Topical Bacteriophage Cocktail Therapy for Biofilm-Associated Chronic Suppurative Otitis Media: An Exploratory Proof-of-Concept Study Targeting *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Project Summary / Abstract

Chronic suppurative otitis media (CSOM) is a persistent middle-ear infection defined by recurrent otorrhea through a perforated tympanic membrane and is a leading cause of acquired hearing loss in low- and middle-income countries and in disadvantaged communities worldwide. Its two dominant pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, are aggressive biofilm formers that colonize the middle-ear mucosa and ossicles, tolerate topical and systemic antibiotics, and are increasingly multidrug-resistant. Because CSOM is localized, biofilm-driven, and—uniquely—anatomically accessible through the tympanic perforation, it is an attractive target for lytic bacteriophage (phage) therapy: phages self-amplify at the infection site, penetrate biofilm via depolymerases and endolysins, and kill antibiotic-resistant strains while largely sparing the commensal microbiome. The most relevant human evidence comes from an adjacent disease: the 2009 Wright et al. randomized, double-blind, placebo-controlled Phase I/II trial of the six-phage Biophage-PA cocktail against antibiotic-resistant *P. aeruginosa* in chronic otitis (a chronic otitis externa population), which showed significant clinical improvement and reduced bacterial counts with no treatment-related adverse events. A 2025 US single-patient report documented resolution of multidrug-resistant *P. aeruginosa* chronic mastoiditis after intratympanic and parenteral custom phage, and a 2024 veterinary study developed a *P. aeruginosa*/*Staphylococcus pseudintermedius* cocktail effective in a mouse otitis externa model and canine otitis externa. No phage cocktail has been developed against the mixed *P. aeruginosa*/*S. aureus* infections that define human CSOM, and no model bridges these data to the perforated middle ear. This R21 will (1) assemble and characterize a dual-pathogen phage cocktail against contemporary CSOM clinical isolates, (2) quantify biofilm eradication and phage–antibiotic synergy in vitro and adapt an in vivo otitis model toward CSOM-relevant endpoints, and (3) define a CSOM-specific otic formulation and the US regulatory path for a future first-in-CSOM pilot. The work establishes the preclinical proof-of-concept and explicit go/no-

go criteria needed to justify a properly powered clinical trial of microbiome-sparing topical phage therapy for the chronically discharging ear.

Specific Aims

CSOM imposes a major global hearing-loss burden through *P. aeruginosa* and *S. aureus* biofilms that resist available antibiotics. The 2009 Biophage-PA trial demonstrated that a *P. aeruginosa* phage cocktail can safely reduce bacterial counts and improve chronic otitis—but in chronic otitis **externa**, addressing *P. aeruginosa* alone, and that single controlled study has never been confirmed or extended to the mixed *P. aeruginosa*/*S. aureus* infections of the perforated middle ear. We propose to build and de-risk a dual-pathogen topical phage cocktail through three exploratory aims, each with explicit success criteria.

Aim 1. Assemble and characterize a dual-pathogen phage cocktail against contemporary CSOM isolates. We will compile a panel of *P. aeruginosa* and *S. aureus* clinical isolates from CSOM otorrhea and screen banked lytic phages to select complementary, broad-host-range members. We will determine host range (efficiency-of-plating), lytic kinetics, and depolymerase/endolysin activity, and design a cocktail intended to suppress phage-resistant escape mutants. *Success criterion:* a defined cocktail with EOP-confirmed lysis of ≥70% of *P. aeruginosa* and ≥70% of *S. aureus* CSOM isolates and reduced in vitro resistance emergence versus single phages.

Aim 2. Quantify biofilm eradication and phage–antibiotic synergy in vitro and in an adapted in vivo otitis model. We will measure cocktail-mediated reduction of mono- and dual-species *P. aeruginosa*/*S. aureus* biofilms and test synergy with sub-inhibitory antibiotics, then evaluate lead formulations in an in vivo otitis model adapted toward CSOM-relevant (middle-ear, post-perforation) endpoints, building on the otitis externa model of Kwon et al. (2024). *Success criterion:* ≥2-log10 dual-species biofilm reduction in vitro, demonstrable phage–antibiotic additivity/synergy, and a statistically significant in vivo bacterial-burden reduction versus vehicle.

Aim 3. Define a CSOM-relevant otic formulation and the US regulatory translation path. We will develop a topically instillable preparation with acceptable titer stability, pH, and osmolality for the middle ear, confirm absence of cytotoxicity in otic-relevant cells, and assemble the regulatory package for a future US pilot under an FDA single-patient/expanded-access IND with IRB oversight. *Success criterion:* a stable (≥6-month), non-cytotoxic candidate formulation meeting endotoxin benchmarks plus a complete pre-IND concept package.

Impact: By generating rigorous dual-pathogen, biofilm-focused preclinical evidence with predefined go/no-go gates, this project provides the proof-of-concept foundation to advance personalized, microbiome-sparing topical phage therapy toward a properly powered CSOM clinical trial and,

ultimately, toward sparing children from progressive hearing loss.

Significance

CSOM is a persistent middle-ear infection that drives acquired hearing loss, particularly among children in resource-limited settings, with downstream effects on language, education, and development. The disease is sustained by *P. aeruginosa* and *S. aureus* biofilms that shelter on the middle-ear mucosa and ossicles, where they tolerate both topical and systemic antibiotics and increasingly carry multidrug resistance. Current management—repeated antibiotic courses and aural toilet—often yields only temporary control, and antibiotic exposure disrupts commensal flora without reliably clearing the biofilm reservoir.

Phage therapy is mechanistically well matched to this problem. Lytic phages bind strain-specific surface receptors (*P. aeruginosa* LPS, type IV pili, or flagella; *S. aureus* wall teichoic acid), replicate inside the host, and lyse it, releasing progeny that propagate through the infected ear so long as host bacteria persist. Many anti-*Pseudomonas* and anti-staphylococcal phages encode depolymerases and endolysins that degrade exopolysaccharide matrix and peptidoglycan, eroding the biofilms that make CSOM recalcitrant. Strain specificity spares commensals but demands cocktails of complementary phages, ideally with pre-treatment host-range matching, to cover heterogeneous mixed infections and suppress resistant escape mutants. Critically, the ear is anatomically reachable: the tympanic perforation permits direct topical instillation of a self-amplifying agent. This fit between an accessible, biofilm-driven, localized infection and the biology of phages is the central rationale for the project and aligns directly with NIDCD's mission to reduce the burden of communication disorders, including hearing loss from chronic ear disease. The most directly analogous human evidence—the Wright et al. (2009) controlled trial—was conducted in chronic otitis **externa** (ear canal) rather than CSOM; extending this safety and mechanistic precedent to the mixed-pathogen, middle-ear setting is the specific knowledge gap this R21 addresses.

Innovation

This project is innovative in three respects. First, it directly targets the *P. aeruginosa*/*S. aureus* pathogen pair that defines human CSOM with a single dual-pathogen cocktail, whereas the only controlled human trial to date (Wright et al., 2009) addressed *P. aeruginosa* alone in otitis externa, and the closest dual-pathogen formulation work exists only in the veterinary literature against *P. aeruginosa* and *S. pseudintermedius* (Kwon et al., 2024)—a canine, not human, staphylococcal species. Second, it centers biofilm eradication and phage–antibiotic synergy—exploiting depolymerase/endolysin activity and documented synergy between sub-inhibitory antibiotics and phages—rather than treating planktonic bacteria, matching the true recalcitrant state of CSOM. Third,

it pairs preclinical development with an explicit, CSOM-specific US regulatory and formulation strategy (otic-compatible drops; single-patient/expanded-access IND), translating compassionate-use experience such as the 2025 intratympanic chronic mastoiditis case (Casazza et al.) into a reproducible, banked-cocktail approach suitable for eventual point-of-care, susceptibility-matched personalized therapy.

Approach

Aim 1 — Assemble and characterize a dual-pathogen phage cocktail against contemporary CSOM isolates

Rationale. Effective CSOM phage therapy requires complementary phages covering heterogeneous *P. aeruginosa* and *S. aureus* strains. The Biophage-PA trial used a six-phage anti-*Pseudomonas* cocktail in otitis externa; human CSOM additionally requires anti-*S. aureus* coverage—coverage that the Kwon et al. veterinary cocktail provided only for the canine species *S. pseudintermedius* and must therefore be rebuilt for the human pathogen.

Experimental design. We will assemble a characterized panel of *P. aeruginosa* and *S. aureus* isolates from CSOM otorrhea (clinical microbiology archives and collaborators), with antibiograms recorded. Candidate lytic phages will be sourced from established banks (e.g., compassionate-use *P. aeruginosa* and *S. aureus* phage collections) and screened by spot assay and efficiency-of-plating for host range. Lead phages will be assessed for one-step growth/lytic kinetics and screened genomically and phenotypically for depolymerase and endolysin activity and for absence of lysogeny, toxin, and antibiotic-resistance genes (a safety screen for any future clinical translation). We will rationally combine complementary phages into candidate cocktails and measure suppression of resistant escape mutants in time-kill cultures.

Expected outcomes & success criterion. A defined dual-pathogen cocktail with EOP-confirmed lysis of ≥70% of *P. aeruginosa* and ≥70% of *S. aureus* CSOM isolates, documented biofilm-relevant enzymatic activity, and reduced in vitro resistance emergence relative to single phages.

Potential pitfalls & alternative approaches. Strain specificity may leave coverage gaps; we will broaden the phage panel, retain host-range matching as a selection criterion, and carry purified endolysins forward as a complementary, largely sequence-independent killing route should phage coverage prove insufficient. If banked phages underperform against contemporary isolates, environmental enrichment isolation against the CSOM panel is a fallback.

Aim 2 — Quantify biofilm eradication and phage–antibiotic synergy in vitro and in an adapted in vivo otitis model

Rationale. CSOM is biofilm-driven; clinical benefit depends on eroding mixed *P. aeruginosa*/*S. aureus* biofilms. Synergy between sub-inhibitory antibiotics and phages is well documented, and a mouse otitis **externa** model has demonstrated phage-cocktail efficacy (Kwon et al., 2024). We explicitly treat the externa-to-media gap as a scientific risk to be managed, not a solved problem.

Experimental design. We will grow mono- and dual-species biofilms on abiotic and mucosa-mimicking substrates and quantify cocktail-mediated reduction by viable counts and biomass imaging. We will test phage + sub-inhibitory antibiotic combinations for additive/synergistic biofilm clearance using checkerboard-style and time-kill designs. Lead cocktails (alone and with antibiotic) will then be evaluated in an in vivo otitis model adapted from the Kwon et al. externa system toward CSOM-relevant endpoints—middle-ear inoculation with post-perforation topical instillation where feasible—measuring middle-ear bacterial burden and clinical signs versus vehicle, with predefined microbiological and clinical endpoints. Both sexes will be included as a biological variable.

Expected outcomes & success criterion. ≥2-log10 reduction of dual-species biofilm in vitro; phage–antibiotic combinations outperforming either agent alone; and a statistically significant in vivo middle-ear bacterial-burden reduction versus vehicle.

Potential pitfalls & alternative approaches. The available models recapitulate otitis externa rather than the human perforated middle ear, and rodent middle-ear anatomy is small and technically demanding; we will (i) anchor decisions on quantitative microbiological endpoints, (ii) adjust dosing/redosing to account for phage self-amplification, (iii) triangulate in vivo results with the in vitro biofilm and synergy data, and (iv) if a reliable middle-ear instillation model proves infeasible within the R21 timeframe, fall back to the validated externa model as a bridging system while explicitly scoping middle-ear validation as the first deliverable of the subsequent R01.

Aim 3 — Define a CSOM-relevant otic formulation and the US regulatory translation path

Rationale. Translation requires a topically instillable, ear-compatible preparation and a defined US regulatory route. The 2025 mastoiditis case used custom phage administered intratympanically and parenterally under single-patient compassionate use in an immunosuppressed recipient; a banked, topically applied CSOM cocktail would follow a related but distinct expanded-access pathway, which this aim defines.

Experimental design. We will formulate the lead cocktail as otic drops, characterizing titer stability, pH, and osmolality compatible with the middle ear, and confirm absence of cytotoxicity in otic-

relevant cell assays (e.g., middle-ear epithelial and, where available, cochlear-relevant lines to flag ototoxicity risk). In parallel, we will assemble the regulatory framework—chemistry/manufacturing considerations, endotoxin/purity benchmarks, a proposed susceptibility-matching workflow, and protocol elements—for a future first-in-CSOM US pilot under an FDA single-patient/expanded-access IND with IRB oversight, and identify a target pre-IND meeting package.

Expected outcomes & success criterion. A stable (≥6-month titer retention), non-cytotoxic candidate otic formulation meeting endotoxin benchmarks, plus a complete pre-IND regulatory/clinical concept package positioning a subsequent properly powered CSOM trial.

Potential pitfalls & alternative approaches. Formulation may reduce phage viability or raise purity concerns; we will optimize buffers/excipients and storage and apply purification to meet endotoxin limits, drawing on established magistral/compassionate-use phage manufacturing precedents. If a single shelf-stable multi-phage product proves unstable, a two-component (separately stored, mixed-at-use) presentation is the fallback.

Timeline

[ILLUSTRATIVE] Months 1–12 (Aim 1): isolate panel assembly, phage screening, cocktail design; **Go/No-Go gate at Month 12** (≥70%/70% EOP coverage). [ILLUSTRATIVE] Months 8–20 (Aim 2): in vitro biofilm/synergy studies, then adapted in vivo otitis efficacy; **Go/No-Go gate at Month 18** (in vitro ≥2-log10 dual-species biofilm reduction before committing in vivo cohorts). [ILLUSTRATIVE] Months 14–24 (Aim 3): otic formulation, cytotoxicity, and regulatory-package assembly. [ILLUSTRATIVE] Total project duration: 24 months, consistent with the R21 exploratory/developmental scope.

Budget Justification (modular R21)

[ILLUSTRATIVE] This R21 is requested within the standard modular cap of [ILLUSTRATIVE] $275,000 total direct costs over [ILLUSTRATIVE] 2 years (e.g., [ILLUSTRATIVE] $150,000 year 1 / [ILLUSTRATIVE] $125,000 year 2), with no single year exceeding $200,000 in direct costs. **Personnel:** [ILLUSTRATIVE] PI (microbiology/phage; ~2.4 calendar months), [ILLUSTRATIVE] co-investigator otolaryngologist (~0.6 months), [ILLUSTRATIVE] one postdoc/technician (~12 person-months/year) for phage screening, biofilm assays, and animal work. **Supplies:** [ILLUSTRATIVE] phage banking/host-range reagents, biofilm and synergy consumables, formulation and endotoxin/cytotoxicity assays. **Animal costs:** [ILLUSTRATIVE] in vivo otitis model per-diem and husbandry (Aim 2). **Other:** [ILLUSTRATIVE] sequencing for phage characterization, regulatory consulting for the pre-IND concept. Indirect costs per the applicant institution's federally

negotiated rate (excluded from the modular direct-cost cap).

Vertebrate Animals

Animal work is proposed in Aim 2. We will use an in vivo otitis model adapted from the otitis externa system of Kwon et al. (2024), advancing where feasible toward middle-ear (post-perforation) inoculation and instillation, to evaluate bacterial-burden and clinical-sign reduction by the phage cocktail versus vehicle. **Justification for species:** rodent otitis models are the in vivo systems used for *P. aeruginosa*/*Staphylococcus* phage-cocktail otitis efficacy in the cited literature; we acknowledge these are validated for otitis externa, and middle-ear adaptation is an explicit objective and risk of this aim. [ILLUSTRATIVE] Group sizes will be the minimum required for statistical power by a priori power analysis, both sexes will be included, and procedures will use predefined humane endpoints, appropriate anesthesia/analgesia, and IACUC approval prior to any procedures. Reduction, refinement, and replacement are addressed by front-loading in vitro biofilm/synergy screening (Aim 2 Go/No-Go gate) so that only lead formulations enter animals.

Human Subjects / Clinical Trial

No human treatment is conducted in this R21. Use of de-identified archived CSOM bacterial isolates will be conducted under IRB review or exemption determination as applicable; no identifiable patient data or interventions are involved. Aim 3 prepares—but does not execute—a future first-in-CSOM US pilot. That subsequent study would administer investigational phage under an FDA single-patient/expanded-access IND, the regulatory family used for recent US compassionate-use phage therapy (e.g., the 2025 intratympanic chronic mastoiditis case), with full IRB oversight, informed consent, pre-treatment phage-susceptibility matching, and predefined safety and microbiological endpoints. The Wright et al. (2009) randomized, double-blind, placebo-controlled trial in chronic otitis externa—approved by the UK regulatory authority—provides the most relevant safety precedent and a methodological template; we note explicitly that extending its design from otitis externa to the perforated middle ear (CSOM) requires the dose, route, and ototoxicity de-risking generated by this R21 before a confirmatory trial.

Team & Environment

- **Principal Investigator (Microbiology/Phage Biology)** — [Name, Institution]: phage isolation, host-range/cocktail design, biofilm assays.
- **Co-Investigator (Otolaryngology)** — [Name, Institution]: CSOM clinical context, isolate sourcing, otic formulation and translational/regulatory design (cf. published US

otolaryngology intratympanic chronic mastoiditis phage experience).

- **Co-Investigator (Animal Models)** — [Name, Institution]: in vivo otitis model execution and middle-ear adaptation (cf. published in vivo otitis externa phage-cocktail work).
- **Consultant (Phage Manufacturing/Regulatory)** — [Name, Institution]: magistral/compassionate-use phage production and single-patient/expanded-access IND strategy.
- **Environment:** [Institution] facilities for BSL-2 microbiology, biofilm imaging, AAALAC-accredited animal care, and formulation/analytical characterization.

Alternate funder note: This proposal fits NIDCD's hearing-loss mission; its antimicrobial-resistance and phage-therapy dimensions also align with NIAID as an appropriate alternate or co-funding home.

References

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2. Casazza J, Tan D, Newcomer M, Sakano H, Isaacson B, Hunter JB. Bacteriophage Therapy for Chronic Mastoiditis. *Otology & Neurotology.* 2025;46(4):e117-e119. https://pubmed.ncbi.nlm.nih.gov/39965256/
3. Kwon J, Kim SG, Kim SW, et al. Tailoring formulation for enhanced phage therapy in canine otitis externa: a cocktail approach targeting *Pseudomonas aeruginosa* and *Staphylococcus pseudintermedius*. *Veterinary Microbiology.* 2024;301:110354. https://pubmed.ncbi.nlm.nih.gov/39740318/