Article/Artigo

Molecular characterization of clinical multiresistant isolates of *Acinetobacter* sp. from hospitals in Porto Alegre, State of Rio Grande do Sul, Brazil

Caracterização molecular de isolados clínicos *Acinetobacter* sp. multirresistentes em hospitais de Porto Alegre, Estado do Rio Grande do Sul, Brasil

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ABSTRACT

Introduction: Hospitals around the world have presented multiresistant *Acinetobacter* sp. outbreaks. The spread of these isolates that harbor an increasing variety of resistance genes makes the treatment of these infections and their control within the hospital environment more difficult. This study aimed to evaluate the occurrence and dissemination of *Acinetobacter* sp. multiresistant isolates and to identify acquired resistance genes. **Methods:** We analyzed 274 clinical isolates of *Acinetobacter* sp. from five hospitals in Porto Alegre, RS, Brazil. We evaluated the susceptibility to antimicrobial, acquired resistance genes from Ambler's classes B and D, and performed molecular typing of the isolates using enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) technique. **Results:** A high (68%) percentage of multiresistant isolates of *Acinetobacter* sp. was observed, and 69% were resistant to carbapenems. We identified 84% of isolates belonging to species *A. baumannii* because they presented the gene bla_{OXA-51}. The gene bla_{OXA-23} was detected in 62% of the isolates, and among these, 98% were resistant to carbapenems. Using the ERIC-PCR technique, we identified clones of *Acinetobacter* sp. spread among the four hospitals analyzed during the sampling period. **Conclusions:** The data indicate the dissemination of *Acinetobacter* sp. isolates among hospitals and their permanence in the hospital after one year.

Keywords: *Acinetobacter* sp. bla_{OXA-23}. Clonal dissemination. ERIC-PCR.

RESUMO

Introdução: Hospitais no mundo todo têm apresentado surtos de *Acinetobacter* sp. multirresistentes. A disseminação destes isolados com uma variedade cada vez maior de genes de resistência torna difícil o tratamento destas infecções e seu controle dentro do ambiente hospitalar. Este trabalho teve como objetivo avaliar a ocorrência e disseminação de isolados de *Acinetobacter* sp. multirresistentes e identificar genes de resistência adquirida. **Métodos:** Foram avaliados 274 isolados clínicos de *Acinetobacter* sp. obtidos de cinco hospitais da Cidade de Porto Alegre, RS, Brasil. Avaliamos o perfil de suscetibilidade a antimicrobianos, genes de resistência adquirida das classes B e D de Ambler e realizamos a tipificação molecular dos isolados utilizando a técnica de *enterobacterial repetitive intergenic consensus-polymerase chain reaction* (ERIC-PCR). **Resultados:** Encontramos uma alta (68%) porcentagem de isolados de *Acinetobacter* sp. multirresistentes e 69% dos isolados apresentaram resistência aos carbapenêmicos. Foram identificados 84% de isolados pertencentes a espécie *A. baumannii*, pois apresentaram o gene bla_{OXA-51}. Em 62% dos isolados, foi detectado o gene bla_{OXA-23}, sendo que 98% destes isolados foram resistentes aos carbapenêmicos. Através da tipificação molecular pela técnica de ERIC-PCR identificamos clones de *Acinetobacter* sp. disseminados entre quatro dos hospitais analisados e nos anos de 2006 e 2007. **Conclusões:** Os dados obtidos indicam a disseminação de isolados de *Acinetobacter* sp. entre hospitais assim como sua permanência no ambiente hospitalar após um ano.

Palavras-chaves: *Acinetobacter* sp. bla_{OXA-23}. Disseminação clonal. ERIC-PCR.

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INTRODUCTION

The genus *Acinetobacter* is widely distributed in nature; they are endogenous organisms in various types of soil and water and are occasionally found in food[1-2]. In recent years, members of this group, particularly *A. baumannii*, have been associated with healthcare-associated infections (HAIs), especially in debilitated patients. The main sites of infection are the respiratory tract, urinary tract, bloodstream, wounds, and burns[3]. Patients with burns and those in intensive care units and/or using mechanical ventilation are at greater risk. According to Jawad et al.[4], *Acinetobacter* isolates can survive desiccation for extended periods. This characteristic increases the incidence of infections at hospitals because it makes these bacteria capable of staying in the skin of patients, doctors, and nurses, as well as remaining for long periods on hospital equipment.

The treatment of multidrug-resistant strains can be difficult. Since 1970, HAIs caused by *Acinetobacter* sp. were successfully treated with aminopenicillins, ureidopenicillins, and cephalosporins of first and second generation. Since then, a steady increase in the prevalence of resistant strains has been seen, which has compromised treatment with penicillins, aminoglycosides, extended spectrum cephalosporins, and most recently, fluoroquinolones[5]. The carbapenems have been the drug of choice against *Acinetobacter* sp., but the number of isolates resistant to these antimicrobial agents has increased considerably. Carbapenem resistance in *A. baumannii* is associated with a variety of combined mechanisms, including the acquiring of β-lactamases, stable desrepression of AmpC, decreased in-membrane permeability, alteration of penicillin binding proteins, and overexpression of efflux pumps. Among the acquired β-lactamases, enzymes of Ambler class B, also called metallo-β-lactamase (MBL), and class D that hydrolyze carbapenems are the most globally identified carbapenem-resistant strains of *Acinetobacter* sp.[6-7].

The aim of this study was to evaluate the occurrence and dissemination of multiresistant *Acinetobacter* sp. strains of clinical origin with acquired resistance genes in five hospitals of Porto Alegre, RS, Brazil.

METHODS

Hospitals studied and bacterial isolates

Acinetobacter sp. was isolated from clinical samples collected in five large hospitals in Porto Alegre, Brazil, and they were obtained after the project was approved by the ethics committee of each hospital involved. The hospitals included in the study were named with letters A to E. Hospital A has an area of 128,338m^2, 897 beds, and 4,499 employees and receives 12,000 people per day. Hospital B, as hospital A, belongs to the Brazilian Health System (SUS), has 603 beds, and receives 18,000 people per day in an area of 49,000m^2. Hospitals C, D, and E belong to the same hospital complex but are located in different regions of the city, and these hospitals receive only SUS patients. Hospital C is a general hospital, and it has the largest emergency unit in the state, with 801 beds and 4,631 employees in an area of 43,030m^2. Hospital D primarily receives victims of traffic and work accidents and violence, as well as burn patients; it has 304 beds, 1,291 employees, and an area of 18,835m^2. Hospital E receives only obstetric and gynecologic emergency patients, has 189 beds, 737 employees, and an area of 12,273m^2. In Hospital A, the sampling period was from February to April 2007 and between July and October 2007. At Hospital B, samples were collected in July 2006 and between July and October 2007. The isolates from hospitals C, D, and E were obtained from October to December 2006 and from May to August 2007. Information on the biological source of the sample from the isolates was also obtained from the laboratories of each hospital.

Identification of the isolates of *Acinetobacter* sp.

The isolates were primarily identified as *Acinetobacter* sp. by the laboratories of the respective hospital. A further confirmation of the genus was performed by amplifying a fragment of 16S rDNA using the primers: Acin16SF (5'-CCT TGA TGC AGA GYT AAT GC-3') and Acin16SR (5'-GTA GCA ACC CTT TGT ACC GA-3'), specific for the genus *Acinetobacter*. These primers were designed by aligning the sequences 16S rRNA gene from 29 species of *Acinetobacter* (GENBANK), using the CLC Program FreeWorkbench 2.2. Amplification reactions were performed in mixtures containing 3.5mM MgCl2, 0.2mM dNTPs, 1µM of each primer, 1 unit of Taq DNA polymerase, 1x reaction buffer of Taq DNA polymerase, and 100ng of bacterial DNA in a final volume of 30µL. The amplification conditions were as follows: an initial cycle of denaturation at 95°C for 5min followed by 25 cycles of denaturation at 95°C for 1min, annealing at 52°C for 1min and extension at 72°C for 1min, and a final extension cycle of 72°C for 8min.

Antimicrobial susceptibility test

The profile of antimicrobial susceptibility of the isolates was provided by each hospital and was determined using the disk diffusion technique according to the standards of the Clinical Laboratory Standards Institute, 2007[8]. We analyzed only the antimicrobial agents tested in common for all hospitals: amikacin (30µg), ceftazidime (30µg), piperacillin-tazobactam (100µg/10µg), imipenem (10µg), meropenem (10µg), cefepime (30µg), and ampicillin/sulbactam (10µg/10µg). The strains resistant to four classes of antibiotics were considered multiresistant.

Phenotypic MBL detection test

Phenotypic triage of MBL production was performed by the disk approximation test using imipenem and ceftazidime as substrates and ethylenediaminetetraacetic acid (EDTA) and 2-mercaptopropionic acid as inhibitors[9-11].

DNA extraction and detection of *bla* genes

The DNA of the isolates was obtained by the boiling method according to Misbah et al.[12]. As *A. baumannii* was identified by the presence of the gene *bla* $_{\text{OXA-51,}}$ its amplification was performed with all isolates used in this study. This gene is found exclusively in isolates of this species and can therefore be used to identify it[13]. Isolates that were resistant to imipenem, meropenem, and/or with a positive triage for MBL production were subjected to polymerase chain reaction (PCR) reaction to assess the presence of the $bla_{\text{OXA-23}}$, $bla_{\text{SPM-1}}$, bla_{VIM}, and bla_{IMP} genes using the primers listed in **Table 1**. The reactions were performed in mixtures containing 1x Taq Go Master Mix (Promega), 1µM of each primer, and 100ng of bacterial DNA in a final volume of 15µL. Amplification conditions were as follows: an initial cycle of denaturation at 94°C for 5min followed by 30 cycles of 94°C for 1min, annealing temperature as in **Table 1**, extension at 72°C for 1min, and a final extension at 72°C for 6min. PCR products were visualized on 1% agarose gel stained with ethidium bromide.

ERIC-PCR

The genetic similarity of isolates of *Acinetobacter* sp. was determined by the enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). The PCR was performed in a final volume of 30µL containing 8L of genomic DNA, 5.5mM of MgCl2, 1.25mM of each dNTP, 400ng of each primer ERIC1 and ERIC2[14], and 2U of Taq DNA polymerase (Invitrogen). The amplification conditions were as follows: an initial denaturation cycle at 95°C for 7min, 30 cycles of 94°C for 1min, 51°C for 1min and 72°C for 1min and 30s, and a final extension at 72°C for 15min. The amplified product was visualized on agarose gel 2% plus the polymer Synergel (BioAmerica) in Tris-borate, stained with ethidium bromide 0.5µg/mL. The banding patterns obtained from ERIC-PCR were converted into a binary matrix, where score 1 means presence of a band and score 0 means absence of a band. In this analysis, each isolate was considered to be

TABLE 1 - Polymerase chain reaction primers and annealing conditions used in this study for detection of the resistance genes.

Gene	Sequence of the primer	Annealing temperature	Expected size(bp)	Reference
$bla_{\text{OXA-23}}$	F1 (5' GAT CGG ATT GGA GAA CCA GA3') R1 (5' ATT TCT GAC CGC ATT TCC AT 3')	51°	570	Woodford et al.[15].
$bla_{\text{OXA-51}}$	F1 (5' TAA TGC TTT GAT CGG CCT TG3') R1 (5' TGG ATT GCA CTT CAT CTT GG 3')	53°	353	Woodford et al.[15].
bla_{IMP}	F2 (5'GGA ATA GAG TGG CTT AAY TCT C 3') R2 (5' CCA AAC YAC TAS GTT ATC T 3')	53°	188	Ellington et al.[16].
bla_{VIM}	F3 (5' GAT GGT GTT TGG TCG CAT A 3') R3 (5' CGA ATG CGC AGC ACC AG 3')	52°	390	Ellington et al.[16].
bla_{SPM}	F (5´TCG GAT CAT GTC GAC TTG CC -3´) R (5´CCT TCG CTT CAG ATC CTC GT - 3´)	53°	344	Fuentefria et al.[17].

one operational taxonomic unit. The results for the fingerprinting were analyzed via SPSS software (version 13 for Windows). The similarity matrix was calculated using the Dice coefficient and clustering of the similarity matrix, via the unweighted pair-group method using arithmetic averages (UPGMAs) clustering. Only the visible bands in the ERIC-PCR fingerprinting were used to construct the similarity matrix and the dendrogram. Isolates with more than 80% similarity were considered closely related and, therefore, were treated as clones; these isolates differed in one to two bands.

RESULTS

We analyzed 274 isolates of *Acinetobacter sp.* obtained from five hospitals in Porto Alegre, Brazil, in 2006/2007. Hospital A presented the largest number of isolates, 100 isolates, followed by Hospital C with 93 isolates. Hospital D had 45 isolates, Hospital B had 32 isolates, and Hospital E had only four isolates. Only one isolate from each patient was included in this study.

Among the isolates, 69% were resistant to carbapenems. The hospitals with higher levels of carbapenem resistance were Hospital C with 87% and Hospital D with 85%. Hospital A had 53% of isolates resistant to carbapenems, and Hospital B had 41%.

In analyzing the data from all hospitals, antimicrobial resistance to cefepime and piperacillin-tazobactam, 88% and 83%, respectively, was found to be higher. The antibiotics ampicillin-sulbactam showed low percentages of resistance in Hospitals A and B, with 29% and 55%, respectively, whereas Hospitals C and D showed the highest rates of antimicrobial resistance, with 100% and 97%, respectively. The four isolates from Hospital E were resistant to all antibiotics tested.

The isolates of *Acinetobacter* sp. were obtained from various biological samples, with tracheal aspirate being the most frequent, at 26% of isolates, followed by sputum at 20%, when all isolates were compared. They were also isolated from blood samples (13%), urine (9%), catheter (8%), secretions (6%), and burns (4%) **(Figure 1).** Other samples containing *Acinetobacter* sp. such as pleural fluid, abdominal fluid, lumbar puncture, scabs, and wound secretion were not detailed in **Figure 1** because of their low percentages. These samples correspond to a total of 14% of the isolates analyzed in this study. As Hospital D has a burn center, it presented seven isolates from burn patients.

Analyzing the data obtained by the antibiogram, the isolates were grouped into 18 distinct profiles based on their susceptibility to seven antimicrobials **(Table 2)**. The greatest diversity of profiles was found in Hospital A, with 16 different profiles, eight of them with just one isolate. In analyzing the five hospitals, the profile with the largest number of isolates showed resistance to seven (61%) antimicrobials, followed by the susceptibility to all drugs, with 12% of isolates. We found 68% of multiresistant isolates, which corresponded to the profiles 1, 2, 3, 4, 5, and 7 of **Table 2**.

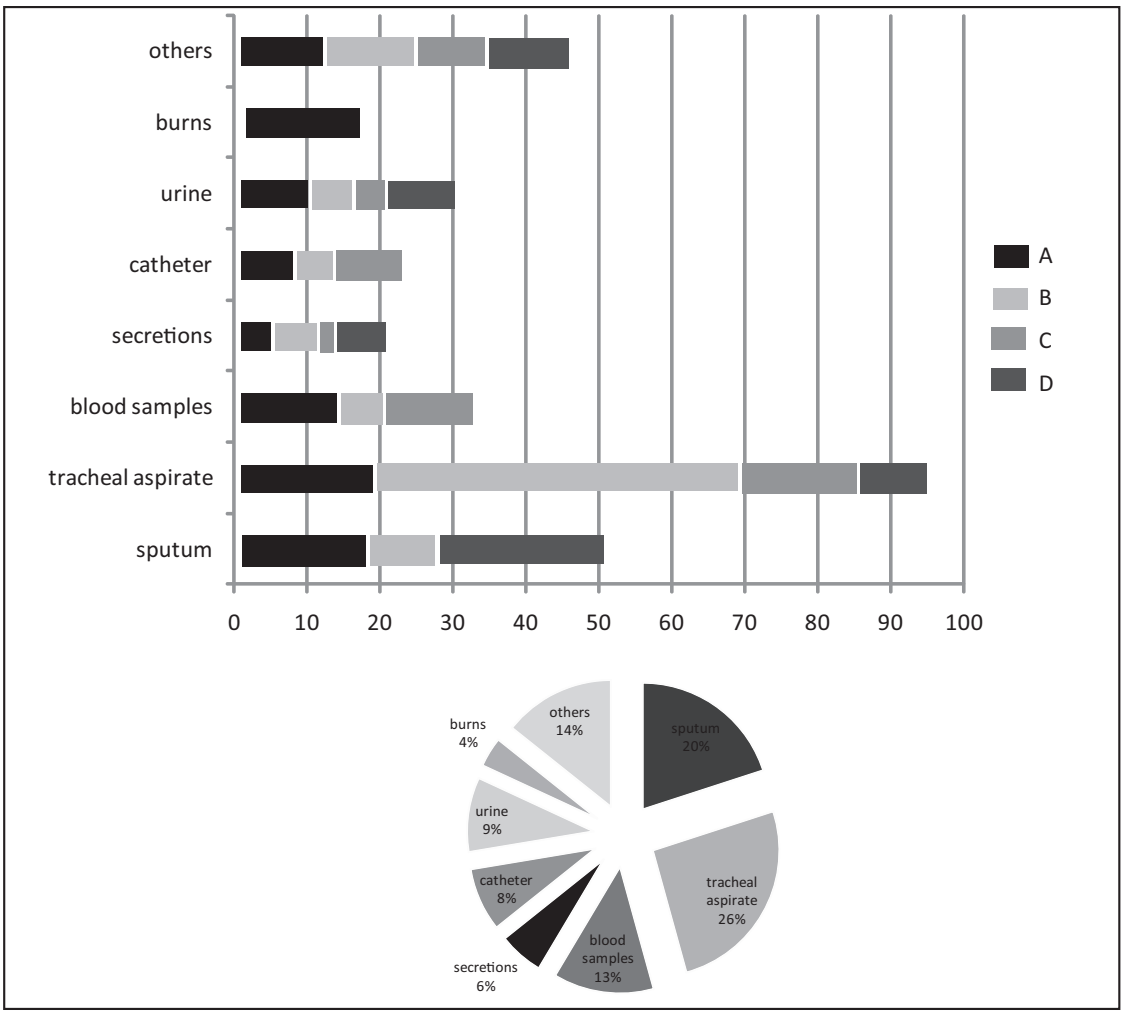

FIGURE 1 - Percentage of isolates for each biological material used in the isolation of *Acinetobacter* sp. per hospital (A, B, C, and D).

TABLE 2 - Profiles of non-susceptibility (resistant and intermediary results) of the *Acinetobacter* sp. isolates in the five hospitals analyzed.

		Hospitals (number of isolates)				
Profile	**Antimicrobial**	**A**	**B**	**C**	**D**	**E**
1	AMI AMP CFP CAZ IMP MER PIP	43	13	73	33	4
2	AMI CFP CAZ IMP MER PIP	5				
3	AMI AMP CFP IMP MER PIP	2		3	5	
4	AMI AMP CFP CAZ IMP PIP	1				
5	AMP CFP CAZ IMP MER PIP			3		
6	AMI AMP CFP CAZ PIP	8	3	6	5	
7	AMP CFP IMP MER PIP	1				
8	AMP CFP CAZ PIP	1				
9	AMI CFP CAZ PIP	9	9			
10	AMI CFP CAZ MER	1				
11	AMI CAZ PIP	1				
12	CFP CAZ PIP	1	1			
13	AMI CFP CAZ	2				
14	AMI CFP	1				
15	AMI	2		1		
16	CAZ	1				
17	AMP		1			
18	Sensitive to all	21	5	4	2	

AMI: amikacin; **AMP**: ampicillin-sulbactam; **CFP**: cefepime; **CAZ**: ceftazidime; **IMP**: imipenem; **MER**: meropenem; **PIP**: piperacillin-tazobactam.

The phenotypic triage for MBL production was carried out with all the isolates. Using the antimicrobial imipenem and the inhibitor EDTA, 10% of the isolates were positive. In the test using the antimicrobial ceftazidime and the inhibitor 2-MPA, the percentage of positive isolates increased to 21%.

The detection of the $bla_{\text{OXA-51like}}$ gene was performed on all isolates analyzed in this study, and 230 (84%) isolates were positive for the presence of this gene. Hospitals A and C had the greatest number of positive isolates, both with 84 isolates. The four isolates from Hospital E were positive for this gene, whereas Hospital B had 27 positive isolates, and Hospital D had 31. It should be mentioned that among the isolates positive for the $bla_{\text{OXA-51}}$ gene, a high percentage of multidrug resistance was also observed. In Hospitals A and B, 16 and 3 isolates, respectively, were not identified as *A. baumannii* because they were negative for the gene $bla_{\text{OXA-51}}$. Of these, 14 were sensitive to all antimicrobial agents analyzed. In Hospitals C and D, 22 isolates were negative for the $bla_{\text{OXA-51}}$ gene, but they showed an opposite behavior when compared with the isolates from the other hospitals because only two isolates were sensitive to all antimicrobials tested.

The presence of the $bla_{\text{SPM-1}}$, bla_{VIM}, and bla_{IMP} genes were analyzed using PCR in the 214 isolates that were resistant to carbapenems and/or positive in the phenotypic test for detection of MBL. None of the isolates was positive for these genes, although there was a large percentage of positive isolates in phenotypic tests (28%). The same 214 isolates were tested for the presence of the $bla_{\text{OXA-23}}$ gene, and it was found in 61.2% of the isolates. The four isolates from Hospital E had the $bla_{\text{OXA-23}}$ gene. The hospital with the greatest number of positive isolates was Hospital C, with 56 (64%) of the 87 positive isolates examined. Hospital B showed the lowest number (12) of tested isolates, and from these, eight (62%) were positive for the gene. Hospital A had 59% (66 tested) of positive isolates, and Hospital D had 46.6% (45 tested). Twenty-one isolates that were susceptible to carbapenems and positive for the phenotypic screening for MBLs were evaluated for the presence of the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes. Eight of these showed the two genes, three isolates from Hospital A, three from Hospital D, and two from Hospital C.

The ERIC-PCR technique was selected for the molecular typing of the isolates that were $bla_{\text{OXA-23}}$ positive. The banding pattern found for these isolates showed high diversity, with patterns of 4 to 17 fragments ranging from 211 to 1,600pb. Twenty-five isolates had a single ERIC-PCR profile, and 124 were grouped into 30 distinct groups (80% similarity as a cutoff) in **Table 3**. The largest group showed nine isolates of *Acinetobacter* sp. belonging to four different hospitals (Group AV), and the isolates were from different sampling periods (five and four isolates from 2006 to 2007). Other groups showed similar results; the isolates were from various hospitals and remained in the hospital for a long period **(Table 3).** Some isolates from the same hospital showed 100% similarity, but this was also observed among isolates from different hospitals, as in Group A. In other groups, isolates with 100% similarity belonged to different hospitals: Hospitals A and B (Group B), Hospitals C and D (Group H), and Hospitals A and E (Group T). This distribution of the isolates in 55 ERIC-PCR profiles indicated that they were grouped neither by the hospital nor by the year of collection. It was not possible to determine any relationship with the susceptibility profiles found. The five isolates that were negative for the $bla_{\text{OXA-51}}$ gene were classified into distinct groups.

TABLE 3 - Distribution of *Acinetobacter* sp. isolates in the groups formed with similarity >80% by the ERIC-PCR and UPGMA analysis.

ERIC-PCR groups*	Number of isolates per hospital				
	A	B	C	D	E
A	1		6		
B	1	1			
H			1	2	
I			1	1	
J	6			1	
K	5		1		
O			2		
P			1	1	
Q			5		
T	1		1		1
U	2			1	
X			1	2	
AC			2		
AD	4				
AE			1	2	
AI		1		1	1
AJ		1	1		
AK			1	1	
AL		1	4	1	1
AM	3		2		
NA			3		
AP	1			1	
AQ			2	1	
AR		1	4		
AT	2				
AU			3		
AV	1	1	4	3	
AY			2	1	
BD	1		1		
BE	1		6	1	

***ERIC-PCR**: enterobacterial repetitive intergenic consensus-polymerase chain reaction; **UPGMA:** unweighted pair-group method using arithmetic averages.

DISCUSSION

In Brazil, as in many parts of the world, outbreaks of infections caused by multidrug-resistant *Acinetobacter* have been reported. In this study, we found a high percentage of multidrug-resistant (68%) and carbapenem-resistant (69%) *Acinetobacter* sp. isolates, which are still the best treatment option in cases of infection by this microorganism. The antimicrobial resistance in this genus is associated with several mechanisms, but the production of β-lactamases has been considered the main one[18].

Although *Acinetobacter* sp. is implicated in a variety of HAIs, including meningitis, urinary tract infections, and surgical infections, it is the predominant etiologic agent of nosocomial pneumonia, especially in patients with ventilator-associated pneumonia, who have been admitted to treatment in intensive care units[19]. This observation concurs with observations obtained in this study regarding isolation of *Acinetobacter* sp., mainly from sputum and tracheal aspirate samples. Other studies also found similar results to ours, such as Papa et al.[20], who found 20% of isolates obtained from tracheal aspirate, 17% from blood culture, and 15% from catheters. Another study found 32% of isolates from the respiratory tract, 19% from wounds, 19% from urine, 16% from blood, and 13% from catheter[21].

Our study found 28% of positive isolates for the phenotypic screening test for MBL, and none of the isolates exhibited the bla_{IMP}, bla_{VIM}, and bla_{SPM} genes. The disk approximation techniques proposed by Arakawa et al.[10] and Lee et al.[12] for Gram-negative bacilli were initially effective for the detection of metallo-enzymes β-lactamases. As the technique was used to detect *Acinetobacter* sp., studies have shown low specificity for this genus[22-23]. Therefore, it was suggested that positive results in the phenotypic test for detection of MBL should be interpreted carefully in samples of *Acinetobacter* sp.

The bla_{OXA-51} gene has been found in all isolates of *A. baumannii* tested and is being considered as a natural component of the species chromosome[8,24]. Thus, the presence of this gene has been used to identify *A. baumannii*. This gene may be associated with resistance to carbapenems when ISAbaI-type insertion sequences, which carry strong promoters, are found upstream to bla_{OXA} genes, resulting in increased expression and concomitant resistance to carbapenems[25]. In our study, we found a large number of isolates positive for the bla_{OXA-51} gene (84%), and results similar to that were found by Wisplinghoff et al.[26], who observed 86% of isolates positive for the bla_{OXA-51} gene. In many studies, the species *A. baumannii* is the most prevalent and associated with HAIs, and it is also the species with the highest rate of resistance to antimicrobials[27-28].

Carbapenem resistance in isolates of *Acinetobacter* sp. is most often mediated by oxacillinase (OXA)-type enzymes and less frequently by MBLs[29]. As in ours, other studies have searched for enzymes like MBLs, but the OXA-type enzymes, such as bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, and bla_{OXA-58}, are the most frequent in this genus[7,30-31]. In another study also held in Porto Alegre, Brazil, with *Acinetobacter* sp., 53 isolates were analyzed, and all of them showed the genes bla_{OXA-23} and bla_{OXA-51}[27]. In another study with *A. baumannii* in Curitiba, Brazil, the bla_{OXA-23} gene was detected in eight strains resistant to carbapenems. They also found that isolates susceptible to carbapenems did not have the $bla_{OXA\ 23}$ gene[32]. In our study, we observed eight strains that were sensitive to carbapenems and had the bla_{OXA-23} and bla_{OXA-51} genes. OXA-type enzymes exhibit a weak hydrolysis of carbapenems and may not always show resistance profile, but when they are associated with insertion elements, they may have an increase in its expression and show resistance to carbapenems[29].

The ERIC-PCR technique is a low-cost, alternative and rapid method that showed good results for the genetic characterization of *Acinetobacter* sp. isolates. By ERIC-PCR, we identified clones of *Acinetobacter* sp. in different hospitals, as well as the permanence of isolates from one year to another in the same hospital. These data can be related to the dissemination characteristics of this microorganism, its permanence for long periods in hospitals, its main form of dissemination, human contact, and the mobility of patients and staff within and between hospitals. In a work carried out by Jeong et al.[33] using the ERIC-PCR in isolates of *A. baumannii*, identical profiles were observed for seven isolates producing OXA-23 in a hospital in Korea. In another study with seven isolates of *A. baumannii* producing OXA-23 from the same hospital, two distinct groups were identified using ERIC-PCR, one group consisting of five isolates with identical profiles and the other consisting of two isolates[6].

With the spread of multiresistant isolates and the diversity of resistance genes, the choice of appropriate treatment becomes increasingly difficult. It is of great importance to study the local

epidemiology of *Acinetobacter* sp. isolates to establish the best treatment and use the correct epidemiological control, to avoid the spread of these microorganisms, because studies like this have shown the dissemination of clones in different hospitals. Efficient control measures such as washing hands, keeping patients in isolation, cleaning equipment properly, and ensuring awareness of the technical staff are needed to reduce the outbreaks of *Acinetobacter* sp. In our study, we can observe the behavior of the isolates from different hospitals analyzed through the antimicrobial resistance and molecular typing profiles and resistance genes found. The five hospitals exhibited dissemination of multiresistant and carbapenem-resistant *Acinetobacter* sp. isolates harboring the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes, probably acting as the main causing agent of the high rate of carbapenem resistance observed in this study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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