Prevalence of SCCmec Type IV in Nosocomial Bloodstream Isolates of Methicillin-Resistant *Staphylococcus aureus*

Priscila de A. Trindade,¹ Renata L. Pacheco,¹ Silvia F. Costa,¹ Flavia Rossi,² Antonio A. Barone,¹ Elsa M. Mamizuka,³ and Anna S. Levin^{1*}

Departments of Infectious Diseases and Hospital Infection Control, LIM 54, Hospital das Clínicas, University of São Paulo, São Paulo, Brazil¹; LIM 03, Hospital das Clínicas, University of São Paulo, São Paulo, Brazil²; and Department of Clinical Analyses, Faculdade de Ciências Farmacêuticas, University of São Paulo, São Paulo, Brazil³

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Over a period of 7 months, 151 consecutive methicillin-resistant *Staphylococcus aureus* blood isolates were evaluated. None was community acquired. Twenty (13%) were susceptible to four or more antimicrobials, and 95% of these isolates were identified as SCCmec type IV. Molecular typing demonstrated four patterns, with one predominant pattern. Although usually community acquired, SCCmec type IV in our setting is clearly nosocomial.

Staphylococcus aureus has long been recognized as an important and versatile human pathogen (11). In Brazil, the frequency of isolation of *S. aureus* is high, mainly as an important cause of nosocomial infection (21). *S. aureus* is responsible for 20% of nosocomial primary bloodstream infections at the Hospital das Clínicas, and 40 to 70% of the isolates are methicillin resistant (E. Girão, personal communication). Methicillin resistance in staphylococci is caused by the expression of PBP2a encoded by the *mecA* gene (15) that is located on a genetic element called the staphylococcal cassette chromosome (SCC). SCCmec is a group of mobile DNA elements of 21 to 67 kb that is integrated into the chromosome of methicillin-resistant *S. aureus* (MRSA) (8, 10). To confer motility, SCCmec carries two specific genes, designated cassette chromosome recombinase A and B (*ccrA* and *ccrB*), that encode recombinases of the invertase/resolvase family (9). In the presence of CcrA and CcrB, SCCmec integrates into the chromosome and is also precisely excised from the chromosome. Hospital-acquired MRSA isolates are typically resistant to multiple antibiotics. Recently, the appearance of community-acquired MRSA strains has been described (6). In contrast to hospital-acquired MRSA, these strains are commonly susceptible to the majority of other non- β -lactam antibiotics, present multiple patterns by pulsed-field gel electrophoresis (PFGE), and have a type IV SCCmec (13). During the last year we observed in our hospital an increasing proportion of MRSA strains that are susceptible to non- β -lactam antimicrobials. These strains, designated as nonmultiresistant oxacillin-resistant *S. aureus* (NORSA), were also reported as nosocomial agents in Australia and the United States (5, 18). The aim of this study was to evaluate the presence of SCCmec type IV and the predominant clones among NORSA bloodstream isolates from our hospital.

One isolate per patient was identified using an automated

system (VITEK; BioMérieux, Durham, NC). Clinical data on the patients were obtained from their records. The infection was then classified as follows: nosocomial, according to definitions of the Centers for Disease Control and Prevention (4); community acquired if culture isolates were positive within 48 h of admission; or healthcare associated if the patient had been admitted to a hospital or to a nursing home or another healthcare-assistance unit within the previous 12 months (3).

MICs were determined by the broth microdilution method according to the Clinical and Laboratory Standards Institutes (formerly the National Committee for Clinical Laboratory Standards) (16). Strains susceptible to four or more antibiotics were considered NORSA and further studied.

The presence of the *mecA* gene was confirmed by amplifying a 214-bp fragment using an NCL-SA-PS kit (Novo Castra, United Kingdom). Molecular typing was done by digesting whole-cell DNA with SmaI macrorestriction enzyme and determining the fragment-size patterns obtained on PFGE using a CHEF DR-II apparatus (Bio-Rad Laboratories) (2). Patterns were analyzed as recommended by Tenover et al. (25). Types were identified using letters, and subtypes were numbered. SCCmec typing was done using a multiplex PCR method as described by Oliveira et al. (20). MRSA strains NCTC 10442, N315, 85/2082, and JSCS 1968 were included as controls for SCCmec types I, II, III, and IV, respectively. The *ccr* gene complex was determined using PCR as described previously (10).

During the study 151 MRSA isolates were obtained. None was community acquired. There were 20 (13%) NORSA isolates: 18 were nosocomial and 2 were healthcare associated. The median age of study subjects was 28 years (range, 3 days to 70 years). These NORSA isolates were selected for further characterization. The oxacillin MICs for the NORSA isolates varied from 4 to >128 mg/liter. All isolates were susceptible to vancomycin (MIC $\leq$ 1 mg/liter), trimethoprim/sulfamethoxazole (MIC $\leq$ 2/38 mg/liter), and ciprofloxacin (MIC $\leq$ 1 mg/liter). Susceptibility was 70% for gentamicin and tetracycline and 20% for erythromycin.

All isolates were positive for the gene *mecA*. PFGE demon-

* Corresponding author. Mailing address: Departments of Infectious Diseases and Hospital Infection Control, LIM 54, Hospital das Clínicas, University of São Paulo, Rua Harmonia 564/52, São Paulo-SP 05435000, Brazil. Phone: 55 11 3069 7066, ext. 3. Fax: 55 11 3069 7066, ext. 3. E-mail: gcih@hcnnet.usp.br.

FIG. 1. (a) Distribution of 17 MRSA isolates in the hospital's main building (the other 3 isolates were from three different buildings). The numbers correspond to the isolates. *, nosocomial infection; #, healthcare-associated infection. Colored circles indicate different PFGE patterns as follows: pink, pattern A; blue, pattern B; yellow, pattern B1; green, pattern C. Colored squares indicate *SCCmec* types as follows: yellow, type IV; red, type IV variant. Colored triangles indicate diagnosis of infection as follows: pink, endocarditis; red, primary bloodstream infection; green, omphalitis; yellow, urinary tract infection; blue, surgical site infection. (b) Molecular typing results with pulsed-field gel electrophoresis identified the following patterns and strains: pattern A (lanes 2 to 11 and 13 to 15), pattern B (lanes 16 to 19), pattern C (lane 21), pattern B1 (lane 22), pattern D (lane 23), Brazilian endemic clone (lane 24), strain NCTC 10442 (lane 25), strain N315 (lane 26), strain 85/2082 (lane 27), strain JSCS1968 (lane 28), and strain JSCS1978 (lane 29). Molecular weight markers (48.5 kb) are in lanes 1, 12, 20, and 30. (c) Results of *SCCmec* typing identified the following types and strains: type IV variant (lanes 2, 4, 20, and 21), type IV (lanes 3, 5 to 13, 16 to 19, and 23), type IIIA (lane 22), strain NCTC 10442 (*SCCmec* type I; lane 24), strain N315 (*SCCmec* type II; lane 25), strain 85/2082 (*SCCmec* type III; lane 26), and strain JSCS 1968 (*SCCmec* type IV; lane 27). Lanes 1, 14, and 15 are DNA molecular size markers (1-kb DNA ladder; Invitrogen); lane 28 is a negative control (water).

strated four major profiles with a predominant one present in 13 (65%) isolates. Fifteen (75%) isolates presented *SCCmec* type IV, four were type IV variant, and one was type IIIA. All isolates presenting *SCCmec* type IV or IV variant presented a type 2 *ccr* complex gene.

SCCmec type IV occurred in patients from different hospital areas. The four *SCCmec* type IV variant isolates were from patients who were in the same hospital unit or had been in the unit, and all of them presented a similar PFGE profile that differed from the predominant one (Fig. 1).

A number of studies have reported the emergence of community-acquired MRSA (6, 12, 14, 17, 24), but in many of these studies the definition of community acquired was not given or varied greatly (22). These community-acquired isolates, although resistant to beta-lactams, are susceptible to other classes of drugs and belong to clones that are different from the nosocomial isolates (1).

Our NORSA isolates presented *SCCmec* type IV, which is

similar to the isolates described in the community (7, 19), and demonstrated four PFGE patterns with one predominant clone. This predominant clone of NORSA was present in different units and is distinct from the Brazilian endemic clone of MRSA. These isolates could represent a new lineage of MRSA emerging in the hospital.

All isolates were susceptible to sulfamethoxazole-trimethoprim, suggesting that this profile could be used as a phenotypic marker. This strategy should be viewed with caution, as the acquisition of other resistance genes by these strains is possible due to the presence of the insertion sequence *IS431* in the *SCCmec* type IV (7). Also, one of the isolates that was susceptible to sulfamethoxazole-trimethoprim presented a type IIIA *SCCmec*, which is characteristic of the multiresistant Brazilian endemic clone (19).

It is possible that these NORSA clones originated in the community and were introduced by patients who were hospitalized. However, during the study period there were no truly

community-acquired cases, suggesting that this is a rare occurrence. In that case, once introduced into the hospital, the SCCmec type IV strains may present a competitive advantage over the predominant endemic multiresistant MRSA clone. It has been suggested that their multiplication and transmission rates could be superior to those of MRSA strains with other SCCmec types (18). Growth regulatory factors (23) or virulence factors (1) may explain their success. Less probably, SCCmec type IV *S. aureus* is originally nosocomial and has spread to the community.

In conclusion, although typically described as a predominantly community-acquired pathogen, SCCmec type IV *S. aureus* in our setting is clearly nosocomial.

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