/phenotype, and microbiome preservation. Enrollment and duration are [ILLUSTRATIVE] pending statistical and regulatory finalization. Unlike the only registered CRE/VRE gut-decolonization program, which evaluates a defined live-microorganism consortium rather than phages (NCT07525089), this study tests an oral *phage* product and adds rigorous pharmacokinetic and microbiome monitoring.

Team & Environment

- **Principal Investigator [NAME, INSTITUTION]** — infectious diseases / phage therapy; overall direction and regulatory strategy.
- **Co-Investigator, Phage Biology & Rational Cocktail Design [NAME, INSTITUTION]** — PhageBank, receptor mapping, matching pipeline.
- **Co-Investigator, Microbial Genomics / Bioinformatics [NAME, INSTITUTION]** — phage and isolate sequencing, microbiome metagenomics.
- **Co-Investigator, Clinical Trials / Hospital Epidemiology [NAME, INSTITUTION]** — Aim 3 conduct, eIND/IRB, DSMB liaison.
- **Collaborators / Resources [NAMES, INSTITUTIONS]** — a US clinical phage center with eIND and clinical-grade production experience for Aim 3.
- **Environment** — gnotobiotic animal facility, BSL-2 microbiology, high-throughput sequencing core, clinical-grade phage production capacity, and an academic medical center with a CRE-carrier patient population and clinical-trials infrastructure.

References

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2. Fang Q, et al. Safety and efficacy of phage application in bacterial decolonisation: a systematic review. *Lancet Microbe.* 2024. PMID 38452780. https://pubmed.ncbi.nlm.nih.gov/38452780/
3. Tan D, et al. Decolonization of carbapenem-resistant *Klebsiella pneumoniae* from intestinal microbiota of mice by phages targeting two surface structures. *Front Microbiol.* 2022;13:877074. https://pmc.ncbi.nlm.nih.gov/articles/PMC9441799/

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