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Emerging Problems in Infectious Diseases

Epidemiological characteristics and antimicrobial susceptibility among carbapenem-resistant non-fermenting bacteria in Brazil

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Abstract

Introduction: Non-fermenting Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are widespread in the environment and are increasingly associated with nosocomial infections. Extensive and indiscriminate use of antibiotics in hospitals has contributed to an increased number of infections caused by these microorganisms, that are resistant to a wide variety of antimicrobials, including β-lactams. This study aimed to isolate and identify carbapenem-resistant *Acinetobacter* spp. and *P. aeruginosa* from hospitalized patients, to determine their antimicrobial susceptibility patterns and to screen for $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-51}}$, $bla_{\text{OXA-58}}$, and $bla_{\text{OXA-143}}$ genes among the isolated bacteria.

Methodology: Antimicrobial resistance patterns were performed using the disk-diffusion method. Genetic markers related to carbapenem resistance were screened by polymerase chain reaction.

Results: Carbapenem-resistant *Acinetobacter* spp. (n = 44) and *P. aeruginosa* (n = 28) samples were isolated from patients admitted to a tertiary hospital. Polymyxin B was the only effective drug for all isolates. Considering the oxacillinase gene screening, genetic markers were observed only in *Acinetobacter* isolates. The most frequent genotype observed was $bla_{\text{OXA-23}}^{+}/bla_{\text{OXA-51}}^{+}$ (45.5%), followed by $bla_{\text{OXA-51}}^{+}/bla_{\text{OXA-143}}^{+}$ (41%). The oxacillinase genes $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-58}}$ were not detected. High mortality rates (> 70%) were observed.

Conclusions: The data suggest the need for rational use of antimicrobials associated with early diagnosis of multidrug-resistant bacteria, especially considering non-fermenting Gram-negative rods, which are widespread in hospitals. The findings of $bla_{\text{oxa-51}}^{-}$ strains suggest the occurrence and spread of non-*A. baumannii* species throughout our hospitals. Effective implementation of surveillance programs in hospitals is needed to reduce infectious and resistant intra- and inter-species bacteria.

Key words: *Pseudomonas*; *Acinetobacter*; carbapenem-resistant bacteria.

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Introduction

Non-fermenting Gram-negative (NFGN) bacteria are widely distributed in the environment and are highly associated with severe opportunistic nosocomial infections, mainly in immunocompromised patients [1,2]. NFGN are resistant to most available antimicrobial drugs, mainly due to their ability to acquire resistance genes through horizontal transfer, but also due to their ability to persist in the open environment for long periods on nosocomial surfaces [3].

The clinical relevance of multidrug-resistant NFGN-associated infections is characterized by their opportunistic nature and their laborious and costly treatment and management, along with their high morbidity and mortality rates [4]. These infections are frequently found in intensive-care patients with pneumonia symptoms associated with mechanical ventilation, bacteremia introduced by central venous catheter, or urinary tract infections [5].

NFGN bacteria may display different mechanisms of intrinsic and acquired resistance to a large number of antimicrobials routinely employed in medical therapy, such as penicillins, cephalosporins, aminoglycosides, and fluoroquinolones. Carbapenems are still the drugs of choice to treat infections associated with the drug-resistant NFGN bacteria [2,6]. Nevertheless, carbapenem resistance among *Pseudomonas* and *Acinetobacter* has emerged and become widespread, demanding increased efforts to treat infection with chemotherapy, using polymyxin B and tigecycline, the last therapeutic option [5,7,8].

Among the NFGN bacteria, *Pseudomonas* and *Acinetobacter* are the main drug-resistant groups

associated with nosocomial infections and are well known for their ability to express several drug-resistant mechanisms, such as, production of β-lactamases and altered surface porins and efflux pumps. β-lactamases production constitutes the major NFGN resistance mechanisms from the epidemiological viewpoint, including production of serine and metallo-β-lactamases, which is related to carbapenem resistance [9].

Serine-β-lactamases (such as class D β-lactamases) are also known as oxacillinases or OXA-type β-lactamases, and display a variable hydrolytic activity against the β-lactam drugs. So far, 121 different variants of oxacillinases have been described, 45 of which have hydrolytic activity on carbapenems [10]. Of these, bla_{OXA} genes are important genetic and epidemiological markers that confirm identification of carbapenem-resistant NFGN bacteria. OXA-23, a clavulanic acid-resistant oxacillinase, is a plasmid-coded enzyme, which hydrolyzes broad-spectrum cefalosporins, aztreonam, and feebly hydrolyzes imipenem and meropenem [11]. OXA-24 is associated with bacterial resistance against third- and fourth-generation cephalosporins as well as carbapenems. In most cases, these microorganisms are multidrug resistant and sensitive to polymyxin B and colistin [12-15]. OXA-51 is a chromosomally coded oxacillinase present in the *Acinetobacter baumannii* species with a distinct hydrolytic action on carbapenems, *i.e.*, slow imipenem hydrolysis and non-meropenem inactivation [16,17]. OXA-58 was first described in 2003 in *A. baumanni*, and rapidly spread to the other countries [18-24]. This enzyme is associated with resistance against carbapenems, third- and fourth-generation cephalosporins, and monobactams [25]. OXA-143 is still little studied and restricted to Brazil. It was identified in a clinical isolate of multidrug-resistant *A. baumanni* [26-29]. β-lactamase OXA-143 hydrolyzes penicillins and carbapenems but does not significantly hydrolyze expanded-spectrum cephalosporins, as observed with other carbapenem-hydrolyzing class D-β-lactamase [26].

Knowledge on the occurrence, distribution, and antimicrobial susceptibility of oxacillinase-producing bacteria may be highly relevant to help prevent a misuse of antimicrobial agents and guide suitable therapy. Thus, this study was focused on the epidemiological characteristics, antimicrobial susceptibility patterns, and genetic screening for oxacillinase genes in carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated from patients admitted in a Brazilian tertiary hospital between January and December of 2012.

Methodology

Study design, patients, and bacteria collection

This was a cross-sectional study based on routinely collected data from patients admitted to a Brazilian tertiary hospital in Juiz de Fora, Minas Gerais, between 1 January and 31 December 2012. None of the sampled patients were diagnosed with infectious diseases at the time of admission. The hospital is a 120-bed private institution, which includes general and specialized surgical units; adult, infant and neonatal intensive care units; a nursery unit; a coronary care unit; male, female and infant wards; outpatient care; and a diagnostic center that includes clinical analysis and pathological anatomy laboratories. The ethics committee of the Federal University of Juiz de Fora (certificate no. 346/2011) approved this study. Bacterial samples isolated from clinical specimens were sent to the hospital's clinical pathology laboratory (mainly urine, blood, tracheal secretion, and catheter tip cultures) in accordance with the guidelines described by the Brazilian Health Surveillance Agency [30].

After initial bacteria were isolated from monomicrobial cultures, all the presumptively identified NFGN rods were identified using the Vitek 2 System (BioMerieux, Marcy l'Etoile, France); *Acinetobacter* spp. and *P. aeruginosa* were selected for further studies, as described below. Patients' demographic and clinical characteristics were recovered from corresponding medical records.

Antimicrobial susceptibility patterns

Antimicrobial susceptibility assays were performed on Mueller-Hinton agar (BD-Difco, São Paulo, Brazil), using the disk-diffusion method. Growth inhibition zones were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) [31] with the exception of tigecycline [32].

Antimicrobial disks of amikacin (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), polymyxin B (300 U), piperacillin-tazobactam (100/10 μg), ceftazidime (30 μg), cefepime (30 μg), meropenem (10 μg), and imipenem (10 μg) were tested against *P. aeruginosa* and *Acinetobacter* spp. Additional disks of sulphazotrim (25 μg), tetracycline (30 μg), and ampicillin-sulbactam (10/10 μg) were tested against *Acinetobacter* spp., and an additional disk of aztreonam (30 μg) was tested against *P. aeruginosa.* All the antimicrobial disks were of commercial grade (Laborclin Ltda, Paraná, Brazil). *Pseudomonas*

aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, and *Staphylococcus aureus* ATCC 25923 were used for quality control.

Screening of bla_{OXA} genes among the carbapenem-resistant bacteria

For the isolated imipenem- and meropenem-resistant *Acinetobacter* spp. and *P. aeruginosa* strains, polymerase chain reactions (PCRs) were carried out targeting the bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, bla_{OXA-58}, and $bla_{OXA-143}$ genes coding for oxacillinase enzymes. The genetic screening was performed using primers and conditions previously described [26,33].

Briefly, bacterial genomic DNA were extracted from 1 mL of overnight cultures in tryptic soy broth (BD-Difco, São Paulo, Brazil) using the Wizard Genomic DNA Purification Kit (Promega Corporation, Wisconsin, USA) following the manufacturer's instructions. The DNA extracts were quantified using NanoDrop (Thermo Fisher Scientific, Massachusetts, USA) and stored at -20°C, to be used as templates in PCRs. The primer sequence, amplicon size, and amplification conditions are described in Table 1. All the PCR experiments were performed in duplicate. The expected amplicons were visualized in 1.5% agarose gel stained with ethidium bromide. The 1 kb DNA ladder was used as molecular weight standard (Life Technologies, California, USA). Positive controls for PCR reactions were carried out by sequencing randomly selected amplicons comprising 10% of the total reactions. The PCR products were sequenced in an ABI Prism 3730 DNA sequencer (Applied Biosystems, California, USA).

Patient chart review

The medical records of the hospital-admitted patients from whose samples carbapenem-resistant *P. aeruginosa* and *Acinetobacter* spp. were isolated were reviewed. The following demographic and clinical data were retrieved: age, sex, clinical specimens for microbiological culture, patient admission unit, and mortality attributable to the infection by carbapenem-resistant NFGN isolates.

Results

Over the sampled period, NFGN bacteria were isolated from 183 hospital-admitted patients, from samples that included urine, blood, tracheal secretion, and catheter tip cultures (49 non-replicated isolates of *Acinetobacter* spp. and 134 isolates of *P. aeruginosa*). Of the isolated bacteria, 44 samples of *Acinetobacter* spp. and 28 samples of *P. aeruginosa* were carbapenem resistant, representative of 72 patients, distributed as follows, according to their admission unit: intensive care unit (n = 42, 58.4%), male and female wards (n = 17, 23.6%), coronary care unit (n = 12, 16.6%), and neonatal intensive care unit (n = 1, 1.4%).

The sociodemographic data, clinical specimens, and the mortality associated with the colonization and infection by carbapenem-resistant NFGN isolates are presented in Table 2. Considering the *Acinetobacter*-associated patients, the mean patient age was 69.6 years old (ranging between 11 days old and 96 years old); 21 (47.7%) bacterial strains were isolated from female patients, while 23 (52.3%) strains were isolated from males. *Pseudomonas*-associated patients had a mean age of 71.7 years (ranging from 30 to 92 years); 20 (71.5%) bacterial strains were isolated from female patients and 8 (28.5%) were isolated from males. Overall, the main clinical specimen for microbiological culture was tracheal secretion followed by urine and catheter tip. Unfortunately, the patients' mortality rate was high, accounting for 77.2% of the cases associated with *Acinetobacter* colonization and infections and 78.5% of the cases associated with *Pseudomonas* colonization and infections.

Table 1. Primers used, expected amplicons, and polymerase chain reaction (PCR) conditions.

Target	Primer sequence (5'-3')	Amplicon size (bp)	PCR conditions	References
bla_{OXA-23}	F-5'-GAT CGG ATT GGA GAA CCA GA-3' R-5'-ATT TCT GAC CGC ATT TCC AT-3'	501	95°C, 5 min; 30x (95°C, 1 min; 52°C, 1 min; 72°C, 1 min); 72°C, 10 min	26
bla_{OXA-24}	F-5'-GGT TAG TTG GCC CCC TTA AA-3' R-5'-AGT TGA GCG AAA AGG GGA TT-3'	246		26
bla_{OXA-51}	F-5'-TAA TGC TTT GAT CGG CCT TG-3' R- 5'-TGG ATT GCA CTT CAT CTT GG-3'	353		26
bla_{OXA-58}	F-5'-AAG TAT TGG GGC TTG TGC TG-3' R-5'-CCC CTC TGC GCT CTA CAT AC-3	599		26
$bla_{OXA-143}$	F-5'-TGG CAC TTT CAG CAG TTC CT-3' R-5'-TAA TCT TGA GGG GGC CAA CC-3'	149		33

Table 2. Patients' sociodemographic and clinical data related to carbapenem-resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa* isolates in a tertiary hospital between January and December of 2012.

Epidemiological parameters	Carbapenem-resistant bacteria	
	***Acinetobacter* spp. (n = 44)**	***Pseudomonas aeruginosa* (n = 28)**
Gender		
Male	23 (52.3%)	8 (28.5%)
Female	21 (47.7%)	20 (71.5%)
Age		
0–20 years	1 (2.2%)	0 (0%)
21–40 years	3 (6.8%)	2 (7.1%)
41–60 years	6 (13.6%)	3 (10.7%)
> 61 years	34 (77.4%)	23 (82.1%)
Clinical specimens		
Urine	5 (9.2%)	13 (31.7%)
Blood	5 (9.2%)	2 (4.8%)
Tracheal secretion	34 (63.2%)	17 (41.6%)
Catheter tip	7 (13%)	6 (14.6%)
Bronchoalveolar lavage	1 (1.8%)	0 (0%)
Ulcer	1 (1.8%)	2 (4.8%)
Abdominal secretion	1 (1.8%)	0 (0%)
Orthopedic implants	0 (0%)	1 (2.5%)
Patient admission unit		
Male and female ward	6 (13.6%)	11 (39.3%)
Intensive care unit	30 (68.2%)	12 (42.9%)
Coronary care unit	7 (16.0%)	5 (17.8%)
Neonatal intensive care unit	1 (2.2%)	0 (0%)
Patient evolution		
Discharge	10 (22.8%)	6 (21.5%)
Death	34 (77.2%)	22 (78.5%)

Table 3. Antimicrobial susceptibility patterns of the carbapenem-resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa* isolated in a tertiary hospital between January and December of 2012.

Antimicrobials	***Acinetobacter* spp. (n = 44)**		***P. aeruginosa* (n = 28)**	
	R (%)	**S (%)**	**R (%)**	**S (%)**
Imipenem	100.0	0	100.0	0
Meropenem	100.0	0	100.0	0
Ampicillin-sulbactam*	83.4	16.6	-	-
Piperacillin-tazobactam	100.0	0	44.0	56.0
Ceftazidime	100.0	0	100.0	0
Cefepime	100.0	0	100.0	0
Aztreonam*	-	-	90.2	9.8
Amikacin	77.8	22.2	63.4	36.6
Gentamicin	87.1	12.9	19.5	80.5
Tetracycline*	55.6	44.4	-	-
Sulphazotrim*	55.6	44.4	-	-
Ciprofloxacin	100.0	0	90.2	9.8
Tigecycline*	0	100.0	-	-
Polymyxin B	0	100.0	0	100.0

*Antimicrobial drugs not tested against both bacterial species; R: resistance including intermediate resistance; S: sensitivity

Table 4. Phenotypic and genotypic characteristics of carbapenem-resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa* recovered from patients in a tertiary hospital between January and December of 2012.

Bacteria	Resistance phenotype	Genotype (*bla*$_{OXA}$ type genes)	Sampling	
			Unit (n; %)	Months (n)
Acinetobacter spp. (n = 44)	MP, IP, TZ, PM, AK, GM, CP, PT, TS	OXA-23	ICU (1; 2.2)	NOV
	MP, IP, TZ, PM, AK, GM, CP, PT, TS, TE	OXA-23	ICU (1; 2.2)	NOV
	MP, IP, TZ, PM, GM, CP, PT, AB, TS, TE	OXA-23	ICU (1; 2.2)	DEC
	MP,IP,TZ,PM,AK,GM,CP,PT,AB,TS,TE	OXA-23	MWAR (1; 2.2)	DEC
	MP, IP, TZ, PM, AK, GM, CP, PT, AB, TS, TE	OXA-51	ICU (1; 2.2)	FEB
	MP, IP, TZ, PM, CP, PT, AB	OXA-23, OXA-51	MWAR (1; 2.2)	APR
	MP, IP, TZ, PM, AK, CP, PT, AB	OXA-23, OXA-51	ICU (1; 2.2)	NOV
	MP, IP, TZ, PM, GM, CP, PT, AB	OXA-23, OXA-51	ICU (1; 2.2)	APR
	MP, IP, TZ, PM, GM, CP, PT, TS	OXA-23, OXA-51	ICU (1; 2.2)	DEC
	MP, IP, TZ, PM, AK, GM, CP, PT, TS	OXA-23, OXA-51	ICU (2; 4.54)	JUN (1) AUG (1)
	MP, IP, TZ, PM, AK, GM, CP, PT, AB	OXA-23, OXA-51	ICU (2; 4.54)	FEB (1) APR (1)
	MP, IP, TZ, PM, AK, CP, PT, AB, TS	OXA-23, OXA-51	CCU (1; 2.2)	MAR
	MP, IP, TZ, PM, CP, PT, AB, TS, TE	OXA-23, OXA-51	CCU (1; 2.2)	JUN
	MP, IP, TZ, PM, CP, PT, AB, TS, TE	OXA-23, OXA-51	MWAR (1; 2.2)	MAR
	MP, IP, TZ, PM, AK, GM, CP, PT, TS, TE	OXA-23, OXA-51	NICU (1; 2.2)	SEP
	MP, IP, TZ, PM, AK, GM, CP, PT, AB, TS	OXA-23, OXA-51	MWAR (1; 2.2)	APR
	MP, IP, TZ, PM, AK, GM, CP, PT, AB, TS	OXA-23, OXA-51	ICU (5; 11.36)	APR (1) MAY (1) JUL (1) SEP (1) OCT (1)
	MP, IP, TZ, PM, AK, GM, CP, PT, AB, TS, TE	OXA-23, OXA-51	CCU (2; 4.54)	MAR (2)
	MP, IP, TZ, PM, CP, PT	OXA-51, OXA-143	ICU (1; 2.2)	DEC
	MP, IP, TZ, PM, CP, PT, AB	OXA-51, OXA-143	CCU (1; 2.2)	OCT
	MP, IP, TZ, PM, AK, CP, PT, AB	OXA-51, OXA-143	ICU (1; 2.2)	OCT
	MP, IP, TZ, PM, AK, GM, CP, PT, AB	OXA-51, OXA-143	FWAR (1; 2.2)	JUL
	MP, IP, TZ, PM, AK, GM, CP, PT, AB	OXA-51, OXA-143	CCU (2; 4.54)	JUL (2)
	MP, IP, TZ, PM, AK, GM, CP, PT, AB	OXA-51, OXA-143	ICU (9; 20.45)	JUL (4) AUG (1) SEP (2) OCT (2)
	MP, IP, TZ, PM, AK, GM, CP, PT, AB, TE	OXA-51, OXA-143	FWAR (1; 2.2)	NOV
	MP, IP, TZ, PM, AK, GM, CP, PT, AB, TS	OXA-51, OXA-143	ICU (1; 2.2)	APR
	MP, IP, TZ, PM, AK, GM, CP, PT, AB, TS, TE	OXA-51, OXA-143	ICU (1; 2.2)	OCT
	MP, IP, TZ, PM, AK, GM, CP, PT, AB	OXA-23, OXA-51, OXA-143	ICU (1; 2.2)	AUG

Table 4 *(continued)*. Phenotypic and genotypic characteristics of carbapenem-resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa* recovered from patients in a tertiary hospital between January and December of 2012.

Bacteria	Resistance phenotype	Genotype (*bla*$_{OXA}$ type genes)	Sampling	
			Unit (n; %)	Months (n)
P. aeruginosa (n = 28)	MP, IP, TZ, PM	*nd**	ICU (1; 3.57)	MAY
	MP, IP, TZ, PM, AT	*nd*	FWAR (1; 3.57)	MAY
	MP, IP, TZ, PM, CP, AT	*nd*	FWAR (1; 3.57)	AUG
	MP, IP, TZ, PM, GM, CP	*nd*	ICU (1; 3.57)	JUN
	MP, IP, TZ, PM, PT, AT	*nd*	ICU (1; 3.57)	APR
	MP, IP, TZ, PM, CP, PT, AT	*nd*	MWAR (1; 3.57) FWAR (1; 3.57)	SEP OCT
	MP, IP, TZ, PM, AK, GM, CP	*nd*	FWAR (3; 10.71)	APR (1) MAY (1) AUG (1)
	MP, IP, TZ, PM, AK, GM, CP	*nd*	ICU (1; 3.57)	APR
	MP, IP, TZ, PM, AK, GM, CP, AT	*nd*	FWAR (1; 3.57) MWAR (1; 3.57)	FEB OCT
	MP, IP, TZ, PM, AK, GM, CP, AT	*nd*	CCU (3; 10.71)	MAY (1) JUL (2)
	MP, IP, TZ, PM, AK, GM, CP, AT	*nd*	ICU (3; 10.71)	JUL (1) OCT (1) NOV (1)
	MP, IP, TZ, PM, AK, GM, CP, PT	*nd*	ICU (1; 3.57)	JAN
	MP, IP, TZ, PM, GM, CP, PT, AT	*nd*	ICU (2; 7.14)	JAN (1) SEP (1)
	MP, IP, TZ, PM, GM, CP, PT, AT	*nd*	FWAR (2; 7.14)	JUL (1) OCT (1)
	MP, IP, TZ, PM, AK, GM, CP, PT, AT	*nd*	CCU (2; 7.14)	MAY (1) AUG (1)
	MP, IP, TZ, PM, AK, GM, CP, PT, AT	*nd*	ICU (2; 7.14)	JUL (1) OCT (1)

*Oxacilinase-type genes (*bla*$_{OXA}$) not detected. MP: meropenem; IP: imipenem; TZ: ceftazidime; PM: cefepime; AK: amikacin; GM: gentamicin; CP: ciprofloxacin; PT: piperacillin-tazobactam; AB: ampicillin-sulbactam; TE: tetracycline; TS: trimethoprim-sulfamethoxazole; AT: aztreonam; FWAR: female ward; MWAR male ward; ICU: intensive care unit; CCI: coronary care unit; NICU: neonatal intensive care unit.

All the studied bacterial samples were resistant to ceftazidime, cefepime, imipenem, and meropenem. With regard to the *Acinetobacter* strains, resistance was also observed against ciprofloxacin (100%), piperacillin-tazobactam (100%), gentamicin (87.1%), ampicillin-sulbactam (83.4%), amikacin (77.8%), tetracycline (55.6%), and sulphazotrim (55.6%). As for *Pseudomonas* strains, resistance was also observed against ciprofloxacin (90.2%), aztreonam (90.2%), amikacin (63.4%), piperacillin-tazobactam (34%), and gentamicin (19.5%). Resistance to tigecycline and polymyxin B was not observed in *Acinetobacter*, and *Pseudomonas* did not appear to be resistant to polymyxin B (Table 3).

At least one of the genetic markers related to oxacillinases $bla_{\text{OXA-23}}$, $bla_{\text{OXA-51}}$, or $bla_{\text{OXA-143}}$ was detected in all *Acinetobacter* strains recovered in this study. The genotype $bla_{\text{OXA-23}}^{+}/bla_{\text{OXA-51}}^{+}$ was the most frequently observed (45.5%), followed by $bla_{\text{OXA-51}}^{+}/bla_{\text{OXA-43}}^{+}$, observed in 41% of the bacterial strains. The genotype $bla_{\text{OXA-23}}^{+}$ was observed in 9% of the isolated bacteria. The genetic markers $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-58}}$ were not observed in any of the *Acinetobacter* samples. With regard to the *P. aeruginosa* isolates, none of the genetic markers were observed (Table 4).

Discussion

As long as antimicrobial resistance against carbapenems in nosocomial strains of *Acinetobacter* and *Pseudomonas* is being reported worldwide, monitoring the drug susceptibility patterns in healthcare-associated bacteria is extremely important to guide the clinical management and chemotherapy of NFGN infections [2,7,8]. In this regard, appropriate laboratory handling of clinical specimens and microbiological procedures is also necessary. In this study, imipenem and meropenem susceptibility were considered for characterizing carbapenem-resistant *Acinetobacter* and *Pseudomonas*. Although there is a phenotypic screening method widely performed (Hodge test), according to the CLSI guidelines, this is only recommended for carbapenem-resistance screening among enterobacteria [31].

According to the literature, colonization and infection by multidrug-resistant bacteria, especially *Pseudomonas* and *Acinetobacter*, is an important and predictive factor for intra-hospital patient death, mainly of immunocompromised individuals [14,34,35]. Our results corroborate data in the literature; high mortality was observed among patients from whom multidrug-resistant *P. aeruginosa* and *Acinetobacter* spp. were isolated.

The mean age of patients harboring *Acinetobacter* spp. strains was 69.6 years and the gender distribution was 47.7% female, whereas for patients harboring *Pseudomonas,* the mean age was 77.1 years and 71.5% were female. Similar age range and gender associated with carbapenem-resistant *Acinetobacter* was previously reported [14], as was a similar patient age range in cases of *Pseudomonas* isolation [35]. Our data may reflect the actual scenario of patients admitted to the evaluated tertiary hospital with high risks of NFGN colonization and infection [34].

Tracheal aspirate was the main clinical specimen associated with multidrug-resistant *Acinetobacter* and *Pseudomonas*, followed by catheter, blood, and urine for *Acinetobacter* isolation and urine for *Pseudomonas*. So far, in Brazil, blood and catheter are among the most frequent clinical specimens associated with carbapenem-resistant NFGN bacteria isolation, followed by respiratory tract secretions, and urine [6,36].

With regard to the antimicrobial susceptibility patterns observed for the isolated carbapenem-resistant NFGN bacteria, a recent study on imipenem-resistant *A. baumannii* in hospital samples showed a result similar to that found in our study, *i.e.*, 100% resistance to ciprofloxacin and piperacillin-tazobactam [37]. Data from a national study carried out by others also corroborate these findings [38]. However, these data revealed a high frequency of isolates that were sensitive to tetracycline (approximately 85%), which were different from our results (44.4%). As long as therapeutic options for treating multidrug-resistant *Acinetobacter* spp. strains are quite limited, our results support the effectiveness of polymyxin B and tigecycline [37-39].

Considering *P. aeruginosa*, as the isolation of carbapenem-resistant strains has been growing increasingly in Brazilian hospitals, literature data corroborate our findings that resistance was observed against piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, and ciprofloxacin, but no resistance was observed against polymyxin B [6,8,34,37]. In this context, our results may support, on a regional scale, the reports by the SENTRY program, which pointed out polymyxin B as an alternative drug against multidrug-resistant *P. aeruginosa* [40].

All isolates found in the present study were characterized as *A. baumannii* by using the automatized method Vitek 2 Compact. However, this method is not able to distinguish the *Acinetobacter baumannii* species from the remaining bacteria such as *A. pitii*, *A. nosocomialis,* and *A. calcoaceticus* [17]. Facing

methodological limitations, the *Acinetobacter* strains were referred as *Acinetobacter* spp. According to the literature, as long as *bla*$_{OXA-51}$ is an intrinsic gene to *A. baumannii*, its screening would be of interest as an additional tool to confirm the species identification. *bla*$_{OXA-51}$ is associated with low expression levels, but hyper expression may occur by the insertion of other genetic elements and also be associated with porin permeability alterations and efflux pumps expression [17]. In this study, although we would suggest that *bla*$_{OXA-51}^{-}$ strains are not *A. baumannii*, additional molecular evaluation should be performed to confirm these *Acinetobacter* spp. identifications.

The oxacillinase gene *bla*$_{OXA-23}$ has been detected all over the world and has also been pointed out as the predominant carbapenemase among *Acinetobacter* species in some geographic regions such as Europe, Australia, Singapore, the Americas, Asia, and Africa. This suggests it has already been widely disseminated [41]. In Brazil, *Acinetobacter* strains harboring *bla*$_{OXA-23}$ have been reported in Porto Alegre (RS), Rio de Janeiro and Niterói (RJ), Belo Horizonte (MG) and São Paulo (SP) [14,37,42-45]. In this study, the data may support the OXA-23 epidemiology in Brazil, as 61.1% of the *Acinetobacter* strains were *bla*$_{OXA-23}^{+}$.

As observed in other Brazilian hospitals, OXA-143 was also observed in this study. In this country, *bla*$_{OXA-143}$ has been reported in frequencies ranging between 8.4% and 76% [29,46,47]. With regard to OXA-24 and OXA-58, although all *Acinetobacter* strains in this study were *bla*$_{OXA-24}^{-}$ and *bla*$_{OXA-58}^{-}$, international literature suggests these oxacillinases are well spread among *A. baumannii* clinical isolates [13-15,28].

In this study, the results may suggest that oxacillinase-type carbapenemases were not associated with the *P. aeruginosa* isolates, since no *bla*$_{OXA}^{+}$ strains were observed. According to the literature, in the absence of enzymes with carbapenem hydrolytic activity such as oxacillinases, carbapenem resistance in NFGN bacteria may be associated with several other mechanisms, which include synthesis of metallo-β-lactamases, increased expression of chromosome-encoded AmpC, reduced outer-membrane porin expression, and efflux systems overexpression [48-50].

Conclusions

Despite empirical chemotherapy not being routinely prescribed to carbapenem-resistant NFGN rod-associated infections, regional data is highly important to monitor bacteria epidemiology and infectious spread into hospitals and the community. It is important to enforce a rational use of antimicrobials together with effective practices of microbial and environmental control, including correct identification of bacterial strains that are resistant to several classes of antimicrobials. Although additional methods were not employed to confirm the species identification of *Acinetobacter* isolates, the findings of *bla*$_{OXA-51}$ strains may suggest that non-*A. baumannii* species are emerging as opportunistic, multidrug-resistant bacteria, currently resistant to carbapenem drugs in Brazilian hospitals.

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