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ORIGINAL ARTICLE

Overview of colistin resistance in Gram-negative carbapenem-resistant bacteria ESKAPE group from patients admitted to intensive care units of a teaching hospital in Cascavel, Brazil

Panorama de resistência à colistina em bacilos Gram-negativos do grupo ESKAPE resistentes a carbapenêmicos oriundos de pacientes internados em unidades de terapia intensiva de um hospital universitário em Cascavel, Brasil

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ABSTRACT

Introduction: The ESKAPE group *(Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.*)* are pathogens associated with hospital-acquired infections and increased resistance to antimicrobial agents, including carbapenems and colistin. **Objective**: This study evaluated the epidemiology and antimicrobial susceptibility profiles of multidrug-resistant Gram-negative bacteria from the ESKAPE group in patients admitted to intensive care units at a teaching hospital in Cascavel, Brazil. **Methods**: Data on age, sex, outcome, and length of hospital stay, in addition to the clinical specimen, origin of the strain, species, and antimicrobial susceptibility test, were obtained from the patients' electronic medical records from January 2019 to December 2023. The presence of carbapenemase (*bla*OXA-23, *bla*NDM or *bla*KPC), and the *mcr1* genes were assessed via Real-Time Polymerase Chain Reaction. Statistical analyses were conducted using chi-square test, Fisher's exact test and binary logistic regression via JASP software ($p \leq 0.05$). **Results**: Among 281 carbapenem-resistant gram-negative bacteria specimens, 80 were also colistin-resistant. *Klebsiella pneumoniae* was the predominant gram-negative bacteria, and *bla*KPC was the most common carbapenemase. Most carbapenem-colistin-resistant strains did not express the *mcr1* gene. Age and length of hospital stay were the only factors influencing clinical outcomes. The mechanisms of colistin resistance in carbapenem-colistin-resistant isolates remain unclear, as most isolates did not express the *mcr1* gene. **Conclusion**: Despite high susceptibility rates to other antibiotics, the institution lacks comprehensive therapeutic protocols for antibiotic combinations and surveillance protocols to manage the spread of multidrug-resistance mechanisms in gram-negative bacteria of ESKAPE group within healthcare environment.

Keywords: Carbapenem-resistance; Colistin; Multidrug-resistance.

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RESUMO

Introdução: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* e *Enterobacter spp.* formam o acrônimo ESKAPE, por serem bactérias associadas a infecções hospitalares dada sua alta resistência a antimicrobianos, incluindo os usados contra cepas multirresistentes, como carbapenêmicos e colistina. **Objetivo:** Tendo isso em vista, buscou-se verificar o perfil de cepas MDR de bacilos Gram-negativos do grupo ESKAPE em pacientes em setores de terapia intensiva num hospital universitário em Cascavel, Brasil. **Métodos:** Dados de gênero, idade, desfecho e tempo de internação hospitalar, além do tipo de amostra, origem da cepa, espécie e seu perfil de bactérias multirresistentes foram obtidos via prontuário eletrônico dos pacientes de janeiro de 2019 a dezembro de 2023. O tipo de carbapenemase expressa, *bla*OXA-23, *bla*NDM ou *bla*KPC, e expressão do gene *mcr1* nos isolados foram avaliadas via RT-PCR. A análise estatística foi feita a partir de testes Qui-Quadrado, exato de Fisher e regressão logística binária realizada via *software* JASP (adotando $p \leq 0{,}05$). **Resultados:** Foram encontradas 281 amostras de bactérias Gram-negativas resistentes a carbapenêmicos; desses, 80 também eram resistentes à colistina, sendo *Klebsiella pneumoniae* a espécie mais frequente e *bla*KPC a carbapenemase mais expressa. Das bactérias resistentes a carbapenêmicos e colistina, a maioria não expressava o gene *mcr1*. Somente as variáveis idade e tempo de internação hospitalar influenciaram os desfechos. **Conclusão**: Como a maioria dos isolados resistentes a carbapenêmicos e colistina não expressava o gene *mcr1*, o mecanismo da resistência à colistina em isolados da instituição ainda precisa ser elucidado. Assim como colistina, outros antibióticos ainda apresentam boas taxas de suscetibilidade nos isolados multirresistentes da instituição, mas carecem protocolos de associação e vigilância para possíveis mecanismos de resistência.

Palavras-chave: Colistina; Resistência microbiana a medicamentos; Resistência a carbapenêmicos.

Conflicts of interest:
The authors declare that they have no conflicts of interest.

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Introduction

Multidrug-resistant (MDR) bacteria have emerged as a significant public health problem globally. In 2021, the Global Burden of Bacterial Antimicrobial Resistance estimated approximately 4.71 million deaths; and by 2050, 8.22 million deaths, associated to bacterial MDR infections[1]. In Brazil, previous studies have reported rising rates of resistance to carbapenem and colistin, two critical last-line therapeutic options for treating multidrug-resistant (MDR) infections caused by Gram-negative bacteria (GNB)[2].

ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) is a group of bacteria commonly associated with nosocomial infections among critically ill patients that is capable of producing several antimicrobial resistance mechanisms encoded by antimicrobial resistance genes harbored on mobile genetic elements (such as plasmids, transposons and integrons) or their chromosomes[3].

The increasing use of carbapenems for treatment of MDR bacterial infections is associated with the bacterial development of β-lactamases, called carbapenemases, capable of hydrolyzing carbapenems which can be encoded, for example, by the *bla*KPC (β-lactamase *Klebsiella pneumoniae* carbapenemase, a serine carbapenemase), *bla*NDM (β-lactamase New Delhi metallo-β-lactamase, a metallo-β-lactamase) or *bla*OXA (β-lactamase oxacillinase, a serine carbapenemase) genes[4].

Polymyxins (cationic antimicrobials that act by increasing membrane permeability), such as colistin, are antibiotics used as the last line of treatment for infections caused by carbapenem- resistant GNB. However, the emergence and dissemination of colistin resistance among GNBs have also been reported[5].

Colistin resistance can be intrinsic (detected in GNB such as *Proteus mirabilis* and *Serratia marcescens*, for example) or acquired (as detected in GNB such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Enterobacter* spp., for example)[6]. Acquired colistin resistance could be derived from the expression of the *mcr1 gene* (mobile colistin resistance gene), which induces a lipopolysaccharide (LPS) modification through the expression 4'-lipid A-phosphoethanolamine transferase, which adds a molecule of phosphoethanolamine (PEtn) to lipid A, decreasing its negative charge and affinity for colistin[7].

In addition to *mcr1*, colistin resistance can be mediated by inhibition of the *mgrB* gene and upregulation of the PhoP/PhoQ pathway which also results in the addition of cationic groups such as PEtn (mediated by expression of the *prmC gene*) and 4-amino-4deoxy-L-arabinose (mediated by expression of the *pmrHFIJKLM operon and pmrE* gene) to LPS[8].

Outer membrane vesicles (OMVs), bilayer structures derived from the GNB outer membrane (periplasmatic lipids and proteins), are associated with antimicrobial resistance though antibiotic-specific enzymes or direct capture (for membrane-active antibiotics, such as colistin). The biosynthesis of OMVs and others virulence factors (such as extracellular polysaccharides) is also modulated by PhoP/PhoQ pathway[9,10].

Mutations in genes related to LPS biosynthesis (*lpxA*, *lpxC* and *lpxD*) leads to LPS absence which confers colistin resistance due to the lack of binding sites for polypeptide antibiotics, as observed in *Acinetobacter baumannii* isolates (although LPS-deficient *Acinetobacter baumannii* were susceptible to several classes of antibiotics, such as glycopeptides)[11].

Considering the importance in therapy against MDR GNB in the healthcare environment, its resistance mechanisms, and its prevalence at a global and national level, in this work, we sought to determine the epidemiological and antimicrobial susceptibility profile of MDR strains of GNB from the ESKAPE group in patients admitted in intensive care units (ICU) and sought to determine its influence on the clinical outcomes of critically ill patients.

Methods

This descriptive, retrospective, cross-sectional study was performed in a tertiary public hospital located in Cascavel, Brazil. The electronic medical records of hospitalized patients in intensive care units (ICUs) with clinical specimens positive for carbapenem-resistant gram-negative bacteria (GNB) in the ESKAPE group (*Acinetobacter baumannii* complex, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* spp.), analyzed from January 2019 to December 2023, were included.

The clinical specimens included were tracheal aspirate, surveillance culture, biopsy specimen, blood culture, central venous catheter (CVC) tip, urine, and tracheal secretion; other specimens were excluded. To avoid duplication, specimens with more than one detected isolate, as well as patients with more than one specimen, were removed from the analysis. Clinical specimens obtained from patients from other hospital wards other than ICUs, isolates positive for gram-positive bacteria or other GNB, as well as extended spectrum β-lactamase (ESBL), were also excluded.

The following patient clinical data were collected: sex, length of hospital stay and age (six age groups were defined: 0 to 15 years, 16 to 30 years, 31 to 45 years, 46 to 60 years, 61 to 75 years and above 75 years). The following data from positive clinical specimens were collected: clinical specimen, antimicrobial susceptibility, expressed carbapenemase, and origin of the isolate (community-associated infection or healthcare-associated infection).

Patients were divided into two groups based on length of hospital stay (less than 30 days X equal to or greater than 30 days), according to the Ministry of Health in the 2nd edition of the Standardization of the Hospital Census Nomenclature[12]. The clinical specimens were divided into two groups according to the origin of the isolate (community-associated infection X healthcare-associated infection) according to the National Health Surveillance Agency (ANVISA) in Technical Note GVIMS/GGTES/DIRE3/ANVISA N° 01/2024[13].

Antimicrobial susceptibility testing

Microbiology analysis (identification of microorganisms and antimicrobial susceptibility tests) was performed using the VITEK II Compact system (bioMérieux, MarcyL'Etoile, France), according to BrCAST Tables of Cutoff Points for Interpretation of MICs and Halo Diameters clinical breakpoints[14].

For the detection of extended-spectrum β-lactamases (ESBLs), bacterial suspensions adjusted to a 0.5 standard on the McFarland scale were inoculated onto Mueller-Hinton agar plates. Discs containing 30 µg of clavulanic acid (Laborclin, Brazil) were placed in the center of the plates, while discs containing 30 µg of aztreonam (Laborclin, Brazil), 30 µg of ceftazidime (Laborclin, Brazil), 30 µg of ceftriaxone (Laborclin, Brazil), and 30 µg of cefotaxime (Laborclin, Brazil) were positioned peripherally. Each ESBL detection test was performed in parallel with positive and negative controls: the positive control was a standard ESBL-producing strain (*Klebsiella pneumoniae* ATCC 70603), and the negative control was a standard strain (*Escherichia coli* ATCC 25922). Isolates showing an increase or deformation of the inhibition zone from the peripheral discs toward the clavulanic acid disc were considered positive for ESBL and subsequently excluded from further analyses.

The GNB isolates were also tested for colistin resistance according to the institution's protocol. Colistin susceptibility tests were performed according to Droptest methodology, based on the deposition of a 10 µL drop of polymyxin solution (16 µg/mL) on a Mueller–Hinton agar plate inoculated with the isolate of interest at 0.5 McFarland. After the incubation period, the isolates were considered susceptible when there was the presence of an inhibition zone and resistant when there was no presence of a halo around the drop or the presence of colonies in the inhibition zone[15]. Colistin susceptibility tests were performed in parallel with negative and positive controls: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

A real-time polymerase chain reaction (RT-PCR) was used to detect carbapenem resistance genes (*bla*OXA-23 and *bla*NDM in the *Acinetobacter baumannii* complex, *bla*NDM and *bla*KPC in *Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterobacter* spp.) and colistin resistance genes (*mcr1*) in stains using TaqMan® probes. Strains harboring carbapenem-resistance (CR) or carbapenem- and colistin-resistance (CR-ColR) genes were categorized as CR stains or CR-ColR stains, respectively.

Statistical analysis

Descriptive statistical analysis was performed using Microsoft Excel® 365 (Microsoft, USA). Inferential statistical analysis of the categorical variables (sex, age, length of stay, clinical specimen, origin of the isolate, harbored carbapenemase and antimicrobial susceptibility) was performed using the Chi-square test and Fisher exact test - with a p-value≤0.05 considered statistically significant - to verify their association with the clinical outcome. Binary logistic regression was performed to evaluate the influence of independent variables on the dependent variable (clinical outcome) – p-value≤0.05 was considered statistically significant. Statistical analysis was performed using JASP software version 0.18.3 (University of Amsterdam, Amsterdam, Netherlands).

Ethical considerations

This study was approved by the Human Research Ethics Committee under CAAE n° 63850222.1.0000.0107.

Results

Medical record analysis

From January 2019 to December 2023, 281 positive results for carbapenem-resistant GNB in the ESKAPE group were recorded: 22 isolates (20 carbapenem-resistant and 2 carbapenem- and colistin-resistant) in 2019; 34 isolates (28 CR and 6 carbapenem- and colistin-resistant) in 2020; 78 isolates (62 carbapenem-resistant and 14 carbapenem- and colistin-resistant) in 2021; 93 isolates (55 CR and 38 carbapenem- and colistin-resistant) in 2022; and 53 isolates (35 CR and 18 carbapenem- and colistin-resistant) in 2023.

Klebsiella pneumoniae was the most prevalent species (255 isolates), followed by *Acinetobacter baumannii* complex (16 isolates), *Enterobacter* spp. (8 isolates carbapenem-resistant), and *Pseudomonas aeruginosa* (2 isolates carbapenem- and colistin-resistant). The distribution of isolates by year according to species, expressing carbapenemase and colistin susceptibility, is shown in Figure 1.

Statistical analysis

The distribution of patients' clinical data according to clinical outcome is shown in Table 1. Most isolates were obtained from male patients (63.70%) aged 46-60 years (55.16%) with LHS equal to or greater than 30 days (29.54%). As shown in Table 1.1, although most patients were male (63.70%), sex did not correlate with the clinical outcome (p value=0.8032). Patients' LHS ranged from 1 to 228 days (mean LHS was 38.67 days, standard deviation was 29.75 days). Most patients stayed for 30 or more days (55.16%) and this was correlated with the observed clinical outcome (p value=0.001), as shown in Table 1.2. The patients' ages ranged from 0.021 years (7 days) to 94 years (mean age was 51.57 years, standard deviation was 21.80 years) and was correlated with the clinical outcome (p value=0.0244). Most patients (29.54%) were aged 46-60 years, as shown in Table 1.3.

No statistical correlation, except age and length of hospital stay (LHS), was found between any of the categorical variables and the clinical outcome. The logistic regression model was statistically significant despite its low predictive power (X^2 = 49.098; p-value = 0.003; Nagelkerke R^2 = 0.214). According to the logistic regression model, older age was associated with an unfavorable outcome (odds ratio=0.985). On the other hand, higher LHS was associated with a favorable outcome (odds ratio = 1.019).

The distribution of the patients' isolates according to clinical outcome is shown in Table 2. Most isolates originated from tracheal secretion culture (21%) and were classified as hospital-associated infections (77.58%). Most isolates harbored *bla*KPC (72.24%) and were colistin susceptible (71.54%). The clinical specimen and origin of the isolate were not correlated with clinical outcomes (p-value = 0.3475 and p-value = 0.7168, respectively), as shown in Table 2.1 and Table 2.2.

Most isolates expressed *bla*KPC (72.24%), but the type of carbapenemase harbored was not correlated with the clinical outcome observed (p-value = 0.2967). Furthermore, 36 isolates harbored *bla*NDM (12.81%), 15 isolates harbored *bla*OXA-23 4 isolates (1.42%) harbored 2 of carbapenemase (*bla*KPC+ *bla*NDM), as shown in Table 2.3

Most isolates were colistin susceptible (71.54%), and the colistin susceptibility pattern of the isolates was not correlated with clinical outcomes (p-value = 0.6787), as shown in Table 2.4. Among carbapenem- and colistin-resistant isolates (28.46%), according to RT−PCR analysis, most isolates (98.75%) did not harbor the *mcr1* gene.

Antimicrobial susceptibility profile

As shown in Table 3, all carbapenem-resistant GNB ESKAPE group isolates were penicillin-, cephalosporin-,

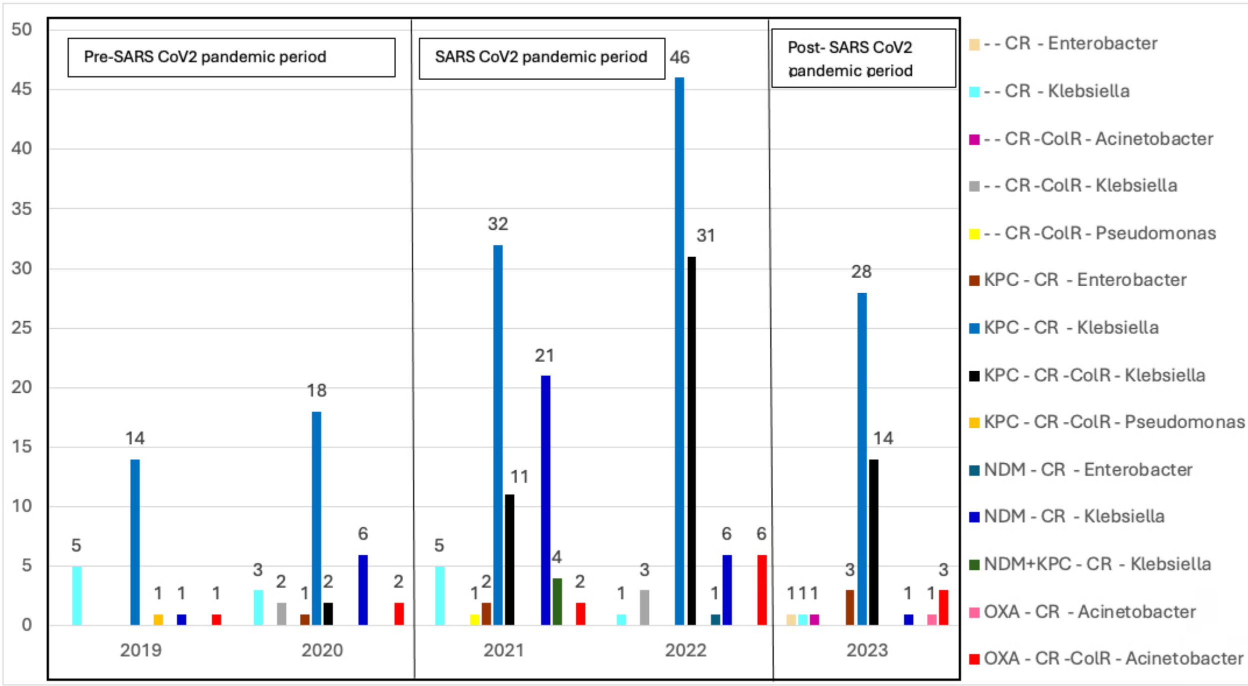

Figure 1. Distribution of isolates by year according to species, expressing carbapenemase and colistin susceptibility.
Legend: CR-ColR = carbapenem- and colistin-resistant; CR = carbapenem-resistant.

Table 1. Distribution of patients' clinical data according to clinical outcome.

Categorical variable	Survival		Death		Total		p value
	AF	RF	AF	RF	AF	RF	
1.1 Sex							
Female	54	19.22%	48	17.08%	102	36.30%	0.8032
Male	92	32.74%	87	30.96%	179	63.70%	
Total	146	51.96%	135	48.04%	281	100%	
1.2. Length of hospital stay (LHS)							
Less than 30 days	49	17.44%	77	27.40%	126	44.84%	
Equal to or greater than 30 days	97	34.52%	58	20.64%	155	55.16%	0.001
Total	146	51.96%	135	48.04%	281	100%	
1.3. Age							
0-15 years old	19	6.77%	7	2.49%	26	9.26%	
16-30 years old	10	3.55%	5	1.78%	15	5.33%	
31-45 years old	26	9.26%	21	7.47%	47	16.73%	
46-60 years old	46	16.37%	37	13.17%	83	29.54%	0.0244
61-75 years old	35	12.46%	45	16.02%	80	28.48%	
Over 75 years old	10	3.55%	20	7.11%	30	10.66%	
Total	146	51.96%	135	48.04%	281	100%	

Legend: *p*-value ≤0.05 was considered statistically significant; AF = absolute frequency; RF = Relative frequency.

and carbapenem- resistant. Most isolates were aztreonam- (81.13%), ciprofloxacin-(92.17%), and gentamicin-resistant (54.44%). Ceftazidime+ avibactam- (1.42%), colistin- (28.47), and amikacin- (35.23%) resistance was less prevalent among CR GNB isolates. All *Enterobacter* spp. isolates were susceptible to colistin.

Table 2. Distribution of patient isolate clinical data according to clinical outcome.

Categorical variable	Survival		Death		Total		p value
	AF	RF	AF	RF	AF	RF	
2.1. Clinical specimen							
Tracheal aspirate	21	7.47%	12	4.27%	33	11.74%	
Surveillance culture	24	8.54%	18	6.40%	42	14.94%	
Biopsy specimen	14	4.99%	11	3.91%	25	8.90%	
Blood culture	28	9.96%	25	8.90%	53	18.86%	0.3475
CVC tip	22	7.83%	18	6.40%	40	14.23%	
Tracheal secretion	23	8.18%	36	12.82%	59	21.00%	
Urine	14	4.99%	15	5.34%	29	10.33%	
Total	146	51.96%	135	48.04%	281	100%	
2.2. Origin of the isolate							
Community-associated	34	12.10%	29	10.32%	63	22.42%	
Healthcare-associated	112	39.86%	106	37.72%	218	77.58%	0.7168
Total	146	51.96%	135	48.04%	281	100%	
2.3. Carbapenemase harbored							
None	12	4.27%	11	3.91%	23	8.18%	
*bla*KPC	109	38.80%	94	33.45%	203	72.24%	
blaNDM	17	6.05%	19	6.77%	36	12.81%	0.297
*bla*KPC+*bla*NDM	0	0%	4	1.42%	4	1.42%	
*bla*OXA-23	8	2.84%	7	2.49%	15	5.33%	
Total	146	51.96%	135	48.04%	281	100%	
2.4. Colistin resistance							
CR	106	37.73%	95	33.81%	201	71.54%	0.6787
CR-ColR	40	14.23%	40	14.23%	80	28.46%	
Total	146	51.96%	135	48.04%	281	100%	

Legend: *p*-value ≤0.05 was considered statistically significant; AF = absolute frequency RF = relative frequency.

Table 3. Comparison of the antibiotic resistance patterns of GNB CR isolated from clinical specimens.

	Nonfermenting		Fermenting		
	Acinetobacter	*Pseudomonas*	*Enterobacter*	*Klebsiella*	*Total*
	n=16	n=2	n=8	n=255	n=281 (%)
AMP	16*	2*	8*	255*	281 (100%)
AMS	16	2*	8*	255	281 (100%)
PIT	16	2	8	255	281 (100%)
CTR	16*	2*	8	255	281 (100%)
CTZ	-	2	7	255	264 (93.95%)
CTV	-	-	-	4	4 (1.42%)
CEP	-	2	7	250	259 (92.17%)
AZT	-	2	6	220	228 (81.13%)
ERT	-	-	8	255	263 (93.59%)
IMI	16	2	8	255	281 (100%)

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	Nonfermenting		Fermenting		
	Acinetobacter	*Pseudomonas*	*Enterobacter*	*Klebsiella*	*Total*
	n=16	n=2	n=8	n=255	n=281 (%)
MER	16	2	8	255	281 (100%)
AMI	12	2	1	84	99 (35.23%)
GEN	5	2	7	139	153 (54.44%)
CIP	6	2	6	245	259 (92.17%)
COL	15	2	0	63	80 (28.47%)

Legend: AMP = ampicillin; AMS = ampicillin+sulbactam; PIT = piperacilin+tazobactam; CTR = ceftriaxone; CTZ = ceftazidime; CTV = ceftazidime+avibactam; CEP = cefepime; AZT = aztreonam; ERT = ertapenem; IMI = imipenem; MER = meropenem; AMI = amikacin; GEN = gentamicin; CIP = ciprofloxacin; COL = colistin. (*) = antibiotics in which the species is intrinsically resistant according to BrCAST. (-) = antibiotics in which BrCAST does not recommend their use for treatment.

Discussion

The institution observed an increase in both carbapenem- and carbapenem- and colistin-resistant isolates between 2019 and 2022, corroborating global trends. Previous studies have reported an increase in carbapenem- and colistin-resistance in MDR Gram-negative bacteria (GNB) among critically ill patients during the SARS-CoV-2 pandemic (2021–2022)[16-18].

In our study, we observed a significant rise in carbapenem-resistant (CR) and CR-colistin-resistant (CR-ColR) isolates during the pandemic period (2021–2022), with 117 CR and 52 CR-ColR isolates, compared to the pre-pandemic period (2019–2020), which had 48 CR and 8 CR-ColR isolates; and the post-pandemic period (2023), which had 35 CR and 18 CR-ColR isolates. Recent data indicate a continued increase in colistin and carbapenem resistance among MDR GNB, although at a slower rate than during the pandemic period. However, these findings require further validation through additional and more recent data, as well as data from other institutions, to confirm this trend.

The effect of other comorbidities, others risk factors for nosocomial infections (mechanical ventilation and corticosteroids use, for instance), previous antibiotic exposure, or the effect of the COVID-19 pandemic and its influence on antibiotic consumption were not investigated (as well as detection for other metallo-β-lactamases or extended spectrum β-lactamases, ESBLs). Hence, it is not possible to verify their contribution to the antimicrobial resistance profile of isolates or to patient outcomes[17,18].

Most of the isolates were identified as *Klebsiella pneumoniae*, which is characterized by the expression of various antibiotic resistance mechanisms [5]. Despite the greater presence of serine carbapenemases (*bla*KPC), the presence of isolates expressing metallo-β-lactamases (*bla*NDM), as well as isolates expressing both carbapenemases, compromises the effectiveness of β-lactams associated with β-lactamase inhibitors, such as ceftazidime+avibactam, in monotherapy.

Furthermore, problems associated with the use of colistin in monotherapy (such as nephrotoxicity, as well as its increasing resistance) and the decreasing available therapeutic options are a growing concern. The development of therapeutic protocols for the combination of antimicrobial agents with synergistic activity, such as aztreonam, ceftazidime+avibactam, and rifampicin[19], associated with surveillance for mechanisms of resistance to antibiotics that still have good susceptibility rates in institution isolates, such as ceftazidime+avibactam and amikacin, for example, could mitigate this problem.

Most carbapenem- and colistin-resistant strains did not harbor the *mcr1* gene, and the search for other mechanisms already mentioned (capsular polysaccharides, loss or mutation of other genes responsible for LPS expression, for example) or other members of the *mcr1* gene family (other members of the *mcr1* gene family have been reported in the country, such as *mcr3*, *mcr5*, *mcr9* and *mcr10*)[20] could be performed to elucidate the form of acquisition of this resistance in the institution's isolates.

Clinical outcomes were influenced by patient age and length of hospital stay (LHS). The correlation between age and clinical outcome has been corroborated by other studies in patients infected with MDR bacteria[21], but the correlation between LHS and clinical outcomes has not been corroborated by other studies[22].

In urine, the respiratory tract, as well surveillance cultures isolates the possibility of colonization cannot be ruled out. Furthermore, in intensive care units, the possibility of using colonized hospital devices cannot be ruled out as a possible source of patient contamination.

In this study, the predominant presence of *Klebsiella pneumoniae* in the institution's isolates, as well as the presence of *bla*KPC among the expressed carbapenemases, the absence of the *mcr1* gene in carbapenem- and colistin-resistant isolates, and the influence of age on clinical outcomes were observed.

However, the effects of other unresearched variables, such as comorbidities and previous antibiotic therapy use, as well as other mechanisms through which colistin resistance can be mediated, could be the subject of future studies. Developing effective therapeutic strategies and robust surveillance protocols to track and manage the dissemination of antimicrobial resistance mechanisms could be essential tools for addressing and mitigating the impact of these MDR pathogens in healthcare environments.

Author's Contributions:

We describe contributions to the paper using the taxanomy (CRediT) provided above:

Conceptualization, Investigation, Methodology, Visualization & Writing – review & editing: APC; FCZV; LCB; NM da RG. *Project administration, Supervision & Writing – original draft*: APC; NM da RG; SBB. *Validation & Software*: LNCM. *Resources & Funding acquisition*: EAC. *Data curation & Formal Analysis*: APC.

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