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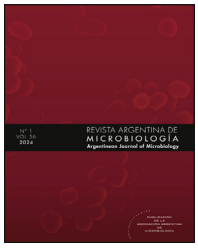

BRIEF REPORT

Predictors of rectal carriage of carbapenemase-producing Gram-negative bacteria in general wards: A case–control study

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KEYWORDS
Carbapenemase-producing *Enterobacterales*;
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Abstract Rectal carriage of carbapenemase-producing Gram-negative bacteria (CP-GNB-RC) increases the risk of invasive infections and horizontal transmission. This study aimed to identify predictors of CP-GNB-RC in hospitalized patients. A case–control study was conducted in a 300-bed hospital in Santa Fe, Argentina, between 2022 and 2023. Cases and controls were randomly selected and included in a 1:2 ratio. A multivariate binary logistic regression model was built using variables significant in univariate analysis ($p < 0.05$). Among 258 patients, 34.5% yielded at least one CP-GNB. Most isolates were *Klebsiella* spp. (79.0%) and 56.0% produced KPC. In the multivariate analysis, intensive care admission in the past 6 months (OR 3.61), patient transfer from another hospital (OR 2.89), antibiotic exposure (OR 2.34), and length of hospital stay (OR 1.05) were the only independent predictors of CP-GNB-RC. These findings may inform infection control strategies and antimicrobial stewardship interventions in our region.

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PALABRAS CLAVE
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Predictores de portación rectal de bacterias gram-negativas productoras de carbapenemasas en salas generales: Estudio de casos y controles

Resumen La colonización rectal por bacterias Gram-negativas productoras de carbapenemasas (CR-BGN-PC) aumenta el riesgo de infecciones invasivas. Se realizó un estudio de casos y controles en un hospital de Santa Fe (Argentina) entre 2022 y 2023 para identificar predictores de CR-BGN-PC. Se seleccionaron casos y controles de forma aleatoria en proporción 1:2. Se construyó un modelo de regresión logística binaria utilizando las variables con $p < 0,05$ en el análisis univariado. El 34,5% del total de pacientes evaluados (n = 258) presentó al menos una BGN-PC. *Klebsiella* spp. predominó entre los aislamientos (79,0%) y el 56,0% produjo KPC. En el análisis multivariado, los predictores de CR-BGN-PC fueron la estadía en cuidados críticos (menor de 6 meses; OR 3,61), la derivación desde otro hospital (OR 2,89), la exposición a antibióticos (OR 2,34) y los días de hospitalización (OR 1,05). Esta información podría orientar las estrategias de control de infecciones y de optimización del uso de antimicrobianos en nuestra región.

Antimicrobial resistance has escalated into a global pandemic with profound implications for healthcare outcomes and costs. Recent data from the Global Burden of Disease group emphasize that progress in reducing the projected 10 million annual deaths by 2050 has stalled, as estimated in 2016[6]. Carbapenemase-producing Gram-negative bacteria (CP-GNB) represent one of the most alarming antimicrobial-resistant threats in healthcare settings[11]. These organisms have limited treatment options due to the scarcity and high cost of newly developed antimicrobials, leading to significantly increased healthcare expenses and poorer patient outcomes[8].

Rectal carriage of CP-GNB (CP-GNB-RC) is recognized as a critical risk factor for developing invasive infections with these resistant organisms[9]. Carriers face an increased risk of CP-GNB infections and pose a threat of horizontal dissemination of carbapenemase genes among bacterial species in the hospital environment, potentially causing in-hospital outbreaks. Given the severity of these infections and their impact on both individual and public health, identifying and managing CP-GNB-RC has become essential to infection prevention strategies in healthcare institutions worldwide.

However, the literature on CP-GNB-RC predictors is highly heterogeneous, largely derived from intensive care settings, and lacks validated predictive tools. This gap is particularly evident in Latin America. Such scarcity of context-specific evidence limits the development of regionally tailored infection-control policies, and research in this area aligns with the World of Health Organization's call for research and development targeting pathogens that pose the highest threat to public health[11]. Therefore, we aimed to identify predictors of CP-GNB-RC among patients hospitalized in general wards.

This was a case–control study conducted in a 300-bed regional hospital in the province of Santa Fe, Argentina, between February 2022 and February 2023. Patients hospitalized in general wards were prospectively identified as part of another study conducted by the research team[1], and provided written informed consent for inclusion.

Patients were selected for CP-GNB screening through random sampling using lists of random numbers. Those selected underwent rectal swabbing to assess colonization. Rectal carriers (cases) and non-carriers (controls) were included, selected by randomization at a 1:2 ratio, excluding repeated swabs with no change in the colonization status.

Rectal swabs (RS) were plated on chromogenic agar (CHROMagar™ KPC, Paris, France) and incubated at 37 °C for up to 48 h. All resulting Gram-negative colonies were subsequently studied. Identification and antimicrobial susceptibility were determined by VITEK2® system (Biomérieux, Marcy-l'Étoile, France) using the AST-GN98 card. Results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines[3]. In those isolates with carbapenem resistance, carbapenemases were phenotypically identified using the double disc synergy test with amino-phenyl boronic acid and EDTA or by the combined disk method (DCM Brit, Britania Lab, Buenos Aires, Argentina). Screening for OXA-48-like carbapenemases was performed using the RESIST-5 O.K.N.V.I. immunochromatographic strip test (Britania Lab, Buenos Aires, Argentina).

Epidemiological data for each patient were extracted from electronic health records and were collected in electronic spreadsheets. Briefly, the variables included were: age, sex, previous contact with healthcare, length of hospitalization, use of invasive devices, in-hospital antibiotic use and duration of use prior to RS, previous surgical procedures, and immunosuppression (Table 2).

SPSS Statistics 27.0v was used to create a database and to perform the statistical analysis. Categorical variables were presented as relative and absolute frequencies [%(n)], and the association between two categorical variables was assessed using the *Chi*-square test for independence. Numerical variables were assessed for normality using the Kolmogorov–Smirnov's test. As all variables were non-normally distributed, data are presented as median and interquartile range (IQR). Median difference was assessed using the Mann–Whitney *U* test. A multivariate binary logistic regression model was built using variables that were

statistically significant ($p < 0.05$) to predict carriage. A spider plot was generated using Python to display all variables associated with CP-GNB-RC in the univariate analysis at a significance level of $p < 0.05$.

This trial was approved by the hospital's Scientific and Teaching Committee and the Provincial Ethics Committee of Santa Fe. The study was conducted in accordance with the Council for International Organizations of Medical Sciences and the International Council of Harmonization Good Clinical Practices guidelines and adhered to the Declaration of Helsinki, the Belmont Report, and the Nuremberg Code. Patient information was kept confidential, and only patients who provided informed consent were included in the study.

A total of 735 patients were selected for CP-GNB-RC screening during the study period. Of these, 12.5% (n = 92) yielded at least one species of CP-GNB. After exclusion for missing data (n = 3), 89 cases were included and 169 controls were selected at random. In total, 91 isolates were identified from the 81 rectal carriers, with *Klebsiella pneumoniae* being the predominant species (75.8%, n = 69). KPC was the most frequently expressed enzyme (56.0%, n = 51), followed by MBL (44.0%, n = 40). One isolate expressed both KPC and MBL, and another isolate was positive for OXA-48-like. The distribution of bacterial isolates and resistance mechanisms is shown in Table 1. Of the participants, 55.8% (n = 144) were male, with a median age of 47 years (IQR 32.8–59.0).

The most commonly used antibiotic with Gram-negative coverage was ampicillin–sulbactam (20.9%, n = 54), followed by piperacillin–tazobactam (18.2%, n = 47) and quinolones (12.8%, n = 33) (Table 2).

To identify variables associated with CP-GNB-RC, a univariate analysis was conducted. Rectal carriers were more frequently transferred from another center; admitted to an ICU either in the previous 6 months or during the current hospitalization; had longer hospital and ICU stays before RS; required mechanical ventilation; resided in long-term care facilities; carried invasive devices (particularly central venous catheters); received in-hospital antibiotics before RS (notably third-generation cephalosporins, carbapenems, and colistin); and underwent longer courses of antibiotic therapy (Table 2 and Fig. 1).

Then, a multivariate analysis was performed to isolate variables independently associated with CP-GNB-RC. ICU admission in the past 6 months (OR 3.61, 95% CI 1.41–9.25), transfer from another hospital (OR 2.89, 95% CI 1.38–6.04), antibiotic exposure (OR 2.34, 95% CI 1.26–4.35) and length of hospital stay (OR 1.05, 95% CI 1.03–1.08) were the only predictors of CP-GNB-RC. The different families of antibiotics were excluded from the multivariate analysis due to collinearity with the variable 'antibiotic exposure', which was deemed to be the best representation of the overall effect of antibiotic administration.

Our study provides valuable evidence on the risk factors associated with CP-GNB-RC in general wards of an acute tertiary-care hospital in Argentina. The two strongest independent predictors identified were: admission to an ICU within the previous six months and transfer from another healthcare facility. Notably, antibiotic exposure and prolonged hospital stay also emerged as highly relevant, given that they represent modifiable risk factors amenable to targeted interventions. Moreover, 36% of RS had been taken from patients that had not been admitted to the ICU nor transferred from another center, two traditional criteria for RS screening. Even more strikingly, 16.8% had not been hospitalized at all in the preceding six months, meaning they met none of the conventional risk criteria. As such, they would have escaped detection under most current screening protocols. These findings are particularly relevant given the current absence of predictive models for CP-GNB-RC with sufficient external validity, despite a substantial body of international literature on the topic. Our findings highlight the complexity of CP-GNB colonization dynamics in general wards and underscore the pressing need for context-specific risk assessment tools to improve early identification and containment of CP-GNB in diverse healthcare settings.

The lack of methodological standardization and consistent inclusion criteria in the current literature significantly hinders the ability to draw generalizable conclusions regarding risk factors for CP-GNB-RC acquisition. Differences abound in study designs, definitions, and statistical approaches[15]. For instance, while some studies adopt case–control designs, others employ cohort methodologies. The primary objective also differs: some studies focus solely on colonization, while others include both colonization and subsequent infections. Similarly, the diagnostic methods for carriage vary, ranging from RS, as in our study, to perineal and groin swabs, either alone or in combination. Furthermore, there is heterogeneity in the microbiological definitions used. While some studies, such as ours, specifically examine CP-GNB, others broaden the scope to include carbapenem-non-susceptible organisms without confirming carbapenemase production. Importantly, some studies consider CP-GNB-RC as a primary outcome, while others analyze it as a subgroup within broader multidrug-resistant bacteria colonization research. Such methodological disparities contribute to the observed heterogeneity in reported risk factors and highlight the challenges in deriving a universally applicable model.

Second, our study stands out for its focus on general wards, a setting that remains underrepresented in the literature. The majority of published studies have been conducted in closed units such as intensive care, hematology, or transplant wards, where patient populations and healthcare dynamics are markedly different. General wards, where patient acuity is lower and exposure to high-risk interventions may be less frequent, present unique challenges and opportunities for understanding CP-GNB colonization dynamics.

Third, our work adds critical insights from a Latin American context, a region where CP-GNB epidemiology, including carbapenemase subtypes, healthcare infrastructure, and patient demographics, differs significantly from other parts of the world. Geographical variability associated with socioeconomic status likely influences the relative frequency and impact of risk factors, highlighting the importance of region-specific studies. Although carbapenem resistance is a global issue, Latin America has been more severely affected compared to other regions[6]; however, data from this area remain limited.

In Argentina, only a few studies have evaluated CP-GNB-RC, therefore evidence on the clinical characteristics of carriers remains limited. Among them, a recently published study reported the epidemiology of a four-month surveillance period in an intensive care unit in Buenos Aires. In that

Table 1 Distribution of carbapenemase-producing Gram-negative bacteria isolates and mechanisms of carbapenem resistance.

Species	n. isolates	% total isolates	% (n) of isolates among each species		
			KPC	MBL	Oxa-48-like
Enterobacterales	85	93.40%	60.0% (51)	40.0% (34)	1.18% (1)
Klebsiella pneumoniae	69	75.80%	60.9% (42)	37.7% (26)	1.45% (1)
Escherichia coli	7	7.69%	14.3% (1)	85.7% (6)	0% (0)
Enterobacter cloacae	5	5.49%	80.0% (4)	20.0% (1)	0% (0)
Klebsiella aerogenes	2	2.20%	100% (2)	0% (0)	0% (0)
Serratia marcescens[a]	2	2.20%	100% (2)	50.0% (1)	0% (0)
NF-GNB	6	6.59%	0% (0)	100% (6)	0% (0)
Acinetobacter lwoffii	2	2.20%	0% (0)	100% (2)	0% (0)
Pseudomonas alcaligenes	2	2.20%	0% (0)	100% (2)	0% (0)
Pseudomonas putida	1	1.10%	0% (0)	100% (1)	0% (0)
Sphingomonas paucimobilis	1	1.10%	0% (0)	100% (1)	0% (0)
Total	91	100%	56.0% (51)	44.0% (40)	1.10% (1)

KPC: *Klebsiella pneumoniae* carbapenemase; MBL: metallo-β-lactamase; NF-GNB: non-fermenting Gram-negative bacilli.
[a] One isolate was a multiple carbapenemase producer (KPC and MBL).

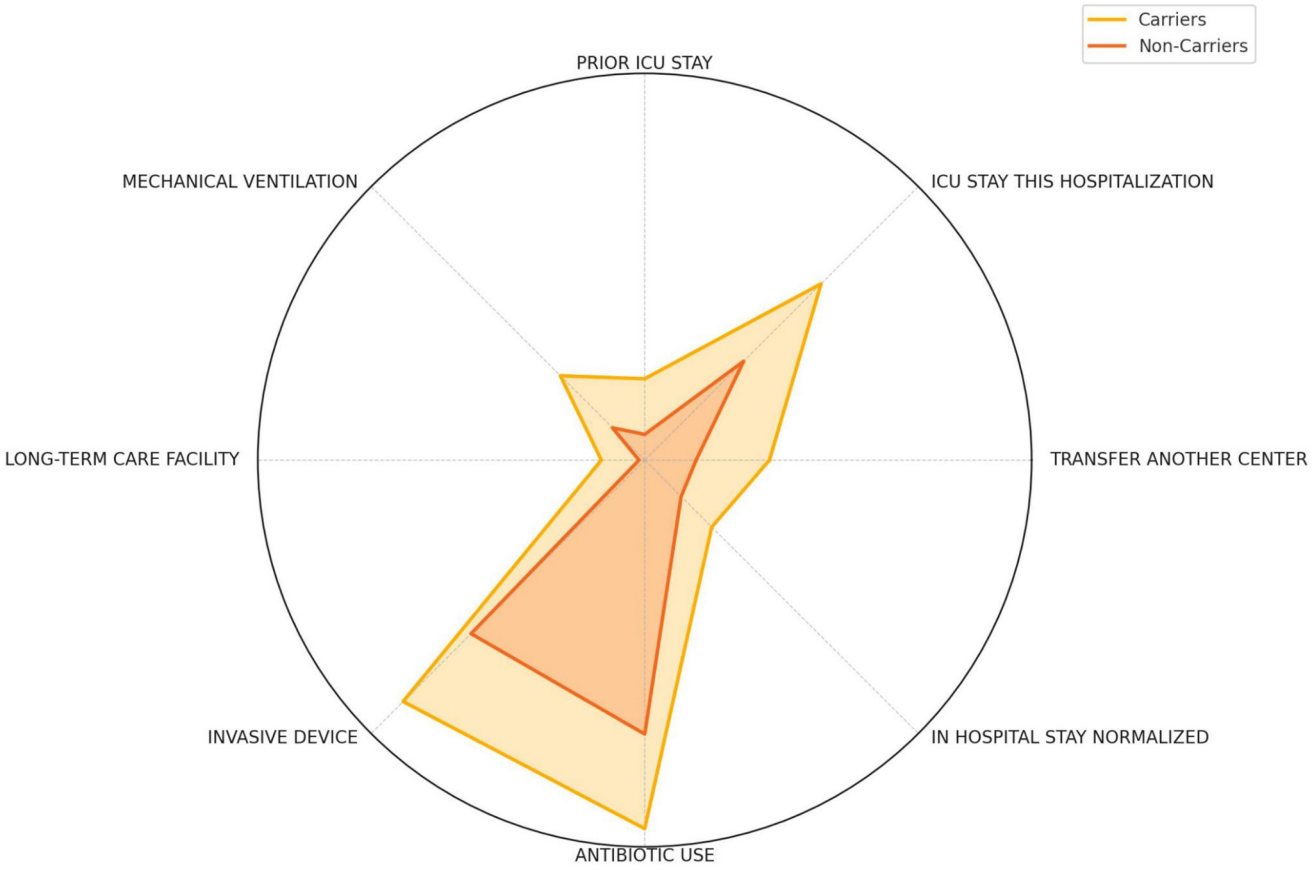

Figure 1 Spider diagram showing the distribution of the variables associated with rectal carriage of carbapenemase-producing Gram-negative bacteria in the univariate analysis. ICU: intensive care unit.

cohort, 40% of isolates harbored bla_{NDM} and 31% $bla_{OXA\text{-}48\text{-}like}$, followed by bla_{KPC} (17%), bla_{VIM} (6%), and $bla_{IMP/VIM}$ (6%). This distribution contrasts with our findings, in which $bla_{OXA\text{-}48\text{-}like}$ isolates were uncommon[13]. This discrepancy is likely driven by differences in local epidemiology, as our carbapenemase distribution more closely mirrors national data from clinical isolates reported through the WHONET registry. Another possible explanation is methodological: the previous study used a real-time PCR capable of detecting $bla_{OXA\text{-}48\text{-}like}$ variants that may remain undetected by phenotypic methods, since a proportion of these strains do not meet phenotypic suspicion criteria.

Table 2 Comparison of the main clinical and epidemiological variables in the univariate and multivariate analysis according to carriage status.

Variables	Total	CP-GNB carriers (n = 89)	Controls (n = 169)	Univariate *p*-value	Multivariate *p*-value	OR	95% CI
Sex (% men)	55.8% (n = 144)	59.6% (n = 53)	53.8% (n = 91)	NS	–	–	–
Age	47.0 (59.0–32.8)	52 (60.5–37.0)	45 (58.0–32.0)	NS	–	–	–
Transfers from another center	16.0% (n = 41)	25.8% (n = 23)	10.7% (n = 18)	**0.02**	**0.005**	2.89	1.38–6.04
Hospitalization in the last 6 months	42% (n = 108)	47.2% (n = 42)	39.5% (n = 66)	NS	–	–	–
ICU stay in the 6 months before hospitalization	9% (n = 24)	16.9% (n = 15)	5.4% (n = 9)	**0.003**	**0.007**	3.61	1.41–9.25
ICU stay during the current hospitalization	37% (n = 95)	51.7% (n = 46)	29.2% (n = 49)	**<0.001**	NS	–	–
In-hospital stay before RS (days)	8 (14–3)	10 (17–6)	6 (13–3)	**<0.001**	**<0.001**	1.05	1.03–1.08
ICU stay before RS (days)	3 (9–0)	8 (18–1)	2 (5–1)	**<0.001**	–	–	–
MV during current hospitalization	15.9% (n = 38)	26.8% (n = 22)	9.82% (n = 16)	**0.001**	–	–	–
Long-term care facility resident	3.90% (n = 10)	9% (n = 8)	1.2% (n = 2)	**0.002**	–	–	–
Invasive devices	57.6% (n = 148)	70.8% (n = 63)	50.6% (n = 85)	**0.002**	NS	–	–
Urinary catheter	39.3% (n = 101)	47.2% (n = 42)	35.1% (n = 59)	NS	–	–	–
Central venous catheter	29.6% (n = 76)	39.3% (n = 35)	24.4% (n = 41)	**0.013**	–	–	–
Hemodialysis catheter	7% (n = 18)	10.1% (n = 9)	5.4% (n = 9)	NS	–	–	–
Drainage catheter	31.1% (n = 80)	36% (n = 32)	28.6% (n = 48)	NS	–	–	–
CP-GNB infection in the last 6 months	12.3% (n = 31)	17.2% (n = 15)	9.6% (n = 16)	NS	–	–	–
In-hospital antibiotic use before RS	63.4% (n = 163)	76.4% (n = 68)	56.5% (n = 95)	**0.002**	**0.007**	2.34	1.26–4.35
Ampicillin–sulbactam	20.9% (n = 54)	27.0% (n = 24)	17.8% (n = 30)	NS	–	–	–
Piperacillin–tazobactam	18.2% (n = 47)	23.6% (n = 21)	15.4% (n = 26)	NS	–	–	–
1st generation cephalosporins	2.30% (n = 6)	3.40% (n = 3)	1.80% (n = 3)	NS	–	–	–
3rd generation cephalosporins	8.90% (n = 23)	14.6% (n = 13)	5.90% (n = 10)	**0.02**	–	–	–
Carbapenems	8.90% (n = 23)	14.6% (n = 13)	5.90% (n = 10)	**0.02**	–	–	–
Quinolones	12.8% (n = 33)	15.7% (n = 14)	11.2% (n = 19)	NS	–	–	–
Trimethoprim–sulfamethoxazole	1.60% (n = 4)	2.20% (n = 2)	1.20% (n = 2)	NS	–	–	–
Colistin	6.20% (n = 16)	14.6% (n = 13)	1.80% (n = 3)	**<0.001**	–	–	–
Aminoglycosides	6.20% (n = 16)	7.90% (n = 7)	5.30% (n = 9)	NS	–	–	–
Other antibiotics	24.8% (n = 64)	31.5% (n = 28)	21.3% (n = 36)	NS	–	–	–
Length of in-hospital antibiotic use (days)	7 (12–4)	9 (15–4)	6 (10–4)	**0.005**	–	–	–
Surgery before RS	47.7% (n = 122)	46.1% (n = 41)	48.5% (n = 81)	NS	–	–	–
Immunosuppressed	16.4% (n = 42)	15.7% (n = 14)	16.8% (n = 28)	NS	–	–	–

Nagelkerke's R^2: 0.218. Model *p*: <0.001. Number of included cases: 256. NS: not statistically significant.
CP-GNB: carbapenemase-producing Gram-negative bacteria; OR: odds ratio; CI: confidence interval; ICU: intensive care unit; RS: rectal swab; MV: mechanical ventilation.
Bold values indicate statistical significance at p < 0.05.

Among our primary findings, a history of ICU admission within the past six months was associated with nearly a fourfold increase in the odds of CP-GNB-RC, consistent with findings reported by other authors, albeit with heterogeneous effect estimates[5]. Transfer from another hospital nearly tripled the odds of CP-GNB-RC. This finding must be considered in the context of the high prevalence of CP-GNB in healthcare settings in Argentina, particularly in the province of Santa Fe. These results are in agreement with previous studies that have also reported an association between CP-GNB-RC and hospital transfers from high-endemic settings[12].

Consistent with findings from other authors, we observed that antibiotic exposure more than doubled the odds of CP-

GNB-RC[5,7,12]. Some antibiotic classes were associated with a higher risk of CP-GNB-RC in the univariate analysis. Among these, the use of cephalosporins has been previously identified as a risk factor for CP-GNB-RC[14], as has the use of carbapenems[2,14]. In contrast, we did not observe an association with other beta-lactams or quinolones, consistent with prior findings[14].

We found that each additional day of hospitalization was associated with a 5% increase in the odds of CP-GNB-RC. Similarly, other studies have reported that longer hospital stays, compared to shorter ones, are linked to an increased risk of CP-GNB-RC[2,5,10,12,14].

Other variables that were associated with CP-GNB-RC in the univariate analysis, but not retained in the multivariate model, included the use of invasive devices, prior hospitalization, residence in long-term care facilities, ICU stay during the current hospitalization, and mechanical ventilation, among others.

Invasive devices have been reported by other authors to increase the odds of CP-GNB-RC[4,14]. Previous hospitalization has been previously associated with CP-GNB-RC in a heterogeneous manner, with variations depending on the length of hospitalization and the timeframes used to define recent hospitalization[2,4,5]. Long-term care facilities represent a heterogeneous group of institutions, ranging from long-term acute care hospitals to low-complexity elder care facilities. These settings have been extensively studied in the international literature due to the higher prevalence of CP-GNB carriage and infections observed among their residents compared to other non-hospitalized populations. In agreement with prior research, we also found in this study that residing in such facilities is associated with CP-GNB-RC[7,10]. ICU stay during the current hospitalization was associated with CP-GNB-RC in the univariate analysis, but not retained in the multivariate model, likely due to interactions with other variables. This is consistent with reports identifying the ICU as the setting with the highest prevalence of CP-GNB and a significant risk factor for both carriage and infection[14]. Notably, half of the rectal carriers in our study had a prior ICU admission. Mechanical ventilation, while excluded from the multivariate analysis due to collinearity with ICU admission, has also been previously reported as a risk factor for CP-GNB-RC[2,14].

We did not observe a relationship between CP-GNB-RC and sex, the presence of catheter drainages specifically among invasive devices, or hemodialysis[10]. Additionally, unlike other studies, we did not find an association with recent hospitalizations unless the patient had a prior ICU admission, as previously mentioned[12].

One potential limitation of this study is that patients were identified through random screening within a prevalence survey, whereas alternative study designs might have provided a stronger foundation for inferring causal relationships between risk factors and CP-GNB-RC. Evaluating prevalence offers the advantage of serving as a practical tool for identifying patients for targeted screening. Additionally, since community acquisition of CP-GNB remains a rare finding, relying primarily on clinical data and healthcare contact history may be sufficient. However, this approach may introduce recall bias if patients had recent interactions with other healthcare institutions.

Moreover, due to the retrospective nature of the study design, it did not allow for the inclusion of variables inherent to hospital epidemiology, such as CP-GNB pressure, defined as the number of colonized individuals relative to the total number of beds during the period when rectal samples were collected. This variable has been clearly associated with an increased risk of CP-GNB-RC in previous studies and is likely tied to factors that facilitate cross-transmission, including staff overload, insufficient cohorting, delayed contact identification, and other similar challenges[14].

Several variables that were not assessed in our study have previously been associated with CP-GNB-RC, including prior VRE carriage, gastrointestinal disease, proton pump inhibitor use, glucocorticoid use, comorbidities, presence of nasogastric tubes, and higher APACHE scores. Additionally, some factors primarily evaluated in ICU settings, such as enteral feeding, pressure ulcers, and morbid obesity, have also been linked to CP-GNB carriage[5,7,14].

Finally, although the inclusion criteria allowed for both *Enterobacterales* and non-fermenting Gram-negative bacilli (NF-GNB), most isolates were *Enterobacterales*. Therefore, extrapolating these findings to settings with a high prevalence of carbapenemase-producing NF-GNB should be done with caution.

In this study, we analyzed isolates only at the phenotypic level, since the objective did not include genotypic characterization of CP-GNB isolates. However, a subset of the patients included in this study had already been evaluated genotypically, and those results have been published elsewhere[1]. Briefly, 43 patients were positive for CP-GNB-RC and yielded 68 isolates, as they had been swabbed in duplicate for that study. Among the 58 *Enterobacterales* isolates, 58.6% carried bla_{NDM} and 43.1% bla_{KPC}; additionally, 44.8% harbored extended-spectrum β-lactamases genes (88.5% bla_{CTX-M}, with the remainder harboring bla_{PER}). Among the 10 NF-GNB, *Pseudomonas putida* (n = 3) and *Pseudomonas alcaligenes* (n = 2) expressed bla_{VIM}, and *Acinetobacter lwoffii* (n = 2) carried bla_{NDM}.

This study identified that, in a general ward of an Argentine tertiary-care hospital, transfer from another hospital, ICU stay within the previous six months, length of hospital stay, and antibiotic use – specifically third-generation cephalosporins and carbapenems – were independent predictors of CP-GNB-RC. These findings help address a critical gap in the literature by providing much-needed evidence from general ward settings in Latin America – where data on predictors of CP-GNB-RC remain limited – and underscore the importance of geographical context in understanding CP-GNB risk factors.

Future efforts should focus on standardizing study designs, definitions, and methodologies, while also considering regional and population-specific factors. This approach will be essential for effectively addressing the global challenge of CP-GNB colonization and infection.

The contrast with existing literature highlights the need for practical, context-specific tools to predict CP-GNB-RC. These tools are necessary not only to optimize resource allocation, but also to address key differences in timing (at hospital admission versus during hospitalization), care settings (general wards versus ICUs), and geographical epidemiology.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT-4.5 and Grammarly to improve the readability and language of the work. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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