Original Article

Prevalence of Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Oral and Nasal Cavities of 4 to 13-year-old Rural School Children: A Cross-sectional Study

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Abstract

Aim: This study aimed to investigate the oral and nasal prevalence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in 4 to 13-year-old rural schoolchildren. **Materials and Methods:** A total of 100 children aged 4 to 13 years were randomly selected and divided into ten groups based on their age (Group 1 = 4-year-old children to Group 10 = 13-year-old children). From each participating child, sampling was done from the anterior nares and dorsum of the tongue. All samples were inoculated into Baird–Parker agar medium and HiCrome™ MeReSa agar medium for the isolation of SA and MRSA. Both the culture plates were checked for the presence of SA and MRSA and overall SA and MRSA carriage. The distribution of SA and MRSA was evaluated. Descriptive statistics were performed using SPSS software (version 17.0). **Results:** Overall SA in 4–13 years' age group was 47%, while CA-MRSA was 35%. On the tongue, 16 children had concomitant MRSA and SA, while only 23.8% (n = 20) of the children comprised the presence of SA when MRSA was absent (P < 0.001). In the nasal cavity, 30 children had concomitant MRSA and SA, while only 21.4% (n = 15) of the children had the presence of SA when MRSA was absent (P < 0.001). When tongue and nose were assessed, 11 children encompassed concomitant MRSA and SA, while only 16.9% (n = 13) of the children had the presence of SA when MRSA was absent in both sites (P < 0.001). **Conclusion:** A significant relation was found between nasal SA and CA-MRSA carriage, with oral SA and CA-MRSA carriage. The study concludes that oral cavity is possibly as important as the nasal area as a zone of SA and MRSA. Dentists dealing with pediatric population should take proper precautions to prevent cross contamination of SA and MRSA in the dental clinic.

Keywords: *Community-associated methicillin-resistant Staphylococcus aureus, methicillin, nasal cavity, oral cavity, Staphylococcus aureus*

Introduction

Staphylococcus aureus (SA) is a Gram-positive, opportunistic bacterium that frequently colonizes the oral cavity, nasal cavity, and skin of the healthy people. This can cause a variety of localized and invasive problems ranging from superficial skin infections to life-threatening pneumonia and bloodstream infections. SA infections have encountered humans since ancient times. The first therapeutic use of penicillin for SA infection in 1940 was followed quickly by the appearance of the first penicillin-resistant strains of SA.[1] Antibiotic-resistant SA strains are considered as a major health problem.[2] Many circumstances in dentistry may contribute to the transmission of microorganisms. The mucosa, skin, environment, and instruments can be contaminated with saliva, blood, or organic debris during routine dental treatment.[3] *Staphylococcus* species and *Viridians streptococci* are the most prevalent microorganisms found on the surfaces of dental equipment.[3] There is a strong evidence suggesting the transmission of SA between patients and the dentist via the clinical environment.[4]

An association between the presence of SA and oral mucosal conditions such as angular chelitis, erythema, swelling, and burning has been demonstrated, suggesting its role in oral mucosal disease.[3] Nasal and oral carriages of methicillin-resistant SA (MRSA) serve as a reservoir for the recolonization of other body sites and cross

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infection between the patient and health-care workers.[5] Moreover, children are more susceptible to MRSA infection. Knowing the prevalence of nasal and oral colonization will provide an indication of the higher risk for subsequent infections, including MRSA. SA colonization varies markedly with demographic characteristics, the highest prevalence was noted among young school-age children.[6] Literature is sparse about the colonization and isolation of SA and MRSA from specimens of oral and nasal cavities in rural schoolchildren of different age groups. The purpose of the present study is to isolate and know the oral and nasal prevalence of MRSA in 4 to 13-year-old rural schoolchildren.

Materials and Methods

The list of schools existing in Vikarabad Mandal was obtained from the Deputy Education Office, Vikarabad. Randomly ten schools were chosen and the upper primary school from Munnuru Somaram village was selected based on the lottery method. The study group comprised of 100 randomly selected children aged 4–13 years and were divided into ten groups. Each group comprised of ten children, with Group 1 = 4-year-old children to Group 10 = 13-year-old children. The ethical clearance was taken from the Institutional Review Board of Sri Sai College of Dental Surgery, Vikarabad, Telangana, India, prior to the study.

Inclusion criteria

Healthy school children residing in the rural area aged 4–13 years.

Exclusion criteria

1. Prolonged antibiotic usage
2. Immunocompromised state/drug abuse
3. Prolonged hospital stay
4. History of recent intravenous medication
5. Nonpurulent cellulitis
6. Children not willing to participate in the study in spite of consent obtained
7. Children with any systemic disease (cystic fibrosis and rheumatoid arthritis)
8. Children with rhinitis at the time of the study
9. History of boils, unhealing wounds, and recurrent abscesses in the oral cavity.

Sample collection

Sampling in the nasal cavity for each participating child was performed by twice rotating a sterile cotton swab, in the vestibule of both anterior nares, while tongue sampling was taken by rotating a sterile swab from the dorsum of the tongue. Each specimen was properly labeled for easy identification. The collected samples were transported to a cold box at a temperature between 4°C and 8°C to the microbiology laboratory at the Central Research Institute of Unani Medicine, Hyderabad, within 4 hrs of collection.

The specimens were diluted with buffered sodium chloride-peptone solution for 24 hrs. 0.1 ml of the sample was transferred to Baird–Parker agar medium (HiMedia Laboratories, Mumbai, India, Ref-MU043) and streaked for isolation of SA. A sample of 0.1 ml was transferred to MeReSa agar medium (HiMedia Laboratories, Ref-M1674) and streaked for isolation of MRSA. Both plates were incubated at 37°C ± 1°C for 24–48 hrs. Colonies were identified by the specific colony morphology and color specific to these media types. All the cultures showing dark black colonies surrounded by translucent hue on Baird–Parker agar medium were recorded as SA. All cultures showing bright blue-colored growth on MeReSa agar medium were taken as MRSA-positive strains. Both the culture plates were checked for the presence of SA and MRSA. Descriptive statistics were performed using SPSS software (version 17.0, Inc., Chicago, Ill., USA) with significant differences of 95% confidence interval ($P < 0.05$).

Results

The prevalence of SA across the 4 to 13-year-old children ranged from 30% to 60%, with highest being in 4-year-olds and lowest in 11-year-olds [Tables 1 and 2]. Gender-wise distribution of SA was noticed and the prevalence in males was 52.7% and that of females was 40% [Table 3]. In the gender-wise distribution of MRSA, the results noticed were as follows: in males, exclusively, oral and nasal cavities comprised a prevalence of 5.5% and 21.8%, respectively, while at both the sites, it was 9.1% [Table 4]. In females, exclusively, oral and nasal cavities showed the prevalence of 4.4% and 15.6%, respectively, while at both the sites, it was 13.3% [Table 4]. Comparison of SA tongue carriage along with MRSA and SA oral carriage without MRSA revealed the following results: on the tongue, a significant number of children ($n = 16$) had concomitant MRSA and SA, while only 23.8% of the children had the presence of SA when MRSA was absent ($P < 0.001$) [Table 5]. Comparison of SA nasal carriage along with MRSA and

Table 1: Distribution of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*

Distribution	Percentage
SA carriage overall	47.0
Total SA oral carriage	36.0
Total SA nose carriage	45.0
Exclusive nasal carriage of SA	11.0
Exclusive tongue carriage of SA	2.0
SA nose and oral carriages	34.0
MRSA carriage overall	35
Total MRSA oral carriage	16.0
Total MRSA nose carriage	30.0
Exclusive nasal carriage of MRSA	19.0
Exclusive oral carriage of MRSA	5.0
MRSA nose and oral carriages	11.0

MRSA: Methicillin-resistant *Staphylococcus aureus*; SA: *Staphylococcus aureus*

SA nasal carriage without MRSA revealed the following results: on the nose, a significant number of children (n = 30) had concomitant MRSA and SA, while only 21.4% of the children had the presence of SA when MRSA was absent (P < 0.001) [Table 6]. Comparison of SA oral and nasal carriages with MRSA oral and nose carriages revealed the following results: on combined oral and nasal carriages, a significant number of children (n = 11) had concomitant MRSA and SA, while only 16.9% of the children had the presence of SA when MRSA was absent from both sites (P < 0.001) [Table 7].

Table 2: Age-wise distribution of *Staphylococcus aureus*

Age	SA	*n* (%)
4	Absent	4 (40.0)
	Present	6 (60.0)
5	Absent	5 (50.0)
	Present	5 (50.0)
6	Absent	5 (50.0)
	Present	5 (50.0)
7	Absent	6 (60.0)
	Present	4 (40.0)
8	Absent	6 (60.0)
	Present	4 (40.0)
9	Absent	4 (40.0)
	Present	6 (60.0)
10	Absent	5 (50.0)
	Present	5 (50.0)
11	Absent	7 (70.0)
	Present	3 (30.0)
12	Absent	5 (50.0)
	Present	5 (50.0)
13	Absent	6 (60.0)
	Present	4 (40.0)

SA: *Staphylococcus aureus*

Table 3: Gender-wise distribution of *Staphylococcus aureus*

Sex	SA	*n* (%)
Male	Absent	26 (47.3)
	Present	29 (52.7)
Female	Absent	27 (60.0)
	Present	18 (40.0)

SA: *Staphylococcus aureus*

Table 4: Gender-wise distribution of methicillin-resistant *Staphylococcus aureus*

Sex	MRSA distribution	*n* (%)
Male	Both absent	35 (63.6)
	Oral only	3 (5.5)
	Nose only	12 (21.8)
	Both present	5 (9.1)
Female	Both absent	30 (66.7)
	Oral only	2 (4.4)
	Nose only	7 (15.6)
	Both present	6 (13.3)

SA: *Staphylococcus aureus*; MRSA: Methicillin-resistant SA

Discussion

Children have particularly shouldered a big burden of infections caused by the emergence of the MRSA pathogen in recent years. In the early global epidemic of MRSA, there was a clear distinction in the susceptibility pattern of CA-MRSA and health care-associated MRSA (HA-MRSA). Recent studies demonstrate that the boundaries between these two pathogens are getting blurred.[5] The enterotoxin Panton–Valentine leukocidin (PVL) genes are often present in CA-MRSA, whereas these genes are absent in HA-MRSA, thus making CA-MRSA more virulent than HA-MRSA.[7]

Table 5: Comparison of *Staphylococcus aureus* oral carriage along with and without methicillin-resistant *Staphylococcus aureus*

Oral carriage	MRSA		*P*
	Absent	Present	
SA			
Absent	64	0	<0.001*
Present	20	16	

McNemar test. *Significant; P<0.05 was considered statistically significant. SA: *Staphylococcus aureus*; MRSA: Methicillin-resistant SA

Table 6: Comparison of *Staphylococcus aureus* nose carriage along with and without methicillin-resistant *Staphylococcus aureus*

Nose carriage	MRSA		*P*
	Absent	Present	
SA			
Absent	55	0	<0.001*
Present	15	30	

SA: *Staphylococcus aureus*; MRSA: Methicillin-resistant *Staphylococcus aureus*; *= significant; P<0.05 considered as significant

Table 7: Comparison of *Staphylococcus aureus* oral and nose carriages, with methicillin-resistant *Staphylococcus aureus* oral and nose carriages

Oral and nose carriages	MRSA				*P*
	Both absent (*n*)	Oral only (*n*)	Nose only (*n*)	Both present (*n*)	
SA					
Both absent	53	0	0	0	<0.001*
Oral only	0	2	0	0	
Nose only	1	0	10	0	
Both present	11	3	9	11	

SA: *Staphylococcus aureus*; MRSA: Methicillin-resistant *Staphylococcus aureus*; *= Significant; P<0.05 was considered statistically significant

The presence of SA nasal and oral colonization can provide an indication of higher risk for subsequent infection, including with MRSA.[6,8] Children have served as the primary patient source for a significant number of these studies.[9] SA colonization varies markedly with demographic characteristics, the highest prevalence was noted among young school-age children. Very few studies have looked at MRSA colonization of the oral cavity. The reported prevalence rates of SA colonization in healthy children range from 6.3% to 72%.[5,6,10-19] The differences observed in the prevalence might depend on the differences in protocols, method of collection of samples, culture media, and methodology used for the isolation of staphylococci. In spite of similar factors favoring the growth of bacteria which are maintained *in vitro* and *in vivo*, bacterial growth may not be similar *in vitro* and *in vivo*. In the present study, the overall prevalence of SA colonization in 4 to 13-year-old children was 47%.

In the present study, the prevalence of SA nasal carriage was 45%. The result of the present study about the prevalence of SA on oral cavity was 36%. These data suggest that oral cavity of children could be a principal reservoir for SA. When the SA tongue carriage along with MRSA and SA tongue carriage without MRSA were compared, a significant number of children (n = 16) had concomitant SA and MRSA, while only 23.8% of the children had the presence of SA with absence of MRSA ($P < 0.001$). Miyake *et al*. reported 33% colonization of SA from the tongue of Japanese children.[20] Airborne transmission is important for dispersal of SA to many different reservoirs, from where they reach the nose and mouth by contaminated hands.[21] CA-MRSA continues to be more prevalent in previously healthy children with no specific predisposing risk. In our study, the overall prevalence of CA-MRSA in 4 to 13-year-old children was 35%.

The prevalence rates of MRSA nasal colonization in healthy children vary from 3.89% to 23%.[11,13,16,19] In the present study, the nasal prevalence of MRSA was 30%. Very few studies have investigated the prevalence rates of MRSA oral colonization in healthy children. According to a Japanese study, the prevalence of MRSA from tongue swabs was 16.3%.[20] In the present study, the prevalence of MRSA on the tongue was 16%.

The prevalence of colonization with SA and MRSA has shown to be age dependent.[4,15] The prevalence of SA across the 4 to 13-year-old children in the present study ranged from 30% to 60%, with highest being in 4-year-olds and lowest in 11-year-olds although differences were not statistically significant ($P > 0.01$). The prevalence of MRSA at both sites of tongue and nose in our study ranged from 0% to 20% and showed no statistically significant difference among 4 to 13-year-old children studied.

A Nigerian study reported that the rate of SA colonization varies in different genders.[22] The authors concluded the rate of SA colonization was 48% for males and 50% for females. The difference in the MRSA colonization in males (20.4%) and females (16.3%) was also not significant. The present study results revealed SA colonization rate of 52.7% for males and 40% for females and MRSA colonization rate of 9.1% for males and 13.3% for females. The results of the present study are similar to that of a Nigerian study and showed that there was no significant difference in the distribution of SA on exclusively tongue, nose, and both sites between males and females. A similar finding was seen with MRSA. This insignificance in the results of the colonization rate of SA and MRSA in the male and female groups indicates that sex is not a remarkable factor in colonization and there is no activity or way of life of any of the groups that predisposes them to SA and MRSA.

In the present study, on comparing the combined SA oral carriage and nasal carriage along with MRSA oral carriage and nasal carriage, a significant number of children (n = 11) had concomitant MRSA and SA, while only 16.9% of the children had the presence of SA when MRSA was absent in both sites, tongue, and nose ($P < 0.001$). A Brazilian study shown the persistence of SA in the oral cavity, especially in children, suggesting that it can serve as a reservoir for MRSA with a potential to spread and cause nosocomial infections.[23] MRSA may reside solely in the oral cavity or can derive from the anterior nares as a result of migration through the oropharynx. Previous studies have demonstrated that the status of MRSA carriers can persist even for 2 years. MRSA clones may colonize in the oral cavity of healthy children for relatively long periods of time (5 years), challenging the hypothesis that SA is a transient member of the oral flora.[24] Moreover, the mouth may represent a portal of entry for staphylococci causing systemic infections. Since Staphylococci is a component of oral flora, oral cavity can play a role as a reservoir of MRSA.[25] However, the risk factors for CA-MRSA carriage in children are not well understood.[23]

Persistent bacteremia was independently associated with MRSA-infective endocarditis. There is a strong relationship among the presence of SA and the occurrence of serious infections, such as infective endocarditis.[1,3,26] SA and MRSA in dentistry implicate acute dentoalveolar infections, angular cheilitis, parotitis, infected jaw cysts, oral mucosal lesions, stomatitis, and staphylococcal mucositis.[4] In a majority of the cases, MRSA causes skin and soft-tissue infections such as boils or abscesses, pustules, cellulitis, impetigo, carbuncle, and furuncle.[1] An association between the presence of SA and oral mucosal alterations such as like angular cheilitis, erythema, swelling, and burning has been demonstrated in a retrospective study of clinical laboratory data.[3,27] Presence of SA in saliva was considered to be a significant risk factor for aspiration pneumonia.[4] High levels of MRSA have been detected on the dental chair and floors of a dental office, suggesting that preventive measures

should be indicated to avoid the dissemination of these microorganisms.[3,28] A high frequency of SA was detected from the operator's hands, mainly with gloves, before the dental appointment. Dentists should use masks and special protective glasses and perform antisepsis procedures on their hands before and after appointments.[3]

Most of the Indian studies have investigated only nasal colonization of SA and MRSA in children. This study contributes that oral presence of SA and MRSA in our study population of rural schoolchildren is very high and is in proportion with the nasal presence of SA and MRSA, which is quite alarming. This "Super Bug" can potentially infect any system of the human body. Given the relationship between nasal carriage, oral carriage, and infection risk, knowledge of the prevalence of MRSA in a community provides a sense of the probability of contracting an MRSA infection in that community. The oral cavity is possibly as important as the nasal area as a zone of staphylococci. Dentists dealing with pediatric population should take proper precautions to prevent cross contamination of SA and MRSA in the dental clinic. Close monitoring by repeated studies will be of importance because knowledge about the prevalence of CA-MRSA will be useful in managing few cases of CA SA infections. More longitudinal studies with continued surveillance are needed in a rural Indian population with different socioeconomic dynamics to correctly identify risk factors in otherwise healthy children.

Conclusion

The following conclusions were drawn from the study:

- Our study showed a high rate of SA colonization (47%) and an alarming rate of CA-MRSA carriage (35%) in 4 to 13-year-old rural schoolchildren
- The prevalence of SA on oral and nasal cavities was 36% and 45%, respectively
- The prevalence of CA-MRSA on oral and nasal cavities was 16% and 30%, respectively
- There is a significant relation between nasal SA and CA-MRSA carriage, with oral SA and CA-MRSA carriage.

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Conflicts of interest

There are no confl icts of interest.

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