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Brief communication

Complete substitution of the Brazilian endemic clone by other methicillin-resistant *Staphylococcus aureus* lineages in two public hospitals in Rio de Janeiro, Brazil

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ABSTRACT

Staphylococcus aureus is an important cause of bloodstream infections. Therefore, the main purpose of this work was to characterize a collection of 139 *S. aureus* isolates from bloodstream infections in two public hospitals in relation to their antimicrobial susceptibility profile, staphylococcal cassette chromosome *mec* types, and clonal relationship. Methicillin resistance and resistance to other 12 agents were accessed by the disk diffusion test. Minimum inhibitory concentration to mupirocin was also determined. The SCC*mec* types were accessed by multiplex PCR, and the clonal relationship was determined by pulsed field gel electrophoresis method and restriction modification system characterization. Besides, multilocus sequence typing was performed for representative methicillin-resistant *S. aureus* isolates. The military hospital showed a dissemination of the New York/Japan (USA100/ST5/CC5/SCC*mec*II) lineage associated to multidrug resistance, including mupirocin resistance, and the teaching hospital presented polyclonal and non-multidrug resistant MRSA isolates. Complete substitution of the Brazilian endemic clone by other lineages was found in both hospitals. These findings can highlight differences in policy control and prevention of infections used in the hospitals and a change in the epidemiological profile of MRSA in Brazilian hospitals, with the replacement of BEC, a previously well-established clone, by other lineages.

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Staphylococcus aureus is considered an important cause of bloodstream infections (BSI), which is associated with high rates of mortality and morbidity.[1] Analysis of molecular characteristics of *S. aureus* isolates have indicated a variety of circulating lineages inside hospitals, according to the geographic area. In United States, the New York/Japan clone (USA100/ST5/CC5/SCC*mec*II) has been replaced by the community-acquired MRSA (USA300/ST8/CC8/SCC*mec*IV) lineage.[1] In China, two pandemic hospital-acquired MRSA (HA-MRSA) clones are disseminated, the Brazilian endemic clone (BEC/ST239/CC8/SCC*mec*III) and the USA100.[2] In Brazil, the BEC lineage remained prevalent inside hospitals,[3] but an increasing presence of the clones USA400 (ST1/CC1/SCC*mec*IV) and the Pediatric clone (USA800/ST5/CC5/SCC*mec*IV) have been reported in the last decade.[3,4] More recently, SCC*mec*II carrying isolates associated to the CC5 were detected replacing, almost completely the BEC lineage among BSI isolates at a hospital located in São Paulo city.[5]

The implementation of a Health Care Associated Prevention and Control Committee (HAIPCC) is mandatory by law in Brazilian hospitals since 1997.[6] These measures apply to the whole health care system, such as the public and the private sector. Public hospitals are responsible for the care of about 75% of the Brazilian population, estimated in 192 millions of habitants (2012 data). However, funding for the Unified Health System (Sistema Único de Saúde – SUS) has not been sufficient to ensure adequate financial resources for the public health system, leading to inappropriate control of dissemination of endemic resistant microorganisms.[6] The aim of the present study was to characterize *S. aureus* isolates from BSI at two public hospitals as their antimicrobial resistance and clonal dissemination associated with clinical aspects.

We evaluated 139 *S. aureus* consecutive isolates from BSI recovered in a 532-bed military hospital (Hospital 1) and in a 490-bed university teaching hospital (Hospital 2), both located in Rio de Janeiro city, between January 2008 and June 2009. This study was approved by the Research Ethics Committee under No. 159/07. Clinical data from patients with *S. aureus* BSI were retrospectively abstracted from the hospital records. *S. aureus* isolates were identified by standard methods. BSIs were classified as hospital-acquired (HA) or community-acquired (CA) according to the Centers for Disease Control (CDC) criteria.

In order to characterize methicillin resistance, cefoxitin disk diffusion test was used according to CLSI.[7] Isolates identified as MRSA were also submitted to antimicrobial susceptibility test for 12 agents by the disk diffusion method.[7] Minimum inhibitory concentration (MIC) to mupirocin was determined by Etest® (AB-Biodisk, Solna, Sweden). The SCC*mec* types were assessed by multiplex-PCR for MRSA isolates.[8] Clonal relationship was determined by pulsed-field gel electrophoresis (PFGE).[9] Restriction modification system characterization (RM test)[10] was used to identify the clonal complexes (CC) of methicillin susceptible *S. aureus* (MSSA) isolates. Besides, multilocus sequence typing (MLST) was performed for representative MRSA isolates.[11] The Fisher's exact test and chi-square test were used to compare categorical data. Significance level was established at 5% ($p < 0.05$).

The distribution of the 139 *S. aureus* isolates and their SCC*mec* types and clonal complexes in each hospital is shown in Table 1. Out of 75 isolates of Hospital 1 (H1), 32 (43%) were characterized as MRSA, whereas in Hospital 2 (H2) from 64 isolates 13 (20%) were MRSA isolates ($p = 0.006$). While at H1 the majority of MRSA isolates carried the SCC*mec* type II (69%), at H2 the SCC*mec* type IV (69%) was the most prevalent. Overall, only one isolate from H2 carried the SCC*mec* type III and was assigned as ST889/CC5. In relation to the CC assignment, the majority of MRSA and MSSA isolates (83%; 62/75) at H1 were related to CC1 and CC5. However, there was a polyclonal distribution of *S. aureus* isolates causing BSI (CCs 1, 5, 8, 30, 45, 221) at H2 regardless of their methicillin resistance.

Characteristics of 45 MRSA isolates from BSI of patients from the two hospitals evaluated are presented in Table 2. Overall, 93% (42 isolates), 75% (34), and 35% (16) of the MRSA isolates were resistant to ciprofloxacin, clindamycin, and mupirocin, respectively. Among the MRSA isolates from H1, resistance to three or more drug classes (multidrug resistance

Table 1 – Distribution of 139 methicillin-susceptible and -resistant *Staphylococcus aureus* isolates, SCC*mec* types and clonal complexes from bloodstream infections.

Hospital/methicillin-resistance (number of isolates)	N (%) of isolates									
	SCC*mec* type			Clonal complexes						
	II	III	IV	1	5	8	30	45	221	ND
Hospital 1										
MRSA (32)	22 (69)	0	10 (31)	9 (28)	23 (72)	0	0	0	0	0
MSSA (43)	–	–	–	14 (32)	16 (37)	1 (3)	2 (5)	4 (9)	0	6 (14)
Total (75)				23 (31)	39 (52)	1 (1)	2 (3)	4 (5)	0	6 (8)
Hospital 2										
MRSA (13)	2 (23)	1[a] (8)	9 (69)	3 (23)	7 (53)	0	1 (8)	1 (8)	1 (8)	0
MSSA (51)	–	–	–	17 (33)	6 (12)	7 (14)	7 (14)	4 (8)	0	10 (19)
Total (64)				20 (31)	13 (20)	7(11)	8 (12.5)	5 (8)	1 (2)	10 (15.5)

MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; SCC*mec*, *Staphylococcal* cassette chromosome *mec*; ND, not determined; N, number.

[a] ST889/CC5.