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Genotyping of methicillin-resistant *Staphylococcus aureus* isolates obtained in the Northeast region of Brazil

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major agent of hospital infections worldwide. In Brazil, a multiresistant MRSA lineage (ST239-SCC*mec*IIIA), the so-called Brazilian epidemic clone (BEC), has predominated in all regions. However, an increase in nosocomial infections caused by non-multiresistant MRSA clones has recently been observed. In the present study, 45 clinical isolates of MRSA obtained from a university hospital located in Natal city, Brazil, were identified by standard laboratory methods and molecularly characterized using staphylococcal chromosome cassette *mec* (SCC*mec*) typing and pulsed-field gel electrophoresis. Antimicrobial susceptibility testing was carried out using CLSI methods. The MRSA isolates studied displayed a total of 8 different pulsed-field gel electrophoresis patterns (types A to H) with predominance (73%) of pattern A (BEC-related). However, MRSA harboring SCC*mec* type IV were also identified, 3 (7%) of which were genetically related to the pediatric clone - USA800 (ST5-SCC*mec*IV). In addition, we found a considerable genetic diversity within BEC isolates. MRSA displaying SCC*mec*IV are frequently susceptible to the majority of non-β-lactam antibiotics. However, emergence of multiresistant variants of USA800 was detected.

Key words: Methicillin-resistant *Staphylococcus aureus*; MRSA; Antimicrobial resistance; Brazilian epidemic clone; Pediatric clone; SCC*mec*IV

Staphylococcus aureus is recognized as one of the most important human pathogens. These versatile bacteria can be involved in nosocomial or community-associated infections (1,2). The increasing incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) has become a serious clinical and therapeutic problem. Hospital-acquired MRSA (HA-MRSA) are generally resistant to many antimicrobial agents such as quinolones, aminoglycosides, tetracyclines, and macrolides, hindering treatment and prolonging hospital stay (3). Methicillin resistance is determined in staphylococci by the staphylococcal cassette chromosome *mec* (SCC*mec*) carrying the *mec*A gene, which encodes the altered penicillin-binding protein 2A or 2', which in turn confers cross-resistance to all β-lactam antibiotics (1).

The increased prevalence of nosocomial infections caused by MRSA during the last two decades throughout the world has been associated with the widespread occurrence of specific international MRSA lineages (3). Based on the genotyping techniques of pulsed-field gel electrophoresis (PFGE), SCC*mec* typing and multilocus sequence typing, various pandemic HA-MRSA clones have been identified, including the Iberian clone (USA500; ST247-SCC*mec*IA), the Brazilian epidemic clone - BEC (ST239-SCC*mec*IIIA), the New York/Japan clone (USA100; ST5-SCC*mec*II), the pediatric clone (USA800; ST5-SCC*mec*IV or SCC*mec*VI), and the EMRSA-16 clone (USA200; ST36-SCC*mec*II) (1,3,4). Studies conducted in Brazil have shown the predominance of BEC, a universally occurring multiresistant clone first described in Brazil in 1992, which has accounted for 70-80% of the total MRSA isolated in Brazilian hospitals

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(5,6). Recently, the number of reported infections caused by non-multiresistant MRSA (nmMRSA) in hospitals has increased (7,8). In contrast to most HA-MRSA, these strains are commonly susceptible to the majority of other non-β-lactam antibiotics and carry SCC*mec* type IV (9). The incidence of nosocomial infections caused by nmMRSA has also increased in Brazil (9,10). Thus, due to recent changes in MRSA epidemiology in healthcare facilities throughout the world, the objective of the present study was to genotype MRSA isolates collected at a university hospital in Natal city, RN, located in the Northeast of Brazil.

The study was carried out using 45 randomly chosen MRSA isolates, 42 obtained from the Onofre Lopes University Hospital, a tertiary reference hospital with about 190 beds distributed over 10 wards of various specialties and one intensive care unit. The remaining 3 isolates were obtained from the maternity unit of the teaching hospital and a private laboratory, both located in the city of Natal. The study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte, according to protocol No. 109/2006. The identification of these isolates was confirmed using Gram stain, catalase and free coagulase tests. The isolates were stored at -70°C in trypticase soy broth (Becton Dickinson, USA) containing 10% (w/v) glycerol. The disk-diffusion test was performed according to the recommendations of the Clinical and Laboratory Standards Institute (11). Methicillin agar screening (25Met) was carried out using trypticase soy agar containing 25 µg/mL methicillin (Sigma, USA) and a heavy bacterial inoculum (10^9-10^{10} cfu) as described previously (12). The methicillin-susceptible strain of *S. aureus* ATCC 29213 and the MRSA isolate BMB9393 were used to control the susceptibility tests. PFGE of total DNA was performed as described elsewhere (5). The BMB9393, AM771 and NY17859 representatives of the BEC, pediatric and New York/Japan clones, respectively, were used to compare the PFGE band patterns. The primary criterion used to define PFGE types was that described by Tenover et al. (13). However, due to the wide variety of BEC subtypes detected among the isolates studied, PFGE patterns were also compared using the GelCompar II software, version 4.01 (Applied Maths, Belgium). Thus, to analyze the correlations of the banding patterns a similarity index was determined for each pair of strains using the Dice coefficient with 0.5% band tolerance. Finally, SCC*mec* typing was performed by the multiplex polymerase chain reaction according to a previously described methodology (14).

The total number of MRSA isolated at Onofre Lopes University Hospital in 2004, 2005, and 2006 was 39, 39, and 38, respectively. MRSA accounted for 49% of the *S. aureus* recovered by the clinical laboratory. Of these isolates, 42 (36%) were selected at random from a single patient each to perform the molecular characterization, along with the three isolates obtained at the maternity unit and at a private laboratory in Natal. These isolates were obtained from different sites, such as secretions (51%), catheters (19%), blood (15%), urine (10%), and others (5%). The mean age of the patients studied was 56 years, ranging from 7 months to 84 years.

The analysis of the 45 isolates by the Tenover criteria revealed 8 different PFGE patterns (types A to H). Eight type A isolates (18%) displayed a pattern identical to that of BMB9393 (pattern A_1), a prototype BEC strain. However, 25 additional isolates were visually classified as BEC subtypes (A_2-A_{16}). Due to the diversity of subtypes A, the genetic relationship among these isolates was also confirmed by plotting the PFGE patterns in a dendrogram. The hierarchical clustering of the patterns confirmed that the BEC subtypes were closer to the BEC prototype strain than the other isolates analyzed (Figure 1). All BEC-related isolates harbored the SCC*mec*IIIA element. The strains belonging to BEC and its subtypes were resistant to most of the antimicrobials tested, except vancomycin and one or two other drugs, and showed homogeneous resistance to methicillin (since they all grew confluently on 25 µg/mL methicillin plates). The BEC strain is endemic in Brazilian hospitals, and its predominance among HA-MRSA has been reported since 1992, when it was first described in the country (5,6,12). This clone has also been isolated in other South American countries, in Europe and in Asia (1,3), demonstrating a superior ability for worldwide dissemination. It was previously reported that BEC has advantageous properties such as enhanced biofilm production, as well as high adhesive and invasive properties, a fact that may explain the bacterial adaptation to the hospital environment and its international propagation (15).

In the present study, we observed a wide variability of PFGE patterns for type A (BEC isolates), with a total of 16 subtypes. It is interesting to note that pattern A_1 was observed in only 24.2% of the BEC isolates. BEC isolates have spread throughout Brazil for more than a decade and previous studies have shown that pattern A_1 accounted for 70-80% of the BEC subtypes (5,12,15). Thus, it seems likely that small genomic changes may have occurred during these years resulting in BEC variants also well-adapted to cause severe hospital infections. It is possible that these genetic changes represent important mechanisms of clonal divergence (expansion) and may have some significance in a specific epidemiological scenario. Previous investigations by our group using microarray technology to study the global genome of BEC isolates showed that these isolates can carry different mobile genetic elements, mainly bacteriophages (Figueiredo AMS, Dunman PM, unpublished results). Genetic variation among isolates within a clone has also been demonstrated by others (16).

The three isolates grouped into cluster B according to Tenover criteria had more than six PFGE band differences from BEC isolate BMB9393 and displayed SCC*mec* type IV. The pattern B (B_1-B_3) isolates were very similar to those of the AM771 strain, a representative of USA800, also known

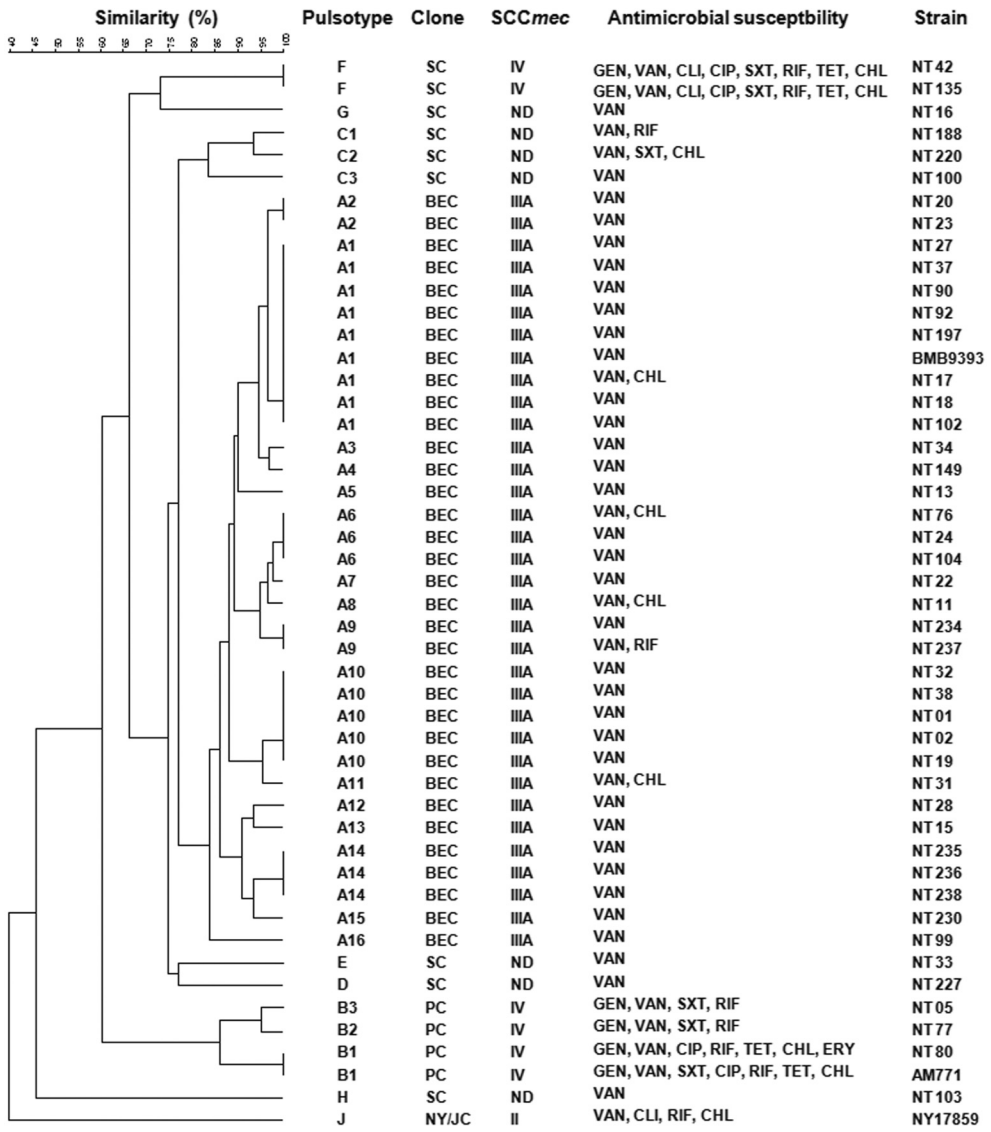

Figure 1. Dendrogram generated by computerized analysis (Gel Compar II, version 4.01) of methicillin-resistant *Staphylococcus aureus* band profiles obtained with pulsed-field gel electrophoresis. Clones: SC = sporadic clone; BEC = Brazilian epidemic clone; PC = pediatric clone; NY/JC = New York/Japan clone. Antimicrobial agents: GEN = gentamicin; VAN = vancomycin; CLI = clindamycin; CIP = ciprofloxacin; SXT = trimethoprim–sulfamethoxazole; RIF = rifampin; TET = tetracycline; CHL = chloramphenicol; ERY = erythomycin.

as the pediatric clone. Finally, nine other isolates differed from BEC, pediatric and New York/Japan clones by more than six bands and were classified as sporadic clones (types C-H). The two isolates displaying type F also carried SCC*mec*IV. Three isolates harboring SCC*mec* type IV (2 type F and 1 type B) were highly susceptible (resistant to the β-lactams only). However, two other B-type isolates (USA800-related) displayed lower susceptibility to antimicrobial drugs. In addition, all SCC*mec*IV isolates showed heterogeneous resistance to methicillin since they formed isolated colonies in 25Met.

Despite the intensive use of antimicrobial agents in hospitals, an increasing incidence of nmMRSA has been reported in different countries (7,9,17). Previous studies have also shown that community-acquired MRSA, which are generally more susceptible to antibiotics than HA-MRSA isolates, have also emerged as healthcare-associated pathogens (2,17). The detection of USA800 (pediatric

clone) in Natal corroborates other studies indicating that this clone type is an important emergent nosocomial agent in Brazil (9,10,12). USA800 isolates have been recently detected in hospitals located in Rio de Janeiro and Recife (10). This clone was also reported to colonize health workers of a home care service in Brazil (18). Although most SCC*mec*IV isolates are highly susceptible to non-β-lactams, in the present study two of the three USA800 isolates were resistant not only to β-lactam antibiotics, but also to erythromycin, clindamycin, tetracycline, and ciprofloxacin, a fact that may indicate a high ability of these SCC*mec*IV isolates to acquire multiple resistant genes.

The first report of USA800 isolates occurred in a pediatric hospital in Portugal (19). However, in the present study, all patients infected with USA800 were adults or elderly persons. Similar results were obtained by de Miranda et al. (10) who described the isolation of USA800 from immunocompromised adult and elderly individuals. The emergence of nmMRSA isolates in nosocomial environments does not support the theory that points to multiresistance as the only factor involved in the success of specific MRSA clones. It is likely that USA800 isolates own particular virulence traits that contribute to the adaptation of these bacteria as international nosocomial pathogens (1,3,10). Laurent et al. (20) have suggested that strains having a non-multiresistant phenotype may display advantageous properties that would enable them to supplant multiresistant MRSA isolates in hospital environments. Other studies have indicated that high growth rate, the ability to form biofilm on inert polystyrene surfaces and the presence of the *ecg* locus, which encodes the enterotoxins SEG, SEI, SEM, and SEO, may have contributed, at least in part, to the performance of these organisms as global nosocomial pathogens (10,20).

Our results demonstrate the predominance of BEC among the nosocomial isolates in Natal, and reveal a considerable genetic diversity within this clone. Also, we report the emergence of multiresistant variants of USA800 in this city. Our data support the importance of genotyping to monitor the dynamics of MRSA epidemiology in hospitals.

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