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Prevalence and molecular characterization of carbapenem-resistant Enterobacterales in patients from a public referral hospital in a non-metropolitan region of Brazil during and post the SARS-CoV-2 pandemic

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Abstract

Antimicrobial resistance (AMR) poses a global threat, with carbapenem-resistant Enterobacterales (CRE) representing a significant concern due to limited therapeutic options. This study investigated the prevalence of carbapenemase genes in CRE strains isolated from tracheal aspirates of patients at a Brazilian university hospital between January 2020 and August 2023. Bacterial identification was conducted using MALDI-TOF, while carbapenemase genes were detected by qPCR. Demographic and clinical data were collected, and univariate analysis was performed using the chi-square test ($p < 0.05$). Variables with $p \leq 0.10$ were further investigated using the chi-square test for linear trend, along with stratified analysis. Out of 1,133 samples, 111 (9.79%) showed CRE growth, with 46 isolates included in the final sample, predominantly comprising *Klebsiella pneumoniae* (65.21%) and *Serratia marcescens* (19.57%). The bla_{KPC} gene was prevalent (78.26%), while bla_{NDM} was detected in 21.74% of cases. The identified population was predominantly male (67.39%), elderly (69.57%), white (56.52%), unmarried (63.04%), and had a low level of education (56.52%). Most patients (69.57%) were in the intensive care unit and remained hospitalized for more than 30 days (76.08%). There was a significant inverse trend between *Klebsiella pneumoniae* and age ($p = 0.045$), as well as a direct linear trend between bla_{NDM} and the annual increase in COVID-19 cases in Brazil ($p = 0.050$). A high probability of finding non-*Klebsiella pneumoniae* bacteria was observed in patients with prolonged hospital stays, independent of COVID-19 ($p = 0.006$) and the type of resistance genes ($p = 0.020$). The persistent prevalence of CRE, especially with bla_{KPC}, underscores the urgency of effective control measures.

Keywords Antimicrobial resistance · Tracheal aspirate · *Klebsiella pneumoniae* · *Serratia marcescens* · bla_{KPC} · bla_{NDM}

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Introduction

In recent years, the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have been sounding the alarm about the escalating threat posed by antimicrobial resistance (AMR) at a global level [1, 2]. In 2014, O'Neill projected that the ongoing rise in antimicrobial resistance until 2050 could lead to an annual mortality rate of 10 million people, coupled with an estimated decline of 2–3.5% in the world's Gross Domestic Product, resulting in staggering costs amounting to up to 100 trillion dollars [3].

The overuse of antimicrobials across various sectors, encompassing human and animal health, food production, and environmental contexts, continues to fuel bacterial resistance, underscoring the imperative to curb the inappropriate utilization of these agents [4, 5]. However, amid the coronavirus disease 2019 pandemic (COVID-19), a sustained surge in antibiotic usage has been noted, often lacking complete confirmation of secondary bacterial infections [6, 7]. Moreover, the sudden shifts in healthcare delivery during this period, including shortages of protective equipment and disruptions to infection prevention protocols, may potentially contribute to heightened rates of resistant infections within hospital settings [8].

Recent research suggests that the COVID-19 pandemic may have substantially contributed to the escalation of AMR worldwide [6, 9–11]. One preponderant mechanism driving antimicrobial resistance is the production of carbapenemases by Gram-negative bacteria (GNB), which leads to carbapenem inactivation, thereby narrowing therapeutic choices [12, 13]. Carbapenem-resistant Enterobacterales (CRE) are classified as critical threats to global health by the WHO, necessitating prioritization of research and the development of novel antibiotics [14].

The WHO and other international bodies stress the significance of multi-sectoral actions to combat AMR. They underscore the One Health approach as the most efficient and cost-effective strategy to confront this escalating challenge. Nevertheless, the dearth of innovation in the development of novel antimicrobials renders the containment of resistance increasingly arduous [4].

Given the global emergency concerning AMR, prevalence studies are of paramount importance, particularly regarding the high-risk bacterial groups highlighted by the WHO. The aim of this study was to examine the prevalence of carbapenem resistance genes, specifically bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{IMP}, and bla_{VIM}, in CRE isolated from tracheal aspirates of Brazilian hospitalized patients between January 2020 and August 2023 (covering both the pandemic and post-pandemic periods), while also identifying potential risk factors associated with resistant infections.

Methods

This retrospective cross-sectional study investigated the presence of bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{IMP}, and bla_{VIM} genes in CRE strains isolated from lower respiratory tract samples (tracheal aspirates) from patients undergoing intubation and/or mechanical ventilation. The study was conducted at a university hospital in Juiz de Fora, Minas Gerais, Brazil, covering the period from January 2020 to August 2023.

The hospital featured in this study is a non-profit institution, accredited as a teaching hospital, and supported by Brazil's Unified Health System (SUS). Situated in the Southeast Region of Minas Gerais, this facility serves as a key referral center within the regional healthcare network, addressing the needs of approximately 2 million residents across 94 municipalities [15]. The hospital provides both high and medium complexity services, with a total capacity of 140 inpatient beds distributed among various specialized areas. These include 17 pediatric beds, 9 in the Intensive Care Unit, 32 in the Women's Medicine ward, 23 in the Men's Medicine ward, 5 dedicated to Bone Marrow Transplantation, 25 in the Men's Surgery ward, 16 in the Women's Surgery ward, 10 in Gynecology, and 3 in Nephrology. This extensive range of specialties enables the hospital to deliver comprehensive and specialized care, effectively meeting the diverse and complex healthcare needs of the regional population.

The Microbiology laboratory of this hospital plays a central role in research, bacterial isolation, and identification stages, as well as being responsible for conducting antimicrobial susceptibility testing (AST) and phenotypic investigation of resistance mechanisms [15].

Tracheal aspirate samples were collected using an aspiration probe [16, 17], then plated on 5% sheep blood agar (Renylab Diagnósticos®) and MacConkey agar (Renylab Diagnósticos®). The plates were incubated aerobically at 35°± 1 °C for 24 to 48 h. Following the incubation period, microbiological tests were conducted to identify the species using biochemical and physiological assays [18, 19]. AST was performed using the disk-diffusion method on Mueller-Hinton agar (Ionlab Equipamentos para Laboratórios e Hospitais LTDA), with disks of imipenem (10 µg), ertapenem (10 µg), and meropenem (10 µg) (Oxoid, UK) and interpreted according to the criteria established by the Brazilian Committee on Antimicrobial Susceptibility Testing [20]. Bacterial strains exhibiting specific resistance mechanisms, including resistance to carbapenems, were inoculated in Trypticasein Soy Broth (TSB) (HIMEDIA®) with 15% glycerol and subsequently stored in a freezer at -10º to -20º C [17].

Exclusion and inclusion criteria

The inclusion criteria employed in this study were as follows: bacterial strains isolated from tracheal aspirate samples of hospitalized patients between January 2020 and August 2023, with a colony count of $\geq 10^5$ CFU/mL, identified as belonging to the order Enterobacterales, and exhibiting phenotypic resistance to at least one of the carbapenem antibiotics, namely: imipenem, meropenem, and/or ertapenem.

Bacteria for which cell viability was not observed after the freezing period were excluded from the final analysis, as well as bacterial isolates of the same species with identical resistance genotypes obtained from the same patient.

During the evaluated period, all CRE strains that could be recovered were included in the study.

Viability testing of frozen isolates

The CRE strains, chosen based on the inclusion criteria established for this study, were inoculated onto Mueller-Hinton (MH) agar medium (TM Media®) and incubated in an aerobiosis oven (FANEM LTDA) at $35° \pm 1$ °C for 24 to 48 h. Subsequently, viability was assessed by observing colony growth [17].

For samples that did not exhibit growth under the aforementioned conditions, a new culture was initiated using Blood agar (Renylab LTDA®) and Brain Heart Infusion (BHI) broth (Renylab LTDA®) from the freezing medium. These cultures were incubated under the same conditions as previously described, and after 48 h, bacteria that failed to display any growth were deemed non-viable [17].

Bacterial identification using matrix-assisted laser desorption ionization– time of Flight

The bacterial strains isolated and identified through phenotypic tests in the microbiology laboratory of the aforementioned hospital underwent analysis using the Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) method to confirm their species.

The mass profiles were obtained using a spectrometer, MALDI-TOF MTB - Smart (Bruker®), and the raw spectra were processed using the MaldiBiotyper program (Bruker Daltonics). This processing step took place at the General Bacteriology Laboratory of the Biological Institute of São Paulo, São Paulo, Brazil. The protein profiles of bacterial colonies were acquired following the ethanol/formic acid extraction protocol described by Freiwald and Sauer (2009), and the methodology for bacterial identification was consistent with that outlined by Bier et al. [21].

Biotyper analyses were classified based on score values recommended by the manufacturer: a score ranging from 2.3 to 3.0 signifies reliable species identification; between 2.0 and 2.29 indicates reliable genus identification with probable species identification; and between 1.7 and 1.99 suggests probable genus identification. Scores below 1.7 are indicative of unreliable identification. For this study, only identifications with a score exceeding 2.3 were deemed acceptable.

Extraction of genetic material from carbapenemase-producing bacterial strains and analysis of the respective genes encoding these enzymes by Real-Time Polymerase Chain Reaction

The obtaining of genetic material from the selected bacterial strains for this study and the subsequent molecular tests aimed at specific detection and differentiation of the carbapenemase-encoding genes (bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{IMP}, and bla_{VIM}) were conducted at the Molecular Biology Laboratory of the Faculty of Pharmacy, Federal University of Juiz de Fora, Minas Gerais, Brazil.

Following viability assessment, during which bacterial strains were cultured on solid MH agar (TM Media®) or Blood agar (Renylab Diagnósticos®) medium, a rapid extraction method was employed to ensure the effective obtaining of nucleic acid. Three to four isolated colonies were transferred using a sterile bacteriological loop to microtubes containing BHI broth (Renylab LTDA®) and incubated at a controlled temperature of $35° \pm 1$ °C overnight. Upon confirmation of bacterial growth, indicated by turbidity in the medium, 40 µL of the sample was combined with 40 µL of Pi-Lysis Nucleic Acid Extraction Reagent (Pi-Biotech Genética Avançada®, Juiz de Fora, Brazil) in a 1:1 (v/v) ratio using another sterile microtube.

The resulting sample was then subjected to a thermal lysis process at 95°C for 5 minutes, followed by a cooling period of 2 minutes and subsequent centrifugation at 3500 rpm for 30 seconds. This sequence of steps was meticulously executed to prepare the sample for Real-Time Polymerase Chain Reaction (qPCR) analysis. The VIASURE Carbapenemase-Producing Enterobacteriaceae Real-Time PCR Detection Kit (Certest Biotec S.L., San Mateo de Cállego, Zaragoza, Spain), based on the 5' exonuclease activity of DNA polymerase, was employed for this purpose.

For the qPCR reaction, 17 µL of buffer and 3 µL of DNA extracted from each bacterial strain were added to each well of the kit strips. The strips contained all necessary components for the multiplex qPCR reaction, including primers, probes, dNTPs, buffer, polymerase, and an internal control to monitor PCR inhibition. The strips were then sealed, briefly centrifuged, and placed into the Bio-Rad thermal

cycler (CFX96 Real-Time System). The qPCR protocol adhered to the manufacturer's recommended conditions: an initial polymerase activation step at 95 °C for 2 min, followed by 45 cycles of denaturation at 95 °C for 10 s, and annealing/extension at 60 °C for 50 s. Result interpretation was conducted with meticulous attention to detail, following the guidelines provided by the manufacturer to ensure accurate detection of carbapenemase-encoding genes [22].

Prior to testing clinical isolates, the qPCR kit and protocol were validated using DNA extracted from bacterial controls, including *Klebsiella pneumoniae* NCTC 13,438 (bla_{KPC}), *Klebsiella pneumoniae* NCTC 13,440 (bla_{VIM}), *Klebsiella pneumoniae* NCTC 13,443 (bla_{NDM}), *Klebsiella pneumoniae* NCTC 13,442 (bla_{OXA-48}), and *Escherichia coli* NCTC 23,476 (bla_{IMP}). The test also included positive and negative controls provided by the kit to ensure the reliability of the results.

Patient's data collection

Following the identification of all tracheal aspirate samples positive for CRE during the period covered by this study at the respective university hospital, a thorough analysis of the patients' medical records was conducted. Patient information was accessed through the hospital's electronic system, known as the Hospital University Management Application. Throughout this process, comprehensive data were collected, including demographic (gender, age, self-declared race, marital status, level of education) and clinical information (hospital sector, hospital stay and clinical outcome), as well as the results of tests confirming COVID-19 infection. These tests included both immunochromatographic tests and qPCR tests (Allplex SARS-CoV-2 Assay by Seegene Inc., Seoul, Republic of Korea).

Statistical and epidemiological analyses

Data obtained from tracheal aspirate cultures and evaluation of genes encoding carbapenemases, along with patients' demographic and hospital information, were subjected to analyses utilizing absolute and relative frequencies. Furthermore, the mean, standard deviation, and median were calculated for the variables of age and length of hospital stay.

The CRE species were categorized into two groups, *Klebsiella pneumoniae* and non-*Klebsiella pneumoniae*. The proportions of each group, along with the presence of associated genes, underwent univariate analysis to assess potential associations with patients' demographic characteristics and hospital data. This comparative evaluation utilized the chi-square test, with a test probability (p) of equal to or less than 0.05 considered as the threshold for statistical significance.

Given the small sample size ($n < 60$), more sophisticated multivariate analyses were not possible due to the lack of statistical power. Alternatively, variables with $p \leq 0.10$ in the univariate analysis were further investigated, including the chi-square test for linear trends and stratified analyses. The chi-square test for linear trend sought to evaluate possible significative linear relationships between two qualitative variables.

This linear trend chi-square was predicated on the hypothesis that with each year of worsening COVID-19 in Brazil (2023, 2020, 2022, and 2021), as evidenced by an increase in cases, there would be a corresponding rise in the proportion of certain carbapenem resistance genes. In other words, we believe that the burden of COVID-19 may have an inversely proportional relationship with the quality of intensive hospital services provided, such as intubation, surgeries, indiscriminate use of antibiotics, etc. Due to the decrease in the quality of intensive hospital services offered due to the burden of COVID-19, there may have been a higher rate of selection of resistant microorganisms and an increase in the dissemination of this resistance.

The stratified analysis, a simpler multivariate analysis, was employed to search for possible associations of two variables, by stratum of a third variable. This analysis assesses whether this third variable may be a confounding factor for the relationship between the other two variables [23]. The stratified analysis aimed to estimate odds ratios pertaining to hospital stays exceeding the median, while controlling for the impact of COVID-19 results and types of associated resistance genes. To explore interactions between variables, a test for stratum homogeneity was conducted [23]. In the absence of interaction and with a significant overall association, the adjusted summary measure of odds ratio was employed based on the Mantel-Haenszel estimate.

The data were initially organized using Microsoft Excel 2010, and the analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 14 and the Epitools software [24].

Results

Between January 2020 and August 2023, a total of 1,133 tracheal aspirate cultures were analyzed. Of the samples tested, 911 (80.40%) were positive for bacterial growth. Among these, non-fermenting Gram-negative bacilli were predominant, accounting for 598 (65.64%) cultures, with species such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* identified. The Enterobacterales group was the second most

frequent, with 248 (27.22%) cultures, including *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Escherichia coli* (Table 1). Lastly, 65 (7.14%) cultures were of Gram-positive cocci, with *Staphylococcus aureus* and coagulase-negative *Staphylococcus* identified.

When examining the resistance profile of isolates within the Enterobacterales order, it was observed that out of the 248 positive cultures, 111 (44.76%) exhibited resistance to carbapenems, including imipenem, meropenem, and ertapenem. Among the 111 resistant strains, 61 were subsequently successfully isolated and, thus, included in this study.

After excluding 15 duplicate isolates, the final sample consisted of 46 isolates, one from each patient, with species and gene distribution detailed in Table 2. A predominance of *Klebsiella pneumoniae* (65.21%) and the presence of the bla_{KPC} gene (78.26%) were observed. The bla_{NDM} gene was identified in 21.74% of the isolates, with an increase in its proportion from 20.00 to 42.86% between 2020 and 2022. The bla_{VIM}, bla_{IMP}, and bla_{OXA-48} genes were not detected, nor was the coexistence of more than one carbapenemase-producing gene. Although the comparative analysis between the years 2020 and 2023 did not show statistically significant differences in the proportions of species ($p = 0.075$) and genes ($p = 0.136$) in the hospital, the increased proportion of the bla_{NDM} gene warrants attention, highlighting a potential emerging trend during the study period.

The demographic and hospital characteristics of the patients (Table 3) revealed that the majority were male (67.39%). The average age of the studied population was 62.00 ± 13.98 years (median = 62 years, range 14 to 84 years), with the most affected age group being between 60 and 69 years (39.13% of cases). Patients who self-declared as white (56.52%), unmarried (63.04%), and with a low level of education (56.52%) predominated.

The intensive care unit (ICU) accounted for the highest number of cases (69.57%), and the length of hospital stay ranged from 5 to 273 days (mean = 78.43 ± 57.32 days, median = 69 days), with a notable proportion of patients remaining hospitalized for more than 30 days (76.08%). The clinical outcome recorded a mortality rate of 58.70%, with *Klebsiella pneumoniae* present in 59.26% of cases.

The predominance of *Klebsiella pneumoniae* and the bla_{KPC} gene was notable in practically all segments analyzed. However, it is important to highlight that the prevalence of the bla_{NDM} gene in this study demonstrated a progressive increase from 2020 to 2022, even though no statistical significance was detected ($p = 0.25$).

COVID-19 infection was confirmed in 34.78% of patients. Upon analyzing official information from the Ministry of Health regarding the number of new COVID-19 cases in Brazil [25], a direct linear trend was observed in this study between the ascending order of the years with the highest COVID-19 burden in Brazil (2023, 2020, 2022 and 2021), and the proportions of CRE carrying the bla_{NDM} gene ($p = 0.050$). This association was even more pronounced in patients with a negative result for COVID-19 ($p = 0.001$) (Fig. 1).

Concerning the different age groups, a significant inverse linear trend was observed between the proportions of *Klebsiella pneumoniae* ($p = 0.045$) (Fig. 2). In other words, the proportions of *Klebsiella pneumoniae* decreased significantly by age group ordered ascendingly.

On the other hand, the proportions of Non-*Klebsiella pneumoniae* bacteria were significantly more elevated in patients with a hospital stay exceeding the median (69 days), irrespective of COVID-19 results ($p = 0.0061$) (Table 4), and the resistance gene types ($p = 0.0200$) (Table 5). The odds of finding Non-*Klebsiella pneumoniae* in the groups with longer hospital stays (> 69 days) were 12.84 (95% CI 2.25–73.21) and 6.47 (95% CI 1.47–28.37) times that of the group with shorter stays, when adjusted for COVID-19 results and types of resistance genes, respectively.

Discussion

The findings of this study revealed a notable prevalence of cultures exhibiting bacterial growth exceeding 10^5 CFU/mL (80.40%), signifying a substantial bacterial burden with potentially significant clinical implications [17]. Prolonged stays in hospital environments, particularly in ICUs, elevate the risk of healthcare-associated infections (HAIs) [26]. The surge in HAI incidence observed in 2019–2020 is

Table 1 Distribution of positive culture results from tracheal aspirates ($n = 911$) of patients at a University Hospital from January 2020 to August 2023

Year	Non-Fermenting Gram-Negative Bacilli	Enterobacterales		Gram-Positive Cocci	Total
		Carbapenem-Susceptible	Carbapenem-Resistant		
2020	201 (22.06%)	34 (3.73%)	44 (4.83%)	13 (1.43%)	292 (32.05%)
2021	180 (19.76%)	34 (3.73%)	31 (3.40%)	25 (2.74%)	270 (29.64%)
2022	131 (14.37%)	31 (3.40%)	23 (2.52%)	17 (1.87%)	202 (22.17%)
2023*	86 (9.44%)	38 (4.17%)	13 (1.43%)	10 (1.10%)	147 (16.14%)
Total	598 (65.64%)	137 (15.04%)	111 (12.18%)	65 (7.14%)	911 (100.00%)

*Data for the year 2023 covers the period up to August 2023

Table 2 Distribution of Carbapenem-Resistant Enterobacterales (CRE) and their carbapenemase-encoding genes ($n=46$), isolated from tracheal aspirate samples of hospitalized patients at a University Hospital

Year	Microorganism										Total per year
	Klebsiella pneumoniae		Serratia marcescens		Enterobacter cloacae		Klebsiella aerogenes		Escherichia coli		
	bla_{KPC}	bla_{NDM}	bla_{KPC}	bla_{NDM}	bla_{KPC}	bla_{NDM}	bla_{KPC}	bla_{NDM}	bla_{KPC}	bla_{NDM}	
2020	6 (13.04%)	3 (6.52%)	5 (10.87%)	0	0	0	1 (2.17%)	0	0	0	15 (32.61%)
2021	9 (19.57%)	2 (4.35%)	0	0	0	1 (2.17%)	0	0	0	1 (2.17%)	13 (28.26%)
2022	1 (2.17%)	1 (2.17%)	2 (4.35%)	0	1 (2.17%)	2 (4.35%)	0	0	0	0	7 (15.22%)
2023	8 (17.39%)	0	2 (4.35%)	0	1 (2.17%)	0	0	0	0	0	11 (23.91%)
Total per species	24 (52.17%)	6 (13.04%)	9 (19.57%)	0	2 (4.35%)	3 (6.52%)	1 (2.17%)	0	0	1 (2.17%)	46 (100.00%)

A comparative analysis of species and genes was conducted during the period from 2020 to 2023. The results indicated the absence of statistically significant differences between the various years, as assessed by the Pearson chi-square test considering the prevalences of species ($p=0.075$) and genes ($p=0.136$)

primarily attributed to the extensive use of invasive devices, such as mechanical ventilation and vascular catheters [9, 11, 27–30]. Assessing the prevalence of carbapenem resistance genes in Enterobacterales isolated from tracheal aspirates of hospitalized patients since the onset of the COVID-19 pandemic could play a pivotal role in understanding the scope of antimicrobial resistance. This assessment could provide essential insights for treating and preventing specific resistance patterns.

Examination of positive cultures, characterized by non-fermenting GNB and predominant Enterobacterales, underscores the microbial diversity within the hospital setting. Further complicating the management of these infections, carbapenem resistance was observed in approximately half of Enterobacterales isolates, aligning with global apprehensions regarding resistance to these antibiotics [1, 2]. It is worth noting that reducing the inappropriate use of antibiotics stands as a central strategy in combating AMR [4, 5]. However, the COVID-19 pandemic has placed an overwhelming burden on health systems worldwide, potentially exacerbating the bacterial resistance scenario [6–8]. Literature underscores that between 1% and 10% of COVID-19 cases manifested secondary infections [31]. Nevertheless, up to 72% of critically ill patients were administered empiric antimicrobial treatment, inclusive of broad-spectrum regimens [7].

The predominance of *Klebsiella pneumoniae* observed in this study aligns with previous research identifying it as one of the primary disseminators of carbapenem resistance [32, 33]. In the Brazilian context, KPC-producing *Klebsiella pneumoniae* strains, particularly the ST437 and ST11 subtypes identified by multilocus sequence typing, are epidemic and pose a significant threat to public health [34–36].

In this study, the bla_{KPC} gene emerged as the most prevalent among the identified species, maintaining its predominance throughout the years under investigation. However, the bla_{NDM} gene was detected in 10 isolates (21.74%), with its proportion escalating from 20.00 to 42.86% between 2020 and 2022. Previous research has documented a surge in the incidence of carbapenem resistance in hospitals in São Paulo, Brazil (from 6.8% in 2011 to 35.5% in 2015). Notably, the bla_{KPC} gene was identified in 96.2% of the carbapenemase-producing *Klebsiella pneumoniae* strains examined [37]. In a more recent study, the detection rates of bla_{KPC} and bla_{NDM} in Enterobacterales were 68.6% (41,301/60,205) and 14.4% (8,377/58,172), respectively, based on data from Brazilian hospitals spanning from 2015 to 2022, integrated into the public laboratory information system [38].

The absence of additional resistance genes and the lack of co-occurrence of multiple carbapenemase-producing genes, coupled with the absence of statistically significant

Table 3 Demographic profile and hospital data of patients with positive tracheal aspirate cultures for Carbapenem-Resistant Enterobacterales (CRE) in a University Hospital from January 2020 to August 2023 ($n=46$)

Patient characteristics	Total	Grouped CRE species			Resistance genes		
		n Klebsiella pneumoniae (%)	n Non-Klebsiella pneumoniae (%)	p-value	n bla_{KPC} (%)	n bla_{NDM} (%)	p-value
Year of diagnosis							
2020	15	9 (60.00)	6 (40.00)	0.084	12 (80.00)	3 (20.00)	0.099
2021	13	11 (84.61)	2 (15.39)		9 (69.23)	4 (30.77)	
2022	7	2 (28.57)	5 (71.43)		4 (57.14)	3 (42.86)	
2023	11	8 (72.72)	3 (27.28)		11 (100.00)	0 (-)	
Gender							
Male	31	21 (67.74)	10 (32.26)	0.605	23 (74.19)	8 (25.81)	0.336
Female	15	9 (60.00)	6 (40.00)		13 (86.66)	2 (13.34)	
Age range (years)							
10–18	1	1 (100.00)	0 (-)	0.045	1 (100.00)	0 (-)	0.987
19–59	13	10 (76.92)	3 (23.08)		10 (76.92)	3 (23.08)	
60–69	18	11 (61.11)	7 (38.89)		14 (77.77)	4 (22.23)	
70–79	10	8 (80.00)	2 (20.00)		8 (80.00)	2 (20.00)	
80 or more	4	0 (-)	4 (100.00)		3 (75.00)	1 (25.00)	
Self-declared race							
White	26	16 (61.54)	10 (38.46)	0.934	21 (80.77)	5 (19.23)	0.257
Brown	10	7 (70.00)	3 (30.00)		7 (70.00)	3 (30.00)	
Black	4	3 (75.00)	1 (25.00)		2 (50.00)	2 (50.00)	
No information	6	4 (66.66)	2 (33.34)		6 (100.00)	0 (-)	
Marital status							
Married	17	11 (64.70)	6 (35.30)	0.471	14 (82.35)	3 (17.65)	0.796
Single	13	9 (69.23)	4 (30.77)		11 (84.61)	2 (15.39)	
Separated	4	1 (25.00)	3 (75.00)		3 (75.00)	1 (25.00)	
Widowed	5	4 (80.00)	1 (20.00)		3 (60.00)	2 (40.00)	
Other	7	5 (71.43)	2 (28.57)		5 (71.43)	2 (28.57)	
Level of education							
None	6	2 (33.34)	4 (66.66)	0.488	5 (83.33)	1 (16.64)	0.367
Incomplete primary education	13	9 (69.23)	4 (30.77)		8 (61.54)	5 (38.46)	
Complete primary education	7	5 (71.43)	2 (28.57)		7 (100.00)	0 (-)	
Complete secondary education	7	4 (57.14)	3 (42.86)		6 (85.71)	1 (14.29)	
Complete higher education	2	2 (100.00)	0 (-)		1 (50.00)	1 (50.00)	
No information	11	8 (72.72)	3 (27.28)		9 (81.81)	2 (18.19)	
Hospital sector							
Intensive Care Unit	32	20 (62.50)	12 (26.09)	0.559	27 (84.37)	5 (15.63)	0.129
Ward	14	10 (71.43)	4 (8.70)		9 (64.29)	5 (35.71)	
Length of hospital stay							
Up to 10	3	2 (66.66)	1 (33.34)	0.173	1 (33.34)	2 (66.66)	0.159
11 to 19	4	3 (75.00)	1 (25.00)		4 (100.00)	0 (-)	
20 to 30	4	4 (100.00)	0 (-)		3 (75.00)	1 (25.00)	
31 to 60	6	6 (100.00)	0 (-)		5 (83.33)	1 (16.67)	
61 to 90	13	8 (61.54)	5 (38.46)		8 (61.54)	5 (38.46)	
91 to 180	13	6 (46.15)	7 (53.85)		12 (92.30)	1 (7.70)	
181 to 360	3	1 (33.34)	2 (66.66)		3 (100.00)	0 (-)	
Length of hospital stay adjusted by the median (69 days)							
Up to 69 days	23	19 (82.60)	4 (17.40)	0.03	16 (69.60)	7 (30.40)	0.280
>69 days	23	11 (47.80)	12 (52.20)		20 (87.00)	3 (13.00)	
Clinical outcome							
Discharge	19	14 (73.68)	5 (26.32)	0.312	14 (73.68)	5 (26.32)	0.528
Deceased	27	16 (59.26)	11 (40.74)		22 (81.48)	5 (13.52)	
COVID-19 diagnosis							

Table 3 (continued)

Patient characteristics	Total	Grouped CRE species			Resistance genes		
		n *Klebsiella pneumoniae* (%)	n Non-*Klebsiella pneumoniae* (%)	*p*-value	n bla_{KPC} (%)	n bla_{NDM} (%)	*p*-value
Yes	16	13 (81.25)	3 (18.75)	0.095	12 (75.00)	4 (25.00)	0.695
No	30	17 (56.66)	13 (43.34)		24 (80.00)	6 (20.00)	

The proportions of the sets (*Klebsiella pneumoniae* and Non-*Klebsiella pneumoniae*) and the presence of associated genes were analyzed concerning the demographic characteristics and hospital data of the patients using the chi-square test, with a test probability (p) below 0.05 as the criterion for statistical significance
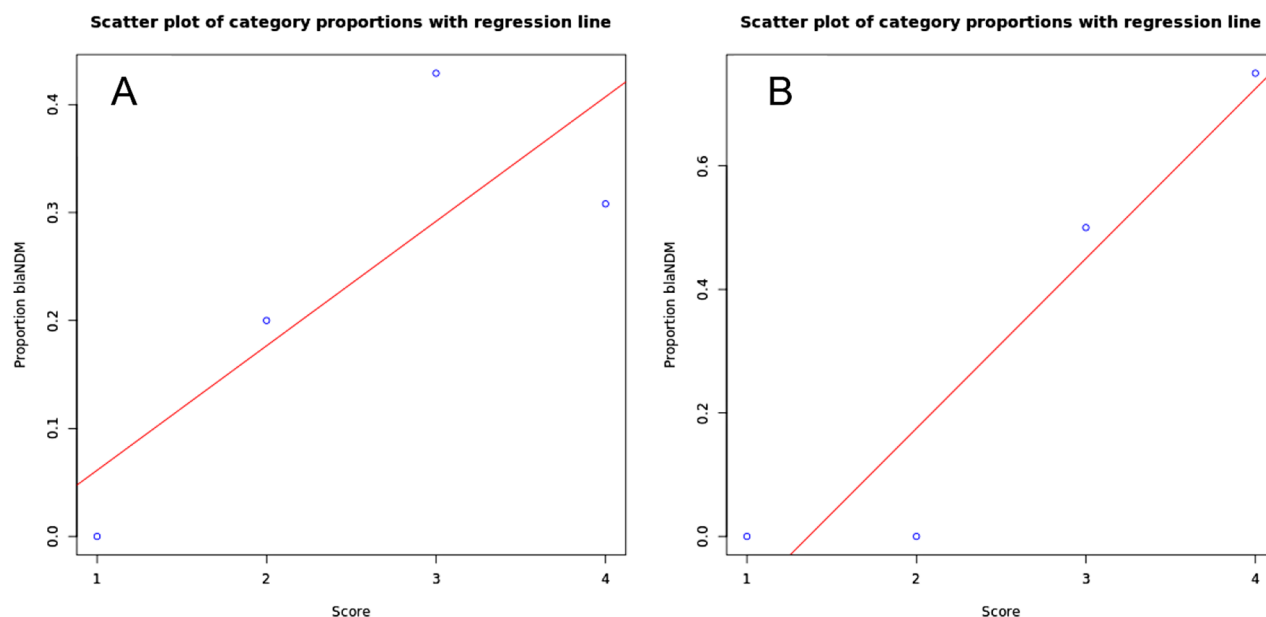
Fig. 1 Relationship between the proportions of CRE carrying the blaNDM gene and the annual evolution of new COVID-19 cases in Brazil [25]. A: CRE isolated from samples from the lower respiratory tract of patients at a University Hospital (n = 46), from January 2020 to August 2023. Chi-square for slope (linear trend) = 3.82; p-value for slope = 0.05; Slope = 0.10; scores 1 (2023), 2 (2020), 3 (2022) and 4 (2021). B: CRE isolated from samples from the lower respiratory tract of patients at a University Hospital who did not test positive for COVID-19 (n = 30) from January 2020 to August 2023. Chi-square for slope (linear trend) = 13.35; p-value for slope = 0.001; Slope = 0.26; scores 1 (2023), 2 (2020), 3 (2022) and 4 (2021)

differences in the prevalence of species and genes over the studied period, may suggest potential lineage-specificity within the analyzed strains in this study. It is crucial to emphasize that this observation does not diminish the significance of ongoing surveillance, as emphasized in the Brazilian plan for the prevention and control of AMR in healthcare settings [5].

The KPC enzyme, produced by CRE via plasmid genes, extends beyond its original *Klebsiella pneumoniae* lineages, as it has been detected in clinical isolates of various bacterial species, elevating its clinical significance [12]. Within the context of this study, it was observed that 19.57% of the isolates belonged to the species *Serratia marcescens*, which harbored the bla_{KPC} gene. The emergence of carbapenemases in *Serratia* imposes considerable therapeutic challenges, given the inherent resistance of this bacterial genus to polymyxins, which are commonly used to treat

CRE infections [39]. This underscores the clinical complexity associated with the dissemination of carbapenem resistance and emphasizes the urgent need for innovative therapeutic strategies to combat infections caused by these resistant strains.

Demographic and hospital analysis revealed that the most affected patients were over 60 years old, corroborating previous studies on AMR [40, 41]. The predominance of cases in male patients, unmarried and with a lower level of school education, in accordance with previous research, emphasizes the importance of considering social and demographic factors in the approach to AMR [41–44].

When analyzing patients' characteristics in relation to bacterial strains or specific genes, a divergent pattern from the literature was observed: the decrease in the proportions of *Klebsiella pneumoniae* was linked to advancing age groups. This observation underscores the necessity for infection

Scatter plot of category proportions with regression line
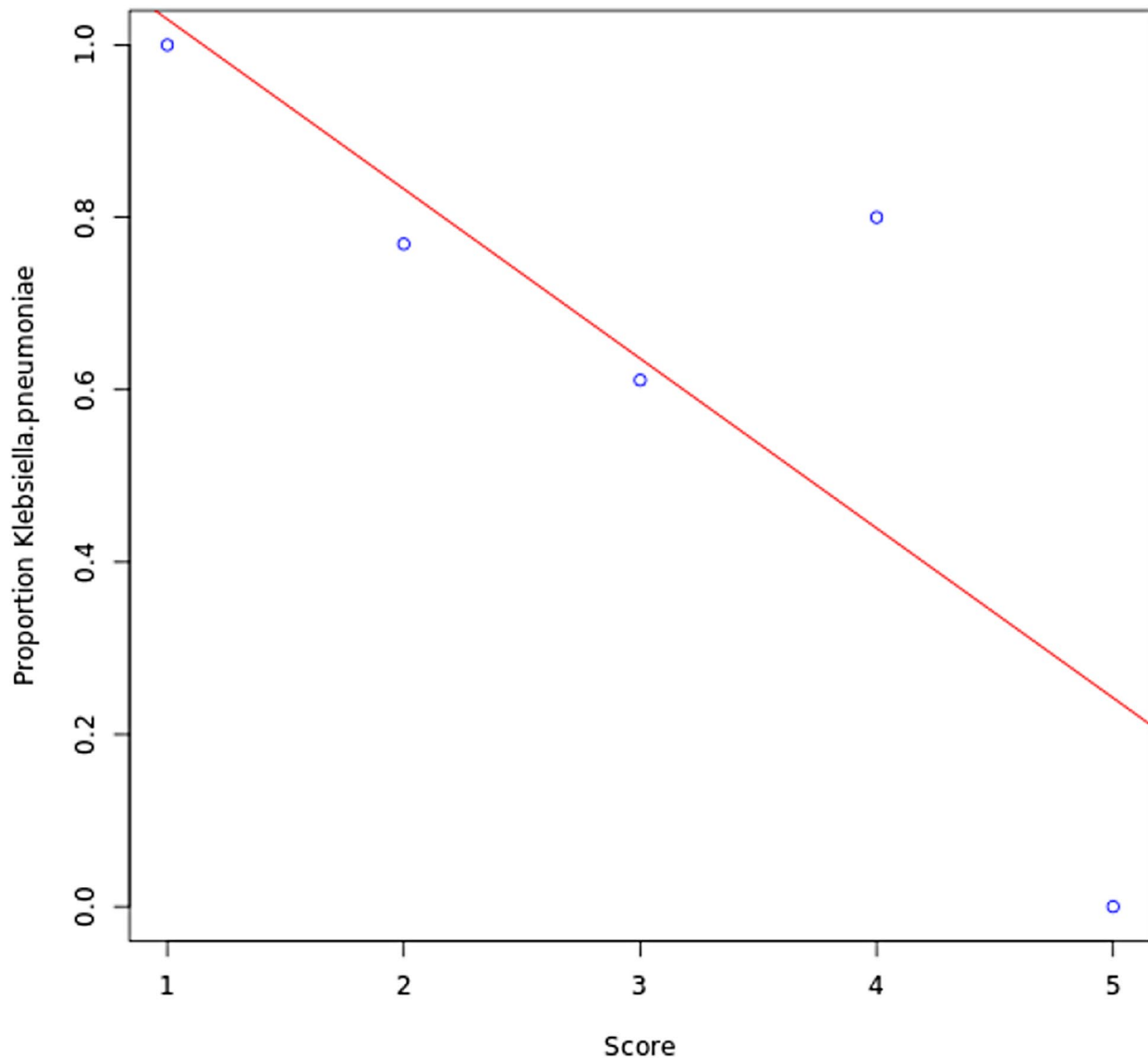
Fig. 2 Inverse linear trend between proportions of *Klebsiella pneumoniae* and patient age groups. CRE strains, including *Klebsiella pneumoniae*, were isolated from lower respiratory tract samples from patients at a University Hospital ($n=46$) from January 2020 to August 2023. Chi-square for slope (linear trend)=3.65; p-value for slope=0.055; Slope = -0.13; scores 1 (10–18 years), 2 (19–59 years), 3 (60–69 years), 4 (70–79 years), and 5 (80 years or older)

prevention and control strategies tailored to both young and elderly populations. However, given the intricate nature of the host-pathogen interaction, widely acknowledged in the literature [44], and the relatively small sample size in this study, the need for more comprehensive studies to comprehend the nuances of this relationship is underscored.

The high prevalence in ICUs, reported in 69.57% of cases, is consistent with studies identifying these units as critical points for the dissemination of resistant bacterial strains [10, 27]. The majority of patients (76.08%) remained hospitalized for more than 30 days, a recognized risk factor for the development of HAIs [9, 33]. Although this study did not investigate the causes of death among patients with CRE isolated from lower respiratory tract samples, it is noteworthy that 58.70% of these patients died. According to a meta-analysis conducted by Martin and colleagues (2018) [45], CRE infections are associated with higher mortality rates, with patients with CRE experiencing mortality rates 2 to 3 times higher than those with carbapenem-susceptible infections.

Table 4 Association between carbapenem-resistant "*Klebsiella pneumoniae*" and "Non-*Klebsiella pneumoniae*" bacterial groups in tracheal aspirate samples ($n = 46$) from patients at a University Hospital and hospital stay time grouped by the median (69 days), stratified by COVID-19 diagnostic test results

COVID-19	Length of hospital stay grouped by median	Total	n*Klebsiella pneumoniae* (%)	n Non-*Klebsiella pneumoniae* (%)	p-value	MHOR* (CI95%)
Positive	Up to 69 days	5	5 (100.00)	0 (-)	0.29	-
	> 69 days	11	8 (72.70)	3 (27.30)		
Negative	Up to 69 days	18	14 (77.80)	4 (22.20)	0.013	-
	> 69 days	12	3 (25.00)	9 (75.00)		
Non-*Klebsiella pneumoniae* rate adjusted by COVID-19 result	Up to 69 days	-	-	-	0.0061	12.84 (2.25–73.21)
	> 69 days	-	-	-		

*Mantel-Haenszel adjusted odds ratio

Table 5 Association between carbapenem-resistant "*Klebsiella pneumoniae*" and "Non-*Klebsiella pneumoniae*" bacterial groups in tracheal aspirate samples ($n = 46$) from patients at a University Hospital and hospital stay time grouped by median (69 days), stratified by types of resistance genes found

Carbapenem resistance genes	Length of hospital stay grouped by median	Total	n*Klebsiella pneumoniae* (%)	n Non-*Klebsiella pneumoniae* (%)	p-value	MHOR* (CI95%)
bla_{KPC}	Up to 69 days	16	14 (87.50)	2 (12.50)	0.04	-
	> 69 days	20	10 (50.00)	10 (50.00)		
bla_{NDM}	Up to 69 days	7	5 (71.40)	2 (28.60)	0.33	-
	> 69 days	3	1 (33.30)	2 (66.70)		
Non-*Klebsiella pneumoniae* rate adjusted by types of resistance genes	Up to 69 days	-	-	-	0.02	6.47 (1.47–28.37)
	> 69 days	-	-	-		

*Mantel-Haenszel adjusted odds ratio

COVID-19 infection was confirmed in 34.78% of the patients. Furthermore, a linear correlation was observed between the presence of the bla_{NDM} gene and the annual increase in new cases of COVID-19 in Brazil, indicating a critical intersection between hospital-acquired respiratory infections and the global pandemic [28, 31]. This relationship has been the subject of investigation, underscoring the need for additional research for a more in-depth understanding. A tentative explanation would be that due to the decrease in the quality of intensive hospital services offered due to the burden of COVID-19, there may have been a higher rate of selection of resistant microorganisms and an increase in the dissemination of this resistance. As this trend occurred in both patient groups with positive and negative COVID-19 results, this strengthens that the pandemic could be indiscriminately affecting the quality of care for both those with positive and negative results.

With the aim of exploring the dynamics of hospital infections based on the data available in this study, a stratified analysis was conducted to investigate associations between the bacterial groups *Klebsiella pneumoniae* and Non-*Klebsiella pneumoniae*. The analysis revealed a significantly higher likelihood of encountering Non-*Klebsiella pneumoniae* bacteria in patients with hospital stays longer than the calculated median (69 days). These results suggest a correlation between this bacterial group and prolonged hospital stays, irrespective of COVID-19 diagnosis or resistance

gene type. These findings carry significant implications for the development of more effective approaches to the management and control of hospital infections, as well as for the formulation of new research proposals.

While this study offers valuable insights into AMR, it's crucial to acknowledge its limitations when interpreting the findings. The retrospective approach, lack of detailed individual risk factor information such as comorbidities and prior antimicrobial exposure, the unique geographic concentration, and potential underreporting of COVID-19 cases, particularly in the early pandemic years, may restrict the generalizability of the results. Moreover, the significant associations identified, which have been less explored in the literature, underscore the necessity for more comprehensive studies to elucidate the individual determinants of resistance. However, the robust methodology, including confirmation of bacterial identification via MALDI-TOF and differentiation of resistance genes using qPCR, bolstered the findings. The exclusion of duplicate isolates and meticulous analysis of medical records enhanced the accuracy and reliability of the results obtained.

Conclusion

The high prevalence of carbapenem resistance, particularly among *Klebsiella pneumoniae* and *Serratia marcescens* strains, underscores the urgent need for more effective control strategies. The predominance of the bla_{KPC} gene across all analyzed species reinforces the importance of targeted preventive and therapeutic interventions, given its widespread dissemination and clinical relevance. Additionally, the concern regarding the rising bla_{NDM} gene highlights the necessity of control measures adapted to the new resistance dynamics. These findings emphasize the importance of developing and implementing effective control measures, as well as conducting more detailed prospective studies to better understand the factors influencing resistance and the transmission of resistant strains within hospitals. Rigorous surveillance and ongoing research are essential to inform targeted interventions aimed at mitigating the negative impacts of antimicrobial resistance on global health.

Declarations

Ethical approval This study adhered to the ethical standards outlined in Resolution no. 466/2012 of the National Health Council for research involving human subjects. Approval was obtained from the Ethics and Research Committee with Human Subjects of the Juiz de Fora University Hospital, under opinion number: 6.008.128, registered with the Certificate of Presentation for Ethical Consideration 62059822.2.0000.513.

Consent to participate Patient consent was not required as this was an anonymized retrospective cross-sectional study.

Conflict of interest All the authors declare that no competing or financial interests exist.

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