Disseminated Clonal Complex 5 (CC5) methicillin-resistant Staphylococcus aureus SCCmec type II in a tertiary hospital of Southern Brazil

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the leading causes of human infections worldwide, with major dominant lineage circulating in particular geographical regions. The Brazilian Epidemic Clone (BEC, SCCmec III, ST 239) has been predominant in most Brazilian hospitals. Here, we report the prevalence of MRSA SCCmec type II exhibiting different STs, most of them belonging to CC5 in a tertiary hospital in Southern Brazil.

KEYWORDS: MRSA. Multi-drug resistance. PVL. Toxic shock syndrome toxin. Methicillin-resistant Staphylococcus aureus.

Staphylococcus aureus can asymptptomatically colonize various human body sites. However, it is also an important human pathogen that causes a wide diversity of infections, ranging from minor skin and soft tissue infection to life-threatening conditions. This bacterium has developed numerous mechanisms of antimicrobial resistance, limiting the treatment options for staphylococcal infections¹. The acquisition of the staphylococcal cassette chromosome mec (SCCmec), carrying the mec (A or C) genes and the site-specific cassette chromosome recombinase (ccr) genes (ccrAB or/and ccrC), plays a pivotal role in the antimicrobial resistance of S. aureus. The mec genes encode a specific penicillin-binding protein (PBP2a or PBP2'') with significantly lower affinity to β-lactams. In addition, multiple antibacterial-resistant and heavy metal-resistant encoding genes can be inserted into this cassette by site-specific recombination¹.

Currently, methicillin-resistant S. aureus (MRSA) is responsible for a high proportion of staphylococcal infections in both community and hospital settings². Particularly in Latin America, MRSA is highly prevalent in hospitals and circulating MRSA clones can vary according to the geographic regions². Remarkably, the Brazilian Epidemic Clone (BEC, ST 239, SCCmec III), first detected in Brazil in the 1990s³, has been predominant in most Brazilian hospitals⁴. However, changes in the population structure of MRSA have also been reported in some hospitals in this country. Caiaffa-Filho et al.⁵ reported the prevalence of MRSA harboring SCCmec type II in a hospital of Sao Paulo. The USA800/Pediatric (ST5, SCCmec IV) and USA400/ MW2/WA-1 (ST1, SCCmec IV) were the most predominant MRSA lineages in five hospitals of Rio de Janeiro⁶. On the other hand, the USA100 (formerly designated as New York/Japan clone/ST5/ CC5/ SCCmec II) associated with multidrug resistance was the predominant MRSA lineage in a military hospital.
whereas polyclonal and non-multidrug resistant MRSA isolates were detected in a teaching hospital of Rio de Janeiro\textsuperscript{7}. Arias et al.\textsuperscript{2} reported prevalence higher than 80% of USA100 in three Brazilian hospitals located in the cities of Sao Paulo and Porto Alegre.

The University Hospital of Londrina (UHL) is a teaching hospital and a major referral center in the North of Parana State, Brazil, for the Sistema Unico de Saude (SUS), a governmental public health system. This is a 313-bed tertiary care center that serves the city of Londrina, besides about 250 localities of Parana State and more than 100 cities from other States, mainly in Sao Paulo, Mato Grosso, Mato Grosso do Sul and Rondonia. The number of MRSA isolates detected in this hospital has increased over the years. Here, we report the prevalence of MRSA CCS/SCCmec II in the UHL.

A total of 59 non-duplicate MRSA isolates isolated from inpatients with diagnosis of bloodstream and respiratory tract infections during 2015-2016 were taken from the bacterial collection of the Clinical Microbiology Laboratory of UHL.

Ethics Committee on Research Involving Human Beings of Universidade Estadual de Londrina (CAAE Nº 78657317.0.0000.5231 CEP-UEL) approved the study protocols. The isolates were recovered from tracheal aspirates (n=44) and blood (n=15). Species identification was based on the phenotypic profile generated by the VITEK\textsuperscript{®} 2 Compact using VITEK\textsuperscript{®} 2 GP ID card and OBSERVA\textsuperscript{®} Integrated Data Management software version 04.03 (bioMérieux- Durham, NC, USA). At the same time, Gram staining, catalase, DNase and mannitol fermentation were also determined. Antimicrobial susceptibility for cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), ciprofloxacin (1 µg), sulfamethoxazole-trimethoprim (23.75/1.25 µg), rifampicin (5 µg), linezolide (10 µg) and tigecycline (15 µg) was determined by disk diffusion assay according to Clinical Laboratory Standards Institute (CLSI)\textsuperscript{6}. The susceptibility breakpoints used were those recommended by the CLSI\textsuperscript{8}, except for tigecycline that was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)\textsuperscript{9}. The minimum inhibitory concentration (MIC) of vancomycin was determined by Etest\textsuperscript{®} (ABBiodisk, Solna, Sweden). Cefoxitin was used to define MRSA phenotypically\textsuperscript{6}.

All isolates were susceptible to rifampicin, sulfamethoxazole-trimethoprim, linezolide and tigecycline. All isolates were resistant to oxacillin, erythromycin, clindamycin and ciprofloxacin, except one that exhibited resistance only to oxacillin. Resistance to gentamicin was detected in two isolates. The vancomycin MIC ranged from 0.5 to 2.0 µg/mL. The mechanism of methicillin resistance is mediated by the meca gene for most isolates (52/59, 88.1%), as judged by the results of multiplex-PCR assay\textsuperscript{10}. Seven (11.9%) isolates did not harbor the meca gene.

Besides the synthesis of modified PBP2a mediated by meca gene, other mechanisms may be related to methicillin resistance in S. aureus: a) the presence of divergent meca homologue known as mecC (formerly meca\textsubscript{1GA255} gene) that was detected in MRSA isolates isolated from animals and humans\textsuperscript{11}; b) the presence of a plasmid-encoded mecB gene that has recently been described in one MRSA recovered from surveillance cefoxitin-based nasal screening in a German hospital\textsuperscript{12}; c) point mutations in genes encoding PBP 1, 2 and 3, resulting in amino acid substitutions in the transpeptidase domains of these proteins\textsuperscript{13}; d) hyperproduction of beta-lactamase that partially hydrolyses penicillinase-resistant penicillin, such as methicillin\textsuperscript{14}.

By using the multiplex-PCR described by Milheiro\textsuperscript{so et al.}\textsuperscript{10}, meca harboring MRSA isolates (n=52) were distributed into four SCCmec types. Remarkably, the predominance of SCCmec type II (34/52, 65.4%) was observed among the MRSA isolates. Eight (15.4%) isolates each were classified as SCCmec type I and IV. One (1.9%) isolate harbored the SCCmec type III and one (1.9%) was untypeable. We used the High-Resolution Melting (HRM) analysis of Single Nucleotide Polymorphisms (SNPs), as described by Lilliebridge et al.\textsuperscript{14} to further investigate the clonal relatedness among SCCmec type II MRSA. Twelve different sequence types (STs) were detected, which include ST5 (n=3), ST6 (n=2), ST9 (n=2), ST27 (n=2), ST53 (n=2), ST63 (n=7), ST99 (n=2), ST306 (n=3), ST445 (n=5), ST835 (n=4), ST1307 (n=1) and ST1502 (n=1). Based on STs similarities, these MRSA were clustered into five clonal complexes (CC) (Table 1). The majority of MRSA SCCmec II isolates belonged to CC5 (52/59, 67.6%), which has been commonly associated with human infections worldwide\textsuperscript{15,16}. Recent studies of our research group have reported the predominance of MRSA harboring SCCmec II elements isolated from different clinical sources in the UHL during 2010 to 2013. Prevalences of 53.7% (66 out of 123 MRSA isolates) and of 43.6% (24 out of 55 MRSA isolates) were described by Oliveira et al.\textsuperscript{16} and Bodnar et al.\textsuperscript{17}, respectively. In both studies, most isolates were also resistant to erythromycin, clindamycin and ciprofloxacin. In contrast to our results, both studies reported MRSA isolates showing intermediate resistance to vancomycin. Of note, there was a substantial proportion of MRSA harboring meca gene exhibiting MIC values equal or higher than 1.5 µg/mL for vancomycin. Although these isolates are reported to be susceptible, data from the literature suggest that patients with MRSA infections presenting these MIC values respond poorly to vancomycin\textsuperscript{1}. 

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We also investigated the presence of genes *lukS*-PV and *lukF*-PV and *tst-1* in SCCmec type II MRSA by PCR as described previously. Seven SCCmec type II isolates (20.6%) harbored the PVL-encoding genes, which contrast with the results of de Oliveira et al. that detected these genes only in MRSA SCCmec type IV (3/123, 2.4%). The *lukS*-PV and *lukF*-PV encode the β-pore-forming cytotoxic Panton-Valentine leukocidin (PVL), a secreted toxin that has been associated with *S. aureus* skin and soft tissue infections, necrotizing pneumonia and septic shock. These genes are encoded by bacteriophages, which can contribute for their dissemination among MRSA strains. In contrast, none MRSA SCCmec type II harbored the *tst-1* gene in this study, whereas this gene was detected in 5.7% (7 out

| Isolate | CCa | STa | PVLb | tst-1b | Antibiotypec | Y eard | Sourcee | Vancomycin µg/mLb |
|---------|-----|-----|------|--------|--------------|-------|---------|------------------|
| 110     | 7   | 306 | -    | -      | II           | 2015  | T       | 1.5              |
| 138     | 5   | 835 | +    | -      | II           | 2015  | T       | 2.0              |
| 160     | 7   | 306 | +    | -      | II           | 2015  | T       | 1.5              |
| 165     | 5   | 63  | +    | -      | II           | 2015  | T       | 1.5              |
| 175     | 80  | 1502| -    | -      | II           | 2015  | T       | 2.0              |
| 176     | 5   | 835 | +    | -      | II           | 2015  | T       | 2.0              |
| 177     | 5   | 63  | -    | -      | II           | 2015  | T       | 1.5              |
| 185     | 5   | 63  | -    | -      | II           | 2015  | T       | 1.5              |
| 193     | 5   | 63  | -    | -      | II           | 2015  | B       | 2.0              |
| 209     | 5   | 5   | -    | -      | II           | 2015  | T       | 1.5              |
| 211     | 5   | 835 | +    | -      | I            | 2015  | B       | 1.5              |
| 215     | 5   | 63  | -    | -      | II           | 2015  | B       | 2.0              |
| 219     | 5   | 63  | -    | -      | II           | 2015  | T       | 1.5              |
| 265     | 7   | 306 | -    | -      | II           | 2015  | T       | 2.0              |
| 332     | 5   | 835 | +    | -      | II           | 2015  | T       | 2.0              |
| 352     | 5   | 6   | -    | -      | II           | 2015  | T       | 1.5              |
| 353     | 5   | 27  | -    | -      | II           | 2015  | T       | 1.5              |
| 356     | 5   | 9   | -    | -      | II           | 2015  | T       | 2.0              |
| 369     | 5   | 27  | -    | -      | II           | 2015  | T       | 1.5              |
| 374     | 5   | 9   | +    | -      | II           | 2015  | B       | 1.5              |
| 411     | 5   | 6   | -    | -      | II           | 2015  | T       | 2.0              |
| 413     | 5   | 99  | -    | -      | II           | 2015  | T       | 1.5              |
| 415     | 5   | 5   | -    | -      | II           | 2015  | B       | 1.5              |
| 419     | 5   | 63  | -    | -      | II           | 2015  | T       | 1.5              |
| 437     | 5   | 5   | -    | -      | II           | 2015  | T       | 2.0              |
| 453     | 5   | 1307| -    | -      | II           | 2016  | T       | 2.0              |
| 465     | 1290| 53  | -    | III    | 2016        | T     | 1.0     |
| 483     | 445 | 445 | -    | II     | 2016        | T     | 2.0     |
| 484     | 1290| 53  | -    | II     | 2016        | B     | 2.0     |
| 492     | 445 | 445 | -    | II     | 2016        | T     | 2.0     |
| 577     | 445 | 445 | -    | II     | 2016        | T     | 1.5     |
| 578     | 445 | 445 | -    | II     | 2016        | T     | 1.5     |
| 631     | 5   | 99  | -    | II     | 2016        | B     | 2.0     |
| 657     | 445 | 445 | -    | II     | 2016        | T     | 1.5     |

aCC: Clonal Complex and ST: Sequence Typing were determined as described by Lilliebridge et al.; bPVL: Panton Valentine Leukocidin encoding gene and *tst-1*: Toxic Shock Syndrome Toxin encoding gene were detected as described in Oliveira et al.; cAntimicrobial resistance profile was determined by disk-diffusion assay according to the CLSI guidelines; I: OXA; II: OXA, ERI, CLI, CIP; III: OXA, ERI, CLI, CIP, GN; OXA: Oxacillin, CLI: Clindamycin, ERI: Erithromycin, CIP: Ciprofloxacin; GN: Gentamicin; dYear of isolation; eSource of isolation, T: Tracheal secretion, B: Blood; fMIC vancomycin determined by E-test; - Absence; + Presence.
of 123) of MRSA harboring the same SCCmec elements in our previous study. The tst-1 gene encodes the toxic shock syndrome toxin (TSST-1), a member of bacterial superantigen family, which induces a massive activation of monocytes/macrophages and T lymphocyte host cells, leading to a potentially fatal toxic shock syndrome. This gene is located in a mobile pathogenicity island which uses bacteriophage-mediated transfer for its mobilization. The contribution of a horizontal gene transfer for the bacterial adaptation in a determined niche has been well-studied. However, the role of gene loss in bacterial fitness has received less attention, thus further studies are needed to understand this phenomenon.

The relatively small number of isolates, the samples origin (tracheal aspirates and blood) and detection of two virulence-encoding genes by PCR are the limitations of our study, which may reduce the generalization of results. Nevertheless, this study reports the predominance of MRSA SCCmec type II exhibiting different STs, most of which belong to CC5 in UHL. The SCCmec type II remained relatively stable over the five years period studied by our research group; however, an increase in the presence of PVL- and the absence of TSST-encoding genes were detected among these strains. Due to the mobility of MGEs, new patterns of antibiotic resistance and virulence can emerge, and then continuous surveillance of S. aureus and of these traits are crucial for the development of preventive and therapeutic approaches for the treatment of infections caused by this bacterium. Corroborating this, a recent study of Wang et al. showed that CC5 isolates, SCCmec type II and erythromycin resistance are independent risk factor associated with 30-day mortality of patients with MRSA infections. In addition, a higher risk of death has been reported in patients with pneumonia caused by PVL-positive S. aureus, compared to non-PVL producing isolates.

CONFLICT OF INTERESTS

The authors report no conflict of interests.

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AUTHORS’ CONTRIBUTIONS

FCD: contributed in all methodological activities and analysis and interpretation of data; TD, MAGR and ERT: performed the microbiological experiments and analyzed the data; LMY: interpretation of data and critical revision of the manuscript; SFYO and MREP: conception, design, analysis and interpretation of data. All authors read and approved the final manuscript.

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