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Isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) not belonging to the Brazilian epidemic clone

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of healthcare-associated infections. Isolates of MRSA not belonging to the Brazilian Epidemic Clone (BEC) are colonizing and infecting patients at the hospital. These include strains carrying *SCCmec type II*, not previously described in the city of São Paulo, showing a trend to a MRSA genetic diversity.

Keywords: MRSA, SCCmec, Genetic diversity, Colonization, Infection

Findings

SCCmec typing revealed that Brazilian clone isolates harbor SCCmec III. We found that is possible a MRSA genetic diversity in Brazil, when strains of MRSA not belonging to the Brazilian Epidemic Clone (BEC) that were isolated in patients at the hospital.

Note

Methicillin-resistant *Staphylococcus aureus* (MRSA) is involved in increasing number of serious infectious with high risk for morbidity and mortality and it is an important cause of healthcare-associated infections [1]. Although the active surveillance and the impact of MRSA colonization on the occurrence of *S. aureus* infections are unclear, some studies described that asymptomatic colonization with MRSA is a risk factor for in a subsequent MRSA infection patients [2, 3].

In Brazil, a considerable number of hospital infections have been caused by an unique multi-resistant MRSA clone designated as the Brazilian epidemic clone (BEC) [4]. *SCCmec* typing revealed that Brazilian clone isolates harbor *SCCmec III* [5].

During a study on hand hygiene compliance, samples were obtained by nasal swab every four days from 446 patients admitted patients at two step-down units (SDUs) of a private tertiary care hospital in São Paulo, Brazil. Blood Culture samples were also obtained at an intensive care unit (ICU) of the same hospital, as part of an antimicrobial resistance surveillance project (unpublished data). The study period was from March 20, 2007 to September 20, 2007 [6]. The open model ICU is a 38-bed medical-surgical unit; where approximately 2,200 patients are admitted annually and the SDUs are medical-surgical 20-bed units each. Nasal samples were plated onto chromagar (CHROMagar, MRSA, CHROMagar, Paris, France) and selective staphylococcal media (Mannitol Salt Agar). Blood cultures were performed at BACTEC 9240 instrument (BACTEC 9240, BD Diagnostics, Sparks, MD) and the positive samples were plated onto chocolate and sheep blood agar.

Twenty-one MRSA strains were isolated from nasal carriers (in the SDUs) and two MRSA strains obtained from patients with bloodstream infections (in the ICU). *Staphylococcus* species were confirmed by an automated (Vitek 2 System,, Hazelwood, MO)) and manual biochemical tests. For the susceptibility profile determination oxacillin agar screening was used with automated system according with Clinical Laboratory Standards Institute (CLSI) recommendations [7].

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SCCmec typing was performed using a polymerase chain reaction (PCR) method [8]. All MRSA isolates from nasal cultures were typed by pulsed-field gel electrophoresis (PFGE). Bacterial cells grown overnight were embedded in agarose, lysed and deproteinated to isolate near intact genomic DNA. The DNA was digested with restriction endonuclease *SmaI* (3 μl/sample) (new England Biolabs, Ipswich, MA) and the restriction fragments were separated using CHEF DR III (BioRad, Hercules, CA) under the following conditions: 1 % agarose, 0.5 × TBE, 200 v and a switch interval ranging from 5-40 sec over 21 h period. The gel was stained in a 1.5 μl/ml ethidium bromide solution [8].

PFGE patterns were compared to MRSA Brazilian Epidemic, Pediatric and New York-Japan clones. Tenover's criteria [9] were used to PFGE analyses, patterns were designated by a capital letter (e.g. A, B, C) when all bands were shown to match. When a difference in one to six bands occurred, the strains were assigned as a subtype or variant of the major type, and were designated with the same capital letter followed by an Arabic number (Ex.: A1, A2, A3). Cluster analysis was provided by BioNumeric software (Applied Maths, Kortrijk, Belgium).

Three clone profiles with different subtypes were found. Clone A defined as the Brazilian Epidemic Clone (BEC) was presented in 13 samples, and included the following subtypes: A1, A2 (two cases each), A3, A4, A5 (two cases), A6, A7, A8, A9, A10 and A11. Clone B was identified in 7 cases: B1, B2 (two cases each), B3, B4 (two cases) and B5. One case exhibited clone C. The dendrogram showed that the clone B is related to New York/Japan clone. Blood culture isolates showed MRSA harbouring *SCCmec type II* with a PFGE profile similar to the New-York Japan clone, also observed in bloodstream isolates from other hospitals (fig 1).

MRSA remains as an important pathogen in Brazilian hospitals, involved in serious infections. While a study was performed in Brazil with strains isolated from 1995 to 1997 in several private and public hospitals showed all isolates with the same PFGE pattern (BEC), in our sample these represent only 61.9 % [10].

Recent studies published in Brazil show *Type III* and even *Type IVSCCmec* MRSA in hospital isolates [11, 12] indicating that the strains obtained from our blood cultures do not represent a typical pattern and are similar to New York-Japan clone.

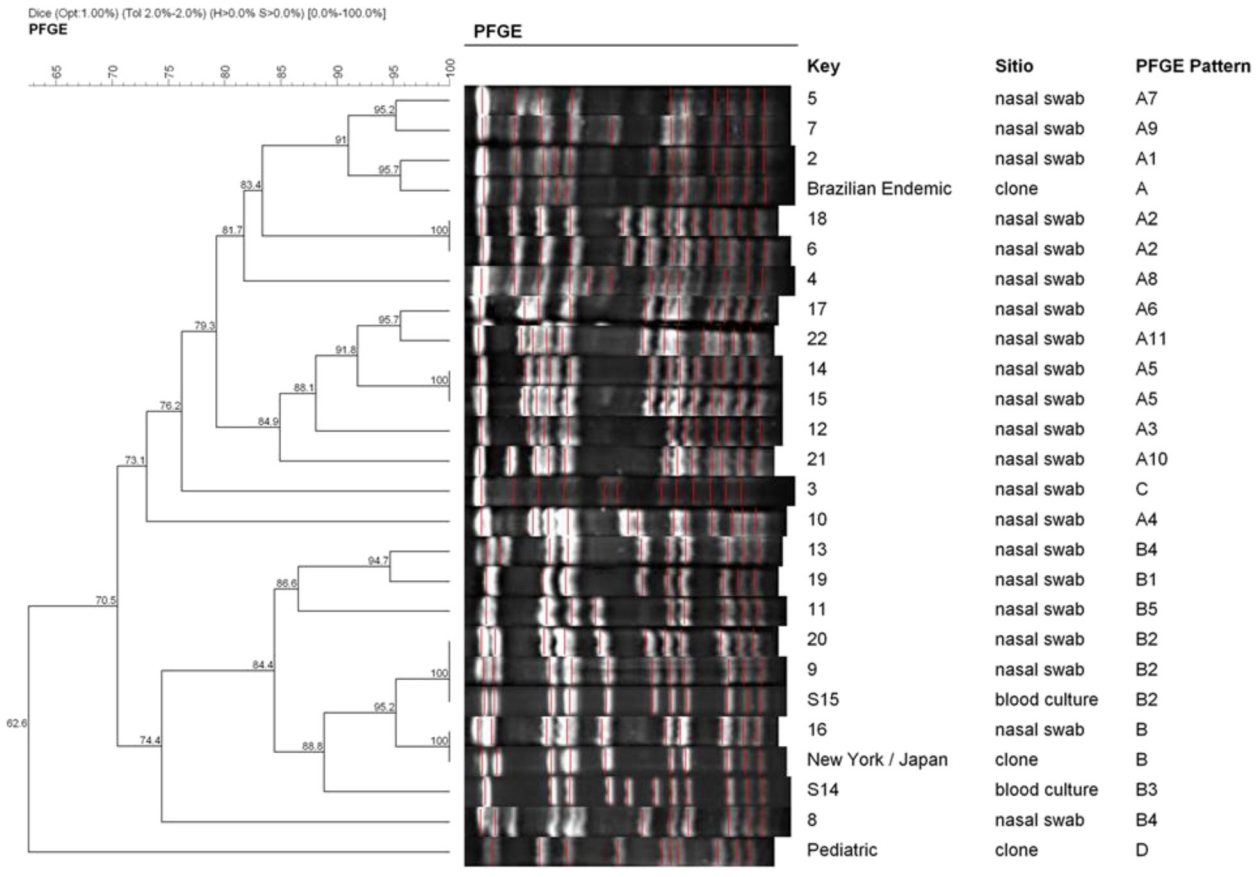

Fig. 1 Dendrogram with clone profiles

In-patient colonization and infection with isolates of MRSA not belonging to the Brazilian Epidemic Clone (BEC) have been observed, and include strains carrying *SCCmec type II*, not previously described in the city of São Paulo, showing a trend toward MRSA genetic diversity.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
MDVM, LC, ACCP, MSJr, IS, FMC, JP, OFPS, and ARM participated in the data collected and data analysis. MDVM, ACCP, OFPS, and ARM participated in the design and coordination. MDVM, ACCP IS, JP, and ARM helped to draft the manuscript and to provide critical review of the manuscript. All authors read and approved the final manuscript.

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