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Brief communication

Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from a Brazilian university hospital

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ABSTRACT

The aim of this study was to perform SCC*mec* typing in *Staphylococcus aureus* isolates and to characterize the clonal profile of these isolates. Forty-six *mec*A gene-positive strains isolated between 2002 and 2006 were submitted to antimicrobial resistance testing by the E-test, SCC*mec* typing by multiplex PCR, and clonal profile analysis by pulsed-field gel electrophoresis. Forty-one (89.1%) isolates were typed as SCC*mec* III and five (10.9%) as SCC*mec* IV. Four circulating clones were detected, one of them comprising isolates related to the Brazilian epidemic clone. This clone was detected throughout the study period. The SCC*mec* III isolates were associated with a high rate of multidrug resistance and clonal dissemination of methicillin-resistant *S. aureus* in the wards of the University Hospital of the Botucatu School of Medicine, Universidade Estadual Paulista.

Staphylococcus aureus is a major human pathogen, which has been related to numerous pathological processes, such as food poisoning, pneumonia, bacteremia, impetigo, folliculitis, and osteomyelitis.[1] At present, this microorganism is the main causative agent of nosocomial infections. In Brazil, approximately 20% of bacteremias are caused by *S. aureus*.[2] Similar rates have been reported in several European countries, indicating that it is important to study the role of this microorganism in healthcare-associated infections.[3,4]

Oxacillin is used as a resistance marker for methicillin-resistant *S. aureus* (MRSA), since strains resistant to oxacillin are often multiresistant. Approximately 30–50% of hospital *S. aureus* isolates are oxacillin-resistant, a fact that leaves few treatment options.[5–7] The *mec*A gene is the main mechanism responsible for methicillin resistance. This gene is carried by the cassette chromosome *mec* (SCC*mec*), a movable genetic element that consists of incision and excision genes (*ccr* complex), in addition to genes that encode resistance to antimicrobials and heavy metals.[8] Eleven different SCC*mec* types have been described so far,[9] which differ in terms of the number of genes that they carry and gene architecture. In addition, different SCC*mec* types are associated with hospital

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or community isolates, a fact making SCC*mec* typing an important epidemiological tool.[10] Some of these SCC*mec* types carry genes encoding multidrug resistance, including resistance to beta-lactams, macrolides, lincosamines, streptogramins, aminoglycosides, and tetracycline. Hence, if a bacterial cell acquires such SCC*mec*, it concomitantly acquires a multidrug resistance phenotype.[11]

In Brazil, oxacillin resistance rates are high, with an incidence of 30–50% depending on the region studied.[12–14] However, little is known about the dissemination of these clonal isolates, although studies on the clonal profile of MRSA infections have been conducted in other countries. Five large pandemic clones of MRSA isolates have been identified by epidemiological techniques. Recent findings permitted us to confirm important changes in the epidemiology of MRSA infections in Brazilian hospitals. At the beginning of the past decade, the Brazilian epidemic clone (BEC)/type III/ST239 was the main lineage in Brazilian hospitals.[15] Over recent years, type IV isolates have emerged in hospital settings and have become a common type of MRSA found in inpatients worldwide. Studies conducted in Rio de Janeiro, Brazil, identified USA400/type IV/ST1 followed by USA800/ST5 as the main lineages.[16–19] Antimicrobial resistance and virulence factors are unique to each clone.

Since oxacillin resistance has become a major problem in hospitals which leaves few therapeutic options and since the presence of pandemic clones indicates clonal dissemination of MRSA isolates, the aim of the present study was to perform molecular characterization of MRSA strains isolated from the University Hospital of the Botucatu School of Medicine (HCFMB), UNESP.

Forty-six *mec*A gene-positive *S. aureus* strains (MRSA) isolated from blood cultures of patients hospitalized in different wards of HCFMB-UNESP between 2002 and 2006 and stored in the culture collection of the Department of Microbiology and Immunology, UNESP, were studied. The strains were isolated using the standard microbiological techniques described by Koneman et al.[20] In this study, the isolates of the 30 HCFMB-UNESP wards were divided into complexes according to specialties: Internal Medicine Wards, Surgery Wards, Pediatric Wards, Gynecology and Obstetrics, Emergency Room, Intensive Care Units, and other wards.

The *in vitro* susceptibility of the isolates to the following drugs was tested: oxacillin, netilmicin, erythromycin, sulfamethoxazole-trimethoprim, and vancomycin. For this purpose, the minimal inhibitory concentration (MIC) of these drugs was determined by the E-test. The isolates were classified as sensitive or resistant according to the cut-off levels (μg/mL) recommended by the CLSI.[21]

SCC*mec* typing was performed by multiplex PCR as described by Milheiriço et al.[22] The *S. aureus* isolates were typed by PFGE according to the modified protocol of McDougal et al.[23] Among the 46 *mec*A gene-positive *S. aureus* isolates, 23 were selected based on a similar profile of MIC determined with the E-test strip. The isolates were divided into groups according to the MIC obtained for all antimicrobial agents tested and one isolate representative of each group was selected. All *S. aureus* strains carrying SCC*mec* type IV were submitted to typing by PFGE. *Sma*I (Fast Digest SmaI, Fermentas Life Science, Canada) was used for genomic DNA restriction. Electrophoresis was performed on 1% agarose gel prepared with 0.5 M TBE (Pulsed Field Certified Agarose, BioRad Laboratories, USA) using the CHEF-DR III System (BioRad Laboratories) under the following conditions: pulse time interval of 5–40 s for 21 h on a linear ramp; 6 V/cm; 120° angle; 14° C; 0.5 M TBE as running buffer. Lambda Ladder PFG Marker (New England BioLabs, UK) was used as a molecular weight marker. The gels were stained with GelRed (10,000× in water, Biotium, USA) for 1 h and photographed under UV transillumination. For similarity analysis, the Dice correlation coefficient was estimated and a dendrogram was created by the UPGMA method (unweighted pair group method using arithmetic averages) using the BioNumerics 6.1 software (Applied Maths, Belgium). Band position tolerance and optimization were set at 1.5 and 1%, respectively. A similarity coefficient of 80% was chosen for cluster definition.

Among the 46 *mec*A gene-positive *S. aureus* strains isolated from blood cultures between 2002 and 2006, 41 (89.1%) were typed as SCC*mec* III and five (10.9%) as SCC*mec* IV. Most ($n = 14$) of the 41 SCC*mec* III isolates were identified in the Internal Medicine complex, followed by nine isolates in other wards, eight isolates in the Surgery complex, seven isolates in the Emergency complex, two isolates in the Intensive Care Unit complex, and one isolate in the Nursery complex. SCC*mec* type IV was detected in two isolates from the Emergency complex and in one isolate each from the Surgery, Internal Medicine and Intensive Care Unit complexes.

Resistance to the drugs tested varied according to SCC*mec* type. A higher percentage of isolates resistant to erythromycin ($n = 40$), netilmicin ($n = 38$) and trimethoprim–sulfamethoxazole ($n = 39$) was observed among SCC*mec* III MRSA. An association was observed between multidrug resistance and presence of the SCC*mec* III cassette, with 38 (92.7%) isolates being resistant to three of the four drugs tested and only one isolate being resistant to two, one and none of the drugs, respectively. Four of the five SCC*mec* IV isolates were susceptible to the drugs tested and one strain was resistant to erythromycin. SCC*mec* III MRSA predominated in all years studied; however, SCC*mec* IV isolates were not detected in 2005.

Susceptibility to vancomycin varied according to SCC*mec* cassette type. The MIC50 and MIC90 of vancomycin for SCC*mec* IV isolates were 2 and 3 μg/mL, respectively. These values were higher than those obtained for SCC*mec* III isolates (1.5 and 2 μg/mL, respectively). No decrease in vancomycin susceptibility was observed in SCC*mec* III and IV isolates over the study period. Among SCC*mec* III isolates, the MIC50 of vancomycin showed a slight decrease from 2002 (2 μg/mL) to 2003 (1 μg/mL), followed by a slight increase in 2004 (1.5 μg/mL), and remained stable in 2005 and 2006 (2 μg/mL). For SCC*mec* IV isolates, MIC values decreased from 2002 to 2003 and remained stable in subsequent years.

Analysis of SCC*mec* III isolates by PFGE revealed the presence of four clones. These clones were divided based on a similarity coefficient ≥80% and identified with capital letters. There was a predominance of clone A, comprising five isolates. When all isolates were analyzed, 16 *S. aureus* strains isolated from the Internal Medicine, Surgery, Emergency and other wards complexes were included in this clone (Table 1