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Prevalence and carbapenem resistance profile of Gram-negative bacteria isolated from clinical samples of patients in an intensive care unit

Prevalência e perfil de resistência aos carbapenêmicos de bactérias Gram-negativas isoladas de amostras clínicas de pacientes em uma unidade de terapia intensiva

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ABSTRACT

Introduction: Healthcare-associated infections caused by multidrug-resistant bacteria, especially carbapenem-resistant Gram-negative bacilli (CRGNB), represent a serious public health problem. **Objective:** To evaluate the prevalence of GNB isolated from different clinical samples of patients hospitalized in an Intensive Care Unit (ICU), as well as their susceptibility profiles to carbapenems. **Materials and Methods:** This is a cross-sectional study, carried out through the collection of culture data from patients hospitalized in the ICU of a teaching hospital, during the year 2023. **Results:** A total of 168 GNB strains were included in this study, 25% belonging to the *Acinetobacter baumanni*i Complex (ABC), 23.2% to *Klebsiella pneumoniae*, and 19.6% to Pseudomonas aeruginosa. Regarding carbapenem-resistant strains, ABC represented 47.1% of the 87 resistant isolates, followed by 28.7% to *K. pneumoniae* and 16.1% to *P. aeruginosa*. Tracheal aspirate was the clinical material with the highest frequency of CRGNB. In addition, a higher number of CRGNB isolates was observed in male patients and those over 51 years of age. **Conclusion:** Greater attention is needed by the competent bodies regarding the occurrence of CRGNB in the hospital environment with a view to implementing measures to prevent and control the spread of these bacteria.

Keywords: Drug resistance bacterial. Cross infection. Intensive care units.

RESUMO

Introdução: As Infecções Relacionadas à Assistência à Saúde causadas por bactérias multidroga-resistentes, especialmente Bacilos Gram-negativos resistentes aos carbapenêmicos (BGN-RC), retratam um grave problema de saúde pública. **Objetivo:** Avaliar a prevalência de BGN isolados de diferentes amostras clínicas de pacientes hospitalizados em uma Unidade de Terapia Intensiva (UTI), bem como seus perfis de suscetibilidade aos carbapenêmicos. **Materiais e Métodos:** Trata-se de um estudo transversal, realizado através de coleta de dados de culturas de pacientes hospitalizados na UTI de um hospital de ensino, durante o ano de 2023. **Resultados:** Foram incluídos neste estudo 168 linhagens BGN, sendo 25% pertencente ao Complexo *Acinetobacter baumannii* (CAB), 23,2% de *Klebsiella pneumoniae*, 19.6% de *Pseudomonas aeruginosa*. Em relação às linhagens resistentes aos carbapenêmicos, o CAB representou 47,1% dos 87 isolados resistentes, seguido de 28,7% de *K. pneumoniae* e 16,1% de *P. aeruginosa*. O aspirado traqueal foi o material clínico com maior frequência de BGN-RC. Além disso, foi observada maior quantidade de isolados de BGN-RC em pacientes do sexo masculino e acima dos 51 anos de idade. **Conclusão:** É necessária maior atenção pelos órgãos competentes sobre a ocorrência de BGN-RC no ambiente hospitalar visando a realização de medidas de prevenção e controle da disseminação destas bactérias.

Palavras-chave: Resistência bacteriana à antibióticos. Infecção hospitalar. Unidade de terapia intensiva.

1. INTRODUCTION

Healthcare-Associated Infections (HAIs), primarily caused by multidrug-resistant (MDR) bacteria, represent a serious public health issue, as they prolong hospital stays, increase public healthcare expenditures, and significantly raise morbidity and mortality rates. Additionally, they are associated with a higher risk of complications and death. (GOMIDES *et al.*, 2014).

The bacterial species frequently isolated in hospital environments belong primarily to the order *Enterobacterales*, including *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., among others. Among the non-fermenting Gram-negative bacilli (NF-GNB), the most relevant are *Pseudomonas aeruginosa*, the *Acinetobacter baumannii* complex, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Alcaligenes*, and *Moraxella*. These microorganisms, often multidrug-resistant, are responsible for severe infections in hospitalized patients, particularly in Intensive Care Units (ICUs), where invasive procedures and the presence of immunocompromised individuals increase the risk of infection (OLIVEIRA & REYGAERT, 2023).

The spread of antimicrobial resistance (AMR) among Gram-negative bacilli (GNB) has turned these microorganisms into one of the leading public health concerns. In ICUs the prevalence of multidrug-resistant Enterobacterales and non-fermenting Gram-negative bacilli poses a serious threat to patients, increasing the risk of severe infections and death (OLIVEIRA, REYGAERT, 2023, WHO, 2024).

Carbapenem resistance among Gram-negative bacilli, such as *Klebsiella pneumoniae, Escherichia coli*, and *Pseudomonas aeruginosa*, has become a growing concern in the healthcare field. These microorganisms are frequently associated with severe infections, including sepsis, pneumonia, and urinary tract infections, particularly in hospitalized or immunosuppressed patients (LOGAN & WEISSER, 2015).

Among Enterobacterales, the *Klebsiella pneumoniae* complex resistant to carbapenems is one of the main causative agents of such infections due to its acquired resistance to multiple antimicrobials, representing a significant challenge to public health. (AMIN *et al.*, 2020). Beta-lactam antimicrobials, such as carbapenems, are widely used in ICUs, increasing the probability of resistance mechanism development by these bacteria. Hydrolysis of the beta-lactam ring by enzymes such as Extended-Spectrum

Beta-Lactamases (ESBLs) and carbapenemases is the main mechanism by which bacteria inactivate these antibiotics (BUSH & BRADFORD, 2019).

Carbapenem resistance is primarily mediated by carbapenemases such as KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo-β-lactamase), and OXA-48 (oxacillinase), which confer a high level of resistance to carbapenems and other β-lactams. These mechanisms can be detected through phenotypic and genotypic laboratory techniques, and the implementation of such technologies is essential to guide treatment and adopt effective infection control strategies. Additionally, the presence of other factors, such as alterations in outer membrane permeability and efflux pumps, also contributes to resistance and further complicates treatment (NORDMANN, NASS & POIREL, 2011).

Given this scenario, early detection and continuous monitoring of bacterial resistance are essential to reduce the transmission of carbapenem-resistant bacteria in healthcare settings. Strategies such as epidemiological surveillance, rational use of antimicrobials, and the strict implementation of infection control measures can significantly contribute to containing the spread of these bacteria. Furthermore, the development of new antimicrobials and alternative therapies is an urgent necessity for the treatment of infections caused by these multidrug-resistant microorganisms (MONTEIRO *et al.*, 2016; TENOVER, WEINSTEIN & PATEL, 2018).

The objective of this study was to evaluate the prevalence of Gram-negative bacilli isolated from clinical samples, as well as their susceptibility profiles to carbapenems, from patients admitted to the Intensive Care Unit.

2. MATERIALS AND METHODS

Study Design and Hospital Characterization

This is an observational, cross-sectional, and retrospective study in which microbiological culture results from hospitalized patients in an Intensive Care Unit were analyzed through databases. These cultures were processed by a clinical analysis laboratory of a university hospital located in the city of Juiz de Fora, Minas Gerais, during the period from January to December 2023.

The hospital in this study is a non-profit institution that provides comprehensive care through the Brazilian Unified Health System. It offers care across various specialties, with

17 pediatric beds, of which 14 are dedicated to the pediatric clinical ward and 3 to the pediatric surgical ward. Regarding adult wards, it has approximately 123 beds, divided into the following sectors: Women's Medicine (WM), Men's Medicine (MM), Bone Marrow Transplant (BMT), Men's Surgery (MS), Women's Surgery (WS), Gynecology (GYN), Nephrology (NEPH), and 9 ICU beds.

Since this was a retrospective study, all positive cultures meeting the eligibility criteria during the study period were included, resulting in a convenience sample of 168 Gram-negative isolates. Therefore, a sample size calculation was not applicable, as the study analyzed the entire population of interest.

Inclusion and Exclusion Criteria

Bacterial cultures from patients hospitalized in the adult ICU of the aforementioned hospital were included in the study. This ICU primarily serves adult patients but may occasionally admit younger patients, as the hospital does not have a pediatric ICU. Both male and female patients of all age groups who presented Gram-negative bacilli isolation and identification in their microbiological exams were included.

Cultures with fungal growth, polymicrobial cultures (with more than three different microorganisms), and epidemiological surveillance cultures were excluded. Positive clinical samples with the same microorganism from the same patient were also excluded.

Identification of Bacterial Strains and Antimicrobial Susceptibility Testing

The microbiology department of the aforementioned hospital identifies bacteria using phenotypic methods, employing biochemical and physiological tests. For the identification of bacteria belonging to the order Enterobacterales, tests such as lactose fermentation on MacConkey agar plates, modified Rugai medium, citrate utilization, decarboxylation of the amino acids lysine, arginine, and ornithine, Voges-Proskauer test, malonate test, intrinsic antimicrobial resistance, and motility were used. For the identification of non-glucose fermenting bacteria, Oxidation-Fermentation tests, motility, oxidase test, growth on MacConkey agar, growth at 42°C and 44°C, and resistance to polymyxin were performed (WINN *et al.*, 2018; OPLUSTIL, 2019).

The Antimicrobial Susceptibility Test was performed using the disk diffusion technique on Mueller-Hinton agar (Ionlab LTDA). Reading and interpretation were conducted

according to the Brazilian Committee on Antimicrobial Susceptibility Testing guidelines (BrCAST, 2023). For these carbapenem-resistant bacterial strains, carbapenemase production was investigated using the Modified Carbapenem Inactivation Method (mCIM) and the EDTA-modified Carbapenem Inactivation Method (eCIM) (OPLUSTIL, 2019; CLSI, 2023).

Data Collection and Ethical Aspects

Data related to the total number of positive cultures for Gram-negative bacteria and their susceptibility profiles to carbapenems (Meropenem and Imipenem) were analyzed. For members of the order Enterobacterales, resistance to Ertapenem was also evaluated.

The data were obtained through patients' online medical records via the University Hospital Management Application, physical medical records, and culture records from the Clinical Analysis and Pathological Anatomy Unit of the hospital.

To ensure data accuracy and minimize potential bias, all data were independently reviewed by two researchers. Each dataset was cross-referenced with the original laboratory records and electronic medical records to confirm the consistency of patient information and microbiological results.

The results were tabulated in spreadsheets, subjected to percentage analysis, and plotted in graphs using Microsoft Excel® version 2016.

This study was approved by the Human Research Ethics Committee of the University Hospital of Juiz de Fora, Minas Gerais, under number: 6.034.608 and ethical review presentation certificate number: 51147021.9.0000.5133

3. RESULTS

A total of 171 GNB strains were isolated, of which 168 were included in this study. Three bacterial strains were excluded due to unidentified bacterial genus.

Of the 168 strains, 25.0% (n=42) were isolated from the *Acinetobacter baumannii* complex, 23,2% (n=39) from the *Klebsiella pneumoniae* complex, 19,6% (n=33) *Pseudomonas aeruginosa*, 11,9% (n=20) *Stenotrophomonas maltophilia*, 6% (n=10) *Escherichia coli*, 5,3% (n=9) *Serratia* sp., 3,6% (n=6) *Enterobacter* sp., 2,4% (n=4) *Proteus* sp., 1,2% (n=2) *Citrobacter* sp., 1,2% (n=2) *Providencia* sp., and 0,6% (n=1) *Morganella morganii*, as shown in Figure 1.

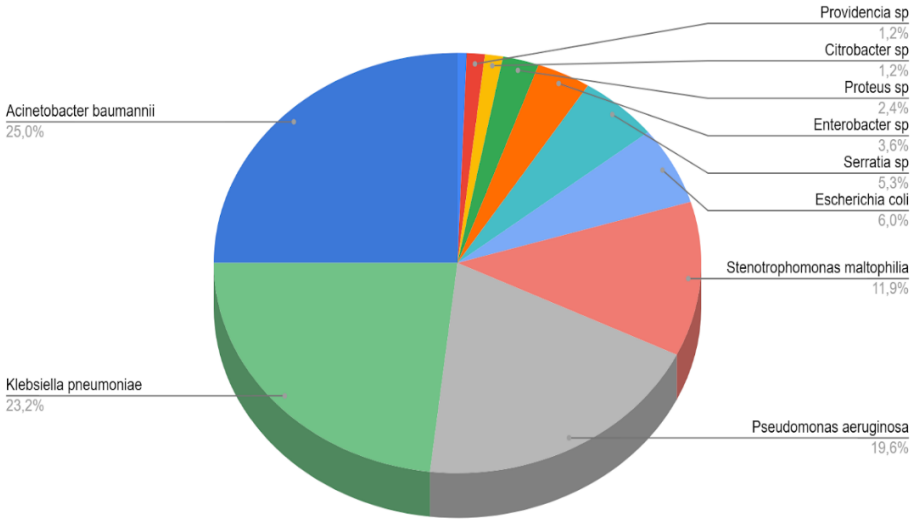

Figure 1. Percentage of Gram-negative bacilli isolated from clinical samples of patients in an intensive care unit in 2023.

For the analysis of acquired resistance to carbapenems, the species *Stenotrophomonas maltophilia* was excluded due to intrinsic resistance. Therefore, the total number (n) of isolated GNB strains was n=148, of which 58,8% (n=87) were resistant to carbapenems (CR). The distribution of resistance among the bacterial isolates is shown in Table 1.

Table 1. Carbapenem-resistant Gram-negative bacilli isolated from clinical samples of patients in an intensive care unit**.**

Isolated bacterial species	**Total isolates** (n e %)	**Carbapenem resistance by species** (n e %)	**Carbapenem resistance in relation to total isolates** (n e %)
Morganella morgannii	1 (0,7%)	0	0
Providencia sp	2 (1,3%)	0	0
Citrobacter sp	2 (1,3%)	0	0
Proteus sp	4 (2,7%)	1 (25,0%)	1 (1,2%)
Enterobacter sp	6 (4,1%)	4 (66,7%)	4 (4,6%)
Serratia sp	9 (6,1%)	2 (22,2%)	2 (2,3%)
Escherichia coli	10 (6,8%)	0	0
Pseudomonas aeruginosa	33 (22,3%)	14 (42,4%)	14 (16,1%)

Klebsiella pneumoniae	39 (26,4%)	25 (64,1%)	25 (28,7%)
Acinetobacter baumannii	42 (28,3%)	41 (97,6%)	41 (47,1%)
Total	148 (100%)	87 (100%)	87 (100%)

Legend: n=number. %=percentage. sp=species.

To determine the number of carbapenem-resistant Enterobacterales strains, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were excluded from the total isolated Gram-negative bacilli, as they do not belong to this group, resulting in 73 Enterobacterales strains.

Of the 73 isolated Enterobacterales strains, 43,8% (n=32) were carbapenem-resistant Enterobacterales (CRE); 56,2% (n=41) were carbapenem-sensitive Enterobacterales (CSE), of which 14 were producers of extended-spectrum beta-lactamase (ESBL), as shown in Table 2.

Table 2. Distribution of Enterobacterales strains isolated from clinical samples resistant to carbapenems or producing ESBL**.**

Genus/species	Total number of isolates	CRE	ESBL
Morganella morgannii	1	0	0
Providencia sp	2	0	1 (50,0%)
Citrobacter sp	2	0	0
Proteus sp	4	1 (25,0%)	1 (25,0%)
Enterobacter sp	6	4 (66,7%)	0
Serratia sp	9	2 (22,2%)	1 (11,1%)
Escherichia coli	10	0	4 (40,0%)
Klebsiella pneumoniae	39	25 (64,1%)	7 (17,9%)
Total	73	32 (43,8%)	14 (19,2%)

Legend: %= percentage. sp=species. CRE: carbapenem-resistant Enterobacterales. ESBL: *Extended-Spectrum Beta-Lactamase.*

Of the 32 CRE isolates, 78,1% (n=25) belonged to the *Klebsiella pneumoniae* complex. Among the 25 CR *Klebsiella pneumoniae* strains, 88,0% (n=22) produced serine-β-lactamase, while 12,0% (n=3) produced metallo-β-lactamases.

The bacterial strains included in this study were recovered from 150 clinical samples from 62 patients and isolated from various biological materials. Tracheal aspirate was the

sample with the highest frequency of GNB isolation, accounting for 68,0% (n=102), followed by urine 12,6% (n=19), blood 8,0% (n=12), catheter tip 2,7% (n=4), and other biological samples 8,7% (n=13), as shown in Figure 2.

The distribution of GNB isolates, both carbapenem-resistant (CR) and non-resistant, by biological sample was as follows: tracheal aspirate (102 samples with GNB isolates, of which 67 (65,6%) were CR), urine (19 samples with GNB isolates, with 8 (42,1%) resistant), blood (12 samples with GNB isolates, of which 5 (41,6%) were resistant), catheter tip (4 samples with GNB isolates, all resistant), and other samples (13 with GNB isolates, with 3 (23,1%) resistant), totaling 150 biological samples with GNB isolation. Figure 2 shows the comparison between the total number of GNB isolates and the number of CR-GNB isolates by clinical sample.

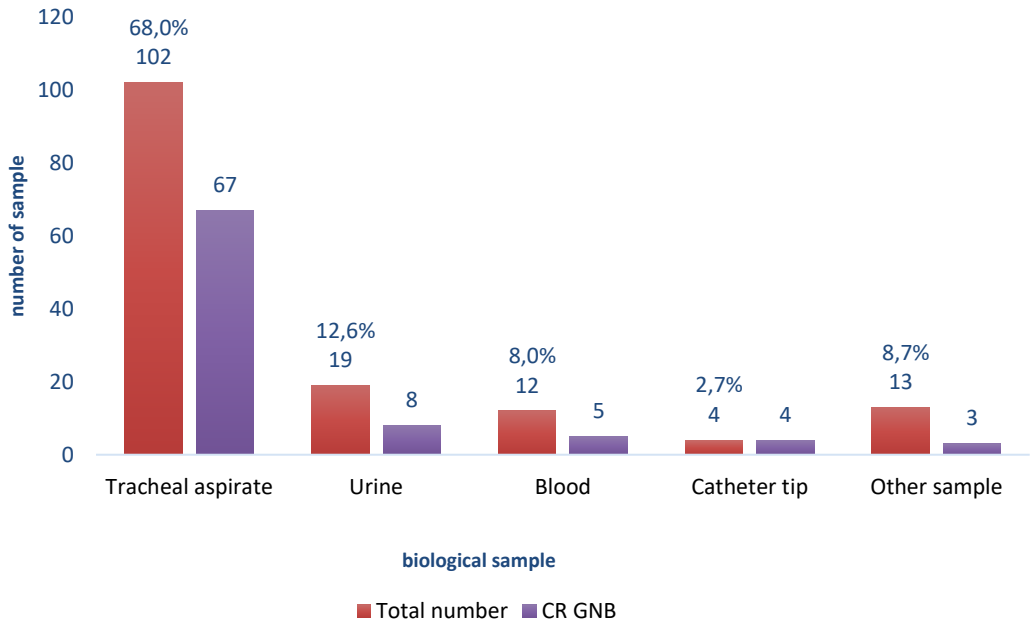

Figure 2. Distribution of biological samples in relation to total Gram-negative bacilli isolates and carbapenem-resistant Gram-negative bacilli isolates.

The distribution of carbapenem-resistant Gram-negative bacilli strains by age group and gender is presented in Table 3.

Table 3. Distribution of carbapenem-resistant Gram-negative bacilli strains by age group and gender.

Age group	n and % total of patients with CR-GNB isolates	n and % female	n and % male
10 a 30 anos	5 (8,1%)	2 (11,1%)	3 (6,8%)
31 a 40 anos	5 (8,1%)	3 (16,7%)	2 (4,6%)
41 a 50 anos	8 (12,9%)	1 (5,6%)	7 (15,9%)
51 a 65 anos	19 (30,6%)	4 (22,2%)	15 (34,1%)
>65 anos	25 (40,3%)	8 (44,4%)	17 (38,6%)
Total	62 (100%)	18 (100%)	44 (100%)

Legenda: n=number. %=percentagem. CR- GNB: Carbapenem-resistant Gram-negative bacilli

4. DISCUSSION

The increase in infections caused by carbapenem-resistant Gram-negative bacilli represents a serious public health issue. Some of these bacteria are prominently featured in the World Health Organization's (WHO) updated 2024 list of critical priority pathogens. Among the critical priority groups are carbapenem-resistant Enterobacterales, third-generation cephalosporin-resistant Enterobacterales, carbapenem-resistant *Acinetobacter baumannii* complex, and rifampicin-resistant *Mycobacterium tuberculosis*. Another important GNB included in the WHO list is *Pseudomonas aeruginosa*, which is classified as a high-priority pathogen. (WHO, 2024).

In this study, the *Acinetobacter baumannii* complex was the most prevalent in the ICU, accounting for 25.0% (n=42) of the total 168 isolated GNB. A critical finding was carbapenem resistance in 97.6% (n=41) of the *Acinetobacter baumannii* strains isolated. Outbreaks of CRAB, particularly in ICUs, have been reported in the literature, especially during the COVID-19 pandemic in Brazil (SELEGATTO *et al.*, 2022).

A study conducted in Minas Gerais analyzed the prevalence of carbapenem-resistant *Acinetobacter baumannii* in various biological samples and hospital units between 2008 and 2014. Among the 420 resistant isolates, the highest prevalences were found in the Adult Emergency Department (26.4%) and the Adult ICU (22.8%) (SILVA & LIMA, 2020).

During the COVID-19 pandemic, an ICU dedicated to COVID-19 patients experienced an outbreak of carbapenem-resistant *Acinetobacter baumannii*. Between June and September 2021, 21 patients presented colonization or infection by this pathogen, with a

mortality rate of 28%. The majority of cases involved the respiratory tract, and 95% of the patients required ventilatory support (SELEGATTO *et al.*, 2022). A study was conducted in an ICU of a tertiary hospital in Porto Alegre to evaluate the 30-day mortality of patients colonized or infected with carbapenem-resistant *Acinetobacter baumannii*. A total of 66 patients were included, with a mortality rate of 47% (PRATES, 2010).

Regarding *Pseudomonas aeruginosa*, 16.1% (n=14) of the isolated GNB strains were resistant to carbapenems. Although this resistance rate may not seem as alarming as that observed for *Acinetobacter baumannii*, non-fermenting Gram-negative bacilli represented by these microorganisms play a crucial role in the dissemination of carbapenem resistance genes to other bacteria, especially through plasmid-mediated genes (GNIADEK, CARROLL & SIMNER, 2016). Carbapenem resistance in *Pseudomonas aeruginosa* within the ICU poses a significant challenge for the treatment of hospital-acquired infections, particularly due to the limited therapeutic options available (SIMNER *et al.*, 2017).

Another pathogen highlighted in this study was *Stenotrophomonas maltophilia*, an emerging pathogen frequently associated with nosocomial infections in the ICU, primarily causing respiratory tract infections, especially in vulnerable patients such as the immunocompromised and debilitated (WANG, TANG & WANG, 2022). It is characterized by intrinsic resistance to various antimicrobials, including β-lactams, aminoglycosides, and quinolones, which limits the available therapeutic options and makes the treatment of *S. maltophilia* infections a considerable challenge for clinicians (MOJICA *et al.*, 2022). The present study focused primarily on carbapenem-resistant microorganisms, prioritizing those with the ability to acquire resistance through genetic transfer. Although *S. maltophilia* does not play this role, its inherent resistance to many antibiotics positions it as a significant concern in ICU outbreaks (ALMEIDA *et al.*, 2021).

In this study, 43.8% of Enterobacterales were carbapenem-resistant, with the majority producing serine β-lactamases. Carbapenemase-producing Enterobacterales have emerged as significant pathogens in ICUs, posing a challenge to the treatment of hospital-acquired infections. Studies indicate a high prevalence of multidrug-resistant strains, with 49.7% resistant to three or more antibiotics and 28% resistant to six or more. Moreover, cross-resistance between carbapenems and other antibiotic classes, such as aminoglycosides and quinolones, has been observed, further limiting available therapeutic options (SILVA *et al.*, 2020).

According to CHIA et al. (2020), carbapenem resistance in Enterobacterales is primarily mediated by the production of β-lactamase enzymes and is readily disseminated among Gram-negative bacteria through the transfer of mobile genetic elements, such as plasmids and transposons (AMBLER *et al.*, 1991; QUEENAN & BUSH, 2007; SILHAVY, KHANE & WALKER, 2010; NORDMANN & POIREL, 2014).

The *Klebsiella pneumoniae* complex was the bacterial group with the highest proportion of carbapenem-resistant isolates (64.1%), with the majority originating from lower respiratory tract samples. Among the 39 isolates, 7 strains were ESBL producers, and 25 were carbapenem-resistant. Of the 25 resistant isolates, 88.0% (n=22) produced serine β-lactamases, while the remaining 12.0% (n=3) produced metallo-β-lactamases. A study conducted in 2021 reported an outbreak of carbapenem-resistant *Klebsiella pneumoniae* in an ICU dedicated to COVID-19 patients in Salvador, Brazil. Twenty-one patients were affected, with 14 episodes of infection and 7 cases of colonization. Central venous catheter-related primary bloodstream infections were the most frequent, followed by lower respiratory tract infections and ventilator-associated pneumonia. The overall mortality rate was 66.7%, with 71% among infected patients and 57% among colonized patients (SILVA *et al.*, 2021).

Early detection and strict control of infections caused by carbapenem-resistant *Klebsiella pneumoniae* are essential to reduce the spread of these strains in intensive care units. Measures such as active epidemiological surveillance, isolation of colonized or infected patients, and rigorous hand hygiene practices are fundamental to preventing outbreaks and protecting vulnerable patients (SILHAVY, KHANE & WALKER, 2010; NORDMANN & POIREL, 2014).

The highest number of carbapenem-resistant GNB isolates in this study were from tracheal aspirate samples, with 35 isolates susceptible and 67 resistant. Some authors highlight that tracheal intubation, especially when associated with postoperative care under general anesthesia, compromises the natural barriers of the upper respiratory tract, increasing the risk of infection by external pathogens and allowing colonization of the lower respiratory tract by oropharyngeal bacteria (KAUR *et al.*, 2020; SHIN *et al.*, 2011).

Among the 62 patients admitted to the ICU, the highest prevalence of carbapenem-resistant GNB isolates was observed in male individuals (71%). The prevalence of infections caused by multidrug-resistant bacteria may vary between men and women, depending on the type of infection and clinical context. In urinary tract infections, studies indicate a higher

incidence in women due to anatomical and physiological factors. However, the prevalence of infections caused by multidrug-resistant bacteria can be influenced by various factors, including the hospital environment and the presence of comorbidities (GARCIA *et al.*, 2013; BRASIL, 2020).

The bacteria isolated in this study were predominantly found in the age group above 51 years in both genders. Studies have shown that the prevalence of infections caused by multidrug-resistant bacteria may vary according to patients' age group, being more frequent in individuals over 60 years old. A study conducted in an ICU of a public hospital in Paraíba revealed that 59.1% of hospital infection cases occurred in patients aged 60 years or older (OLIVEIRA et al., 2020). Another study carried out in a hospital in northern Minas Gerais observed that the highest frequency of hospital infections caused by multidrug-resistant bacteria occurred in the age group of 60 to 69 years (GARCIA *et al.*, 2013).

The primary concern regarding infections in patients over 60 years of age lies in the high mortality rate caused by resistant GNB strains, particularly in ICUs, which is further exacerbated by the natural frailty of elderly patients (KARVOUNIARIS et al., 2022).

Multidrug-resistant bacteria represent a growing threat to global public health, transforming infections that were once treatable into significant therapeutic challenges. This reality underscores the urgent need for effective infection control strategies and rigorous epidemiological surveillance to curb the spread of these resistant bacteria. The implementation of preventive measures, such as rational use of antibiotics, strict hygiene and disinfection practices, as well as investments in research for the development of new antimicrobials, are essential to confront this escalating challenge (MELLO & OLIVEIRA, 2019; BRAZIL, 2020).

5. STUDY LIMITATIONS

This study has limitations that should be considered when interpreting the results. First, it was a retrospective, cross-sectional study, which may be subject to reporting bias due to the use of previously recorded data and the limited availability of certain clinical variables. Second, the study was conducted at a single university hospital located in southeastern Brazil, which may limit the generalizability of the findings to other institutions or regions with different patient populations and local epidemiology. Finally, the analysis was restricted to a single year (January to December 2023), which prevents the assessment of temporal trends

and long-term changes in the prevalence of Gram-negative bacteria and their resistance patterns.

Despite these limitations, the study provides valuable insights into the epidemiology of carbapenem-resistant Gram-negative bacteria in critically ill patients and highlights the urgent need for ongoing surveillance and effective infection control strategies.

6. FINAL CONSIDERATIONS

The prevalence of carbapenem-resistant Gram-negative bacilli was primarily observed in *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, especially in lower respiratory tract samples, acting as causative agents of ventilator-associated pneumonia in patients over 65 years of age.

Carbapenem-resistant Gram-negative bacilli represent a serious global public health concern. Prevalence studies are essential to provide epidemiological data on bacterial resistance and to raise awareness for preventive measures and control of the spread of these bacteria, particularly within hospital settings.

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