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Review Article

Diversity of Scc*mec* types in Methicillin Resistant *Staphylococcus* Spp. Causing Hospital-Associated Infections

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Abstract

The determination of SCC*mec* types among staphylococcal strains causing infections in the hospital environment is crucial for understanding the basis of genetic transfer, tracking the emergence and dispersion routes of the strains involved, and defining control strategies. The aim of this study was to determine the SCC*mec* types of methicillin-resistant (MR) *Staphylococcus* spp. causing hospital-associated infections and to compare the diversity of SCC*mec* types between *Staphylococcus aureus* (*S. aureus*) and coagulase-negative *Staphylococcus* (CoNS) clinical MR isolates. SCC*mec* types were assessed via a PCR-based scheme using sets of primers targeting the *ccr* and *mec* complexes and other structures. The results revealed differences in the distribution of SCC*mec* types between the analyzed *S. aureus* and CoNS isolates. *SCCmec* types II, III and IV were evenly distributed among the *S. aureus* strains. Among the CoNS isolates, the *SCCmec* types occurred in different proportions: the highest frequency was observed for SCC*mec* IV, followed by type III, while type V, which was absent from the *S. aureus* strains, occurred in a lower percentage of the CoNS strains. A number of isolates could not be categorized and were designated as non-typable (NT). The frequency of NT isolates was higher among the *S. aureus* strains than the CoNS strains, but the difference was not statistically significant (p-value = 0.2485). We hope that this study will contribute to improving the understanding of the molecular epidemiology of *SCCmec* types among hospital infection-associated *S. aureus* and CoNS strains in the study region.

Keywords: *Staphylococcus aureus;* Coagulase-Negative *Staphylococcus;* Scc*mec* Types; Hospital Infection.

Introduction

A large proportion of hospital-acquired staphylococcal infections are caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, methicillin-resistant coagulase-negative *Staphylococcus* (MRCoNS) nosocomial infections, particularly those associated with prosthetic devices, are increasing [1-3].

Methicillin resistance (MR) in staphylococci is conferred by an alternative penicillin-binding protein known as PBP2a or PBP2', which is essential for cell wall synthesis and bacterial growth in the presence of β-lactam antibiotics. PBP2a is encoded by the *mec*A gene, which is located on a mobile genetic element (MGE) referred to as staphylococcal cassette chromosome *mec* (SCC*mec*) [3,4]. Structurally, SCC*mec* is composed of two major complexes (the *mec* gene complex and the *ccr* gene complex) and several nonessential components: three joining (J1-J3) regions and a few other genes or pseudogenes that may carry various other MGEs, such as insertion sequences, transposons and integrated plasmids carrying additional determinants of antimicrobial resistance to non-β-lactam antibiotics or heavy metals [5].

There are currently 11 major SCC*mec* types (I-XI) that have been identified among staphylococci based on the combination of the structures of the *ccr* and *mec* gene complexes. The distribution and prevalence of SCC*mec* types varies throughout the world depending on the source (related to the host species, hospital or community environment, antibiotic use, geographical origin and isolation period) [1, 5-14]: *SCCmec* types I-V appear to be globally distributed, while the other types are limited to the country of origin; SCC*mec* types I, II and III are hospital associated (HA), while types IV, V, VI and VII are community associated (CA) [15, 16]. However, changes are occurring worldwide [1, 5- 6, 8-14].

Methicillin resistance is more prevalent in CoNS than in *S. aureus* [17]. Hence, CoNS strains are considered to represent a large SCC*mec* reservoir [17]. CoNS are regarded as potential SCC*mec* donors, and it is believed that they serve as donors of SCC*mec* to *S. aureus* [2]. In fact, new MRSA clones are continuously emerging, spreading and adapting to the environment [6,11,17].

Therefore, it is necessary to determine the distribution of SCC*mec* types among staphylococcal strains causing hospital infections to understand the basis of genetic transfers, to track the emergence and dispersion routes of the strains involved and to define control strategies.

In Brazil, most of the available information on this topic is limited to the south and southeast regions [18-23], while studies carried out in the northeast region [24,25] have not yet fully addressed this subject. Thus, with the aim of addressing this issue, we performed an analysis of nosocomial infection-associated MR *S. aureus* and CoNS isolates through multiplex PCR (M-PCR) and long-range PCR using primers targeting the *ccr* and *mec* complexes and associated structures.

Materials and Methods

Bacterial isolates and growth conditions

The study included 39 clinical *S. aureus* and 45 CoNS *mecA* PCR-positive isolates from the bacteriotheque of the Department of Microbiology, CPqAM/FIOCRUZ-PE. The isolates originated from a university hospital in Recife, PE, Brazil, between 2002-2010 and were stored at -80ºC in brain heart infusion (BHI) broth (HiMedia Laboratories Pvt Ltd 23, Vadhani Industrial Estate, LBS Marg, Ghatkopar West, Mumbai, Maharashtra 400086, India) / 25% glycerol.

The sources of the strains are shown in Tables 1 and 2. The infections from which the isolates were obtained were characterized as nosocomial infections according to the Centers for Disease Control and Prevention criteria [26], and the isolates were identified using the conventional bacteriological catalase test, the coagulase tube test, the thermonuclease growth test, and the mannitol salt agar test. All of the samples were methicillin resistant, as determined by the presence of the *mec* gene via PCR [27]. The absence of coagulase in the strains was confirmed by a PCR test negative for the *coa* gene [28].

Table 1. Origin of the analyzed methicillin-resistant *Staphylococcus aureus* isolates causing hospital-associated infections and the assigned SCC*mec* types.

Number/year	Source	*SCCmec* type
014/02	Hemoculture	IV
038/02	Hemoculture	III
047/02	Catheter	NT
055/02	Hemoculture	II
066/02	Hemoculture	II
075/02	Hemoculture	II
121/02	Hemoculture	II
126/02	Catheter	IV
157/02	Hemoculture	III
202/02	Hemoculture	II
227/02	Hemoculture	IV
236/02	Hemoculture	III
663/03	Hemoculture	NT
666/03	Pulmonary fluids	NT
676/03	Hemoculture	NT
687/03	Hemoculture	NT
692/02	Hemoculture	NT

722/03	Catheter	NT
741/03	Hemoculture	II
749/03	Hemoculture	II
794/03	Catheter	III
807/03	Pulmonary fluids	II
808/03	Pulmonary fluids	III
835/03	Hemoculture	NT
853/03	Catheter	IV
859/03	Pulmonary fluids	NT
038/04	Catheter	II
464/04	Hemoculture	III
599/04	Surgical wound	IV
827/04	Hemoculture	II
862/04	Surgical wound	IV
969/04	Catheter	III
990/04	Hemoculture	IV
995/04	Pulmonary fluids	NT
038/09	Hemoculture	III
043/09	Hemoculture	IV
049/10	Hemoculture	III
050/10	Hemoculture	IV
054/10	Hemoculture	IV

NT: non-typable.

Table 2. Origin of the analyzed methicillin-resistant coagulase-negative *Staphylococcus* strains causing hospital-associated infections and the assigned SCC*mec* types.

Number/year	Source	*SCCmec* type
39/02	Hemoculture	IV
81/02	Hemoculture	III
105/02	Hemoculture	III
109/02	Hemoculture	III
124/02	Catheter	III
162/02	Hemoculture	IV
237/02	Surgical wound	NT
009/04	Hemoculture	II
012/04	Hemoculture	V
013/04	Hemoculture	NT
70/04	Catheter	IV
412/04	Hemoculture	II
522/04	Catheter	V
525/04	Catheter	IV
942/04	Catheter	NT
976/04	Hemoculture	NT
992/04	Hemoculture	IV
018/09	Hemoculture	IV
019/09	Hemoculture	III
020/09	Hemoculture	III
021/09	Hemoculture	NT
022/09	Hemoculture	II
023/09	Hemoculture	IV
024/09	Hemoculture	III
026/09	Hemoculture	III
027/09	Hemoculture	III
028/09	Hemoculture	NT
029/09	Hemoculture	IV
030/09	Hemoculture	IV
032/09	Hemoculture	II
033/09	Hemoculture	IV
34/09	Hemoculture	IV
035/09	Hemoculture	III
036/09	Hemoculture	III
037/09	Hemoculture	IV
40/09	Hemoculture	IV
41/09	Hemoculture	IV
42/09	Hemoculture	IV
44/09	Hemoculture	V
46/10	Hemoculture	IV
47/10	Hemoculture	III
48/10	Hemoculture	III
51/10	Hemoculture	IV
52/10	Hemoculture	IV
55/10	Hemoculture	IV

NT: non-typable.

The following reference strains were used as controls: MRSA05166 and MRSA05279 (SCC*mec* type II), MRSA04673 and MRSA05616 (SCC*mec* type III); MRSA01171 and MRSA03343 (SCC*mec* type IV); and MRSA02928 and MRSA03231 (SCC*mec* type V).

Unless otherwise stated, all cultures were grown on sheep blood agar plates overnight at 37º C.

Bacterial DNA extraction

DNA samples were extracted following a protocol based on Ausubel et al. [29]. Briefly, 1 ml of each bacterial culture in BHI was centrifuged for 10 min at 20,000 x g. The resulting pellet was washed with 500 µL of Tris: EDTA (10:1), and 10 µg of

lysostaphin and 5 µg proteinase K were added to lyse the cells. The samples were subsequently incubated at 60°C for 20 minutes, and 100 µL of STE (2.5% SDS, 0.25 M EDTA, 10 mM Tris pH 8.0) was added. The samples were next incubated at 60°C for 15 minutes, then at room temperature for 5 minutes and in an ice bath for 5 minutes, after which 130 µL of 7.5 M sodium acetate was added, followed by incubation in an ice bath for 15 minutes and centrifugation for 3 minutes at 20,000 x g. At this point, 700 µL of the supernatant was transferred to a new tube; 420 µL of isopropanol was added; and the samples were then incubated at -80°C for 30 minutes and centrifuged at 20,000 x g for 10 minutes. The supernatant was discarded, and the pellet DNA was vacuum dried and resuspended in 10 µL of 0.02% RNAse. The obtained DNA was quantified using a NanoDrop 2000 (Thermo Fisher Scientific Inc. 81 Wyman Street, Waltham, MA 02451 USA).

Determination of SCC*mec* types

SCC*mec* types were determined via multiplex PCR (M-PCR) following Kondo et al. [30] using primers targeting the *mec* gene complex, the *ccr* gene complex and other structures, including the mercury resistance operon (SCC_{Hg}) and the IS1272 and IS431 elements (Table 3). The reactions were prepared in a final volume of 50 µL containing 5 µL of l Green Go Taq buffer (Promega Corporation. 2800 Woods Hollow Road, Madison, WI 53711 USA), 2.5 mM of each deoxynucleotide, 25 mM $MgCl_2$, 1.5 U of Go Taq DNA polymerase (Promega), 20 ng of DNA from each sample and the primers at 10 µM. Thermocycler amplifications were performed as follows: 30 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 minutes. The amplification products were electrophoresed on 2% agarose gels stained with the GelRed Nucleic Acid Stain (Biotium Inc. 3159 Corporate Place, Hayward, CA 94545 USA), visualized in a UV transilluminator and digitalized using KODAK 1D software, version 3.5.2 (Eastman Kodak Company, 1669 Lake Avenue, Rochester, NY 14652 USA).

DNA sequencing

Each *mec* class element was amplified via long-range PCR using previously described primers [31, 32] and other primers designed specifically for this study (Table 3). The reactions were performed in a final volume of 50 µL, containing 5 µL of 10 × PfuUltra ™ II buffer (Stratagene, 1834 W. Hwy 71, Cedar Creek, TX 78612 USA), 200 mM dNTPs, 10 mM primers, 1 µL of PfuUltra ™ II fusion HS DNA polymerase (Stratagene) and 10 ng of DNA. The reactions were performed in a Mastercycler ep gradient thermal cycler (Eppendorf, HQ Barkhausenweg 1 22339 Hamburg, Germany) under the conditions recommended by the manufacturer. The amplicons were purified using ExoSAP-IT® (Affymetrix, 3420 Central Expressway, Santa Clara, CA 95051 USA) and sequenced via the Sanger method. The obtained sequences were processed using SeqMan II (Lasergene software, DNASTAR, Inc., Madison, WI), assembled using ClustalW and compared with GenBank sequences using BLAST (http://www.ncbi.nih.gov/BLAST).

Table 3. Primers used in this study.

Multiplex PCR		Sequences (5'-3')	Target	References
ccr Complex	MA1	TGCTATCCACCCTCAAACAGG	*mec*A	[30]
	MA2	AACGTTGTAACCACCCCAAGA	*mec*A	[30]
	α1	AACCTATATCATCAATCAGTACGT	*ccr*A1	[30]
	α2	TAAAGGCATCAATGCACAAACACT	*ccr*A2	[30]
	α3	AGCTCAAAAGCAAGCAATAGAAT	*ccr*A3	[30]
	βC	ATTGCCTTGATAATAGCCITCT	*ccr*B1, *ccr*B2, *ccr*B3	[30]
	γR	CCTTTATAGACTGGATTATTCAAAATAT	*ccr*C	[30]
	γF	CGTCTATTACAAGATGTTAAGGATAAT	*ccr*C	[30]
mec Complex	MI6	CATAACTTCCCATTCTGCAGATG	*mec*I	[30]
	IS7	ATGCTTAATGATAGCATCCGAATG	IS*1272*	[30]
	IS2	TGAGGTTATTCAGATATTTCGATGT	IS*431*	[30]
	MA7	ATATACCAAACCCGACAACTACA	*mec*A	[30]
Long-range PCR				
	mec_F1	CCAATTCCACATTGTTTCGGTCTAA	*mec*A	[32]
	mec_R1	GTAGAAATGACTGAACGTCCGATAA	*mec*A	[32]
	mec_F2	AGTTCTGCAGTACCGGATTTGC	*mec*A	[31]
	mec_R2	GGTCCCATTAACTCTGAAG	*mec*A	[31]
	SA10	GACTTGATTGTTTCCTCTGTT	*mec*I	[31]
	SA09	AATGGCGAAAAAGCACAACA	*mec*I	[31]
	SA17	CGCTCAGAAATTTGTTGTGC	*mec*R1	[31]

	SA18	ATCCTCCTTATATA-AGACTAC	*mec*R1	[31]
	SA19	CATATCGTGAGCAAT-GAACTG	*mec*R1	[31]
	SA13	GTCTCCACGTTAATTC-CAATT	*mec*R1	[31]
	SA14	GTCGTTCATTAAGATAT-GACG	*mec*R1	[31]
	SA15	CAAGCACCGTTAC-TATCTGC	*mec*R1	[31]
	SA[20]	ATAAGTAAAACAAT-GATGGCAAT	*mec*R1	This study
	SA21	TGTTTATC-CCATTGTTGTTCAG	*mec*R1	This study
	SA22	TGATTAAGGCATTCCG-ACAAA	*mec*R1	This study
	IS8	ATCGAACGCCACT-CATAACA	IS*1272*	This study

Statistical analysis

The data were analyzed with Pearson's chi-square statistic test using R software [33]. For the purpose of this work, a p-value of ≤0.05 was considered statistically significant.

Results

SCC*mec* types were determined based on the classes of the *mec* gene and *ccr* gene complex structures (Table 3). According to the amplification results, out of the 84 isolates, 68 were assigned to SCC*mec* types II (*mec* gene complex class A / *ccr* gene complex class *ccr*AB2), III (*mec* gene complex class A / *ccr* gene complex class *ccr*AB3), IV (*mec* gene complex class B / *ccr* gene complex class *ccr*AB2) and V (*mec* gene complex class C / *ccr* gene complex class *ccrC*). Overall, 16.7% of the isolates were classified as SCC*mec* type II; 26.2% as SCC*mec* type III; 34.5% as SCC*mec* type IV; and 3.6% as SCC*mec* type V.

The remaining 16 (19%) isolates (10 S. *aureus* and six CoNS), could not be typed according to the scheme that was employed and were designated as SCC*mec* non-typable (NT). The NT isolates showed amplification of the *ccr* gene complex class *ccr*-AB2, but there was no PCR amplification of the targeted *mec* complex genes.

The percentage of NT isolates (Table 4) was higher among the *S. aureus* strains (25.6%) than the CoNS strains (13.3%). However, this difference was not statistically significant (p-value = 0.2485).

The 39 *S. aureus* strains harbored SCC*mec* types II, III and IV in similar proportions, and the percentage of NT isolates was also similar (p-value = 0.99). The SCC*mec* type V element was not found among the analyzed strains (Table 4).

Table 4. Distribution of SCCmec types among the analyzed methicillin-resistant Staphylococcus spp. causing hospital-associated infections.

S. aureus	**II**	**III**	**IV**	**V**	**NT**	**Total**
Total	10	9	10	0	10	39
%	25.6	23.2	25.6	0	25.6	100.00
CoNS	**II**	**III**	**IV**	**V**	**NT**	**Total**
Total	4	13	19	3	6	45
%	8.9	29.0	42.2	6.7	13.3	100.00

NT: non-typable

The 45 CoNS isolates harbored SCC*mec* types II, III, IV and V and NT in significantly different proportions (p-value = 0.000368) (Table 4). SCC*mec* type IV was the most prevalent (42.2%), followed by SCC*mec* type III (29.0%).

To obtain further insight into the structure of the SCC*mec* elements, the presence of the SCC_{Hg} operon was investigated in the 22 isolates harboring type III SCC*mec*, and all of them (9 *S. aureus* and 13 CoNS) yielded amplification products using the primers targeting the *ccr*C element (Table 3). The amplified segments were sequenced via long-range PCR and were found to show homology with the sequence of the SCC_{Hg} operon, confirming the identity of the segment.

For further confirmation, the SCC*mec* type IV strains were analyzed with primers targeting the IS1272 and IS431 elements. Both sequences were amplified in all 29 SCC*mec* type IV isolates (10 *S. aureus* and 19 CoNS), and the segments were confirmed via sequencing.

Analysis of the amplified sequences revealed 99% identity with the sequences deposited in GenBank: accession numbers D86934 for SCC*mec* type II, AB037671 for SCC*mec* type III, AB063172 for SCC*mec* type IV and AB121219 for SCC*mec* type V.

Discussion

In this study, we addressed the occurrence of the various SCC*mec* types in MR *Staphylococcus* spp. causing hospital-associated infections. Previous studies have reported the occurrence of SCC*mec* types I, II, III, IV and V in Brazilian hospitals in the south and southeast regions [18-23]. Thus far, *SCCmec* types VI, VII, IX, X and XI have not been reported in Brazil. In the

present study, the isolates were classified as SCC*mec* types II, III, IV, V and NT (non-typable).

The failure to determine the SCC*mec* types of some strains was previously attributed to the presence of new structures and rearrangements or recombination within the *mec* complex [1,6,9,13,21]. New variants of the *ccr* genes continue to be identified, which cannot be typed using the currently available schemes, and new schemes for the classification of SCC*mec* are in need of improvement [14,34-36].

SCC*mec* types IV, V, VI and VII are traditionally categorized as community associated (CA) and types I, II and III as hospital associated (HA) [15,16]. However, changes in this epidemiological profile are being observed worldwide, with an increase in the prevalence of CA clonal lineages within hospital environments, abolishing the separation between traditional CA and HA clones [15,16].

As previously reported in hospitals from southern and southeastern Brazil [19,20], in our study, SCC*mec* types III and IV occurred at a higher frequency than SCC*mec* types II and V (Table 4). SCC*mec* type I was not identified, despite being reported in other studies conducted in southern and southeastern Brazil [19-22]. The frequency of SCC*mec* type II was higher among the *S. aureus* strains (25.6%) than the CoNS strains (8.9%) (Table 4).

The frequency of SCC*mec* type III in *S. aureus* (23.2%) was quite close to that in the CoNS isolates (29.0%). SCC*mec* type III encodes the largest number of resistance genes, and strains harboring this type are important pathogens in hospitals, where they cause severe infections, and should therefore be considered in infection control policies [7]. It was observed that the SCC*mec* type III element is a composite of two smaller SCC elements integrated in tandem: the $SCC_{mercury}$ (SCC_{Hg}) operon and the type III SCC*mec* element [5,35]. The presence of SCC_{Hg} was confirmed in the isolates through PCR and sequencing.

Although SCC*mec* type IV is categorized as CA, in our study this type was the most prevalent among the CoNS strains (42.2%), and it was also found at a high frequency (25.6%) among the *S. aureus* strains (Table 4). The occurrence of SCC*mec* type IV in Brazilian nosocomial isolates was previously reported [37-38]. The higher prevalence of SCC*mec* type IV could be attributed to the small size of this element, which is likely to increase in prevalence over time due to the low metabolic cost of its transfer [7].

SCC*mec* type V, which is seldom reported in Brazil [18,19], was found in a low percentage of the CoNS isolates (6.7%) and was absent among the *S. aureus* strains (Table 4) in the present study.

Rather than representing a mere contaminant, as was previously indicated, CoNS is now recognized as an important nosocomial infectious agent acting as a reservoir of virulence genes in the hospital environment, in addition to serving as a reservoir and donor of resistance genes [2,17,39]. A previous study on methicillin-resistant *Staphylococcus* spp. causing hospital-associated infections [40] revealed the toxigenic potential of CoNS strains. It was observed that out of 43 CoNS isolates analyzed, 18 (41.86%) showed amplification of at least one toxigenic gene. Carrying staphylococcal toxigenic genes indicates the ability of CoNS to incorporate toxigenic genes, which increases their toxigenic potential as well as their ability to invade sterile sites and their ability to cause serious hospital-acquired infections.

In conclusion, differences were observed in the distribution of SCC*mec* types between *S. aureus* and CoNS isolates obtained from hospital-associated infections in this study (Table 4). In general, the CoNS strains displayed an absence of SCC*mec* type I; a low frequency of SCC*mec* type II; and a higher frequency of SCC*mec* type IV. In contrast, SCC*mec* types II, III, IV and NT were evenly distributed among the *S. aureus* strains. Among the CoNS strains, the *SCCmec* elements occurred in different proportions: the highest frequency was observed of SCC*mec* type IV (42.2%), followed by SCC*mec* type III (29.0%). SCC*mec* type V was absent from *S. aureus* and displayed a low frequency (6.7%) among the CoNS strains. The frequency of NT (25.6%) was higher among the *S. aureus* strains than the CoNS strains (13.3%), but this difference was not statistically significant. This study contributes to reducing the gap in our knowledge of the molecular epidemiology of *SCCmec* types among hospital infection-associated *S. aureus* and CoNS strains in the study region.

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Conflict of interest

There is no conflict of interest to declare.

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