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 (SCCmec) 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 SCCmec 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 ermR, ermB and ermC genes, type III was the most prevalent, followed by subtype IIa. 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; SCCmec; 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, PB2. This gene is inserted into a mobile genetic element called staphylococcal cassette mec (SCCmec). Eleven types of SCCmec 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 ermR, ermB and ermC genes, which encode methylases [10]. Resistance to lincosamide and streptogramin antibiotics is also attributed to the presence of ermR 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-de-O-2 phosphotransferase activities. The presence of this enzyme in pathogenic gram-positive bacteria mediates resistance to gentamicin, tobramycin, netilmicin and amikacin, via chromosomal or plasmid-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...
Materials and Methods

Bacterial strains, identification and susceptibility testing

This study evaluated 56 strains of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) isolated from blood cultures from December 2008 to June 2009, in five hospitals in 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 \textit{Staphylococcus aureus} ATCC 25923 and \textit{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 minutes 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 (\textit{coa}), according to Gandre et al. 2005 [19], for \textit{S. aureus} species identification (Table 1). In order to detect resistance genes and pathogenicity markers of \textit{S. aureus}, multiplex-PCR assays (mPCR) were performed for the following genes: \textit{blaZ}, \textit{mecA}, \textit{ermA}, \textit{ermB}, \textit{ermC}, and \textit{aacA-aphD1} (Table 1), and for the \textit{SCCmec} cassette typing as previously described in methodology (Table 2).

Characterization of the genetic profile of the isolates

The characterization of the genetic profile 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 \textit{S. aureus}, the ERIC-PCR primers, ERIC1 (5'-ATGTAAGCTCCTGGGAGATAC-3') and ERIC-2 (5'-AAGTAAGTGACTGGGGGTAGCG-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% 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.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
<th>Primers sequence (5’-3’)</th>
<th>Size in base pairs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{coa}</td>
<td>coagulase production</td>
<td>AAT CTT TGT CGG TAC ACG ATATTC TTC ACG</td>
<td>173 bp</td>
<td>[19]</td>
</tr>
<tr>
<td>\textit{blaZ}</td>
<td>resistance to β-lactam agents</td>
<td>ACTTCAACACCTGCTTTC TGACCACCTTTATCGCAACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{mecA}</td>
<td>resistance to oxacillin</td>
<td>AACAGGTGAATTATTAGCACCTTGTA AG ATTGCTGTTAATATTTTTTGAGTTGA A</td>
<td>174 bp</td>
<td>[40]</td>
</tr>
<tr>
<td>\textit{ermA}</td>
<td>resistance to erythromycin and clindamycin</td>
<td>AAGCGGTAAACCCCTCTGA TTCGCAAATCCCTTCTCAAC</td>
<td>190 bp</td>
<td>[11]</td>
</tr>
<tr>
<td>\textit{ermB}</td>
<td>resistance to erythromycin and clindamycin</td>
<td>CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTTGTTTGTGAAGAATT</td>
<td>142 bp</td>
<td>[40]</td>
</tr>
<tr>
<td>\textit{ermC}</td>
<td>resistance to erythromycin and clindamycin</td>
<td>AATCGTCAATTCCTGACATGT TAATCGTGGAATACGGGTTTG</td>
<td>299 bp</td>
<td>[11]</td>
</tr>
<tr>
<td>\textit{aacA-aphD1}</td>
<td>resistance to aminoglycosides</td>
<td>TAATCCAAGAAGCAATAGGGGC GCCACACTATCATACCCACTA</td>
<td>227 bp</td>
<td>[11]</td>
</tr>
</tbody>
</table>
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].

<table>
<thead>
<tr>
<th>SCCmec type primers</th>
<th>Sequence (5'-3')</th>
<th>Size in base pairs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CIF2 F2, CIF2R2</td>
<td>TCGAGTTGCTGATGAAGAAGG ATTACCAACAGGCTGACACC</td>
<td>495bp</td>
</tr>
<tr>
<td>II</td>
<td>KDP F1, KDP R1</td>
<td>AATCATCTGGCAATGAGTATGC CGATGAGGAGGAAAGTGG</td>
<td>284bp</td>
</tr>
<tr>
<td>II - III</td>
<td>MECI P2, MECI P3</td>
<td>ATCGACCTTGCATTCCAGGC GCAGTTTCAATTCACTTGTCC</td>
<td>209bp</td>
</tr>
<tr>
<td>I- II-III</td>
<td>DCS F2, DCS R1</td>
<td>CATCCTATGAATGGTATGTC CTATATCATTGAACTGACCG</td>
<td>342bp</td>
</tr>
<tr>
<td>III</td>
<td>RIF4 F3, RIF4 F9</td>
<td>GTGATTGTTGCGATATGTTGG CGCTATTGCTGATATTCTGCC</td>
<td>243bp</td>
</tr>
<tr>
<td>III</td>
<td>RIF5 F10, RIF5 R13</td>
<td>TTCTTAAGTACACCTGTAATCG GTCCACGTTACCTAATGCG</td>
<td>414bp</td>
</tr>
<tr>
<td>ISA431P4, Pub110 R1</td>
<td>CAGGTCATTTCCAGATTCCAGG GAGGCTACAAACCAGGACCC</td>
<td>381bp</td>
<td></td>
</tr>
<tr>
<td>ISA431 P4, P181 R1</td>
<td>CAGGTCATTTCCAGATTCCAGG GAAGATGGGAGGAACTTAC</td>
<td>303bp</td>
<td></td>
</tr>
<tr>
<td>mecA</td>
<td>MEC A4, MEC P7</td>
<td>TCCAGATTACACCTCGACAGG CCACCTCATCATTGTAAGC</td>
<td>162bp</td>
</tr>
<tr>
<td>V</td>
<td>Tipo V-F, Tipo V-R</td>
<td>GAACATTTCTCTCAAATGAGG TGAAAGTTGCAACGTTTGTACCC</td>
<td>325bp</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>HOSPITAL</th>
<th>%</th>
<th>OXA MIC (µg/ml)</th>
<th>CFO MIC (µg/ml)</th>
<th>PEN MIC (µg/ml)</th>
<th>CLI MIC (µg/ml)</th>
<th>CLO MIC (µg/ml)</th>
<th>GEN MIC (µg/ml)</th>
<th>ERY MIC (µg/ml)</th>
<th>LEV MIC (µg/ml)</th>
<th>RIF MIC (µg/ml)</th>
<th>SMT MIC (µg/ml)</th>
<th>TET MIC (µg/ml)</th>
<th>VAN MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1 (n=16)</td>
<td></td>
<td>50 512</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;256</td>
<td>16</td>
<td>2</td>
<td>&gt;128</td>
<td>4</td>
<td>0.5</td>
<td>&lt;4.75</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>512</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;256</td>
<td>16</td>
<td>16</td>
<td>&gt;128</td>
<td>8</td>
<td>1</td>
<td>9.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>H2(N=5)</td>
<td></td>
<td>50 256</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;256</td>
<td>16</td>
<td>4</td>
<td>&gt;128</td>
<td>8</td>
<td>2</td>
<td>&lt;4.75</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>512</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;256</td>
<td>16</td>
<td>4</td>
<td>&gt;128</td>
<td>128</td>
<td>2</td>
<td>&gt;608</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>H3(N=13)</td>
<td></td>
<td>50 64</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;256</td>
<td>16</td>
<td>2</td>
<td>&gt;128</td>
<td>8</td>
<td>&lt;0.25</td>
<td>9.5</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
S. aureus, with seven fragments in common. Of these isolates, 91.3% showed the mecA gene and 90% 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 strain’s resistance profile to 70% of the antimicrobials tested.

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 strain’s resistance profile to 70% of the antimicrobials tested.

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).
isolates. However, these were distinguished from each other by the data not shown. For the analysis of identical, showing a single band of 100bp, but this was between 100 bp to 900 bp for all isolates from different movement of these isolates in these two hospitals.

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 ancestor.

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 H1 were identical, showing a single band of 100bp, but this was different from the others, suggesting the existence of a new clonal ancestor.

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

<table>
<thead>
<tr>
<th>Profile</th>
<th>Total (n)</th>
<th>Size of fragments</th>
<th>Phenotypic-MIC (% positive)</th>
<th>Genotypic-MIC (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>OXA</em> CFO PEN ERY CLI LEV GEN TET RIF SMT mecA bla Z aacAa phD erm A erm B erm C</td>
<td></td>
</tr>
<tr>
<td>P1 14</td>
<td>705-620-565-480-425</td>
<td>100 100 100 100 100 85.7 7.1 0 0 7.1</td>
<td>92.8 57.1 7.1 100 0 21.4</td>
<td></td>
</tr>
<tr>
<td>P2 2</td>
<td>705-565-480-425</td>
<td>100 100 100 100 100 50 0 0 0 0 50 100 100 100 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3 3</td>
<td>705-620-565-480-390</td>
<td>100 100 100 66.6 100 33.3 33.3 0 0 33.3</td>
<td>100 100 75 100 25 75</td>
<td></td>
</tr>
<tr>
<td>P4 23</td>
<td>705-620-565-500-480-425-390</td>
<td>100 100 100 100 100 95.3 26 21.7 17.4 4.3</td>
<td>78.3 78.3 74 82.6 0 69.3</td>
<td></td>
</tr>
<tr>
<td>P5 5</td>
<td>705-620-565-500-390</td>
<td>100 100 100 100 100 0 0 0 0 20</td>
<td>100 60 100 100 0 80</td>
<td></td>
</tr>
<tr>
<td>P6 4</td>
<td>705-620-565-500-480-425</td>
<td>100 100 100 100 100 75 50 50 0 0 50</td>
<td>100 100 50 100 0 100</td>
<td></td>
</tr>
<tr>
<td>P7 3</td>
<td>565-500-425-390</td>
<td>100 100 100 100 33.3 100 66.6 33.3 0 0 33.3</td>
<td>66.6 66.6 100 66.6 33.3 33.3</td>
<td></td>
</tr>
<tr>
<td>P8 1</td>
<td>535-425-390</td>
<td>100 100 100 100 100 0 0 0 100 100 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

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)
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 a 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, tetracyclines, 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 (aac(6)-Iβ), 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 more 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 agents [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 genotypically 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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