Molecular and phenotypic characteristics of methicillin-resistant Staphylococcus aureus isolated from hospitalized patients

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Abstract
Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is one of the leading causes of infections acquired in both community and hospital settings. In this study, MRSA isolated from different sources of hospitalized patients was characterized by molecular and phenotypic methods.

Methodology: A total of 123 S. aureus isolates were characterized according to their genetic relatedness by repetitive element sequence based-PCR (REP-PCR), in vitro antimicrobial susceptibility profile, SCCmec typing and presence of seven virulence factor-encoding genes. Results: REP-PCR fingerprinting showed low relatedness between the isolates, and the predominance of one specific lineage or clonal group was not observed. All isolates were susceptible to teicoplanin and linezolide. All isolates were resistant to cefoxitin and penicillin, and the majority were also resistant to one or more other antimicrobials. Fifty isolates (41.7%) were intermediately resistant to vancomycin. Most isolates harbored SCCmec type II (53.7%), followed by type I (22.8%), type IV (8.1%) and type III (1.6%). All isolates harbored at least two virulence factor-encoding genes, and the prevalence was as follows: coa, 100%; icaA, 100%; hla, 13.0%; hlb, 91.1%, hld, 91.1%; lukS-PV and lukF-PV, 2.4%; and tst, 34.1%. A positive association with the presence of hla and SCCmec type II, and tst and SCCmec type I was observed.

Conclusion: This study showed the high virulence potential of multidrug-resistant MRSA circulating in a teaching hospital. A high prevalence of MRSA showing intermediate vancomycin resistance was also observed, indicating the urgent need to improve strategies for controlling the use of antimicrobials for appropriate management of S. aureus infections.

Key words: antimicrobial resistance; intermediate vancomycin resistance; MRSA; SCCmec typing; virulence factors.


(Received 08 September 2014 – Accepted 16 December 2014)

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Introduction

Staphylococcus aureus can be found as a harmless colonizer of skin and mucosa, mainly the anterior nares, in 20%-60% of the population [1-3]. Colonization by this bacterium is one important predisposing factor for staphylococcal infections [1]. In fact, as an opportunistic pathogen, S. aureus can cause diseases ranging from superficial skin and soft tissue infections to life-threatening disseminated infections [4]. Currently, S. aureus is one of the leading causes of healthcare-associated infection worldwide. Most importantly, a substantial proportion of staphylococcal infections are caused by methicillin-resistant S. aureus (MRSA), which also exhibits resistance to several other antimicrobials [5,6], contributing to its persistence as a human pathogen for decades.

The acquisition of the mecA gene, which encodes a penicillin-binding protein (PBP) with low affinity for the antimicrobial, called PBP2’ or PBP2a, is the most common mechanism of methicillin-resistance. The gene mecA is inserted into a mobile genetic element (MGE) known as staphylococcal cassette chromosome mec (SCCmec), and currently, eleven types (SCCmec I to XI) have been described in MRSA strains isolated from various sources [7-10].
The virulence potential of \textit{S. aureus} is extensive, with it being represented by both structural and secreted products, whose encoding genes are mostly located in MGEs, which also contribute to bacterial genome plasticity and evolution [11]. This diverse array of virulence factor-encoding genes facilitates the adhesion of bacterial cells to biotic or abiotic surfaces, resistance to host defenses, invasion and cell injury [12]. The differential expression of these genes may enhance \textit{S. aureus} virulence, enabling the bacterium to cause specific clinical presentation [13]. Furthermore, the virulence of this bacterium may vary between isolates from different geographical regions [14].

Continuous efforts to understand the biological basis of MRSA antimicrobial resistance and virulence are therefore necessary. This knowledge may contribute not only to the adoption of effective measures to control the infection, but also to the development of new anti-infective drugs that inhibit bacterial growth plus control virulence [12,15].

In this study, MRSA strains isolated from different sources of patients seen at the University Hospital of Londrina, Paraná, Brazil were characterized by phenotypic and molecular methods. The \textit{in vitro} antimicrobial susceptibility profile, genetic relatedness and occurrence of virulence genes \textit{icaA} from the intercellular adhesion \textit{locus} (encoding N-acetylmuramyltransferase), \textit{hla}, \textit{hld} and \textit{hld} (encoding α-, β- and δ-hemolysin, respectively), \textit{lukS-PV} and \textit{lukF-PV} (encoding the β-pore-forming Panton-Valentine leukocidin) and \textit{tst} (encoding toxic shock syndrome toxin) were also evaluated.

\section*{Methodology}

\textbf{Microorganisms}

The University Hospital of Londrina, Paraná, Brazil is a 353-bed tertiary care center that serves the city of Londrina, in addition to several localities in the states of Paraná, São Paulo, and Mato Grosso do Sul. This is the major referral center for the Sistema Único de Saúde (a governmental healthcare assistance program) of northern Paraná. A total of 123 non-duplicate \textit{S. aureus} isolates from patients seen in this hospital between June 2010 and June 2013 were randomly taken from the bacterial collection of the Laboratory of Clinical Microbiology of Universidade Estadual de Londrina (UEL). The isolates were classified according to CDC definitions of healthcare-associated infections [16]. The study protocol was approved by the Ethics Committee (CAAE no. 3346.0.000.268.09/protocol 186/09 CEP-UEL) and all assays were carried out in the Laboratory of Molecular Biology of Microorganisms, Department of Microbiology, UEL. Bacterial isolates were identified to the species level by standard phenotypic methods on the basis of colony morphology, Gram staining, catalase, DNase and mannitol fermentation after growing in Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 5% sheep blood at 37°C for 24 hours. Bacteria were kept at -80°C in tryptone soya broth (TSB, Oxoid) containing 30% glycerol. Species identification was also performed by a polymerase chain reaction (PCR)-based method using specific primers for \textit{coa} gene (encoding coagulase) regions according to Tiwari et al. (2008) [17].

\section*{Antimicrobial susceptibility pattern}

Bacterial isolates were tested for antimicrobial susceptibility to cefoxitin (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), linezolid (10 μg), oxacillin (1 μg), penicillin (10 U), rifampicin (5 μg), sulfamethoxazole-trimethoprim (23.75/1.25 μg), teicoplanin (30 μg) and tetracycline (30 μg) using the disk-diffusion assay. Cefoxitin and oxacillin were used to define methicillin-resistant \textit{S. aureus} (MRSA) isolates. The minimal inhibitory concentration (MIC) for vancomycin was determined by the broth-dilution method. MIC was determined as 100% growth inhibition. Both methods were performed and interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2013) [18]. \textit{S. aureus} ATCC 29213 and \textit{Enterococcus faecalis} ATCC 51299 were used as controls.

\section*{DNA extraction}

A single bacterial colony was transferred to 3 mL TSB and incubated at 37°C for 24 hours. The bacterial pellets obtained after centrifugation at 10,000 x g for 5 minutes were washed once with sterile 0.15 M phosphate-buffered saline (PBS), pH 7.2, resuspended in 300 μL sterile solution (10 mM Tris-HCl and 1 mM EDTA, pH 8.0, and 1.0 mg/mL lysozyme). Genomic DNA was extracted following the procedure described by Ausubel et al. (1991) [19], and a 2-μL aliquot was used in all amplification reactions.

\section*{MRSA typing}

The identification of SCC\textit{mec} type of all MRSA isolates was performed by multiplex PCR assay as described by Milheiro et al. (2007) [20]. Non-typeable isolates were designated NT.NCTC10442 (type I), N315 (type II), 85/2082 (type III) and 81/108 (type IV) strains were used as control. The genetic
relatedness of all MRSA was analyzed by repetitive element sequence based-PCR (REP-PCR) using the primer RW3A as described by Del Vecchio et al. (1995) [21]. Banding patterns were categorized using the UPGMA algorithm and Jaccard coefficient [22] of the Bionumerics v.4.6 software (Applied Mathematics, Kortrijk, Belgium), with the band tolerance set at 3% and the threshold cutoff value set at 85%.

Detection of virulence factor-encoding genes

The detection of nucleotide sequences corresponding to genes encoding virulence factors of *S. aureus* was performed by PCR. The genes *icaA*, *lukS-PV* and *lukF-PV* and *tst* were amplified as described by Campbell et al. (2008) [23]. The genes *hla*, *hlf* and *hld* were amplified as described by Jarraud et al. (2002) [24]. All PCRs were performed in a Veriti 96-well thermal cycler (Applied Biosystems, Waltham, Massachusetts, USA), and reactions without any template DNA were carried out simultaneously as negative control.

Statistical analysis

The categorical variables were compared with the chi-square test or Fisher’s exact test using the BioEstat Software version 5.3. *P* values less than 0.05 were considered significant.

Results

### Table 1. Relationship between phenotypic antimicrobial resistance profile and SCC*mec* types of MRSA isolates.

<table>
<thead>
<tr>
<th>Group</th>
<th>Antimicrobial resistance profile</th>
<th>SCC<em>mec</em> - Number of isolates (%)</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
<th>NT</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>FOX, P</td>
<td></td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
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<td>FOX, OX, P</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
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<td>FOX, OX, P, SXT</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>V</td>
<td>FOX, P, CN, SXT</td>
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<td>1</td>
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<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
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<td>VII</td>
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<td></td>
<td>1 (3.6)</td>
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<td></td>
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<tr>
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<td>3 (10.7)</td>
<td>47</td>
<td></td>
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<tr>
<td>IX</td>
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<td>1</td>
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<tr>
<td>X</td>
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<td>22 (78.6)</td>
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<td>XI</td>
<td>FOX, OX, P, CIP, DA, E, RD</td>
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<td>8</td>
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<td></td>
<td></td>
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<tr>
<td>XVII</td>
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<tr>
<td>XVIII</td>
<td>FOX, OX, P, CIP, DA, E, TE, SXT, RD</td>
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<td>1</td>
<td>2</td>
<td></td>
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<td></td>
<td>100</td>
</tr>
</tbody>
</table>

*Among the total isolates in each group; *Among all isolates analyzed. FOX, cefoxitin; OX, oxacillin; P, penicillin; E, erythromycin; STX, sulphamethoxazole-trimethoprim; CN, gentamycin; CIP, ciprofloxacin; DA, clindamycin; RD, rifampicin; TE, tetracyclin.

Patients, MRSA identification and genotyping

The age of the patients enrolled in this study ranged from three months to 87 years (median of 50 years old), and the majority of them were men (n = 86, 70.0%). The isolates were recovered from various clinical sources as follows: blood (n = 43, 35.0%), tissue fragment (n = 21, 17.1%), general discharge (n = 18, 14.6%), tracheal aspirates (n = 15, 12.2%), central venous catheter line (n = 10, 8.1%), urine (n = 7, 5.7%) and general swab (n = 9, 7.3%). In 17 patients (13%), MRSA infection was identified less than 48 hours after hospital admission. All isolates harbored the gene *coa* and exhibited resistance to cefoxitin, although seven (5.7%) isolates were classified as susceptible to oxacillin.

Cluster analysis and visual observation of bands generated by REP-PCR typing revealed low relatedness between the isolates. By using a cutoff value of 95% and 85% similarity, a total of 65 different genotypes were respectively identified among the isolates, indicating their high diversity. To compare the REP-PCR pattern with other features of MRSA, those isolates showing 65% similarity were clustered in the same group, and the analysis of the dendrogram resulted in 17 different genotypes, named A to Q. The groups with similar REP-PCR profiles consisted of 50 (E, 40.7%), 16 (F, 13.0%), 14 (H, 11.4%), 13 (A, 10.6%), 8 (I, 6.5%), 5 (B, 4.1%) and 4 (G, 3.3%) isolates each. Three genotypes (D, M, N, 28 (22.8)) were more frequently observed in our study.
1.6% each) consisted of two isolates each. The other seven (5.6%) isolates had unique banding profiles.

**Phenotypic and genotypic characterization of antimicrobial susceptibilities**

The *in vitro* susceptibility patterns of MRSA to various antimicrobials are given in Table 1. All isolates were susceptible to teicoplanin and linezolid. Besides being resistant to cefoxitin, all isolates showed resistance to penicillin. MRSA isolates were distributed into four SCCmec types, according to the multiplex PCR used in this study, and type II (66/123, 53.7%) was the most frequent, followed by type I (28/123, 22.8%), type IV (10/123, 8.1%) and type III (2/123, 1.6%). Seventeen (13.8%) isolates were classified as NT (Table 1).

Concerning the phenotypic resistance profile, the MRSA isolates were classified into 18 groups (Table 1). Three isolates were resistant only to β-lactam antimicrobials, and harbored the SCCmec type IV. Most isolates (50/123, 40.7%) displayed the group VIII phenotypic antimicrobial resistance profile (cefoxitin, oxacillin, penicillin, ciprofloxacin, clindamycin, and erythromycin) and harbored the SCCmec type I (3/50) and type II (47/50). Thirty (24.4%) out of 123 isolates were clustered in group X (cefoxitin, oxacillin, penicillin, ciprofloxacin, gentamycin, clindamycin, and erythromycin) and harbored the SCCmec types I (22/30), II (3/30), IV (3/30) and NT (2/30). Most isolates classified as NT were those with resistance to a greater number of antimicrobials.

Most isolates were susceptible to vancomycin (73/123, 59.4%), and among them 1.4% (1/73), 13.7% (10/73) and 84.9% (62/73) had MIC values of 0.25, 1.0 and 2.0 µg/mL, respectively. For all isolates, the MIC50 and MIC90 values were 2.0 and 4.0 µg/mL, respectively. Fifty (40.7%) isolates out of 123 isolates were intermediately resistant to vancomycin, according to CLSI (2013) criteria. Among these, 92% (46/50) and 8% (4/50) of isolates showed MIC values of 4 and 8 µg/mL, respectively. However, patients with intermediate MIC values did not have higher in-hospital mortality than those with susceptible MIC values.

**Relationship between SCCmec types and REP-PCR genotyping**

Eighteen MRSA SCCmec type I (18/28, 64.3%) isolates were grouped in genotype E, and four (14.3%) and two (7.1%) in genotypes F and H, respectively. One isolate each (3.6%) was grouped in genotypes I, I.

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**Figure 1.** REP-PCR fingerprinting pattern and SCCmec types obtained from 123 methicillin-resistant *Staphylococcus aureus* isolates from different sources of patients seen at the University Hospital of Londrina.

Cluster analysis was performed using UPGMA algorithm and Jaccard coefficient of the Bionumerics v. 4.6 software, with band tolerance set at 3% and threshold cutoff value set at 85%. SCCmec typing was performed by multiplex PCR assay as described by Milheiro et al. (2007). All *S. aureus* isolates were resistant to cefoxitin. NT: non-typeable; BL: blood; TF: tissue fragment; GD: general discharge; TA: tracheal aspirates; CVCL: central venous catheter line; UR: urine; GS: general swab.
K, L, and P. The SCCmec type II isolates were mainly distributed into genotype E (28/66, 42.4%), followed by genotype H (11/66, 16.7%), F (8/66, 12.1%), I (6/66, 9.1%) and G (4/66, 6.0%). Two isolates each (3.0%) were grouped in genotypes M and N, and one isolate each (1.5%) in genotypes A, B, C, O and Q. One isolate each of SCCmec type III displayed the genotypes A and B. Three isolates each (30%) of SCCmec type IV were grouped in genotypes A, and the other isolates were distributed into genotypes B (2/17, 11.8%), D (2/17, 11.8%), E (1/17, 5.9%), F (1/17, 5.9%) and I (1/17, 5.9%) (Figure 1).

Detection of virulence factor-encoding genes

The presence of the genes coa, icaA, hla, hlb, hld, lukS-PV, lukF-PV and tst in MRSA isolates was detected by PCR. The overall prevalence was as follows: coa, 100%; icaA, 100%; hla, 13.0%; hlb, 91.1%; hld, 91.1%; lukS-PV and lukF-PV, 2.4%; and tst, 34.1%, and no significant association with the origin of the isolates was observed (Table 2). All isolates harbored at least two virulence markers (Table 3), and a significant association with the presence of hla gene and the SCCmec type II (p < 0.005), and tst and SCCmec type I (p < 0.001) was observed. Although no statistical correlation was found, it was also observed that: a) most isolates (59/123, 48.0%) harbored the combination of coa, icaA, hlb and hld genes, which were distributed into SCCmec types II (46/66, 69.7%), III (2/2, 100%), IV (1/10, 10%) and NT (10/17, 58.8%); b) isolates with the gene hla harbored the SCCmec types I, II, IV and NT; c) the presence of PVL-encoding genes was detected only in isolates belonging to SCCmec type IV. A larger number of isolates may corroborate these findings.

Discussion

In this study, MRSA isolates harboring four SCCmec types (I, II, III and IV) were identified. The predominance of one specific lineage or clonal group (as assigned by REP-PCR fingerprinting) was not

<table>
<thead>
<tr>
<th>Table 2. Distribution of virulence encoded genes according to the origin of 123 methicillin-resistant Staphylococcus aureus isolates from hospitalized patients of University Hospital of Londrina.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical source</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Blood (n=43)</td>
</tr>
<tr>
<td>Tissue fragment (n=21)</td>
</tr>
<tr>
<td>General discharge (n=18)</td>
</tr>
<tr>
<td>Tracheal aspirates (n=15)</td>
</tr>
<tr>
<td>Venous central catheter line (n=10)</td>
</tr>
<tr>
<td>Urine (n=7)</td>
</tr>
<tr>
<td>General swab (n=9)</td>
</tr>
<tr>
<td>Total (%)*</td>
</tr>
</tbody>
</table>

*Among the total isolates in each group; *Among all isolates analyzed. coa: coagulase; icaA: intercellular adhesion locus encoding N-acetylglucosaminyltransferase; hla: α-hemolysin, hlb: β-hemolysin; hld: δ-hemolysin; tst: toxic shock syndrome toxin; PVL: lukS-PV and lukF-PV of the β-pore-forming Panton-Valentine leucocidin. -: absence.

<table>
<thead>
<tr>
<th>Table 3. Methicillin-resistant Staphylococcus aureus isolates harboring clusters of virulence encoded genes according to their SCCmec typing.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virulence markers*</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>coa, icaA</td>
</tr>
<tr>
<td>coa, icaA, hla, tst</td>
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<tr>
<td>coa, icaA, hlb, hld</td>
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<tr>
<td>coa, icaA, hlb, hld, PVL</td>
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<tr>
<td>coa, icaA, hla, hlb, hld</td>
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<tr>
<td>coa, icaA, hlb, hld, tst</td>
</tr>
<tr>
<td>coa, icaA, hla, hlb, hld, tst</td>
</tr>
<tr>
<td>Total (%)</td>
</tr>
</tbody>
</table>

*Among the total isolates in each group; *Among all isolates analyzed. coa: coagulase; icaA: intercellular adhesion locus encoding N-acetylglucosaminyltransferase; hla: α-hemolysin, hlb: β-hemolysin; hld: δ-hemolysin; tst: toxic shock syndrome toxin; PVL: lukS-PV and lukF-PV of the β-pore-forming Panton-Valentine leucocidin.
observed, indicating that many different strains circulated in the hospital during the period analyzed here. These SCCmec types were also detected in MRSA surveys conducted in different Brazilian hospitals. However, the prevalence of SCCmec types varied between different regions [3,25-31]. The Brazilian epidemic clone (BEC) carrying the SCCmec type III has been the predominant clone in several hospitals in Brazil [28,29,32]. However, in this study, a prevalence of SCCmec type II was observed, as in Caiaffa-Filho et al. (2013) [30], who detected a high prevalence of this SCCmec type in a tertiary care teaching hospital in São Paulo, Brazil.

Not surprisingly, most MRSA strains harboring the SCCmec types I, II and III were resistant to more than three other non-β-lactam antimicrobial classes. Among SCCmec type IV isolates, most were susceptible to almost all non-β-lactam antimicrobials, which is a common feature of these strains. However, four isolates harboring SCCmec type IV showed resistance to more than three antimicrobial classes. This study also detected NT-SCCmec isolates, and except for one, all displayed resistance to a greater number of antimicrobial classes. Of note was a high proportion of MRSA strains showing intermediate resistance to vancomycin, one of the last therapeutic choices for the treatment of invasive infections. In fact, glycopeptide treatment failure and poor clinical outcomes have been reported in infections caused by vancomycin-intermediate S. aureus [33].

Besides being resistant to many antimicrobial agents, S. aureus possesses a number of virulence determinants, which makes it highly adaptive and versatile. The expression of the virulence factor-encoding genes seems likely to be dependent on the site of infection [34,35]. In this study, the genes coa,icaA, hlb and hld were highly conserved among MRSA isolates, independently from the clinical origin. Coagulase plays a role in intravascular coagulation, facilitating the aggregation of S. aureus in blood, which in turn promotes bacterial survival [35]. The gene icaA encodes the transmembrane protein N-acetylglucosaminyl transferase, which is involved in the biosynthesis of polysaccharide intercellular adhesin (PIA). This adhesin promotes cellular aggregation during the maturation stage of PIA-dependent biofilm formation by S. aureus [36]. The gene hld encodes the δ-Hemolysin (Hld or δ-toxin), a cytotoxic protein of phenol-soluble modulins family [37] that can also contribute in structuring and detachment stages of biofilm development [38]. The gene hlb encodes a magnesium-dependent sphingomyelin-specific phospholipase called β-hemolysin (Hlb), which can lyse erythrocytes, neutrophils and lymphocytes [39].

The pore-forming hemolysin-α (Hla or α-toxin), encoded by hla gene is virtually produced by all strains [34]. However, this gene was detected in only 13% of the isolates in this study, and its presence was strongly associated with the SCCmec type II-harbor ing isolates. The mechanism of cell lysis mediated by Hla is dependent on the initial interaction with a specific receptor on host membranes, often targeting a particular cell type [40]. It can damage epithelial cells, fibroblast, erythrocytes, lymphocytes, monocytes and macrophages, but not neutrophils [41]. The expression of Hla contributes to the pathogenesis of sepsis, skin infections and pneumonia in murine experimental infection [35,40,42]. Furthermore, the role of this exotoxin during biofilm formation in vitro and in a mucosal model of S. aureus infection has been shown elsewhere [43,44].

It has been previously shown that SCCmec type IV harboring the gene encoding the bi-component PVL was characteristic of community-acquired MRSA, which is a highly virulent strain [26,31]. However, PVL has also been detected in other SCCmec strains [45]. This pore-forming toxin, whose genes lukF-PV and lukS-PV are lysogenic bacteriophage-encoded (MGE), can lyse macrophages, monocytes and neutrophils and cause tissue necrosis [46]. PVL has been associated with S. aureus skin and soft tissue infections, and necrotizing pneumonia and septic shock [35,47]. In this study, the PVL-encoding genes were detected in three SCCmec type IV-harboring isolates, and except for one that showed resistance to erythromycin, the other two isolates were susceptible to all non-β-lactam antimicrobials analyzed here. In addition, in two patients who were outpatients, MRSA infection was identified less than 48 hours after hospital admission, and none was using medical devices during the time of cultures. Altogether, these data indicate that these two cases may be epidemiologically characterized as community-associated MRSA infection [10].

The tst gene, which encodes the pyrogenic toxic shock-syndrome toxin 1 (TSST-1), is present in only a small number of S. aureus strains, and this can be explained by its genomic location in a pathogenicity island (MGE). In this study, a low prevalence of tst gene was also observed among MRSA isolates, and its presence was associated with SCCmec type I. TSST-1, a non-cytolytic toxin, is a member of superantigen (SAgs) family, which induces a potent activation of T
cell and macrophages with large production of cytokines that can interfere with the host immune responses. In addition, the expression of tsf or other SAg has been associated with enhanced susceptibility to endotoxic shock syndrome development, with high mortality rates [48].

Limitations of this study, which may reduce generalization of the results, are a) the number of isolates of each clinical source; b) the evaluation of a single hospital; and c) the presence or absence of a particular virulence factor encoding gene being evaluated only by PCR.

Conclusion
This study showed the high virulence potential of multidrug-resistant MRSA circulating in our hospital. Besides, a high prevalence of MRSA showing intermediate vancomycin resistance was observed. These results corroborate the importance of continuous monitoring of antimicrobial susceptibility profiles and potential virulence of S. aureus. In addition, they will contribute to improving strategies for controlling the use of antimicrobials for appropriate management of infections caused by this bacterium.

Acknowledgements
This study was supported by grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)–PROAP, Programa de Pós-Graduação em Microbiologia da Universidade Estadual de Londrina. C.F. Oliveira was awarded a student scholarship from CAPES. We thank Dr. A. Leyva for English editing of the manuscript.

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Conflict of interests: No conflict of interests is declared.