Nasal Carriage and Methicillin Resistance of *Staphylococcus Aureus* Among Schoolchildren in Sana'a City, Yemen

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Research

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Abstract

Background: *Staphylococcus aureus* (*S. aureus*) is a frequent cause of serious health problems with high morbidity and mortality. The risk of *S. aureus* infections is increased with the emergence of methicillin-resistant *S. aureus* (MRSA). The aim of this study is to determine the nasal carriage rate of both *S. aureus* and MRSA among schoolchildren in Sana'a city.

Methods: This is a cross-sectional study conducted from January 2018 to May 2020. Five hundred and eighty eight students were enrolled. Nasal swabs were collected from each student for culturing and methicillin susceptibility testing.

Results: Out 588 nasal swab, 536 yielded bacterial growth. Students with positive culture were 271(51%) males and 265(49%) females. Their age ranged from 5 to 19 years old with mean age and standard deviation equaled to 13.3±3.5 years. *S. aureus* was isolated from 129 (24%) students whereas the overall prevalence of MRSA was 8(1.5%). *S. aureus* was significantly recovered from students at age group 10-14 years (χ^2 = 7.02, p = 0.03), females than males (OR= 1.96, χ^2 = 10.75, p = 0.001), and students who were admitted into hospitals (OR= 1.6, χ^2 = 4.89, p = 0.03). Nevertheless, there were no significant differences between MRSA carriage and students' age (χ^2 = 2.3, p = 0.32), gender (OR= 1.02, χ^2 = 0.001, p = 0.63), and hospital admission (OR= 1.4, χ^2 = 0.25, p = 0.62).

Conclusions: The prevalence of MRSA is low among schoolchildren in Sana'a city. Age, gender and previous hospital admission were statistically associated with nasal carriage of *S. aureus* but not MRSA nasal carriage.

Background

Staphylococcus aureus (*S. aureus*) is a Gram-positive, round-shaped, catalase- and coagulase-positive bacterium. *S. aureus* is a frequent cause of serious health problems with high morbidity and mortality. *S. aureus* colonizes skin and nasal mucosa and thus can be regarded as a human commensal as well. The anterior nares appear as the main reservoir site for *S. aureus* replication and spread to other body sites. Approximately, 25–30% of healthy individuals are nasal carriers of *S. aureus*[1,2]. Any damage to the epithelial barrier such as trauma, medical or surgical interventions can lead to tissue invasion by *S. aureus* [3,4].

S. aureus displays rising virulence and resistance to antibiotics complicating therapeutic measures in recent years. Overuse and/or misuse of antibiotics led to the appearance of strains of *S. aureus* that are resistant to many current used antibiotics. Penicillin-resistant *S. aureus* produces penicillinase, which hydrolyzes the β-lactam ring of penicillin leading to penicillin resistance. Later on, scientists developed a novel semisynthetic penicillin called methicillin, which is resistant to staphylococcal β-lactamase[5,6]. Methicillin successfully controlled the infection caused by penicillin-resistant *S. aureus* strains. Nevertheless, only 2 years later after methicillin use in treatment, isolation of methicillin resistant *S.*

aureus (MRSA) strains was reported. Resistance to methicillin is due to acquisition of the methicillin resistance (*mecA*) gene integrated into the chromosomal element. The *mecA* gene encodes an altered penicillin binding protein 2a or 2' (PBP2a or PBP2') which permits *S. aureus* to grow in the presence of methicillin and other β-lactam antibiotics[7].

MRSA is recognized as one of the main causes of infections in human beings. MRSA strains have spread internationally and caused numerous nosocomial outbreaks, occurring in both the hospital and the community. MRSA carriers are predisposed to skin infections, wound infections, bone and joint infection, pneumonia, septicemia, endocarditis and occasionally toxic shock syndrome. Infections from either hospital-acquired or community-acquired MRSA have posed a substantial challenge[8–10].

In a study conducted by AL-Haj *et al*, 2017, the nasal carriage of *S. aureus* was reported to be 23.1% among public school children. However, no population-based study had been carried out on nasal carriage of MRSA[11]. Therefore, the aim of this study was to determine the nasal carriage rate of both *S. aureus* and MRSA among schoolchildren in Sana'a city.

Methods

Study design and area

A cross-sectional study was conducted from January 2018 to June 2020. Six primary and secondary schools at Sana'a city were randomly chosen by a cluster sampling method. These schools included four public schools (Al-Hassen Bin Ali, Roqaya, Omer Bin Al-khtab, and Khaled Bin Al-Waleed) and two private schools (Al-Amjad, and Al-Elmyeh).

Study population

Five hundred and eighty-eight students were enrolled in this study. Simple random sampling was used to choose students who would participate in the study.

Inclusion Criteria

All randomly selected schoolchildren who agreed to participate were included.

Exclusion criteria

Students who were on antibiotic therapy for one week before sample collection or those who had ulceration or pus at the nares or skin were excluded. Diabetic students were also excluded.

Sample size determination

Based on study conducted by Al-Haj *et al*, 2017, who reported the prevalence of *S. aureus* among schoolchildren to be 23.1%,[11] and on Central Statistical Organization at Ministry of Planning and Inter Coop (2005-2006) which stated the number of schoolchildren in Sana'a city to be about 2240000

students, calculated sample size was 471 students at confidence level 99%. However, because many students liked to participate, 588 students were enrolled in this study.

Specimen collection and examinations

Samples were obtained from students by using sterile dry-cotton swabs from anterior nares. The swab was inserted 2-3 centimeter in the nasal cavity and rotated 4-5 times both clockwise and anticlockwise before swab withdrawal. Samples were labeled and transported in Ameis transport media to the microbiology laboratory at the Faculty of Medicine and Health Sciences within 5-6 hours. At the microbiology laboratory, nasal swabs were inoculated on mannitol salt agar (HiMedia, India). The inoculated agar plates were incubated at 35-37 °C for 24-48h. After incubation, plates were investigated for mannitol-fermenter colonies which appear as yellow colonies on mannitol salt agar. Yellow colonies was subcultured on nutrient agar. Golden-yellow colonies on nutrient agar were further examined by Gram stain, catalase and coagulase tests. Gram positive cocci, arranged in grape-like clusters, catalase and coagulase positive were recorded as *S. aureus*[12,13].

Antibiotic susceptibility test

All colonies confirmed to be *S. aureus* were tested for methicillin (5 mcg) susceptibility by Modified Kirby-Bauer disc diffusion method. Using a sterile loop, colonies from nutrient agar which confirmed to be *S. aureus* were picked up, suspend in a sterile saline and mixed to an even turbidity. The turbidity intensity of bacterial suspension was adjusted in compare with 0.5 McFarland turbidity standard by adding saline or more bacteria. A sterile cotton swab was dipped into the bacterial suspension. Then, Mueller- Hinton agar plate (HiMedia, India) was inoculated by swabbing in three directions to evenly distribute the inoculum and make sure there were no gaps between streaks. Five µg methicillin disc (HiMedia, India) was applied using a sterile needle to come in contact with agar surface. Inoculated Muller-Hinton plates were incubated at 35-37 °C for 24h[14]. Resulted inhibition zones were measured using a ruler. Inhibition zone less than or equaled to 9 mm indicated *S. aureus* to be MRSA, inhibition zones of 10-13 mm indicated intermediate resistance while inhibition zones ≥ 14 were sensitive (Zone Size Interpretative Chart, HiMedia).

Statistical analysis

Data analysis was done using SPSS program version 20 (SPSS Inc., Chicago, IL, USA). Descriptive measures (mean±standard deviation) were used for quantitative variables. Frequencies and percentages were used to present qualitative variables. Chi-square (χ^2) test was used for verifying existence of associations. Probability (*P*) values ≤0.05 were considered statistically significant.

Results

Out of 588 collected nasal swabs, 536 yielded bacterial growth whereas 52 nasal swabs showed no bacterial growth or contamination. The age of the 536 students ranged from 5–19 years old with mean

age and standard deviation equaled to 13.3 ± 3.5 years. They were divided into three age groups: the first age group was from 5–9, the second from 10–14 years, the third group was from 15–19 years old. Out of 536, 271 (51%) students were males and 265 (49%) students were females. Number of students per class ranged from 3-159 students with mean and standard deviation equaled to 71 ± 34 students. Four hundred (75%) students studied at public schools while 136 (25%) studied at private schools, table 1.

Table 1:
Sociodemographic features of schoolchildren in Sana'a city, Yemen

	No	%
Age groups of students:		
5–9 years	98	18
10–14 years	210	39
15–19 years	228	43
Mean age ± SD*	13.3 ± 3.5	
Gender		
Males	271	51
Females	265	49
Number of students		
Public schools	400	75
Private schools	136	25
Mean per class ± SD	71 ± 34	
*SD: standard deviation		

S. aureus was isolated from the anterior nares of 129 (24%) students. It was recovered more frequently from students at age group 10–14 years (63, 30%) than from students of age group 5–9 years (22, 22%) and students at age group 15–19 years (44, 19%). This difference was statistically significant difference ($\chi^2 = 7.02$, $p = 0.03$). *S. aureus* was commonly isolated from females (80, 30%) than males (49, 18%). Sex difference was statistically significant ($\chi^2 = 10.75$, $p = 0.001$). *S. aureus* was recovered from 48(30%) students who were admitted into hospitals for either medical treatment or surgery whereas *S. aureus* was found among 81(21%) students who reported no hospital admission. This difference was statistically significant ($\chi^2 = 4.89$, $p = 0.03$). Twenty-eight (21%) students who were *S. aureus* carries had family members who worked at hospitals or any other health centers compared to 101(25%) students had no family members worked in hospitals or health centers. This difference was statistically non-significant ($\chi^2 = 1.1$, $p = 0.3$), table 2.

Table 2:
Risk factors for nasal carriage of *S. aureus* among schoolchildren in Sana'a city, Yemen

	S. aureus carriers		Non-carriers		χ^2	*p*
	No.	%	No.	%		
Age groups (years)						
5–9	22	22	76	78	7.02	0.03
10–14	63	30	147	70		
15–19	44	19	184	81		
Gender						
Males	49	18	222	82	10.75	0.001
Females	80	30	185	70		
History of hospitalization						
Yes	48	30	110	70	4.89	0.03
No	81	21	297	79		
Family member works at hospitals or health centers						
Yes	28	21	101	25	1.09	0.3
No	107	79	300	75		

Out of 129 isolated *S. aureus*, 8(6.2%) were resistant to the methicillin disc with overall prevalence of 1.5% among school students. Table 3 showed that 3(1.4%) students at age group 10–14 years old and 5(2.2%) students at age group 15–19 years but no student at age group of 5–9 years were carriers for MRSA. this age difference was statistically non-significant ($\chi^2 = 2.3$, $p = 0.32$). MRSA was equally isolated from both males and females (4, 1.5%) with no significant difference, ($\chi^2 = 0.001$, $p = 0.63$). MRSA was isolated from 3(1.4%) students who were admitted to hospitals while it was recovered from 5(2.2%) who were not admitted to hospitals. This difference was statistically non-significant ($\chi^2 = 2.25$, $p = 0.62$). Seven (1.7%) students who were MRSA carriers had no family members work at hospitals or health centers but 1(0.7%) student who had MRSA at his nares reported a family member works at a health center. This difference was statistically non-significant ($\chi^2 = 0.69$, $p = 0.41$), table 3.

Table 3:
Risk factors for nasal carriage of MRSA among schoolchildren in Sana'a city, Yemen

	MRSA*		Other bacteria		χ^2	*p*
	No.	%	No.	%		
Age groups (years)						
5–9	0	0	98	100	2.25	0.32
10–14	3	1.4	207	98.6		
15–19	5	2.2	223	97.8		
Gender						
Males	4	1.5	267	98.5	0.001	0.63
Females	4	1.5	261	98.5		
History of hospitalization						
Yes	3	1.9	155	98.1	0.25	0.63
No	5	1.3	373	98.7		
Family member works at hospitals or health centers						
Yes	1	0.7	134	99.3	0.69	0.41
No	7	1.7	394	98.3		
*MRSA: methicillin resistant *S. aureus*						

Discussion

Studies performed on nasal colonization of *S. aureus* reported approximately 20–30% of healthy persons to be permanent nasal carriers with high colonization rates among children[15,16]. Currently, the emergence of MRSA infections has become a worrying problem in a clinical field because MRSA strains are resistant to many antibiotics particularly β-lactam classes[17]. The purpose of this study was to estimate the nasal carriage and methicillin resistance of *S. aureus* among schoolchildren in Sana'a city.

In the present study, the prevalence of *S. aureus* among primary and secondary schoolchildren was 24% (129/536) whereas the prevalence of MRSA was 1.5% (8/536). This finding is consistent with a study conducted by Al-Haj *et al*, 2017, who found the colonization rate of *S. aureus* among public school children in Sana'a city to be 23.1% but they did not test isolated *S. aureus* for methicillin susceptibility[11]. Our finding was also similar to those reported from other countries[18–20]. However, the nasal carriage rate of *S. aureus* in this study tends to be lower than that reported in Nigeria (56.3%), India (46.67%), United State of America (39.6%; age group 1–19 years old), Netherland (36%; age group 1–19 years, and Nepal

(31%; age group less than 15 years old)[21–25]. On the other hand, the prevalence of *S. aureus* among school children in our study was higher than that reported in China (5.1%), Serbia (2.59%), and Iraq (17.75%)[26–28]. Variation in the *S. aureus* nasal carriage from country to another might be attributed to differences in geographical distribution, sampling, culturing, and diagnostic techniques used by the researchers.

The current study showed nasal carriage of *S. aureus* in age group 5–9 years to be 22% which increased significantly to 30% in age group 10–14 years, and then decreased to 19% in age group 15–19 years. Our finding is in agreement with a study conducted by Esposito *et al*, 2014, who reported nasal carriage of *S. aureus* to decrease while oropharyngeal carriage to increase with age[29]. Decreased nasal carriage in older students may be due to that their immune system is well-developed and becomes stronger.

With respect to gender, our study revealed significantly higher prevalence of *S. aureus* among females than males but no difference between nasal carriage of MRSA among females and males. Al-haj *et al*, 2017, reported a higher nasal carriage among female students than males but the difference was non-significant[11]. Moreover, Okwu *et al*, 2012, found *S. aureus* and MRSA to be non-significantly higher among female than male students[30]. Nevertheless, many studies conducted on schoolchildren found the frequency of nasal carriage of *S. aureus* and MRSA to be non-significantly higher in male students than females[25,29,31]. Tigabu *et al*, 2018, described a higher prevalence of *S. aureus* among males than females but a slightly higher MRSA prevalence among females than males[20]. Statistically significant nasal colonization of *S. aureus* among females than males in our study might be due to wearing veil among females as an obligatory custom. Wearing veil might make anterior nares environment warmer and more humid which in turn may favor *S. aureus* colonization.

Regarding hospitalization, this study showed highly significant association between nasal colonization of *S. aureus* with hospitalization; however, nasal carriage of MRSA among school children showed a non-significant association with hospitalization. Nevertheless, no association was found between presence of first-degree relatives who worked in hospitals or health centers and nasal colonization with *S. aureus* and MRSA. Our study in disagreement with study conducted in Argentina by Gardella *et al*, 2011, who reported no statistical difference between nasal carriage of *S. aureus* and hospitalization[32]. However, our study is in consistent with study performed in Ethiopia by Reta *et al*, 2014, who reported no association between MRSA prevalence and hospitalization[31]. This finding might imply *S. aureus* acquisition during hospital admission. On the other hand, the low frequency of isolated MRSA makes it difficult to conclude whether MRSA is HA-MRSA or CA-MRSA.

Declarations

Ethical approval and consent to participate

The study was approved by the Faculty of Medicine and Health Sciences, Sana'a University and heads of schools. Before samples collection, students' parents gave a written informed consent. School children

gave a verbal consent which was approved for children by the ethical committee after their parents gave a written informed consent.

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available. Anyone interested can get upon reasonable request from corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AMO, BSA, RMA, HZA and contributed equally to the design, implementation, statistical analysis and manuscript drafting. All authors read and approved the final manuscript.

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