DOI: 10.7860/NJLM/2021/45862.2506

Original Article

Microbiology Section

Nasal Carriage of *Staphylococcus aureus* in Different Age Groups among Healthy School Children and its Antimicrobial Susceptibility Pattern

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ABSTRACT

Introduction: *Staphylococcus aureus* (*S.aureus*) is associated with increased risk for acquiring invasive disease. There exists an increasing prevalence of resistant community acquired *S.aureus* infections due to the nasal colonisation of *S.aureus* and Methicillin-Resistant *S.aureus* (MRSA) worldwide.

Aim: To evaluate the prevalence rate of *S.aureus* and MRSA nasal colonisation among healthy school children and to determine its antibiotic susceptibility rate of the *S.aureus* isolates.

Materials and Methods: The community based cross-sectional study was conducted among 344 school going children of 5-16 years of age. Samples were obtained from the anterior nares from each child and cultured on Blood Agar (BA) and Mannitol Salt Agar (MSA). Antibiotic susceptibility pattern of *S.aureus* was done by Kirby-Bauer disc diffusion method. MRSA detection was done through Cefoxitin 30 µg discs along with MIC for Oxacillin and Vancomycin simultaneously according to Clinical Laboratory Standard Institute (CLSI).

Results: Of the 344 children, 26 (7.55%) were found to be with nasal carriage for *S.aureus* of which MRSA was found to be in 12 (3.48%) isolates. *S.aureus* isolates exhibited resistance to multiple classes of antibiotics including Penicillin (100%), Co-trimoxazole (50%), Ciprofloxacin (15.4%), Vancomycin (7.7%), Clindamycin (7.7%), Gentamycin (7.7%). No resistance to Linezolid was observed.

Conclusion: A relatively high rate of nasal carriage of *S.aureus* in children of age group 5-10 years was observed when compared to children of age group 11-15 years. With the risk involved transmission of infection and resistance to many classes of antibiotics among *S.aureus* strains exists and hence, emphasis needs to be laid to design control measures, continued surveillance and its eradication.

Keywords: Cefoxitin, Community associated-methicillin resistant *S.aureus*, Nasal colonisation

INTRODUCTION

S.aureus is a major cause for community as well as nosocomial infections in different countries including India [1,2]. Nasal colonisation 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. *S.aureus* can survive in anterior nares for months asymptomatically in different regions of healthy individual [3]. In addition, spreading of *S.aureus* and MRSA to both community and hospital environments may also be because of children who serve as reservoir [4].

Although the infections were easy to treat earlier even after the development of resistance to penicillin, the problem arises with the emergence of MRSA. There has been an increasing prevalence of MRSA infections in India due to pressure of infections of *S.aureus* increasing [5]. MRSA is considered as a major cause of nosocomial infections and associated with high morbidity and mortality. Earlier MRSA infections were only associated with hospitalised patients known as Hospital-Associated MRSA (HA-MRSA). However, now-a-days it is also spreading among healthy individuals without any contact with healthcare workers, especially in children known as Community-Associated MRSA (CA-MRSA) without conventional risk factors for MRSA [6].

There is need for active surveillance of the organism because of huge disease burden due to increase in CA-MRSA infection. The carrier who is the reservoir of MRSA is asymptomatic, this is of public health importance. To prevent its transmission of MRSA in community and its early detection of the colonisation among children can be done by screening and decolonising the children so that primary care physicians may play a pivotal role [7]. Thus, serious infections caused by MRSA strains can be prevented by early detection of carriers. To understand the transmission among the healthy individuals as well as the diseased surveillance, it is necessary to evaluate the prevalence of nasal carriage of MRSA in community. Therefore, the present study was done to determine the epidemiology of MRSA in nasal carriage among 344 healthy school children.

MATERIALS AND METHODS

This study was a prospective, cross-sectional and observational study conducted in Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India from March 2018 to June 2019. Ethical clearance was obtained from the Institutional Review Board of Santosh Medical University. The objectives as well as the nature of the study were explained to the school community for the purpose of their consent.

Sample size calculation: Considering prevalence of nasal carriage of MRSA among healthy school children as 27.92% [5] and allowable error of 20%, at level of significance of 95%, the sample size was calculated using the standard formula:

$$N=4PQ/L^2,$$

where N is the sample size to be taken, P is the 27.92%,

Q=1-prevalence, L=Relative allowable error, Type 1 error=5%, Power=80%.

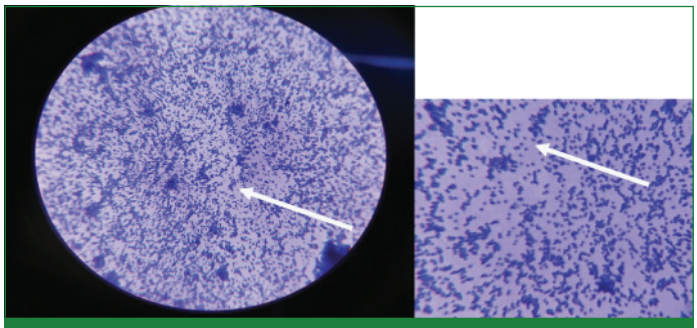
The desirable sample was obtained 344, based on the current population school going children of the age group of 5-15 years and only one school was included in the study.

Inclusion criteria: School children of age group of 5-15 years, who were asymptomatic were included in the study.

Exclusion criteria: Children who had history of hospitalisation in the past one year, oral antibiotics use in the past three days and intramuscular use in the past 28 days or presence of other illness was excluded from the study.

After obtaining informed consent, children were enrolled for the study based on the inclusion and exclusion criteria. The information was recorded on a self-structured pretested questionnaire (Sample questionnaire of 18 questions were given to 20 parents for validation and based on their answers, it was modified to 12 questions based on the study). It was administered in the local languages (English and Hindi as applicable) to the guardian of each participant to collect demographic. The information collected included identification details, demographic variables, present medical history, physical examination and previous medical history. Sampling for each participating child was performed with a single swab from both anterior nares by twice rotating 4-5 times both clockwise and anticlockwise before withdrawal. The collected samples were transported to the laboratory using Stuart transport medium.

Swabs were cultured on 5% sheep Blood Agar (BA) and Mannitol Salt Agar (MSA) media and incubated aerobically overnight at 37°C. After 24 hour of incubation, identification of isolates was done on the basis of colony characteristic, gram staining [Table/Fig-1] and biochemical tests which were performed based on standard microbiological procedures.



[Table/Fig-1]: Gram stain of *Staphylococcus aureus*.

Antimicrobial susceptibility patterns of the isolates were determined according to Kirby-Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines [8]. Antibiotic discs included were Penicillin (10 units), Gentamycin (10 µg), Clindamycin (2 µg), Erythromycin (15 µg), Vancomycin (30 µg), Linezolid (30 µg), Co-trimoxazole (1.25/23.75 µg), and Ciprofloxacin (5 µg) (HiMedia India). Inducible clindamycin resistance was detected by placing the Clindamycin and Erythromycin disc at a distance of 15-26 mm apart by disc diffusion method [8]. Flattening of the zone of inhibition adjacent to the erythromycin disk indicates inducible clindamycin resistance.

Detection of MRSA was carried out using Cefoxitin disc as per CLSI guidelines [9]. A 30 µg cefoxitin disc was used for disc diffusion test for all the isolates. Plates were incubated at 33-35°C for 16-18 hours and inhibition zone diameters (mm) were measured [Table/Fig-2]. An inhibition zone diameter of ≤21 mm was reported as mecA positive (oxacillin resistant) and ≥22 mm was considered as mecA negative (oxacillin sensitive). QC strains used were *S.aureus* ATCC 25923 - mecA negative (cefoxitin zone 23-29 mm) and *S.aureus* ATCC 43300-mecA positive (zone ≤21 mm). CLSI guidelines were used for the determination of MIC of oxacillin and vancomycin [8]. A range from 0.5 to 64 µg/mL and 0.5-128 µg/mL was prepared for doubling dilution of oxacillin and vancomycin, respectively. After incubation, the presence of >1 colony or light film of growth was considered as resistant.

[Table/Fig-2]: Mannitol Salt Agar with Cefoxitin Disc showing MRSA.

STATISTICAL ANALYSIS

Data was entered in Microsoft excel and descriptive statistics were used.

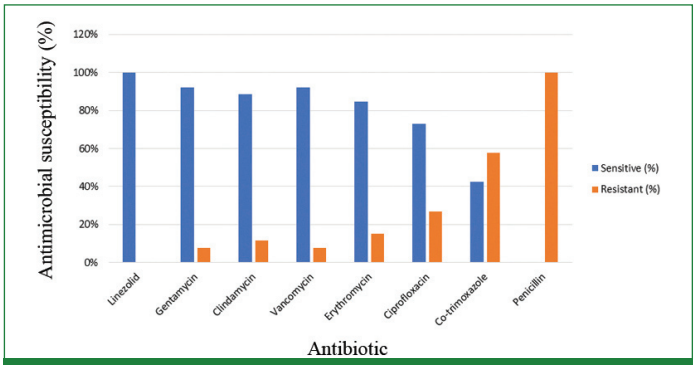
RESULTS

Of the 344 (samples collected), 166 (48.2%) samples were from the age group of 5-10 years and 178 (51.7%) samples were from the age group of 11-15. Total 177 (51.4%) were males and 167 (48.5%) were females. Frequency of *S.aureus* was 26 (7.6%) that were culture positive and 318 (92.4%) were culture negative. Among the 26 culture positive cases, 12 (3.48%) were males and 14 (4%) were females. Fifteen(4.36) isolates belong to age group 5-10 years while 11(3.20) isolates belong to 11-15 years [Table/Fig-3].

Variables	*Staphylococcus aureus* (%)	MRSA (%)	
		Positive	Negative
Sex			
Male	12 (3.49)	4 (1.16)	8 (2.33)
Female	14 (4.07)	8 (2.33)	6 (1.74)
Total	26 (7.56)	12 (3.49)	14 (4.07)
Age (years)			
5-10	15 (4.36)	15 (4.36)	8 (2.33)
11-15	11 (3.20)	5 (1.45)	6 (1.74)
Total	26 (7.56)	12 (3.48)	14 (4.07)

[Table/Fig-3]: Distribution of nasal carriage of *Staphylococcus aureus* and Methicillin Resistant *S.aureus* on the basis of age and sex (n=26).
Parenthesis indicates percentage out of total number of children; MRSA: Methicillin-resistant *S.aureus*

Inducible clindamycin resistance was determined while performing antimicrobial susceptibility testing by D-zone test showed that none of the *S.aureus* isolates had positive D-zone test. On antibiotic susceptibility testing of all the 26 isolates, it was found that all (100%) strains were sensitive to Linezolid followed by 92.3% to Gentamycin, 88.4% to Clindamycin, 92.3% to Vancomycin, 73% to Ciprofloxacin while 42.3% resistant to cotrimoxazole and 100% resistant were to Penicillin [Table/Fig-4].



[Table/Fig-4]: Antimicrobial susceptibility pattern of *S.aureus* (n=26).

Of the 26 *S.aureus* strains isolated from school children, 12 (3.48%) were found to be resistant to Methicillin/Cefoxitin and confirmed as MRSA while 14 (4.0%) were sensitive to Methicillin and confirmed as MSSA. Among the school children highest positivity rate of isolated MRSA was observed in the age group 5-10 years (2.04%) followed by 11-15 years (1.45%) and in females it was (2.33%) followed by males (1.16%).

MICs of Oxacillin and Vancomycin for *S.aureus* determined by agar dilution method. [Table/Fig-5] documented that 92.3% *S.aureus* isolates were sensitive to Vancomycin with MIC value <2 µg/mL, whereas 12 (3.49%) isolates of *S.aureus* were found resistant to oxacillin with MIC value >4 µg/mL similar to Cefoxitin disc diffusion test [Table/Fig-5]. Two MRSA isolates were also resistant to Vancomycin both by disc diffusion and MIC method.

Bacterial isolates	Oxacillin		Vancomycin		
	≤2 µg/mL (S)	≥4 µg/mL (R)	≤2 µg/mL (S)	4-8 µg/mL (I)	≥16 µg/mL (R)
Staphylococcus aureus	14	12	24	-	2
MRSA	-	12	10	-	2

[Table/Fig-5]: Minimum inhibitory concentrations of oxacillin and vancomycin for Staphylococcus aureus and Methicillin-Resistant *Staphylococcus aureus*.
S: Sensitive; I: Intermediate; R: Resistant; MRSA: Methicillin-resistant *S.aureus*

DISCUSSION

In the present study, an attempt was made to determine the carrier rate of methicillin resistant Stapylococcus aureus among healthy school children. Nasal carriage among healthy school children may act as a reservoir of infections, which may be transmitted to the other healthy children and to the adults living in close contact.

In the present study, a total of 344 nasal swabs were collected from school going children of which 177 (51.4%) were males and 167 (48.5%) were females in the age group 5-16 years. Of the 344 school going children, 26 (7.55%) were culture positive for *S.aureus* and 318 (92.4%) were culture negative for *S.aureus* isolates. Similar studies found culture positivity for *S.aureus* from 16-52% [11-13]. A study by Ranjeeta and Hogade SA have found a higher carriage rate of 30.3%, in healthy school children [14]. The prevalence of MRSA in various studies is depicted in [Table/Fig-6] [5,10-16].

Author	Year of publication	*S.aureus* nasal carriage rate	MRSA carriage rate
Rasheed N et al., [15]	2020	37.6%	-
Ranjeeta and Hogade SA [14]	2018	30.3%	-
Reta A et al., [12]	2015	41%	13.8%
Govindan S et al., [16]	2015	29.3%	17%
Shetty V et al., [13]	2014	25%	3.06%
Fomda BA et al., [5]	2014	27.92%	1.83%
Dey S et al., [17]	2013	35%	29%
Ramana KV [11]	2009	16%	12%
Present study	2020	7.6%	3.5%

[Table/Fig-6]: Comparison of prevalence of nasal carriage of *Staphylococcus aureus* and MRSA reported in similar studies [5,10-16].

In the present study, among the 26 isolates subjected to antibiotic susceptibility testing, it was found that 100% of maximum strains were sensitive to Linezolid and maximum (100%) resistance to penicillin. Similarly, Ramana KV et al., have shown only 14.3% resistance to cotrimoxazole but 100% isolates to be resistant to penicillin [10]. A similar result is found by Pathak A et al., who have found resistance in children <5 years of age with 49% to cotrimoxazole and 90% resistance to ampicillin [17]. The indiscriminate use of antibiotics by the unregistered medical practitioners and over-the-counter sale of the drugs are the main cause of high level of resistance to few antibiotics among the rural population.

In this study, *S.aureus* isolates were significantly susceptible to linezolid, gentamycin, clindamycin, vancomycin and ciprofloxacin. This finding is similar with the study conducted by Reta A et al., who also reported 100% sensitivity to gentamycin, 92.3% in the present study, and >73% sensitivity of other antibiotics except Penicillin which shows 0% sensitivity and 42.3% for cotrimoxazole, used in the present study [11], but Ramana KV et al., have reported with gentamycin showing the highest susceptibility rate followed by cotrimoxazole, but in the present study cotrimoxazole were sensitive in 42.3% isolates while all the isolates were sensitive to linezolid. This is consistent with some other studies who have found 100% isolates to be susceptible to linezolid [12,17].

Of the 26 culture positive cases for *S.aureus*, 12 (3.48%) isolates were methicillin resistant while 14 (4%) isolates were methicillin sensitive *S.aureus*. Nasal carriage of MRSA was found in 12 (3.48%) school children in our present study and this finding is consistent with reports of various other similar studies conducted in India by Shetty V et al., Ramana KV et al., and Chatterjee SS et al., who have found carriage rate of 3%, 3.06% and 3.89%, respectively in an Indian community setting of rural, urban and semi-urban slums [10,12,18]. Studies in the developed world suggests that factors associated with risk of CA-MRSA carriage contribute to poor socioeconomic conditions, contact with healthcare facility, prior antibiotic usage, and overcrowding [18,19]. With reflections of the local endemicity, sanitary standard, environmental conditions, timing, seasonal differences and personal hygiene, these variables results in the prevalence of MRSA. However, a much higher rate (13.8%) of MRSA colonisation was found in the study conducted by Reta A et al., [11], whereas, Adhikari S et al., found lower MRSA nasal carriage prevalence of 2.9% [20].

In the present study, of the 12 MRSA nasal carriage, prevalence was highest in female children (66.6%) compared to male children (33.3%) which was non significant (p=0.45). Similarly, Rijal KR et al., reported higher prevalence of MRSA in female children (68.7%) than males (31.3%) [21]. This finding is similar to the study conducted by Yildirim M et al., who reported higher prevalence of MRSA among female children than males [22]. Patil AK et al., study found prevalence of MRSA in males 21.8%, to be higher when compared to females, 15.6% [23].

In this study *S.aureus* and MRSA was highest among students belonging to age group of 5-10 years followed by 11-16 years. In a comparable study, highest rate of *S.aureus* and MRSA carriage found to be in age group of 6-10 (62.06%) years [21].

Cefoxitin is a good indicator for determination of MRSA isolates in routine laboratories. Thus, the present study determined 12 isolates detected as MRSA by cefoxitin disc diffusion method showing MIC value <2 for oxacillin whereas 92.3% isolates of *S.aureus* have a MIC value <2 for vancomycin.

It is necessary to highlight the antibiotic crisis as a result of the emergence of many genera of bacteria that are multidrug resistant [24].

Both the detected VRSA strains were also methicillin-resistant but sensitive to linezolid. On further eliciting of history, it was found that one of the children with VRSA had history of hospitalisation of 18 months back due to pneumonia while in another child one of the close family members had a history of hospital stay four months back. It is entirely possible that the VRSA strains in the present study were acquired in a healthcare setting. With the emergence of VanA-mediated vancomycin resistance among MRSA strains that are well adapted to transmission in community settings as has been demonstrated with USA300 were not done which may have to heath linked with VRSA [25,26].

Inadequate hand hygiene and management of MRSA patients are the main reasons for the increasing prevalence of MRSA around the world [27].

Strategies to interrupt transmission of *S.aureus* by elimination of nasal carriage and thereby, preventing subsequent infection should be implemented. A resilient and stringent continued surveillance is of utmost importance. Several preventive measures have been recommended for the control of MRSA, such as prospective microbiological surveillance, contact precautions for colonised or hand hygiene, cleaning of the environment, control of microbes, and preventing colonisation among the children as well as the community.

Limitation(s)

Advanced molecular techniques like PCR were not done, this may have led to underestimation of the true prevalance in the study population. Molecular test for genotyping or Staphylococcal cassettee chromosome mec gene (SCCmec) typing and similarly, MIC test for MRSA confirmation also was not included due to lack of resources and time-bound factors. Neither risk factor analysis of MRSA carrier nor longitudinal data of MRSA trend was generated due to the limitation of current study settings and expertise in our network. Also, a larger study population obtained over a longer period of time can give a more accurate representation of antibiotic susceptibility and resistance amongst various strains today.

CONCLUSION(S)

The present study indicates higher nasal carriage rate of *S.aureus* among school going children with the significant colonisation of MRSA. Baseline colonisation rate and continued surveillance of this population is necessary to assess the ongoing risk posed by *S.aureus* to this community. There is a growing urgency to promote activities in order to improve the hygienic behavior of school children. Measures to be taken to control the spread of MRSA infection should include school-based surveillance, isolation of colonised and infected children, use of barrier precautions and basic infection control measures, and screening and treatment of MRSA-positive children.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jul 25, 2020
- Manual Googling: Dec 03, 2020
- iThenticate Software: Mar 04, 2021 (25%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Jul 20, 2020**
Date of Peer Review: **Aug 26, 2020**
Date of Acceptance: **Feb 03, 2021**
Date of Publishing: **Jul 01, 2021**