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Predictive value of extra-rectal colonization and the *Giannella* risk score for subsequent carbapenem-resistant *Enterobacterales* infections in ICU patients: an Argentinean retrospective cohort study in a mixed KPC and NDM setting

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SUMMARY

Background: The increasing incidence of carbapenem-resistant *Enterobacterales* (CRE) infections, driven by carbapenemase-producing *Klebsiella pneumoniae* (KPC) and New Delhi metallo-β-lactamase (NDM) dissemination, demands reliable predictive tools to optimize empiric therapy. The Giannella risk score (GRS), initially developed in a CRE-KPC-dominant setting, may not perform equally in heterogeneous contexts. This study aimed to evaluate the predictive value of extra-rectal CRE colonization and the GRS for overall CRE infections, specific CRE-KPC and CRE-NDM infections, and bacteraemia in patients rectally colonized with CRE.

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Risk assessment
Colonization
Intensive care units



Methods: We conducted a retrospective cohort study of adults with rectal CRE-KPC and/or CRE-NDM colonization admitted to an intensive care unit in Argentina during a *Providencia stuartii*-NDM outbreak in a setting with endemic CRE-KPC circulation. Patients were followed up for up to 90 days. Associations were analysed using bivariate statistics, and logistic regression and predictive performance were evaluated through receiver operating characteristic (ROC) curves.

Results: Among 327 CRE rectal-colonized patients, 49.2% carried CRE-KPC, 30.3% CRE-NDM, and 20.5% were co-colonized. Overall, 10.7% developed CRE infections. Extra-rectal colonization was independently associated with infection (adjusted odds ratio: 3.5 [1.53—7.94], $P \leq 0.01$), with high specificity (93.9%) but low sensitivity (20.0%). The GRS showed moderate predictive performance for overall infection (area under the ROC curve, 0.66) with a threshold of $\geq$3 points. However, when applied specifically to CRE-KPC- or CRE-NDM-colonized patients, neither extra-rectal cultures nor GRS calculations reliably predicted infection or bacteraemia.

Conclusions: In this cohort, extra-rectal CRE colonization was the only independent predictor of subsequent infection. These findings highlight the need for carbapenemase-independent dynamic risk models better suited to mixed resistance environments.

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Introduction

The global increase of carbapenem-resistant *Enterobacterales* (CRE) infections, driven by the dissemination of carbapenemase-producing *Klebsiella pneumoniae* (KPC) and other resistance determinants such as New Delhi metallo-β-lactamase (NDM) [1—3], underscores the urgent need for reliable predictive tools to guide empiric antimicrobial therapy [4—7]. While novel β-lactam/β-lactamase inhibitor combinations targeting CRE-KPC, such as ceftazidime—avibactam, meropenem—vaborbactam, or imipenem/cilastatin—relebactam, have demonstrated clinical efficacy [8—12], their selective pressure on the epidemiology of other CRE subtypes remains a critical concern. Notably, the introduction of these agents has been associated with a global shift favouring CRE strains harbouring class B carbapenemases, including NDM, Verona Integron-encoded Metallo-β-lactamase (VIM), and Imipenemase Metallo-β-lactamase (IMP) [2,3,13], raising concerns about the evolving resistance landscape.

In this context, predictive tools like the Giannella risk score (GRS), which combines results from extra-rectal CRE colonization with patient history and evolving clinical data from those with rectal colonization by CRE, hold significant potential for optimizing empiric treatment strategies [5,14—16]. However, their applicability across diverse clinical and epidemiological settings remains uncertain. Although the GRS was designed to predict CRE infections broadly, its validation was primarily conducted in regions where *K. pneumoniae*-KPC predominated, vastly outnumbering other carbapenemase types such as NDM and OXA 48-like enzymes, as well as other *Enterobacterales* species. This epidemiological imbalance may affect its predictive accuracy in settings with a higher prevalence of alternative carbapenemases and non—*K. pneumoniae* CRE species.

The risk of developing an active infection, particularly bacteraemia, in colonized CRE patients remains a subject of ongoing debate [4—7,14,17,18]. Inadequate empiric therapy is frequently observed in this population, as definitive treatment is often delayed until susceptibility results become available, potentially leading to worse clinical outcomes [4,6,7,9,10,16,19—22]. Consequently, validating the GRS across diverse patient populations is essential to enhance its clinical usefulness. This is even more critical in settings where economic constraints limit access to rapid microbiological diagnostic and antimicrobial susceptibility testing methods [23].

This study aimed to assess, in a hospital with limited resources, the impact of extra-rectal CRE colonization and the GRS on the prediction of CRE infections, including those caused by CRE-KPC strains, CRE-NDM strains, and the combined group of both, among patients with rectal CRE colonization. Additionally, the effectiveness of these factors in forecasting bacteraemia was evaluated as the secondary outcome.

Methods

Study design and population

CRE extra-rectal colonization and the GRS were assessed in a retrospective observational cohort comprising all adult patients ($\geq$18 years) with rectal colonization by CRE-KPC and CRE-NDM detected at the intensive care unit (ICU) of a 60-bed emergency and trauma hospital in Buenos Aires Province, Argentina, with experience in clinical infections and colonization by CRE-KPC. The study was conducted between July 2016 and September 2019 during an outbreak of *Providencia stuartii*-NDM.

Rectal CRE colonization was identified through weekly rectal surveillance cultures, systematically performed on all patients transferred from other healthcare facilities, admitted to the ICU, or sharing rooms with known CRE-colonized individuals during the outbreak. Extra-rectal samples were obtained from non-rectal sites following a clinical suspicion that prompted microbiological evaluation and were subsequently classified as colonization when no clinical, radiological, or laboratory evidence of active infection was identified. These samples included skin and soft-tissue or surgical wound swabs, respiratory samples (tracheal aspirates or oropharyngeal swabs), urine samples, and isolated catheter

cultures (venous, abdominal, urinary, or neurosurgical drainage catheters) collected in the absence of criteria suggesting catheter-related infections. Patients undergoing intestinal decontamination with oral, non-absorbable antibiotics were excluded from the study.

CRE-colonized patients were followed up from the date of their first positive CRE surveillance culture to the occurrence of a CRE infection or until 90 days after their last recorded positive culture or in-hospital mortality. Patients with an initial negative rectal swab underwent weekly additional rectal swabs until discharge or death.

Data collection and variables'definition

Comorbidities were documented based on Charlson comorbidity index. Risk factor variables were assessed from the time of colonization diagnosis until the onset of CRE infection (for cases) or hospital discharge (for controls). Invasive hepatobiliary procedures encompassed hepatic surgery, liver transplantation, endoscopic retrograde cholangiopancreatography, percutaneous biliary drainage, and biliary stent placement. Invasive abdominal procedures included both open and laparoscopic abdominal surgeries, as well as percutaneous drainage. Any additional invasive procedures not specified in these categories were also recorded.

Corticosteroid therapy was defined as the administration of prednisone or its equivalent at a dose of $\geq$16 mg per day for more than 15 days. Neutropenia was classified as an absolute neutrophil count $\leq$500 cells/mm of blood for at least 7 days. Chemotherapy and/or radiation therapy refers to the use of cytotoxic antineoplastic agents or ionizing radiation, either for curative or palliative cancer treatment.

Rectal colonization was defined as the presence of CRE in a surveillance culture without clinical signs of infection. As described earlier, extra-rectal colonization was defined as the CRE isolation from non-blood, non-rectal clinical samples not fulfilling criteria for active infection. CRE infections were microbiologically confirmed and classified according to the criteria outlined by the Centers for Disease Control and Prevention and national guidelines. Bacteraemia was diagnosed when CRE was isolated from blood cultures in the presence of systemic inflammatory response syndrome.

To assess the GRS, the following criteria were considered: ICU admission (2 points), invasive abdominal procedures (3 points), receiving chemotherapy/radiation therapy (4 points), and extra-rectal CRE colonization, with each additional site contributing 5 points [14].

Microbiological studies and molecular biology

All clinical samples were transported to the microbiology laboratory without delay. Rectal surveillance swabs were directly inoculated on to MacConkey agar plates (Britania™, Buenos Aires, Argentina) supplemented with an ertapenem (10 μg) disk (Britania™) to facilitate the recovery of suspected CRE. After incubation at 37°C for 24 h, Gram-negative bacilli (GNB) were identified using a nephelometry-based system (Phoenix™, Becton Dickinson, Franklin Lakes, New Jersey, USA). Other clinical samples were processed using conventional culture media for the recovery of GNB. Recovered isolates from all sample types were identified using the same system, and antimicrobial susceptibility testing was performed following the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines [24]. Additionally, all suspected isolates underwent real-time polymerase chain reaction (rt-PCR) for the detection of bla_{KPC}, bla_{NDM}, and $bla_{OXA\ 48\text{-}like}$ genes according to Pasterán *et al*. previous publications [25,26]. CRE was defined according to CLSI criteria for reduced susceptibility or resistance to carbapenems, supported by phenotypic patterns suggestive of carbapenemase production as inferred from automated susceptibility testing (Phoenix™) [24], in combination with the detection of carbapenemase genes by rt-PCR [26,27].

Statistical analysis

A comparison was made between extra-rectal CRE cultures and GRS results among patients who developed CRE infections (including KPC, NDM, or both) and those who did not. The comparative analysis was conducted using the χ^2 test and Fisher's exact test for categorical variables, and either an unpaired *t*-test or the Mann—Whitney—Wilcoxon test for continuous variables, as appropriate. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the GRS were calculated for each cutoff. Receiver operating characteristic (ROC) curves were used to evaluate the predictive efficiency of the GRS, and an optimal breakpoint was assigned using Youden's J statistic. The association between rectal and extra-rectal colonization by CRE and the onset of infection probability was analysed using Kaplan—Meier survival curves and the log-rank test. Multi-variable analysis was conducted using logistic regression, including variables previously identified as risk factors for bloodstream infection in the original study by Giannella *et al*. and subsequent validation studies [7,14,15]. Statistical analysis was performed using R Statistics version 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria), with a *P*-value $<$0.05 considered statistically significant.

Results

Population characteristics

Throughout the study period, rectal colonization was identified in 327 individuals. Of the colonized patients, 134 (41%) were female, with a median age of 66 years (interquartile range [IQR], 21 years). Among them, 161 (49.2%) carried CRE-KPC, 99 (30.3%) carried CRE-NDM, and 67 (20.5%) were co-colonized. All CRE-KPC isolates corresponded to *K. pneumoniae*, and all CRE-NDM isolates to *P. stuartii*. Additionally, 21 (6.42%) had at least one extra-rectal site colonized by CRE, including 12 (3.65%) with CRE-KPC, six (1.8%) with CRE-NDM, and three (0.9%) with both. No other types of CRE were detected.

A total of 35 patients (10.7%) developed CRE infection, with a median time from rectal colonization to infection of 24.5 days (IQR: 9.0—76.0). The probability of infection at 90 days was higher among rectal-colonized patients than among non-colonized patients (10.1% vs 5.4%), although this difference did not reach statistical significance (log-rank $P = 0.08$; Supplementary Fig A1). Of these patients, 21 (60%) presented with CRE bacteraemia. CRE-NDM accounted for eight cases (22.8%), while the remaining 27 (77.2%) were due to CRE-KPC. The characteristics of the patients according to CRE infection status are detailed in Table I. Significant differences were

Table I
Characteristics of CRE rectal-colonized patients

	No CRE infection ($N = 292$)		CRE infection ($N = 35$)		*P*-value
Characteristics					
Age, years, median [IQR]	66.5	[54−74]	60	[45.5−67.5]	≤0.05
Male sex, *N* (%)	170	58%	23	65.7%	0.47
Surgery in the previous 3 months, *N* (%)	93	31%	12	34.3%	0.85
Neutropenia, *N* (%)	0	0%	0	0%	
GRS, median [IQR]	2	[0−2]	2	[2−5]	≤0.01
Chronic kidney disease, *N* (%)	10	3.4%	4	11.4%	≤0.05
Solid organ transplantation, *N* (%)	0	0%	0	0%	
Charlson comorbidity index, median [IQR]	2	[1−4]	3	[2−4]	≤0.05
Immunosuppression, *N* (%)	32	11%	2	5.7%	0.56
CRE extra-rectal positive cultures, *N* (%)	16	5.5%	5	14.3%	≤0.01
Ward of admission					
Medical, *N* (%)	95	32.5%	8	22.9%	0.32
Surgical, *N* (%)	30	10.3%	2	5.7%	
Intensive care unit, *N* (%)	167	57.2%	25	71.4%	
Source of CRE infection					
Abdominal, *N* (%)	-	-	6	17.1%	-
Catheter, *N* (%)	-	-	3	8.6%	-
Bone and joint, *N* (%)	-	-	1	2.9%	-
Primary, *N* (%)	-	-	5	14.3%	-
Respiratory, *N* (%)	-	-	15	42.9%	-
Urinary, *N* (%)	-	-	3	8.6%	-
Profound ischaemia, *N* (%)	-	-	2	5.7%	-
CRE bacteraemia, *N* (%)	-	-	21	60%	-
Clinical outcome					
Died during follow-up, *N* (%)	146	50%	13	37.1%	0.16

CRE, carbapenem-resistant *Enterobacterales*; GRS, Giannella risk score; IQR, interquartile range.

observed in age ($P \leq 0.05$), with CRE-infected patients being younger. Both the GRS ($P \leq 0.01$) and the Charlson comorbidity index ($P \leq 0.05$) were significantly higher in CRE-infected patients. Likewise, extra-rectal CRE-positive cultures were highly associated with CRE infection ($P \leq 0.01$). No significant differences were found regarding sex, chronic kidney disease, prior surgery, or immunosuppression. The majority of CRE-infected patients (71.4%) were admitted to the ICU.

The most frequently stated infection sources were respiratory (42.9%), abdominal (17.1%), and urinary (11.4%). CRE bacteraemia was present in 60% of cases. During follow-up, mortality was 37.1% among infected patients and 50% among non-infected patients ($P = 0.16$).

Extra-rectal cultures and GRS as predictors of CRE infection and bacteraemia

Extra-rectal cultures, encompassing both CRE-KPC and CRE-NDM isolates, in patients carrying rectal CRE were associated with the development of CRE infections. After adjustment for

Table II
GRS (in any CRE rectal-colonized patient and including any CRE extra-rectal culture) as a predictor of CRE infection

	Proportion of patients (%)	OR [95% CI]	*P*-value	Sensibility (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Score ≥ 2	69.42	2.82 [1.14−8.61]	0.03	85.71	32.53	13.22	95	38.23
Score ≥ 3	24.16	3.05 [1.46-6.31]	≤0.01	45.71	78.42	20.25	92.34	74.92
Score ≥ 4	18.96	3.38 [1.57−7.11]	≤0.01	40	83.56	22.58	92.08	78.9
Score ≥ 5	18.65	3.47 [1.61−7.3]	≤0.01	40	83.9	22.95	92.11	79.2
Score ≥ 6	8.56	3.25 [1.18−8.09]	≤0.01	20	92.81	25	90.64	85.02
Score ≥ 7	8.26	3.42 [1.23−8.57]	≤0.01	20	93.15	25.93	90.67	85.32
Score ≥ 8	3.98	1.64 [0.22−6.56]	0.64	5.71	96.23	15.38	89.49	86.54
Score ≥ 10	3.36	2.01 [0.27−8.4]	0.33	5.71	96.92	18.18	89.56	87.16
Score ≥ 12	2.45	3.01 [0.39−14.21]	0.21	5.71	97.95	25	89.66	88.07
Score ≥ 15	0.61	-	-	5.71	100	100	89.85	89.91
Score ≥ 17	0.61	-	-	5.71	100	100	89.85	89.91
Score ≥ 25	0.31	-	-	2.86	100	100	89.57	89.6

GRS, Giannella risk score; CRE, carbapenem-resistant *Enterobacterales*; OR, non-adjusted odds ratio; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

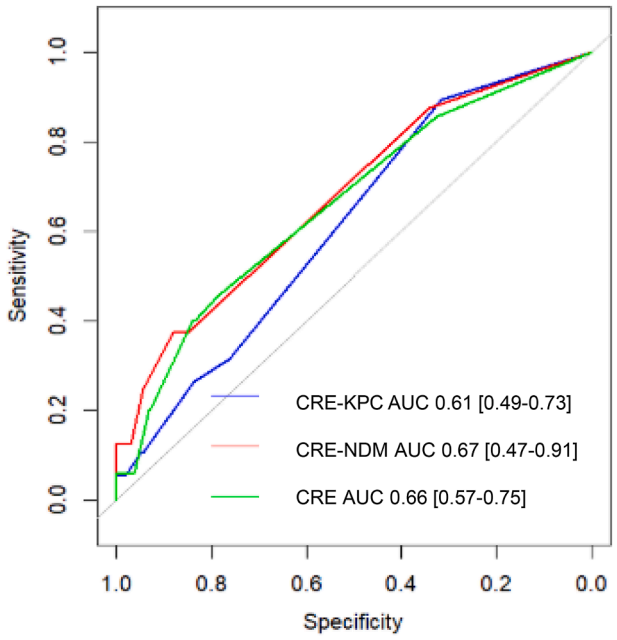


Figure 1. Receiver operating characteristic curve analysis showing the performance of the GRS for predicting CRE (green line), CRE-KPC (blue line), and CRE-NDM (red line) infections in CRE rectal-colonized patients. CI95%: [ ]; GRS, Giannella risk score; CRE, carbapenem-resistant *Enterobacterales*; KPC, carbapenemase-producing *Klebsiella pneumoniae*; NDM, New Delhi metallo-β-lactamase; CI, confidence interval; AUC, area under the ROC curve.

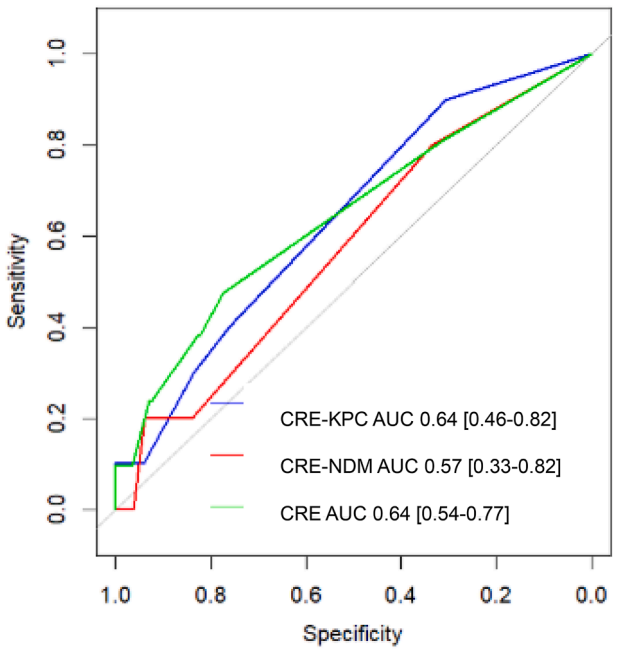


Figure 2. Receiver operating characteristic curve analysis showing the performance of the GRS for predicting CRE (green line), CRE-KPC (blue line), and CRE-NDM (red line) bacteraemia in CRE rectal-colonized patients. CI95%: [ ]; GRS, Giannella risk score; CRE, carbapenem-resistant *Enterobacterales*; KPC, carbapenemase-producing *Klebsiella pneumoniae*; NDM, New Delhi metallo-β-lactamase; CI, confidence interval; AUC, area under the ROC curve.

Charlson comorbidity index, ICU admission, invasive abdominal procedures, chemotherapy/radiotherapy, and extra-rectal CRE colonization, the latter remained the only independent risk factor for infection (adjusted odds ratio [aOR]: 3.5 [1.53—7.94], $P \leq 0.01$; Supplementary Table A1), showing a sensitivity of 20.0% and a specificity of 93.9%, with a PPV of 25.0% and an NPV of 92.0%. Thus, the 90-day probability of infection was significantly higher in patients with extra-rectal colonization than in those without (22.5% vs 7.3%, log-rank $P = 0.001$; Supplementary Fig A2). For patients colonized by any type of CRE, without distinguishing between specific types, a GRS with a threshold $\geq$3 provided the highest predictive efficacy for subsequent CRE infection. At this cutoff, sensitivity was 45.7%, specificity was 78.4%, with a PPV of 20.3%, an NPV of 92.3%, an overall accuracy of 74.9%, and an odds ratio (OR) of 3.1 (Table II). The area under the ROC curve (AUC) was 0.66 [0.57—0.75], indicating that the score had low-to-moderate predictive performance (Figure 1).

When assessing only CRE bacteraemia, and after adjustment for the previously described factors, extra-rectal colonization remained associated with infection (aOR: 3.3 [1.25—8.77], $P \leq$

Table III
GRS (in any CRE rectal-colonized patient and including any CRE extra-rectal culture) as a predictor of CRE bacteraemia

	Proportion of patients (%)	OR [95% CI]	*P*-value	Sensibility (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Score $\geq$ 2	69.42	1.89 [0.67—6.88]	0.33	80.95	31.37	7.49	96	34.56
Score $\geq$ 3	24.16	3.11 [1.23-7.77]	$\leq$0.01	47.62	77.45	12.66	95.56	75.54
Score $\geq$ 4	18.96	2.88 [1.08—7.25]	$\leq$0.05	38.1	82.35	12.9	95.09	79.51
Score $\geq$ 5	18.65	2.94 [1.1—7.42]	$\leq$0.02	38.1	82.68	13.11	95.11	79.82
Score $\geq$ 6	8.56	3.89 [1.16—11.11]	$\leq$0.01	23.81	92.48	17.86	94.65	88.07
Score $\geq$ 7	8.26	4.08 [1.21—11.71]	$\leq$0.01	23.81	92.81	18.52	94.67	88.38
Score $\geq$ 8	3.98	2.96 [0.4—12.37]	0.20	9.52	96.41	15.38	93.95	90.83
Score $\geq$ 10	3.36	3.63 [0.48—15.79]	0.15	9.52	97.06	18.18	93.99	91.44
Score $\geq$ 12	2.45	5.44 [0.68—26.53]	0.09	9.52	98.04	25	94.04	92.35
Score $\geq$ 15	0.61	-	-	9.52	100	100	94.15	94.19
Score $\geq$ 17	0.61	-	-	9.52	100	100	94.15	94.19
Score $\geq$ 25	0.31	-	-	4.76	100	100	93.87	93.88

GRS, Giannella risk score; CRE, carbapenem-resistant *Enterobacterales*; OR, non-adjusted odds ratio; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table IV
GRS best cut-off characteristics for CRE infection and bacteraemia

	GRS < 3 (*N* = 248)		GRS ≥3 (*N* = 79)		*P*-value
Age, years, median [IQR]	66	[54–74]	65	[46.5–71.5]	0.21
Male sex, *N* (%)	147	59.3%	46	58.2%	0.97
Surgery in the previous 3 months, *N* (%)	50	20.2%	55	69.6%	≤0.01
Chronic kidney disease, *N* (%)	10	4%	4	5.1%	0.75
Charlson comorbidity index, median [IQR]	3	[1–4]	3	[1–4]	0.38
Immunosuppression, *N* (%)	23	9.3%	11	13.9%	0.33
Bacteraemia, *N* (%)	11	4.4%	10	12.7%	0.33
Infection, *N* (%)	19	7.7%	16	20.3%	0.33
Ward of admission					
Medical, *N* (%)	83	33.5%	20	25.3%	≤0.01
Surgical, *N* (%)	17	6.9%	15	19%	
Intensive care unit, *N* (%)	148	59.7%	44	55.7%	
Clinical outcome					
Died during follow-up, *N* (%)	120	48.4%	39	49.4%	0.98

GRS, Giannella risk score; CRE, carbapenem-resistant *Enterobacterales*; IQR, interquartile range.

0.05), with a sensitivity of 21.43% and a specificity of 93.44% (PPV 16.67%, NPV 95.10%; Supplementary Table A2). Similarly, the GRS performance in predicting CRE bacteraemia presented the best threshold at ≥3 points. With this cutoff, sensitivity, specificity, PPV, and NPV were 47.6%, 77.4%, 12.7%, and 95.6%, respectively. The overall accuracy was 75.5%, with an OR of 3.11 ([1.23–7.77], $P \leq 0.01$) (Table III). In this case, the AUC reached 0.64 [0.54–0.77], demonstrating that the score offers a predictive performance that ranges from low to moderate (Figure 2).

Patients categorized based on the optimal GRS cut-off point of 3 displayed some differences in their characteristics and clinical outcomes, as detailed in Table IV. Notably, patients with a GRS ≥3 were more likely to have undergone surgery in the previous 3 months ($P \leq 0.01$) and were more frequently admitted to the ICU ($P \leq 0.01$). However, no significant differences in mortality were observed between the two groups during follow-up (48.4% vs 49.4%, $P = 0.98$).

Extra-rectal cultures and GRS as predictors of CRE-KPC infection and bacteraemia

After stratifying by colonization type, only extra-rectal cultures positive for CRE-KPC were included for GRS estimation and prediction of CRE-KPC infections and bacteraemia. The ORs for infection and bacteraemia were 2.79 ([0.6–9.03], $P = 0.17$) and 3.57 ([0.49–14.28], $P = 0.18$), respectively (Supplementary Table A3). The AUCs were 0.61 ([0.49–0.73]) for infection and 0.64 ([0.46–0.82]) for bacteraemia (Figures 1 and 2), indicating low-to-moderate predictive performance.

For predicting CRE-KPC infection, a GRS ≥2 achieved 35.1% accuracy, 88.89% sensitivity, 30% specificity (PPV 10.74% and NPV 96.61%), and an OR of 3.21 ([0.86–22.59], $P = 0.1$) (Supplementary Table A4). For bacteraemia, a GRS ≥3 yielded 73.08% accuracy, 44.4% sensitivity, 74.37% specificity (PPV 7.27% and NPV 96.73%), and an OR of 2.33 ([0.53–9.46], $P = 0.2$) (Supplementary Table A5).

Patients with GRS values above the respective thresholds had significantly higher Charlson comorbidity scores, recent surgical history (<3 months), and ICU admission rates. However, no significant association was observed between GRS above the threshold and the infection occurrence (Supplementary Table A6) or bacteraemia (Supplementary Table A7).

Extra-rectal cultures and GRS as predictors of CRE-NDM infection and bacteraemia

Similarly, for patients colonized with CRE-NDM, only extra-rectal cultures positive for CRE-NDM were considered for GRS calculation and prediction of CRE-NDM infections and bacteraemia. In both cases, extra-rectal cultures did not show a statistically significant association with the development of infections or bacteraemia. The ORs for CRE-NDM infection and bacteraemia were 3.72 ([0.51–14.92], $P = 0.16$) and 2.89 ([0.11–16.63], $P = 0.41$), respectively (Supplementary Table A3). The AUCs were 0.67 ([0.47–0.91]) for infection and 0.57 ([0.33–0.82]) for bacteraemia (Figures 1 and 2), indicating low-to-moderate predictive performance.

For predicting CRE-NDM infection, a GRS ≥5 achieved 85.9% accuracy, 42.9% sensitivity, and 87.8% specificity (PPV 13.6% and NPV 97.2%), with an OR of 5.37 ([0.93–27.66], $P = 0.056$) (Supplementary Table A8). To predict CRE-NDM bacteraemia, a GRS ≥7 yielded 6.7% accuracy, 20% sensitivity, 93.7% specificity (PPV 9.1% and NPV 97.4%), and an OR of 3.98 ([0.14–32.42], $P = 0.30$) (Supplementary Table A9).

When the CRE-NDM-colonized population was stratified by GRS thresholds (≥5 for infection and ≥7 for bacteraemia), the only significant association identified was a history of surgery within the previous 3 months in patients with a GRS ≥5. As with CRE-KPC, no significant association was found between a GRS above the threshold and the presence of CRE-NDM infection (Supplementary Table A10) or bacteraemia (Supplementary Table A11).

Discussion

Our study represents one of the first evaluations of the GRS in a clinical setting characterized by the coexistence of CRE-KPC and CRE-NDM colonization, including an outbreak of *P. stuartii*-NDM [28]. This context is epidemiologically distinct from those in which the GRS was originally developed and

validated, which were mainly CRE-KPC dominant [14], and thus provides novel insights into the applicability of the GRS in increasingly heterogeneous resistance landscapes.

We observed a CRE infection rate of 10.7% among rectal-colonized patients, aligning with prior findings: 7.8% bacteraemia in the original Giannella *et al*. study and 14.4% bacteraemia among CRE-KPC-colonized patients in Salazar *et al*. publications [14,15]. In contrast, Cano *et al*. reported a markedly higher overall infection rate of 44.7%, possibly reflecting a population with higher baseline risk or the influence of more virulent outbreak strains [7]. The high ICU admission rate (92.8%) in the Salazar cohorts likely contributed to their elevated prevalence, while differences in clinical context may also explain the variation [15]. Our hospital, a regional trauma referral centre, presents distinct demographic and clinical characteristics that could have influenced infection rates compared with transplant centres or institutions managing more diverse patient populations.

The predictive performance of the GRS varies considerably across studies. Giannella *et al*. reported an AUC of 0.80 for predicting CRE-KPC bacteraemia and proposed a low threshold ($\geq$2), yielding 93% sensitivity and NPV. In contrast, Cano *et al*. found an AUC of 0.92 for any CRE-KPC infection and identified a higher optimal cutoff ($\geq$7), with 84.8% of patients above this threshold developing infection compared with only 6.3% below. Salazar *et al*., validating the GRS for bacteraemia, reported an AUC of 0.76 and also identified a cutoff of $\geq$7; patients above this score had about seven times higher risk of developing CRE-KPC infection [7,14,15].

In our setting, the GRS showed only modest predictive capacity for overall CRE infections (AUC 0.66, threshold $\geq$3), and this performance was even lower for different subgroups: AUC 0.61 for CRE-KPC infection, 0.64 for CRE-KPC bacteraemia, 0.67 for CRE-NDM infection, and 0.57 for CRE-NDM bacteraemia. No specific threshold consistently achieved good discrimination. These results, even when focusing solely on CRE-KPC-colonized patients, suggest that a tool developed in a CRE-KPC dominant environment may not translate well to settings with significant CRE-NDM co-circulation. Epidemiological diversity and differences in carbapenemase distribution likely play a key role in diminishing the predictive strength of the GRS.

Differences in patient characteristics may also influence the findings. In the original study, control subjects were older and exhibited higher Charlson comorbidity scores than infected cases [14], In contrast, other validation studies observed the reverse trend, with infected patients being younger and less comorbid [7]. Our findings were comparable to the last mentioned: infected patients were younger, and higher GRS values were associated with ICU admission, recent surgery, and higher Charlson comorbidity index. These inconsistencies highlight how risk factors may interact differently depending on the population studied.

A consistent finding across studies is the critical role of extra-rectal colonization [5,7,14,15]. In our cohort, it emerged as the sole independent predictor of subsequent infection or bacteraemia, irrespective of the carbapenemase type. These findings are in line with previous reports, where extra-rectal (particularly multi-site) colonization has been associated with an increased risk of CRE infection and contributes substantially to GRS performance. In our study, the presence of extra-rectal colonization, regardless of carbapenemase type, appeared to be more informative than the GRS itself. However, this observation should be interpreted with caution, as in our cohort (as well as in the original study by Giannella *et al*. and subsequent validations by Cano *et al*. and Salazar *et al*.) [7,14,15], extra-rectal sampling was performed opportunistically, often driven by clinical suspicion of infection. This may introduce selection bias and potentially overestimate its predictive value. Therefore, prospective studies incorporating systematic extra-rectal screening protocols would be essential to more accurately define the true predictive role of this variable, particularly in settings with diverse epidemiological profiles.

Our unique setting, characterized by the co-circulation of *K. pneumoniae*-KPC and *P. stuartii*-NDM, also warrants consideration [28]. The occurrence of a *P. stuartii*-NDM outbreak, less common than *K. pneumoniae*-KPC, may influence colonization and infection dynamics. Additionally, colonization with one type of CRE does not preclude infection with another, particularly in contexts where surveillance sensitivity is suboptimal. This may partly explain the reduced predictive performance of the GRS, even in CRE-KPC-associated events [4,7,14,17].

Taken together, our findings highlight the challenges of applying predictive tools developed in homogeneous epidemiological settings to more diverse real-world contexts. Although the GRS remains a valuable tool in *K. pneumoniae*-KPC-dominant environments, its reduced performance in our cohort underscores the need for adaptable and dynamic risk models. Incorporating colonization by any CRE (regardless of carbapenemase type) may provide a more robust alternative. This is particularly relevant given the global spread of class B carbapenemases, such as NDM, likely accelerated by selective pressure from newer β-lactam/β-lactamase inhibitor combinations that primarily target class A enzymes [1–3,8,12,13,16,28,29]. Finally, it is important to note that our study was conducted before the widespread availability of newer agents such as ceftazidime–avibactam in Argentina. This contrasts with more recent studies, such as that by Cano *et al*., in which the use of these agents may have influenced both clinical outcomes and risk patterns [7,16,21,22].

This study has several limitations inherent to its retrospective design. Detection of extra-rectal colonization was opportunistic and likely underestimated, reflecting the surveillance approach commonly applied in previous observational cohorts from comparable healthcare settings [7,14,15]. Although our sample size was relatively large, external validation is necessary to confirm these findings. Additionally, the absence of chromogenic media for routine CRE screening, due to constraints imposed by local guidelines [25,27,30], may have led to under detection of colonization, though this is unlikely to have introduced bias in outcome-related analyses.

In conclusion, our study highlights the limitations of traditional risk prediction tools when applied to complex and evolving epidemiological settings marked by the co-circulation of multiple carbapenemase types. Although the GRS proved useful in CRE-KPC-dominant settings, its performance was suboptimal in our mixed CRE environment. Extra-rectal colonization by CRE, regardless of carbapenemase type, was the only independent predictor of subsequent infection or bacteraemia. These findings underscore the urgent need for updated carbapenemase-independent risk models that more accurately reflect the current, multi-faceted landscape of CRE dissemination.

CRediT authorship contribution statement

P. Favier: Writing — original draft, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **P. Suárez-Urquiza:** Formal analysis, Data curation. **C. Raffo:** Data curation. **D. Torres:** Data curation. **F. Pinilla-Huayta:** Data curation. **C. Muñoz-Soto:** Data curation. **M. Ravelli:** Data curation. **E. Serio:** Data curation. **L. Kumar:** Data curation. **P. Johanna:** Investigation. **Ivana Primost:** Methodology, Investigation. **M.I. Gallino:** Methodology, Investigation. **J. Pemán-García:** Writing — review & editing, Supervision, Conceptualization. **A. Valentín-Martín:** Writing — review & editing, Supervision, Conceptualization. **E.M. González-Barbera:** Writing — review & editing, Supervision, Data curation, Conceptualization.

Ethical approval

After analysing the presented results, the members of the ethics and research committee of the healthcare system of the Malvinas Argentinas County (Buenos Aires, Argentina) considered that the study did not require the approval of an ethics committee, as it was an intervention within the framework of an epidemiological surveillance program with necessary action to ensure the health of the participating subjects. Resolution 1480/2011 'Guidelines for Research with Human intervention', the Argentine Ministry of Health, stipulates in section A2.b that this type of intervention is exempt from the approval of an ethics research committee, as long as the personal data of the participating individuals are not involved.

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Conflict of interest statement

Patricio Favier has received financial compensation for consulting and speaking engagements from bioMérieux Argentina. Javier Pemán-García has received financial compensation for consulting, speaking, and research activities from Pfizer, Gilead, and MSD. Diego Torres has received financial compensation for consulting and speaking engagements from MSD, Pfizer, Knight, GSK, and Gilead. Amparo Valentín-Martín has received financial compensation for speaking engagements from Werfen. All other authors declare no conflicts of interest, financial or non-financial, relevant to this work. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.infpip.2026.100545.

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