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Molecular Epidemiology and Risk Factors for Nasal Carriage of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* in Infants Attending Day Care Centers in Brazil[▽]

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Investigations regarding *Staphylococcus aureus* carriage among Brazilian children are scarce. We evaluated the determinants of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) nasal carriage in infants attending day care centers (DCCs) and the molecular features of the MRSA strains. A total of 1,192 children aged 2 months to 5 years attending 62 DCCs were screened for *S. aureus* and MRSA nasal carriage. MRSA isolates were characterized by pulsed-field gel electrophoresis, multilocus sequence typing, *spa* typing, staphylococcal cassette chromosome (SCC) *mec* typing and the presence of the Panton-Valentine leukocidin gene. Logistic regression was performed to determine risk factors associated with *S. aureus* and MRSA colonization. *S. aureus* and MRSA carriage were detected in 371 (31.1%) and 14 (1.2%) children, respectively. Variables found to be independently associated with an increased risk for *S. aureus* carriage included being older than 24 months (odds ratio [OR], 1.8; 95% confidence interval [CI], 1.3 to 2.6) and previous DCC attendance (OR, 1.5; 95% CI, 1.0 to 2.2). Having a mother with a high level of education was a protective factor for nasal colonization (OR, 0.4; 95% CI, 0.2 to 0.8). Moreover, we observed that more children carrying MRSA had younger siblings than children not colonized by MRSA. Among the 14 MRSA strains, three SCC*mec* types (IIIA, IV, and V) were detected, together with a multidrug-resistant dominant MRSA lineage sharing 82.7% genetic similarity with the Brazilian clone (ST239-MRSA-IIIA; ST indicates the sequence type determined by multilocus sequence typing). Although SCC*mec* type V was recovered from one healthy child who had been exposed to known risk factors for hospital-associated MRSA, its genetic background was compatible with community-related MRSA. Our data suggest that DCC attendees could be contributing to MRSA cross-transmission between health care and community settings.

Staphylococcus aureus is an important human pathogen that causes community- and health care-associated infections worldwide in all age groups (43). Nasal colonization by *S. aureus* is common in children, and genetic evidence has supported a causal relationship between nasal carriers of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) and invasive staphylococcal disease (8, 35, 38). In addition, children may act as vectors for spreading *S. aureus* and MRSA to both community and hospital environments (15). Several determinants of *S. aureus* carriage in healthy children have been suggested, examples of which are the number of siblings, family size, and day care center (DCC) attendance (31). DCCs constitute reservoirs of MRSA where children are at increased risk of nasal colonization by *S. aureus* (24, 25).

The emergence and dissemination of MRSA are a global concern in both health care and community settings (13). Although MRSA has initially been recognized as a purely health care-associated pathogen, its epidemiology is changing, and it has been increasingly found in healthy individuals without conventional risk factors for MRSA acquisition (37). MRSA strains carry the *mecA* gene that encodes PBP2A, the central determinant of methicillin resistance, and is carried by a mobile genetic element designated staphylococcal cassette chromosome *mec* (SCC*mec*) (18). SCC*mec* types I, II, III, and VI have been mostly linked to health care-associated MRSA strains (HA-MRSA) while types IV and V have been commonly associated with community-associated (CA-MRSA) isolates (10, 28). Recent studies in DCCs conducted on different continents have found mainly SCC*mec* type II and type IV MRSA strains (15, 41).

Little is known about the extent of *S. aureus* and MRSA carriage in children, and there are many gaps in the epidemiology and pathogenesis of CA-MRSA strains in Brazil. What is well established is that a single multidrug-resistant HA-MRSA clone (ST239-MRSA-IIIA clone; ST indicates the sequence type determined by multilocus sequence typing [MLST]) ac-

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counts for the vast majority of MRSA infections (34, 38, 42). In a previous survey of children with acute respiratory tract infections and meningitis, we found the prevalence of nasal *S. aureus* and MRSA to be 13.5% and 1.0%, respectively. The MRSA isolates were multidrug resistant, and all of them were classified as SCC*mec* type III (21). The aim of the present study was to assess the prevalence of *S. aureus* and MRSA nasal carriage in a large population of healthy infants and children attending DCCs and to determine the potential risk factors for its acquisition. We also describe the molecular features of MRSA strains circulating among DCCs.

MATERIALS AND METHODS

Study population. The study was conducted from August to December, 2005, in Goiânia (1,201,007 inhabitants), located in central Brazil. As part of a major investigation, nasal carriage of *S. aureus* was determined by a cross-sectional survey conducted among children less than 5 years old attending 62 out of the 70 municipal DCCs. Children who had taken antibiotics in the previous 7 days were not considered eligible for the study. The study protocol was approved by the Regional Ethical Committee of the Federal University of Goiás. Written informed consent was obtained from each child's parents or legal representative before nasal specimen collection or interviews.

Data collection. Individual variables, sociodemographic characteristics of the families, and potential risk factors for *S. aureus* nasal carriage were obtained by interviews with the guardians immediately after consent was obtained and before swabs were collected.

Sampling and sample size. The overall population attending DCCs was considered to be the target population for sampling purposes. The number of children sampled per DCC was proportional to the number of children attending each center. Based on previous studies in healthy children (7, 23), we estimated the rate of *S. aureus* carriage to be 30%. Therefore, we calculated that a sample size of 1,100 children would be necessary to estimate risk factors for *S. aureus* and MRSA carriage (estimated prevalence ranging from 1 to 3%) with a 95% confidence interval ([CI] design effect, 1.5). Study participants were recruited sequentially within each DCC until the estimated recruitment number was met.

Collection of nasal swabs and microbiological procedures. A single specimen was obtained per child from one nasal side by two trained nurses using swabs that were placed into Stuart transport medium tubes (Medical Wire, Corsham, United Kingdom). The swabs were transferred to the Laboratory of Bacteriology of the Federal University of Goiás within 6 h of collection and processed immediately according to standard procedures. The nasal swabs were streaked on mannitol salt agar (Difco, Detroit, MI) and incubated at 37°C for 24 h. Colonies with suggestive morphology of staphylococci that also fermented mannitol were submitted to Gram staining, catalase, coagulase, DNase tests, and PCR amplification of *femB*. Susceptibility tests were performed by disk diffusion (Oxoid Ltd., Basingstoke, Hampshire, England) for erythromycin, trimethoprim-sulfamethoxazole, tetracycline, clindamycin, chloramphenicol, rifampin, teicoplanin, oxacillin, cefoxitin, ciprofloxacin, and gentamicin. Etest was used to test isolate sensitivity to vancomycin (6). *S. aureus* ATCC 25923 and ATCC 29213 strains were used as quality controls.

MRSA definition. The presence of the *mecA* gene was used to define MRSA isolates. PCR amplification of the *mecA* gene (27) was performed in all isolates that showed an oxacillin (1 μg) inhibition zone of >13 mm.

Molecular typing. Pulsed-field gel electrophoresis (PFGE) with SmaI was performed as described previously (5). The following international epidemic MRSA clones were included as controls: ST5-II (New-York/Japan), ST239-III (Brazilian), ST5-VI (pediatric), ST22-IV (epidemic MRSA-15), ST45-IV (Berlin), ST247-IA (Iberian), ST8-IV (USA300), ST1-IV (USA400), and ST30-IV (Oceania, Southwest Pacific). SCC*mec* typing was done by multiplex PCR (26). MLST, *spa* typing, and Panton-Valentine leukocidin (PVL) gene detection were performed as reported elsewhere (9, 14, 22). The MLST sequences obtained were submitted to the international public database (http://www.mlst.net) to generate an allelic profile and to assign the ST. The *spa* types were determined using Ridom Staphtype software, version 1.5.13 (Ridom, Germany).

Data analysis. Risk factors were assessed using SPSS software, version 15.0 (SPSS Inc., Chicago, IL). The data were stratified into results for two age groups, ≤23 months and >23 months. Multidrug-resistant *S. aureus* isolates were defined as isolates resistant to three or more antimicrobial classes. Logistic regression was used to analyze risk variables associated with *S. aureus* carriage. Results were presented as odds ratios (OR) with 95% CIs. All variables with *P* values of <0.20 in univariate analyses were included in the final multivariable model. The significant variables were selected based upon likelihood ratio tests. A probability level of 0.05 (two-tailed) was used to determine the statistical significance.

PFGE band patterns were analyzed by visual inspection according to the Tenover criteria (39), followed by computer analysis using Bionumerics software (version 4.0; Applied Maths, Gent, Belgium). *S. aureus* strain NCTC8325 (GenBank accession number CP000253) was used for intragel normalization and intergel comparison purposes. The dendrogram was constructed using the unweighted pair group method for arithmetic averages and the Dice band-based similarity coefficient with a band position tolerance of 1.0% and an optimization of 0.7%. Isolates were defined as epidemiologically related if they shared ≥80% similarity on the dendrogram. Band-based patterns were compared with the PFGE patterns of the major international MRSA clones.

RESULTS

Nasal swabs were collected from 1,192 children attending 62 public DCCs in Goiânia. The median age of the participants was 39 months, and 645 (54.1%) were male. Overall, 80.5% of the children had taken antibiotics in the previous 6 months, 88.5% had attended other DCCs, and 10.3% had been admitted to a hospital in the previous 6 months. The annual median household income was $2,643.17 (U.S. dollars), which is below the Brazilian poverty line. The prevalence of *S. aureus* and MRSA nasal colonization was 31.1% (95% CI, 28.50 to 33.84) and 1.2% (95% CI, 0.64 to 1.96), respectively.

Sociodemographic and clinical characteristics of the children are presented in Tables 1 and 2. The variables significantly associated with *S. aureus* carriage in univariate analysis were the following: being older than 23 months, previous DCC attendance, antibiotic consumption, and mother's level of education. After multivariate analysis (Table 3), being older than 23 months (OR, 1.8; 95% CI, 1.3 to 2.6; $P = 0.001$) and previous DCC attendance (OR, 1.5; 95% CI, 1.0 to 2.2; $P = 0.037$) remained as the child factors independently associated with an increased risk for *S. aureus* carriage. The risk for *S. aureus* carriage was inversely associated with the mother's degree of schooling. Having a mother with a high level of education was a protective factor for child nasal colonization compared to mothers who were illiterate (OR, 0.4; 95% CI, 0. 2 to 0.8; $P = 0.007$). No interaction was detected between the mother's education and the number of siblings ($P < 0.841$). Previous DCC attendance, previous fever in the last 30 days, the number of younger siblings, and use of an antibiotic by a family member in the previous 6 months were all associated with MRSA carriage in univariate analyses. After adjusting for confounders, only a larger number of younger siblings and not the absence of younger siblings ($P = 0.022$) remained associated with MRSA nasal carriage.

All MRSA isolates were susceptible to vancomycin, teicoplanin, chloramphenicol, and rifampin. Seven (50%) of the MRSA isolates were multidrug resistant. According to Fig. 1, among the 14 MRSA strains, six PFGE types, six *spa* types, five STs, and three SCC*mec* types were detected. The majority of the isolates ($n = 8$; 57%) belonged to a single clone (PFGE type A, *spa* types t037/t275, ST239, and SCC*mec* IIIA). The dendrogram showed that PFGE A shares 82.7% similarity with the representative Brazilian clone. The majority (75%) of the ST239-IIIA strains were multidrug resistant. Three MRSA strains showed the same SCC*mec*, ST, and *spa* type (type IV/ST121/t645) although they belonged to different pulso-types (B and D). Only a single strain presented an unusual association

TABLE 1. Demographic and clinical characteristics of *S. aureus* carriage among infants attending DCCs in Brazil[a]

Study characteristic	Value of the parameter (no. of participants [%]) for the group					
	S. aureus carriage			MRSA carriage		
	Yes (n = 371)	No (n = 821)	P	Yes (n = 14)	No (n = 1,178)	P
Child factors						
Age (mos.)						
≤23	44 (11.9)	166 (20.2)	<0.001	2 (14.3)	208 (17.7)	0.735
24–59	327 (88.1)	655 (79.8)		12 (85.7)	970 (82.3)	
Sex						
Male	206 (55.5)	439 (53.5)	0.510	9 (64.3)	636 (54.0)	0.438
Female	165 (44.5)	382 (46.5)		5 (35.7)	542 (46.0)	
Previous DCC attendance						
Yes	58 (15.6)	79 (9.6)	0.003		137 (11.6)	0.064
No	313 (84.4)	742 (90.4)		14 (100)	1,041 (88.4)	
Admitted to hospital[b]						
Yes	34 (9.2)	89 (10.8)	0.375	2 (14.3)	121 (10.3)	0.641
No	337 (90.8)	731 (89.2)		12 (85.7)	1,056 (89.7)	
Use of an antibiotic[b]						
Yes	303 (89.1)	657 (86.8)	0.276	13 (92.9)	948 (87.5)	0.513
No	37 (10.9)	100 (13.2)		1 (7.1)	136 (12.5)	
Having recurrent AOM[c]						
Yes	14 (3.8)	28 (3.4)	0.759		42 (3.6)	0.314
No	357 (96.2)	791 (96.6)		14 (100)	1,134 (96.4)	
Previous pneumonia[d]						
Yes	5 (1.4)	12 (1.5)	0.878		17 (1.4)	0.525
No	366 (98.6)	809 (98.5)		14 (100)	1,161 (98.6)	
Previous fever[d]						
Yes	94 (25.3)	251 (30.6)	0.063	1 (7.1)	344 (29.2)	0.04
No	277 (74.7)	569 (69.4)		13 (92.9)	833 (70.8)	
Previous flu-like symptoms[d]						
Yes	205 (55.3)	476 (58.1)	0.367	7 (50.0)	674 (57.3)	0.587
No	166 (44.7)	344 (41.9)		7 (50.0)	503 (42.7)	
Previous cough[d]						
Yes	175 (47.3)	383 (46.7)	0.850	5 (35.7)	553 (47.0)	0.395
No	195 (52.7)	437 (53.3)		9 (64.3)	623 (53.0)	
Having asthma						
Yes	14 (3.8)	21 (2.6)	0.253		35 (3.0)	0.359
No	357 (96.2)	798 (97.4)		14 (100)	1,141 (97.0)	
Household factors						
Use of an antibiotic by a child of the household[d]						
Yes	24 (6.6)	31 (3.9)	0.041		55 (4.8)	0.243
No	339 (93.4)	771 (96.1)		14 (100)	1,096 (95.2)	
Use of antibiotics by a family member[b]						
Yes	303 (81.7)	672 (81.9)	0.940	14 (100)	877 (74.4)	0.004
No	68 (18.3)	149 (18.1)			301 (25.6)	
Hospitalization of a family member[b]						
Yes	57 (15.4)	137 (16.7)	0.567	1 (7.1)	193 (16.4)	0.304
No	314 (84.6)	684 (83.3)		13 (92.9)	985 (83.6)	
Mother's schooling						
Illiterate	4 (1.1)	4 (0.5)	0.012[e]		8 (0.7)	0.959[f]
Elementary	206 (55.7)	490 (60.6)		9 (64.3)	687 (59.0)	
High school	145 (39.2)	256 (31.6)		4 (28.6)	397 (34.1)	
College	15 (4.1)	59 (7.3)		1 (7.1)	73 (6.3)	

[a] Missing values for any predictor were excluded from the analysis, so the numbers may not add to totals.
[b] Previous 6 months.
[c] AOM, acute otitis media (three episodes of AOM in 6 months or more than three episodes in 12 months).
[d] Previous 30 days.
[e] P value corresponds to a chi-square with 3 degrees of freedom equal to 10.81.
[f] P value corresponds to a chi-square with 3 degrees of freedom equal to 0.30.

(ST12-MRSA-IIIA). It is interesting that we detected one SCC*mec* type V (ST1120), classified as a single-locus variant of ST45, which we submitted to the MLST database. The PVL gene was not detected in any MRSA strain. Among the 62 DCCs participating in the study, 11 (17.7%) were found to have at least one child colonized with MRSA. The ST239/MRSA-IIIA clone was distributed among five different DCCs. Four out of the eight children that were carriers of the ST239-IIIA lineage attended the same DCC (code 35), and two of them shared the same DCC room. All MRSA carriers had at least one health care-associated risk factor for MRSA acquisition (previous hospitalization or use of antibiotics).

TABLE 2. Other demographic data of the study population

Characteristic of participant's family	Value of the parameter for the indicated group (mean ± SD)					
	S. aureus carriage			MRSA carriage		
	Yes (n = 371)	No (n = 821)	P	Yes (n = 14)	No (n = 1,178)	P
No. of siblings	1.2 ± 1.2	1.4 ± 1.2	0.102	1.4 ± 1.9	1.3 ± 1.2	0.923
No. of younger siblings	0.4 ± 0.5	0.4 ± 0.6	0.826	0.8 ± 0.4	0.4 ± 0.6	0.022
No. of older siblings	1.4 ± 1.1	1.4 ± 1.1	0.3571	1.3 ± 2.3	1.4 ± 1.1	0.807
Annual household income (U.S. dollars)	3,160.2 ± 1,975.03	3,152.4 ± 2,331.09	0.955	3,549.40 ± 2,530.39	3,150.05 ± 2,222.15	0.505

DISCUSSION

To the best of our knowledge, this is the first comprehensive survey of *S. aureus* nasal carriage conducted in Brazil, and it included 1,192 children attending 62 out of a total of 70 public DCCs in Goiânia. The prevalence of *S. aureus* nasal colonization in this population (31.1%) was higher than previously detected in *S. aureus* carrier children at the moment of hospital admission (13.5%) in the same municipality (21). Our findings are consistent with those of studies conducted in other DCCs, especially in Asia (15, 17, 25), and they corroborate the fact that DCCs are a favorable environment for *S. aureus* transmission.

We found that children older than 23 months, those who previously attended other DCCs, and those whose mother had a lower level of education were at higher risk to be colonized by *S. aureus*. A possible explanation for the increase in *S. aureus* colonization with age may be due to pneumococcal competition at an early age of life, leading to a negative correlation for the cocolonization of *S. aureus* and *Streptococcus pneumoniae*, as observed by others (2). In fact, in a previous study among day care attendees in Goiânia, we have shown that children under 24 months of age were preferentially colonized by *S. pneumoniae* in contrast to older children, who were colonized by *S. aureus* (11).

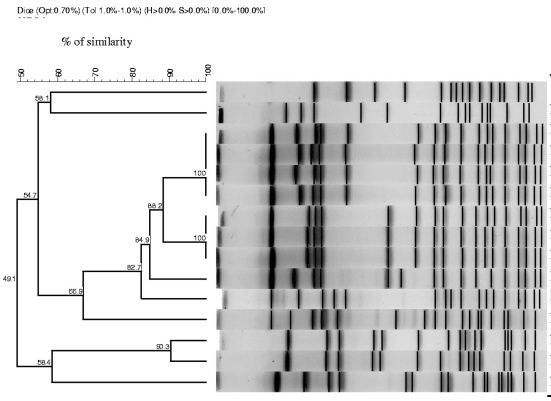
A mother's high level of education was found to be an independent protective factor for *S. aureus* nasal colonization. This association could not be explained by the number of siblings because no interaction was observed between this variable and the mother's schooling. Education level is a proxy of socioeconomic status, and a low level of maternal education may interfere with the mother's compliance with control measures (such as hand washing) that minimize the spread of *S. aureus* and MRSA.

It is well recognized that DCCs are efficient settings for the acquisition and transmission of pathogens due to crowded conditions, frequent close physical contact, breakdown in appropriate hygiene, and intensive exposure to antimicrobials. In addition, children with longer time periods of child care exposure are more prone to be colonized by MRSA than children who are attending DCCs for the first time (15, 23). One intriguing point of this survey is the low prevalence of MRSA in an environment that otherwise would be considered very conducive to spread. Therefore, one may question why the MRSA strains that have entered this population did not spread widely in this DCC environment. Among the 14 MRSA strains found, 8 belonged to clone ST239-III, which is an international epidemic nosocomial clone that is widely spread in hospitals in many countries all over the world. ST239-III is not common in the community unless people attend a health care facility recurrently, which was not the case in our sample of healthy children attending a DCC. Most of the children enrolled in the present study have attended an outpatient service, which explains the acquisition of nosocomial MRSA clones. Even though HA-MRSA clones have been frequently found in the community and though DCCs are considered to be very conducive to the spread of many bacterial pathogens due to close contact, sharing of secretions, heavy antibiotic use, etc., HA-MRSA does not seem to be able to spread in this environment. This is consistent with the fact that CA-MRSA clones are phenotypically and genotypically different from HA-MRSA.

TABLE 3. Risk factors independently associated with *Staphylococcus aureus* nasal carriage in infants from DCC in Brazil

Variable	Univariate analysis			Multivariate analysis		
	OR[a]	95% CI	P	OR	95% CI	P
Child factors						
Age ≥24 mo	1.9	(1.3–2.7)	<0.001	1.8	(1.3–2.6)	0.001
Previous DCC attendance	1.7	(1.2–2.5)	0.003	1.5[a]	(1.0–2.2)	0.037
Previous fever[b]	1.3	(1.0–1.7)	0.063	1.3[a]	(0.9–1.7)	0.124
Household factors						
Mother's schooling						
Illiterate	1			1[a]		
Elementary	0.2	(0.6–1.1)	0.07	0.2	(0.04–0.8)	0.031
High school	0.6	(0.3–1.1)	0.09	0.5	(0.3–1.0)	0.040
College	0.4	(0.2–0.8)	0.01	0.4	(0.2–0.8)	0.007
Use of an antibiotic by a child of the household[b]	1.8	(1.0–3.0)	0.04	1.7[a]	(1.0–3.1)	0.058

[a] The OR was adjusted by age as a continuous variable and number of siblings.
[b] In the past 30 days.



Strain	Pulso type	SCC*mec* type	ST	*spa* type	PVL	Age months	DCC code	DCC Room	Previous DCC attendance	Admitted to hospital	Use of antibiotic	MDR
BRZ33r	E	IV	ST30	t012	-	56	69	3	no	no	yes	no
BRZ15r	F	V	ST1120	t065	-	33	62	4	no	yes	yes	no
BRZ34r	A1	IIIA	ST239	t037	-	49	39	4	no	no	yes	yes
BRZ28	A1	IIIA	ST239	t037	-	21	35	2	no	yes	no	yes
BRZ41	A1	IIIA	ST239	t037	-	59	35	4	no	no	yes	yes
BRZ58	A1	IIIA	ST239	t037	-	49	25	2	no	no	yes	no
BRZ12r	A2	IIIA	ST239	t037	-	34	51	3	no	no	yes	no
BRZ17r	A2	IIIA	ST239	t037	-	43	68	1	no	no	yes	yes
BRZ42	A2	IIIA	ST239	t037	-	58	35	5	no	no	yes	yes
BRZ40	A3	IIIA	ST239	t275	-	36	35	4	no	no	yes	yes
HU25	A	IIIA	ST239	t138	-	NA	NA	NA	NA	NA	NA	yes
BRZ26	D	IV	ST121	t645	-	40	34	2	no	no	yes	yes
BRZ9r	B	IV	ST121	t645	-	54	53	4	no	no	yes	no
BRZ39r	B1	IV	ST121	t645	-	25	38	1	no	no	yes	no
BRZ37	C	IIIA	ST12	t213	-	22	61	1	no	no	yes	no

FIG. 1. Molecular properties of the 14 MRSA isolates found among infants attending DCCs in Goiânia, Brazil, in 2005. Use of antibiotic was considered for the previous 6 months. All of the strains were PVL negative (−). Strain HU25 is a representative strain of the Brazilian HA-MRSA clone (35). MDR, multidrug resistant; NA, not applicable.

The prevalence of MRSA nasal colonization in this population was estimated to be 1.2%. This rate is within the reported range (0.2 to 13%) of MRSA carriers among healthy children (17, 36). The majority of MRSA strains were identified as belonging to the most common MRSA clone in Brazil (ST239-III), which is endemic in health care settings all over the country (38, 42). This cluster is epidemiologically related because half of the isolates were obtained from children attending the same DCC, supporting cross-transmission. In this way, our findings suggest that day care children are acquiring and spreading SCC*mec* type III MRSA clones associated with the health care environment into the pediatric community as colonizing pathogens. In fact, studies have indicated that SCC*mec* type III is escaping the hospital environment and is adapting to the community (4).

In the present study we found a new association, ST12-MRSA-IIIA; ST12 has been previously found in association with SCC*mec* type IV only (19, 40). So far, few reports have detected the presence of ST12 strains. Strains representing ST12 have been described as an uncommon MRSA genetic background, and studies have suggested that these strains come from a sporadic and diverse lineage (3). Whether these ST12 strains are in a transitional state is an open issue.

Three MRSA strains belonged to ST121. As far as we know, no other ST121-IV isolates from Brazil have been reported. This clone has been found in both health care- and community-associated methicillin-susceptible *S. aureus* (MSSA) and in some cases carrying the PVL gene (12, 20). The emergence of ST121-IV may be the result of a local SCC*mec* acquisition by an ST121 MSSA strain (30). Moreover, ST121 isolates that are also PVL positive have been reported all over the world mainly as MSSA. The chronological order of acquisition of the SCC*mec* and PVL genes by the same *S. aureus* strain is currently still unclear. It may be that in the present ST121-IV strain, it is a question of fitness cost, with the SCC*mec* acquisition occurring preferentially in a background without PVL. A few cases of infection caused by CA-MRSA ST30-IV have been reported in Brazil (32, 33). These strains were PVL positive, but they were responsible for infection instead of colonization. Because our study addressed only colonization, this may explain (at least partially) the absence of this toxin's genes.

A novel finding of this investigation was the detection of the SCC*mec* type V in Brazil, which was assigned to ST1120, a single-locus variant of ST45 differing at the *aroE* locus. This is the first report of a Brazilian isolate of SCC*mec* V. The strain showed a PFGE profile very similar to the first SCC*mec* type V isolate (ST45) recovered in Portugal (1) and was grouped into clonal complex 45, previously associated with both health care and community environments (16, 19, 29). Both the Brazilian and Portuguese SCC*mec* type V isolates were PVL negative. Although this SCC*mec* type V strain was recovered from a healthy child who had been exposed to known risk factors for HA-MRSA (hospitalization and antimicrobial use), its SCC*mec* type was compatible with CA-MRSA. Thus, it is not possible to establish the epidemiology of this MRSA strain acquisition. Our findings emphasize the need for continued molecular surveillance of MRSA, with special concern for the dissemination of CA-MRSA into the Brazilian hospital setting.

Limitations of this work should be mentioned. We failed to detect a positive association between carriage of MRSA and variables currently acknowledged as risk factors. A possible explanation could be the small number of MRSA strains isolated in this study, which thus lacks sufficient statistical power for detecting such an expected association. Moreover, the collection of nasal swabs from a single nare may have diminished the detection of MRSA strains.

In our study, no significant association was found between antibiotic usage in the past 6 months and MRSA carriage although more than 80% of the participants had taken antibiotics in the previous 6 months. This indicates that this population is itself a high-risk one, as the participants have attended an outpatient service, which explains the acquisition of nosocomial MRSA clones. Also, the questionnaire did not evaluate the presence of a health care worker in the household as a potential risk factor for both *S. aureus* and MRSA carriage. However, proxy variables were assessed, such as having any household member admitted to the hospital and family morbidity in the past 6 months, and these could indicate a connection between a household member and the health care environment. Another point to be considered is the fact that this investigation occurred 4 years ago, and the epidemiology of MRSA may have changed significantly since then.

In conclusion, this survey showed that the prevalence of MRSA in a large sample of healthy Brazilian children is still low. However, the horizontal spread of HA-MRSA clones, such as the Brazilian strain of MRSA, may be expected within the pediatric community due to the two-way flow of MRSA dissemination. Moreover, the detection of three isolates belonging to ST121, a clone frequently associated with PVL genes, is of special concern in a young population. Thus, continued monitoring of *S. aureus* and MRSA in our municipality is advisable in order to establish appropriate educational and infection control measures to disrupt transmission to susceptible hosts.

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