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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 In United States, the New York/Japan clone (USA100/ST5/CC5/SCC*mec*II) has been replaced by the community-acquired MRSA (USA300/ST8/CC8/SCC*mec*IV) lineage.[1] In China, two pandemic hospital-acquired MRSA (HA-MRSA) clones are disseminated, the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.

Table 2 – General characteristics of 45 methicillin-resistant *Staphylococcus aureus* isolates from bloodstream infections.

Hospital/genotype (no of isolates)	Isolate number	Isolation date (mm/dd/yy)	Unit or floor	Acquisition mode	SCC*mec* type	PFGE subtype	Clonality	ST/CC	Antimicrobial resistance profile
Hospital 1 (32)									
A (22)	1223a	01/10/2008	11	HA	II	A1	USA100	5/5	cip cli ery mup tec
	1224a	01/12/2008	ICU	HA	II	A1	USA100	5/5	cip cli ery mup
	1255a	09/02/2008	ICU	HA	II	A1	USA100	5/5	cip cli ery mup
	1258a	09/16/2008	ICU	HA	II	A1	USA100	5/5	cip cli ery mup
	1265a	09/23/2008	9	HA	II	A1	USA100	5/5	cip cli ery mup
	1266a	09/24/2008	9	HA	II	A1	USA100	5/5	cip cli ery mup
	1276a	02/12/2008	9	HA	II	A1	USA100	5/5	cip cli ery mup
	1288a	03/12/2009	ICU	HA	II	A1	USA100	5/5	cip cli ery mup
	1289a	03/13/2009	11	HA	II	A1	USA100	5/5	cip cli ery mup
	1309a	03/14/2009	ND	HA	II	A1	USA100	5/5	cip cli ery mup clo rif
	1290a	03/19/2009	11	HA	II	A1	USA100	5/5	cip cli clo ery
	1291a	03/31/2009	9	HA	II	A1	USA100	5/5	cip cli ery mup
	1305a	06/04/2009	11	HA	II	A1	USA100	5/5	cip cli ery mup clo rif
	1308a	06/15/2009	10	HA	II	A1	USA100	5/5	cip cli ery mup clo rif
	1260a	09/17/2008	10	HA	II	A2	USA100	5/5	cip cli ery
	1263a	09/22/2008	11	HA	II	A2	USA100	5/5	cip cli ery
	1275a	12/02/2008	ICU	HA	II	A2	USA100	5/5	cip cli ery
	1238a	05/26/2008	8	HA	II	A3	USA100	5/5	cip cli ery mup
	1240a	05/27/2008	Em	HA	II	A3	USA100	5/5	cip cli ery mup
	1284a	02/02/2009	Em	HA	II	A4	USA100	5/5	cip cli ery
	1285a	02/16/2009	11	HA	II	A4	USA100	5/5	cip cli ery
	1301a	05/27/2009	IU	HA	II	A5	USA100	5/5	cip cli clo ery
B (9)	1229a	02/09/2008	11	HA	IV	B1	USA400	1/1	cip cli ery
	1237a	05/20/2008	11	HA	IV	B1	USA400	1/1	cip cli ery
	1307a	06/12/2009	ICU	HA	IV	B1	USA400	1/1	cip clo
	1231a	02/13/2008	11	HA	IV	B2	USA400	1/1	cip
	1282a	01/22/2009	ICU	HA	IV	B2	USA400	1/1	cip clo
	1268a	09/26/2008	10	HA	IV	B3	USA400	1/1	cip cli ery gen
	1283a	01/22/2009	ICU	HA	IV	B3	USA400	1/1	cip clo
	1295a	05/25/2009	9	HA	IV	B4	USA400	1/1	–
	1302a	05/21/2009	ICU	HA	IV	B5	USA400	1/1	cip clo
F (1)	1306a	06/04/2009	10	HA	II	F	ND	105/5	cip cli clo ery
Hospital 2 (13)									
A (1)	1087a	01/20/2008	11	HA	II	A1	USA100	5/5	cip cli ery mup
B (3)	1094a	01/16/2008	9	CA	IV	B1	USA400	1/1	cip cli ery
	1187a	06/12/2008	8	HA	IV	B1	USA400	1/1	cip cli clo ery
	1100a	01/27/2008	Em	CA	IV	B2	USA400	1/1	cip cli ery
C (5)	1214a	06/26/2009	7	HA	IV	C1	USA800	5/5	–
	1318a	08/16/2008	Em	HA	IV	C2	USA800	5/5	cip
	1324a	08/08/2008	Em	HA	IV	C3	USA800	5/5	cip
	1328a	12/23/2008	Em	HA	IV	C4	USA800	5/5	cip cli ery
	1326a	12/25/2008	9	HA	IV	C4	USA800	5/5	–
D (1)	1314a	11/08/2008	7	HA	IV	D	ND	484/30	cip cli ery
E (1)	1092a	02/22/2008	9	HA	III	E	ND	889/5	cip clo ery gen sut tec
G (1)	1212a	06/02/2009	ICU	HA	II	G	ND	3050/45	cip cli ery
H (1)	1219a	06/06/2009	8	HA	II	H	ND	221/221	cip cli ery

ICU, intensive care unit; Em, emergency; ND, not determined; HA, hospital acquired; CA, community acquired; SCC*mec*, *Staphylococcal* cassette chromosome *mec*; PFGE, pulsed field gel electrophoresis; ST, sequence type; CC, clonal complex; cip, ciprofloxacin; cli, clindamycin; ery, erythromycin; mup, mupirocin; tec, teicoplanin; clo, chloramphenicol; rif, rifampin; gen, gentamicin; sut, sulfamethoxazole/trimethoprim.

– MDR) was verified in 59.3% (19/32), while among the MRSA isolates from H2, MDR was found in only 23% (3/13) ($p = 0.05$). Moreover, 97% of the MRSA isolates from H1 were related to only two disseminated lineages, USA100/ST5/CC5/SCC*mec*II (69%) and USA400/ST1/CC1/SCC*mec*IV (28%), all of them causing hospital-acquired BSI. Furthermore, 94% (15/16) of mupirocin-resistant isolates were found at H1 and it was associated to the USA100 lineage. Two USA100 isolates (1238a and 1240a) showed high levels of mupirocin resistance (MIC > 1024 μg/mL) (data not shown). In this hospital, the prevalent USA100/ST5/SCC*mec*II lineage was found widely disseminated. At H2, 62% of MRSA isolates carried the SCC*mec* IV and were related to USA800 (38%) or USA400 (23%) lineages. Besides, the polyclonal presence of sporadic lineages (STs 484, 889, 3050, and 221) was identified in this hospital, the ST3050 being described for the first time in this study.

In our study, 139 *S. aureus* isolates from BSI obtained at two different public hospitals in Rio de Janeiro city were characterized regarding their antimicrobial resistance and clonal profile. We verified that almost 70% of the BSI isolates from the military public hospital (H1) carried the SCC*mec*II and were related to the USA100 lineage. All but one isolate were assigned as USA400 lineage. This lineage appears to have survival and growth advantage since it has remained for years as a major hospital-associated lineage in USA and Japan[1,12] showing the good adaptability of such clone, even in different geographic areas.

At H2, a reference teaching public hospital, a polyclonal profile was observed for the MRSA isolates. Interestingly, we previously found a similar higher clonal diversity among MRSA isolates at a private hospital.[3] These findings may be a reflection of the occurrence of fewer outbreaks due to adequate infection control measures at this particular institution, as found in this teaching public hospital. Padoveze et al.[6] conducted a cross-sectional study evaluating a collection of 153 hospitals from five different Brazilian regions. The authors showed that a minimal structure is necessary for an effective prevention of hospital infections, specially the presence of an active HAIPCC, as well as sterilization services, hand hygiene resources, and a microbiology laboratory.

Caboclo et al.[3] compared *S. aureus* isolates from two health institutions in Rio de Janeiro between 2004 and 2007. One of these institutions was the same military hospital (H1) of the current study. The authors showed a dissemination of the USA100, USA400, USA800 and BEC lineages at this military institution. Moreover, around 60% of the isolates were from the BEC lineage, showing that at the time this lineage was still highly present. Simultaneously, similar results were found in a study conducted at a private tertiary care hospital in São Paulo where 40% (13/33) of MRSA isolates belonged to BEC and 21% were related to the USA100 lineage.[13] At H2, Cavalcante et al.[14] showed that the BEC lineage was responsible for around 30% of all MRSA isolates, between 2005 and 2006. In the present study, carried out between 2008 and 2009, complete absence of the BEC lineage was verified in both public institutions evaluated, showing that certain global MRSA lineages are replacing BEC. Caiaffa-Filho et al.[5] recently evaluated a collection of 50 consecutive MRSA BSI isolates in a São Paulo tertiary care teaching hospital, between October and December 2010, and found that a single PFGE clone related to the USA100 lineage was disseminated, almost replacing the BEC in that institution during the study period. This finding highlights a possible change in the epidemiological profile of MRSA in Brazilian hospitals.

MRSA isolates were more frequently found at H1 than in the teaching hospital (H2) being associated to a MDR profile and mupirocin resistance. In Brazil, despite the legislation mandating the implementation of HAIPCC in the health system, the lack of qualified professionals, the growing health costs, and limited availability of financial resources are of great impact in the infection control.[6] Therefore, the differences regarding resistance rates observed between MRSA isolates from the H1 and H2, as well as the prevalence of specific SCC*mec* types, may be a reflection of the policy for control and prevention of infections and use of antimicrobials in the hospitals evaluated. Interestingly, the prevalent USA100 clone presenting MDR profile isolated from H1 had already been described as a MRSA-daptomycin-resistant isolate with vancomycin MIC of 4 μg/mL, also causing BSI,[15] confirming the ability of this lineage to acquire resistance determinants. Moreover, this lineage was also associated with mupirocin resistance, a drug used for decolonizing MRSA nasal carriage.[2] Although only two isolates have showed high levels of mupirocin resistance (MIC > 1024 μg/mL), both high- and low-level resistance have been associated with *S. aureus* decolonization failure.[2] As showed in a study conducted in China,[2] USA100/ST5 isolates associated with mupirocin resistance lead to possible outbreaks. The dissemination of mupirocin-resistant MRSA isolates among H1 patients in our study also highlights the importance of the judicious use of mupirocin among the hospitalized patients.

MRSA, as well as MSSA isolates, have been reported as important causes of nosocomial infections, such as BSI.[1] The present study showed that *S. aureus* isolates from the CCs 1 and 5, regardless their methicillin-resistance status, had similar clonality at both hospitals. According to Diep and Otto,[16] the emergence of the MRSA lineages around the world may be related to the successful conversion of certain MSSA isolates into MRSA isolates by the acquisition of SCC*mec*. The presence of MSSA and MRSA isolates presenting the same CCs and/or STs have already been observed in Rio de Janeiro hospitals,[4] indicating the ability of certain lineages to acquire the *mec* cassette thus providing an advantage to their spreading in health institutions.

In conclusion, although both institutions evaluated in the present study are public hospitals, the military hospital showed dissemination of the USA100/ST5/CC5/SCC*mec*II lineage associated to multidrug resistance, including to mupirocin. On the other hand, the teaching hospital presented polyclonal and non-multidrug resistant MRSA isolates. These differences may reflect the policy of control and prevention of infections and/or use of antimicrobials employed in each hospital evaluated. Moreover, complete substitution of the BEC/ST239/SCC*mec*III by other lineages was found in both hospitals, highlighting a change in the epidemiological profile of MRSA in Brazilian hospitals.

Ethics statement

The present study was approved by the Research Ethics Committee under No. 159/07.

Conflicts of interest

The authors declare no conflicts of interest.

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