Personalized, Depolymerase-Armed Bacteriophage Cocktails to Treat Carbapenem-Resistant *Acinetobacter baumannii*: Rapid Capsule-Typed Host-Range Matching, Phage–Antibiotic Synergy, and an eIND-Ready Translational Path

Project Summary / Abstract

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a top-tier antimicrobial-resistance priority pathogen. As an opportunistic, frequently extensively drug-resistant (XDR) nosocomial organism, it causes ventilator-associated pneumonia, bloodstream infections, and wound, burn, and device-related infections in critically ill ICU patients, often leaving only colistin/polymyxin or sulbactam–durlobactam as last-line therapy — agents constrained by toxicity and emerging resistance. Lytic bacteriophages kill bacteria through a mechanism orthogonal to antibiotics, so carbapenem or colistin resistance does not confer phage resistance. Many *A. baumannii* phages additionally encode capsule depolymerases that enzymatically strip the protective capsular polysaccharide, dismantle biofilm, and re-sensitize the organism to antibiotics and to host serum/immune killing. The first modern Western phage-therapy success was a disseminated XDR *A. baumannii* infection cleared with a personalized 9-phage cocktail delivered intravenously and percutaneously, with documented additive activity between the phage cocktail and sub-lethal minocycline (Schooley et al., 2017) — establishing CRAB as the prototype indication for personalized phage cocktails.

This proposal develops and rigorously characterizes personalized, depolymerase-armed phage cocktails for CRAB across three aims. We will (1) build a capsule-typed, genomically vetted phage bank and a rapid host-range matching workflow that nominates multi-phage cocktails against patient isolates within a clinically actionable window; (2) define phage–antibiotic synergy (PAS) and phage-resistance trade-offs in vitro, in biofilm, and in a murine CRAB bloodstream model, prioritizing depolymerase-driven potentiation of colistin/polymyxin B; and (3) establish release specifications, a matching standard operating procedure (SOP), and a prospective compassionate-use case series under

FDA single-patient emergency IND (eIND). Each aim carries pre-specified, quantitative go/no-go gates. The work advances the near-term goal of moving CRAB phage therapy from heroic last-resort rescue toward a stocked, antibiogram-style ICU formulary.

Specific Aims

CRAB causes life-threatening, often XDR infections in ICU patients for whom antibiotic options are nearly exhausted. Bacteriophages kill by a mechanism orthogonal to antibiotics, and many *A. baumannii* phages encode capsule depolymerases that strip capsular polysaccharide, dismantle biofilm, and re-sensitize the organism to antibiotics and immunity. Personalized phage cocktails have already cleared disseminated XDR *A. baumannii* in compassionate-use settings, with phage activity potentiated by sub-lethal antibiotic (Schooley et al., 2017; Aslam et al., 2020). Yet the field lacks standardized capsule-typed host-range matching, mechanistic characterization of phage–antibiotic synergy specific to CRAB, and a disciplined translational path. We propose three aims to close these gaps.

Aim 1 — Build a capsule-typed CRAB phage bank and a rapid host-range matching workflow. We will assemble and whole-genome–sequence a panel of lytic *A. baumannii* phages, annotate capsule depolymerases, and confirm absence of integrase, known toxin, and antibiotic-resistance genes. Against a contemporary CRAB clinical-isolate collection, we will capsule (K-) type isolates and run standardized host-range screening (spot assays + quantitative efficiency-of-plating, EOP) to map coverage. A matching algorithm will nominate multi-phage cocktails that maximize coverage while combining complementary receptors to suppress single-step phage-resistant escape. *Go/no-go:* a 2–4-phage cocktail covers ≥70% of contemporary isolates (EOP ≥0.1) **[ILLUSTRATIVE threshold]**.

Aim 2 — Define phage–antibiotic synergy and phage-resistance trade-offs. Using matched cocktails from Aim 1, we will quantify, in vitro (planktonic + biofilm) and in a murine CRAB bloodstream model, whether depolymerase-armed phages potentiate colistin/polymyxin B and sulbactam–durlobactam, and whether phage-resistant escape forces fitness-costly capsule loss that re-sensitizes the organism to serum and antibiotics. *Go/no-go:* ≥1 cocktail–antibiotic pair achieves synergy (FIC index ≤0.5 or ≥2-log time-kill advantage over the better single agent) and a statistically significant survival or bacterial-burden benefit in vivo **[ILLUSTRATIVE]**.

Aim 3 — Establish the translational and regulatory framework for a compassionate-use case series. We will define release specifications (titer, sterility, endotoxin limits), a host-range-matching SOP, and a prospective clinical data-capture protocol, then treat a small number of CRAB-infected patients under FDA single-patient eIND with IRB oversight — capturing safety, microbiologic clearance, clinical response, and serial isolates for emergent-resistance monitoring. *Deliverable gate:*

a finalized, reproducible eIND/matching package usable to power a subsequent controlled trial, independent of accrual rate.

Impact: By coupling rapid capsule-typed matching with mechanistically defined phage–antibiotic synergy and a disciplined eIND pathway, this work lays the evidentiary and operational foundation for CRAB phage therapy to graduate from last-resort rescue toward a stocked ICU formulary.

Significance

CRAB is among the highest-priority antimicrobial-resistance threats recognized by the WHO and CDC. It is an opportunistic, frequently XDR nosocomial pathogen responsible for ventilator-associated pneumonia, bloodstream infections, wound and burn infections, and device-related infections in ICU patients, where therapeutic options are often reduced to colistin/polymyxin or sulbactam–durlobactam. Both last-line strategies face dose-limiting toxicity and emerging resistance, defining an urgent unmet need squarely within NIAID's antimicrobial-resistance remit and directly relevant to combat-wound *Acinetobacter* of interest to the DoD.

Bacteriophages are uniquely suited to this target for three reasons. First, lytic phages kill through a mechanism orthogonal to antibiotics, so resistance to carbapenems or colistin does not confer phage cross-resistance. Second, many *A. baumannii* phages encode **capsule depolymerases** that degrade capsular polysaccharide — both enabling phage adsorption and dismantling biofilm that shields bacteria on catheters, ventilators, and abscess walls. A phage-derived depolymerase (Dpo71) has been shown to strip the *A. baumannii* capsule, destabilize the outer membrane, potentiate **colistin**, and re-sensitize MDR strains to serum/host-immune killing (Chen et al., 2022) — the mechanistic crux of our adjuvant strategy. Third, **antibiotic potentiation of phage** is clinically observed: the index disseminated XDR *A. baumannii* case documented additive activity between the personalized phage cocktail and sub-lethal minocycline (Schooley et al., 2017).

The clinical proof of concept is established but narrow. The 2016 UC San Diego case used IV and percutaneous personalized 9-phage cocktails (Navy/DoD-derived) to clear a disseminated XDR *A. baumannii* infection (Schooley et al., 2017). UC San Diego's IPATH subsequently reported its first 10 consecutive IV phage cases, of which **2 were *A. baumannii*** (one full recovery, one uninterpretable owing to comorbidity), with phage resistance emerging in 3 of 10 cases and overcome by introducing new phages, and phage-neutralizing serum noted in 1 case (Aslam et al., 2020). Most recently, a single CRAB-bloodstream-infection phage (vB_AbaP_CV1) combined with polymyxin B/colistin showed checkerboard and time-kill synergy and superior outcomes versus monotherapy in a BALB/c tail-vein (bloodstream) model (Wang et al., 2026), directly validating phage+polymyxin combinations in vivo for CRAB bacteremia.

Despite these advances, CRAB phage therapy remains predominantly investigational/compassionate-use under single-patient eIND, and no controlled efficacy trial specific to CRAB has reported. The field needs standardized capsule-typed host-range matching, rigorous mechanistic characterization of phage–antibiotic synergy, and a disciplined translational path. This proposal addresses each, generating the data and operational infrastructure to advance CRAB phage therapy toward a controlled, formulary-style intervention.

Innovation

This proposal is innovative in four respects, each tied to a specific published precedent.

- **Rapid, capsule-typed host-range matching as a standardized workflow.** We operationalize K-type–informed matching as a diagnostic-to-therapeutic pipeline returning a candidate cocktail within hours of a positive culture, rather than the ad hoc, weeks-long rescue procedures behind prior compassionate-use successes (Schooley et al., 2017; Aslam et al., 2020).
- **Depolymerase-armed phages as the mechanistic centerpiece.** We deliberately exploit capsule degradation to dismantle biofilm and drive synergy with colistin/polymyxin, extending the demonstration that a depolymerase strips the capsule, potentiates colistin, and restores serum killing (Chen et al., 2022) from a single isolated enzyme to a banked, phage-delivered, cocktail-scale strategy.
- **Phage-resistance evolution as a therapeutic lever.** Because phage-resistant escape often forces *A. baumannii* to shed or alter its capsule at a fitness cost, the design combines complementary-receptor phages with antibiotics to convert resistance into an exploitable trade-off (re-sensitization to serum/colistin; Chen et al., 2022), rather than treating resistance only as a failure mode (Aslam et al., 2020).
- **Mechanistic science fused to a disciplined eIND path.** Release specifications, the matching SOP, and combination regimens are defined prospectively — anchored to an in vivo phage+polymyxin efficacy benchmark for CRAB bacteremia (Wang et al., 2026) — rather than reconstructed after the fact.

Approach

Overarching design & rigor. All in vitro endpoints use biological and technical replicates with pre-registered analysis plans; synergy is defined a priori (fractional inhibitory concentration index ≤0.5; or ≥2-log10 CFU reduction over the more active single agent in time-kill). In vivo studies use predetermined group sizes from power analysis (Aim 2), randomization, and blinded enumeration. Each Aim carries explicit go/no-go gates (Specific Aims). Sex as a biological variable will be

incorporated in animal studies. We report negative and isolate-/K-type–dependent results.

Aim 1 — Capsule-typed CRAB phage bank and rapid host-range matching workflow

Rationale. Therapeutic *A. baumannii* phages are highly strain-specific, frequently binding the capsule (K-type), so cocktails must be matched to a patient's isolate and combined to broaden coverage and suppress escape mutants (Schooley et al., 2017; Aslam et al., 2020). A standardized, genomically vetted bank is the prerequisite for everything downstream.

Experimental design. We will isolate and acquire lytic *A. baumannii* phages, propagate them on safe production hosts, and perform whole-genome sequencing to (i) annotate capsule-depolymerase genes, (ii) confirm strictly lytic lifestyle, and (iii) exclude integrase, known toxin, and antibiotic-resistance genes. A contemporary CRAB clinical-isolate collection will be capsule (K-) typed (genomic K-locus typing) and screened by standardized spot assays and quantitative EOP to build a phage×isolate coverage matrix. A transparent matching algorithm will nominate 2–4-phage cocktails that maximize coverage while requiring ≥2 distinct receptor classes per cocktail to limit single-step resistance. We will pilot a rapid-turnaround path (isolate in → candidate cocktail out) and benchmark the wall-clock time.

Expected outcomes. A genomically characterized, capsule-typed phage bank; a validated matching workflow; and coverage statistics across contemporary CRAB isolates, including the fraction matchable by a 2–4-phage cocktail and the turnaround time achieved.

Potential pitfalls & alternatives. Some isolates may be unmatchable with the existing bank; we will expand by targeted isolation against gap isolates and, where lytic coverage fails, prioritize depolymerase candidates as enzybiotic adjuvants (Chen et al., 2022). If lytic activity is weak, sub-lethal antibiotic conditioning (Aim 2) may enhance propagation. If K-typing throughput limits screening, we will triage by prevalence-weighted K-types first.

Aim 2 — Phage–antibiotic synergy and phage-resistance trade-offs

Rationale. Capsule depolymerase activity destabilizes the outer membrane and improves colistin binding and serum killing (Chen et al., 2022); sub-lethal antibiotic can potentiate phage activity (Schooley et al., 2017); and phage+polymyxin combinations reduce CRAB burden in vivo (Wang et al., 2026). Combinations therefore plausibly outperform either agent alone. Separately, phage-resistant escape frequently incurs a capsule-associated fitness cost that re-sensitizes the strain to serum and antibiotics — a trade-off worth quantifying.

Experimental design. Using matched cocktails from Aim 1, we will run checkerboard and time-kill

assays combining phages with colistin/polymyxin B and with sulbactam–durlobactam against representative CRAB isolates spanning multiple K-types, including static and dynamic biofilm models on abiotic surfaces. We will isolate phage-resistant mutants, characterize capsule status (genotype + phenotype), and assay their susceptibility to serum killing and to antibiotics to test the re-sensitization hypothesis (Chen et al., 2022). Leading combinations will be evaluated in a **murine CRAB bloodstream/systemic challenge model**, comparing phage alone, antibiotic alone, and the combination on bacterial burden and survival — directly mirroring and extending the BALB/c tail-vein phage+polymyxin benchmark (Wang et al., 2026).

Expected outcomes. Quantitative synergy profiles (FIC, time-kill) identifying combinations that beat monotherapy; evidence that depolymerase-armed phages potentiate polymyxins and disrupt biofilm; and a determination of whether phage resistance forces capsule loss with antibiotic/serum re-sensitization.

Potential pitfalls & alternatives. Synergy may be isolate- and K-type-dependent; we will test across multiple K-types and report the conditions under which synergy holds rather than a single point estimate. If in vivo phage neutralization or pharmacokinetics limit monotherapy efficacy — as anticipated from neutralizing-serum observations in compassionate use (Aslam et al., 2020) — we will emphasize combination regimens where antibiotics suppress residual escape mutants, and adjust dose/route. Animal-model variability will be mitigated by power-based group sizes, randomization, blinding, and predefined humane endpoints.

Aim 3 — Translational and regulatory framework for a compassionate-use case series

Rationale. Clinical CRAB phage therapy remains predominantly investigational/compassionate-use under single-patient eIND (Schooley et al., 2017; Aslam et al., 2020). A disciplined framework — defined specifications plus prospective data capture — is needed to convert anecdotal rescue into trial-enabling evidence.

Experimental design. We will define release specifications (titer, sterility, endotoxin limits), a host-range-matching SOP linking the patient isolate to a selected cocktail, and a clinical data-capture protocol (safety, microbiologic clearance, clinical response). Working with the IRB and FDA, we will prospectively treat a small number of CRAB-infected patients under single-patient eIND, with cocktails matched per Aim 1 and, where appropriate, combined with antibiotics informed by Aim 2 — mirroring the personalized IV/percutaneous approach of the index case (Schooley et al., 2017) and the single-center IV case series reported by IPATH (Aslam et al., 2020). Serial isolates will be banked to monitor microbiologic response and emergent phage resistance, which arose in 3 of 10 prior IV cases (Aslam et al., 2020).

Expected outcomes. A documented, reproducible eIND pathway and matching SOP; preliminary safety and microbiologic/clinical outcome data from a CRAB case series; and a protocol scaffold to power a future controlled trial.

Potential pitfalls & alternatives. Compassionate-use cohorts are heterogeneous, concurrent antibiotics confound attribution, and some outcomes are uninterpretable — as IPATH explicitly observed (Aslam et al., 2020). We will pre-specify microbiologic endpoints, collect serial isolates, and treat clinical response as supportive rather than primary. If clinical accrual is slow, the finalized specifications and SOP remain a stand-alone, trial-de-risking deliverable. Manufacturing constraints will be addressed by prioritizing bank phages with robust, well-characterized production.

Timeline

Year 1 [ILLUSTRATIVE]: Assemble and sequence the phage bank; annotate depolymerases; capsule-type the isolate collection; establish host-range screen (Aim 1). **Years 2–3 [ILLUSTRATIVE]:** Finalize matching workflow and coverage benchmarking (Aim 1); execute in vitro synergy, biofilm, and resistance-trade-off studies (Aim 2). **Years 3–4 [ILLUSTRATIVE]:** Murine combination-efficacy studies (Aim 2); finalize release specifications, matching SOP, and eIND/IRB framework (Aim 3). **Years 4–5 [ILLUSTRATIVE]:** Prospective compassionate-use case series under eIND; analysis and trial-protocol scaffolding (Aim 3). Go/no-go gates are evaluated at the end of Years 1 (Aim 1 coverage), 3 (Aim 2 synergy/in vivo), and 4 (eIND package).

Budget Justification (modular R01-style sketch)

This is a modular R01 request of approximately **$250,000 direct costs per year [ILLUSTRATIVE]** for **5 years [ILLUSTRATIVE]**. **Personnel:** PI (microbiology/phage biology) at ~~**2.4 person-months [ILLUSTRATIVE]**~~; ~~Co-Investigators in infectious diseases and clinical microbiology (~~**1.2 person-months each [ILLUSTRATIVE]**); one postdoctoral scientist and one research technologist (**12 person-months each [ILLUSTRATIVE]**) for phage isolation, genomics, and assays; partial study-coordinator effort in Years 4–5 [ILLUSTRATIVE] for the case series. **Supplies/services:** whole-genome sequencing, media and consumables, antibiotics (colistin, polymyxin B, sulbactam–durlobactam), biofilm-assay materials, and endotoxin/sterility testing. **Animal costs:** per-diems and procedures for the murine CRAB bloodstream model (Aim 2). **Other:** GMP-aligned production/quality testing for clinical-grade cocktails and regulatory/eIND submission costs (Aim 3). Final figures are placeholders to be set with institutional budgeting.

Vertebrate Animals

Animal work is proposed in Aim 2. We will use a **murine CRAB bloodstream/systemic challenge model** to compare phage alone, antibiotic alone, and combination regimens on bacterial burden and survival. *Justification:* in vivo testing is required to establish phage–antibiotic synergy and pharmacologic relevance not captured in vitro; a BALB/c tail-vein CRAB model has demonstrated superior outcomes for phage+polymyxin B combinations versus monotherapy (Wang et al., 2026), providing a validated benchmark for our design. (Mechanistic depolymerase/colistin re-sensitization data to date derive from a *Galleria mellonella* invertebrate model and in vitro serum-killing assays (Chen et al., 2022); the murine model is therefore needed to extend those findings to a mammalian host.) Group sizes will be the minimum required for statistical rigor, set by formal power analysis, with randomization, blinded enumeration, predefined humane endpoints, and analgesia/anesthesia and euthanasia per approved IACUC protocol. Specific species/strain, numbers, and endpoints will be finalized with the IACUC. Approximate animal numbers and group sizes are **[ILLUSTRATIVE]** until protocol approval.

Human Subjects / Clinical Trial

Aim 3 involves human subjects in a small prospective compassionate-use case series. Investigational phage cocktails will be administered under the **FDA single-patient emergency IND (eIND)** pathway — the current regulatory route for individualized phage therapy in the United States — with full **IRB oversight**, informed consent, and predefined safety and microbiologic/clinical endpoints. This personalized, isolate-matched approach follows the index UC San Diego case (Schooley et al., 2017) and the IPATH single-center IV case series (Aslam et al., 2020). Enrollment is anticipated to be a small number of CRAB-infected patients **[ILLUSTRATIVE]**; because phage resistance emerged in 3 of 10 prior IV cases and treatment failure can occur despite in vitro susceptibility (Aslam et al., 2020), serial isolates will be collected to monitor microbiologic response and emergent resistance, and concurrent-antibiotic confounding will be documented in analysis. This work is intended to inform, not substitute for, a future controlled trial.

Team & Environment

- **Principal Investigator [NAME, INSTITUTION]** — phage biology/microbiology; overall direction, bank development, and genomics (Aim 1).
- **Co-Investigator, Infectious Diseases [NAME, INSTITUTION]** — clinical lead for eIND/IRB strategy and the case series (Aim 3).
- **Co-Investigator, Clinical Microbiology [NAME, INSTITUTION]** — capsule typing, host-range screening, susceptibility testing.

- **Co-Investigator, Antimicrobial Pharmacology / In Vivo Models [NAME, INSTITUTION]** — synergy assays and murine efficacy (Aim 2).
- **Collaborators / Resources [TO FILL]** — established US phage-therapy infrastructure and DoD-derived phage expertise (e.g., IPATH at UC San Diego; Walter Reed Army Institute of Research / Naval Medical Research Center phage program) and a clinical-grade phage-banking partner. *Environment:* BSL-2 microbiology and genomics cores, an AAALAC-accredited animal facility, a CLIA clinical-microbiology laboratory, and institutional IRB/IACUC and regulatory-affairs support.

Preliminary data: this proposal is written as concept-stage; where the assembled team holds pilot phage-isolation, K-typing, or synergy data, it should be inserted to strengthen feasibility for each Aim **[ILLUSTRATIVE]**.

References

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