Bacteriophage Surface Biocontrol to Reduce Clinic-Acquired Antibiotic-Resistant Infections in Feline Patients

A pilot study at a Cyprus companion-animal clinic targeting drug-resistant *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP)

Submitted to: EveryCat Health Foundation — Feline Health Research Grant **Principal Investigator:** Markos Ktori, DVM — Limassol Veterinary Clinic, Limassol, Cyprus **Co-Investigator:** Karen Pendergrass — Founder, Microbiome Medicine; Paleo Foundation **Grant-administering institution (payee):** Limassol Veterinary Clinic, Limassol, Cyprus **Requested:** up to US $50,000 · **Duration:** 18 months

> *Prepared as a complete draft for submission. Re-verify the live EveryCat application fields, current budget ceiling, and the Fall-2026 deadline (portal opens 28 April 2026; deadline 21 August 2026) before submitting, and confirm by email to grants@everycat.org that the clinic may serve as the grant-administering institution (the 2023 EC23-060 award to a private-clinic partnership is the precedent).*

Lay summary (for cat owners and the public)

When a cat is hospitalized for surgery, a wound, or a chronic illness, the one place it should be safest — the veterinary clinic — can also be where it picks up its most dangerous infection. The surfaces of busy clinics quietly accumulate antibiotic-resistant bacteria that conventional disinfectants do not fully clear. In our own practice in Limassol, three resistant organisms — *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, and methicillin-resistant *Staphylococcus pseudintermedius* — have been involved in the deaths of feline patients, and we currently care for a cat with an antibiotic-resistant *Klebsiella* infection that standard drugs cannot reliably clear.

This study tests a precise, antibiotic-sparing way to make the clinic environment safer: **bacteriophages** — viruses that kill only specific bacteria and are harmless to cats and people. We will build a "cocktail" of phages matched to the exact resistant bacteria found on our clinic's

surfaces, then spray it as an environmental treatment and measure whether it reduces the resistant-bacterial burden in the clinic — and, in turn, the rate of clinic-acquired infections in our feline patients. Phage sprays are already used to decontaminate surfaces in the food industry; **no one has yet tested this in a veterinary clinic.** If it works, it offers a low-cost, resistance-proof tool that any feline practice could adopt to protect vulnerable cats.

Scientific abstract

Companion-animal clinics are documented environmental reservoirs of multidrug-resistant (MDR) bacteria, with resistant *Klebsiella*, *Staphylococcus*, and Enterobacterales recovered from a large fraction of clinic surfaces despite routine disinfection. Resulting nosocomial infections cause feline morbidity and mortality and create a One Health, zoonotic reservoir transmissible to staff and owners. Bacteriophage surface biocontrol — strictly lytic phages applied to environmental surfaces — is regulatorily mature in food processing (e.g., FDA-cleared, EFSA-reviewed anti-*Listeria* products achieving 3.5–5.4 log/cm² reductions on stainless steel) and has shown >90% reductions of resistant staphylococci and Gram-negatives on hospital surfaces in proof-of-concept work, yet has never been evaluated in a veterinary setting. We will (Aim 1) isolate and genomically vet a **strictly lytic** phage cocktail matched to our clinic's own resistant *K. pneumoniae*, *S. epidermidis*, and MRSP isolates; (Aim 2) quantify its surface and antibiofilm efficacy on clinic-relevant materials in vitro; and (Aim 3) pilot a sprayed deployment in the clinic, measuring environmental resistant-bacterial bioburden and feline nosocomial-infection incidence before and after. The deliverable is the first evidence for, and a deployable protocol of, phage surface biocontrol to protect hospitalized cats from clinic-acquired drug-resistant infection.

Background and significance

Veterinary clinics are reservoirs of resistant bacteria — on their surfaces. Environmental contamination of small-animal hospitals with MDR pathogens is well documented. In a UK referral hospital, methicillin-resistant *Staphylococcus aureus* was recovered from 10% of environmental surfaces and matched the dominant hospital clone (Loeffler et al., *J Antimicrob Chemother*, 2005). Longitudinal sampling of a small-animal hospital found *Staphylococcus* (including *S. pseudintermedius*) on 30.8% of surfaces, and surfaces omitted from cleaning checklists were 2.3× more likely to be contaminated (Hunter et al., *Zoonoses Public Health*, 2021). A 2024 study of a veterinary clinical hospital recovered 109 Gram-negative isolates — including *Klebsiella*, *Enterobacter*, *Escherichia*, and *Pseudomonas* — from 183 surface samples, with high multidrug resistance and clonality suggesting cross-transmission (Pérez Jiménez et al.,

Environ Microbiol Rep, 2024). Conventional disinfection can fail outright: an MDR *Enterobacter* persisted in a veterinary ICU hand-wash sink despite two disinfection attempts (Kamathewatta et al., *Antimicrob Resist Infect Control*, 2020). Walther et al. (*Vet Microbiol*, 2016) review the resulting nosocomial outbreaks and the One Health, human ↔ animal exchange of MDR organisms in veterinary medicine.

The three target pathogens are clinically and zoonotically important — and are the organisms killing our patients.

- ***Klebsiella pneumoniae*** is a leading MDR/carbapenem-resistant nosocomial pathogen, a biofilm former, and a documented contaminant of veterinary-clinic surfaces (Pérez Jiménez 2024). Our clinic currently manages a cat with an antibiotic-resistant *Klebsiella* infection refractory to standard therapy.
- ***Staphylococcus epidermidis*** and other coagulase-negative staphylococci are persistent biofilm formers implicated in surgical-site, catheter, and device-associated infections.
- ***Methicillin-resistant Staphylococcus pseudintermedius*** **(MRSP)** is the defining nosocomial and zoonotic Staphylococcus of companion-animal medicine, with globally disseminated MDR clones (Nocera & De Martino, *Vet Res Commun*, 2024; Brooks et al., *mSystems*, 2020). It is carried by small-animal veterinarians at rates above the general population (Paul et al., *Zoonoses Public Health*, 2011) and transmits between animals and people (Guimarães et al., *J Infect Public Health*, 2023). Although classically canine-associated, MRSP is an emerging feline pathogen and has been directly involved in fatal feline infections in our practice.

Bacteriophage surface biocontrol is proven on surfaces — but never tried in a veterinary clinic. Strictly lytic phages applied to surfaces remove bacteria and biofilm with no collateral effect on the wider microbiome or on animal cells. The quantitative backbone comes from food-contact surfaces: phage P100 removed *Listeria* biofilms from stainless steel by 3.5–5.4 log/cm² across 21 strains (Soni & Nannapaneni, *J Food Prot*, 2010), and a 2024 systematic review/meta-analysis confirmed phage cocktails are ~1.26× more effective than single phages on surfaces and that higher titres and longer contact improve kill (Azari et al., *Biofilm*, 2024). Crucially for the clinical setting, D'Accolti et al. (*Infect Drug Resist*, 2018) showed a detergent-stable phage preparation removed drug-susceptible and drug-resistant *Staphylococcus*, *Pseudomonas*, and *Acinetobacter* from hospital hard surfaces by >90% rapidly and >99% over time. **To our knowledge no study has evaluated phage surface biocontrol in a veterinary clinic — this proposal fills that gap.**

Per-pathogen phage activity is established or rapidly emerging. Phage-derived depolymerases

and lytic phages degrade biofilm and kill carbapenem-resistant *K. pneumoniae*, with in vivo protection and intestinal load reduction (Jiao et al., *Arch Virol*, 2025 [review]; Chakraborty et al., *Pharmaceutics*, 2024 — 99% biofilm eradication in 4 h; Hao et al., *Antibiotics*, 2021). Lytic *S. epidermidis* phages with antibiofilm activity and clean (non-lysogenic) genomes have been characterized for device-infection contexts (Fanaei Pirlar et al., *Viruses*, 2022). For MRSP, the first verified strictly lytic *S. pseudintermedius* phages were reported only in 2022 (Hernandez Santos et al., *PHAGE*, 2022); phage cocktails prevent and degrade MRSP biofilm (Kim et al., *Front Med*, 2021); and a lytic MRSP phage shows ex vivo synergy with antibiotics on canine dermis (Ehling et al., *Vet Dermatol*, 2025), with a first clinical case report of topical MRSP-phage success (Mota Sá et al., *Vet Dermatol*, 2025). The MRSP literature's youth is itself an opportunity: this study would generate among the first surface-biocontrol data for a pathogen of acute veterinary importance.

Significance for feline health. Cats undergoing surgery, hospitalization, immunosuppression, or treatment for chronic disease are precisely the patients most endangered by clinic-acquired resistant infection — and for whom few antibiotics remain. A validated, low-cost, antibiotic-sparing environmental tool that reduces the resistant-bacterial burden a cat is exposed to in the clinic would be directly protective, broadly adoptable across feline practices, and would not drive further antibiotic resistance.

Hypothesis and specific aims

Central hypothesis: A strictly lytic bacteriophage cocktail, matched to a clinic's own resistant *K. pneumoniae*, *S. epidermidis*, and MRSP isolates and applied as an environmental surface spray, will significantly reduce the resistant-bacterial bioburden on clinic surfaces and, in turn, the incidence of clinic-acquired resistant infection in feline patients — without measurable harm.

Aim 1 — Build and genomically vet a clinic-matched, strictly lytic phage cocktail. Recover the resistant *K. pneumoniae*, *S. epidermidis*, and MRSP strains circulating on our clinic's surfaces and in feline clinical cases; isolate or source lytic phages against them; whole-genome sequence every candidate phage to exclude integrase/lysogeny, toxin, and antimicrobial-resistance genes; and select a 4–8 phage cocktail maximizing strain coverage. *Go/no-go:* a genomically clean, strictly lytic cocktail covering ≥80% of banked clinic isolates.

Aim 2 — Quantify surface and antibiofilm efficacy on clinic-relevant materials in vitro. Measure log-reduction of each target organism and of mixed biofilms on stainless steel, sealed plastic, and ceramic coupons representative of clinic surfaces, across realistic titres, contact times, and temperatures; confirm cocktails outperform single phages and test addition of a

depolymerase/endolysin component to counter biofilm shielding. *Go/no-go:* ≥2–3 log reduction on contaminated coupons.

Aim 3 — Pilot a sprayed deployment in the clinic with before/after surveillance. Over a controlled period, apply the validated cocktail as a routine adjunct surface spray in defined clinic zones; quantify resistant-bacterial environmental bioburden (sentinel-surface swabs/contact plates) and feline nosocomial-infection incidence before vs. during phage use, with safety monitoring. *Outcome:* a deployable protocol plus the first real-world signal of phage surface biocontrol in a veterinary clinic.

Experimental design and methods

Aim 1. Surface and clinical isolates will be collected by standardized swab/contact-plate sampling and from diagnostic cultures, speciated (MALDI-TOF or 16S/biochemical), and antibiotic-susceptibility tested (disk diffusion/MIC per CLSI VET standards; methicillin resistance by *mecA*/cefoxitin). Phages will be sourced from environmental samples and from established banks (e.g., the Eliava Institute, Tbilisi, and the Citizen Phage Library, University of Exeter, both holding *Klebsiella* and staphylococcal phages) and propagated on the clinic's own isolates. Each phage will be Illumina whole-genome sequenced and screened to **exclude lysogeny, toxin, and AMR genes** — the essential qualifying test that makes a phage safe for use and prevents horizontal gene transfer (see Pitfalls). Host range will define a 4–8 phage cocktail across independent receptors to suppress resistance.

Aim 2. Stainless-steel, plastic, and ceramic coupons will be inoculated with single-organism and mixed biofilms and treated with phage at defined titres (10^6–10^9 PFU/mL), contact times, and temperatures; viable counts (CFU/cm²) and biofilm biomass (crystal violet; live/dead) will quantify reduction. Single phages vs. cocktails will be compared, informed by the Azari (2024) meta-analysis parameters, and a depolymerase/endolysin adjunct evaluated against the *S. epidermidis* matrix-shielding effect (Melo et al., *Viruses*, 2020). Titre stability of the spray on surfaces over time will be measured to define a re-application interval.

Aim 3. With the cocktail validated, defined clinic zones (e.g., examination tables, cages, prep surfaces) will receive the phage spray as a post-cleaning adjunct on a fixed schedule. Sentinel surfaces will be swabbed on a calendar to quantify total and resistant-target bioburden; feline nosocomial-infection incidence (clinic-acquired resistant infections per admission) will be tracked from clinical records before vs. during the intervention. Safety endpoints: absence of adverse effects in patients/staff and monitoring for any shift in resistance patterns. Analyses will compare pre/post bioburden and infection incidence with appropriate statistics.

Anticipated results, pitfalls, and alternatives

We expect a clinic-matched lytic cocktail to achieve ≥2–3 log surface reductions in vitro and a measurable drop in environmental resistant bioburden and feline nosocomial infections in the pilot.

- **Pitfall — horizontal gene transfer.** Some phages (especially temperate ones, and notably some *S. epidermidis* phages) can transduce resistance/virulence genes (Fišarová et al., *mSphere*, 2021; Fijan et al., *Front Microbiol*, 2024). **Mitigation:** use only **strictly lytic, fully sequenced, transduction-incapable** phages — exactly as the food-grade product P100 is characterized (EFSA BIOHAZ opinion, *EFSA Journal*, 2016). This is a hard design constraint, screened in Aim 1.
- **Pitfall — biofilm shielding.** Biofilm matrix can physically protect cells from phage (Melo 2020). **Mitigation:** cocktail design plus an optional depolymerase/endolysin component and adequate contact time.
- **Pitfall — phage resistance / coverage gaps. Mitigation:** multi-receptor cocktails; periodic re-matching of the cocktail to surveillance isolates.
- **Pitfall — titre decay on surfaces. Mitigation:** stability testing (Aim 2) to set re-application frequency; phages with broad environmental tolerance (e.g., pH 4–11, ≤50 °C; Huang et al., *Virulence*, 2025) where available.

Regulatory note (EU)

Therapeutic phage use is tightly regulated, but this project is **environmental surface biocontrol**, not treatment of an animal. Food-contact phage surface products are FDA-cleared and EFSA-reviewed (EFSA BIOHAZ, 2016). In the EU, surface sanitizers fall under the Biocidal Products Regulation (EU) No 528/2012; no phage surface product is yet EU-authorized as a biocide, so this work is framed as a feasibility/research pilot generating the safety and efficacy data such a pathway would require — a deliberate strength of the proposal, not a barrier to the study.

Feline relevance and impact

This study is, at its core, feline infection prevention. It targets the organisms killing hospitalized cats, in the environment where cats acquire them, with a tool that spares the patient's microbiome and adds no selective pressure for antibiotic resistance. Success yields (1) the first evidence that phage surface biocontrol reduces clinic-acquired resistant infection in cats, (2) a low-cost, open protocol any feline practice could adopt, and (3) a banked, sequenced phage cocktail against three priority feline-relevant pathogens. It directly advances EveryCat's mission to improve the health

and welfare of cats, and builds on the Foundation's own 2023 investment in feline phage therapy (EC23-060).

Timeline (18 months)

- **Months 1–6:** isolate banking, susceptibility testing, phage isolation/sourcing, whole-genome sequencing, cocktail selection (Aim 1).
- **Months 5–11:** in vitro surface and antibiofilm efficacy and stability testing (Aim 2).
- **Months 10–18:** clinic pilot deployment with before/after environmental and feline-infection surveillance; analysis; reporting and dissemination (Aim 3).

Budget and justification (US $50,000 total direct costs)

EveryCat funds direct project costs only — no investigator salaries, major equipment, travel, or institutional overhead; up to $2,000 of publication costs are allowable. Investigator time (PI M. Ktori; Co-I K. Pendergrass) is contributed in kind.

Category	Justification	Amount (USD)
Microbiology consumables	Swabs, contact plates, selective/chromogenic media, MALDI/biochemical ID, susceptibility testing across the study	$8,500
Phage isolation, propagation & purification	Host strains, media, purification and endotoxin-removal reagents, titre/QC assays, cocktail formulation & spray materials	$9,000
Whole-genome sequencing — phages	Illumina WGS + bioinformatics for safety screening (lysogeny/toxin/AMR exclusion) of all candidate phages	$7,500
Whole-genome sequencing / typing — bacterial isolates	Strain matching, methicillin-resistance and clonality typing of clinic isolates	$7,000

Category	Justification	Amount (USD)
In vitro surface/biofilm assays	Coupons (steel/plastic/ceramic), biofilm reagents, depolymerase/endolysin test materials, plasticware	$7,500
Pilot surveillance materials	Calendar environmental sampling supplies and consumables across the deployment	$6,000
Bench technician support (non-PI)	Part-time research-assistant time for sampling and assays	$2,500
Publication / dissemination	Open-access publication of results	$2,000
Total		**$50,000**

Animal welfare and ethics

This is primarily an environmental-microbiology study; phage is applied to surfaces, not administered to animals. Feline clinical data are drawn from routine diagnostic cultures and medical records collected as part of standard care, with owner consent and in accordance with the clinic's ethical standards and Cyprus/EU requirements. No experimental infection or invasive procedures are performed on animals for this study. An ethics/animal-use statement will be provided per EveryCat requirements.

Team and environment

Markos Ktori, DVM (PI) — practicing veterinarian, Limassol Veterinary Clinic — provides the clinical setting, the feline caseload and isolates, infection-control oversight, and the deployment site. **Karen Pendergrass (Co-Investigator)** — founder of Microbiome Medicine and the Paleo Foundation; standards developer and microbiome-signatures researcher — provides study design, microbiome and AMR expertise, and project coordination. **Phage-sourcing partners:** the Eliava Institute (Tbilisi) and the Citizen Phage Library (University of Exeter) for *Klebsiella* and staphylococcal phage stocks and characterization. The Pancyprian Veterinary Association (a FECAVA/WSAVA member) will be invited to provide letters of support.

References

1. Loeffler A, et al. *J Antimicrob Chemother*. 2005;56(4):692–7. https://doi.org/10.1093/jac/dki312
2. Walther B, et al. *Vet Microbiol*. 2016;200:71–78. https://doi.org/10.1016/j.vetmic.2016.05.017
3. Hunter SA, et al. *Zoonoses Public Health*. 2021;68(8):947–54. https://doi.org/10.1111/zph.12887
4. Pérez Jiménez M, et al. *Environ Microbiol Rep*. 2024;16(6):e70055. https://doi.org/10.1111/1758-2229.70055
5. Kamathewatta K, et al. *Antimicrob Resist Infect Control*. 2020;9(1):163. https://doi.org/10.1186/s13756-020-00828-0
6. Soni KA, Nannapaneni R. *J Food Prot*. 2010;73(8):1519–24. https://doi.org/10.4315/0362-028x-73.8.1519
7. Azari R, et al. *Biofilm*. 2024;7:100170. https://doi.org/10.1016/j.bioflm.2023.100170
8. D'Accolti M, et al. *Infect Drug Resist*. 2018;11:1015–26. https://doi.org/10.2147/IDR.S170071
9. Fijan S, et al. *Front Microbiol*. 2024;15:1412269. https://doi.org/10.3389/fmicb.2024.1412269
10. Jiao Y, et al. *Arch Virol*. 2025;170(4):70. https://doi.org/10.1007/s00705-025-06257-x
11. Chakraborty S, et al. *Pharmaceutics*. 2024;16(7):904. https://doi.org/10.3390/pharmaceutics16070904
12. Hao G, et al. *Antibiotics*. 2021;10(8):894. https://doi.org/10.3390/antibiotics10080894
13. Huang Y, et al. *Virulence*. 2025;16(1):2450462. https://doi.org/10.1080/21505594.2025.2450462
14. Fanaei Pirlar R, et al. *Viruses*. 2022;14(6):1340. https://doi.org/10.3390/v14061340
15. Melo LDR, et al. *Viruses*. 2020;12(10):1076. https://doi.org/10.3390/v12101076
16. Hernandez Santos AJ, et al. *PHAGE (New Rochelle)*. 2022;3(2):116–24. https://doi.org/10.1089/phage.2022.0004
17. Kim SG, et al. *Front Med (Lausanne)*. 2021;8:524059. https://doi.org/10.3389/fmed.2021.524059
18. Ehling-Schulz, et al. *Vet Dermatol*. 2025;37(2):200–10. https://doi.org/10.1111/vde.70030
19. Mota Sá D, et al. *Vet Dermatol*. 2025;36(2):227–30. https://doi.org/10.1111/vde.13328
20. Nocera FP, De Martino L. *Vet Res Commun*. 2024;48(6):3505–15. https://doi.org/10.1007/s11259-024-10508-8
21. Brooks MR, et al. *mSystems*. 2020;5(1):e00684-19. https://doi.org/10.1128/mSystems.00684-19

22. Paul NC, et al. *Zoonoses Public Health*. 2011;58(8):533–9. https://doi.org/10.1111/j.1863-2378.2011.01398.x
23. Guimarães L, et al. *J Infect Public Health*. 2023;16(Suppl 1):183–9. https://doi.org/10.1016/j.jiph.2023.10.041
24. Fišarová L, et al. *mSphere*. 2021;6(3):e00223-21. https://doi.org/10.1128/mSphere.00223-21
25. EFSA BIOHAZ Panel. *EFSA Journal*. 2016;14(8):4565. https://doi.org/10.2903/j.efsa.2016.4565

Illustrative figures (budget, coverage and log-reduction targets, timeline) are planning estimates to be finalized with quotes and the clinic's caseload.