Genetics and Molecular Research

Characterization and molecular epidemiology of extensively prevalent nosocomial isolates of drug-resistant *Acinetobacter* spp

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ABSTRACT. *Acinetobacter* sp isolates deserve special attention once they have emerged globally in healthcare institutions because they display numerous intrinsic and acquired drug-resistance mechanisms. This study assessed the antibiotic susceptibility profile, the presence

of the genetic marker *bla*$_{OXA-23}$, and the clonal relationship among 34 nosocomial isolates of *Acinetobacter* spp obtained at a hospital in southeastern Brazil. Antibiotic sensitivity analysis was performed by the standard disc-diffusion method. All isolates were found to be extensively resistant to several drugs, but sensitive to polymyxin B. A polymerase chain reaction (PCR) assay was used to detect the *bla*$_{OXA-23}$ gene, which is associated with carbapenem resistance. The genetic profile and the clonal relationship among isolates were analyzed via enterobacterial repetitive intergenic consensus (ERIC)-PCR. The *Acinetobacter* spp were divided into four groups with 22 distinct genetic subgroups. ERIC-PCR analysis revealed the genetic diversity among isolates, which, despite having a heterogeneous profile, displayed 100% clonality among 56% (19/34) of them.

Key words: *Acinetobacter* spp; Nosocomial infections; *bla*$_{OXA-23}$ gene; ERIC-PCR; Genetic diversity; Clonality

INTRODUCTION

Acinetobacter spp are Gram-negative bacteria belonging to the Moraxellaceae family. The genus *Acinetobacter* is widely distributed in nature (Baumann, 1968; Ferreira et al., 2011) and includes 37 different species (Peleg et al., 2008). Among these, *Acinetobacter baumannii* is the most clinically relevant species (Martins and Barth, 2013) and has emerged as a major problem in health institutions due to its ubiquity, difficult eradication (resistance to desiccation, chemical sanitizers, and ultraviolet) (Cherkaoui et al., 2015), and high tendency to acquire resistance to antibiotics (Peleg et al., 2008; Chang et al., 2015). This species is responsible for nosocomial infections, especially by cross transmission, and has a propensity to cause outbreaks (Khorsi et al., 2015). *A. baumannii* has spread globally and hence, has become one of the major causes of healthcare-associated infections (HCAIs) (Gulen et al., 2015).

With high mortality, morbidity, and hospitalization costs (Gulen et al., 2015), infections caused by multiple drug-resistant *A. baumannii* are extremely difficult to treat (de Sá Cavalcanti et al., 2013). Treating these infections has been a challenge, due to the resistance of these microorganisms to β-lactam antibiotics, which are highly-effective antimicrobial agents with low toxicity, that are widely used in the treatment of Gram-negative bacterial infections (Peleg et al., 2008; Chang et al., 2015).

Resistance to β-lactam antibiotics results mainly from the production of β-lactamases. Enzymes belonging to every class of β-lactamase from the Ambler molecular classification have been described in *A. baumannii*: class A, extended-Spectrum β-lactamases; class B, metallo-β-lactamases (MβL); class C, *Acinetobacter*-derived cephalosporinases, and class D, carbapenem-hydrolyzing class D β-lactamases (Hammoudi et al., 2015). Among these, the emergence of carbapenem resistance in *A. baumannii* has become a current global issue (Peleg et al., 2008; Chang et al., 2015). The molecular basis of carbapenem resistance is mainly due to the presence of Ambler class D oxacillinases, including the phylogenetic groups *bla*$_{OXA-23}$-like, *bla*$_{OXA-40}$-like, and *bla*$_{OXA-58}$-like oxacillinases, or to the overproduction of intrinsic enzymes of the group *bla*$_{OXA-51}$-like (Queenan and Bush, 2007; Abbott et al., 2013;), where the promoter sequences are associated with this gene, such as IS*Aba1* (de Sá Cavalcanti et al., 2013). An

endemic dissemination of *A. baumannii* expressing enzymes belonging to β-lactamase Class B has also been widely reported (Peleg et al., 2008; Abbott et al., 2013).

The bla_{OXA-23} gene, together with IS*Aba1*, is linked to carbapenem resistance, and is the most widely disseminated among *A. baumannii* isolates worldwide, and is also the most prevalent among isolates in Brazil (Werneck et al., 2011; Fonseca et al., 2013; Pagano et al., 2013).

The objective of this study was to track the presence of the bla_{OXA-23} gene among carbapenem-resistant nosocomial isolates of *Acinetobacter* spp obtained from patients admitted to a hospital in southeastern Brazil, as well as to investigate the clonal relationship among the strains involved, by characterizing the molecular epidemiological profile of the isolates.

MATERIAL AND METHODS

Bacterial isolates and antibiotic sensitivity profile

This study was performed in a 377-bed hospital, classified as a third-tier general hospital, in the southeastern region of Brazil. From November 2014 to April 2015, we obtained 34 non-repetitive clinical isolates of carbapenem-resistant *Acinetobacter* spp from different hospital sectors (intensive care unit, semi-intensive care unit, surgical unit, internal medicine clinic, transplantation, cardiology, and other units). Clinical isolates were obtained from samples of blood, urine, tracheal aspirate, catheter, secretions, and tissue fragments from patients. Genus-level identification of the isolates was through biochemical and enzymatic tests performed in the clinical laboratory of the hospital, according to the Clinical Laboratory Standard Institute (CLSI) guideline (Wayne, 2014). Sensitivity to several antibiotic classes was determined through the disc-diffusion test, according to the CLSI guideline. *Acinetobacter* sp isolates resistant to imipenem and meropenem were stored at -20°C in BHI medium (brain heart infusion - BD®) with 20% glycerol.

DNA extraction and polymerase chain reaction (PCR) for detection of gene bla_{OXA-23}

The cryopreserved isolates were reactivated by seeding in BHI medium (Laborclin®), and were incubated at 37°C. After growing for 24 h, microbial suspensions were seeded on 90 mm-dishes with blood agar (Laborclin®) and incubated for 24 h. Colonies were isolated from the dishes and DNA was extracted with the KAPAExtract® kit, according to the manufacturer instructions. The DNA was eluted into a final volume of 100 µL, quantified by 1.5% agarose gel electrophoresis, and used for PCR and enterobacterial repetitive intergenic consensus (ERIC)-PCRs. The presence of the bla_{OXA-23} gene was determined by PCRs with primers OXA23F 5'-GATGTGTCATAGTATTCGTCG-3' and OXA23R 5'-TCACAACAACTAAAAGCACTG-3', resulting in a 1057-base pair amplicon (Fonseca et al., 2013). Primers were synthesized by Integrated DNA Technology, USA. Reactions took place in a mix containing 1X Taq buffer from the Kappa PCR kit, 2.5 mM $MgCl_2$, 1 µM deoxynucleotides, 0.5 U Kappa Taq Polymerase, 1.25 µM of each primer, and 1 µL (50 ng/µL) bacterial DNA, for a final reaction volume of 25 µL. Amplification conditions were as follows: an initial denaturation cycle at 95°C for 3 min, followed by 35 denaturation cycles at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension of 5 min. Amplicons were viewed on a 1.5% agarose gel stained with ethidium bromide and were photographed. As a positive control for the PCR, we used a strain of *A. baumannii* isolated

from the hospital and genetically identified by a Brazilian reference laboratory (Ezequiel Dias Foundation) as *A. baumannii* coding the bla_{OXA-23} gene. As a negative control, we used a strain of *Escherichia coli*, ATCC 25922.

ERIC-PCR

Characterization of the genetic profile and clonal relationship among the *Acinetobacter* sp isolates was performed through genomic DNA polymorphism analysis. The conserved primers ERIC-1 5'-TGTAAGCTCCTGGGGATTAAC-3' and ERIC-2 5'-AAGTAAGTGACTGGGGTGAGCG-3' were used in a PCR as sequences for the ERIC region described in the literature (Duan et al., 2009). Reactions took place in a mix containing 1X Taq buffer from the Kappa PCR kit, 2.5 mM $MgCl_2$, 1 µM deoxynucleotides, 0.5 U Kappa Taq polymerase, 1 µM of each primer, and 2 µL (50 ng/µL) bacterial DNA with a final reaction volume of 50 µL. Amplification conditions were as follows: an initial denaturation cycle at 95°C for 3 min, followed by 40 denaturation cycles at 92°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 8 min, and a final extension of 16 min. Amplicons were viewed on a 1.5% agarose gel stained with ethidium bromide and were photographed. Amplification profile analysis was visually performed by two observers, and was transformed into binary data in a matrix, according to the presence (1) or absence (0) of bands. To determine the genetic relationship among the isolates, the matrix was subjected to the multivariate analysis "Cluster Analysis" with the complete linkage method for calculation of the Euclidean distance and generation of a dendrogram using the Minitab v.16 statistic software.

Access and analysis of patient records

With hospital authorization, and in conformity with ethical rules, the records of 34 patients from whom the extensively drug-resistant *Acinetobacter* spp strains had been isolated were accessed and analyzed. We looked for data regarding the principal complaint that had caused patient admission, as well as co-morbidities, age, gender, admission date, date of isolation of resistant bacteria, admission sector, and duration of hospital admission.

Ethical aspects

This study was approved by the research Ethics Committee (CEP) of the participating hospital, as well as by the CEP of Universidade Estadual de Montes Claros (No. 855.002/2014).

RESULTS AND DISCUSSION

Epidemiological profile of the patients and their *Acinetobacter* sp isolates

Every one of the 34 carbapenem-resistant *Acinetobacter* sp isolates analyzed in this study exhibited extensive drug resistance, in agreement with what has been commonly reported worldwide (Khorsi et al., 2015). The isolates were obtained from a variety of sources, such as blood (N = 12), tracheal aspirate (N = 9), urine (N = 6), liquor (N = 2), catheter (N = 2), secretions (N = 2), and tissue fragment (N = 1) (Table 1).

Table 1. Clinical characteristics of the 34 nosocomial isolates of carbapenem-resistant *Acinetobacter* spp.

Gender	Total number	Source of isolation	Total number	Admission sector	Total number
Male	20 (58.8%)	Blood	12 (35.3%)	Intensive Care Unit	13 (38.2%)
Female	14 (41.2%)	Tracheal aspirate	9 (26.5%)	Semi-Intensive Care Unit	6 (17.6%)
Age		Urine	6 (17.6%)	Internal Medicine Clinic I	2 (5.9%)
0-20	3	Liquor	2 (5.9%)	Internal Medicine Clinic II	6 (17.6%)
21-50	11	Catheter tip	2 (5.9%)	Surgical Unit	3 (8.8%)
51-60	5	Secretion	2 (5.9%)	Cardiology	1 (3%)
61-70	8	Tissue fragment	1 (2.9%)	Transplantation	1 (3%)
71 or older	7			Other units	2 (5.9%)

Epidemiological analysis of the 34 patients with carbapenem-resistant *Acinetobacter* spp indicated that 7 were ≥71 years old, 8 were between 61 and 70 years old, 5 were between 51 and 60 years old, 11 were between 21 and 50 years old, and 3 were ≤20 years old, with an average age of 47.6 years (SD = 21.31), ranging from 17-86 years, which was comparable to what was observed in other studies (48, 54, and 53.2 years; Wisplinghoff et al., 2000; Sunenshine et al., 2007; Godoy, 2012). In the patient sample, 20 were male (58.8%) and 14 were female (41.2%), supporting reports by Chang et al. (2015) that males have a higher risk of infection than females. Analyzing the principal complaint related to patient admission, nine had hematological or solid neoplasms, eight had suffered trauma, five had neurological diseases, four had skin lesions (of which two were large burns), six had assorted clinical complaints (cardiac, respiratory, renal, digestive, metabolic), and two had been admitted due to post-surgery complications. The average number of days between patient admission and the date the bacteria were isolated was 27 days. As for the hospital sector to which they were admitted, 19 (55.8%) were in the intensive care unit or in the semi-intensive care unit, eight (23.5%) were in the internal medicine clinic, and the rest are summarized in Table 1. All of these results agree with the expected profile for patients afflicted with infection by an opportunistic microorganism of low virulence and multi-resistance to drugs, such as CRAb (carbapenem-resistant *A. baumannii*), that is: older patients, immunosuppressed or gravely ill, debilitated, those with co-morbidities, those with a prolonged hospital stay, and those staying in critical hospital sectors, such as the ICU.

The mortality rate by infections caused by multi-resistant *A. baumannii* is high, with different percentages having been reported in the literature (26-68%) (Kwon et al., 2007; Sunenshine et al., 2007). However, the mortality rate attributed to these infections is hard to measure, considering that afflicted patients usually have other serious diseases and different co-morbidities that may cause a bias in the analysis (Martins and Barth, 2013; Vanegas-Múnera et al., 2014). In this study, the observed mortality rate was 50%. Gulen et al. (2015) reported, in a controlled study, a 53% rate in a group of case patients similar to ours.

Phenotypic analysis by antibiotic sensitivity profile

In our study, the antibiotic sensitivity test performed with the *Acinetobacter* spp revealed a high degree of resistance to the drugs tested, characterizing them as extensively drug-resistant (Falagas and Karageorgopoulos, 2008; Manchanda et al., 2010). Of the isolates, 100% (34/34) were resistant to the following antibiotics: amikacin, ampicillin + sulbactam, cefepime, ceftazidime, ceftriaxone, ciprofloxacine, gentamicin, imipenem, meropenem, piperacillin + tazobactam, sulfamethoxazole + trimethoprim, and tobramycin (Table 2). Of all

the tested antibiotics, sensitivity was only observed to Polymyxin B. These data are equivalent to those found in Brazil by Oliveira dos Santos et al. (2014) and Guimarães (2011).

Table 2. Phenotypic characterization of antibiotic resistance, bla_{OXA-23} gene tracking, and genetic profile analysis of the 34 nosocomial isolates of *Acinetobacter* spp.

Isolate	Isolation date	Sector[a]	Antibiotic resistance profile[b]	bla_{OXA-23} gene	Genetic profile- ERIC-PCR	
					Group	Genotype
ISO 1	11/11/2014	SICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	A
ISO 2	11/21/2014	SU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	N
ISO 3	12/11/2014	SICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	C
ISO 4	12/15/2014	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	P
ISO 5	12/24/2014	T	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	III	U
ISO 6	12/26/2014	O	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	H
ISO 7	01/06/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	J
ISO 8	01/10/2015	O	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	B
ISO 9	01/11/2015	SICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	R
ISO 10	01/15/2015	SU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	B
ISO 11	01/20/2015	MC2	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	P
ISO 12	01/22/2015	SICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	A
ISO 13	01/22/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	I
ISO 14	01/31/2015	SU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	A
ISO 15	02/05/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	S
ISO 16	02/06/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	Q
ISO 17	02/13/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	C
ISO 18	02/14/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	O
ISO 19	02/14/2015	MC2	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	A
ISO 20	02/15/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	T
ISO 21	02/25/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	II	T
ISO 22	02/27/2015	SICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	C
ISO 23	03/16/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	K
ISO 24	03/20/2015	C	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	IV	V
ISO 25	03/24/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	D
ISO 26	03/31/2015	SICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	L
ISO 27	04/01/2015	MC2	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	E
ISO 28	04/06/2015	MC2	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	G
ISO 29	04/14/2015	MC1	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	F
ISO 30	04/13/2015	MC2	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	G
ISO 31	04/11/2015	MC2	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	E
ISO 32	04/15/2015	MC1	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	E
ISO 33	04/18/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	E
ISO 34	04/25/2015	ICU	AMI, ASB, CPM, CAZ, CRO, CIP, GEN, IPM, MER, PPT, SXT, TOB	+	I	M

[a]ICU = intensive care unit; SICU = semi-intensive care unit; SU = surgical unit; T = transplantation; MC1 = medical clinic 1; MC2 = medical clinic 2; C = cardiology; O = others (hospital room). [b]AMI = amikacin; ASB = ampicillin + sulbactam; CPM = cefepime; CAZ = ceftazidime; CRO = ceftriaxone; CIP = ciprofloxacin; GEN = gentamicin; IPM = imipenem; MER = meropenem; PPT = piperacillin + tazobactam; SXT = sulfamethoxazole-trimethoprim; TOB = tobramycin.

PCR identification of the gene bla_{OXA-23}

Resistance to carbapenems in the genus *Acinetobacter* spp is often significantly related to the production of class D β-lactamases (OXA carbapenemases) and, less frequently, to the production of class B β-lactamases (MβL). Resistance related to the loss of porins may also occur, but less frequently (Martins and Barth, 2013). Of the OXA carbapenemase types, the worldwide dissemination of OXA-23 in *A. baumannii* is particularly noteworthy (Cherkaoui et al., 2015). Chang et al. (2015) reported in their study that most *A. baumannii* isolates from China hospital contained OXA-23-like and OXA-51-like carbapenemase genes. In Brazil, *A. baumannii* producing OXA-23 was detected for the first time in the city of Curitiba and spread quickly thereafter (Werneck et al., 2011; Martins et al., 2014). In this study, we tried to

identify the presence of the gene bla_{OXA-23} as a factor responsible for *Acinetobacter* resistance to carbapenems. As a result, all 34 isolates of carbapenem-resistant *Acinetobacter* spp (100%) amplified in the PCR exhibited the expected 1057-base pair fragment corresponding to gene bla_{OXA-23}, related to carbapenem resistance (Figure 1 and Table 2), a result that was expected in agreement with the literature.

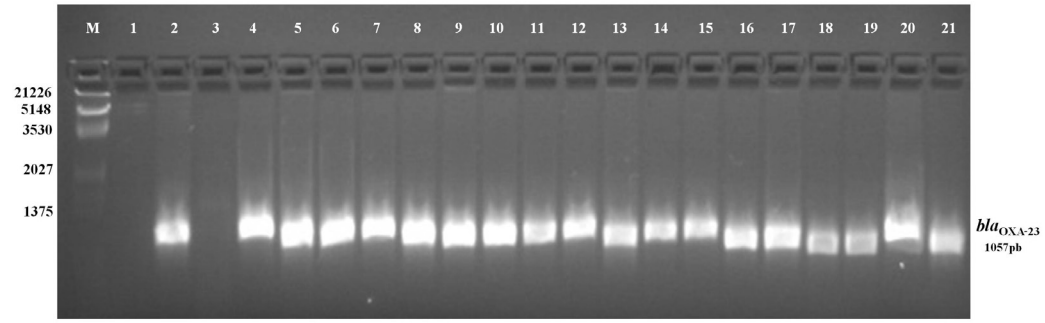

Figure 1. PCR for detection of the bla_{OXA-23} gene among carbapenem-resistant *Acinetobacter* spp nosocomial isolates on 1.5% agarose gel. *Lane M* = Molecular mass marker Lamda *Eco*RI/*Hind*III DNA (Thermo Fisher Scientific); *lane 1* = Negative control (absent DNA). *lane 2* = Positive control (*Acinetobacter baumannii* reference strain carrying bla_{OXA-23} gene). *lane 3* = Negative control (*Escherichia coli* strain BL21DE3+). *lanes 4* to *21* = *Acinetobacter* spp nosocomial isolates (ISO 17 to ISO 34). The size of the Lamda *Eco*RI/*Hin*dIII DNA marker, in base pairs, is indicated to the left. The expected 1057-base pair amplified fragment corresponding to the bla_{OXA-23} gene is designated to the right of the gel.

Genetic profile analysis and clonal relationship among the carbapenem-resistant *Acinetobacter* spp isolates carrying the bla_{OXA-23} gene

Genotypic analysis of the 34 isolates carrying the bla_{OXA-23} gene, with the ERIC-PCR technique, resulting in 21 fragments of different sizes (Figure 2). For band-sharing analysis, bands in the range of 130 to 2000 base pairs were considered. The binary matrix, created by visual observation of the presence or absence of these bands, after submitting to Cluster analysis/Euclidean distance calculation, generated a dendrogram showing the clonal relationship among the isolates. The 34 isolates of carbapenem-resistant *Acinetobacter* spp carrying the bla_{OXA-23} gene were divided into 4 different groups (I, II, III, and IV). In addition, according to the degree of similarity, it was possible to determine 22 different genotypes (A to V), revealing a heterogeneous profile (Figure 3 and Table 2).

However, seven genotypes (IA, IB, IC, IE, IG, IIP, and IIT) had 100% similarity among their isolates. Based on the combined analysis of the genotypes, the data regarding the sector where the patient was staying and the date of the sample collection revealed that the same strain of *Acinetobacter* spp was present in different patients (clone IC = isolates 3 and 22; clone IE = isolates 27 and 31; clone IG = isolates 28 and 30) at the same time and place, suggesting the hypothesis that cross-transmission of the bacteria, from one patient to the other, may have happened. Also regarding the 100% similarity in genetic profile among the isolates, the presence of the same clone (clone IA = isolates 1 and 12; clone IIT = isolates 20 and 21) in patients that had been in the same sector, but during different periods, demonstrated the colonization of that hospital sector with the clones (Table 2 and Figure 3).

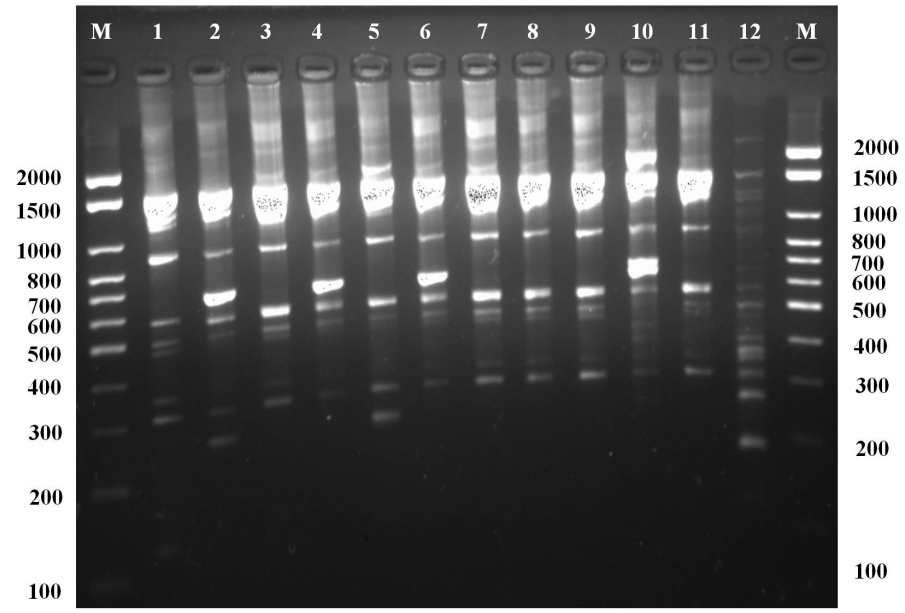

Figure 2. ERIC-PCR for determination of the genetic profile of *Acinetobacter* spp nosocomial isolates containing the bla_{OXA-23} gene on 1.5% agarose gel. *Lane M* = Molecular mass marker 100 base pair DNA (Norgen Biotech). *Lanes 1* to *10* = *Acinetobacter* spp nosocomial isolates positive for the bla_{OXA-23} gene (ISO 25 to ISO 34). *Lane 11* = Isolate 33 repeated. *Lane 12* = Isolate 24. The size of the 100 base pair DNA marker is indicated to the left and to the right of the gel.

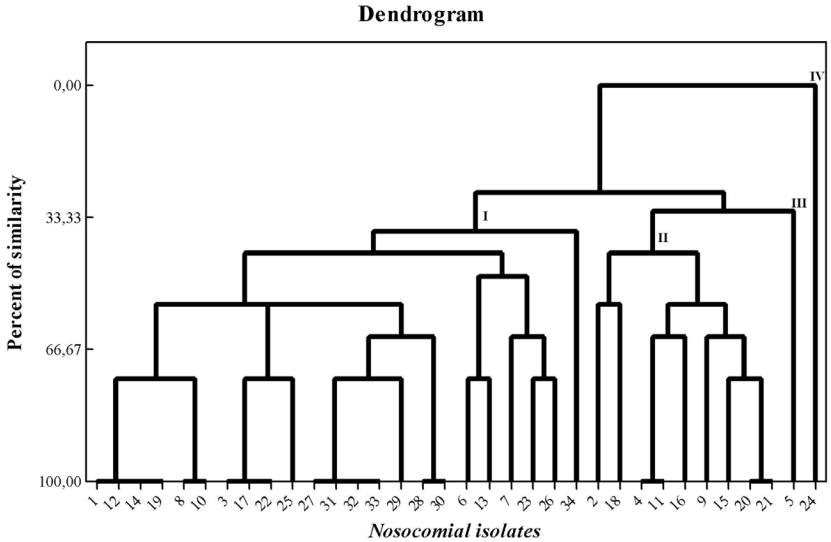

Figure 3. Dendrogram of the genetic relationship of the bla_{OXA-23}-positive *Acinetobacter* spp nosocomial isolates obtained by ERIC-PCR. Nosocomial isolates (ISO 1 to ISO 34) distributed in four groups (I, II, III, and IV) according to degree of similarity are shown.

Profiles with 70% similarity were found among strains of *Acinetobacter* spp isolated from patients that had stayed in the same sector of the hospital, in the same period of time [clone IB (isolate 10) and clone IA (isolate 14); clone IF (isolate 29) and clone IE (isolate 32); clone IIS (isolate 15) and clone IIT (isolate 20)], or in different periods of time [clone ID (isolate 25) and clone IC (isolate 17); clone IIS (isolate 15) and clone IIT (isolate 21)]. With 60% similarity, clones IG (isolates 28 and 30) and clone IE (isolates 27 and 31), clone IIP (isolate 4) and clone IIQ (isolate 16), clone IJ (isolate 7) and clone IK (isolate 23) were present in the same place. Band analysis among these isolates revealed the presence or absence of one, two, or three discordant bands, leading us to the hypothesis of a possible mutation among the isolates.

Isolate 24, belonging to group IV, genotype IVV, was the only one which did not display a clonal relationship with the other isolates of *Acinetobacter* spp carrying the gene $bla_{\text{OXA-23}}$ (Table 2 and Figure 3), and displayed a band profile quite different from the others, possibly corresponding to a species of the *A. baumannii-calcoaceticus* complex other than *A. baumannii.*

Another significant data obtained from the similarity analysis was that 3 sectors of the hospital - general ICU, semi-intensive care unit, and internal medicine clinic, each with very peculiar characteristics, both in relation to the patient profile as well as the physical structure - were the likely places of acquisition/transmission of the bacteria for most of the patients.

Considering the diversity of the resistance genes, and the easy dissemination of *A. baumannii*, the study of the local epidemiology of isolates of *Acinetobacter* spp is of great importance to guide epidemiological control and to determine the best treatment, preventing intra-, or even inter-hospital dissemination of these microorganisms (Ferreira et al., 2011). In this regard, the ERIC-PCR technique emerges as a fast and low-cost alternative method that shows good results for genetic characterization of *Acinetobacter* spp isolates (Ferreira et al., 2011).

In conclusion, this study reported the high prevalence of the gene $bla_{\text{OXA-23}}$ among nosocomial clinic isolates of extensively drug-resistant *Acinetobacter* spp in a hospital in the southeastern region of Brazil. ERIC-PCR analysis allowed us to ascertain the genetic diversity among the isolates, which, albeit with a heterogeneous profile, in some cases displayed 100% clonality. This data lead us to important considerations, as well as to adopt intervention measures at several levels. In revealing cross transmission and environmental colonization in some of the hospital units, they indicate the need to review and improve actions to prevent HCAIs: precautions regarding contact and isolation of patients to reduce the opportunities for cross transmission; improvement of the physical environment and clinical practices, based on adherence to best practices for control of hospital infections; identification of germ sources; and assessment of the quality of the methods adopted for environmental hygiene.

At the same time, the heterogeneous profile found in the samples, indicating the presence of several strains in the hospital environment, suggests a situation of high selective pressure through the use of antibiotics, and therefore, the need to curtail inappropriate use of antibiotics. In that sense, key elements for controlling their use may be: differentiating between colonization and infection, and limiting treatment to cases of infection; and knowledge of the local sensitivity profile of the microorganisms to antibiotics, in order to rationalize their use.

Conflicts of interest

The authors declare no conflict of interest.

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