Original Article

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Microbiology Section

Prevalence of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization Among Children

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ABSTRACT

Background: Invasive infections from community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are increasingly being encountered in healthy children. Nasal colonization of MRSA is associated with increased risk for acquiring invasive disease. The objective of this study was to determine prevalence and risk factors for CA-MRSA nasal colonization among a healthy paediatric population and to determine antibiotic susceptibilities of *S. aureus* isolates.

Materials and Methods: Using a cross-sectional study design, children aged 1mnth-17y attending well-child clinic at an academic hospital and a local public school in Mangalore, India were screened for *S. aureus* colonization via nasal swabs. A questionnaire was administered and data on risk factors for nasal colonization was collected. Samples were obtained from the anterior nares and cultured quantitatively. *S. aureus* isolates were confirmed by growth on selective media and coagulase testing. Disk diffusion antibiotic susceptibility tests were performed according to Clinical and Laboratory Standard Institute guidelines.

Results: Of the 500 children included in the study, *S. aureus* was isolated from the anterior nares in 126 (25%) children; four (3%) isolates were classified as CA-MRSA. Factors associated with *S. aureus* nasal colonization were children <6 y old (p=0.030) and members of joint families (p=0.044). Resistance to many classes of antibiotics were noted among *S. aureus* isolates including trimethoprim-sulfamethoxazole (39%), ciprofloxacin (16%), erythromycin (19%) and clindamycin (5%). Inducible clindamycin resistance (positive D test) was detected in 11 of the erythromycin-resistant strains not already classified as resistant to clindamycin. No resistance to vancomycin was observed.

Conclusion: Children in India have a high rate of nasal colonization of *S. aureus*. Nasal colonization of community-associated methicillin-resistant *S. aureus* exists but is still low among healthy children. The high rate of resistance to many classes of antibiotics among *S. aureus* strains is of great concern warranting continued surveillance and antimicrobial stewardship.

Keywords: Children, Disk diffusion test, Karnataka, Nasal carriage, *Staphylococcus aureus*

INTRODUCTION

In recent years, the prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections has increased among healthy children [1-3]. The CDC has created a case definition for a CA-MRSA infection: any MRSA infection diagnosed for an outpatient or within 48 h of hospitalization if the patient lacks the following health care-associated MRSA risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient [4]. Nasal colonization of MRSA has been correlated with increased risk for acquiring invasive disease [5]. In the U.S. and other resource rich settings, many studies have documented the prevalence of nasal colonization of MRSA in children in the community without any established risk factors [1-3]. Rates of nasal colonization of CA-MRSA have been increasing in the U.S; a study conducted in Nashville, TN reported increasing rates of nasal carriage of MRSA (from 0.8% in 2001 to 9.2% in 2004) [2,3].

Although the epidemiology of CA-MRSA colonization and infection in the U.S. community has been extensively studied, data is limited on the extent of CA-MRSA colonization in India [6-8]. With a human population of over 1 billion and growing, the microbial milieu within India should be closely monitored, especially with recent reports of global spread antimicrobial resistance [9]. Few studies from India have reported the prevalence of nasal carriage of *S. aureus* among the healthy preschool children below age four years and two prevalence studies have been conducted in children aged 5 to 15 y of age [10-13].

The purpose of this study was to assess rates of CA-MRSA nasal colonization among children. Risk factors for colonization and antimicrobial susceptibility characteristics among *S. aureus* isolates were also studied.

MATERIALS AND METHODS

Setting and Population

The study was conducted at the K.S. Hegde Medical Academy (KSHEMA), an academic tertiary care teaching hospital located in Deralakatte, a suburb of Mangalore, Karnataka, India. A total of 500 participants between the ages of 1 mnth to 17 y old were enrolled over July- September 2010. If the child had earlier history of hospitalization within a year, indwelling catheters, or a history of skin and soft-tissue infection in the previous year were excluded from the study. Participants were enrolled upon presentation to the paediatric clinic for well-child visits including immunizations. In addition, participants were also enrolled from a local public school.

Questionnaire

After obtaining informed consent, a trained research assistant administered a questionnaire in the local language to the guardian of each participant to collect demographic data, to measure risk factors for colonization, and to evaluate whether the colonization was community or health care associated.

Sample Collection and Culture Methods

Specimen collection and laboratory procedures were primarily modeled after previous techniques used by Nakamura et al., and

Factor	Positive no(%)	Negative no(%)	p-value	95%CI
Gender: Male Female	88(36.5) 102(39.4)	153(63.5) 157(60.6)	0.509	(0.671, 1.271)
Age: <6 years ≥ 6 years	43(30.5) 147(40.9)	98(69.5) 212(59.1)	0.030	(0.418, 0.959)
Dwelling : Urban Rural	65(35.9) 124(39.2)	116(64.1) 192(60.8)	0.462	(0.594, 1.267)
Family Income: <5000 Indian rupees ≥5000 Indian rupees	 148(37.1) 42(43.8)	 251(62.9) 54(56.2)	0.229	(0.483, 1.191)
Family type: Joint(≥6) Nuclear(≤5)	95(43.2) 93(34.3)	125(56.8) 178(65.7)	0.044	(1.009, 2.098)
Chronic disease: Absent Present	177(37.7) 13(43.3)	125(56.8) 178(65.7)	0.535	(0.375, 1.665)
Hospitalization : Yes No	21(38.2) 169(38)	34(61.8) 276(62.0)	0.977	(0.567, 1.796)
Surgery: Yes No	11(47.6) 180(37.6)	21(52.4) 299(62.4)	0.353	(0.629, 3.627)
Visits to Hospitals/Clinics ≤2 ≥2	 105(37.2) 82(38.9)	 177(62.8) 129(61.1)	0.712	(0.646, 1.347)
Contact with health care worker Yes No	 28(39.4) 160(37.5)	 43(60.6) 267(62.5)	0.752	(0.649, 1.818)
Day care attendance: Yes No	8(40) 182(37.90)	12(60) 298(62.1)	0.851	(0.438, 2.721)

[Table/Fig-1]: Risk factors for nasal colonization of *S. aureus* among 500 children (n=500)

Mainous et al., [2,14]. Samples were obtained from the anterior nares of each child participant using a sterile Culturette swab (BBL Culture Swab; BD Diagnostics, Franklin Lakes, NJ), pre-moistened with sterile water. The swab was inserted into each nostril, rotated for 5 sec, and placed immediately into the Culturette tube. The sample was plated on mannitol salt agar and incubated for 48 h at 37°C. Mannitol-fermenting colonies appeared yellow and signified the presence of *S. aureus*. The samples that were positive for mannitol fermentation were then inoculated onto 5% sheep blood agar and incubated at 37°C for 24 h. The isolates were identified based on colony morphology, gram staining, catalase and tube coagulase tests.

Antimicrobial Susceptibility Testing

A single colony was chosen from each coagulase-positive *S.aureus* isolate and streaked onto a Mueller-Hinton agar plate for susceptibility testing. Kirby-Bauer disc diffusion on Mueller-Hinton medium was used to determine antimicrobial susceptibility to oxacillin, penicillin-G, clindamycin, erythromycin, gentamicin, vancomycin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole (TMP-SMX) . The plates were incubated for 24 h at 37°C and zone diameters were measured, recorded, and classified as sensitive, intermediate, or resistant according the CLSI interpretation table [15]. The isolates resistant to oxacillin were confirmed to be MRSA by Cefoxitin disc incubated at 35°C for 24 h. Quality control organism *S.aureus* ATTC 25923 was used according to standard procedures recommended by CLSI. Isolates resistant to cefoxitin were classified as MRSA. Inducible resistance to clindamycin was tested by 'D-test' as per CLSI guidelines [16]. The erythromycin (15 µg) and clindamycin discs were placed at a distance of 15 mm (edge to edge) from each other on a Mueller-Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspensions. Following incubation at 37°C, for 18- 24 h, flattening of zone (D-shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance

STATISTICAL ANALYSIS

The data were analyzed using SPSS software package (Statistical Package for the Social Sciences; SPSS for Windows, Inc., Chicago, IL). Questionnaire data were recorded on the questionnaire forms and entered into a Microsoft Excel spreadsheet. Laboratory results were entered into the spreadsheet along with the corresponding participant's information as they become available. Descriptive statistics (including means, standard deviations, frequencies and percentage) were calculated for the socio-demographic variables. Stepwise logistic regression was used to evaluate independent associations. All tests were 2-tailed, and a $p<0.05$ was considered statistically significant.

S. No.	Antibiotics	Resistant	Sensitive
1	Cefoxitin	4(3%)	122(97%)
2	Oxacillin	4(3%)	122(97%)
3	Ciprofloxacillin	20(16%)	106(84%)
4	Clindamycin (constitutive)	6(5%)*	120(95%)
5	Co-trimoxazole	49(39%)	77(61%)
6	Erythromycin	24(19%)	102(81%)
7	Gentamicin	4(3%)	122(97%)
8	Penicillin	58(46%)	68(54%)
9	Tetracyclin	1(1%)	125(99%)
10	Vancomycin	0	126(100%)

[Table/Fig-2]: Antibiotic susceptibility patterns of 126 *Staphylococcus aureus* isolates

Ethical Considerations

Written informed consent was obtained from each participant and the study was approved by the Institutional Review Boards at Wake Forest University Health Sciences and the Ethics Committee at K. S. Hegde Medical Academy.

RESULTS

Demographic characteristics, prevalence and risk factors of the study population. Among the 500 children included in the study the statistically significant risk factors for nasal colonization of *S. aureus* were children younger than 6 y of age and members of joint families [Table/Fig-1].

One hundred twenty six children were found to be colonised with *S.aureus*. Of the 126 *S.aureus* only 4 isolates were MRSA. Among 126 the *S.aureus* colonised children 71 (56.35%) were girls and 55 (43.65%) were boys. Their dwelling was noted down as 86 (68.25%) rural and 40 (31.75%) urban. We found that among the colonised children 14.9% children had visited hospital on Well child visits, 2.4% children had come for Immunization, 11.11% children had come for Sick visit, 26.2% children had come for a Dental preventative visit, 45.23% children attended a Dental health camp. The age group of the colonised varied over a wide range, It was found 6% children were ≤ 1 y, 14.3% children belonged to the age group >1 y - ≤6 y, 71.7% children in > 6 -≤13y of age, and 7.5% children in the age group of > 13 - ≤ 17. Of these 126, 15.07% children were found to have taken antibiotics in the past one month.

Antimicrobial susceptibility

Based on the antimicrobial susceptibility reports among the 126 *S. aureus* isolates, 122 (97%) were classified as MSSA and 4 (3%) were MRSA [Table/Fig-2]. Resistance to many classes of antibiotics were noted among *S. aureus* isolates including trimethoprim-sulfamethoxazole (TMP-SMX) (39%), ciprofloxacin (16%), erythromycin (19%) and constitutive clindamycin resistance (5%). Constitutive MLS B phenotype was labeled for those *Staphylococcal isolates*, which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14 mm) with circular shape of zone of inhibition if any around clindamycin. Inducible MLSB(iMLSB) phenotype was labeled for those *Staphylococcal isolates*, which showed resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm)

and giving D-shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (positive D-test) [16]. Inducible clindamycin resistance (positive D-test) was detected in 11 of the erythromycin-resistant strains not already classified as resistant to clindamycin. Isolates did not exhibit any resistance to vancomycin by disc diffusion method. Resistance to many classes of antibiotics were noted among MSSA isolates.

DISCUSSION

This study from South India reports the nasal carriage of *S. aureus* among healthy children in the community in the age group of 1 mnth to 17 y. Our study showed that 25% of healthy children between the age group of 1 mnth -17 y attending primary care clinic were colonized with *S. aureus* in the anterior nares. Our findings are consistent with previous reports from India [8,10,11].The prevalence of nasal carriage of *S. aureus* in healthy preschool children aged 1 mnth through 59 mnth was 6.3% in a large study (n=1,562) from Ujjain, India [10]. In another study from Andhra Pradesh, India, a carriage rate of 16% for *S. aureus* (19% MRSA) was documented [11]. Studies from Taiwan and the U.S. have documented prevalence of nasal carriage of *S. aureus* among children ranging from 16% - 23% respectively [14,15]. In contrast, Chaterjee, SS et al., studied 489 school children aged 5-15 years by PCR and found nasal colonization of *S. aureus* in 256 (52.5%) of children, which is much higher compared to our study and other reports [12,13]. The comparatively higher prevalence rate may be attributed to the characteristics of the study population, although other factors (e.g., sampling and culture techniques) may have played a contributory role.

The low prevalence of MRSA isolates, 4 (3%) was found to be consistent with studies from the U.S., India, Turkey and Taiwan, reporting low rates of MRSA colonization among healthy children in the community ranging from 0.3% to 7.8% [12,17,18]. In contrast, other studies from India and other countries have documented higher rates of MRSA colonization ranging from 13.2% to 22% [13,19-21]. In one study from the U.S., the nasal colonization rate of MRSA among healthy children increased from 0.8% in 2001 to 9.2% in 2004 [2,3]. Data from our study and other reports suggests that healthy school going children under the age of 16 y of age are potential carriers of *S. aureus* including MRSA. The differences in the carriage rate of *S. aureus* (and the percentages of MRSA isolates) from different regions suggesting geographical differences in the prevalence of MRSA colonization.

The prevalence of colonization with *S. aureus* has previously been shown to be age dependent [5,6,22]. In our study, the prevalence varied across different age groups, with the lower prevalence in the first 12 months of life. This may be due to the fact that the children do not mingle in the community on their own and are bound to be closer to their parents. Statistically significant risk factors for colonization included children below 6 y of age and members belonging to joint families. School going children were shown to have significantly higher prevalence of carriage. The finding is consistent with the fact that large family size with 10 or more members had higher carriage prevalence as compared to families with less than or equal to 4 members. This might be due to poor hygiene and overcrowding. Recent studies have demonstrated the importance of close contacts within households and with parents in spread of *S. aureus* carriage among children residing in the same household [23,24]. In our study, history of hospitalization prior to one year, visits to hospitals or clinics and contact with the health care workers were not statistically significant factors associated with nasal carriage of *S. aureus*, as in contrast to other studies [5,6,25-27]. In our study, the *S. aureus* isolates exhibited resistance to multiple classes of antibiotics including TMP-SMX resistance (39%), ciprofloxacin (16%), erythromycin resistance (19%), and constitutive clindamycin resistance (5%); inducible clindamycin resistance (positive D test) was detected in 11 (55%) of the erythromycin-resistant strains. Other studies have also found similar results [11,27,28]. In a recent study from Ujjain, MSSA isolates were found to be resistant to many classes of antibiotics including ampicillin (90%), amoxicillin-clavulanate(54%), TMP-SMX (49%), ciprofloxacin (23%) and erythromycin (11%); of the erythromycin resistant strains of MSSA 15% were clindamycin inducible [10]. A study from Portugal found that among the 36 *S. aureus*, only 11.5% of isolates were susceptible to all antibiotics tested; a higher non-susceptibility rate (88.5%) to penicillin was detected,whereas it was much lower (46%) in our study [25].Compared to our study, a comparatively lower rate of resistance was found in the study conducted by Oguzkaya-Artan M, et al., where erythromycin resistance was noted in 6 of the 36 isolates (16.7 %) and clindamycin resistance was present in 3 of the 36 isolates (8.3% total, 6.2 consecutive and 2.1% inducible); All tested isolated were susceptible to vancomycin, as noted in our study [29].

In our study we found a comparatively low level of resistance to penicillin by disc diffusion method, in contrast to other reports [28]. This may be due to the fact that TMP-SMX (Cotrimaxazole) is prescribed in the outpatient department as the first drug of choice instead of penicillin. Resistance to clindamycin and TMP-SMX in our setting is a concern since these antibiotics are routinely used to treat common infections due to *S. aureus* in the outpatient setting and often prescribed to hospitalized patients upon discharge to complete a course of outpatient therapy. Judicious antimicrobial use should be implemented by the physicians in this setting given the increasing prevalence of drug resistant microbes.

Our study has several limitations. First, the sample size was relatively small. Second, we did not investigate the colonization at other body sites (such as, axillae, pharynx, and rectum) thereby underestimating the true prevalence in our study population. We elected to sample the nasal cavity due to easy of collection, adherence and consistency with other studies; in addition, studies have reported a relatively high sensitivity (~66%) of nose swabs in detecting MRSA carriage [30]. Third, this observational, cross-sectional study was performed at a single location involving relatively healthy children, limiting the generalization of our results throughout the country. Fourth, we did not study the persistence of *S.aureus* nasal colonization, which would warrant a community based cohort design with repeated sampling of children over a period of time. Finally, we did not perform molecular typing of the strains due to lack of funding.

CONCLUSION

Children in Mangalore, India have a high rate of colonization with *S. auerus*. Nasal colonization of CA-MRSA exists but is still low among healthy children lacking traditional risk factors for MRSA infection. This study has demonstrated the baseline colonization rate and continued surveillance of this population is necessary to assess the ongoing risk CA-MRSA poses to this community. The high rate of antibiotic resistance to TMP-SMX and other classes of frequently used antibiotics to *S. aureus* is a major concern warranting continued surveillance and antimicrobial stewardship programs to promote judicious use of antimicrobials in the hospital and ambulatory settings.

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