Study Protocol

Official Title

Correlation Between Carbapenemase-Producing Enterobacterales Load, Environmental Contamination, and Transmission in Critical Care Units

ClinicalTrials.gov Identifier (NCT Number Pending **)**

Protocol Version

Version 1.0

Document Date

26 June 2026

Sponsor

Hospital Italiano de Buenos Aires

Principal Investigator

Maria Ines Staneloni, MD

Correlation Between Carbapenemase-Producing Enterobacterales Load, Environmental Contamination, and Transmission in Critical Care Units

Authors

Maria Ines Staneloni
Infection Control Committee, Hospital Italiano de Buenos Aires
Infectious Diseases Section
Department of Internal Medicine, Hospital Italiano de Buenos Aires
maria.staneloni@hospitalitaliano.org.ar
0000-0001-8259-7215

Emilio Felipe Huaier Arriazu
Infection Control Committee, Hospital Italiano de Buenos Aires
emilio.huaier@hospitalitaliano.org.ar
https://orcid.org/0000-0003-1138-7792

Analia M De Cristófano
Infection Control Committee, Hospital Italiano de Buenos Aires
analia.decristofano@hospitalitaliano.org.ar
0009-0007-3508-2543

Sol Burokas
Infection Control Committee, Hospital Italiano de Buenos Aires
maria.burokas@hospitalitaliano.org.ar
0000-0002-1349-6354

Mariángeles Visús
Microbiology, Hospital Italiano de Buenos Aires
mariangeles.visus@hospitalitaliano.org.ar
0000-0002-9749-585X

Julieta Trinks
IMTIB, CONICET–UHIBA–Hospital Italiano de Buenos Aires
julieta.trinks@hospitalitaliano.org.ar

Marina Soledad Medina
Molecular Biology, Hospital Italiano de Buenos Aires
soledad.medina@hospitalitaliano.org.ar
0009-0005-3336-1050

Santiago Manuel Pedernera
Molecular Biology, Hospital Italiano de Buenos Aires
santiago.pedernera@hospitalitaliano.org.ar
0009-0008-8374-6188

María Victoria Bergoglio
Bacteriology, Hospital Italiano de Buenos Aires
victoria.bergoglio@hospitalitaliano.org.ar

Giovana Leiva
Bacteriology, Hospital Italiano de Buenos Aires
giovana.leiva@hospitalitaliano.org.ar

Table of Contents

Authors 1

1. Abstract 3

2. Introduction 4

1.1. Research question 4

1.2. Purpose 5

3. Objectives 5

3.1. Primary objective 5

3.2. Secondary objectives 5

4. Methodology 6

4.1. Study design 6

4.2. Study setting 6

4.3. Population 7

4.3.1. Inclusion criteria 7

4.3.2. Exclusion criteria 7

4.3.3. Definitions 7

4.4. Intervention and data collection 8

4.4.1. Baseline screening 8

4.4.2. Rectal swabs in patients with CPE 8

4.4.3. Environmental sampling 8

4.4.4. Molecular characterization 9

4.5. Variables 10

4.5.1. Primary variable (independent) 10

4.5.2. Dependent variables 10

4.5.3. Confounding or effect-modifying variables 10

5.3.4. Descriptive and characterization variables 11

5.3.5. Derived variables (secondary analysis) 12

4.6. Statistical considerations 12

4.6.1. Sampling and sample size calculation 12

4.6.2. Statistical analysis plan 13

4.7. Ethical considerations 13

4.8. Funding 14

4.9. Budget 15

I. Per-patient analyses 15

II. Environmental samples 15

III. Genomic sequencing studies 15

IV. Correlation between Ct values and resistance gene copy numbers 16

V. Publication 16

Total budget 16

1. Abstract

The spread of carbapenemase-producing Enterobacterales (CPE) represents one of the most significant threats to global public health, with a profound impact on intensive care units (ICUs). In these settings, intestinal colonization serves as the primary reservoir and source of environmental transmission. However, not all carriers contribute equally to transmission; certain individuals, often termed 'super-spreaders,' harbor high bacterial loads and cause extensive contamination of the hospital environment.

While bacterial load can be measured through quantitative cultures or molecular techniques, a specific cycle threshold (Ct) value in real-time PCR assays to practically identify these high-risk patients has not yet been established. Determining such a correlation could optimize infection prevention and control strategies by prioritizing interventions for individuals with the highest dissemination potential.

The present study aims to evaluate the correlation between the bacterial load of CPE in rectal swabs and the Ct value obtained by PCR, as well as to explore its relationship with environmental contamination and intrahospital transmission. A prospective observational study will be conducted in patients admitted to the ICU of Hospital Italiano de Buenos Aires, an institution that has an active and systematic surveillance program for CPE carriers, according to the institutional protocol.

In patients with positive detection of CPE, quantitative culture and PCR with Ct recording will be performed on the same samples obtained within the framework of routine surveillance. In addition, swabs will be collected from five high-touch environmental surfaces. In selected cases, genomic sequencing (WGS or PFGE) will be performed to confirm transmission events between index and secondary patients.

The primary variable will be the CPE bacterial load (CFU and Ct); dependent variables will include environmental contamination and transmission of genetically related strains. Clinical and exposure variables (length of hospital stay, prior antimicrobial use, fecal incontinence, among others) will be incorporated to adjust the analyses using multivariable models.

The study does not involve additional procedures or risk for patients, as samples derive from routine institutional surveillance. Therefore, and in accordance with CIOMS Guideline 10 (2019), a waiver of informed consent will be requested from the Ethics Committee.

The results will allow determination of whether the Ct value can be used as an indirect marker of bacterial load and environmental dissemination, identifying “super-spreaders” within routine clinical practice. This knowledge will provide evidence to implement targeted prevention strategies, optimizing infection control effortsand reducing the transmission of multidrug-resistant microorganisms in critical care settings.

Keywords: Carbapenemase-producing Enterobacterales, Super-spreaders, Real-time PCR cycle threshold (Ct), Environmental contamination, Nosocomial transmission, Infection control, Antimicrobial resistance.

2. Introduction

According to the World Health Organization, the dissemination of carbapenemase-producing Enterobacterales (CPE) constitutes a global public health threat. Gastrointestinal colonization in patients is recognized as the main reservoir of these bacteria. Previous investigations have shown that there are patients with a higher CPE bacterial load, referred to as “super-spreaders,” with a greater potential to contaminate the hospital environment [1].

It has been observed that approximately 24% of carriers do not disseminate CPE into the environment, with fecal continence being the only independent predictor of this lack of dissemination [2]. Among patients who did disseminate, approximately 20% were identified as “super-spreaders,” being responsible for 80% of the colony-forming units detected in the environment surrounding these patients. As mentioned, “super-spreaders” are characterized by presenting high rectal concentrations of CPE in cultures. In addition, bacterial load expressed as RNA copy numbers has been correlated in fecal cultures obtained from rectal swabs.

The identification of “super-spreaders” has been attempted using molecular and culture-based methods. It has been proposed that a resistance gene ratio (blaKPC/16S rDNA) greater than 0.05, or a ratio of CPE to total aerobic bacteria greater than 0.2, respectively, could define them. However, these methods are complex to implement in daily clinical practice. To date, no threshold value (Ct) has been established in commonly used real-time PCR assays that would allow precise identification of these patients. Defining such a value could optimize infection control strategies, allowing prevention and containment efforts to focus on environmental contamination driven by “super-spreaders.”

Although current findings suggest a possible association between high bacterial load in rectal swabs, increased environmental dispersion, and potentially greater transmission to other patients, this relationship has not been conclusively demonstrated. To establish a robust link between “super-spreaders” and increased CPE transmission, genomic sequencing studies are required to analyze the traceability of isolates.

Therefore, this research protocol proposes to determine whether Ct values in rectal swabs correlate with the CPE bacterial load in a cohort of critically ill patients. In addition, it aims to evaluate whether patients with a higher CPE load, indicated by a lower Ct value, contribute more significantly to contamination of the hospital environment and to the transmission of carbapenemases to other patients.

1.1. Research question

Is there a correlation between the PCR cycle threshold (Ct) value and the bacterial load measured by culture in perianal surveillance swab samples for CPE in patients hospitalized in an Intensive Care Unit?

Is there a correlation between the microbiological load in surveillance cultures, the PCR Ct value, and the number of environmental samples surrounding the patient that test positive, as an indicator of environmental contamination?

Are patients with higher microbiological load and/or lower PCR Ct values those who transmit CPE strains to newly colonized individuals within the same ICU?

1.2. Purpose

This study will provide crucial evidence on the feasibility of identifying patients with high bacterial load through a method that is more accessible at the clinical care level. This would allow overcoming the limitations of current diagnostic approaches for identifying patients at high risk for CPE dissemination.

In addition, it will provide evidence regarding the role of CPE bacterial load in environmental dissemination and intrahospital transmission. The findings may enable the identification of “super-spreader” patients (with high bacterial load) and the implementation of targeted and personalized infection control interventions, particularly in lower-resource settings. This could strengthen CPE prevention measures, such as contact precautions, environmental hygiene, and hand hygiene in this population, or allow exploration of specific decolonization strategies for these patients in order to reduce carbapenemase transmission in clinical settings.

3. Objectives

3.1. Primary objective

To evaluate the correlation between the bacterial load of CPE in rectal swabs from colonized patients, measured by the number of colony-forming units in culture, and the Ct value obtained by PCR.

3.2. Secondary objectives

To describe the relationship between the Ct value of colonized patients and the magnitude of environmental contamination with CPE in their rooms.

To identify, through genomic sequencing, whether patients with higher CPE bacterial load are the main transmitters of carbapenemases to other patients within the same unit.

4. Methodology

4.1. Study design

A prospective, observational, and analytical study will be conducted, aimed at evaluating the correlation between the bacterial load of carbapenemase-producing Enterobacterales (CPE), environmental contamination, and intrahospital transmission in critically ill patients.

The study will be carried out in the Adult Intensive Care Unit (AICU) of Hospital Italiano de Buenos Aires (HIBA), between January 1, 2026, and June 1, 2026.

4.2. Study setting

HIBA is an integrated healthcare network composed of two hospitals, the main campus and the San Justo campus (HISJ). HIBA is a high-complexity university hospital located in the Autonomous City of Buenos Aires. It has 789 beds, 246 of which are dedicated to critical care; approximately 2.5 million outpatient visits per year, 41,000 discharges, and performs approximately 45,000 surgical procedures in its 57 operating rooms [3]. In addition, it has a prepaid healthcare system called *Plan de Salud*, with approximately 184,000 members.

Care across all settings is centralized in a single Electronic Health Record (EHR). This data repository contains administrative patient information (such as diagnostic studies, appointment requests, outpatient visits, hospitalizations, and medication use) as well as all clinical information (health problems, clinical diagnoses, medical progress notes, and test results, among others). All patient problems or comorbidities are automatically coded through a terminology server with a local thesaurus that maps and encodes information using the SNOMED controlled vocabulary [4].

The Adult Intensive Care Unit of Hospital Italiano de Buenos Aires is a high-complexity service with 38 beds, of which 30 are single rooms and 8 are located in an open area, with 6 beds specifically designated for solid organ transplant patients. This multidisciplinary service specializes in quality-of-care programs, organ transplantation, shock, sepsis, trauma, neurocritical care, and respiratory care. In addition to its clinical focus, it stands out as a national reference center for academic training of critical care specialists, including physicians, nurses, pharmacists, and physical therapists.

The infection control program (ICP) in the Adult Intensive Care Unit of Hospital Italiano de Buenos Aires focuses on the active prevention of healthcare-associated infections (HAIs) and on preventing and containing the emergence and dissemination of multidrug-resistant microorganisms. This program is based on daily surveillance by an infection control nurse who monitors the incidence of device-associated infections (catheters, mechanical ventilation), follows culture results and implements with local staff preventive measures. The ICP promotes and audits compliance with bundles of specific measures to prevent HAIs, together with the rigorous application of contact precautions, hand hygiene, and environmental hygiene to control transmission of these pathogens.

Complementarily, surveillance is performed through rectal swabs to detect colonization by carbapenemase-producing Enterobacterales upon admission of patients with risk factors and weekly in those hospitalized for more than 48 hours in the unit. Risk factors considered at admission include a history of hospitalization in any institution within the previous year for more than 24 hours, referral from another center, and being a patient listed for transplantation (hematopoietic stem cell or solid organ).

4.3. Population

Individuals hospitalized in the ICU of HIBA during the period 01/06/2026 to 31/12/2026 who meet the following criteria:

4.3.1. Inclusion criteria

- Positive surveillance swab for carbapenemase-producing Enterobacterales (CPE).

4.3.2. Exclusion criteria

- Patients colonized or infected with CPE who remained in the same room for less than 48 hours.

4.3.3. Definitions

CPE-colonized patient:
An individual who carries carbapenemase-producing Enterobacterales in the gastrointestinal tract or other body surfaces, without necessarily presenting symptoms of infection.

CPE-infected patient:
An individual who presents a disease or clinical symptoms caused by the presence and multiplication of carbapenemase-producing Enterobacterales in tissues or fluids and is receiving antimicrobial treatment for CPE.

“Super-spreader” patient:
A subgroup of patients colonized or infected with CPE characterized by having a higher bacterial load in rectal swab cultures and a greater capacity to contaminate the hospital environment with CPE, defined as a value greater than 50 colony-forming units (CFU) in the environment.

Index patients:
Adult hospitalized patients with known or recent colonization by CPE (confirmed by culture and/or prior PCR in rectal swabs) who represent the first case with their genotype in the Adult Intensive Care Unit of Hospital Italiano de Buenos Aires (ICU) during the study period.

Contact patients:
Hospitalized patients who have remained for more than 48 hours in adjacent rooms or in the

same unit as index patients, without known CPE colonization at the beginning of the study, who will be evaluated with rectal swabs for CPE according to the routine weekly surveillance protocol in the ICU.

Secondary cases:
Hospitalized patients in adjacent rooms or in the same unit as index patients, without known CPE colonization at the beginning of the study, who become positive during the study period with the same genotype as an index patient.

4.4. Intervention and data collection

4.4.1. Baseline screening

At the beginning of the study period (T0), a unit-wide rectal surveillance screening will be performed for all patients hospitalized in the Adult Intensive Care Unit (AICU), regardless of previous colonization status. This screening will be conducted within the framework of the institutional infection control surveillance program and aims to establish the baseline prevalence of carbapenemase-producing Enterobacterales (CPE) in the unit.

Patients identified as CPE-positive in this baseline screening will constitute the initial cohort of index cases.

Following this initial assessment, routine weekly rectal surveillance will continue in accordance with the standard institutional protocol of the Infection Control Committee. Any newly identified CPE-positive patients during the study period will be incorporated as incident cases and analyzed as new index or secondary cases, depending on genomic and epidemiological findings.

4.4.2. Rectal swabs in patients with CPE

Patients with positive surveillance results for CPE will be classified as index or secondary cases according to the timing of detection and genomic relatedness analyses.

For each CPE-positive patient, two rectal swabs will be collected simultaneously during routine surveillance procedures: one for quantitative culture (Microbiology) and one for molecular analysis (Molecular Biology).

For culture-based quantification, rectal swabs will be inoculated onto CHROMagar™ KPC plates, and the number of colony-forming units (CFU) will be counted to estimate bacterial load. Results will be expressed as CFU per swab and $\log_{10}$-transformed for analysis when appropriate.

For molecular quantification, DNA will be extracted directly from rectal swab samples and analyzed by real-time PCR (BD MAX™ system) targeting carbapenemase genes (blaKPC, blaNDM, blaOXA-48, blaVIM, and blaIMP). The cycle threshold (Ct) value will be recorded and interpreted as an inverse measure of bacterial load (lower Ct values indicating higher bacterial load).

To allow more precise quantification, Ct values may be correlated with absolute gene copy numbers using standard curves constructed from serial dilutions of plasmids containing the respective carbapenemase genes (blaKPC, blaNDM, blaOXA-48, blaVIM, and blaIMP) [5].

Simultaneously, environmental samples will be collected from the immediate surroundings of the patient as described in Section 4.4.3.

4.4.3. Environmental sampling

- Simultaneously with rectal swab collection, samples will be obtained from five high-touch surfaces in the rooms of patients colonized or infected with CPE who have remained in the same room for more than 48 hours, always following the same sampling protocol. Surfaces include bed linen (around the pillow, groin area, and legs), the personal bedside table, and the infusion pump, selected based on previous scientific studies, a pilot sample, and prior environmental hygiene monitoring.

- Samples will be collected in the morning, prior to cleaning and linen change, and immediately transported to the laboratory. Sampling will be performed using appropriately supplemented CHROMagar™ KPC contact plates. Environmental contamination will be quantified by the total colony count across the five plates.

- From each positive environmental site, one suspected colony will be isolated and processed. Bacterial identification and isolates will also be analyzed for carbapenemase genes blaKPC, blaNDM, blaOXA-48, blaIMP, and blaVIM, according to previously described methodologies [18].

- All environmental samples will undergo quantitative culture on chromogenic media and PCR analysis for the detection of CPE.

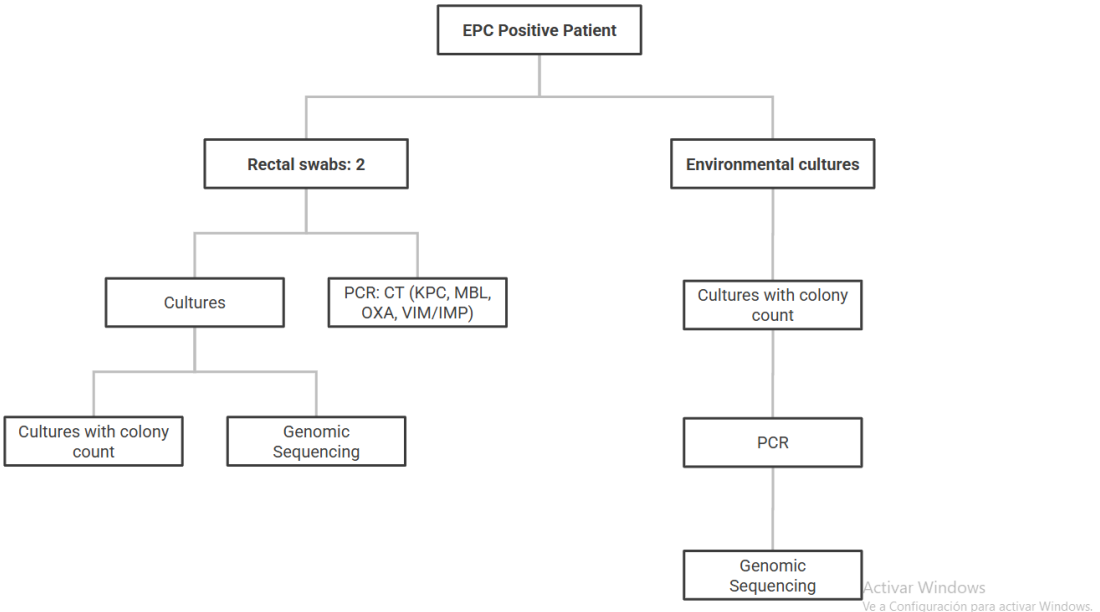
4.4.4. Molecular characterization

- **Genetic amplification and automated sequencing:**
  DNA will be extracted from CPE culture samples obtained from rectal swabs of index patients, environmental samples, and contact patients using the MagCore Genomic DNA Bacterial Kit and the MagCore Plus II platform (RBC Bioscience). The genes blaKPC, blaNDM, blaOXA-48, and blaVIM will be amplified by PCR using specific primers. PCR products will be purified using a Wizard® SV gel and PCR Clean-Up System kit (both from Promega Corporation, Madison, WI, USA), according to the manufacturer's instructions. Purified PCR products will be bidirectionally sequenced on an ABI 3730XL sequencer (Applied Biosystems, Carlsbad, CA, USA).
- **Sequence quality analysis and alignment:**
  The quality of the electropherograms obtained during sequencing will be analyzed using ChromasPro software (http://www.technelysium.com.au/chromas.html). At least two sequences of each carbapenemase type will be selected from databases to perform alignments using MEGA 4.0. Similarities among the obtained nucleotide sequences will be verified using BLASTN [6,7].
- **Identification of blaKPC variants:**
  To identify blaKPC gene variants, all sequences will be translated into amino acids

using ExPASy software (translation tool: http://web.expasy.org/translate/) in all six reading frames. The correct translation will be selected based on available GenBank data. The deduced amino acid sequences will be compared with GenBank KPC protein sequences using BLASTX.

- **Pulsed-field gel electrophoresis (PFGE):**
  PFGE is a molecular technique used to separate large DNA fragments and is particularly useful for bacterial typing. PFGE of genomic DNA digested with XbaI will be performed using a CHEF-DR III system (Bio-Rad, Hercules, CA), as previously reported by Gómez et al. [8]. *Salmonella enterica* serotype Braenderup (H9812) digested with XbaI will be used as the molecular weight standard.

PFGE gels will be analyzed and interpreted according to Tenover et al. [9]. Plasmid content and estimated sizes will be determined by digestion of genomic DNA with the S1 endonuclease, followed by PFGE (S1-PFGE), as described by Barton et al. [10].



4.5. Variables

4.5.1. Primary variable (independent)

Variable	Conceptual definition	Operational definition	Type / Unit
CPE bacterial load	Concentration of carbapenemase-producing Enterobacterales present in the gastrointestinal tract of a colonized patient, reflecting the degree of colonization and dissemination potential.	Measured using two complementary methods: (1) real-time PCR (BD MAX™ system), expressed as cycle threshold (Ct), inversely proportional to bacterial DNA quantity; and (2) quantitative culture on CHROMagar™ KPC, expressed as colony-forming units per milliliter (CFU/mL).	Continuous quantitative (Ct and $\log_{10}$ CFU/mL)

4.5.2. Dependent variables

Variable	Conceptual definition	Operational definition	Type / Unit
Environmental contamination by CPE	Presence and concentration of CPE on surfaces in the immediate environment of a colonized patient.	Quantitative cultures performed on five high-touch surfaces (bed linen, bedside table, infusion pump, etc.) using CHROMagar™ KPC. Expressed as: (a) number of positive surfaces and (b) total CFU per room.	Discrete quantitative (number of positive surfaces) and continuous (total CFU)
CPE transmission	Acquisition of CPE by other patients in the unit attributable to the same genotype detected in an index patient.	Identification of new positive cases (secondary cases) with genomic concordance (WGS or PFGE) with the index patient during the same hospitalization period and physical space.	Dichotomous categorical (yes/no)

4.5.3. Confounding or effect-modifying variables

Variable	Conceptual definition	Operational definition	Type / Unit
Length of stay in the same room	Duration of environmental exposure and potential accumulation of microorganisms.	Days elapsed from patient admission to the room until swab collection or discharge.	Continuous quantitative (days)
Fecal incontinence or diarrhea	Clinical condition that increases environmental dispersion of microorganisms.	Documentation in the medical record or nursing notes of fecal incontinence or liquid stools during the sampling period.	Dichotomous categorical (yes/no)
Prior antimicrobial use (previous 30 days)	Recent antibiotic exposure that may modify bacterial load or select for CPE.	Electronic record of systemic antibiotic prescriptions within the 30 days prior to CPE detection.	Dichotomous categorical (yes/no)
Contact isolation for other pathogens	Preventive measure that may alter sampling frequency or environmental conditions.	Active contact isolation at the time of detection due to pathogens other than CPE (e.g., MRSA, VRE).	Dichotomous categorical (yes/no)

Immunosuppression status	Degree of immune compromise that may influence colonization and bacterial clearance.	History of solid organ or hematopoietic stem cell transplantation, active immunosuppressive therapy, or hematologic malignancy.	Dichotomous categorical (yes/no)

5.3.4. Descriptive and characterization variables

Variable	Conceptual definition	Operational definition	Type / Unit
Age	Chronological age of the patient.	Years completed at the time of the positive swab.	Continuous quantitative
Sex	Sex recorded in the medical record.	Male / Female / Other.	Nominal categorical
Primary diagnosis at admission	Reason for ICU admission.	SNOMED coding extracted from the electronic health record.	Nominal categorical
Type of CPE	Carbapenemase-producing gene or enzymatic group.	Molecular detection by PCR (blaKPC, blaNDM, blaOXA-48, blaVIM, blaIMP).	Nominal categorical
Date of positive swab	Date of sample collection with CPE detection.	Laboratory record.	Temporal (dd/mm/yyyy)
Hospitalization location	Physical location within the ICU.	Assigned unit and bed.	Nominal categorical

5.3.5. Derived variables (secondary analysis)

Variable	Conceptual definition	Operational definition	Type / Unit
Super-spreader	Patient with high bacterial load and high environmental contamination.	Defined post hoc as Ct values below the 25th percentile and ≥2 positive environmental surfaces with >50 CFU each.	Dichotomous categorical (yes/no)

4.6. Statistical considerations

4.6.1. Sampling and sample size calculation

Item	Description
Sampling strategy	Consecutive random sampling of all individuals admitted to the ICU of HIBA between January 1, 2026, and June 1, 2026, with positive surveillance for CPE.
Primary outcome	Correlation between CPE bacterial load measured by quantitative culture (CFU/mL) and cycle threshold (Ct) value obtained by real-time PCR.
Statistical test for sample size estimation	Bivariate correlation test (Pearson or Spearman), two-tailed.
Assumed correlation coefficient	Moderate inverse correlation ($r = -0.40$), based on previous studies demonstrating an inverse relationship between bacterial load and Ct values in clinical samples.
Significance level (α)	0.05
Statistical power	80%
Required sample size	47 patients with positive rectal swabs for CPE.
Expected losses / exclusions	Approximately 15% (technical failures, duplicate samples, incomplete data).
Final projected sample size	55 colonized patients.
Additional considerations	This sample size is sufficient for the primary correlation analysis and allows exploratory estimation of interindividual variability in Ct values.

4.6.2. Statistical analysis plan

Analysis objective	Variables involved	Statistical method
Correlation between Ct value and bacterial load	Ct value vs CFU/mL	Linear models and Spearman or Pearson correlation coefficients, depending on data distribution.

Association between bacterial load and environmental contamination	Ct value / CFU vs number of positive environmental surfaces and total CFU	Logistic regression or linear regression models.
Analysis of secondary cases (transmission)	Time to acquisition of CPE in contact patients vs exposure to index patients with different bacterial loads	Survival analysis using Cox proportional hazards models.
Genetic relatedness of isolates	Index, environmental, and secondary isolates	Phylogenetic analysis and reconstruction of transmission clusters using WGS and/or PFGE.
Ct vs CFU quantitative relationship	Ct value vs CFU counts from rectal swab cultures	Regression models (linear or logistic, as appropriate).
Transmission dynamics	Genomic data	Phylogenetic reconstruction to identify transmission clusters and direction of dissemination.

4.7. Ethical considerations

The present study is prospective and observational in nature and will be conducted exclusively using practices that are part of the routine epidemiological surveillance of Hospital Italiano de Buenos Aires (HIBA), in accordance with the institutional protocol approved by the Infection Control Committee for the prevention and control of carbapenemase-producing Enterobacterales (CPE) (2nd Revision, 2021) [11].

Such surveillance—through cultures and molecular testing of rectal swabs—constitutes a systematic healthcare activity in critical care units and in patients with risk factors, with the objective of identifying carriers and preventing the nosocomial transmission of multidrug-resistant microorganisms.

Within this context, the study does not introduce any additional procedures nor does it modify routine patient care. When an individual colonized with CPE is detected, the sample already obtained as part of routine surveillance will be used to perform complementary analyses (quantitative culture and PCR with Ct determination) in order to evaluate the correlation between both methodologies. In addition, environmental samples will be prospectively collected from the immediate surroundings of the patient. This procedure will be performed by members of the Infection Control Committee, who are routinely responsible for environmental surface surveillance and for monitoring compliance with isolation measures.

All data will be handled in a strictly confidential and coded manner, without including personal identifiers or sensitive information. Databases will be stored on restricted-access devices and will be deleted upon completion of the statistical analysis.

As the study is based on information and samples obtained within the framework of routine clinical practice, it does not entail additional risk for participants, does not alter their clinical care, and does not require direct contact with patients or the collection of new consent forms. Therefore, and in accordance with CIOMS Guideline 10 (2019) regarding modifications and waivers of informed consent, a waiver of written informed consent will be requested from the Ethics Committee on the grounds that:

- The research would not be feasible without such a waiver, as it involves patients hospitalized in intensive care units who may be unconscious or otherwise unable to provide informed consent, from whom data and samples are obtained routinely as part of institutional infection control activities.

- The study has high social and scientific value, as it may provide evidence to optimize strategies for controlling CPE transmission and rationalizing the use of prevention resources.

- The risks to participants are minimal and limited to the handling of clinical data under strict confidentiality safeguards.

The study will be conducted in accordance with national and international regulations governing human health research, including Resolution 1480/2011 of the Argentine Ministry of Health; Law 3301/09 of the City of Buenos Aires; the Declaration of Helsinki and its amendments; and the International Council for Harmonisation Good Clinical Practice guidelines (ICH E6).

The processing of personal data will comply with Argentine Law No. 25.326 (Personal Data Protection Act) and Law No. 26.529 (Patient Rights Act). All study data will be treated with the highest level of confidentiality, with access restricted exclusively to authorized study personnel in accordance with current legal regulations. Personal identifiers will be replaced by a randomly generated numeric code, and the identifying data will be removed from the analytical database in accordance with the 18 identifiers specified by HIPAA standards. The database will be stored on a password-protected computer accessible only to the principal investigator and will be permanently deleted after completion of the statistical analysis.

Approved by CEPI. Protocol approval No. 7703, PRIISA 18096.

4.8. Budget

Calculated based on USD exchange rate as of 02/05/2026

I. Patient Analysis (N=60 patients: 50 index cases and 10 potential secondary cases)

Rectal Swabs:

- **Validation Costs:**
    - Validation using media with NDM, KPC, OXA, VIM, and IMP strains.
    - **Chromogenic media total**: 84 plates x $1,992.20 = $167,344.80 (ARS) = **115 USD**
    - **CLED media total**: 63 plates x $766.49 = $48,288.87 (ARS) = **33 USD**
    - **Total PCR**: 30 x 48 USD = **1,440 USD**
    - **Total PCR (with donated kits)**: 30 x 18 USD = **540 USD**
- **Total Validation Cost (as of 02/05/26) including full PCR cost**: **1,585 USD**
- **Total Validation Cost (with donated PCR kits)**: **685 USD**

Patient Culture Costs:

- **Carbapenemase screening**: 19.5 USD x 60 patients = **1,172 USD**

PCR for Rectal Swabs:

- **Cost per sample**: 48 USD
- **Cost with donated PCR kits**: 18 USD
- **Total cost (60 patients) at full price**: 48 USD/sample x 60 patients = **2,880 USD**
- **Total cost (60 patients) with donated kits**: 18 USD/sample x 60 patients = **1,080 USD**

II. Environmental Samples (5 samples per index and secondary patient)

Quantitative Culture of Environmental Samples:

- **Total number of environmental samples**: 5 samples/patient x 60 patients = **300 samples**
- **Culture cost using contact plates (convex) for environmental surfaces**: **19.5 USD**
- **Total cost (300 samples)**: 19.5 USD/sample x 300 samples = **5,850 USD**
- **Total cost (150 samples)**: 19.5 USD/sample x 150 samples = **2,925 USD**

PCR for Environmental Swabs:

- **Cost per sample**: 48 USD
- **Cost with donated PCR kits**: 18 USD
- **Total cost (maximum 300 samples) at full price**: 48 USD/sample x 300 samples = **14,400 USD**
- **Total cost (minimum 150 samples) at full price**: 48 USD/sample x 150 samples = **7,200 USD**
- **Total cost (maximum 300 samples) with donated kits**: 18 USD/sample x 300 samples = **5,400 USD**
- **Total cost (minimum 150 samples) with donated kits**: 18 USD/sample x 150 samples = **2,700 USD**

III. Whole Genome Sequencing (WGS) Studies (Index and Secondary Cases)

Genomic Sequencing (including logistics/transport):

- **Sample extraction cost**: 10 USD
- **Sequencing cost**: 10 USD
- **Estimated 60 patients for sequencing**: 20 USD x 60 cases (maximum) = **1,200 USD**
- **Maximum environmental samples (assuming 100% positivity)**: 300 x 20 USD = **6,000 USD**
- **Minimum environmental samples (assuming 50% positivity)**: 300 x 20 USD = **3,000 USD**

IV. Correlation between Ct and Resistance Gene Copy Number

- Specific PCR for genes (bla_KPC, bla_NDM, bla_OXA-48, bla_VIM, bla_IMP): 4,000 USD
- Cloning into pGEM-T Easy vector: 1,500 USD
- Purification of recombinant clones: 450 USD
- Subtotal: 5,950 USD

V. Publication

- **Estimated Publication Fee (APC)**: **2,000 USD**

Total Budget

- **Total (assuming 50% environmental sample positivity)**: **34,912 USD**
- **Total (assuming 50% environmental sample positivity + donated PCR kits)**: **20,212 USD**

References

1. Lerner A, Adler A, Abu-Hanna J, Cohen Percia S, Kazma Matalon M, Carmeli Y. Spread of KPC-producing carbapenem-resistant Enterobacteriaceae: the importance of super-spreaders and rectal KPC concentration. Clin Microbiol Infect. 2015;21(5):470.e1–470.e7.
2. Lerner A, Romano J, Chmelnitsky I, Navon-Venezia S, Edgar R, Carmeli Y. Rectal swabs are suitable for quantifying the carriage load of KPC-producing carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother. 2013;57(3):1474–1479.
3. Hospital Italiano de Buenos Aires. Memoria institucional 2022. Buenos Aires: HIBA; 2022. Available from: https://trovare.hospitalitaliano.org.ar/greenstone/collect/instit/index/assoc/D1828.dir/intr-memoria-hiba-2022.pdf
4. Centro Nacional de Terminología en Salud. SNOMED CT en Argentina. Buenos Aires: Ministerio de Salud de la Nación; 2021. Available from: https://www.argentina.gob.ar/salud/terminologia
5. Wei M, Chen X, Liu J, Li T, Wang P, Wang S, et al. Development and validation of a novel multiplex real-time PCR assay for rapid detection of carbapenemase genes in carbapenem-resistant Enterobacterales isolates and clinical samples. Infect Drug Resist. 2024;17:3451–3462.
6. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–410.
7. De Belder D, Lucero C, Rapoport M, Rosato A, Faccone D, Petroni A, et al. Genetic diversity of KPC-producing Escherichia coli, Klebsiella oxytoca, Serratia marcescens, and Citrobacter freundii isolates from Argentina. Microb Drug Resist. 2018;24(7):958–965.
8. Gomez SA, Pasteran FG, Faccone D, Tijet N, Rapoport M, Lucero C, et al. Clonal dissemination of Klebsiella pneumoniae ST258 harbouring KPC-2 in Argentina. Clin Microbiol Infect. 2011;17(10):1520–1524.
9. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33(9):2233–2239.
10. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. Anal Biochem. 1995;226(2):235–240.
11. Hospital Italiano de Buenos Aires. Protocolo institucional para la prevención y control de enterobacterias productoras de carbapenemasas (2ª revisión). Buenos Aires: HIBA; 2021. Available from: https://hiba.hospitalitaliano.org.ar/archivos/noticias_archivos/74/archivos/KPC%202021%20compilado%20final.pdf