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Brief communication

Community-acquired methicillin-resistant *Staphylococcus aureus* carrying SCCmec type IV and V isolated from healthy children attending public daycares in northeastern Brazil



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ABSTRACT

Nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) have increasingly been reported in healthy communities. This study aimed to assess the rate of *S. aureus* in general and MRSA in particular from nasal secretion of children in daycare centers in Vitória da Conquista, Brazil. The isolates were identified based on morphology, biochemical tests and by PCR. Detection of virulence genes, biofilm production, and susceptibility test by disk diffusion agar were performed. MRSA isolates were characterized by *spa*, SCCmec, and multilocus sequence typing (MLST). *S. aureus* were recovered from 70 (47.3%) of 148 children. Among the 11 MRSA strains (15.7%), two SCCmec types (IV and V) were detected. MLST identified four STs related to three clonal complexes (CC): 5, 45, and 398. Four *spa* types were found circulating in this setting. Resistance of *S. aureus* isolates to ampicillin, erythromycin, ciprofloxacin, clindamycin, and tetracycline was 80%, 32.8%, 7.1%, 7.1% and 4.3%, respectively. One isolate presented intermediate resistance to vancomycin detected by Etest methodology. All strains were biofilm producers. The virulence genes *seb*, *sec*, *spa*, and *pvl* were detected in some isolates. This study revealed a high rate of children carrying MRSA among healthy attendees in daycare centers in Vitória da Conquista, Brazil.

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Staphylococcus aureus may cause various infections with considerable morbidity and mortality in healthy and immunocompromised hosts.¹ Methicillin-resistant *Staphylococcus aureus* (MRSA) is commonly associated with severe nosocomial infections (HA-MRSA); however, it has been detected in individuals without risk factors for infection, referred to as community-acquired MRSA (CA-MRSA).² Methicillin resistance is carried on a staphylococcal cassette chromosome *mec* (*SCCmec*). HA-MRSA usually carry *SCCmec* types I, II, or III, whereas CA-MRSA strains commonly carry *SCCmec* types IV and V.³

The molecular characterization is important, since it enables studying the relatedness of MRSA strains, their genetic diversity, and clonal distribution. Various molecular typing techniques have been developed, including the multilocus sequence type (MLST), as well as the *SCCmec* typing, *spa* typing.³⁻⁵

Nasal colonization of *S. aureus* is common in children, but the genetic findings suggested a causal relationship between carriers, chromosome cassettes of those harboring MRSA, and invasive staphylococcal disease.⁶ In addition, the incidence of pediatric infections due to CA-MRSA, including children with no identifiable risk factors, has increased worldwide.⁷ Daycare centers (DCCs) are reservoirs of *S. aureus* in general and MRSA in particular, and these children may spread these bacteria to the community and hospitals.⁶

In Brazil little is known about the distribution and characteristics of *S. aureus* and MRSA in children as nasal carriers, particularly in those attending DCCs. The aim of the present study was to assess the rate of *S. aureus* and MRSA nasal carriage in healthy children attending public DCCs in Vitória da Conquista – Bahia State (BA), Brazil, and to identify the resistance profile, genotypic characterization, and pathogenicity.

The study was conducted from October to December 2012, in four public DCCs in Vitória da Conquista, a city in Northeastern Brazil. One hundred and forty-eight samples of nasal swabs were obtained from healthy children ranging from one to six years attending DCCs. Children treated with antibiotics in the last 30 days were not included. The isolates obtained were incubated at 37 °C for 48 h; Gram-positive cocci colonies, positive for catalase and coagulase tests were selected as presumptive *S. aureus* and identified by PCR.

MRSA isolates and reduced vancomycin activity were screened for all *S. aureus* isolates by microdilution method following the recommendations of the CLSI guidelines.⁸ Strains of *S. aureus* ATCC 29213 and ATCC 43300 were used as controls. Vancomycin-resistant isolates were confirmed using the E-test[®] (bioMérieux's, Brazil) and the bacterial growth at $\geq 16 \mu\text{g/mL}$ was indicative of resistance. Susceptibility to erythromycin, tetracycline, clindamycin, ciprofloxacin, and ampicillin were performed by disk diffusion.⁸ Inducible resistance to clindamycin was tested by 'D test'.⁸ Multidrug resistance was considered when the strain was resistant to two or more antibiotics.

The genomic DNA of all strains were extracted using the Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA), and all isolates were characterized as *SCCmec* types (I-V),³ and for the presence of virulence genes, including *sea*, *seb*, *sec*, *pvl*, *clfA*, and *spa* by PCR.⁹ Moreover, MRSA isolates were subjected to MLST and SPA typing techniques, as described previously.^{4,5}

Biofilm assays were performed in 96-well polystyrene microplates using trypticase soy broth (TSB/Difco) with 1% (w/v) glucose (TSB-1% Glc).¹⁰ The samples were compared with cultures of *Streptococcus pyogenes* ATCC75194 (non-biofilm producer sample). *S. aureus* isolates were classified as non-biofilm producers, weak producers, moderate producers, producers, and strong producers. Statistical analysis was performed by chi-square test and a *p*-value lower than 0.05 was considered statistically significant.

Out of 148 children analyzed, *S. aureus* was recovered from 70 (47.3%). Eleven (15.7%) isolates were MRSA, the majority ($n=8$; 72.7%) were *SCCmec* IVA type and three (27.2%) were *SCCmec* V type.

Resistance rates of *S. aureus* isolates to ampicillin, erythromycin, clindamycin, ciprofloxacin, and tetracycline were 80%, 32.8%, 7.1%, 7.1%, and 4.3%, respectively. One methicillin-susceptible *S. aureus* (MSSA) isolate showed reduced susceptibility to vancomycin by E-test and was confirmed with a MIC of 6 $\mu\text{g/mL}$. There were no significant differences in resistance between MRSA and MSSA isolates. The rate of resistant isolates to more than two antibiotics was 47.1% (33/70), and only 15.7% (11/70) of the isolates were susceptible to the studied antibiotics. A total of 63.6% of MRSA isolates were multidrug resistant (Table 1).

MLST performed on MRSA isolates identified four STs, related to three clonal complexes (CC): 5 (63.5%), 45 (18.2%), and 398 (18.2%). Among the four STs identified, the ST5 clone (63.6%, 7/11) was the most prevalent (Table 2). Protein A was characterized in isolates of four *spa* types. The t242 (72.7%, 8/11) was dominant among all isolates (Table 1).

Two MRSA isolates (2.8%) had the *seb* gene, and all had *spa* genes. Two strains of MSSA (2.8%) showed the *sec* gene, three (4.2%) were positive for *pvl* gene, and all had *spa* (Table 1). All *S. aureus* isolates produced biofilm (Table 2) and there was no statistical difference in biofilm production between MRSA and MSSA isolates ($p > 0.05$).

The detection of *S. aureus* (47.3%) and MRSA (15.7%) in nasal swabs of the studied children demonstrated a higher rate of these staphylococci than in studies conducted in other DCCs with rates ranging from 17% to 31.1% and 0.8 to 13.2%, respectively.^{7,11,12}

Although the prevalence of MRSA among healthy children was high, no correlation was found between carriage of MRSA and antibiotic use. In addition, the close contact among DCC attendees has also been considered a risk factor for carrying MRSA.⁶ A similar Brazilian study showed a MRSA prevalence of 1.2%¹² confirming the variations between regions. The high incidence of MRSA in healthy children is the most relevant and worrying finding for the susceptible hosts and even for the healthy carrier children if immunosuppressed. This context involves at least the risk for coagulase-positive staphylococcal infections in elderly hosts, hospitalized individuals, neonates, and the community. Furthermore, most CA-MRSA reported worldwide have been shown to carry *SCCmec* types IV and V, as found in our results.^{11,13} The isolates detected by Ho et al. had *SCCmec* IV (46.4%) or V (53.6%),¹³ unlike Lamarco-Cardoso et al. who found *SCCmec* IIIA (57%) in most Brazilian children, which is widespread in hospitals in many countries worldwide, and only three MRSA

Table 1 – Microbiological aspects and genetic characteristics of 11 MRSA isolates carriage from infants attending DCCs in Vitória da Conquista, Brazil.

Isolates (MRSA)	SCCmec typing	Resistance profile ^a	MIC (µg/mL)		Phenotype ^b	Virulence genes profiles	ST/CC	spa type
			OXA	VAN				
C18	V	amp, ery	4	–	Moderate	spa	398/398	t242
C48	IVA	amp, ery	4	2	Strong	spa	5/5	t242
C54	IVA	amp	4	2	Strong	spa	5/5	t242
C60	IVA	amp	4	2	Strong	seb, spa	2228/45	t004
C77	IVA	amp, ery	4	–	Strong	spa	5/5	t242
C80	IVA	–	4	–	Strong	spa	5/5	t002
C83	V	amp, ery	4	2	Producer	seb, spa	398/398	t242
C94	IVA	–	8	2	Strong	spa	5/5	t242
C137	V	amp, cli, ery	4	–	Strong	spa	45/45	t371
C138	IVA	amp, ery, tet	4	–	Strong	spa	5/5	t242
C152	IVA	amp, ery, cip	4	–	Strong	spa	5/5	t242

^a amp, ampicillin; ery, erythromycin; cip, ciprofloxacin; cli, clindamycin.

^b Moderate, moderate biofilm producer; producer, biofilm producer; strong, strong biofilm producer.

strains showed SCCmec IV and one SCCmec type V genes.¹² Therefore the distribution of the MRSA clone varies throughout the world.

The SCCmec IV has been strongly associated with strains causing MRSA infections in patients without risk factors in Brazil¹⁴ and elsewhere.¹⁵ Our findings point out the potential of this specific DCCs to be a reservoir of emerging MRSA genotypes and highlight the need to enhance surveillance of these bacteria and control their transmission.

The MLST and spa typing revealed a small diversity between CA-MRSA isolates. ST5 clones have emerged in hospital and community isolates, while ST45 is most often found in the community carrying SCCmecIV.^{16,17} The Clonal complexes – CC5 and CC45 – which represented 81.8% of CA-MRSA isolates in this study, are among the clonal groups known to be involved in global pandemic caused by MRSA. Meanwhile, invasive infection by CC398 rarely occurs.¹⁸

High rates of MRSA and MSSA isolates resistant to ampicillin (80%) and erythromycin (32.8%) were found, as well as low resistance rates to clindamycin, tetracycline, and ciprofloxacin. Our findings agree with previous studies in the literature, except for higher susceptibility to erythromycin elsewhere (14–20%).⁷ Thus, isolates of CA-MRSA had limited resistance to non-β-lactams. The majority of MRSA isolates (63.6%) detected herein were resistant to more than two antibiotics and this is a challenge in controlling these infections. One isolate (1.43%) with reduced susceptibility to vancomycin among MSSA strains is an important finding.

Most cases of reduced vancomycin susceptibility in *S. aureus* (vancomycin-intermediate *S. aureus*, or VISA) reported in the literature are also methicillin-resistant. However, the development of VISA in MSSA isolates have been reported.^{19,20} Thus, reduced vancomycin susceptibility can occur in *S. aureus* irrespective of background methicillin susceptibility. There are no data in the literature of CA-MRSA strains susceptible to or with reduced susceptibility to vancomycin, so this fact is of particular concern in a healthy young population. Therefore, additional studies and continuous monitoring of *S. aureus* and MRSA are recommended.

Herein, all *S. aureus* isolates were able to produce biofilm, and the percentage of MRSA and MSSA strains producing biofilm was similar. There are no studies on biofilm production of *S. aureus* recovered from healthy individuals. There is no direct relationship of the methicillin resistance profile with greater or lesser ability to produce biofilm by the strains. The majority of biofilm producer isolates were classified as strong producers. This feature may act as an additional barrier to control an infection with antimicrobials.²

Protein A encoded by spa gene is a hallmark of *S. aureus*.¹⁵ Other virulence genes such as seb, sec, and pvl were detected in a few isolates. In a study of strains isolated from healthy students in Turkey, the sec gene was found to be the most frequent, followed by sea and none of the *S. aureus* isolates had pvl.²¹ The pvl gene has been shown to occur less frequently in CA-MRSA isolates associated with asymptomatic nasal colonization, consistent with the results of the present

Table 2 – Biofilm production by MRSA and MSSA isolates recovered from nasal secretion of healthy children attending daycare centers in Vitória da Conquista, Bahia, Brazil.

Samples	n	Non-producers		Weak producers		Moderate producers		Producers		Strong producers	
		n	%	n	%	n	%	n	%	n	%
MRSA	11	0	0	0	0	1	9.1	1	9.1	9	81.8
MSSA	59	0	0	4	6.7	7	11.8	12	20.3	36	61
Total	70	0	0	4	5.7	8	11.4	13	18.5	45	64.2

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

study.²² The genes of staphylococcal enterotoxins have been implicated as important determinants of *S. aureus* virulence²; however, they have been poorly studied in healthy children in the community.

In conclusion, this study showed high detection rates of *S. aureus* and MRSA (7.4%) in healthy Brazilian day care children. Moreover, the characterized isolates with reduced sensitivity to vancomycin and biofilm production are of special concern. Thus, continuous surveillance of *S. aureus* and MRSA in Vitória da Conquista should be encouraged to gain a better understanding of the circulating staphylococci in order to establish better control of possible infections.

Conflicts of interest

The authors declare no conflicts of interest.

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