

Nasal Colonization of *Staphylococcus aureus* and their Antibiograms among School Children in Bharatpur, Nepal

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ABSTRACT

Background: Nasal carriage of *Staphylococcus aureus* has been linked to several community-acquired and nosocomial infections. The present study was undertaken to determine the prevalence and antibiotic susceptibility patterns of *S. aureus* colonizing the anterior nares of school children in Bharatpur, Nepal. **Materials and Methods:** In a cross-sectional study, 206 school children aged 3-15 years from fourteen different government schools in Bharatpur, Nepal were enrolled from January to May 2017. Prior to collecting sample, permission was sought from the administration of each school. All the participants were informed about the purposes of the study and the method of sample collection. Isolation of *S. aureus* and their antimicrobial susceptibility testing were performed by standard microbiological procedures. **Results:** *S. aureus* was isolated in 35 (16.9%) cases of which 6 (17.1%) were methicillin-resistant *S. aureus* (MRSA) and 7 (20.0%) were multi-drug resistant (MDR). Prevalence of *S. aureus* and MRSA was higher in the age group 10-15 years (24.1% and 3.6%) compared to the age group 3-9 years (8.5% and 2.1%) ($p=0.003$). All the isolates were sensitive towards vancomycin and amikacin. **Conclusions:** Promotion of good hygienic practices among school going children can be suggested to abate the risk of spread and infections by *S. aureus*.

Keywords: antibiotic resistance; MRSA; nasal carriage; *S. aureus*; school children.

INTRODUCTION

Staphylococcus aureus is the most frequently faced species of clinical importance.^{1,2} *S. aureus* has also been identified as an important human pathogen.³ In addition *S. aureus* colonizes huge proportion of human population.⁴ About 20.0% to 30.0% of human population is estimated to be the long term carrier of *S. aureus*.⁵ The nose is considered to be the main ecological niche of this pathogen.⁶

Nasal carriage of *S. aureus* has been recognized as a risk factor for community-acquired and nosocomial infections.⁷ Nasal carriage among hospital personnel and patients is much greater (60.0%-70.0%) as compared to those among community carriers.⁸ Relationship between nasal carriage of *S. aureus* and MRSA and subsequent invasive staphylococcal infections is supported by genetic evidence.⁹ Young children have a higher carriage of *S. aureus* than adults.¹⁰

One of the seriously feared strains of *S. aureus* is methicillin-resistant *S. aureus* (MRSA), which shows resistant to virtually all β -lactam

antimicrobials including penicillin, cephalosporin, carbapenem and monobactam.¹¹ Treatment of infections caused by *S. aureus*, has become more difficult since the development of MRSA. Longer hospital stay, prolonged antibiotic usage and higher costs to treat for the infections caused by methicillin-susceptible *S. aureus* (MSSA) strains are associated with the infections caused by MRSA.¹² Prevalence of MRSA ranges from 5.0% to 73.0%.^{13,14} Some strains of MRSA are epidemic in character (EMRSA). The drug of choice for treating MRSA infections is vancomycin. However, the emergence of vancomycin-intermediate or vancomycin-resistant strains of *S. aureus* has become a very great challenge for clinicians.¹⁵

The nasal carriage of *S. aureus* among the children in some cities in Nepal like Kathmandu and Pokhara have been previously assessed, however there's no any reports regarding nasal colonization of *S. aureus* among the children in Bharatpur. In this regard, the present study was conducted to determine the prevalence of nasal carriage of *S. aureus* in school

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children in Bharatpur city and to observe the susceptibility of the recovered isolates against various antibiotics.

MATERIALS AND METHODS

Study design and specimen collection

A cross-sectional study was conducted among 206 school children aged 3-15 years from fourteen different government schools in Bharatpur, Nepal from January to May 2017. All the participants were informed about the purpose of the study and the procedure of sample collection. The study volunteers seemed healthy on physical examination and none of them had nasal abnormalities. Nasal swab specimens were collected by using sterile dry cotton-wool swabs, and both the anterior nares (left and right) were swabbed by rubbing the swab several times around the inside of each nostril and rotating the swab without any interruption.

Specimen processing and identification

All the nasal swabs were placed in buffered peptone water and then transported to and processed at Microbiology laboratory of Birendra Multiple Campus, Bharatpur, within an hour. The collected samples were inoculated on Blood Agar and Mannitol Salt Agar plates and incubated at 37°C for 24 hours. Culture-positive plates were further taken into macroscopic and microscopic examination. The organism identified as Gram-positive cocci in bunch on Gram's staining was further confirmed by catalase and coagulase (slide and tube) tests.¹⁶

Antibiotics susceptibility testing

The antibiotic susceptibility testing was performed by using modified Kirby-Bauer disc diffusion technique against different antibiotics (HI-MEDIA, Mumbai, India) such as amikacin (30 mcg), cefoxitin (30 mcg), ceftriaxone (30 mcg), ciprofloxacin (5 mcg), cloxacillin (5 mcg), co-trimoxazole (25 mcg), erythromycin (15 mcg), gentamicin (10 mcg), tetracycline (30 mcg) and vancomycin (30 mcg). Suspension of the isolates were adjusted to 0.5 MacFarland solutions and streaked on MHA plates. Antibiotics discs were placed on the streaked plates and incubated at 35°C for 18-24 hours and the interpretation was made based on Clinical Laboratory Standard Institute (CLSI 2016) guidelines.¹⁷ Cefoxitin (30 mcg) disc was used for screening of MRSA. Isolates showing inhibition zone ≤ 21 mm around cefoxitin disc were identified as MRSA strain. *S. aureus* ATCC-25923 was used as positive control for the tests.

Data analysis

All the obtained data were analyzed using SPSS version 20. Chi-square test and Fischer's exact test were used to determine the possible risk factors for nasal carriage of *S. aureus*. P-value ≤ 0.05 was considered to be statistically significant, at 5.0% level of significance. Finally, all the obtained results were presented in tabulated form.

Ethical approval

Permission to conduct this research was granted from the Department of Microbiology, Birendra Multiple Campus. The purpose and procedure were clearly stated to the participants prior to specimen collection. The participants were selected only after they were sufficiently counseled and verbal informed consent was obtained from each participant and also from the school administration of each school. Only those children willing to participate in this study were included.

RESULTS

During the study period, 206 school children from 14 different government schools were screened for nasal carriage of *S. aureus*. The participants were categorized into two age groups: 3-9 years and 10-15 years. Ninety four (45.6%) children belonged to 3-9 years of age and 112 (54.3%) belonged to 10-15 years of age. Altogether 99 (48.0%) boys and 107 (51.9%) girls were recruited in the study. Table 1 shows the sex and age-wise distribution of the study participants.

Category	Division	Size
Sex	Male	99
	Female	107
	Total	206
Age	3-9 years	94
	10-15 years	112
	Total	206

S. aureus was isolated from 35 (17.0%) study subjects of which 6 (17.1%) were screened as MRSA. Nasal carriage of *S. aureus* amongst boys was found to be 17.2% (17/99) whereas amongst girls was 16.8% (18/107). MRSA was detected from 2 (2.0%) boys and 4 (3.7%) girls. Prevalence of *S. aureus* and MRSA in the age group 3-9 years was 8.5% and 2.1% respectively whereas their prevalence in the age group 10-15 years was 24.1% and 3.6% (Table 2).

The antibiotics susceptibility testing was performed on all the 35 isolates of *S. aureus*, out of which, 7 (20.0%) showed resistance to two or more classes of

Table 2: Sex and Age-wise distribution of *S. aureus* and MRSA

Study Characteristics	<i>S. aureus</i>		P-value	MRSA		P-value	
	Present No. (%)	Absent No. (%)		Present No. (%)	Absent No. (%)		
Sex	Male	17 (17.2)	82 (82.8)	0.947	2 (2.0)	97 (98.0)	0.658
	Female	18 (16.8)	89 (83.2)		4 (3.7)	103 (96.3)	
	Total	35 (17.0)	171 (83.0)		6 (2.9)	200 (97.1)	
Age	3-9 years	8 (8.5)	86 (91.5)	0.003	2 (2.1)	92 (97.9)	0.602
	10-15 years	27 (24.1)	85 (75.9)		4 (3.6)	108 (96.4)	
	Total	35 (17.0)	171 (83.0)		6 (2.9)	200 (97.1)	

antibiotics tested (MDR). Among 35 *S. aureus*, 3 (8.6%) were resistant towards ciprofloxacin and cefoxitin, 2 (5.7%) were resistant towards ciprofloxacin and co-trimoxazole, 1 (2.9%) was resistant towards ciprofloxacin and erythromycin, and 1 (2.9%) was resistant towards cefoxitin, ciprofloxacin and erythromycin. All the MRSA isolates were sensitive towards tetracycline, gentamicin, vancomycin and amikacin whereas intermediately resistant towards cloxacillin. Higher percentage of resistance was observed amongst MRSA isolates against ciprofloxacin (66.0%) compared to other antibiotics used. Similarly, 1 (16.1%) MRSA isolate showed combined resistance towards ciprofloxacin and erythromycin. Vancomycin and amikacin were the most effective antibiotics used as all the isolates (100.0%) were sensitive against these antibiotics whereas ciprofloxacin was found to be the least effective drug used as only 14(40.0%) isolates were resistant to it (Table 3).

study by Soysal et al, in Turkish children (17.3%) and by Okwa et al, in Nigerian school children (18.3%).^{21,22} The present study reports fairly a higher nasal carriage of *S. aureus* in the school children in Bharatpur city in comparison to 11.7% nasal carriage among healthy school children in the capital city, Kathmandu.²³ On the other hand, prevalence of *S. aureus* found in this study is lower than that of the study conducted in Pokhara, Nepal and in Jimma, Ethiopia which were 31.0% and 47.3% respectively.^{24,25} The variation in the prevalence of *S. aureus* among different studies could be due to the several factors such as difference in personal hygiene and lifestyle among different population characteristics, crowded living environment, direct skin to skin contact, hospitalization, sharing of individual objects etc.²⁶

Among thirty-five *S. aureus* isolates, 6 (17.2%) were found to be MRSA, in this study. The finding of this study is nearly similar to the study conducted

Table 3. Antibiotics susceptibility pattern of *S. aureus* and MRSA isolates.

Antibiotic	<i>S. aureus</i> (N=35), n (%)			MRSA (N=6), n (%)		
	S	I	R	S	I	R
Cefoxitin	29 (82.9)	0 (0.0)	6 (17.1)	0 (0.0)	0 (0.0)	6 (100.0)
Cloxacillin	15 (42.9)	20 (57.1)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)
Ciprofloxacin	17 (48.6)	4 (11.4)	14 (40.0)	1 (16.7)	1 (16.7)	4 (66.7)
Tetracycline	34 (97.1)	1 (2.9)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)
Erythromycin	20 (57.2)	9 (25.7)	6 (17.1)	3 (50.0)	2 (33.3)	1 (16.7)
Gentamicin	33 (94.2)	1 (2.9)	1 (2.9)	6 (100.0)	0 (0.0)	0 (0.0)
Ceftriaxone	27 (77.1)	8 (22.9)	0 (0.0)	2 (33.3)	4 (66.7)	0 (0.0)
Cotrimoxazole	25 (71.4)	6 (17.1)	4 (11.4)	4 (66.7)	2 (33.3)	0 (0.0)
Amikacin	35 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)
Vancomycin	35 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)

DISCUSSION

S. aureus is considered to be a versatile pathogen, since it is one of the most common causes of nosocomial and community-acquired infections.¹⁸ The most consistent location for *S. aureus* is the anterior nares of a person. Although this colonization is a typical phenomenon, it may be a source for invasive infections.¹⁹ *S. aureus* carriage is likely to decrease with age.²⁰ In our study, 17.0% of the school children were found to be the nasal carriers of *S. aureus*. This result is in tune with the

in Urmia, which reported the prevalence of MRSA as 14.9% among the *S. aureus* isolates in the preschool and school children of age below 14 years.²⁷ Other studies in different countries show variation in MRSA colonization. The nasal colonization rates of MRSA reported in the same year in two different cities in Nepal were quite varying i.e. 5.2% in Kathmandu²³ and 56.1% in Pokhara.²⁴ A recent study in Iraq reported that nasal colonization of MRSA among the primary school children was 13.3%.²⁸

In the present study, the rate of isolation of *S. aureus* in male school children was 17.2% and 16.8% in female school children. In a similar study conducted by Reta et al, nasal carriage of *S. aureus* was found to be 23.7% in male children and 17.3% in female children, which shows that the prevalence of *S. aureus* is more in female than in male,²⁹ just like the result obtained from this study. On the other hand, a study conducted in Pokhara reported that the rate of isolation of *S. aureus* from male was 35.1% and 64.9% from female.²⁴ Similarly, the study conducted in Urmia showed that the rate of isolation of *S. aureus* in male was 17.0% and in female was 23.5%.²⁷ These results indicate that the nasal colonization of *S. aureus* in female is higher than the male counterparts which is in contrary to the findings of our study. The present study showed the nasal carriage of MRSA amongst male and female school children was 2.0% and 3.7% respectively. Our finding is similar to another study by Assafi et al, in Iraq who reported the prevalence of MRSA in male as 3.8% and in female as 4.4%.²⁸

Nasal carriage of *S. aureus* was also studied in two different age-groups: 3-9 years and 10-15 years. In the study, 8.5% of the children belonging to the age group 3-9 years and 24.1% belonging to the age group 10-15 years were found to harbor *S. aureus*. The present study showed a higher colonization of *S. aureus* in the age group 10-15 years. A significant association was noted between the age group and the nasal colonization of *S. aureus* (p-value 0.003). In a similar study conducted in Ethiopia, the children were divided into two different age groups: 6-9 years and 10-12 years and a higher prevalence of *S. aureus* was found in the age group 10-12 years (45.2%) compared to the age group 6-9 years (36.4%).²⁹ In this study, nasal carriage of MRSA in 3-9 years age group was found to be 2.1% and its carriage in the age group 10-15 was found to be 3.6%. Different studies show variations in the rate of colonization of MRSA. In Ethiopia, Reta et al, further reported colonization of MRSA was 12.7% and 15.4% among 6-9 years old children and 10-12 years old children respectively.²⁹ According to a previous study, among 4-6 years age group of healthy children in Turkey, the nasal carriage of MRSA was 0.3%.³⁰ While, among the children of 1-5 years age in Philippines, 1.3% were the carriers of MRSA.³¹ Similarly, Dey et al, reported the carriers of MRSA as 10.2% among the children of 1-5 years in India.³²

In the antibiotic sensitivity testing, we observed that all the isolates were sensitive towards amikacin and vancomycin, which is similar to the findings reported by Habeeb et al,³³ in Iraq and by Paulino et al, in Philippines.³¹ Higher number of isolates were resistant towards ciprofloxacin (40.0%), followed by erythromycin (17.1%), co-trimoxazole (11.4%) and gentamicin (2.9%). In a similar study at Ujjain, India, children of 1-6 years were studied. In this particular study, 4.0% of the total *S. aureus* isolates were resistant to vancomycin, 28.0% resistant to co-trimoxazole, 32.0% resistant to gentamicin, 41.0% resistant to tetracycline, 16.0% resistant to ciprofloxacin and 17.0% resistant to ceftriaxone.³² Emergence of MDR strains of *S. aureus* have been problematic to treat infections caused by them. This study revealed that 7(20.0%) of the *S. aureus* isolates were MDR. In a study in India, 11.0% *S. aureus* showed combined MDR to ciprofloxacin and co-trimoxazole.³² In this study, MRSA strains showed resistance towards ciprofloxacin (66.7%) and towards erythromycin (16.7%). These findings are different than the study conducted by Reta et al, in Ethiopia, where none of the MRSA isolates were resistant towards these two antibiotics,²⁹ whereas according to the results obtained from the study conducted by Rijal et al., in Pokhara, MRSA strains showed resistance towards these two antibiotics.²⁴

CONCLUSIONS

The present study showed that the rate of nasal carriage of *S. aureus* was 16.9% (35/206) and MRSA nasal carriage prevalence was 2.9% (6/206). *S. aureus* colonization was higher in male children than in female children, while MRSA colonization was higher in female than in male. This study also revealed that the children of higher age are more prone to be the carriers of *S. aureus* and MRSA. The most effective antibiotics against the isolates were amikacin and vancomycin, while ciprofloxacin was found to be the least effective antibiotic. There is a growing urgency to promote activities in order to improve the hygienic behavior of school children in Bharatpur.

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