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Potential Spread of Methicillin-Resistant *Staphylococcus aureus* Recovered from Patients with Bloodstream Infection

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Abstract

Bloodstream infection (BSI) caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide public health problem, and is associated with high morbidity and mortality. Our aim was to evaluate antimicrobial resistant genes, to characterize the Staphylococcal cassette chromosome elements (SCC*mec*) and the genetic diversity of MRSA strains recovered from the BSI of five Hospitals in Belo Horizonte, Brazil. Fifty-six MRSA isolates were identified by the Vitek II system, and by the agar dilution method to determine the minimum inhibitory concentration. Polymerase chain reaction (PCR) was performed to detect coagulase (*coa*), methicillin (*mecA*) aminoglycosides (*aaca-aphD*), macrolides, lincosamides (*ermA/ermB/ermC*) and beta-lactams (*blaz*) genes, as well as chromosomal SCC*mec* type. The genetic diversity was carried out by ribotyping and intergenic repetitive sequences ERIC/PCR analysis. The *mecA* gene was detected in 84% of strains. At least one of the genes was present in the isolates from hospitals studied; the more frequent combinations were *ermA/mecA* and *ermA/ermB/ermC* (78.6% of samples). The *SCCmec* studies have shown that such bacteria may be carriers of the *ermA, ermB and ermC* genes, type III was the most prevalent, followed by subtype IIIa. Ribotyping and ERIC-PCR results showed a variety of MRSA strains and suggest that certain clonal populations are circulating among the hospitals studied for different routes that should be better investigated.

Keywords Methicillin-resistant *Staphylococcus aureus*; Resistance marks; Clonal diversity; SCC*mec*; Bloodstream infection

Introduction

Nosocomial infections by methicillin-resistant *Staphylococcus aureus* (MRSA) are a global challenge to public health due to the spread of bacterial clones, and increased resistance to multiple classes of antimicrobials [1,2]. Among the nosocomial infections caused by *S. aureus*, bloodstream infections (BSI) are highlighted, which are associated with high morbidity and mortality, especially in critical, newborns and immuno compromised patients [1,3].

It is estimated that 250,000 cases of BSI occur per year in the United States, with a high mortality rate [4]. In Europe, BSI account for 54% of nosocomial infections [5]. A study of hospitals in China showed that, from January 2006 to May 2011, 63.6% of *S. aureus* recovered in BSI were MRSA [6]. Most Latin American public hospitals have found 30-50% of BSI related to MRSA [7].The number of nosocomial MRSA infections in Brazil is also high, corresponding to between 40% and 80%. Studies have shown high mortality rates in patients who developed MRSA bacteremia (49% to 55%), with these indices being larger than those involving methicillin-sensitive *S. aureus* (MSSA), with rates of 20% to 32% [8].

The methicillin resistance is associated with the presence of the *mecA* gene, which encodes a protein binding to penicillin, PBP2. This gene is inserted into a mobile genetic element called staphylococcal cassette *mec* (SCC*mec*). Eleven types of SCC*mec* have been described for *S. aureus* based on the class of *mec* complex and *ccr* gene [9].

The azithromycin and erythromycin resistance in bacteria of the genus *Staphylococcus* is often associated with resistance to other macrolides. Studies have shown that such bacteria may be carriers of the *ermA, ermB* and *ermC* genes, which encode methylases [10]. Resistance to lincosamide and streptogramin antibiotics is also attributed to the presence of *ermA* and *ermC* genes [11, 12].

Genes encoding enzymes inactivating aminoglycosides are also frequently seen in *S. aureus.* These genes encode the AAC (6')-APH (2"), an enzyme with 6'-N-acetyltransferase and 2"-O-2 phosphotransferase activities. The presence of this enzyme in pathogenic gram-positive bacteria mediates resistance to gentamycin, tobramycin, netilmicin and amikacin, via chromosomal or plasmid-4 mediated pathways [11,12].

The emergence, in 2002 of resistant strains of *S. aureus* to vancomycin (VRSA) is further aggravating this public health problem; however, due to its high efficacy, vancomycin and teicoplanin are widely used in the treatment of serious infections caused by MRSA [13,14]. Alternative drugs as daptomycin, rifampin or gentamicin, when combined with vancomycin, can improve its effect on infections [14].

Comparative studies using the techniques of random amplified polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus (ERIC-PCR) showed that the ERIC-PCR has a high

discriminatory index for the molecular typing of *S. aureus* compared to RAPD [8,15].

The aim of this study was to characterize the resistance profiles and the clonal diversity of methicillin-resistant *Staphylococcus aureus* lineages, recovered from patients with BSI at different hospitals in Belo Horizonte, state of Minas Gerais, Brazil.

Materials and Methods

Bacterial strains, identification and susceptibility testing

This study evaluated 56 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from blood cultures from December 2008 to June 2009, in five hospitals in of general assistance, emergency and outpatient care, in Belo Horizonte city, Minas Gerais State, Brazil. All samples were previously identified using the GP Card of the bioMerieuxVITEK2® System.

Antimicrobial susceptibility assays

The agar dilution methods was used to determine the minimum inhibitory concentration (MIC) and to study the antimicrobial susceptibility profiles to: oxacillin (OXA), penicillin (PEN) cefoxitin (CFO), vancomycin (VAN), erythromycin (ERI), clindamycin (CLI), gentamicin (GEN), chloramphenicol (CLO), tetracycline (TET), rifampicin (RIF), levofloxacin (LVX), and sulfamethoxazole-trimethoprim (SUT). The antimicrobial agents were obtained by the Sigma Chemical Co. (St. Louis, MO, USA). Results were interpreted according to the critical points recommended by the Clinical and Laboratory Standards Institute/CLSI guidelines [16]. The reference strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 strains were used as controls for all phenotypic tests.

Molecular characterization of the isolates

The bacterial DNA was extracted by the thermal lysis method followed by centrifugation under refrigeration at 4°C for 30 seconds at 9000 g [17,18]. The supernatant DNA was quantified using a nanodrop and stored in the freezer at-70°C until use.

Polymerase chain reaction (PCR) assays were used to detect the presence of the gene encoding coagulase (*coa*), according to Gandre et al. 2005 [19], for *S. aureus* species identification (Table 1). In order to detect resistance genes and pathogenicity markers of *S. aureus,* multiplex-PCR assays (mPCR) were performed for the following genes: *blaZ, mecA, ermA, emrB, ermC,* and *aacA-aphD1*(Table 1), and for the *SCCmec* cassette typing as previously described in methodology (Table 2).

Gene	Phenotype	Primers sequence (5'- 3')	Size in base pairs	Reference
coa	coagulase production	AAT CTT TGT CGG TAC ACG ATATTC TTC ACG CGT AAT GAG ATT TCA GTA GTAAAT ACA ACA	420 bp	[19]
blaZ	resistance to β-lactamics agents	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173 bp	[40]
mecA	resistance to oxacillin	AACAGGTGAATTATTAGCACTTGTA AG ATTGCTGTTAATATTTTTTGAGTTGA A	174 bp	[40]
ermA	resistance to erythromycin and clindamycin	AAGCGGTAAACCCCTCTGA TTCGCAAATCCCTTCTCAAC	190 bp	[11]
emrB	resistance to erythromycin and clindamycin	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142 bp	[40]
ermC	resistance to erythromycin and clindamycin	AATCGTCAATTCCTGCATGT TAATCGTGGAATACGGGTTTG	299 bp	[11]
aacA- aphD1	resistance to aminoglycosi des	TAATCCAAGAGCAATAAGGGC GCCACACTATCATAACCACTA	227 bp	[11]

Table 1: Antibiotic resistance, gene-specific and specific primers used in PCR assays.

PCR assays were performed in a final volume of 25 µl with 12.5 µl of Master Mix (Promega®, Madison, USA), 4 µM of each primers, 100 ng of bacterial DNA and nuclease-free water. All amplification assays were performed according to the conditions described by the author for each primer used (Tables 1 and 2). The amplicons were analyzed on the 2% agarose gel electrophoresis in TBE buffer (2 mM EDTA, 10 mM Trisborate, pH 8.0) at 100 volts for 2 h. The gels were stained with GelRedTM (Biotium Glowing Products for ScienceTM,USA) according to the manufacturer's recommendations.

Characterization of the genetic profile of the isolates

The characterization of the genetic profiles of MRSA strains was performed by ribotyping analysis with primers: G1 (5'-GAAGTCGTAACAAGG-3') and L1 (5'-CAAGGCATCCACCGT-3') using the PCR conditions described by Jensen et al. (1993) [20]. In order to produce genetic fingerprints of *S. aureus*, the ERIC-PCR primers, ERIC1 (5′-7 ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC-2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′), were used in PCR assays under conditions previously described by Versalovic et al. (1991) [21]. PCR products were analyzed by electrophoresis on a 1.5%

gel at 90 volts for approximately 2 h. The banding pattern was used to form a binary matrix 1 (presence) or 0 (absence) of each band and construct a dendrogram in order to determine the genetic relationships between the isolates using numerical taxonomy and multivariate analysis system for PC (NTSYS-pc, version 2.1)/(Exeter Software, New York, NY, USA) by DICE similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA) [22,23].

SCCmec type primers		Sequence (5'-3')	Size in base pairs	Reference
I	CIF2 F2 CIF2R2	TTCGAGTTGCTGATGAAGAAGG ATTTACCACAAGGACTACCAGC	495bp	[27]
II	KDP F1 KDP R1	AATCATCTGCCATTGGTGATGC CGAATGAAGTGAAAGAAAGTGG	284bp	[27]
II - III	MECI P2 MECI P3	ATCAAGACTTGCATTCAGGC GCGGTTTCAATTCACTTGTC	209bp	[27]
I- II-III	DCS F2 DCS R1	CATCCTATGATAGCTTGGTC CTAAATCATAGCCATGACCG	342bp	[27]
III	RIF4 F3 RIF4 F9	GTGATTGTTCGAGATATGTGG CGCTTTATCTGTATCTATCGC	243bp	[27]
III	RIF5 F10 RIF5 R13	TTCTTAAGTACACGCTGAATCG GTCACAGTAATTCCATCAATGC	414bp	[27]
	ISA431P4 Pub110 R1	CAGGTCTCTTCAGATCTACG GAGCCATAAACACCAATAGCC	381bp	[27]
	IS431 P4 Pt181 R1	CAGGTCTCTTCAGATCTACG GAAGAATGGGGAAAGCTTCAC	303bp	[27]
mecA	MECA P4 MECA P7	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	162bp	[27]
V	Tipo V-F Tipo V-R	GAACATTGTTACTTAAATGAGG TGAAAGTTGTACCCTTGACACC	325bp	[27]

Table 2: Used primers and size of the multiplex PCR products expected in the study of *SCCmec* gene in *S. aureus* strains recovered from blood cultures.

Ethical aspects

This study was approved by the Research Ethics Committees of the participating hospitals and the COEP/UFMG (ETIC 614/08).

Results

All 56 Staphylococci isolates evaluated by PCR were confirmed as *S. aureus* by the presence of the gene coa. Considering the agar dilution methods, resistance to cefoxitin and oxacillin were observed in 93% (52/56) of the isolates, respectively. Resistance to the beta-lactam agents OXA, CFO and PEN ranging between 90 and 100%. Regarding ERY and CLIN, the found rates varying from 89% to 100% and 82% to 100% for LEV. The drug concentration able to inhibit 50% and 90% of the samples was high for most classes of antimicrobial used, especially in relation to PEN, CFO, OXA, ERY and CLI, for which higher levels were observed at critical points for all hospitals (Table 3).

HOSPITAL	%				ANTIMICROBIALS* (MIC µg/ml)								
		OXA	CFO	PEN	CLI	CLO	GEN	ERY	LEV	RIF	SMT	TET	VAN
H1 (n=16)	50	512	>128	>128	>256	16	2	>128	4	0.5	<4.75	0.5	1
	90	512	>128	>128	>256	16	16	>128	8	1	9.5	0.5	1
H2(N=5)	50	256	>128	>128	>256	16	4	>128	8	2	<4.75	0.5	1
	90	512	>128	>128	>256	16	4	>128	128	2	>608	4	2
H3(N=13)	50	64	32	>128	>128	16	2	>128	8	<0.25	9.5	4	1

	90	>512	>128	>128	>128	64	64	>128	32	2	38	64	2
H4(N=18)	50	256	>128	>128	>128	16	2	>128	4	<0.25	<4.75	0.5	2
	90	>512	>128	>128	>128	128	>256	>128	32	2	>608	64	4
H5(N=4)	50	256	>128	>128	>128	8	1	>128	8	<0.25	<4.75	4	1
	90	>512	>128	>128	>128	128	>256	>128	32	4	>608	64	1

Table 3: Values of minimum inhibitory concentration (MIC) of antimicrobial agents able to inhibit 50% and 90% of *S. aureus* isolates recovered from hemoculture. *Antimicrobial agents - OXA: Oxacillin; CFO: Cefoxitin PEN: Penicillin; CLI: Clindamycin; CLO: Chloramphenicol; GEN: Gentamicin; ERY: Erythromycin; LEV: Levofloxacin; RIF: Rifampicin; SMT: Sulfamethoxazole-Trimethoprim; TET: Tetracycline; VAN: Vancomycin.

Table 4 shows the correlation between the studied genes and phenotypic MIC results with related antimicrobial agents, where it was observed that all samples were resistant to PEN, and the *blaz* gene was present in 86% of these. Similarly, all strains were resistant to CLI, and 91% showed the *ermA* gene. Although 26.8% were resistant to GEN, the *aac-aphD* gene was present in 43% of isolates. The most frequent genes combination identified in *S.aureus* isolates were *ermA/mecA and ermA/ermB/ermC* (both combinations evidenced in 78.6% of the strains), followed by ermA/blaZ in 76.7% of cases. The simultaneous identification of all genes evaluated occurred in only one strain, H3-35, from the intensive care unit. This strains resistance profile to 70% of the antimicrobials tested.

Phenotypic and genotypic antimicrobial resistance (%)												
Hospitals		**MIC**		**GENES**			**MIC**				**GENES**	
*OXA		CFO	PEN	*mecA*	*blaZ*	ERY	CLIN	GEN	*ermA*	*ermB*	*ermC*	*aacAaphD*
H1 (n=16)	100	100	100	87.5	100	94	100	81	100	0	62.5	62.5
H2 (N=5)	100	100	100	100	100	100	100	0	100	0	80	60
H3(N=13)	76.9	76.9	100	61.5	77	92	100	30.8	69	0	31	23
H2(N=18)	94.5	94.5	100	88.8	77.7	100	100	27.5	94.4	5.5	94.4	33
H3(N=4)	100	100	100	100	75	100	100	75	100	25	75	50
Total (n=56)	92.9	92.9	100	84	86	96.5	100	26.8	91	4	68	43

Table 4: Correlation between susceptibility testing and PCR assays for S. aureus strains from hemoculture by hospitals.*Antimicrobials agents - OXA: Oxacillin; CFO: Cefoxitin PEN: Penicillin; CLI: Clindamycin; CLO: Chloramphenicol; GEN: Gentamicin; ERY: Erythromycin; LEV: Levofloxacin; RIF: Rifampicin; SMT: Sulfamethoxazole-Trimethoprim; TET: Tetracycline; VAN: Vancomycin.

PCR ribotyping and genotypic characterization of *S. aureus* isolates showed different profiles intra-species. The observed fragments measured and strains with similar profiles were grouped into eight groups (patterns 1 through 8) (Table 5) and correlated with the results of tests that are traditionally used for *S. aureus* phenotyping. The fourth pattern (P4) was the most common, appearing in 23 strains of *S. aureus*, with seven fragments in common. Of these isolates, 91.3% were resistant to OXA, 82.6% to CFO and 100% to ERY, CLI and LEV. Regarding the gene research, 78.3 showed the *mecA* and *blaZ* genes, and *aacA-aphD* and *ermB* genes were present in 74% and 82.6%, respectively (Table 5).

The mPCR assays were used to detect the mecA gene and characterize the staphylococcal cassette chromosome mec(*SCCmec*). The presence of a specific fragment of 162 nucleotides corresponding to the *mecA* gene was detected in 84% (47/56) of strains. The mPCR was able to differentiate the types and variants of SCCmec in the isolates positive for the *mecA* genes, showing that type III was present in most of the strains (29/47) in the five participating hospitals. All strains in this group were resistant to PEN and CLI; 97% to OXA, CFO and ERY; 81% to LEV. It was also observed that 97% of these strains carried the *blaz* and *ermA* genes and 87% *mecA* gene.The SCCmec type IIIa was the second most prevalent, being found in 27.6% (13/47) of these strains. In this group, all strains were resistant to OXA, PEN, ERY and CLI; 80% to CFO and 70% to LEV. Most of these strains (90%) carried the *ermA* gene and 80% the *mecA* and *blaz* genes.

In an isolate of H4, type I was found, in two strains of H5 and H4, type Ia was detected, and in three strains of H4, type II and IV were reported (Data not shown).

Groups		**Ribotyping**	**Antimicrobial susceptibility**

Profile	Total (n)	Size of fragments	Phenotypic-MIC (% positive)										Genotypic-MIC (% positive)					
			*OXA	CFO	PEN	ERY	CLI	LEV	GEN	TET	RIF	SMT	*mecA*	*bla Z*	*aacAa phD*	*erm A*	*erm B*	*erm C*
P1	14	705-620-565-480-425	100	100	100	100	100	85.7	7.1	0	0	7.1	92.8	57.1	7.1	100	0	21.4
P2	2	705-565-480-425	100	100	100	100	100	100	50	0	0	0	50	100	100	100	0	0
P3	3	705-620-565-480-390	100	100	100	66.6	100	33.3	33.3	0	0	33.3	100	100	75	100	25	75
P4	23	705-620-565-500-480-425-390	91.3	82.6	100	100	100	69.5	26	21.7	17.4	4.3	78.3	78.3	74	82.6	0	69.3
P5	5	705-620-565-500-390	100	100	100	100	100	100	0	0	0	20	100	60	100	100	0	80
P6	4	705-620-565-500-480-425	100	100	100	100	100	75	50	50	0	50	100	50	100	100	0	100
P7	3	565-500-425-390	100	100	100	33.3	100	66.6	33.3	0	0	33.3	66.6	66.6	100	66.6	33.3	33.3
P8	1	535-425-390	100	100	100	100	100	0	0	0	100	100	100	0	100	100	0	0

Table 5: Comparative analysis between phenotypic and genotypic profiles of S. aureus stains recovered from hemoculture *Antimicrobials agents - OXA: Oxacillin; CFO: Cefoxitin PEN: Penicillin; CLI: Clindamycin; CLO: Chloramphenicol; GEN: Gentamicin; ERY: Erythromycin; LEV: Levofloxacin; RIF: Rifampicin; SMT: Sulfamethoxazole-Trimethoprim; TET: Tetracycline; VAN: Vancomycin.

The profiles of amplification of DNA from isolates H1(16), H2(13), H3(18), H4(5) and H5(4) obtained in gels by ERIC-PCR technique produced, on average, three to seven fragments per *S. aureus* isolate (data not shown). For the analysis of profiles, fragments ranging between 100 bp to 900 bp for all isolates from different hospitals of evaluated patients were considered. The matrix of the DICE similarity coefficient generated a dendrogram constructed with these profiles (Figure 1).

In this analysis, it was showed that two different clonal populations of *S. aureus* are circulating among patients from H1 hospital. The strain distribution was concentrated in the first clade, suggesting that clonal populations (samples H1:1 to13 and 28) have a common ancestor, as the fragments of 100, 180, 240 and 290bp are present in all isolates. However, these were distinguished from each other by the presence or absence of a fragment. Isolates 14 and 15 from H1 were identical, showing a single band of 100bp, but this was different from the others, suggesting the existence of a new clonal population.

Regarding the grouping of H2 hospital isolates, the dendrogram shows three different clonal populations circulating in this hospital; the first population comprised 11 strains (45 to 53 an 55-56), which were grouped with strains from the H1 hospital in the first clade, but in different branches. The isolates 44 and 54 from H2 hospital are in the last clade of the tree, but in different branches. These data suggest a co-movement of these isolates in these two hospitals.

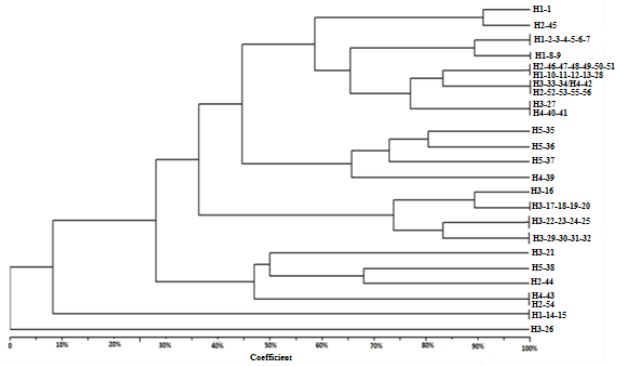

Figure 1: Dendrogram of genetic similarity derived from DICE similarity coefficient showing the relationship between *staphylococcus aureus* strains recovered from blood cultures from different hospitals BH/MG. Construction made using grouping with unweighted pair group method with arithmetic mean (UPGMA)

Considering the grouping of isolates from H3 hospital, the dendrogram showed that strains 33- 34 and 27 were distributed in different branches of the first clade grouped with strains from the H1 hospital, suggesting the co-movement of these strains in two hospitals. Most isolates of H3 were grouped in different branches of third clade, suggesting a common clonal ancestor with bands of 180, 240, 310bp and being separated from each other by the presence or absence of a fragment. In contrast, the third population is composed of isolate 21, in which the 310bp fragment was absent, suggesting that a new clonal population exists. The fourth and new clonal population circulating in

H3 is composed of isolate 26, with a profile of 400, 450, 500 and 600bp fragments, completely different from the others, suggesting the existence of a new clonal population.

The analysis of dendrogram for the five isolated from H4 hospital showed the existence of three clonal populations with the isolates 40-41 and 42 grouped in the first clade with other strains from H1, H2 and H3 hospitals. The isolates 39 and 43 were individually grouped in the second and third clades, respectively.

Regarding the four isolates from hospital H5, the existence of two clonal populations was shown in the dendrogram: one formed by H5 isolates 35-36 and 37 samples, which have a common ancestor, shown by the presence of conserved fragments (240 and 300bp), and being separated from each other by the presence or absence of a fragment; the other comprising sample 38, well differentiated profile from the other, but with pooled isolate 21 of hospital H3, having common fragments of 240, 290 and 600bp, suggesting that it was a clonal population circulating in both hospitals.

Finally, it is important to note that last clade of the dendrogram showed a grouping of isolates from the five hospitals analyzed (H1 to H5), strains 14, 15, 21, 26, 38, 43, 44 and 54, a co-movement of these isolates in all hospitals.

Discussion

Staphylococcus aureus is a versatile pathogen that causes a wide range of human infections and is a major cause of community and nosocomial infections worldwide [6,24]. Due to its pathogenic potential, effective methods are being used for the identification of clinically significant strains [10]. Thus, in this work, classical phenotypic and genotypic methods were used for the determination of antibiotic susceptibility and genes associated with resistance in *S. aureus* strains recovered from bloodstream infection in five hospitals in Belo Horizonte city, as well to characterize the clone circulating in these institutions.

The results obtained in the present study showed high rates of resistance to oxacillin and cefoxitin. The drug concentration able to inhibit 50% and 90% of the strains was high for most of the classes of antimicrobial drugs used, especially with regard to penicillin, cefoxitin, oxacillin, erythromycin and clindamycin, which have been observed at higher levels than critical points in all strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are associated with considerable morbidity and mortality, as well as high costs of treatment [25]. In addition to their worldwide dissemination, MRSA strains represent a major challenge in the treatment of nosocomial infections, not only because they carry a resistance mechanism that confers protection against all β- lactam antimicrobials [26,27], but because they may also promote resistance to other classes of antimicrobials, such as macrolides, aminoglycosides, tetracycline, rifampin, and quinolones [9].

In Brazil, the increased incidence of *S. aureus* with intermediate resistance to vancomycin profile (VISA) has been reported [28]. *S. aureus* strains with intermediate or full resistance to vancomycin profile were not found in the present study. However, due to the increasing use of this antimicrobial agent in recent years, the isolation of these variants is expected, and should be monitored thoroughly.

Molecular methods were used for the detection of aminoglycosides (*aaca-aphD*), macrolides, lincosamides (*ermA, ermB, ermC*) and beta-lactams (*blaZ*) resistance genes, as well as presence of chromosomal *SCCmec* type characterization and genetic diversity of the MRSA strains included in this study. mPCR assays showed the presence of them in isolates from patients in all of the hospital units studied; 84% of the strains presented the *mecA* gene, 86% *blaz*, 91% *ermA* and *ermB*, 68% *ermC* and 43% *aac-aphD*.

The *SCCmec* typing is one of the most important tools in studies of the epidemiology of MRSA and their clonal relationships [29]. In the present study, we evaluated the presence of variants *SCCmec* in all isolates, and the type III was identified in most of them. *SCCmec* type IIIa was the second most prevalent. The data found are in agreement with those obtained in Brazil, which indicate the prevalence of *SCCmec* III in MRSA strains isolated from hospitals [30-35].

The results of this study show that all isolates harboring *SCCmec* III were PEN and CLI resistant. It is important to note that MRSA strains harboring *SCCmec* types I, II or III often have simultaneous resistance to multiple classes of antimicrobial agentes [36]. On the other hand, the MRSA strains with *SCCmec* types IV, V, VI, are often more susceptible to macrolides, quinolones, tetracyclines, trimethoprim, sulfamethoxazole, and lincosamides [36-38].

The elements *SCCmec* types I, II, III, VI and VIII are generally associated with hospitals. Types IV, V or VII have been widely disseminated among community samples of MRSA, called CA-MRSA. Despite these differences, there were few epidemic MRSA clones found in the hospital environment, which reinforces the theory that successive genomic alterations led to the evolution of only those clones that had a better combination of factors, including antimicrobial resistance, virulence and transmissibility [32,39]. The resistance of *S. aureus* remains a worrying problem in Latin America. This resistance can vary widely between hospitals, even within the same Country. Because of this, active epidemiological surveillance systems, such as SENTRY, have incorporated techniques of molecular typing and resistance genotyping, providing additional information that is useful to understanding the pathogenicity of microorganisms worldwide [7].

Ribotyping, which was used for genotypic characterization of isolates of *S. aureus*, showed a significant degree of intra-species variation, with eight patterns (P1 to P8) of resistance profiles being observed. P3 was the most standard and it was found that all were resistant to OXA and CFO had the mecA gene. Thus, the results of the PCR assay with specific primers correlated with the results of the phenotypic antibiotic resistance determination.

ERIC-PCR assays allowed the clonal distribution of *S. aureus* isolates from the different hospitals researched, showing the variety of methicillin-resistant strains that are circulating among patients in these hospitals, disseminated by different routes, such as via health professionals and patients transferred between hospitals. The ERIC-PCR method has been shown to be valuable for the identification of genetically similar but not identical organisms [34].

It is emphasized that the results obtained in this study are in agreement with those in the literature, since molecular epidemiological studies of *S. aureus* in humans have shown populations that consist of several prevalent lineages [35]; this is best illustrated by the Hospital- and Community-acquired methicillin-resistant S. aureus strains where specific clonal complexes are dominant [25]. Thus, nosocomial infections by MRSA continue to pose a major challenge to physicians and microbiologists, due to the spread of bacterial clones with reduced sensitivity against various classes of antimicrobials.

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References

1. Otto M (2010) Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. Annu Rev Microbiol 64: 143-162.
2. Malachowa N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, et al. (2011) Global changes in *Staphylococcus aureus* gene expression in human blood. PLoS One 6: e18617.
3. Wang LX, Hu ZD, Hu YM, Tian B, Li J, et al. (2013) Molecular analysis and frequency of *Staphylococcus aureus* virulence genes isolated from bloodstream infections in a teaching hospital in Tianjin, China. Genet Mol Res 12: 646 - 654.
4. National Nosocomial Infections Surveillance System (2004) National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2001. Am J Infect Control 32: 470-485.
5. Cosgrove SE, Fowler VG Jr (2008) Management of methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis 46 Suppl 5: S386-393.
6. Rong C, Zhong-Qiang Y, Dan F, Yan-Ping L, Lei-Li W, et al. (2012) Nosocomial bloodstream infection in patients caused by *Staphylococcus aureus*: drug susceptibility, outcome, and risk factors for hospital mortality. Chin Med J 2: 226 - 229.
7. Sader HS, Gales AC, Pfaller MA (2004) SENTRY antimicrobial surveillance program report: Latin American and Brazilian results for 1997 through 2001. Braz J Infect Dis 8: 25-79.
8. Rossi F (2011) The challenges of antimicrobial resistance in Brazil. Clin Infect Dis 52: 1138-1143.
9. Zong Z, Peng C, Lü X (2011) Diversity of SCC*mec* elements in methicillin-resistant coagulase-negative staphylococci clinical isolates. PLoS One 6: e20191.
10. Flui AC, Carpaii N, Majoor EA, Bonten MJ, Willems RJ (2013) Shared reservoir of *ccrB* gene sequences between coagulase-negative staphylococci and methicillin-resistant *Staphylococcus aureus*. J Antim Chem 68: 1707-1713.
11. Strommenger B, Kettlitz C, Werner G, Witte W (2003) Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J Clin Microbiol 9: 4089 - 4094.
12. Saderi H, Emadi B, Owlia P (2011) Phenotypic and genotypic study of macrolide, lincosamide and streptogramin B (MLSB) resistance in clinical isolates of *Staphylococcus aureus* in Tehran, Iran. Med Sci Monit 17: BR48-53.
13. Deresinski S (2009) Vancomycin in combination with other antibiotics for the treatment of serious methicillin-resistant *Staphylococcus aureus* infections. Clin Infect Dis 49: 1072 -1079.
14. Howden BP, Davies JK, Johnson PDR, Timothy P, Stinear TP, et al. (2010) Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev 23: 99-139.
15. Yingwang Y, Qihuan J, Qingping W, Jumei Z, Lu J, et al. (2012) The characterization and comparison of *Staphylococcus aureus* by antibiotic susceptibility testing, enterobacterial repetitive intergenic consensus–polymerase chain reaction, and random amplified polymorphic DNA–polymerase chain reaction. Foodb Pathog Dis 9: 168-171.
16. CLSI - Clinical and Laboratory Standards Institute (2013) Performance standards for antimicrobial susceptibility testing; 23th informational supplement. CLSI M100-S20U. Clinical and Laboratory Standards Institute, Wayne, PA.
17. Mendes RE, Castanheira M, Pignatari ACC, Gales AC (2006) Metalo-ß-lactamases. J Bras Patol Med Lab 42: 103-113.
18. Martins HS, Bomfim MR, França RO, Farias LM, Carvalho MA, et al. (2014) Resistance markers and genetic diversity in Acinetobacter baumannii strains recovered from nosocomial bloodstream infections. Int J Environ Res Public Health 11: 1465-1478.
19. Gandre EA, Silva JA, Macedo MRP, Araújo MR, Mata MM, et al. (2005) Differentiation between *Staphylococcus aureus*, S. intermedius and S. hyicus using phenotypical tests and PCR. Alim Nutr16: 99-103.
20. Jensen MA, Webster JA, Straus N (1993) Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Appl Environ Microbiol 4: 945-952.
21. Versalovic J, Koeuth T, Lupski JR (1991) Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 19: 6823-6831.
22. Rohlf FJ (1990) NTSYS Numerical taxonomy and multivariate analysis system, version 1.60; Exeter Software 1990. New York, NY, USA.
23. Iorio NL, Caboclo RF, Azevedo MB, Barcellos AG, Neves FP (2012) Characteristics related to antimicrobial resistance and biofilm formation of widespread methicillin-resistant *Staphylococcus* epidermidis ST2 and ST23 lineages in Rio de Janeiro hospitals, Brazil. Diagn Microbiol Infect Dis 72: 32–40.
24. Jamrozy DM, Fielder MD, Butaye P, Coldham NG (2012) Comparative genotypic and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* ST398 isolated from animals and humans. PLoSOne 7: 7 e40458.
25. Crisóstomo MI, Westh H, Tomasz A (2001) The evolution of methicillin resistance in *Staphylococcus aureus*: Similarity of genetic backgrounds in historically early methicillin susceptible and resistant isolates and contemporary epidemic clones. Proc Natl Acad Sci U S A 17: 9865-9870.
26. Oliveira GA, Dell'Aquila AM, Masiero RL, Levy CE, Gomes MS, et al. (2001) Isolation in Brazil of nosocomial *Staphylococcus aureus* with reduced susceptibility to vancomycin. Infect Control Hosp Epidemiol 22: 443-448.
27. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM (2005) Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 10: 5026-5033.
28. Lowy FD (2003) Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest 111: 1265-1273.
29. Amaral MM, Coelho LR, Flores P, Souza RR, Silva-Carvalho MC, et al. (2005) The predominant variant of the Brazilian epidemic clonal complex of methicillin-resistant *Staphylococcus aureus* has an enhanced ability to produce biofilm and to adhere to and invade airway epithelial cells. J Infect Dis 192: 801-810.
30. Vivoni AM, Diep BA, Magalhães ACG, Santos KR, Riley LW, et al. (2006) Clonal composition of *Staphylococcus aureus* isolates at a Brazilian University Hospital: identification of international circulating lineages. J Clin Microbiol 44: 1686 -1691.
31. De Miranda OP, Silva-Carvalho MC, Ribeiro A, Portela F, Cordeiro RP, et al. (2007) Emergence in Brazil of methicillin-resistant *Staphylococcus aureus* isolates carrying SCCmec IV that are related genetically to the USA800 clone. Clin Microbiol Infect 13: 1165-1172.
32. Shorr AF (2007) Epidemiology of *staphylococcal* resistance. Clin Infect Dis 45 Suppl 3: S171-176.
33. Costa MCS, Carvalho MAR, Cisalpino EO, Damasceno CAV, et al. (1986) Epidemiological aspects of *Staphylococcus* isolated in the Hospital das Clínicas in UFMG: determination of antimicrobial sensitivity standards. Rev Bras Med 4: 92-99.

34. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, et al. (2003) How clonal is Staphylococcus aureus? J Bacteriol 185: 3307-3316.

35. Deurenberg RH, Stobberingh EE (2008) The evolution of *Staphylococcus aureus*. Infect Genet Evol 8: 747-763.

36. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, et al. (2003). Comparison of community and health care-associated methicillin resistant *Staphylococcus aureus* infection. JAMA 290: 2976-84.

37. Cocchi P, Cariani L, Favari F, Lambiase A, Fiscarelli E, et al. (2011). Molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in Italian cystic fibrosis patients: a national overview. J Cyst Fibros 6: 407-11.

38. Zhang K, Mc Clure JA, Elsayed S (2009) Novel staphylococcal cassette chromosome mec type, tentatively designated type VIII, harboring class A mec and type 4 ccr gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 53: 531- 540.

39. Martineau F, Picard FJ, Lansac N, Menard C, Roy PH, et al. (2000) Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrobial Agents and Chemotherapy 44: 231-238.