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**INSTITUTO DE CIÊNCIAS BIOMÉDICAS**
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*Projeto de Pesquisa de Pesquisa FAPESP-ANID 2035:*

# Latin American Antarctic Research Consortium on Antimicrobial Resistance and Emerging Contaminants (LARCARE)


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## Abstract

**Background:** The Latin American Antarctic Research Consortium on Antimicrobial Resistance and Emerging Contaminants (LARCARE) is a collaborative Chilean-Brazilian effort focused on supporting and promoting scientific research in the Antarctic region, involving various institutions (Universidad de Concepción, Universidad Católica de Valparaíso, Universidad de la Frontera, Universidad Mayor, Universidad de O'Higgins, Universidade de São Paulo, Instituto de Pesca, Laboratório Nacional de Luz Síncrotron, e Universidade Federal de São Paulo), and researchers dedicated to understanding the microbial biodiversity, the bioprospection, identification of emerging contaminants (chemical and microplastics), emerging pathogens, and the antimicrobial resistance status in the South Atlantic Ocean and the Antarctic continent. **Objectives:** This proposal aims to: i) investigate the presence, concentration, and ecotoxicological effects of plastic particles—with an emphasis on nanoplastics—in filter-feeding organisms of Antarctic benthic fauna, particularly bivalve mollusks, as sentinel indicators of contamination. Additionally, comparative sampling will be conducted in tropical and subtropical coastal areas of Brazil and Chile to establish latitudinal patterns of plastic pollution and its biological effects; ii) to identify and characterize antibiotic-resistant bacteria present in Antarctic wildlife species, focusing on seabirds, pinnipeds, and benthic invertebrates; iii) to map antimicrobial resistance genes, with particular attention to those associated with mobile genetic elements (e.g., $bla_{\text{carbapenemases}}$, $bla_{\text{ESBL}}$, *mcr*, *vanA*); iv) to assess the microbial diversity associated with wildlife, correlating it with ecological and behavioural variables; v) identification and characterization of biodiversity, bacteriophages, bacteriocins and probiotics with clinical potential; vi) to model potential routes of introduction and dissemination of resistance genes in the Antarctic environment, including connections to sub-Antarctic and continental regions via migratory species; vii) to foster the development of integrated public policies based on the *One Health* approach, incorporating wildlife into AMR surveillance and control programs. **Methodology:** The project will employ integrated approaches combining classical microbiology, molecular biology (qPCR, metagenomic sequencing), omics methods, microbial ecology, and ecological modelling. Samples of tissues, feces, and secretions will be collected from marine birds and mammals, along with sediment and water from sites with high wildlife activity or human presence (e.g., research stations and landing zones). Analyses will include both phenotypic and genotypic screening for resistance, plasmid characterization, and phylogenetic comparisons using international reference databases. Collaboration with Chilean institutions will provide logistical access to Antarctica and promote technical-scientific exchange focused on the use of advanced analytical tools, such as vibrational microspectroscopy (micro-FTIR and micro-Raman), and the Sirius (a new Brazilian synchrotron light source in the Laboratório Nacional de Luz Síncrotron), enabling precise characterization of plastic and chemical contaminants. This project aligns with global efforts to monitor emerging plastic pollutants, emergent and antimicrobial-resistant pathogens, contributing unprecedented and relevant data on the extent and impacts of these pollutants and microorganisms across different ecological zones in the Southern Hemisphere. Omics methods will be conducted in the CEFAP facility (https://cefap.icb.usp.br/). All genomic data will be publicly available on the OneBR platform (http://www.onehealthbr.com/), under the description of Antartic Genomic Microbial Data "LARCARE Genome". **Expected Impact:** This project aims to address a critical gap in the global understanding of AMR and emerging contaminants by integrating Antarctic wildlife and the environment into existing surveillance frameworks, generating novel data on the presence and ecological roles of Antarctic organisms as vectors of antimicrobial resistance. The findings will provide essential scientific input for biosafety measures in polar environments, support evidence-based policy development, and reinforce the implementation of the One Health approach at the international level. Bioproducts could be investigated in depth in next collaborative projects, to consolidate technological innovation products. Finally, postgraduate, undergraduate, PhD researchers and senior researchers will be favoured with the exchange of knowledge and visits and internships at the respective laboratories that make up the consortium.

## 1. Justification

Antimicrobial resistance (AMR) is one of the most serious global public health threats, with increasingly severe implications for the effectiveness of medical treatments and the sustainability of healthcare systems. Wildlife, as an integral component of ecosystems, can act as a natural reservoir of antibiotic-resistant bacteria, actively contributing to the dissemination of these microorganisms even in remote and seemingly pristine environments such as Antarctica, where direct human presence is minimal, yet indirect introduction of resistant bacteria can occur via migratory birds, ocean currents, and scientific or touristic activities. Although Antarctica is protected and human activity is restricted, the increased human presence appears to be impacting terrestrial and aquatic ecosystems and the environment. Regarding emerging contaminants, micro- and nanoplastic pollution has intensified as one of the most severe threats to marine ecosystems, including regions considered remote and presumably pristine, such as Antarctica. On the other hand, chemical pollutants including antimicrobial agents, biocides, heavy metals and drug residues have been identified in coastal environments and marine wildlife, in Brazil and the northern Antarctic Peninsula, where the main source of contaminants that pose a high risk for the Antarctic environment has been the wastewater. Microbial biodiversity in the Antarctic is an important source for identification of bioproducts including bacteriophages, bacteriocins and probiotics with clinical potential, or microorganism with potential for bioremediation.

## 2. Objectives

*Main*

To Stablish the Latin American Antarctic Research Consortium on Antimicrobial Resistance and Emerging Contaminants (LARCARE)

*Specifics*

i) To investigate the presence, concentration, and ecotoxicological effects of plastic particles—with an emphasis on nanoplastics—in filter-feeding organisms of Antarctic benthic fauna, particularly bivalve mollusks, as sentinel indicators of contamination;

ii) To identify and characterize antibiotic-resistant bacteria present in Antarctic wildlife species, focusing on seabirds, pinnipeds, and benthic invertebrates;

iii) To map antimicrobial resistance genes, with particular attention to those associated with mobile genetic elements (e.g., $bla_{\text{carbapenemases}}$, $bla_{\text{ESBL}}$, *mcr*, *vanA,* etc);

iv) To assess the microbial diversity associated with wildlife, correlating it with ecological and behavioural variables;

v) Identification and characterization of biodiversity, bacteriophages, bacteriocins and probiotics with clinical potential;

vi) To model potential routes of introduction and dissemination of resistance genes in the Antarctic environment, including connections to sub-Antarctic and continental regions via migratory species;

vii) To foster the development of integrated public policies based on the One Health approach, incorporating wildlife into AMR surveillance and control programs.

## 3. Methods

*Sampling sites*

Sampling will be conducted at strategic sites in the Antarctic Peninsula and surrounding islands, selected according to their accessibility, wildlife presence, and logistic feasibility (Figure 1). Two fixed sampling points with laboratory infrastructure for immediate processing will be prioritized (i) Base Profesor Julio Escudero (INACH, King George Island; 62°12′S, 58°57′W) and (ii) Base General Bernardo O'Higgins (Chilean Army, Antarctic Peninsula; 63°19′S, 57°54′W). Additional sampling campaigns in coordination with INACH will be carried out aboard Antarctic vessels (e.g., Almirante Viel, Betanzos, Karpuj), allowing access to complementary sites (iii) Base Gabriel González Videla (Chilean Navy, Paradise Bay; 64°49′S, 62°51′W), (iv) Base Yelcho (INACH, Doumer Island; 64°52′S, 63°35′W), (v) Two Hummock Island (63°22′S, 57°56′W), (vi) Avian Island (67°46′S, 68°54′W), (vii) Base Comandante Ferraz (Brazilian Antarctic Program, King George Island; 62°05′S, 58°24′W).

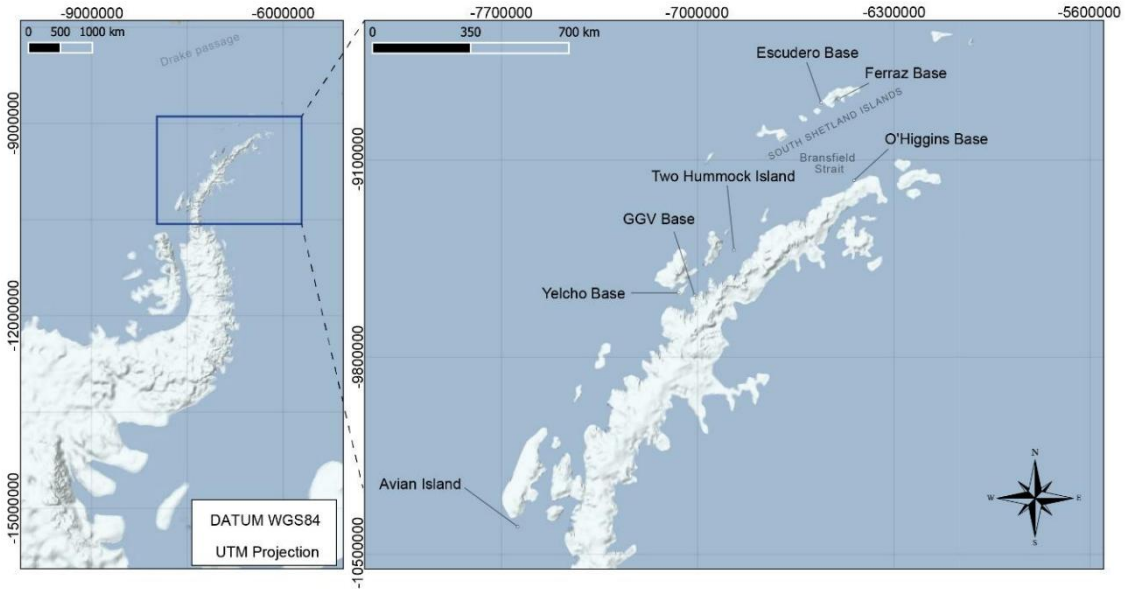


**Figure 1.** Map of collection points in Antarctic Scientific Expedition.

At each site, sampling will target penguin colonies, seabird rookeries, pinniped haul-out areas, and intertidal habitats for marine invertebrates. Fecal samples and invertebrate specimens will be preserved and processed on-site whenever laboratory facilities are available, ensuring integrity prior to transfer to central laboratories.

*Sample collection from Antarctic wildlife*

To assess sentinel species for AMR and emerging contaminants, we will focus on fresh fecal sampling from key Antarctic wildlife. Target groups include: i)Sphenisciformes (*Pygoscelis* spp.) fresh fecal samples will be collected primarily from three penguin species of the genus *Pygoscelis*: Gentoo (*P. papua*), Adelie (*P. adeliae*), and Chinstrap (*P. antarcticus*). These species are selected due to their large biomass, relatively small colony sizes, and proximity to human settlements, facilitating systematic sampling and ensuring sufficient numbers for analysis. Opportunistic sampling may include other Sphenisciformes visiting the study sites; ii) Charadriiformes and Procellariformes: Brown skuas (*Stercorarius antarcticus*), south polar skuas (*S. maccormicki*), kelp gulls (*Larus dominicanus*), Antarctic terns (*Sterna vittata*), snowy sheathbills (*Chionis albus*) and southern giant-petrel (*Macronectes giganteus*) recognized AMR bioindicators due to feeding ecology and interactions with anthropogenic environments; iii) Pinnipeds: When accessible, fresh fecal samples will be collected from leopard seal (*Hydrurga leptonyx*), Antarctic fur seal (*Arctocephalus gazella*), southern elephant seal (*Mirounga leonina*), crabeater seal (*Lobodon carcinophaga*) or Weddell seal (*Leptonychotes weddellii*). These species are commonly observed near human facilities or in accessible coastal haul-out areas, facilitating opportunistic yet representative sampling; iv) Marine invertebrates: Marine invertebrates as crustaceans and mollusks will be included as complementary bioindicators, given their trophic roles and exposure pathways. Target taxa include Antarctic krill (*Euphausia superba*), amphipods (e.g., *Orchomene* spp.), and benthic mollusks such as limpets (*Nacella* spp.) and bivalves (*Laternula elliptica*).

Sampling cap per species: to minimize ecological impact, collection will not exceed up to 10 individuals per species per site per sampling event, with a campaign-wide cap of ≤30 individuals per species (or a lower number if required by permits).

Population safeguard rules: no sampling from visibly depleted patches; avoid brooding/ovigerous females and juveniles; prioritize naturally dislodged or moribund individuals when scientifically acceptable; rotate micro-sites to prevent local depletion.

Refinement & humane handling: handling time will be minimized; anesthesia/euthanasia (if required for laboratory analyses) will follow the approved

institutional protocol and permit conditions (e.g., rapid cooling/ice-slurry for small crustaceans; validated molluscan protocols), with immediate specimen processing or preservation (e.g., RNAlater/ethanol as appropriate for downstream genomics/microbiology).

Non-lethal alternatives: whenever feasible, hemolymph swabs, mucus, gut contents from naturally deceased individuals, or shell biofilm scrapings will be preferred over whole-organism sacrifice, without compromising scientific endpoints.

*Sampling procedure*

Daylight surveys will be conducted in penguin colonies, bird rookeries, pinniped haul-out areas, and intertidal/coastal habitats. Fresh fecal deposits will be identified and collected using sterile tools; invertebrates will be captured by hand or fine nets in the intertidal/shallow subtidal, following the caps and safeguards above.

*Transport/preservation*

Fecal samples will be placed in Amies transport medium with charcoal and maintained at ambient environmental temperature until field-lab processing. Invertebrates or their tissues will be transferred into sterile containers and preserved according to the analytical pipeline (culture, WGS, resistome/metallome assessments). Chain-of-custody and sample labeling will follow GLP-compatible procedures.

*Compliance and permits*

All activities will comply with the Protocol on Environmental Protection to the Antarctic Treaty, the SCAR Code of Conduct, Chilean INACH permits and station-specific rules, and institutional animal care approvals (PICUA/CEUA/IACUC as applicable). Field personnel will adhere to site-specific biosecurity (e.g., footwear decontamination, tool sterilization) and minimum-disturbance guidelines (no off-trail walking in sensitive areas, buffer distances to wildlife)

*Bacterial isolation (antimicrobial resistant and emerging pathogens), identification and antimicrobial susceptibility testing*

Collected samples will be seeded on specific agar plates for psychrotrophic, mesophilic and halophilic bacteria and incubated overnight in refrigerator temperatures and or 35 °C. Bacterial taxonomy will be assessed using MALDI-TOF spectrophotometry (Bruker, Daltonics). Antimicrobial susceptibility testing will be performed using the disc-diffusion

method or the microdilution inhibition concentration test, in accordance with the CLSI guidelines. A biobank will be created, catalogued, and maintained at ICB-USP, Universidad de Concepción, and/or Instituto Adolfo Lutz or Fiocruz (Fundação Oswaldo Cruz) repositories.

*Whole-Genome Sequencing and Data Analysis.*

Genomic DNA will be extracted using the PureLink® Genomic Quick Gel Extraction and PCR Purification Kit (Thermo Fisher Scientific®). Further, DNA quality and concentration will be analyzed by spectrophotometry using the DeNovix DS-11 instrument. The precise quantification and integrity of the obtained DNA will be assessed with the Qubit® fluorometer, using the dsDNA HS (High Sensitivity) assay kit, according to the manufacturer's protocol. A paired-end library will be prepared using the Nextera XT DNA Library Preparation Kit (Illumina), as per the manufacturer's instructions. The fragment size obtained from the library preparation will be determined by capillary electrophoresis using the Agilent Bioanalyzer DNA 1000 system, and the final library quantification will be assessed with the Qubit® using the dsDNA HS assay kit. Genomic sequencing will be performed on the Illumina NextSeq platform (150 bp) (http://cefap.icb.usp.br/genial) at the Center for Research Support Facilities (CEFAP-GENIAL) of the Institute of Biomedical Sciences at the University of São Paulo. The processing of the files generated by sequencing will be performed using FASTQC 0.11.3 and Trimmomatic v0.32 using PHRED quality score threshold ≥ 20. Genome assembly will be performed using Unicycler v0.4.8. Gene prediction and protein classification will be performed using Prokka v1 and Blast2GO, respectively. Prediction of antimicrobial resistance, virulence and plasmid incompatibility groups will be performed using Abricate v.1. The prediction of genes for resistance to disinfectants, heavy metals, and herbicides will be performed using the BacMet database. The core genome will be determined using Panaroo v1.5.1, and phylogenetic inference based on core-genome single nucleotide polymorphisms (cgSNPs) will be performed using raxml-ng v.1.2.2 and the GTR+G model with 1000 bootstraps. All genome data will be publicly available on the OneBR platform (http://www.onehealthbr.com/). Additionally, the LARCARE genomic platform will be constructed.

*Biofilm Formation Experiment*

Commonly found MP compositions will be used, namely polystyrene (PS), polyethylene (PE), and polypropylene (PP). PS, PE, and PP particles will be produced in the laboratory

by fragmenting plastic items. MPs with sizes between 500 μm and 1000 μm will be selected using steel sieves. The particles will be rinsed in 70% ethanol, air-dried, and placed in sterilized organza bags. A mixture of different MPs will be prepared using equal quantities of PP, PE, and PS. An adapted mimicked aquatic environment will be constructed. Seawater or water from highly polluted sites will be placed in a previously sterilized Erlenmeyer flask. The water will be filtered using a mesh sieve (pore size: 100 μm) to remove larger particles. MPs of different types will be added to each Erlenmeyer flask containing 100 mL of the filtered water. Each experiment will have a maximum exposure time of five weeks to determine the optimal duration for biofilm formation on the MPs, which will be assessed over time. Analysis of Biofilm Formation Using Optical Density Optical density (OD) measurements will be used to monitor biofilm formation on MPs. MPs from each flask will be collected at pre-established time intervals, rinsed with sterile water, and inoculated into 10 mL of nutrient broth. The flasks will be incubated for 24h. Subsequently, the flasks containing MPs will be vortexed for 1 minute to detach the biofilms. OD will be measured at 660 nm using a spectrophotometer.

*Scanning Electron Microscopy*

Scanning electron microscopy (SEM) analysis will be performed to confirm the adhesion of microorganisms to the MP surfaces after exposure to the polluted and/or marine water medium. The MPs will be fixed in 2.5% glutaraldehyde for 8 h at 4 °C. After fixation, the samples will be rinsed with 0.1 M sodium phosphate buffer (pH 7.2) and dehydrated through an alcohol series consisting of 30%, 50%, 70%, 90%, 95%, and 100% alcohol (10 min each). To minimize distortion of the microplastics prior to SEM analysis, critical point drying will be performed. The particles will be mounted on a stub, secured with carbon adhesive, and coated with gold-palladium. The stub will be placed in the SEM, and images will be recorded and stored.

*Micro- and Nanoplastic Analysis*

Analysis will be performed at the Laboratório Nacional de Luz Síncrotron, in the Centro Nacional de Pesquisa em Energia e Materiais (CNPEM, Campinas Brazil), using enzymatic or mild chemical digestion (e.g., KOH), filtration and analysis via micro-FTIR and micro-Raman, confirmation by SEM/EDX.

Complementary ecotoxicological assessments will be performed in the Universidad de Concepción (Chile), including histopathological analysis (liver, gills, digestive tract),

antioxidant enzymes and oxidative stress biomarkers, and gene expression analysis of cellular stress, inflammation, and detoxification-related genes.

Comparative and Statistical Analysis Multivariate statistical models and PCA for contamination patterns. Ecological models for correlation with environmental data (temperature, salinity, urbanization). Integrated discussion with international literature and IPCC data.

*Microbiological analysis of surface water for Antartic region, Pacific and Atlantic Oceans*

Surface water samples will be collected using a 10 L aluminum bucket, previously sanitized. Sieving will be performed on-site using a stacked system of three sieves in the following order: a 5000 μm mesh sieve on top, a 1000 μm mesh sieve in the middle, and a 100 μm mesh sieve on the bottom. After sampling, each sieve will be rinsed with distilled water, covered with aluminum foil, and stored in a cardboard box. Microbiological and genomic analysis will be performed as above.

*Probiotic identification*

Different culture media will be used for assess antibacterial activity of bacterial strains and production of antimicrobial metabolites [Mitis Salivarius Agar (MSA), Nutrient Agar (NA), Brain Heart Infusion Agar (BHI), Luria-Bertani Agar (LB), M9 Minimal Salts Agar (M9A), Reasoner's 2A Agar (R2A), and Tryptic Soy Agar (TSA). Each medium will be tested at three incubation temperatures, 4°C, 28°C and 37°C. Inhibition of multidrug-resistant bacteria will be evaluated. The analysis of secondary metabolites will be predicted by antiSMASH for gene clusters for antimicrobial compounds like hydrogen cyanide, pyoluteorin, pyrrolnitrin, NRP-metallophore, arylpolyene, and betalactone.

*Brazil–Chile Symposium on Antarctic Pollution*

Brazilian and Chilean researchers, working in scientific collaboration, will organize a symposium dedicated to the issue of pollution in Antarctica, based on a recently developed joint project between the two teams. The meeting aims to discuss preliminary results and challenges related to the presence of emerging contaminants—particularly micro- and nanoplastics—in Antarctic ecosystems, as well as to assess their potential impacts on marine biodiversity and ecosystem services. The symposium will bring together experts in ecotoxicology, oceanography, marine biology, and environmental policy, creating a forum for the exchange of knowledge, advanced analytical

methodologies, and field experiences carried out in different areas of the Antarctic continent and in coastal environments of the Southern Cone. Beyond its scientific scope, the event seeks to strengthen integration between Brazilian and Chilean institutions, expanding opportunities for cooperation in polar research. The initiative also aims to contribute to the international debate on Antarctic environmental protection, in line with the principles of the Antarctic Treaty and the guidelines of multilateral organizations. It is expected that the symposium's results and discussions will help inform policy recommendations and mitigation strategies to safeguard one of the planet's most sensitive and emblematic ecosystems.

## 4. Chronogram

| **Activities** | **Semesters** | | | | | |
|---|---|---|---|---|---|---|
| | **2025** | **2026** | | **2027** | | **2028** |
| Literature review | x | x | x | x | x | x |
| Sampling (water, animal and invertebrates) | x | x | x | x | | |
| Bacterial isolation and identification | | x | x | x | | |
| Antimicrobial susceptibility testing | | | x | x | | |
| Whole genome sequencing and bioinformatics | | | x | x | x | |
| Biofilm Formation Experiment | | | x | x | x | |
| Scanning Electron Microscopy | | | x | x | x | |
| Nano- and Microplastic analysis | | | x | x | x | |
| Histological and toxicological analysis | | | x | x | x | |
| Microbiological analysis of surface water | | x | x | x | x | |
| Probiotic identification | | | x | | x | |
| Brazil–Chile Symposium on Antarctic Pollution | | | x | x | x | |
| Publications | | x | x | x | x | x |
| Partial report | | | x | | x | |
| Final report | | | | | | x |

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