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SHORT COMMUNICATION

Diversity of SCC*mec* types in methicillin-resistant *Staphylococcus aureus* clinical isolates in southern Brazil

Diversidade dos tipos de SCCmec em isolados clínicos de Staphylococcus aureus resistente à meticilina no Sul do Brasil

Diversidad de tipos de SCCmec en aislados clínicos de Staphylococcus aureus resistentes a meticilina en el sur de Brasil

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Methicillin-resistant *Staphylococcus aureus* is one of the most frequent causes of community- and healthcare-associated infections[1]. This resistance is conferred by the acquisition of the staphylococcal chromosomal cassette *mec* (SCC*mec*), carrying the *mecA* or *mecC* gene, which encodes an altered penicillin binding protein (PBP2a or PBP2'), presents low affinity for most beta-lactams.[1,2] Until now, there are thirteen SCC*mec* types (I-XIII), highly diverse in their structural organization and genetic content, being widely used in MRSA molecular typing.[2] MRSA is considered to be healthcare-associated (HA-MRSA) if the isolates are recovered after 48 h of hospitalization and community-associated (CA-MRSA) if recovered in up to 48 h after hospital admission. Commonly, HA-MRSA are associated with invasive infections and belongs to SCC*mec* types I, II or III, while CA-MRSA are associated with skin and soft tissue infections and carries SCC*mec* type IV or V.[3,4] Since MRSA are in constant evolution and geographic spread, the SCC*mec* typing is useful to understand the epidemiology and clonal relatedness of these multiresistant strains.[2] The aim of this study was to determine the diversity of SCC*mec* types and subtypes in MRSA strains obtained from Hospital Mãe de Deus (HMD), Hospital Moinhos de Vento (HMV), Hospital Ernesto Dornelles (HED) and Hospital Nossa Senhora da Conceição (HNSC) in Porto Alegre, Brazil, between 2014 and 2019. This cross-sectional observational study was conducted with 217 non-repetitive MRSA clinical isolates, under Institutional Ethics Committee number 2.770.338. They were recovered from the respiratory tract (34.6%), blood (25.3%), skin and soft tissue (19.4%), bone and connective tissue (11.1%), sterile cavity liquids (5.5%) and medical devices (4.1%). The isolates were identified as *S. aureus* using conventional bacteriological methods, such as colony morphology on sheep blood agar, Gram staining, catalase activity, coagulase production and mannitol fermentation. The methicillin resistance was determined by cefoxitin disk diffusion method and polymerase chain reaction (PCR) to detect the *mecA* gene, according to Clinical and Laboratory Standard Institute (CLSI) guidelines.[5,6] The bacterial DNA was extracted using Chelex®100 (Bio-Rad, Richmond, CA) and Proteinase K (Sigma-Aldrich, Poole, UK), and SCC*mec* types I-X (including subtypes IVa, IVb, IVc and IVd) was determined by multiplex-PCR as previously described,[5,7] with some modifications (Table 1). The three multiplex-PCR contained 0.2 mM of

dNTPs, 2 mM of $MgCl_2$, 1 X of PCR buffer, 5 µL of primer mix, 1.5 U of Taq DNA polymerase and 1.5 µL of DNA template in a total volume of 25 µL. Amplifications were performed in a LifePro Thermal Cycler (Hangzhou Bioer Technology Co. Ltd., Hangzhou, China), and the PCR products were analyzed by electrophoresis in 2.0 % agarose gel (Sigma-Aldrich, United States), stained with ethidium bromide and observed on UV transilluminator. As PCR positive controls, NCTC 10442 (SCC*mec* I), N315 (SCC*mec* II), 85/2082 (SCC*mec* III), JCSC 4474 (SCC*mec* I Va), JCSC 2172 (SCC*mec* IVb), JCSC 4488 (SCC*mec* IVc), JCSC 4469 (SCC*mec* IVd), WIS (SCC*mec* V), HDE 288 (SCC*mec* VI), JCSC 6082 (SCC*mec* VII), JCSC 6943 (SCC*mec* I X) and JCSC 6945 (SCC*mec* X) were included and, as negative control a tube containing all components of the mixture, except template DNA was used. In relation to SCC*mec* typing, SCC*mec* type IV was the most common type, with a frequency of 57.1% (124/217), being 77 of subtype IVa (62.1%), 3 of IVb (2.4%), 42 of IVc (33.9%) and 2 of IVd (1.6%), followed by type III, with frequency of 17.1% (37/217); type I, with a frequency of 13.4% (29/217); type II, with a frequency of 9,2% (20/2017) and type V, with a frequency of 1.4% (3/217). None of the isolates showed SCC*mec* types VI, VII and X. The distribution of SCC*mec* types between 2014 and 2019 is presented in figure 1. Interestingly, over the years, we observed a decrease of types I, II, and III and an increase of subtypes IVa and IVc among HA-MRSA. Epidemiological studies have demonstrated that SCC*mec* I, II, and III are the most common in HA-MRSA, whereas SCC*mec* IV and V predominate in CA-MRSA.[3,4] The results of our analysis show that SCC*mec* IV and III were the most prevalence types among the HA-MRSA strains, followed by type I, II and V. These findings corroborate other studies, which presented that SCC*mec* IV and III were the most common types identified among HA-MRSA isolates, in varying proportions.[8,9] The presence of SCC*mec* types IV and V in HA-MRSA isolates suggests clearly the circulation of clones of MRSA in the hospital setting that carry SCC*mec* types from the community as well.[8] However, the distinction between CA-MRSA and HA-MRSA is becoming increasingly difficult, with migration of CA-MRSA being observed in health-care settings.[1,2] This differentiation is important to understand their pathogenicity, antimicrobial resistance, molecular characteristics and clinical implications. Although

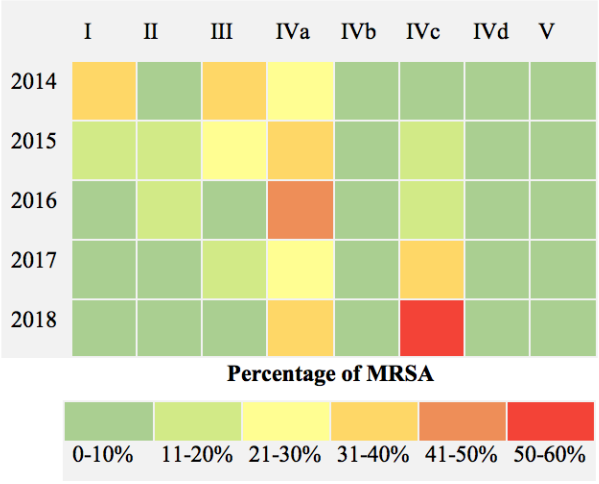

Figure 1. Heat map showing the distribution of SCC*mec* types among 217 MRSA clinical isolates, according to the year of collection.

Table 1. The primers and amplification conditions used to detect SCC*mec* types.

Primers	Sequences (5'-3')	Amplicon (bp)	PCR conditions
First reaction[5]			
mcABC1-F	ATTTTGAATCGCCATGAACA		Pre cycle 95°C 5 min
mcA-R	CGCATTGTCTTCGCCTTTTA	936	35 cycles: 95°C 30 s,
mcB-R	TTTGGGTTTCACTCGGATGT	676	61°C 45 s,
mcC1-R	GGGTTCAAGAATATGCACCAA	791	72°C 1 min
ccrB2-F	TCCAAAGTATGTTCGGCAAAC	563	Last cycle 72°C 10 min
ccrB2-R	TGCACTTCGTCGAGTTGTTT		
ccrB4-F	TGCAAACGGATGGTTACAGT	394	
ccrB4-R	CGTTGTCTTTGGCCATTGTA		
Second reaction[5]			
mcC2-F	TCAGTTCATTGCTCACGATATG	411	
mcC2-R	ATGTCCCTCTGCATCAATGG		
ccrC-F	CCGGTCGTGTTTTAGGCTAC	677	
ccrC-R	CACAYTTGACGCAATCTGCT		
ccrA1/3-F	GGMGAACAAGTCAAAAATGG		Pre cycle 95°C 5 min
ccrA1-R	TTCACAGACAAAACGAGATGC	519	35 cycles: 95°C 30 s,
ccA3-R	TTCGTTGCCGAAACAATAGG	326	57,5°C 45 s,
mecA-F	CCACCCTCAAACAGGTGAAT	796	72°C 1 min
mecA-R	CCCAATTTGTCTGCCAGTTT		Last cycle 72°C 10 min
Third reaction[7]			
IVa-F	GCCTTATTCGAAGAAACCG	776	Pre cycle 94°C 5 min
IVa-R	CTACTCTTCTGAAAAGCGTCG		10 cycles: 94°C 45 s,
IVb-F	TCTGGAATTACTTCAGCTGC	493	63°C 45 s,
IVb-R	AAACAATATTGCTCTCCCTC		72°C 1.5 min
IVc-F	CCTGAATCTAAAGAGATACACCG	200	25 cycles: 94°C 45 s,
IVc-R	GGTTATTTTCATAGTGAATCGC		53°C 45 s,
IVd-F	CTCAAAATACGGACCCCAATACA	881	72°C 1.5 min
IVd-R	TGCTCCAGTAATTGCTAAAG		Last cycle 72°C 10 min

studies suggest a blurring of the line between CA-MRSA and HA-MRSA,[10] continuous monitoring of CA- and HA-MRSA is necessary to understand the epidemiology and spread of these multiresistant pathogens.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. *Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nat Rev Microbiol 2019;17:203-18. doi: 10.1038/s41579-018-0147-4*
2. *Lakhundi S, Zhang K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev 2018;31:e00020-18. doi: 10.1128/CMR.00020-18*
3. *Lee AS, de Lencastre H, Garau J, et al. Methicillin-resistant Staphylococcus aureus. Nat Rev Dis Primers 2018;4:18033. doi: 10.1038/nrdp.2018.33*
4. *Sowash MG, Uhlemann A-C. Community-associated methicillin-resistant Staphylococcus aureus case studies. Methods Mol Biol 2014;1085:25-69. doi: 10.1007/978-1-62703-664-1_2*
5. *Lawung R, Chuong LV, Cherdtrakulkiat R, et al. Revelation of staphylococcal cassette chromosome mec types in methicillin-resistant Staphylococcus aureus isolates from Thailand and Vietnam. J Microbiol Methods 2014;107:8-12. doi: 10.1016/j. mimet.2014.08.024*
6. *Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: twenty-fourth informational supplement. Document M100-S24. Wayne, PA: CLSI; 2019. p.55-69.*
7. *McClure-Warnier J-A, Conly JM, Zhang K. Multiplex PCR Assay for Typing of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-resistant Staphylococcus aureus. J Vis Exp 2013;79:e50779. doi: 10.3791/50779*
8. *Bashir Y, Bali N, Sharma S, et al. SCCmec Type IV and V Methicillin-Resistant Staphylococcus aureus Intrusion in Healthcare Settings. Am J Clin Microbiol Antimicrob 2019;2:1032. doi: 10.1016/j.bjid.2019.04.005*
9. *Sit PS, The CSJ, Idris N, et al. Methicillin-resistant Staphylococcus aureus (MRSA) bacteremia: Correlations between clinical, phenotypic, genotypic characteristics and mortality in a tertiary teaching hospital in Malaysia. Infect Genet Evol 2018;59:132-41. doi: 10.1016/j.meegid.2018.01.031*
10. *Bal AM, Coombs GW, Holden MTG, et al. Genomic insights into the emergence and spread of international clones of healthcare-, community- and livestock-associated meticillin-resistant Staphylococcus aureus: Blurring of the traditional definitions. J Glob Antimicrob Resist 2016;6:95-101. doi: 10.1016/j.jgar.2016.04.004*