Disarming the Vegetation: Adjunctive Intravenous Bacteriophage-Antibiotic Therapy for *Staphylococcus aureus* and *Enterococcus faecalis* Native and Prosthetic-Valve Endocarditis

Funder / Mechanism: NHLBI · R01 (Research Project Grant) — cardiovascular infection, with NIAID antimicrobial-resistance co-interest.

Project Summary / Abstract

Infective endocarditis (IE) caused by *Staphylococcus aureus* and *Enterococcus faecalis* remains among the deadliest cardiovascular infections, with in-hospital mortality on the order of 20–30% despite weeks of high-dose intravenous antibiotics and frequent valve surgery. The core obstacle is biological: these organisms build dense biofilm vegetations on native and prosthetic valves, where antibiotic penetration is poor and metabolically dormant "persister" cells survive to drive relapse and persistent bacteremia. Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE) narrow already limited options. Lytic bacteriophages are a rational adjunct: they self-amplify at the infection focus, penetrate and disrupt biofilm matrix (often via depolymerases), and kill through a mechanism orthogonal to antibiotics, so antibiotic cross-resistance does not apply. Phage-antibiotic synergy (PAS) is repeatedly observed, including reviewed enterococcal prosthetic-valve biofilm models in which a lytic phage combined with a beta-lactam outperforms either agent alone (Hetta et al., 2023). Clinical safety evidence is strongest for *S. aureus*: an Australian single-arm study of intravenous AB-SA01 in severe *S. aureus* infection — including bacteremia and endocarditis — reported no major adverse events (Petrovic Fabijan et al., 2020); adjunctive IV phage produced rapid blood-culture clearance in a *S. aureus* prosthetic-valve endocarditis case (Gilbey et al., 2019); and the randomized, double-blind, placebo-controlled diSArm Phase 2a trial of IV AP-SA02 in complicated *S. aureus* bacteremia reported a day-12 clinical response of 88% versus 58% with placebo (Miller et al., 2026). Enterococcal evidence remains preclinical. This proposal advances a precision phage-antibiotic strategy for valve IE by (1) building and characterizing matched anti-staphylococcal and anti-enterococcal cocktails against contemporary IE isolates; (2) defining PAS and biofilm-

eradication pharmacodynamics on prosthetic-valve biomaterials; and (3) executing a small randomized adjunctive-IV-phage trial under an FDA expanded-access/emergency IND framework. The work directly serves NHLBI's cardiovascular mission with NIAID antimicrobial-resistance relevance.

Specific Aims

Infective endocarditis on native and prosthetic valves is fundamentally a biofilm disease: even with optimal antibiotics, relapse and surgical referral are common because drug-tolerant persisters survive within the vegetation matrix. Bacteriophages offer a complementary, self-amplifying, biofilm-active killing mechanism, and early clinical data in *S. aureus* bacteremia and endocarditis support safety and a promising efficacy signal, while enterococcal evidence remains preclinical. We will systematically build, characterize, and clinically test matched phage cocktails as adjuncts to standard antibiotics for *S. aureus* and *E. faecalis* IE.

Central hypothesis: Adding host-range-matched lytic phage cocktails to best-available antibiotics will degrade valve-vegetation biofilm, kill persister and resistant subpopulations, and accelerate blood-culture clearance without compromising safety.

Aim 1 — Assemble and characterize precision phage cocktails against contemporary IE isolates. We will curate a banked panel of clinical *S. aureus* (including MRSA) and *E. faecalis* (including VRE) bloodstream/explanted-valve isolates and host-range-match lytic phages to assemble two cocktails on a GMP-trackable path. We will define receptor usage, host range, lytic kinetics, and resistance-emergence frequency, prioritizing phages encoding depolymerases and confirming a strictly lytic, toxin-/resistance-gene-free genotype. *Go/no-go (end of Year 2):* each cocktail covers a pre-specified majority of banked isolates [ILLUSTRATIVE: ≥80%] with a validated host-range assay.

Aim 2 — Define phage-antibiotic synergy and biofilm eradication on prosthetic-valve biomaterials. Using checkerboard, time-kill, and *in vitro*/*ex vivo* valve-biomaterial and pericardial-patch biofilm models, we will quantify phage-plus-antibiotic activity (anti-staphylococcal cocktail with cefazolin/vancomycin; anti-enterococcal cocktail with ceftriaxone) against planktonic, biofilm-embedded, and persister populations, across dose ratios and phage-first versus simultaneous sequencing. *Go/no-go:* identify ≥1 combination per organism achieving significantly greater biofilm-viable-count reduction than antibiotic alone while suppressing resistance, defining the dosing/pairing rules carried into Aim 3.

Aim 3 — Conduct a randomized adjunctive IV phage cocktail trial in *S. aureus*/*E. faecalis* IE. We will perform a small randomized, double-blind, placebo-controlled trial of IV cocktail plus best-

available antibiotics versus placebo plus antibiotics in adults with definite IE, with blood-culture clearance and safety as co-primary endpoints and day-12 clinical response, relapse, valve-surgery requirement, and mortality as secondary endpoints, under an FDA expanded-access/emergency IND (eIND) pathway with central IRB and DSMB oversight. This yields the first IE-focused efficacy and safety estimates needed to power a definitive trial.

Impact: Success would establish phage cocktails as a standardized companion therapy that strips biofilm armor from valve vegetations, reducing relapse and the need for high-risk valve replacement in the most drug-resistant IE.

Significance

IE due to *S. aureus* and *E. faecalis* carries roughly 20–30% in-hospital mortality despite prolonged high-dose IV antibiotics, and a large fraction of patients require valve surgery. Conventional therapy fails for a reason that is mechanistic and central to cardiovascular medicine: the organisms encase themselves in biofilm vegetations on native and prosthetic valves, where antibiotic penetration is poor and dormant persister cells tolerate even bactericidal drugs, seeding relapse and persistent bacteremia. MRSA and VRE compress the usable drug list further. This is squarely an NHLBI problem — valve infection, valve destruction, and valve surgery — with direct NIAID antimicrobial-resistance relevance.

Bacteriophages are mechanistically suited to this niche. They self-amplify where bacteria are densest; many anti-staphylococcal and anti-enterococcal phages encode depolymerases that degrade biofilm matrix and reach embedded, stationary-phase cells; and their killing is orthogonal to antibiotics, so resistance to one class does not confer resistance to the other (Hetta et al., 2023). Critically, the human evidence is no longer purely theoretical. IV AB-SA01 was administered safely to patients with severe *S. aureus* infection including bacteremia and endocarditis, with no major adverse events (Petrovic Fabijan et al., 2020); adjunctive IV phage produced rapid blood-culture clearance in *S. aureus* prosthetic-valve endocarditis (Gilbey et al., 2019); and the randomized diSArm Phase 2a trial of AP-SA02 in complicated *S. aureus* bacteremia reported an 88% versus 58% day-12 clinical response favoring the phage arm (Miller et al., 2026). For enterococcal IE the evidence remains preclinical, including reviewed VRE prosthetic-valve biofilm models in which a lytic phage plus a beta-lactam outperformed either agent alone (Hetta et al., 2023). A program that rigorously builds matched cocktails, defines their biofilm and synergy pharmacodynamics on valve biomaterials, and tests them clinically would fill the single largest gap blocking phage therapy from becoming a regulated adjunct for IE.

Innovation

This proposal is innovative in four respects. **First**, it targets the prosthetic and native valve vegetation directly as a biofilm compartment, using valve-biomaterial and pericardial-patch models rather than planktonic assays alone. **Second**, it pairs *S. aureus* and *E. faecalis* in one precision framework, extending the comparatively mature anti-staphylococcal clinical experience to enterococcal IE, where only preclinical data currently exist (Hetta et al., 2023). **Third**, it operationalizes phage-antibiotic synergy as an explicit design principle — exploiting sub-lethal beta-lactam effects that can resensitize otherwise resistant enterococci to companion drugs — rather than treating phage as a standalone agent. **Fourth**, it embeds rapid clinical phage microbiology (sequence the isolate, match a cocktail within days) into a randomized adjunctive-therapy trial executed through the FDA eIND pathway already used for investigational phage. We deliberately do not claim phage will replace antibiotics; the innovation is a standardized companion therapy of natural lytic phages that disarms the biofilm so conventional drugs and host immunity can finish the job.

Approach

Aim 1 — Assemble and characterize precision phage cocktails against contemporary IE isolates

Rationale. Therapeutic phages bind species- and strain-specific surface receptors (e.g., wall teichoic acid on *S. aureus*; surface polysaccharides on *E. faecalis*), so cocktails must be matched to the patient's isolate to broaden coverage and pre-empt receptor-mutation escape (Hetta et al., 2023). A defined, host-range-characterized cocktail is the prerequisite for all downstream work.

Experimental design. We will assemble a biobank of contemporary clinical *S. aureus* (MSSA/MRSA) and *E. faecalis* (including VRE) bloodstream and explanted-valve isolates [ILLUSTRATIVE: ~120 isolates per species]. Candidate lytic phages will be screened for host range by spot and efficiency-of-plating assays. We will characterize receptor usage, one-step growth/lytic kinetics, whole-genome sequencing to confirm a strictly lytic lifestyle and the absence of toxin, virulence, and antimicrobial-resistance genes, and presence of depolymerases. Resistance-emergence frequency will be measured for single phages versus multi-phage cocktails to select complementary, non-overlapping-receptor combinations.

Rigor & reproducibility. Isolates will be genotyped and provenance-tracked; assays run in biological and technical replicate with pre-registered acceptance thresholds and blinded plaque scoring; phage stocks endotoxin-tested and titer-controlled. Sex of the source patient will be recorded as a biological variable and tracked through all downstream analyses.

Expected outcomes. Two defined cocktails (anti-staphylococcal, anti-enterococcal) with documented coverage [ILLUSTRATIVE: ≥80% of banked isolates], confirmed lytic/depolymerase-positive genomes, and a reproducible host-range susceptibility assay for trial screening.

Potential pitfalls & alternatives. Coverage gaps or rapid resistance are possible; we will broaden phage diversity, add depolymerase-rich phages, and design cocktails with non-overlapping receptors. If enterococcal phage breadth is limiting, we will prioritize PAS-dependent activity (Aim 2), where a beta-lactam restores susceptibility, consistent with reviewed enterococcal models (Hetta et al., 2023).

Aim 2 — Define phage-antibiotic synergy and biofilm eradication on prosthetic-valve biomaterials

Rationale. The lethal feature of IE is the biofilm vegetation harboring persisters. Sub-lethal beta-lactams can elongate cells and boost phage replication, and reviewed VRE prosthetic-valve models show a lytic phage plus a beta-lactam yielding greater biofilm reduction than either alone (Hetta et al., 2023). Quantifying these effects on valve biomaterials defines clinical pairing and sequencing.

Experimental design. Using checkerboard and time-kill assays plus *in vitro*/*ex vivo* biofilm models on prosthetic-valve materials and pericardial-patch substrates, we will test cocktails alone and combined with standard antibiotics (anti-staphylococcal cocktail with cefazolin/vancomycin; anti-enterococcal cocktail with ceftriaxone). Endpoints include planktonic and biofilm viable counts, persister survival, matrix disruption, and resistance emergence under combination versus monotherapy, across dose ratios and phage-first versus simultaneous sequencing.

Rigor & reproducibility. Synergy will be scored by predefined criteria; biofilm assays validated under both static and flow/dynamic conditions to mitigate static-model artifacts; multiple strains per species (including MRSA/VRE) tested to assess strain dependence; analyses powered and pre-specified.

Expected outcomes. Quantified PAS with combinations achieving greater biofilm-vegetation reduction than antibiotics alone, plus preferred pairings/sequencing and dosing rules to carry into Aim 3.

Potential pitfalls & alternatives. Antagonism or strain-dependent synergy may occur; we will screen multiple antibiotic partners and ratios and select per-isolate combinations. An optional small-animal IE model may confirm top combinations *in vivo* if institutionally approved (see Vertebrate Animals).

Aim 3 — Conduct a randomized adjunctive IV phage cocktail trial in *S. aureus*/*E. faecalis* IE

Rationale. Safety of IV phage in severe *S. aureus* infection including endocarditis is established (Petrovic Fabijan et al., 2020; Gilbey et al., 2019), and randomized AP-SA02 data in complicated *S. aureus* bacteremia show a promising efficacy signal (Miller et al., 2026). No IE-dedicated randomized trial exists, and none for enterococcal IE.

Experimental design. A randomized, double-blind, placebo-controlled trial of IV cocktail (dosed per Aim 2; administration schedule informed by prior IV phage regimens such as AB-SA01) plus best-available antibiotics versus placebo plus antibiotics, in adults with definite *S. aureus* or *E. faecalis* IE [ILLUSTRATIVE: ~40–60 participants across two strata]. Isolates undergo Aim 1 host-range matching before enrollment. **Co-primary endpoints:** blood-culture clearance and safety. **Secondary:** day-12 clinical response (per diSArm), relapse, valve-surgery requirement, and mortality. Conducted under an FDA expanded-access/emergency IND (eIND) framework with central IRB oversight and an independent DSMB; sex/gender and minority representation will be tracked and reported.

Expected outcomes. Preliminary efficacy and safety estimates for adjunctive IV phage in valve IE sufficient to design a definitive Phase 3 trial.

Potential pitfalls & alternatives. Enterococcal IE enrollment may be slow and matched phage may be unavailable for some isolates; we will permit personalized single-patient eIND expanded access, allow stratum-specific stopping rules, and weight enrollment toward *S. aureus*, where the evidence base and cocktail breadth are strongest. If accrual lags, the trial defaults to a *S. aureus*-primary design with enterococcal IE as an exploratory stratum.

Timeline

[ILLUSTRATIVE] **Years 1–2:** Aim 1 isolate banking, phage characterization, cocktail assembly; begin Aim 2; Aim 1 go/no-go. **Years 2–3:** Aim 2 PAS/biofilm pharmacodynamics; eIND/IRB preparation and GMP cocktail manufacturing readiness; Aim 2 go/no-go. **Years 3–5:** Aim 3 trial conduct, analysis, and Phase 3 design. Manufacturing and regulatory milestones are front-loaded into Years 1–3 so that a release-tested clinical cocktail and active eIND precede first enrollment.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] We request modular direct costs of [ILLUSTRATIVE: $250,000/year for 5 years]. **Personnel:** PD/PI (cardiology/infectious disease), co-investigator phage microbiologist, clinical-trial coordinator, research technician, and biostatistician effort. **Other significant costs:**

clinical isolate banking and sequencing; phage characterization and host-range assays; valve-biomaterial/biofilm modeling; GMP-grade cocktail manufacturing and release testing for Aim 3; eIND/IRB/DSMB and trial monitoring; participant clinical/microbiology costs. Years 3–5 shift toward trial conduct and manufacturing. No equipment over the modular cap is anticipated.

Vertebrate Animals

[ILLUSTRATIVE] An optional confirmatory rabbit or rat infective-endocarditis model may be incorporated in Aim 2 to validate the highest-performing phage-antibiotic combinations *in vivo* prior to the trial. If included, procedures, anesthesia/analgesia, humane endpoints, and IACUC approval will follow institutional policy, and animal numbers will be justified by power analysis. If *in vitro*/*ex vivo* valve-biomaterial models prove sufficient, animal work will not be conducted.

Human Subjects / Clinical Trial

Aim 3 is an interventional, randomized, double-blind, placebo-controlled clinical trial in adults with definite *S. aureus* or *E. faecalis* infective endocarditis. Investigational phage cocktails will be administered intravenously as adjuncts to best-available antibiotic therapy under an FDA expanded-access/emergency IND (eIND) pathway, with provision for single-patient expanded-access eINDs where personalized matching is required. The protocol will operate under central IRB oversight with informed consent and an independent DSMB. Safety monitoring is informed by prior IV phage experience showing no major adverse events in severe *S. aureus* infection (Petrovic Fabijan et al., 2020) and the favorable safety profile reported in the diSArm trial (Miller et al., 2026). Enrollment will include appropriate sex/gender and minority representation; isolates undergo host-range matching (Aim 1) before randomization.

Investigators & Environment

[Template — fill with real names/institutions.] **PD/PI:** [Cardiologist or infectious-disease physician-scientist] with prior leadership of bacteremia/endocarditis cohorts and investigational-IND trials. **Co-Investigators:** [Phage microbiologist / clinical phage microbiology lead] with phage-banking and host-range expertise; [Cardiothoracic surgeon] providing explanted-valve specimens and prosthetic-valve biomaterials; [Clinical pharmacologist] leading PAS/PK; [Biostatistician] for adaptive design and analysis. The assembled team is intended to span the three competencies the project requires — clinical IE trials, phage microbiology/manufacturing liaison, and valve biomaterials — so no single aim depends on a single individual. **Consultants/partners:** academic phage-therapy programs with documented IV-phage clinical experience (e.g., the Westmead Bacteriophage Therapy Team) and a

GMP phage manufacturing/release-testing partner. **Environment:** [Institution] provides BSL-2 phage and biofilm laboratories, a clinical microbiology/sequencing core, GMP or GMP-liaison manufacturing access, and an established cardiovascular clinical-trials infrastructure with IRB and DSMB support.

References

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