

## CLONAL TYPES AND ANTIMICROBIAL RESISTANCE PROFILES OF METHICILLIN-RESISTANT *Staphylococcus aureus* ISOLATES FROM HOSPITALS IN SOUTH BRAZIL

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### SUMMARY

In the present study were evaluated the DNA macrorestriction profile and SCC*mec* types for nine multi-resistant MRSA selected. Also antimicrobial susceptibility testing by disk diffusion method was evaluated for 68 MRSA isolates against 12 antimicrobial agents. The isolates were recovered from blood culture collected from hospitalized patients in three hospitals of Porto Alegre, Brazil. PFGE and PCR for *mecA* and SCC*mec* I, II, III, IV types genes were done on selected nine isolates with susceptibility only to vancomycin, teicoplanin and linezolid. Two clone profiles, with five subtypes, were demonstrated among multi-resistant MRSA analyzed. Eight isolates showed harbor SCC*mec* type III and one isolate was not typeable. The knowledge of SCC*mec* type, clone and antimicrobial profiles among *S. aureus* is essential mainly to prevention and control of dissemination of the antimicrobial resistance.

**KEYWORDS:** *Staphylococcus aureus*; MRSA; SCC*mec*; Typing; Drug resistance

### INTRODUCTION

*Staphylococcus aureus* is an important agent of nosocomial and community-acquired infections. The methicillin resistance characterization is crucial for therapeutic and epidemiologic surveillance programs<sup>11</sup>. Methicillin resistance is characterized through exogenous acquisition of a mobile genetic element called staphylococcal chromosomal cassette *mec* (SCC*mec*)<sup>4,5</sup>. In Brazilian hospitals, MRSA are responsible for about 37% of staphylococcal infections. A single MRSA clone, called Brazilian endemic clone MRSA, is responsible by great majority of infections<sup>8,14</sup>. However, studies have reported the occurrence of community-acquired MRSA (CA-MRSA) infections in south Brazil that equally to others CA-MRSA harboring the particular SCC*mec* type IV<sup>7,10</sup>.

In this study we investigated antimicrobial susceptibility and molecular (typing and SCC*mec* types) characteristics associated with MRSA infections in hospitalized patients of Porto Alegre City, south Brazil.

### MATERIAL AND METHODS

**Bacterial isolates:** A total of 68 consecutive MRSA isolates were recovered from blood cultures of patients admitted in three hospitals (Hospital de Clínicas - HCPA, Irmandade Santa Casa de Misericórdia - ISCMPA and Grupo Hospitalar Conceição - GHC) located in Porto Alegre City. The isolates were collected from August/2004 to

December/2004. Only one isolate of each patient was included in the study. All samples were frozen and stored at -20 °C in skim milk with 10% glycerol. All isolates were identified as *S. aureus* by Gram stain, tube coagulase production and mannitol fermentation. All were characterized as methicillin-resistant by polymerase chain reaction (PCR) for detection of the *mecA* gene, as previously described<sup>7</sup>.

**Antimicrobial susceptibility testing:** Antimicrobial susceptibility was evaluated by disk diffusion method according to Clinical Laboratory and Standards Institute 2006 (formerly NCCLS) recommendations<sup>3</sup>. The following antimicrobial agents and concentrations (µg) (Becton Dickinson, Sparks, MD) were tested: vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), trimethoprim/sulfametoxazol (23.75/1.25 µg), penicillin (10 U), chloramphenicol (30 µg), tetracycline (30 µg), and rifampin (5 µg).

**Pulsed Field Gel Electrophoresis:** Molecular typing was realized by pulsed field gel electrophoresis (PFGE) after *SmaI* digestion as previously described<sup>9</sup>. Banding profile was analyzed by visual inspection. Isolates were considered as different genotypes when presented more than six band differences<sup>12</sup>. The choice criterion for typing was susceptibility only to vancomycin, teicoplanin and linezolid.

**SCC*mec* determination type:** Characterization of SCC*mec* type was performed by PCR procedure, as described by OLIVEIRA & DE

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LENCASTRE<sup>8</sup>. The choice criterion of MRSA isolates was the same adopted for PFGE submitting.

## RESULTS AND DISCUSSION

Higher resistance rates (intermediate and full) were noted for gentamycin and erythromycin 94.11% (64/68) followed by clindamycin 91.17% (62/68), ciprofloxacin 89.7% (61/68), trimethoprim/sulfametoxazol 88.23% (60/68), penicillin 97.05% (66/68), chloramphenicol 47.05% (32/68), tetracycline 83.8% (57/68) and rifampin 76.47% (52/68). Nine isolates (13.23%, 9/68) presented resistance to all antimicrobial agents except vancomycin, teicoplanin and linezolid.

The knowledge of the DNA macrorestriction profiles of MRSA is crucial to establish the prevalence of multiresistant isolates among the distinct medical centers also to control the dissemination horizontal of this resistance. Besides, the character "methicillin-susceptible" among staphylococci is easily transferable for "methicillin-resistant" due to an element genetic called *SCCmec*<sup>2</sup>. This element highly mobile is a 21-67 kb fragment of DNA that, for movement, carries two specific genes designated as cassette chromosome recombinase A and B (*ccrA* and *ccrB*), which encode recombinases of the invertase/resolvase family, with great capacity of dissemination<sup>13</sup>.

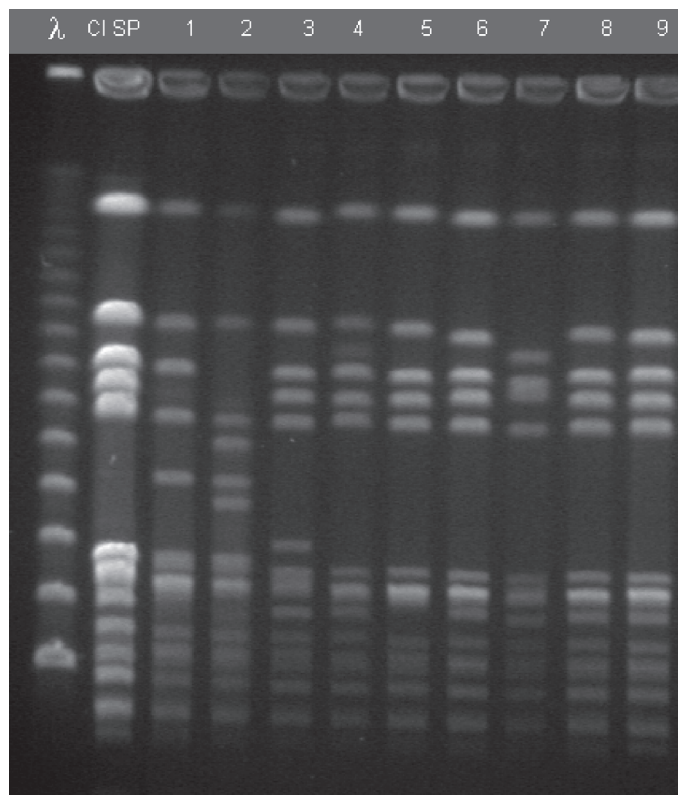
The *SCCmec* type III, also type II, confers characteristics of multi-resistance to MRSA<sup>6</sup>. Differently, elements designated *SCCmec* type IV, no harboring drug-resistance genes other than *mecA*, which was consistent with the most notable characteristic of CA-MRSA - susceptibility to many non- $\beta$ -lactam antimicrobials<sup>6</sup>. In report by RIBEIRO *et al.*<sup>10</sup>, CA-MRSA harbored *SCCmec* type IV was described in Porto Alegre City.

Although usually community acquired, TRINDADE *et al.*<sup>13</sup> show the *SCCmec* type IV *S. aureus* occurrence as clearly nosocomial. For that reason, monitoring of dissemination of MRSA isolates is an element important for study of the epidemiologic scenario.

In this study, *SCCmec* typing revealed that eight MRSA isolates harbored *SCCmec* type III and one other isolate harbored a non-typeable *SCCmec*. This is in accordance with multi-resistant character among isolates analyzed.

Nine multi-resistant MRSA isolates were submitted to PFGE typing. The clone profiles are shown in the Fig. 1. PFGE profiles showed two distinct biotypes, termed A and B, divided in five subtypes, A1, A2, A3, B1 and B2. The MRSA Brazilian clone (A1) was recovered in 2/3 and 3/5 of the isolates from HCPA and ISCMPA institutions, respectively. A clone "B" no-relatedness to the MRSA Brazilian clone was recovered from ISCMPA (subtype B2) and GHC (subtype B1) institutions. Clones relatedness to the MRSA Brazilian clone, A2 and A3 subtypes, were recovered from ISCMPA and HCPA institutions, respectively.

Out of nine DNA macrorestriction profiles obtained, four were clonally related and these isolates, strongly related with MRSA Brazilian clone, are from three distinct centers. Results from an international survey indicate that the Brazilian clone has a large capacity



**Fig. 1** - PFGE profiles of *SmaI*-digested genomic DNAs of MRSA isolates recovered from hospitalized patients in Porto Alegre City, Brazil. Lane  $\lambda$ , molecular weight marker (48.5 kb); lane CISP, Brazilian endemic clone MRSA; line 1 MRSA isolate from Grupo Hospitalar Conceição; lanes 2-6, MRSA from Irmandade Santa Casa de Misericórdia (ISCMPA) and lanes 7-9, MRSA isolates from Hospital de Clínicas (HCPA).

for geographic expansion and persistence<sup>9</sup>. However, a clone no-relatedness to the MRSA Brazilian clone was recovered in 2/4 institutions, GHC and ISCMPA (clones B1 and B2 subtypes, respectively). Thus, we think that these findings could be illustrating the presence of other clonal types among MRSA isolates in our hospitals. Studies with more samples are necessary to establish a clonal tendency among MRSA isolates causing nosocomial-acquired infections.

The same isolates submitted to the PFGE were examined for characterization of *SCCmec* type. *SCCmec* type III was detected in eight MRSA isolates. The *SCCmec* type characterization was not possible for one MRSA. All MRSA isolates included for *SCCmec* typing that presented *SCCmec* type III were resistant to sulfamethoxazole-trimethoprim, showing that this is not a characteristic of multi-resistant Brazilian endemic clone<sup>1</sup>. This showed that a unique phenotypic profile is poorly sufficient for a clonal characterization.

In conclusion, we have demonstrated that genetic determinant of the methicillin resistance (*mecA* gene) among multi-resistant MRSA isolates from hospitals of Porto Alegre City, south Brazil, is carried by *SCCmec* type III. In spite of the low number of isolates analyzed, there was a variation in DNA macrorestriction profiles of MRSA, with

the presence of two types, A and B. The knowledge of the occurrence of SCCmec types as well as clonal and phenotypic profiles determination among *S. aureus* and other staphylococcal species are essentially helpful to prevent the spread of these agents and to supervise the resistance in the local scenario.

## RESUMO

### Tipos de clones e perfis de resistência antimicrobiana de *Staphylococcus aureus* resistentes à meticilina isolados de hospitais no sul do Brasil

No presente estudo foram avaliados o perfil de macrorrestrição do DNA e tipos de SCCmec para nove MRSA multirresistentes selecionados. Além disso, susceptibilidade a 12 agentes antimicrobianos pelo teste de disco-difusão foi avaliada para 68 isolados de MRSA. Os isolados foram obtidos de hemoculturas de pacientes hospitalizados de três hospitais de Porto Alegre, Brasil. PFGE e PCR para detecção do gene *mecA* e para os tipos genéticos SCCmec I, II, III e IV foram realizados em nove isolados selecionados que apresentaram susceptibilidade somente a vancomicina, teicoplanina e linezolida. Dois perfis clonais, com cinco subtipos, foram demonstrados entre os isolados analisados. Oito isolados apresentaram SCCmec tipo III e um isolado não foi caracterizado quanto ao tipo de SCCmec. O conhecimento do tipo de SCCmec bem como dos perfis clonais e de susceptibilidade aos antimicrobianos entre isolados de *S. aureus* é essencial, principalmente, para a prevenção e controle da disseminação da resistência antimicrobiana.

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