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One Health: Determinants of Rectal Colonization by Extended-Spectrum Beta-Lactamase-Producing Enterobacterales in Community-Dwelling Adults in Argentina

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One Health: Relationship Between Antimicrobial Resistance, Microbiota, and Pesticides in Territorial Areas of Argentina

ABSTRACT

The One Health approach considers human beings as ecological spaces existing in continuous interdependence with microorganisms, environmental resources, and surrounding habitats. This perspective promotes the understanding of health and disease as emergent properties of complex interactions among biological, social, cultural, occupational, environmental, and political determinants. Consequently, the study of antimicrobial resistance (AMR) requires integrated and systemic methodologies capable of combining quantitative and qualitative approaches.

This project aims to investigate the prevalence of antimicrobial resistance in community-dwelling populations through the study of intestinal colonization by extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E), a recognized proxy indicator of antimicrobial resistance. Beyond estimating prevalence, the study seeks to characterize the relationships between resistant microorganisms, gut microbiota composition, and contextual bio-socio-environmental determinants.

A population-based quota sampling strategy will be implemented among individuals attending selected sentinel healthcare facilities in participating territorial regions. Based on previous studies conducted by our research groups, we anticipate a ratio of approximately 1:4 between colonized and non-colonized individuals.

Each participant will provide urine and fecal samples for biological analyses and complete a comprehensive health and environmental questionnaire designed to capture individual, clinical, occupational, social, cultural, and environmental exposures. In-depth qualitative interviews will also be conducted in selected participants to explore contextual determinants not adequately captured through conventional epidemiological methods.

The first phase of the project will be conducted in two sentinel institutions: Hospital J. B. Iturraspe (Santa Fe Province) and Hospital Italiano de Buenos Aires. Biological specimens will be incorporated into a strategic biobank collection established under a One Health framework. Urinary glyphosate concentrations will be measured in a subset of participants identified as having high socio-environmental vulnerability according to a composite risk score derived from questionnaire data.

Subject to funding availability and strategic collaborations, fecal samples will subsequently undergo integrated multi-omics analyses, including microbiome and resistome characterization. These investigations aim to explore the relationship between microbial ecosystem composition, environmental exposures, and antimicrobial resistance.

The project adopts a participatory action research perspective to integrate microbiological, environmental, epidemiological, molecular, and qualitative findings into a systemic causal framework. Ultimately, the study seeks to generate actionable evidence capable of informing

community-based interventions, public health policies, and legislative initiatives addressing antimicrobial resistance through a One Health perspective.

RELEVANCE OF THE PROBLEM

Antimicrobial resistance (AMR) is currently recognized as one of the most important global public health threats. Despite international action plans and stewardship initiatives, the burden of antimicrobial resistance continues to increase worldwide. AMR is a planetary health problem that transcends political borders and requires coordinated actions across countries, sectors, and disciplines.

The landmark Review on Antimicrobial Resistance estimated that, in the absence of effective interventions, annual deaths attributable to antimicrobial resistance could increase from approximately 700,000 to 10 million by 2050. The greatest burden is expected to occur in countries with lower Human Development Index scores, where weaker regulatory frameworks and health systems may facilitate the emergence and dissemination of resistant organisms.

Traditionally, the development of AMR has been associated with inappropriate antibiotic use in human medicine and the extensive use of antimicrobials in food-producing animals. Approximately 70% of global antibiotic consumption occurs in animal production systems, where these agents have historically been used for growth promotion, disease prevention, and treatment. More recently, environmental contaminants such as pesticides have emerged as additional contributors to antimicrobial resistance.

Glyphosate (N-phosphonomethylglycine), the most widely used herbicide worldwide, has become particularly relevant in this context. Argentina is among the countries with the highest glyphosate utilization rates, with annual applications estimated at 180–200 million liters. Emerging evidence suggests that glyphosate may exert selective pressure on bacterial communities, potentially contributing to the emergence of antimicrobial resistance while simultaneously altering microbiome composition.

Resistance to third-generation cephalosporins, particularly through the production of extended-spectrum beta-lactamases (ESBLs), is among the most important drivers of the global burden of AMR. ESBL-producing Enterobacterales are associated with increased morbidity, mortality, healthcare utilization, and antibiotic consumption. For these reasons, the World Health Organization and the European Centre for Disease Prevention and Control have recommended ESBL-producing Escherichia coli as a key surveillance indicator for integrated One Health monitoring systems.

From a One Health perspective, intestinal colonization by ESBL-producing Enterobacterales is especially relevant because it reflects interactions among human behavior, environmental exposures, animal reservoirs, food production systems, and healthcare practices. Colonized individuals constitute a reservoir for subsequent infections and transmission, making colonization an important epidemiological endpoint.

Particular attention is warranted regarding occupational exposure to food-producing animals. Previous studies have demonstrated substantial rates of ESBL colonization among workers

involved in intensive livestock production, especially poultry and swine farming. Several investigations have shown that occupational exposure, inadequate hygiene practices, and contact with farm animals may contribute to the acquisition and dissemination of resistant microorganisms.

Furthermore, structural determinants such as agricultural regulations, antimicrobial policies, environmental governance, and socioeconomic inequalities influence patterns of antimicrobial resistance. Evidence suggests that interventions exclusively targeting antibiotic prescribing may not be sufficient to reverse resistance trends, highlighting the need for broader systemic approaches capable of addressing the multiple interacting determinants involved in AMR emergence.

Within this framework, antimicrobial resistance is conceptualized not merely as a microbiological phenomenon but as an emergent property of complex ecological systems involving humans, animals, microorganisms, and the environment. The One Health approach therefore provides an appropriate conceptual framework for investigating the interconnected determinants that shape antimicrobial resistance in contemporary societies.

 # PRELIMINARY RESULTS AND CONTRIBUTIONS OF THE RESEARCH GROUP

The Global Epidemiology Research Group, based at the Institute of Translational Medicine and Biomedical Engineering (IMTIB-CONICET), has extensive experience conducting multidisciplinary investigations in Global Health, Environmental Health, and One Health. The group integrates expertise from clinical medicine, public health, epidemiology, microbiology, environmental sciences, sociology, and health systems research, applying systemic and multilevel approaches to complex health problems.

Since 2013, the group has developed multiple research initiatives focused on environmental determinants of health, environmental exposure assessment, participatory action research methodologies, and the development of systemic models linking biological, environmental, and social determinants. Among its achievements is the validation of a liquid chromatography–tandem mass spectrometry method for the measurement of glyphosate in human urine, which subsequently enabled population-based studies evaluating environmental exposure to pesticides in agricultural regions of Argentina.

A recent participatory action research project conducted in rural communities demonstrated the presence of glyphosate in human urine and generated evidence supporting local public health interventions and environmental regulations. These experiences established the methodological foundations for integrating biological measurements with social, environmental, and community-level determinants.

The microbiome research team participating in the present project is part of the International Microbiome Network and has contributed to several international collaborative initiatives investigating the role of the microbiome in colorectal cancer and other chronic diseases. These projects have demonstrated the feasibility of conducting large-scale microbiome studies across diverse socioeconomic and geographical settings and have generated valuable expertise in microbiome sampling, biobanking, sequencing technologies, and bioinformatic analyses.

In parallel, the antimicrobial resistance research group at Hospital J. B. Iturraspe has been conducting investigations on antimicrobial resistance since 2022 within the framework of multidisciplinary infection prevention and antimicrobial stewardship programs.

In 2023, a cross-sectional study was conducted among randomly selected adults hospitalized for less than 24 hours. Rectal swabs were cultured on selective chromogenic media for ESBL-producing Enterobacterales. Preliminary analyses including 260 participants demonstrated an intestinal colonization prevalence of 25.4% (95% CI 20.4–30.9). Most isolates corresponded to Escherichia coli, followed by Klebsiella pneumoniae and Citrobacter freundii.

Colonized individuals more frequently reported hospitalization during the previous year and had lower access to potable water. A trend toward higher colonization prevalence among healthcare workers was also observed. These preliminary findings support the feasibility of community surveillance and suggest that both healthcare-related and environmental factors may contribute to ESBL colonization.

The integration of microbiome science, environmental health research, and antimicrobial resistance surveillance creates a unique opportunity to investigate the biological and contextual determinants of antimicrobial resistance within a comprehensive One Health framework.

 # HYPOTHESIS AND CONCEPTUAL FRAMEWORK

The One Health perspective considers antimicrobial resistance as an emergent ecological phenomenon arising from interactions among humans, animals, microorganisms, and environmental systems. Under this framework, resistant microorganisms are not viewed solely as pathogens but also as indicators of ecological imbalance generated by human activities and environmental pressures.

The present study is based on the hypothesis that intestinal colonization by ESBL-producing Enterobacterales, used as a community-level marker of antimicrobial resistance, is associated with socioeconomic conditions, occupational exposure to food-producing animals, environmental exposure to pesticides (particularly glyphosate), and alterations in gut microbiota composition, independently of individual antibiotic consumption.

We further hypothesize that disruptions in microbiota diversity and composition may represent an intermediate mechanism linking environmental and occupational exposures to antimicrobial resistance. Changes in microbial ecology may facilitate colonization by resistant organisms and increase susceptibility to the acquisition and persistence of antimicrobial resistance genes.

Consequently, understanding antimicrobial resistance requires simultaneous investigation of biological factors, environmental exposures, social determinants, occupational activities, cultural practices, and healthcare-related variables. This multidimensional perspective justifies the integration of quantitative epidemiology, microbiological analyses, molecular techniques, environmental measurements, and qualitative research methodologies.

 # GENERAL OBJECTIVE

To determine the prevalence of intestinal colonization by extended-spectrum beta-lactamase-producing Enterobacterales and to identify biological, clinical, sociocultural, occupational, environmental, and microbiome-related determinants associated with colonization among community-dwelling adults from selected territorial regions of Argentina.

 # SPECIFIC OBJECTIVES

1. To estimate the prevalence of intestinal colonization by ESBL-producing Enterobacterales according to recruitment center.

2. To quantify the frequency of known and exploratory risk factors associated with ESBL colonization.

3. To identify independent variables associated with intestinal colonization by ESBL-producing Enterobacterales.

4. To compare occupational exposure to food-producing animals between colonized and non-colonized participants.

5. To establish a strategic biobank collection of fecal samples, urine samples, bacterial isolates, and extracted DNA for future One Health research.

6. To evaluate environmental exposure to glyphosate according to colonization status and occupational exposure profiles.

7. To characterize gut microbiota composition and associated antimicrobial resistance genes using advanced molecular approaches.

8. To compare microbiome profiles according to ESBL colonization status, glyphosate exposure, and other environmental determinants.

9. To identify epidemiological clusters associated with ESBL colonization, emphasizing socioeconomic conditions, geographical origin, dietary habits, water access, occupational activities, and animal contact.

10. To develop predictive models for intestinal colonization by ESBL-producing Enterobacterales.

11. To explore behavioral, cultural, occupational, and environmental dimensions potentially associated with antimicrobial resistance through qualitative methodologies.

12. To integrate biological, environmental, and social findings into a systemic conceptual model capable of explaining the emergence of antimicrobial resistance from a One Health perspective.

13. To document participatory processes and community-based actions derived from the interpretation of study findings.

14. To develop and validate a Bayesian model for identifying individuals with a high probability of environmental pesticide exposure.

STUDY DESIGN AND METHODS

Study Design

This project is designed as a multicenter cross-sectional observational study conducted under a One Health framework. The primary objective is to estimate the prevalence of intestinal colonization by extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) among community-dwelling adults and to investigate biological, clinical, occupational, environmental, and sociocultural determinants associated with colonization.

The study constitutes the first phase of a broader research program aimed at integrating epidemiological, microbiological, environmental, molecular, and qualitative data. Future stages will incorporate additional territorial regions and advanced molecular analyses to support ecological and multilevel investigations of antimicrobial resistance.

Study Setting

The initial phase of the study will be conducted in two sentinel healthcare institutions located in distinct geographical and socio-environmental regions of Argentina:

- Hospital Italiano de Buenos Aires (Buenos Aires City)
- Hospital J. B. Iturraspe (Santa Fe Province)

These institutions were selected because they represent different territorial contexts and possess the infrastructure required for participant recruitment, biological sample collection, microbiological processing, and long-term collaboration within a One Health research network.

Study Population

Target Population

The target population consists of adults aged 18 years or older residing in territorial regions of Argentina.

Accessible Population

Participants will be recruited among:

- Individuals attending outpatient clinics;
- Individuals attending emergency departments;
- Individuals hospitalized for less than 24 hours;
- Family members or caregivers accompanying eligible participants.

Participants must reside within the territorial area corresponding to the participating healthcare institution.

Sampling Strategy

A systematic quota-based sampling strategy will be employed to ensure representation according to:

- Geographic area;
- Age group;
- Sex.

Sampling will be conducted in three stages:

1. Selection of territorial area.
2. Selection of participating healthcare facility.
3. Selection of eligible participant.

Within each institution, participants will be consecutively recruited until predefined quotas are achieved for each demographic stratum. This strategy aims to improve representativeness while maintaining operational feasibility.

The study adopts a non-probability sampling approach because recruitment is based on predefined demographic quotas rather than random population sampling.

Sample Size Calculation

The sample size was calculated to estimate an expected prevalence of intestinal colonization by ESBL-producing Enterobacterales of approximately 15%, with:

- 95% confidence level;
- 5% margin of error.

Using OpenEpi software, the required sample size was estimated at 196 participants per center. Assuming a 10% loss rate or incomplete sampling, the final recruitment target was increased to 216 participants per center.

The total planned sample size for the first phase is therefore 432 participants.

Participant Recruitment

Potential participants will be identified by trained healthcare professionals at participating institutions.

Eligible individuals will receive verbal and written information regarding the study objectives, procedures, risks, benefits, confidentiality protections, and future use of biological specimens.

Participation will be entirely voluntary. Written informed consent will be obtained prior to any study procedure.

Participants may withdraw from the study at any time without affecting their medical care or relationship with the participating institutions.

Data Collection Procedures

Each participant will complete a standardized epidemiological questionnaire administered by trained healthcare personnel.

The questionnaire will collect information regarding:

Sociodemographic Characteristics

- Age
- Sex
- Educational level
- Occupation
- Household characteristics
- Socioeconomic indicators

Clinical Variables

- Medical history
- Chronic diseases
- Medication use
- Previous antibiotic exposure
- Healthcare utilization
- Previous hospitalizations

Environmental Exposures

- Water sources
- Sanitation conditions
- Housing characteristics
- Environmental pollution indicators
- Exposure to pesticides and agrochemicals

Occupational Exposures

- Agricultural activities
- Livestock production
- Poultry production
- Swine production
- Veterinary activities
- Healthcare-related occupations

Animal Contact

- Companion animals
- Farm animals

- Occupational exposure to animals
- Household animal ownership

Dietary Habits

- Food consumption patterns
- Consumption of animal-derived products
- Food sourcing practices

Qualitative Research Component

Selected participants will be invited to participate in in-depth semi-structured interviews.

Particular emphasis will be placed on individuals presenting combinations of:

- ESBL colonization;
- Environmental vulnerability;
- Occupational exposure;
- Evidence of pesticide exposure.

Interviews will explore:

- Health beliefs;
- Environmental perceptions;
- Occupational practices;
- Behavioral factors;
- Community-level determinants;
- Cultural dimensions related to health and environmental exposures.

Interviews will be audio-recorded, transcribed, anonymized, and analyzed using qualitative research methodologies.

Geographic and Population-Level Data

To contextualize individual-level findings, population-level indicators will be obtained from governmental databases and publicly available sources.

Variables may include:

- Demographic structure;
- Socioeconomic indicators;
- Healthcare resource availability;
- Agricultural production indicators;
- Livestock density;
- Poultry density;
- Environmental characteristics;
- Land use patterns;
- Water resource distribution.

These variables will support future ecological and multilevel analyses investigating contextual determinants of antimicrobial resistance.

Data Management

Study data will be recorded using REDCap electronic data capture systems.

Each participant will receive a unique study identification code.

Personally identifiable information will be stored separately from research databases and will only be accessible to authorized study personnel.

Data quality procedures will include validation rules, range checks, monitoring procedures, and periodic audits.

BIOLOGICAL SAMPLE COLLECTION, PROCESSING, AND BIOBANKING

Biological Specimens

All participants will be asked to provide:

1. One fecal sample.
2. One urine sample.

These specimens will be used for microbiological, molecular, environmental, and future multi-omics analyses conducted within the framework of the study and associated biobank.

Biological samples will be collected following standardized operating procedures to ensure specimen quality, reproducibility, and long-term usability for future research.

FECAL SAMPLE COLLECTION AND PROCESSING

Sample Collection

Participants will provide a fecal specimen equivalent to approximately one tablespoon of stool.

Samples will be collected using sterile collection containers and transported according to institutional biosafety procedures.

Upon arrival at the processing facility, samples will be divided into aliquots and transferred into cryovials for long-term preservation.

Microbiological Analysis

Fecal specimens will undergo microbiological processing for the detection of intestinal colonization by ESBL-producing Enterobacterales.

Selective culture media and standard microbiological techniques will be used to identify bacterial isolates and determine ESBL production through phenotypic methods.

Microorganisms isolated during the study may subsequently undergo molecular characterization, including the identification of antimicrobial resistance genes and resistance mechanisms.

Future Multi-Omics Analyses

Subject to funding availability and collaborative agreements, stored fecal samples may undergo advanced molecular analyses including:

* Metagenomic sequencing;
* Metatranscriptomic profiling;
* Metabolomic analyses;
* Microbiome characterization;
* Resistome characterization.

These approaches will allow comprehensive evaluation of:

* Microbial community composition;
* Microbial diversity;
* Functional metabolic pathways;
* Antimicrobial resistance gene profiles;
* Relationships between environmental exposures and microbial ecosystem structure.

Molecular Analyses

DNA extracted from fecal samples may be analyzed using polymerase chain reaction (PCR) and sequencing-based methodologies to detect and characterize antimicrobial resistance genes.

These analyses will contribute to the identification of resistome patterns and their association with environmental and clinical determinants.

URINE SAMPLE COLLECTION AND PROCESSING

Sample Collection

Participants will be asked to provide approximately 20 mL of urine.

Whenever possible, first-morning urine specimens will be preferred to improve standardization and biomarker detection.

Samples will be aliquoted into multiple sterile tubes for:

1. Glyphosate determination;
2. Creatinine measurement;
3. Long-term storage for future environmental exposure studies.

Glyphosate Determination

Glyphosate concentrations will be measured in a subset of participants selected according to environmental vulnerability indicators and probabilistic exposure models.

Measurements will be performed using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method previously developed and validated by the research group.

Urinary glyphosate concentrations will be normalized using urinary creatinine levels and reported as micromoles per mole of creatinine or equivalent standardized units.

Future analyses may include additional environmental contaminants depending on funding availability and scientific priorities.

TRANSPORTATION AND STORAGE OF BIOLOGICAL SAMPLES

Bacterial Isolates

Following microbiological identification and characterization, ESBL-producing isolates will be preserved for future analyses.

Isolates will be cultured and stored under appropriate laboratory conditions before transfer to centralized facilities.

Upon arrival at the central laboratory, isolates may undergo:

* DNA extraction;
* Molecular characterization;
* Long-term cryopreservation.

Bacterial stocks and extracted DNA will be maintained at -80°C according to institutional procedures.

Fecal and Urine Samples

Fecal and urine specimens will initially be stored at local facilities at temperatures appropriate for temporary preservation.

Samples will subsequently be transported in batches using specialized biological specimen transportation systems with temperature monitoring and cold-chain maintenance.

Upon arrival at the central biobank facility, specimens will be transferred to ultra-low temperature freezers (-80°C) for long-term preservation.

BIOBANK COLLECTION

Establishment of a Strategic One Health Biobank

The study includes the development of a strategic biological collection within the institutional biobank of Hospital Italiano de Buenos Aires.

The purpose of this collection is to create a high-quality repository of biological specimens suitable for current and future investigations related to:

* Antimicrobial resistance;
* Microbiome science;
* Environmental health;
* Molecular epidemiology;
* One Health research.

Stored materials may include:

* Fecal samples;
* Urine samples;
* Bacterial isolates;
* Extracted DNA;
* Future molecular derivatives generated during analyses.

Governance and Quality Standards

The biobank will operate according to international best practices and institutional regulations governing human biological repositories.

All specimens will be coded and stored without direct personal identifiers.

Access to samples and associated data will require approval from the corresponding scientific and ethical oversight committees.

Future studies using stored specimens must comply with participant consent, applicable regulations, and institutional governance policies.

QUESTIONNAIRES AND QUALITATIVE INTERVIEWS

The epidemiological questionnaire has been developed using validated instruments and national survey frameworks, including household, environmental, occupational, and health-related indicators.

The questionnaire will be implemented through REDCap electronic data capture systems.

Participants identified as presenting combinations of antimicrobial resistance, environmental vulnerability, or environmental exposure biomarkers may be invited to participate in in-depth qualitative interviews.

These interviews will be conducted by researchers with expertise in environmental health, sociology, and qualitative methodologies.

Interview guides will be pilot-tested and refined before implementation.

Audio recordings will be transcribed, anonymized, and analyzed using established qualitative research methods.

STATISTICAL ANALYSIS

Descriptive Analysis

Descriptive analyses will be performed to characterize the study population and estimate the prevalence of intestinal colonization by ESBL-producing Enterobacterales.

Continuous variables will be summarized as:

* Mean and standard deviation (SD) for normally distributed variables;
* Median and interquartile range (IQR) for non-normally distributed variables.

Categorical variables will be presented as:

* Frequencies;
* Percentages;
* 95% confidence intervals (95% CI), when appropriate.

The prevalence of ESBL colonization will be estimated overall and stratified according to recruitment center and relevant demographic, clinical, environmental, and occupational characteristics.

Statistical analyses will be performed using STATA software. A two-sided p-value <0.05 will be considered statistically significant.

Geographic information systems and georeferencing techniques may be used to visualize the spatial distribution of colonization and environmental exposures.

Inferential Analysis

Univariable Analysis

Potential determinants of ESBL colonization will first be evaluated individually using appropriate statistical tests according to variable type and distribution.

Associations between explanatory variables and colonization status will be explored using:

* Chi-square or Fisher's exact tests for categorical variables;
* Student's t-test or non-parametric alternatives for continuous variables.

Variables demonstrating statistical significance or considered clinically relevant will be considered for multivariable modeling.

Multivariable Logistic Regression

Independent predictors of intestinal colonization by ESBL-producing Enterobacterales will be identified using multivariable logistic regression models.

Potential explanatory variables include:

Demographic Variables

* Age
* Sex
* Educational level
* Socioeconomic indicators

Clinical Variables

* Previous hospitalization
* Antibiotic exposure
* Comorbidities
* Healthcare-related exposures

Occupational Variables

* Livestock production
* Poultry production
* Swine production
* Veterinary activities
* Healthcare occupations

Environmental Variables

* Water access
* Sanitation conditions
* Housing characteristics
* Environmental vulnerability indicators
* Pesticide exposure indicators

Biological Variables

* Gut microbiota composition
* Microbial diversity measures
* Glyphosate concentrations
* Resistance gene profiles

Adjusted odds ratios (ORs) with corresponding 95% confidence intervals will be reported.

Future stages involving multiple territorial regions may incorporate hierarchical or multilevel regression models to evaluate the influence of contextual and area-level determinants.

QUALITATIVE ANALYSIS

Qualitative interviews will be analyzed using principles derived from grounded theory methodologies.

The analysis will focus on identifying:

* Emerging concepts;
* Behavioral patterns;
* Cultural practices;
* Occupational experiences;
* Environmental perceptions;
* Community-level determinants.

Audio recordings will be transcribed verbatim and anonymized before analysis.

Coding procedures will include:

1. Open coding;
2. Axial coding;
3. Selective coding.

The objective is to identify novel explanatory dimensions that may not be adequately captured through quantitative epidemiological methods.

Qualitative data management and analysis will be conducted using specialized software such as Atlas.ti or equivalent platforms.

BAYESIAN MODEL FOR ENVIRONMENTAL EXPOSURE ASSESSMENT

Because urinary glyphosate measurements may only be feasible in a subset of participants, a Bayesian predictive model will be developed to identify individuals with a higher probability of environmental pesticide exposure.

Candidate predictors may include:

* Residential characteristics;
* Occupational activities;
* Agricultural exposures;
* Environmental history;
* Socioeconomic indicators;
* Lifestyle characteristics;
* Household environmental conditions.

The long-term objective is to develop a practical decision-support tool capable of identifying individuals who may benefit from environmental exposure assessment in clinical and public health settings.

SYSTEMIC CONCEPTUAL MODEL

One of the distinguishing features of this project is the integration of biological, environmental, occupational, sociocultural, and epidemiological findings into a systemic conceptual framework.

This model seeks to explain antimicrobial resistance as an emergent phenomenon arising from interactions across multiple levels of organization, including:

Structural Determinants

* Policies
* Regulations
* Agricultural systems
* Economic factors

Intermediate Determinants

* Community environments
* Occupational contexts
* Food production systems
* Local ecological conditions

Individual Determinants

* Behaviors
* Health conditions
* Medication use
* Environmental exposures

Biological Determinants

* Microbiota composition
* Microbial diversity
* Antimicrobial resistance genes
* Colonization by resistant organisms

The resulting framework will be used to facilitate interpretation of study findings and support the development of interventions consistent with One Health principles.

FEASIBILITY AND AVAILABLE INFRASTRUCTURE

Hospital Italiano de Buenos Aires is one of the largest tertiary-care academic medical centers in Argentina and provides the infrastructure required for the successful execution of this project.

The institution includes:

* More than 750 inpatient beds;
* Comprehensive outpatient services;
* Advanced microbiology and molecular biology laboratories;
* Next-generation sequencing facilities;
* Biostatistics and bioinformatics support;
* A certified electronic health record system;
* An established institutional biobank.

The Institute of Translational Medicine and Biomedical Engineering (IMTIB) provides specialized facilities for:

* Molecular biology;
* Genomics;
* Microbiome research;
* Bioinformatics;
* Biobanking.

Available equipment includes:

* Ultra-low temperature freezers (-80°C);
* Automated nucleic acid extraction systems;

* Real-time PCR platforms;
* High-throughput sequencing technologies;
* Advanced computational infrastructure for multi-omics analyses.

Hospital J. B. Iturraspe contributes expertise in antimicrobial resistance surveillance, microbiological processing, and community-based epidemiological research.

The combined capabilities of these institutions provide a strong foundation for conducting integrated One Health research.

ETHICAL CONSIDERATIONS

ETHICAL CONSIDERATIONS

The study will be conducted in accordance with:

* The Declaration of Helsinki;
* Council for International Organizations of Medical Sciences (CIOMS) Guidelines;
* International Conference on Harmonisation Good Clinical Practice (ICH-GCP) principles applicable to observational research;
* Applicable Argentine regulations governing human subjects research, personal data protection, and biobanks.

Participation will be entirely voluntary.

Written informed consent will be obtained from all participants prior to enrollment.

Participants may refuse participation or withdraw from the study at any time without affecting their medical care or relationship with participating institutions.

Risks associated with participation are considered minimal and are primarily related to biological specimen collection procedures and the potential risk of loss of confidentiality.

To minimize these risks:

* Personal identifiers will be stored separately from research data;
* Biological specimens will be coded using unique study identification numbers;
* Access to identifiable information will be restricted to authorized study personnel;
* Electronic databases will be protected through institutional security measures and restricted-access systems.

Biological samples will be stored in the institutional biobank according to approved governance procedures and applicable regulations. Future use of stored specimens will be limited to research purposes consistent with participant consent and subsequent ethical approvals when required.

The study protocol, informed consent forms, questionnaires, and participant-facing materials have been reviewed and approved by the Research Ethics Committee of Hospital Italiano de Buenos Aires (Comité de Ética de Protocolos de Investigación, CEPI).

Ethics Approval Number: CEPI No. 7067.

The study will be conducted under the oversight of the approved ethics committee, and confidentiality, privacy, and protection of participant rights will be maintained throughout all phases of the research.

STUDY TIMELINE

Study Start Date: September 2025

Estimated Primary Completion Date: December 2027

Estimated Study Completion Date: December 2030

Phase 1:
Cross-sectional recruitment, microbiological analyses, questionnaire administration, and biobank establishment.

Phase 2:
Glyphosate quantification, microbiome characterization, resistome analyses, and environmental exposure assessment.

Phase 3:
Integrated multi-omics analyses, predictive modeling, and development of a systemic One Health framework for antimicrobial resistance.

DISSEMINATION OF RESULTS

Study findings will be disseminated through peer-reviewed scientific publications, presentations at national and international conferences, technical reports, and community engagement activities.

Results will be communicated regardless of the direction, magnitude, or statistical significance of findings.

Whenever possible, aggregate results will be shared with participating communities and healthcare institutions to support evidence-based interventions addressing antimicrobial resistance and environmental health.

Authorship of scientific publications will follow the recommendations of the International Committee of Medical Journal Editors (ICMJE).

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