JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2011, p. 1041–1047 Vol. 49, No. 3
0095-1137/11/$12.00 doi:10.1128/JCM.02235-10

Prevalence and Risk Factor Analysis for Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization in Children Attending Child Care Centers▽

Melissa B. Miller,[1]* David J. Weber,[2] Jennifer S. Goodrich,[3] Elena B. Popowitch,[1] Michele D. Poe,[4] Viet Nyugen,[5] Timothy R. Shope,[6] David T. Foster,[6] James R. Miller,[6] and Jonathan Kotch[5]

Departments of Pathology and Laboratory Medicine[1] and Medicine,[2] University of North Carolina School of Medicine, Clinical Microbiology-Immunology Laboratory, University of North Carolina Health Care,[3] Frank Porter Graham Child Development Institute,[4] and Department of Maternal and Child Health, Gillings School of Global Public Health, University of North Carolina at Chapel Hill,[5] Chapel Hill, North Carolina, and Department of Pediatrics, Naval Medical Center Portsmouth, Portsmouth, Virginia[6]

Received 5 November 2010/Returned for modification 14 December 2010/Accepted 20 December 2010

Children attending child care centers (CCCs) are at increased risk for infections, including those caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Nasal colonization often precedes infection, and MRSA colonization has been associated with increased infection risk. Community-associated MRSA (CA-MRSA) has caused increased MRSA infections in the general population, including children. Little is known about the frequency of MRSA nasal colonization in young children, particularly in those attending CCCs where disease transmission is common. We sampled the nares of 1,163 children in 200 classrooms from 24 CCCs in North Carolina and Virginia to assess *S. aureus* colonization. MRSA strains were molecularly analyzed for staphylococcal cassette chromosome *mec* (SCC*mec*) type, Panton-Valentine leukocidin status, and multilocus sequence type. A case-control study was performed to identify risk factors for MRSA colonization. We found that 18.1% children were colonized with *S. aureus* and 1.3% with MRSA. Molecular analysis of the MRSA strains identified 47% as CA-MRSA and 53% as health care-associated MRSA (HA-MRSA). Although two centers had multiple children colonized with MRSA, genotyping indicated that no transmission had occurred within classrooms. The case-control study did not detect statistically significant risk factors for MRSA colonization. However, MRSA-colonized children were more likely to be nonwhite and to have increased exposure to antibiotics and skin infections in the home. Both CA-MRSA and HA-MRSA strains were found colonizing the nares of children attending CCCs. The low frequency of colonization observed highlights the need for a large multicenter study to determine risk factors for MRSA colonization and subsequent infection in this highly susceptible population.

Staphylococcus aureus is a common cause of serious community- and health care-associated infections. The numbers of both community-associated and health care-associated staphylococcal infections have increased in recent decades (25). Methicillin was introduced into clinical use in 1960, and this introduction was closely followed by the first reports of methicillin-resistant *S. aureus* (MRSA), with their resistance arising through the production of a supplementary penicillin-binding protein (PBP) known as PBP2a or PBP2′ (40). The prevalence of health care-associated MRSA (HA-MRSA) infection has increased dramatically since the mid-1980s (2). In 1974, MRSA infections accounted for 2% of the total number of *S. aureus* infections; in 1995, it was 22%, and in 2004, it was 63% (4).

Beginning in the early 1990s in the United States, case reports and case series documented the increasing problem of community-associated MRSA (CA-MRSA). At-risk populations for CA-MRSA have included children, athletes, injection drug users, military personnel, persons living in correctional facilities or shelters, African-Americans, and veterinarians (2). Published reports using both population-based surveillance (29) and laboratory-based surveillance (12) have documented that CA-MRSA infections are more frequent in children, especially children less than 2 years of age.

Approximately one-third of healthy persons harbor *S. aureus* in their nose at any time (14, 21, 22). The nose appears to be the primary reservoir for replication and spread to other body areas (26). The fact that colonization precedes infection is supported by studies that have demonstrated that nasal *S. aureus* isolates are often identical to strains later causing clinical infection (38, 39) and that when colonization is eradicated the risk of clinical infection is reduced (21). The frequency of nasal colonization with MRSA has been less well described, but in general it has varied from approximately 1 to 12% (1, 14, 22). However, colonization with MRSA as opposed to methicillin-sensitive *S. aureus* (MSSA) has been associated with a 4-fold increase in the risk of infection (35).

In the United States in the summer of 2006, approximately 32.2% of the 19 million children younger than 5 years of age attended a child care center (CCC) or other organized out-of-home care on a regular basis (37). Of these, approximately 36% attend CCCs, defined as an arrangement where, at any

* Corresponding author. Mailing address: UNC School of Medicine, Department of Pathology and Laboratory Medicine, Campus Box 7525, Chapel Hill, NC 27599. Phone: (919) 966-3723. Fax: (919) 966-0486. E-mail: mbmiller@unch.unc.edu.

▽ Published ahead of print on 29 December 2010.

one time, there are three or more unrelated preschool-age children receiving care for more than 4 h a day (37). Children attending child care centers have an increased risk for a variety of infections (6, 28) because they are immunologically naïve and vulnerable to infection. Complicating the issue is their tendency to contaminate the environment with respiratory tract secretions, urine, and feces, all of which can spread disease.

Given that young children are at higher risk for CA-MRSA and that attendance in a CCC is a well-described risk factor for respiratory, skin, and gastrointestinal infections, we undertook the following study of a large sample of children attending CCCs in North Carolina and Virginia. The goals of our study were to define the prevalence of MSSA and MRSA nasal colonization, microbiologically and molecularly characterize all isolated strains of MRSA, and perform a case-control study (in which the cases were children colonized with MRSA and the controls were noncolonized children and children colonized with MSSA) to assess risk factors for MRSA colonization.

MATERIALS AND METHODS

Study design. This study was conducted between March 2007 and October 2009. In North Carolina, CCCs were eligible for participation if they were in New Hanover County and had access to a Child Care Health Consultant or public health nurse. The 17 centers recruited in North Carolina by the Child Care Health Consultants were based on their previous working relationship with the county health department. In Virginia, all Navy CCCs in the Hampton Roads area were eligible and participated. The inclusion criteria for subjects were as follows: child is enrolled in a designated CCC, child's age is between 0 and 5 years, family plans to remain in the CCC throughout the academic year, guardian signs informed consent and agrees to participate (if selected) in case-control questionnaire by telephone, and family is English speaking. Colonization was assessed by use of a nasal swab (see below) obtained by a study nurse or physician. Demographic information on North Carolina subjects was available from classroom lists provided by the CCC director but was not obtained in Virginia due to Navy-specific Institutional Review Board (IRB) requirements. Classroom- and CCC-level data were provided by the CCC director. Star-rated licenses ranging from one star (meets minimum qualification) to five stars (exceeds minimum qualifications) were available for North Carolina CCCs. Star ratings reflect indicators of a program's quality based on an objective evaluation of program standards and staff education (31). All military CCCs in Virginia are accredited by the National Association of Education of Young Children; this accreditation corresponds to the highest star rating. Additional demographic information and risk factors for staphylococcal colonization used in the case-control study were obtained via a telephone questionnaire of the child's primary caregiver.

All subjects from whom a culture was obtained were used in the analysis to determine the prevalence of colonization by MRSA and MSSA. An evaluation of risk factors for MRSA colonization was assessed by means of a case-control study. Cases consisted of all children colonized with MRSA. Two groups of controls were used: non-staphylococcally colonized children (control group 1) and children colonized with MSSA (control group 2). The ratio of cases to controls (cases/control group 1/control group 2) was 1:2:2. Controls were chosen using a random number generator from all eligible subjects in each control group.

Consent for CCC participation was obtained from the CCC director. Consent for subject participation was obtained from the child's guardian. This study was approved by the IRB of the University of North Carolina at Chapel Hill and the Naval Medical Center Portsmouth IRB.

Microbiology. Nasal swabs were collected by swabbing both nares using a Copan (Brescia, Italy) liquid Stuart minitip transport swab. Swabs were shipped at ambient temperature via overnight Federal Express to the University of North Carolina at Chapel Hill for analysis. The swabs were plated to sheep blood agar and mannitol salt agar plates. Isolates were identified as *S. aureus* based on a positive tube coagulase test or a positive BactiStaph (Remel, Lenexa, KS) latex agglutination test. *S. aureus* isolates were identified as MRSA using oxacillin screening agar (Remel) and confirmed using cefoxitin disk diffusion (Becton Dickinson, Franklin Lakes, NJ). Susceptibilities of MRSA strains were tested using Kirby-Bauer disk diffusion for all drugs except daptomycin and vancomycin, which were assessed by Etests (AB bioMérieux, Sweden). All interpretations were based on CLSI guidelines (7). Antibiotics tested included clindamycin, daptomycin, doxycycline, erythromycin, gentamicin, levofloxacin, linezolid, trimethoprim-sulfamethoxazole, and vancomycin. The D-test was performed to detect inducible clindamycin resistance; D-test-positive organisms were reported as clindamycin resistant (19).

Molecular analyses. DNA extraction was performed as previously described (34). The presence of Panton-Valentine leukocidin was assessed using real-time multiplex PCR assays as previously described (13, 30). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed by the method of Oliveira et al. (32). SCC*mec* typing classified as indeterminate by the Oliveira et al. method was performed as described by Boye et al. (3). Multilocus sequence typing (MLST) was performed and analyzed as outlined by Enright et al. (10, 11). MRSA strains were defined as health care associated or community associated based on MLST type cluster analysis along with SCC*mec* type.

Statistical analyses. All data were entered into a Microsoft Access database. All analyses were performed using SAS 9.1 (SAS, Cary, NC). Descriptive measures were compared between the North Carolina and Virginia sites to evaluate differences between the CCCs in the two states. Logistic mixed models were fitted at the child level to test for relationships between positive swab status for MRSA and MSSA and six child-, classroom-, and center-level variables, using GLIMMIX. A total of 12 models were therefore fit. Odds ratios (OR), 95% confidence intervals, and *P* values were calculated. Multivariable models were not fit because of missing data which would have resulted in listwise deletion of all cases that contained any missing data. In the case-control study predictor, variables analyzed included hours per week in a CCC, playing sports, underlying diseases, chronic skin conditions, previous staphylococcal infections, pet(s) in the home, military persons or health care workers in the home, ethnicity, and household income (see Table 4 for complete list). Logistic regression models were fit for binary (i.e., yes/no) variables, and linear regression models were fit for scale or continuous variables. F-tests (two degrees of freedom [d.f.]) or χ^2 tests were used to test for group differences.

RESULTS

Subject and child care center demographics. The study population consisted of 1,163 subjects (811 in North Carolina and 352 in Virginia), from 200 classrooms (131 in North Carolina and 69 in Virginia) distributed among 24 centers (17 in North Carolina and 7 in Virginia), who had nasal cultures. Approximately 40% of children participated, with individual center participation ranging from 20% to 58%. Of note, some guardians did not consent for their child's participation because the child was already known to be positive for MRSA. The mean age of children in North Carolina was 3.3 years (standard deviation [SD], 1.4). Overall, gender distribution was similar for North Carolina (51% male) and Virginia (45% male).

Center-level and classroom-level data for the subjects are displayed in Table 1. Center-level data include number of classrooms per center, center type, percentage of children in center financially subsidized, and star rating for North Carolina CCCs. Since the Virginia CCCs were all Navy associated, they were all government-based centers, while North Carolina centers were a range of center types. The Virginia CCCs also had a higher percentage of subjects subsidized (mean, 29%; SD, 49%) than did North Carolina CCCs (mean, 18%; SD, 19%). Classroom-level data, including number of children in classroom by age group and child-to-teacher ratio by age group, were similar for North Carolina and Virginia classrooms.

MRSA prevalence. Overall, 210 children (18.1%) were colonized with *S. aureus*. Of these, 195 (16.8% of total) were colonized with MSSA while 15 (1.3% of total) were colonized with MRSA (Table 2). The proportions were not different

TABLE 1. Subject and child care center demographics[a]

Parameter	Result for location										Result for both locations				
	North Carolina					Virginia									
	n	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max
Age (yr)	549	3.3	1.4	<1	5						549	3.3	1.4	<1	5
Gender															
Male	414					160					574				
Female	386					153					539				
Unknown	11					39					50				
Avg no. of classrooms/center	17	7.7	2.8	4	13	7	9.9	6.9	1	21	24	8.3	4.4		21
Center type															
Church operated	3					0					3				
Government	0					7					7				
Other nonprofit	2					0					2				
Profit, franchise	2					0					2				
Profit, independent	9					0					9				
Work site, employer provided	1					0					1				
Star rating	14	3.9	1.1	1	5						14	3.9	1.1	1	5
Subsidized children/center	17	18%	19%	0%	56%	7	29%	49%	0%	100%	24	21%	30%	0%	100%
No. children/classroom															
Birth to 12 mo	109	9.2	1.7	4	12	30	8.3	3.0	4	16	139	8.1	2.7	4	16
1 to 2 yr	97	10.5	2.9	5	18	61	10.9	2.3	10	20	158	10.5	3.1	5	20
2 to 3 yr	140	14.9	3.9	8	20	77	14.7	3.6	10	21	217	13.9	3.3	8	21
2 to 4 yr	12	26.0	0.0	26	26						12	26.0	0.0	26	26
3 to 4 yr	137	16.7	4.5	10	25	37	21.0	2.0	18	24	174	16.6	4.7	10	25
3 to 5 yr						98	22.4	2.6	16	24	98	20.9	4.2	12	24
4 to 5 yr	161	19.8	6.7	10	34	19	24.0	0.0	24	24	180	17.8	6.3	9	34
5 to 6 yr	29	18.3	5.6	12	27						29	17.2	5.1	12	27
Child/teacher ratio															
Birth to 12 mo	109	4.5	0.7	3	5	30	4.0	0.0	4	4	139	4.4	0.7	3	5
1 to 2 yr	97	5.5	1.2	4	9	37	5.0	0.0	5	5	134	5.3	1.0	4	9
2 to 3 yr	76	7.8	1.3	6	9	77	6.6	0.8	5	7	153	7.2	1.2	5	9
2 to 4 yr	12	9.0	0.0	9	9						12	9.0	0.0	9	9
3 to 4 yr	137	9.3	1.9	7	17	37	12.0	0.0	12	12	174	9.9	2.0	7	17
3 to 5 yr						98	10.9	3.1	5	14	98	10.9	3.1	5	14
4 to 5 yr	172	11.1	2.9	6	17	19	12.0	0.0	12	12	191	11.2	2.7	6	17
5 to 6 yr	29	11.5	3.3	8	15						29	11.5	3.3	8	15

[a] Min, minimum; Max, maximum.

between North Carolina (MSSA, 16.8%; MRSA, 1.4%) and Virginia (MSSA, 16.8%; MRSA, 1.1%) subjects.

MRSA was isolated from children attending 10 different CCCs. Eight centers had one positive child each, one center (A) had four positive children, and one center (B) had three positive children. Center A children were found in two classrooms (two children each), and center B positivity involved three different classrooms (one child each). However, no transmission within classrooms was documented (see below).

Analysis of center-level and classroom-level variables based on subject colonization status (i.e., noncolonized, MSSA colonized, and MRSA colonized) is shown in Table 2. MSSA-colonized subjects were older ($P < 0.001$). However, there was no other statistically significant characteristic associated with MRSA or MSSA colonization. Although not reaching statistical significance, the child-to-teacher ratio of the MSSA-colonized classrooms was lower ($P = 0.08$), but the number of children in the classrooms of MSSA-colonized children (mean, 16.3; SD, 6.4) was higher ($P = 0.14$) than the number of children in classrooms of noncolonized children (mean, 15.4; SD, 6.1). The number of children in MRSA-colonized classrooms was higher as well (mean, 16.3; SD, 5.2). The MRSA-colonized children more often came from centers with a higher percentage of subsidized children (MRSA, 39%; MSSA, 21%; noncolonized, 20%), but this did not reach statistical significance (OR, 4.76, $P = 0.15$).

MRSA molecular analyses and antibiotic susceptibility patterns. The 15 MRSA isolates were analyzed for SCC*mec* type, Panton-Valentine leukocidin (PVL) status, antibiotic susceptibilities, and multilocus sequence type (Table 3). The sequence type determined by MLST was used to define strains as either CA-MRSA or HA-MRSA based on their clonal complex (CC). Of the 15 MRSA strains, 7 (47%) were CA-MRSA (CC8, $n = 6$, and CC97, $n = 1$) and 8 (53%) were HA-MRSA (CC5). The five CC8 CA-MRSA isolates were PVL positive and SCC*mec* type IV, and the CC97 isolate was PVL negative and SCC*mec* type IV. The eight CC5 HA-MRSA isolates were PVL negative ($n = 8$) and SCC*mec* types II ($n = 2$) and IV

TABLE 2. Prevalence study[a]

Parameter	Result for group																
	Negative (control)					MSSA						MRSA					
	n	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max	OR (95% CI)	*P*	*n*	Mean	SD	Min	Max	OR (95% CI)	*P*
Location of study											1.03 (0.65, 1.62)	0.9						0.74 (0.14, 3.86)	0.73
NC	664					136							11						
VA	289					59							4						
Total	953					195							15						
Age (yr)	470	3.1	1.4	<1	5.8	85	3.9	1.5	<1	5.8	1.48 (1.22, 1.78)	<0.001	15	3.3	1.3	1	4.8	1.04 (0.72, 1.50)	0.82
Gender											0.94 (0.68, 1.30)	0.71						1.41 (0.50, 3.98)	0.52
Male	472					93							9						
Female	443					90							6						
Star rating	570	3.9	1.1	1	5	101	4.1	1	1	5	1.17 (0.88, 1.54)	0.3	10	3.4	1.1	1	5	0.62 (0.28, 1.40)	0.27
Subsidized children/center	953	20%	31%	0%	100%	195	21%	33%	0%	100%	1.11 (0.59, 2.14)	0.75	15	39%	36%	0%	100%	4.76 (0.61, 37.0)	0.15
No. children/classroom	856	15.4	6.1	4	34	167	16.6	6.4	4	34	1.02 (0.99, 1.04)	0.14	15	16.3	5.2	9	26	1.06 (0.97, 1.16)	0.22
Child/teacher ratio, by age											0.93 (0.86, 1.01)	0.08						1.10 (0.85, 1.42)	0.48
Birth to 12 months	122	4.4	0.7	3	5	16	4.5	0.6	3	5			1	5.0	0.0	5	5		
1 to 2 yr	118	5.4	1.1	4	9	13	5.2	0.4	5	6			3	5.7	0.6	5	6		
2 to 3 yr	135	7.3	1.2	5	9	17	6.8	0.8	5	8			1	9.0	0.0	9	9		
2 to 4 yr	10	9.0	0.0	9	9	2	9.0	0.0	9	9									
3 to 4 yr	140	10.0	2.1	7	17	31	9.1	1.4	7	12			3	10.7	1.2	10	12		
3 to 5 yr	78	10.9	3	5	14	19	11.2	3.1	5	14			1	5.0	0.0	5	5		
4 to 5 yr	139	11.3	2.7	6	17	48	10.8	2.9	6	17			4	12.5	0.6	12	13		
5 to 6 yr	17	11.7	3.3	8	15	12	11.2	3.4	8	15									

[a] Min, minimum; Max, maximum; CI, confidence interval.

TABLE 3. Microbiological and molecular analyses of MRSA isolates

Parameter	Result
No. of MRSA isolates	15
Percent susceptible	
Clindamycin	67
Erythromycin	20
Levofloxacin	27
No. with SCC*mec* type	
II	2
IV	13
No. with PVL result	
Positive	6
Negative	9
No. with sequence type (clonal complex)	
ST5 (CC5)	6
ST8 (CC8)	5
ST1532 (CC5)	1
ST231 (CC5)	1
ST97 (CC97)	1
SLV[a] ST5 (CC5)	1

[a] SLV, single-locus variant.

($n = 6$). A dendrogram showing the relatedness of the MRSA strains and their corresponding identification as CA-MRSA or HA-MRSA is shown in Fig. 1. MLST also revealed that none of the MRSA isolates obtained from children in the same classroom were identical.

All 15 MRSA strains were uniformly susceptible to daptomycin, doxycycline, gentamicin, linezolid, trimethoprim-sulfamethoxazole, and vancomycin. The various susceptibilities to clindamycin, erythromycin, and levofloxacin are shown in Table 3.

Case-control study. The case-control study did not reveal any statistically significant differences between the MRSA-colonized or MSSA-colonized children and the noncolonized children (Table 4). Interestingly, there was no increased risk for MRSA colonization associated with living on a military base or having a military person or health care worker in the home. However, children who were MRSA positive were 20 to 30% more likely to be nonwhite than those in the control groups (MRSA, 40%; MSSA, 10%; noncolonized, 20%), but this difference was not statistically significant ($P = 0.07$). MRSA-colonized children reported a history of antibiotic use 10 to 26% more frequently than those in the control groups (MRSA, 93%; MSSA, 83%; noncolonized, 67%; $P = 0.09$) and were 24 to 30% more likely to have skin infections in the home (MRSA, 47%; MSSA, 23%; noncolonized, 17%; $P = 0.13$). Of the eight MRSA-colonized subjects that had a history of skin infections, half were colonized with CA-MRSA and half with HA-MRSA.

DISCUSSION

S. aureus is a human pathogen which primarily colonizes the nose (25). In the past few decades, methicillin-resistant strains have come to predominate in both health care-associated and

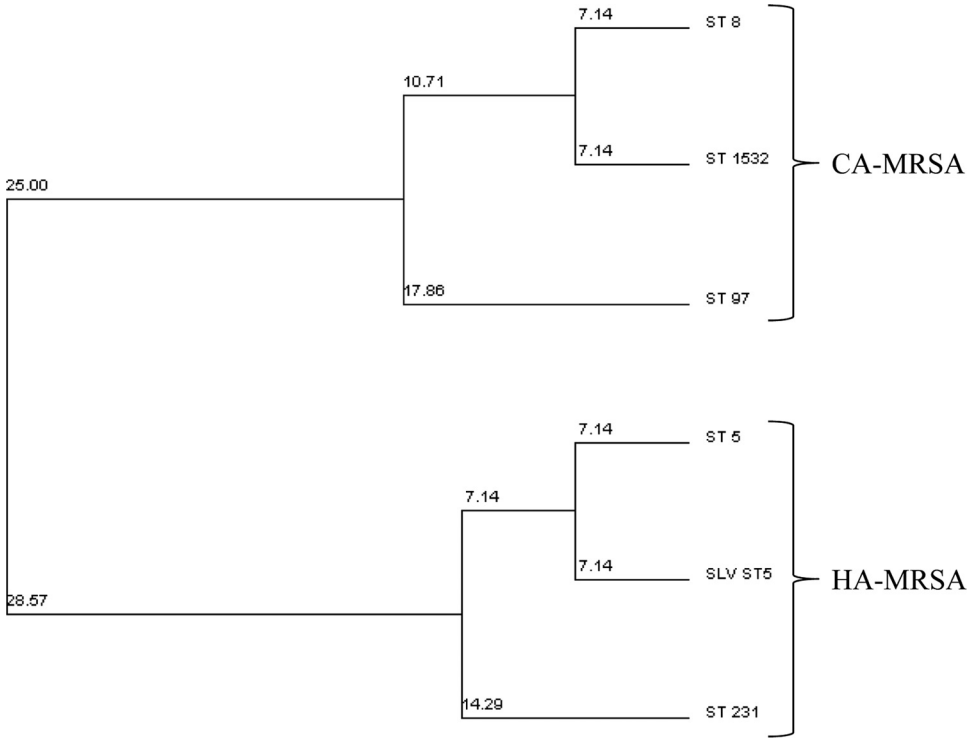

FIG. 1. Cluster analysis of MRSA isolates. Shown is a dendrogram displaying the relatedness of MRSA strains isolated from children attending childcare centers. Two clusters are evident that separate CA-MRSA isolates from HA-MRSA isolates. ST, sequence type; SLV, single locus variant.

community-associated *S. aureus* infections (40). However, the epidemiology and microbiology of CA-MRSA and HA-MRSA strains have differed (8). CA-MRSA has involved younger patients and caused predominantly skin and soft tissue infections, while HA-MRSA has involved older patients who have typical health care-associated risk factors (e.g., indwelling catheter or percutaneous device). CA-MRSA strains have most commonly been SCC*mec* type IV and Panton-Valentine leukocidin positive, while HA-MRSA strains have most commonly been SCC*mec* types I, II, and III and Panton-Valentine leukocidin negative. However, recent studies describing CA-MRSA strains in the hospital setting (17, 27, 33) suggest that clinical presentation and epidemiologic risk factors are no longer sufficient to reliably define molecular strain types.

CA-MRSA infections have been demonstrated to be more common in children, especially children less than 2 years of age (12). Outbreaks of MRSA have been noted in CCCs (18), and CCC attendance has been identified as a risk factor for CA-MRSA infections in the United States (9). Only limited information is available regarding the prevalence of colonization of healthy children attending CCCs in the United States. Single child care center prevalence studies have reported frequencies of MRSA colonization in children of 1.2% (2 of 164) (36) and 6.7% (7 of 104) (15). These results are similar to the 2.5% (3 of 122) (16) and 1.7% (5 of 291) (5) frequencies of MRSA colonization found in healthy children attending outpatient pediatric clinics in Chicago and to the 0.9% prevalence in children in Boston communities (24).

Since all previous studies of MRSA colonization in children attending CCCs in the United States were single center based and small, we undertook our study to further define the prevalence of MRSA colonization, characterize the MRSA strains, and determine the risk factors for colonization. Our study of 1,163 subjects from 200 classrooms in 24 centers revealed that overall 18.1% were colonized with *S. aureus* and 1.3% were colonized with MRSA. The frequency of nasal colonization was lower than the 31.1% reported among 1,192 children under 5 years of age attending child care centers in Brazil but similar to the 1.2% frequency of MRSA colonization (23). The prevalence we determined may also be underestimated since some potential subjects had already been identified as MRSA positive and did not consent for participation in our study. Importantly, our study which used molecular typing did not reveal evidence of person-to-person transmission of MRSA strains within classrooms. This is in contrast to the transmission observed by Hewlett et al. in a single university-based facility (15).

The molecular analysis of the colonizing MRSA strains revealed that six isolates were both PVL positive and SCC*mec* type IV, which is the classic molecular definition of CA-MRSA. Of those that were PVL negative ($n = 9$), only two were SCC*mec* type II—the molecular classification for HA-MRSA. When multilocus sequence typing with subsequent cluster analysis was applied (Fig. 1), the identification of one CA-MRSA strain was PVL negative and six HA-MRSA strains were SCC*mec* type IV. The latter isolates are likely related to the USA 800 pediatric clone. Interestingly, there was no difference in proportions of children with a history of hospitalization (Table 4)

TABLE 4. Case-control study

Parameter	*P*	Result for group											
		Negative (control)				MSSA				MRSA			
		n	%	Mean	SD	*n*	%	Mean	SD	*n*	%	Mean	SD
Age (yr)	0.37	30		4.5	1.3	30		5	1.6	15		4.5	1.4
Males	0.65	14	47			14	47			9	60		
Time (h/day) in child care center[a]	0.68	16		34.4	11.2	10		34.7	11.8	9		30.2	15.5
Sport participation (h/wk)	0.66	30		1	1.6	30		1.2	1.8	15		0.7	1.7
Underlying disease	0.47	2	7			1	3			2	13		
Hospitalization	1.00	4	13			4	13			2	13		
Antibiotics	0.09	20	67			25	83			14	93		
Chronic skin condition	0.10	2	7			7	23			3	20		
Skin infections	0.37	10	33			13	43			8	53		
Eczema	0.62	6	20			7	23			5	33		
History of staphylococci	0.11	3	10			0	0			1	7		
Pet(s) (cat, dog, or rodent)	0.23	18	60			23	77			11	73		
Single-family home	0.84	26	87			25	83			12	80		
Home on military base or military person in home	0.83	10	33			8	27			4	27		
Health care worker in home	0.06	11	37			3	10			2	13		
Skin infections in home	0.13	5	17			7	23			7	47		
No. of people in household	0.71	30		4	1	30		3.8	1.1	15		4.1	1.0
<12 yr old		30		1.9	0.8	30		1.7	0.6	15		1.7	0.5
12–18 yr old		30		0.2	0.5	30		0.3	0.7	15		0.3	0.6
>18 yr old		30		2	0.3	30		1.9	0.4	15		2.1	0.7
No. of rooms in home	0.45	30		7.5	1.7	30		7.3	2.2	15		6.7	2.2
Ethnicity	0.07												
White		24	80			27	90			9	60		
Nonwhite		6	20			3	10			6	40		
Household income	0.61												
<$50,000		5	17			7	23			5	33		
$50,001–$75,000		9	30			7	23			5	33		
$75,001–$100,000		11	37			7	23			1	7		
>$100,000		5	17			9	30			4	27		

[a] Number of hours at the time of case-control questionnaire.

among those who were molecularly classified as CA-MRSA versus HA-MRSA. Our study suggests that the terms "community associated" and "health care associated" no longer apply in the CCC setting based on their traditional epidemiologic definitions (20). Similarly, molecular typing beyond SCC*mec* and PVL determination is necessary to accurately categorize MRSA strains and therefore the types of infections they are likely to cause (e.g., CA-MRSA and skin and soft tissue infections).

Multiple factors have been associated with CA-MRSA infection, including participation in contact sports, pet ownership, concurrent skin and soft tissue infections, and close contact (in the same household) with a person colonized or infected with MRSA. Our case-control study was not able to demonstrate any risk factors specific for MRSA colonization in our study population. However, this was likely due to our small number of colonized subjects. If the data from our study and that conducted in Brazil are generalizable to the United States, then determining risk factors for MRSA colonization would require an enormously larger study. Based on our MRSA prevalence of 1.3%, we would need 3,909 subjects to detect an odds ratio of 2.0 and over 12,000 subjects to detect an odds ratio of 1.5.

Additional potential limitations of the current study should be acknowledged. First, the participation rate among eligible children was 40%, which may make it difficult to generalize our results to a larger population of healthy children. However, the cross-section analysis of children enrolled in each center was demographically representative of the center in general based on center-level demographic data obtained (data not shown). Another limitation was the difference in demographic information obtained in children enrolled in North Carolina versus those enrolled in Virginia due to IRB-specific guidelines. This resulted in the lack of demographic information available for Virginia children making it impossible to compare the population of children in North Carolina and Virginia and to analyze the relationship between age and colonization status in Virginia, unless the subject was in the case-control study. Due to missing data for some of the variables, only bivariate associations could be analyzed in both the prevalence data and the case-control study (Tables 2 and 4).

In summary, we have demonstrated that approximately 1% of young children attending child care centers are colonized with MRSA. We did not document any person-to-person transmission of MRSA strains within classrooms. We were unable to statistically document risk factors associated with

MRSA colonization but were limited by the small number of colonized children. It will be important for much larger multicenter studies to be performed to identify risk factors associated with MRSA colonization and subsequent infection in this highly susceptible population.

ACKNOWLEDGMENTS

The views expressed in this article are those of the author(s) and do not necessarily reflect the official policy or position of the Department of the Navy, the Department of Defense, or the United States Government.

We gratefully acknowledge Cubist Pharmaceuticals for its financial support of this study.

REFERENCES

1. **Acton, D. S., M. J. Plat-Sinnige, W. van Wamel, N. de Groot, and A. van Belkum.** 2009. Intestinal carriage of *Staphylococcus aureus*: how does its frequency compare with that of nasal carriage and what is its clinical impact? Eur. J. Clin. Microbiol. Infect. Dis. **28:**115–127.
2. **Boucher, H. W., and G. R. Corey.** 2008. Epidemiology of methicillin-resistant *Staphylococcus aureus.* Clin. Infect. Dis. **46**(Suppl. 5)**:**S344–S349.
3. **Boye, K., M. D. Bartels, I. S. Andersen, J. A. Moller, and H. Westh.** 2007. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I-V. Clin. Microbiol. Infect. **13:**725–727.
4. **Centers for Disease Control and Prevention.** 3 March 2010, posting date. Healthcare-associated methicillin resistant *Staphylococcus aureus* (HA-MRSA). http://www.cdc.gov/ncidod/dhqp/ar_mrsa.html.
5. **Cheng Immergluck, L., et al.** 2004. Prevalence of *Streptococcus pneumoniae* and *Staphylococcus aureus* nasopharyngeal colonization in healthy children in the United States. Epidemiol. Infect. **132:**159–166.
6. **Churchill, R. B., and L. K. Pickering.** 1996. Infections in child care centers. Curr. Opin. Infect. Dis. **9:**176–180.
7. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement. M100-S19, p. 52-59. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Diederen, B. M., and J. A. Kluytmans.** 2006. The emergence of infections with community-associated methicillin resistant *Staphylococcus aureus.* J. Infect. **52:**157–168.
9. **Dietrich, D. W., D. B. Auld, and L. A. Mermel.** 2004. Community-acquired methicillin-resistant *Staphylococcus aureus* in southern New England children. Pediatrics **113:**e347–e352.
10. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
11. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 11 May 2010, posting date. The seven loci and the primers and conditions used for PCR. http://saureus.mlst.net/misc/info.asp.
12. **Fridkin, S. K., et al.** 2005. Methicillin-resistant *Staphylococcus aureus* disease in three communities. N. Engl. J. Med. **352:**1436–1444.
13. **Goodrich, J. S., et al.** 2009. Prevalence of community-associated methicillin-resistant *Staphylococcus aureus* in patients with cystic fibrosis. J. Clin. Microbiol. **47:**1231–1233.
14. **Graham, P. L., III, S. X. Lin, and E. L. Larson.** 2006. A U.S. population-based survey of *Staphylococcus aureus* colonization. Ann. Intern. Med. **144:** 318–325.
15. **Hewlett, A. L., P. S. Falk, K. S. Hughes, and C. G. Mayhall.** 2009. Epidemiology of methicillin-resistant *Staphylococcus aureus* in a university medical center day care facility. Infect. Control Hosp. Epidemiol. **30:**985–992.
16. **Hussain, F. M., S. Boyle-Vavra, and R. S. Daum.** 2001. Community-acquired methicillin-resistant *Staphylococcus aureus* colonization in healthy children attending an outpatient pediatric clinic. Pediatr. Infect. Dis. J. **20:**763–767.
17. **Jenkins, T. C., et al.** 2009. Epidemiology of healthcare-associated bloodstream infection caused by USA300 strains of methicillin-resistant *Staphylococcus aureus* in 3 affiliated hospitals. Infect. Control Hosp. Epidemiol. **30:**233–241.
18. **Jensen, J. U., et al.** 2006. Control of a methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak in a day-care institution. J. Hosp. Infect. **63:**84–92.
19. **Jorgensen, J. H., S. A. Crawford, M. L. McElmeel, and K. R. Fiebelkorn.** 2004. Detection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing. J. Clin. Microbiol. **42:**1800–1802.
20. **Klevens, R. M., et al.** 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA **298:**1763–1771.
21. **Kluytmans, J., A. van Belkum, and H. Verbrugh.** 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. **10:**505–520.
22. **Kuehnert, M. J., et al.** 2006. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001–2002. J. Infect. Dis. **193:**172–179.
23. **Lamaro-Cardoso, J., et al.** 2009. Molecular epidemiology and risk factors for nasal carriage of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in infants attending day care centers in Brazil. J. Clin. Microbiol. **47:**3991–3997.
24. **Lee, G. M., et al.** 2009. Epidemiology and risk factors for Staphylococcus aureus colonization in children in the post-PCV7 era. BMC Infect. Dis. **9:**110.
25. **Lowy, F. D.** 1998. *Staphylococcus aureus* infections. N. Engl. J. Med. **339:** 520–532.
26. **Miller, L. G., and B. A. Diep.** 2008. Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Clin. Infect. Dis. **46:**752–760.
27. **Milstone, A. M.** 2010. Community-associated methicillin-resistant *Staphylococcus aureus* strains in pediatric intensive care unit. Emerg. Infect. Dis. **16:**647–655.
28. **Mink, C. M., and S. Yeh.** 2009. Infections in child-care facilities and schools. Pediatr. Rev. **30:**259–269.
29. **Naimi, T. S., et al.** 2003. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA **290:**2976–2984.
30. **Nakagawa, S., et al.** 2005. Gene sequences and specific detection for Panton-Valentine leukocidin. Biochem. Biophys. Res. Commun. **328:**995–1002.
31. **North Carolina Department of Health and Human Services, North Carolina Division of Child Development.** 28 January 2011, accession date. Star rated license overview. http://ncchildcare.dhhs.state.nc.us/providers/pv_sn2_ov_sr.asp.
32. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:** 2155–2161.
33. **Orendi, J. M., et al.** 2010. Community and nosocomial transmission of Panton-Valentine leucocidin-positive community-associated methicillin-resistant *Staphylococcus aureus*: implications for healthcare. J. Hosp. Infect. **75:**258–264.
34. **Reischl, U., H. J. Linde, M. Metz, B. Leppmeier, and N. Lehn.** 2000. Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR. J. Clin. Microbiol. **38:**2429–2433.
35. **Safdar, N., and E. A. Bradley.** 2008. The risk of infection after nasal colonization with *Staphylococcus aureus*. Am. J. Med. **121:**310–315.
36. **Shahin, R., et al.** 1999. Methicillin-resistant *Staphylococcus aureus* carriage in a child care center following a case of disease. Toronto Child Care Center Study Group. Arch. Pediatr. Adolesc. Med. **153:**864–868.
37. **U.S. Census Bureau.** 28 January 2011, accession date. Who's minding the kids? Child care arrangements: summer 2006 detailed tables. http://www.census.gov/population/www/socdemo/child/tables-2006.html.
38. **von Eiff, C., K. Becker, K. Machka, H. Stammer, and G. Peters.** 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N. Engl. J. Med. **344:**11–16.
39. **Wertheim, H. F., et al.** 2004. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet **364:**703–705.
40. **Woodford, N., and D. M. Livermore.** 2009. Infections caused by Gram-positive bacteria: a review of the global challenge. J. Infect. **59**(Suppl. 1)**:** S4–S16.