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Major Article

The role of the genetic elements *bla*$_{oxa}$ and IS*Aba*1 in the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex in carbapenem resistance in the hospital setting

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Abstract

Introduction: Members of the *Acinetobacter* genus are key pathogens that cause healthcare-associated infections, and they tend to spread and develop new antibiotic resistance mechanisms. Oxacillinases are primarily responsible for resistance to carbapenem antibiotics. Higher rates of carbapenem hydrolysis might be ascribed to insertion sequences, such as the IS*Aba*1 sequence, near *bla*$_{OXA}$ genes. The present study examined the occurrence of the genetic elements *bla*$_{OXA}$ and IS*Aba*1 and their relationship with susceptibility to carbapenems in clinical isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. **Methods:** Isolates identified over 6 consecutive years in a general hospital in Joinville, Southern Brazil, were evaluated. The investigation of 5 families of genes encoding oxacillinases and the IS*Aba*1 sequence location relative to *bla*$_{OXA}$ genes was conducted using polymerase chain reaction. **Results:** All isolates presented the *bla*$_{OXA\text{-}51\text{-}like}$ gene (n = 78), and 91% tested positive for the *bla*$_{OXA\text{-}23\text{-}like}$ gene (n = 71). The presence of IS*Aba*1 was exclusively detected in isolates carrying the *bla*$_{OXA\text{-}23\text{-}like}$ gene. All isolates in which IS*Aba*1 was found upstream of the *bla*$_{OXA\text{-}23\text{-}like}$ gene (n = 69) showed resistance to carbapenems, whereas the only isolate in which IS*Aba*1 was not located near the *bla*$_{OXA\text{-}23\text{-}like}$ gene was susceptible to carbapenems. The IS*Aba*1 sequence position of another *bla*$_{OXA\text{-}23\text{-}like}$-positive isolate was inconclusive. The isolates exclusively carrying the *bla*$_{OXA\text{-}51\text{-}like}$ gene (n = 7) showed susceptibility to carbapenems. **Conclusions:** The presence of the IS*Aba*1 sequence upstream of the *bla*$_{OXA\text{-}23\text{-}like}$ gene was strongly associated with carbapenem resistance in isolates of the *A. calcoaceticus-A. baumannii* complex in the hospital center studied.

Keywords: *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. *bla*$_{OXA}$. IS*Aba*1. Carbapenem antibiotics.

INTRODUCTION

Healthcare-associated infections (HAI) are increasingly affecting public and private healthcare systems and raising not only hospital costs but also the morbidity and mortality rates of affected patients[1,2,3]. The bacterial genus *Acinetobacter* is recognized as a key HAI-causing pathogen that is associated with high mortality rates in hospital settings[2,4]. *Acinetobacter* has considerable capacity to spread and develop new antibiotic resistance mechanisms[5]. Consequently, numerous HAI outbreaks caused by multidrug-resistant (MDR) representatives of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex have been reported in several countries, including Brazil[2,5,6].

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Oxacillinase-type (OXA) carbapenemases (Ambler class D β-lactamases) are the primary factors responsible for resistance to carbapenem antibiotics worldwide[7,8]. Approximately 150 variants have already been described, and at least 45 show carbapenem-hydrolyzing activity[7]. OXAs are genetically divided into 6 subgroups. The most commonly identified examples are *bla*$_{OXA\text{-}23\text{-}like}$ (OXA-23, OXA-27, and OXA-49), *bla*$_{OXA\text{-}24\text{-}like}$ (OXA-24, OXA-25, OXA-26, OXA-40, and OXA-72), *bla*$_{OXA\text{-}58\text{-}like}$, *bla*$_{OXA\text{-}143\text{-}like}$ (OXA-143, OXA-231, and OXA-253), *bla*$_{OXA\text{-}235}$, and *bla*$_{OXA\text{-}51\text{-}like}$. The latter subgroup is intrinsic to *A. baumannii* species[8,9]. Higher carbapenem hydrolysis rates have been reported to result from the acquisition of insertion sequences (ISs) that affect the expression of the *bla*$_{OXA}$ genes encoding oxacillinases[10,11,12,13,14]. These elements are the smallest and most abundant transposable elements and are capable of causing mutations and rearrangements in the genome, thereby contributing to the spread of resistance and virulence determinants in various bacterial species[15].

Different ISs have been described in the *A. calcoaceticus-A. baumannii* complex in association with specific genes;

these sequences play a key role in the development of carbapenem resistance[15]. Insertion sequence *Acinetobacter baumannii* 1 (IS*Aba*1) belongs to the IS4 family and has been identified in association with several antibiotic resistance genes in the *A. calcoaceticus-A. baumannii* complex[14][15]. The role of IS*Aba*1 in increasing gene expression was demonstrated for the bla_{ampC} gene encoding cephalosporinase and the $bla_{OXA\text{-}23\text{-}like}$ genes encoding an OXA that hydrolyzes carbapenems[15]. Some studies have also linked the presence of the IS*Aba*1 sequence upstream of the $bla_{OXA\text{-}51\text{-}like}$ gene to carbapenem resistance phenotypes[4][15].

The diversity of the genetic elements involved in the resistance of the *A. calcoaceticus-A. baumannii* complex to antibiotics has spurred research on these determinants of resistance[1][2][4][16][17][18][19]. On the basis of the findings of these studies, the present study examined the occurrence of the genetic elements bla_{OXA} and IS*Aba*1 in clinical isolates belonging to the *A. calcoaceticus-A. baumannii* complex and their association with phenotypic susceptibility to carbapenem antibiotics.

METHODS

Selection of clinical isolates and ethical aspects

The study was conducted at Dona Helena Hospital, a private general hospital with 189 beds, and at the Molecular Biology Laboratory of the University of Region of Joinville [*Universidade da Região de Joinville* (UNIVILLE)], both located in Joinville, Santa Catarina, Brazil. Clinical isolates belonging to the *A. calcoaceticus-A. baumannii* complex identified over 6 consecutive years, starting in March 2009, were included in the study. Isolates related to HAI (healthcare-associated infection after 48h of admission), colonization (presence of the microorganism without clinical signs of related infection), and surveillance culture upon admission (detection of colonization in patients referred from other healthcare institutions at the time of hospital admission) according to the criteria defined by the Hospital Infection Control Service of the hospital center studied were included in the study. Only the first isolate obtained from patients with 2 or more isolates derived from the same anatomical site during the same period of hospitalization was considered for this study. All isolates were identified by the Microscan Walkaway automated system (Siemens, Munich, Germany) complemented by Gram staining.

This study was evaluated and approved by the Research Ethics Committee of UNIVILLE (Opinion 788.455).

Antibiotic susceptibility analysis

The determination of the antibiotic susceptibility profile was performed using the disc-agar diffusion method (Kirby and Bauer)[20]. The inhibition halos were interpreted according to the current recommendations of the Clinical and Laboratory Standards Institute (CLSI)[21] for each corresponding year.

Imipenem and meropenem discs with 10µg of each antibiotic were used. Simultaneously, other classes of antibiotics were tested in the following amounts indicated by the CLSI: amikacin (30µg), ampicillin/sulbactam (10/10µg), ceftazidime (30µg), ciprofloxacin (5µg), doxycycline (30µg), gentamicin (10µg), piperacillin/tazobactam (100/10µg), polymyxin B (300 units), sulfamethoxazole/trimethoprim (1.25/23.75µg), and tobramycin (10µg). All discs were from the same manufacturer (Oxoid, Cambridge, UK).

Bacterial DNA preparation and evaluation

The heat shock method was used to prepare bacterial deoxyribonucleic acid (DNA) from a pure culture grown in MacConkey agar[22]. Initially, 3 colonies collected using a bacteriological loop were resuspended in 100µL of ultrapure sterile water (Milli-Q, São Paulo, Brazil). The suspension was subjected to a boiling water bath for 5 min and then cold shocked in an ice bath for 5 min. The boiling and cooling procedures were repeated, followed by centrifugation at 8,000 ×*g* for 10 min. The supernatant containing the bacterial DNA was evaluated through spectrophotometric readings at 260 and 280nm and then stored at -20°C.

The extracted bacterial DNA was assessed by polymerase chain reaction (PCR) to determine its viability for use in the subsequent genotypic analyses. A pair of primers specific to the *A. calcoaceticus-A. baumannii* complex [gltA-F (5′-AATTTACAGTGGCACATTAGGTCC-3′) and gltA-R (5′-GCAGAGATACCAGCAGAGATACACG-3′)] was used to amplify a single 722-bp fragment of the *gltA* gene encoding bacterial citrate synthase[23]. The thermocycling conditions were as follows: initial denaturation at 94°C for 3 min; 30 cycles of 94°C for 1 min, 62°C for 30 s, and 72°C for 1 min; and final extension at 72°C for 10 min[23].

Investigation of the genetic elements associated with oxacillinase production

The occurrence of the $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, and $bla_{OXA\text{-}143\text{-}like}$ genes in the selected isolates was investigated using multiplex PCR with the primers outlined in **Table 1**[24][25]. The reactions were conducted in a final volume of 50µL containing approximately 50-500ng of extracted DNA and a reagent mixture with 1U Platinum® Taq DNA polymerase (Invitrogen, São Paulo, Brazil), 200µM dNTPs mix (GE Healthcare, Little Chalfont, United Kingdom), 1× PCR buffer (Invitrogen), 20 pmol of each primer (DNA Express, São Paulo, Brazil), and 1.5mM of $MgCl_2$ (Invitrogen). The reactions were performed in an XP Cycler device (BIOER Technology, Tokyo, Japan) at the following conditions: initial denaturation at 94°C for 3 min; 30 cycles of 1 min at 94°C, 30s at 52°C, and 1 min at 72°C; and final extension at 72°C for 10 min[24].

The presence of the IS*Aba*1 promoter sequence in the isolates and its association with the $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}51\text{-}like}$ genes were investigated by PCR[12][26][27]. The primer sequences and the sizes of the expected reaction products are outlined in **Table 1**. Excluding the primers (10 pmol of each primer per reaction), all other components were used in quantities identical to those described for the multiplex PCR, and the reactions were performed using the same thermocycler. The initial denaturation was performed at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 1 min, hybridization at 50°C for 1 min, and extension at 72°C for 2 min. The final extension was performed at 72ºC for 5 min.

TABLE 1

Primers used to detect genes encoding oxacillinases and IS*Aba*1.

Target	Sequence (5′ to 3′)	Product (bp)	Reference
$bla_{\text{OXA-23-like}}$	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	501	Woodford et al.[24]
$bla_{\text{OXA-24-like}}$	GGTTAGTTGGCCCCCTTAAA AGTTGAGCGAAAAGGGGATT	246	Woodford et al.[24]
$bla_{\text{OXA-51-like}}$	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	353	Woodford et al.[24]
$bla_{\text{OXA-58-like}}$	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	599	Woodford et al.[24]
$bla_{\text{OXA-143-like}}$	TGGCACTTTCAGCAGTTCCT TAATCTTGAGGGGGCCAACC	149	Higgins et al.[25]
IS*Aba*1	CACGAATGCAGAAGTTG CGACGAATACTATGACAC	548	Segal et al.[12]
IS*Aba*1 $_{+}$ $bla_{\text{OXA-23-like}}$	GTGTCATAGTATTCGTCG ATTTCTGACCGCATTTCCAT	875	Turton et al.[27]
IS*Aba*1 $_{+}$ $bla_{\text{OXA-51-like}}$	CAAGGCCGATCAAAGCATTA GTGTCATAGTATTCGTCG	359	Turton et al.[27]

IS*Aba*1: insertion sequence *Acinetobacter baumannii;* **bla_{OXA}:** oxacillinase encoding gene**.**

The PCR products were subjected to submerged electrophoresis (TBE buffer, 10 V/cm) in a 1% agarose gel with 0.5µg/mL ethidium bromide. The expected fragments were confirmed by exposure to ultraviolet light (300nm) in a transilluminator (MiniBis-Pro Photodocumentation System – DNR Bio-Image Systems Ltd., Jerusalem, Israel), followed by scanning.

Standard strains of the *A. calcoaceticus-A. baumannii* complex from the collection of cultures of the Oswaldo Cruz Foundation [*Fundação Oswaldo Cruz* (FIOCRUZ), Rio de Janeiro, Brazil] labeled 7892 ($bla_{\text{OXA-24-like}}$), 7740 ($bla_{\text{OXA-58-like}}$), and 7572 ($bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-143-like}}$) were used as positive controls. The carbapenem-susceptible *A. calcoaceticus-A. baumannii* American Type Culture Collection (ATCC) strain 19606 was used as the negative control.

Data analysis

A database was created using Excel 2013 (Microsoft Corp., Redmond, WA, USA) for phenotypic and genotypic data collection and analysis. Appropriate percentages were calculated, and data were analyzed descriptively.

RESULTS

Seventy-eight clinical isolates belonging to the *A. calcoaceticus-A. baumannii* complex derived from 60 patients (12 individuals contributed 2 or more isolates) were employed in the phenotypic and genotypic analyses established for the study. All isolates were positive for amplification of the $bla_{\text{OXA-51-like}}$ gene fragment. These isolates were identified in clinical samples from patients with HAI (66.7%), surveillance culture upon admission (15.4%), and colonization (17.9%).

The isolates were collected from bronchoalveolar lavage (38.5%), wound secretions (12.8%), rectal swabs (12.8%), blood (9%), urine (6.4%), and other types of samples (20.5%). The distribution of isolates by hospital department showed that 53.8% were derived from the intensive care unit (ICU); 29.5%, from the inpatient units; 10.3%, from the emergency room; and 6.4%, from the surgical center. The temporal evolution in relation to the sectors and types of samples corresponding to the analyzed isolates is shown in **Table 2.**

Phenotypic analysis

Evaluation of antibiotic susceptibility profiles showed that 88.5% (n = 69) of the isolates were resistant to the carbapenems imipenem and meropenem (**Figure 1**). Simultaneously, the proportions of resistance to the other antibiotics tested were distributed as follows: ciprofloxacin, 88.3% (68/77); piperacillin/tazobactam, 87.2% (68/78); sulfamethoxazole/trimethoprim, 74.3% (52/70); ceftazidime, 68.4% (52/76); ampicillin/sulbactam, 41% (32/78); amikacin, 41% (32/78); tobramycin, 28.4% (21/74); doxycycline, 13.7% (10/73), and gentamicin, 10.8% (8/74). All isolates exhibited full susceptibility to polymyxin B.

Genotypic evaluation

All extracted DNA samples enabled the amplification of the *gltA* gene segment and were considered viable for subsequent genotypic analyses. **Figure 2** depicts the electrophoretic pattern resulting from the PCR analysis of the main subgroups of genes encoding oxacillinases and IS*Aba1* in the clinical isolates of the present study.

TABLE 2

Distribution of clinical isolates according to sample type and hospital department over 6 years (2009-2015).

	Year						
	1	**2**	**3**	**4**	**5**	**6**	**Total**
Isolates [n (%)]	16	12	7	12	20	11	78
Healthcare-associated infections	14 (87.5)	10 (83.4)	5 (71.4)	11 (91.6)	9 (45.0)	3 (27.3)	52
colonization	2 (12.5)	2 (16.6)	2 (28.6)	1 (8.4)	4 (20.0)	3 (27.3)	14
surveillance	np	np	-	-	7 (35.0)	5 (45.4)	12
Type of sample [n (%)]							
bronchoalveolar lavage	8 (50.0)	4 (33.3)	2 (28.6)	8 (66.7)	6 (30.0)	2 (18.2)	30
wound secretion	-	3 (25)	1 (14.3)	1 (8.3)	2 (10.0)	3 (27.2)	10
rectal swab	-	-	-	-	6 (30.0)	4 (36.4)	10
blood	1 (6.2)	2 (16.7)	1 (14.3)	2 (16.7)	1 (5.0)	-	7
urine	3 (18.8)	1 (8.3)	-	-	1 (5.0)	-	5
others:	4 (25.0)	2 (16.7)	3 (42.8)	1 (8.3)	4 (20.0)	2 (18.2)	16
cutaneous fistula	1	-	-	-	-	-	1
tissue fragment	-	-	1	-	1	1	3
bone fragment	-	-	1	-	-	-	1
abdominal fluid	-	-	1	-	-	-	1
peritoneal fluid	1	-	-	-	-	-	1
pleural fluid	1	-	-	-	1	-	2
cerebrospinal fluid	-	1	-	1	-	-	2
surgical secretion	-	-	-	-	1	-	1
abscess secretion	1	1	-	-	-	1	3
trochanter secretion	-	-	-	-	1	-	1
Hospital department [n (%)]							
ICU	12 (75)	6 (50.0)	2 (28.6)	11 (91.7)	8 (40.0)	3 (27.3)	42
inpatient units	3 (18.8)	4 (33.4)	3 (42.8)	1 (8.3)	6 (30.0)	6 (54.5)	23
emergency room	-	1 (8.3)	-	-	5 (25.0)	2 (18.2)	8
surgical center	1 (6.2)	1 (8.3)	2 (28.6)	-	1 (5.0)	-	5

np: not performed; **ICU:** intensive care unit.

Seventy-one of the isolates phenotypically identified as belonging to the *A. calcoaceticus-A. baumannii* complex had the genes $bla_{OXA-23-like}$ and $bla_{OXA-51-like}$, whereas 7 exclusively amplified $bla_{OXA-51-like}$. The genes $bla_{OXA-24-like}$, $bla_{OXA-58-like}$, and $bla_{OXA-143-like}$ were not detected.

All isolates carrying the $bla_{OXA-23-like}$ gene (n = 71) also had the IS*Aba*1 promoter sequence; this sequence was located upstream from the $bla_{OXA-23-like}$ gene in 69 isolates. A single isolate carried IS*Aba*1, which was not associated with the location of $bla_{OXA-23-like}$, whereas another isolate proved inconclusive in terms of the insertion position. The IS*Aba*1 sequence was not observed upstream of the $bla_{OXA-51-like}$ gene in the analyzed isolates.

Evaluation of the association between genetic markers and phenotypic resistance to carbapenems

All isolates with IS*Aba*1 located upstream of the $bla_{OXA-23-like}$ gene (n = 69) showed resistance to imipenem and meropenem and were identified during the 6 consecutive years of observation (93.7%, 91.7%, 100%, 91.7%, 85%, and 72.7% of the resistant isolates in each year). Conversely, the only isolate in which IS*Aba*1 was not coupled to $bla_{OXA-23-like}$ was susceptible to carbapenems and was identified in year 1. The isolates exclusively containing $bla_{OXA-51-like}$ (n = 7; 1 in year 2, 1 in year 4, 2 in year 5, and 3 in year 6) also showed full susceptibility to imipenem and meropenem.

DISCUSSION

Carbapenems are the antibiotics of choice for the treatment of infections caused by β-lactam-resistant bacteria of the *A. calcoaceticus-A. baumannii* complex. However, resistance to carbapenems has increased, thereby limiting the use of this class of antibiotics[15]. The present study demonstrated that the presence of the IS*Aba*1 promoter sequence associated with the $bla_{OXA-23-like}$ gene in *A. calcoaceticus-A. baumannii* was strongly associated with resistance to carbapenem antibiotics in the hospital setting.

The species *A. baumannii* is the most important and representative member of the *A. calcoaceticus-A baumannii* complex, and it is considered the leading pathogen of the *Acinetobacter* genus in health institutions worldwide[10]. Small genomic differences exist among the species of this genus and

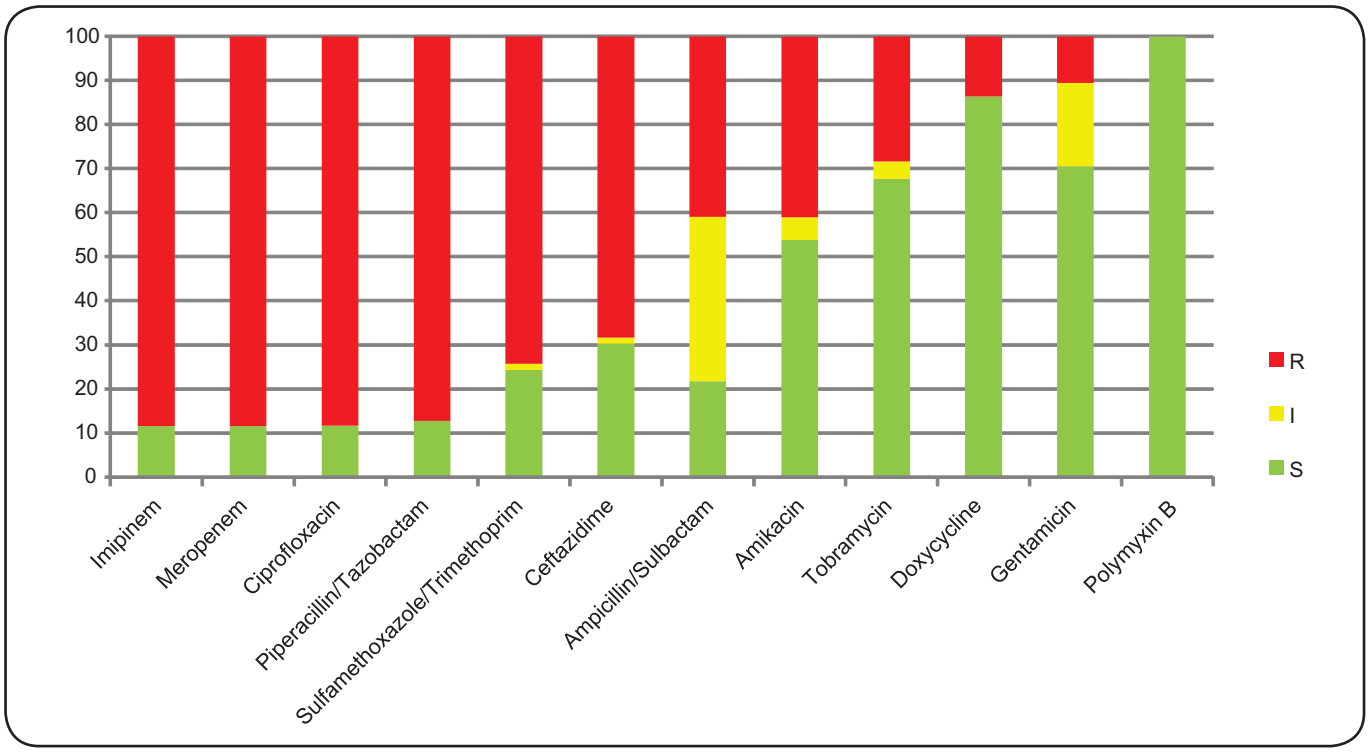

FIGURE 1. *In vitro* antibiotic susceptibility profile of *Acinetobacter calcoaceticus-Acinetobacter baumannii* clinical isolates (%): **R:** resistant; **I:** intermediate and **S:** susceptible.

have been reported by several authors; however, *A. calcoaceticus*, *A. baumannii, A. pittii,* and *A. nosocomialis* are closely related and are collectively termed the *A. calcoaceticus-A. baumannii* complex owing to the difficulty in separating them using conventional tests[16] [28]. *A. baumannii* species identification may be confirmed by detecting the presence of the $bla_{\text{OXA-51}}$ gene because it naturally occurs in the *A. baumannii* chromosome[29] [30]. This gene is intrinsic to the species and has been identified in a wide collection of isolates of the *A. calcoaceticus-A. baumannii* complex collected from various geographic areas[13] [28] [29] [31] [32]. Thus, phenotypically identified isolates that possess the $bla_{\text{OXA-51-like}}$ gene were considered members of the complex in the present study. Conversely, recent studies have reported the plasmid-encoded carbapenemase OXA-51 in clinical isolates of *Acinetobacter nosocomialis* and in other genospecies not corresponding to *A. baumannii*[17] [33] [34] [35].

Acinetobacter baumannii has become particularly problematic due to its increased prevalence and resistance to carbapenem antibiotics[36]. In the present study, 88.5% (69/78) of the isolates showed resistance to carbapenems. According to the Antimicrobial Surveillance Program in Latin America, the resistance rates of *Acinetobacter* spp. to carbapenems increased from 6.4%, 12.6%, and 0% between 1997 and 1999 to 84.9%, 71.4%, and 50.0% between 2008 and 2010 in Argentina, Brazil, and Chile, respectively[36] [37]. A cohort study conducted in 5 ICUs of tertiary hospitals of the metropolitan area of the

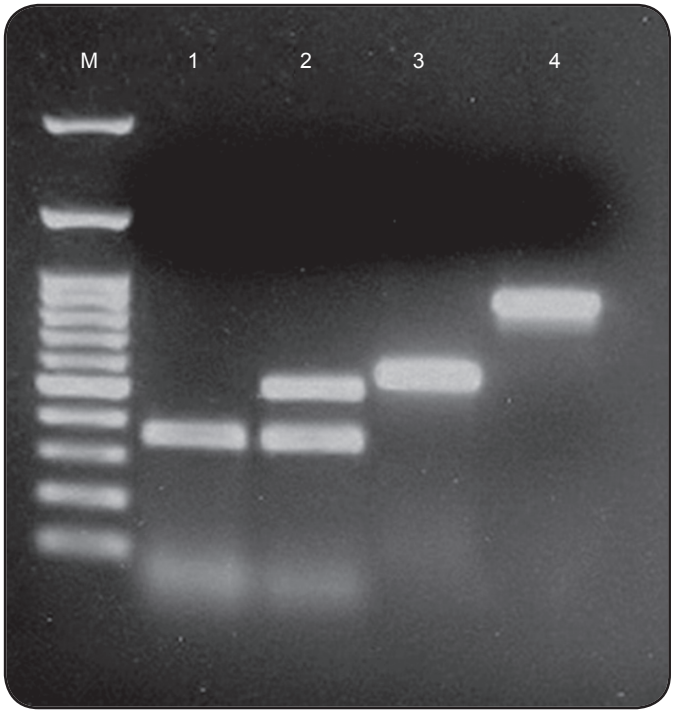

FIGURE 2. Identification of genetic elements associated with the production of oxacillinases by PCR. M: molecular marker (100bp; Fermentas, Ontario, Canada); 1: $bla_{\text{OXA-51-like}}$ (353bp); 2: $bla_{\text{OXA-51-like}}$ (353bp) and $bla_{\text{OXA-23-like}}$ (501bp); 3: IS*Aba*1 (548bp) and 4: IS*Aba*1 + $bla_{\text{OXA-23-like}}$ (875bp). **PCR:** polymerase chain reaction.

City of Goiânia, Goiás, Brazil, showed a high rate of *A. baumannii* resistance to carbapenems (73.4%), which is higher than the mean values reported by other Brazilian institutions (25-45%)[38].

Oxacillinase production corresponding to $bla_{OXA\text{-}23\text{-}like}$ expression is the most common carbapenem resistance mechanism in *A. calcoaceticus-A. baumannii* reported in various countries, including Bulgaria, China, Iraq, Afghanistan, France, and Polynesia[39]. In the present study, 91% (71/78) of the isolates were positive for $bla_{OXA\text{-}23\text{-}like}$ amplification. A study conducted by Carvalho et al.[17] in 10 public and private hospitals in the City of Rio de Janeiro from 2005 to 2007 showed similar results, with the detection of $bla_{OXA\text{-}23\text{-}like}$ in 96% of the isolates. This rate is higher than the rates observed in studies conducted in other countries, both nationwide, such as in Taiwan (21.1%), or restricted to a few local hospitals, such as in Romania (84.6%), Tunisia (82%), Greece (72.4%), and Italy (40%)[33].

The expression of bla_{OXA} genes might be increased by the presence of the IS*Aba*1 sequence upstream of the enzyme-encoding gene, which provides higher levels of resistance to carbapenems[14]. The presence of IS*Aba*1 was detected in all isolates positive for $bla_{OXA\text{-}23\text{-}like}$ gene amplification in the present study (n = 71). Furthermore, all isolates wherein IS*Aba*1 was located upstream of the bla_{OXA} gene (n = 69) showed resistance to carbapenems. This profile of a strong association between IS*Aba*1 and $bla_{OXA\text{-}23\text{-}like}$ was repeated during the 6 years of observation, which suggests the occurrence of cross-infection, dissemination, and long-term permanence of this resistance mechanism in the hospital center studied. The only isolate wherein IS*Aba*1 was not located upstream of the $bla_{OXA\text{-}23\text{-}like}$ gene showed susceptibility to carbapenems. Thus, the presence of $bla_{OXA\text{-}23\text{-}like}$ without the IS*Aba*1 promoter sequence might not be sufficient to cause carbapenem hydrolysis, as reported by Carvalho et al.[40] OXA-51 carbapenemases encoded by intrinsic (chromosomal) genes have a weak capacity to hydrolyze carbapenems[11] [41]. The presence of IS*Aba1* may provide a strong promoter for the $bla_{OXA\text{-}51\text{-}like}$ gene, thereby increasing its expression and causing resistance[42] [43] [44]. A study conducted in the United Kingdom by Turton et al.[27] showed that carbapenem hydrolysis by isolates carrying the $bla_{OXA\text{-}51\text{-}like}$ gene only occurred in bacteria in which IS*Aba*1 was upstream of the gene, suggesting that this IS acted as a promoter for the expression of this gene. Antonio et al.[26] reported that, in their study performed in hospitals of the Southeast and South regions of Brazil from 2004 to 2008, IS*Aba*1 was not observed upstream of the $bla_{OXA\text{-}51\text{-}like}$ gene; furthermore, none of the isolates analyzed showed the carbapenem-resistant phenotype. The results in our study were similar because only 9% (7/78) of the isolates exclusively amplified $bla_{OXA\text{-}51\text{-}like}$, and none amplified IS*Aba*1 concomitantly; moreover, all these isolates were susceptible to carbapenems. This finding reinforces the hypothesis that the absence of IS*Aba*1 or its presence in a region not upstream of the $bla_{OXA\text{-}51\text{-}like}$ gene renders a very low or nonexistent ability to hydrolyze carbapenems in *A. baumannii* isolates without other bla_{OXA} genes.

The emergence of carbapenem resistance has limited the treatment of HAIs caused by *A. baumannii*[36] [38]. In the present study, 41% of the isolates showed resistance to ampicillin/sulbactam, which is nearly half that of the 85.2% rate reported by Carvalho et al.[45] from 2002 to 2010 in the ICU of a public institution in the City of Teresina, Piauí, Brazil. The use of ampicillin/sulbactam has been effective for the treatment of systemic infections caused by MDR *A. baumannii*; however, a steady decline in susceptibility rates has been reported in recent decades[46] [47]. Furthermore, in Latin America, *A. baumannii* has been commonly reported as being MDR, with high rates of resistance to other antibiotics in addition to carbapenems, including ceftazidime, piperacillin/tazobactam, and ciprofloxacin. Thus, Latin America appears to be the highest-ranking region in the world for the prevalence of MDR *A. baumannii*[38]. In a study conducted using 155 *A. baumannii* isolates from 11 Brazilian states from 2008 to 2011, Chagas et al.[36] demonstrated that most isolates also showed resistance to more than 5 classes of antibiotics in addition to carbapenems. The highest rates were observed for ciprofloxacin, cefepime, and piperacillin/tazobactam, with 99.4% resistant isolates, followed by ceftazidime (97.4%), sulfamethoxazole/trimethoprim (23.9%), and amikacin (11.6%); however, the isolates did display susceptibility to polymyxin B. In the present study, 88.3%, 87.2%, and 68.4% of the isolates showed resistance to ciprofloxacin, piperacillin/tazobactam, and ceftazidime, respectively, and all analyzed isolates were susceptible to polymyxin B. However, higher rates of resistance were observed for sulfamethoxazole/trimethoprim (74.5%) and amikacin (41%). These data suggest that the use of polymyxins should be highlighted as a therapeutic option for HAIs caused by *A. baumannii*[48]. Polymyxin B and colistin have long been used as antibiotic agents, but they cause nephrotoxicity and neurotoxicity. Nevertheless, they are considered effective in treating infections caused by carbapenem-resistant *A. baumannii*[49]. Unfortunately, resistance to these antibiotics is also emerging[36] [50].

Acinetobacter baumannii also stands out because it is an opportunistic pathogen in HAI episodes, especially in ICUs[34]. In particular, invasive mechanical ventilation – a life support method widely used in ICUs – is usually associated with nosocomial infection by *A. baumannii*[51]. In a study performed using 500 isolates from 30 hospitals of 10 Cuban provinces from 2010 to 2012, Quiñones et al.[37] demonstrated that the hospital unit with the highest number of cases of *A. calcoaceticus-A. baumannii* infection was the ICU (60.2%) and that the respiratory tract had the highest rate of positive cultures (38.6%). Godoy et al.[38] showed that the lungs were also the most common site of infection with *A. baumannii* among patients monitored in 5 ICUs of Goiânia (53.1% of cases). Similar results were observed in our study, in which 53.8% (42/78) of the isolates were derived from the ICU, and the majority of positive cultures were derived from bronchoalveolar lavage (66.7%; 28/42).

Acinetobacter calcoaceticus-A. baumannii colonization should be analyzed because it may precede cases of infection and contribute to the endemicity of this agent in healthcare institutions, in addition to being a key microorganism related to HAIs[52]. In the present study, 17.9% (14/78) of the isolates were related to patient colonization, a prevalence higher than

the 9.4% *A. baumannii* colonization rate reported by Godoy et al.[38]. Surveillance cultures of patients transferred from other institutions were adopted in the hospital studied during year 3. A total of 12 cases positive for *A. baumannii* were confirmed, including 7 in year 5 (6 isolates from rectal swabs and 1 from wound secretion) and 5 in the following year (4 isolates derived from rectal swabs and 1 from tissue fragments). All the cases were identified within 48h of admission.

Our study had some limitations. First, the number of isolates was relatively small despite the period of 6 years covered by the analyses, and the study was performed at a single hospital center. Second, the included patients had several comorbidities, and some underwent multiple hospital admissions, which complicated the evaluation of the impact on the numbers related to colonization. Therefore, we recommend conducting similar multicenter studies involving a greater number of hospitals and evaluating the clinical and epidemiological characteristics associated with HAIs and the risk factors. Furthermore, we propose an investigation of the association between genetic profiles related to resistance and the severity and clinical outcomes of the affected patients using standardized clinical scores. Finally, we consider it pertinent to conduct the genotyping of isolates identified in the hospital to examine the clonal relationship between resident strains and possible community-acquired strains, thereby highlighting possible flaws in infection control.

In conclusion, this study provides data on the prevalence of isolates belonging to the *A. calcoaceticus-A. baumannii* complex carrying genetic elements associated with carbapenem resistance. The emergence and spread of variants of the bla_{OXA} gene encoding carbapenemases and of ISs capable of increasing bla_{OXA} expression in *A. baumannii* in Brazil underscore the need for the medical community and health managers to remain alert to the potential threat of outbreaks of carbapenem-resistant isolates, especially those with the IS*Aba*1 sequence upstream of the $bla_{OXA\text{-}23\text{-}like}$ gene.

Conflicts of interest

The authors declare no conflicts of interest.

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