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Disseminated Clonal Complex 5 (CC5) methicillin-resistant *Staphylococcus aureus* SCC*mec* 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, SCC*mec* III, ST 239) has been predominant in most Brazilian hospitals. Here, we report the prevalence of MRSA SCC*mec* 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 asymptomatically 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 infections[1]. The acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*), 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 recombination[1].

Currently, methicillin-resistant *S. aureus* (MRSA) is responsible for a high proportion of staphylococcal infections in both community and hospital settings[2]. Particularly in Latin America, MRSA is highly prevalent in hospitals and circulating MRSA clones can vary according to the geographic regions[2]. Remarkably, the Brazilian Epidemic Clone (BEC, ST 239, SCC*mec* III), first detected in Brazil in the 1990s[3], has been predominant in most Brazilian hospitals[4]. 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 MRSAs harboring SCC*mec* type II in a hospital of Sao Paulo. The USA800/ Pediatric (ST5, SCC*mec* IV) and USA400/ MW2/WA-1 (ST1, SCC*mec* IV) were the most predominant MRSA lineages in five hospitals of Rio de Janeiro[6]. On the other hand, the USA100 (formerly designated as New York/Japan clone/ST5/ CC5/ SCC*mec* 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[7]. Arias *et al*.[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 CC5/SCC*mec* II in the UHL.

A total of 59 non-duplicate MRSAs 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® 2 Compact using VITEK® 2 GP ID card and OBSERVA® 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 (5 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)[8]. The susceptibility breakpoints used were those recommended by the CLSI[8], except for tigecycline that was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[9]. The minimum inhibitory concentration (MIC) of vancomycin was determined by *Etest*® (ABBiodisk, Solna, Sweden). Cefoxitin was used to define MRSA phenotypically[8].

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 to 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[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 $mecA_{LGA251}$ gene) that was detected in MRSAs isolated from animals and humans[1]; 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[11]; c) point mutations in genes encoding PBP 1, 2 and 3, resulting in amino acid substitutions in the transpeptidase domains of these proteins[12]; d) hyperproduction of beta-lactamase that partially hydrolyses penicillinase-resistant penicillin, such as methicillin[13].

By using the multiplex-PCR described by Milheiriço *et al*.[10], *mecA* harboring MRSA isolates (*n*=52) were distributed into four SCC*mec* types. Remarkably, the predominance of SCC*mec* type II (34/52, 65.4%) was observed among the MRSA isolates. Eight (15.4%) isolates each were classified as SCC*mec* type I and IV. One (1.9%) isolate harbored the SCC*mec* 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*.[14] to further investigate the clonal relatedness among SCC*mec* type II MRSAs. 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 MRSAs were clustered into five clonal complexes (CC) (Table 1). The majority of MRSA SCC*mec* II isolates belonged to CC5 (*n*=23, 67.6%), which has been commonly associated with human infections worldwide[2,15].

Recent studies of our research group have reported the predominance of MRSA harboring SCC*mec* II elements isolated from different clinical sources in the UHL during 2010 to 2013. Prevalences of 53.7% (66 out of 123 MRSAs isolates) and of 43.6% (24 out of 55 MRSAs isolates) were described by Oliveira *et al*.[16] and Bodnar *et al*.[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 MRSAs 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[1].

Table 1 - Molecular characteristics and antimicrobial susceptibility profile of 34 methicillin-resistant *Staphylococcus aureus* SCC*mec* type II isolated in 2015-2016

Isolate	CC[a]	ST[a]	*PVL*[b]	*tst-1*[b]	Antibiotype[c]	Year[d]	Source[e]	Vancomycin µg/mL[g]
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

[a]CC: Clonal Complex and ST: Sequence Typing were determined as described by Lilliebridge *et al.*[14]; [b]PVL: Panton Valentine Leukocidin encoding gene and *tst-1*: Toxic Shock Syndrome Toxin encoding gene were detected as described in Oliveira *et al.*[16]; [c]Antimicrobial resistance profile was determined by disk-diffusion assay according to the CLSI guidelines[8]- I: OXA; II: OXA, ERI, CLI, CIP; III: OXA, ERI, CLI, CIP, GN; OXA: Oxacillin, CLI: Clindamycin, ERI: Erithromycin, CIP: Ciprofloxacin; GN: Gentamicin; [d]Year of isolation; [e]Source of isolation, T: Tracheal secretion, B: Blood; [f]MIC vancomycin determined by *E-test*; - Absence; + Presence.

We also investigated the presence of genes *lukS*-PV and *lukF*-PV and *tst-1* in SCC*mec* type II MRSAs by PCR as described previously[15]. Seven SCC*mec* type II isolates (20.6%) harbored the PVL-encoding genes, which contrast with the results of de Oliveira *et al.*[15] that detected these genes only in MRSA SCC*mec* 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[18]. In contrast, none MRSA SCC*mec* type II harbored the *tst-1* gene in this study, whereas this gene was detected in 5.7% (7 out

of 123) of MRSA harboring the same SCC*mec* elements in our previous study[16]. 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[19]. 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 SCC*mec* type II exhibiting different STs, most of which belong to CC5 in UHL. The SCC*mec* 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*.[15] showed that CC5 isolates, SCC*mec* 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[20].

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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