

# ***Staphylococcus aureus* Nasal and Hand Carriers and the Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) Among School Children in Gharyan City**

**Salem MA<sup>1,2\*</sup>, At Thar AD<sup>1</sup>, Belkher MS<sup>1</sup> and Shaban TM<sup>1</sup>**

<sup>1</sup>Department of Zoology, Gharyan University, Gharyan, Libya

<sup>2</sup>Molecular diagnostics unit, Medical Laboratory, Saint Jamis Hospital, Tripoli, Libya

**\*Corresponding author:** Milud Ahmed Salem, Department of Zoology, Gharyan University, Gharyan, Libya, Tel: +218-91-7871624; +218-92-5690258; Email: milud.salem@jgu.edu.ly

## **Research Article**

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## **Abstract**

**Background:** *Staphylococcus aureus* infections are increasingly reported from both health institutions and communities around the world. In particular, infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been detected worldwide. If MRSA becomes the most common form of *S. aureus* in a community, it makes the treatment of common infections much more difficult. But, reports on the current status of community acquired MRSA in the study area are non-existent.

**Aim:** To determine nasal and hand carriage of *S. aureus* and the prevalence of MRSA isolates among school children in Gharyan city.

**Methods:** This study was a cross sectional descriptive study carried out over a period of three months in three basic education schools in Gharyan city. Children were recruited into the study after getting consent from schools headmasters. Nasal swabs and fingerprints were collected from 120 children and taken to Microbiology laboratory of science faculty, Gharyan University, where conventional culture techniques, characterization of *S. aureus* and determination of MRSA using Kirby Bauer Disc Diffusion method were performed.

**Results:** Out of a total of 240 nasal swabs and fingerprints, 48 (20%) *S. aureus* isolates were identified. *S. aureus* carriage rate was the highest among males, higher at hands than nares and lower among age group 11-15 years than 5-10 years. The overall prevalence of MRSA among *S. aureus* isolates was 39.6 %. MRSA carriage rate was lower among females than males, the lowest at hands and higher among age group 11-15 years than 5-10 years.

**Conclusion:** This is the first study to assess *S. aureus* carriage and the MRSA prevalence among children aged 5-15 years in Gharyan city schools. *S. aureus* was isolated from only 20% of the nares and hands of children included in this study.

MRSA is moderately prevalent 39.6% among the *S. aureus* isolates. Therefore the prevalence of MRSA among school children needs close attention to prevent its transmission.

**Keywords:** *Staphylococcus aureus*; Nasal and Hand Carriers; MRSA; School Children

## Introduction

*Staphylococcus aureus* is a Gram-positive bacteria commonly found on the skin and in the nose of 30% of healthy people [1,2]. It is a leading cause of human bacterial infections worldwide [1]. Person-to-person transmission is the usual form of spread and occurs through contact with secretions from infected skin lesions, nasal discharge or spread via hands [3]. The increasing frequency of antimicrobial resistance among infectious organisms is of great concern to both medical providers and the general public [4]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is an emerging type of *S. aureus* implicated in skin and soft tissue infections [3]. Nowadays, MRSA is the hottest research area in most developed countries due to increased mortality and morbidity [2].

The role of health care workers (HCWs) in the nosocomial transmission of MRSA has been widely discussed, with many studies citing HCWs as a source of nosocomial transmission of MRSA in developing countries including Libya [5]. So, there are great chances of MRSA to be transmitted outside hospitals, because there is no checkup for MRSA decolonization when patients are discharged from hospitals. This necessitates the need for evaluation of whether the pathogen is restricted in hospitals or already disseminated to the healthy community members including children.

This study was initiated to assess the nasal and hand carriage of *S. aureus* and to determine the prevalence of MRSA among healthy school children from three basic education Schools in Gharyan city.

## Materials and Methods

### Specimens Collection

A total of one hundred and twenty school children i.e. 5 children from each class were included in this study, which was carried out in three basic education schools (Alkmeshat, Alahed Aljaded and Alawael schools) in Gharyan city located about 100 km southwest Tripoli,

during March to May 2017. Consent was taken from the headmaster of each school before initiation of the research activity.

The specimen was collected from both the anterior nares by sterile disposable cotton swab stick after moistening it with sterile normal saline. The swab was rubbed very well 5 times over the inner wall nose and then placed back into its container. Otherwise fingerprints of right hand of the same child were collected on Mannitol salt agar (MSA) plate, where MSA medium surface was touched with four fingers and then moved right and left lightly, a thumb was printed on unprinted area on the same plate as well. Swabs and plates were brought to the microbiology laboratory for further processing without any delay.

### Bacterial Inoculation and Isolation

All swabs and MRSA positive control strain (ATCC 43300) were aseptically streaked on MSA media. Two swabs each from different children were inoculated on a single plate, each swab occupying half of the plate. The plates of swabs and fingerprints were incubated at 37°C for 18-24 hours to get growth. After incubation period, the plates were read and the staphylococcal colonies were identified on the basis of colonial morphology (yellow, large and concave colonies), cultural characteristics (reduced pH turned medium Colour from red to yellow), catalase test (release of O<sub>2</sub> after mixing of suspected colonies with H<sub>2</sub>O<sub>2</sub>) and coagulase test (clot of plasma after mixing of suspected colonies with blood plasma) [6]. Isolates which gave a positive coagulase test were taken as *S. aureus* [7].

### Detection of MRSA

All isolates of *S. aureus* and MRSA positive control strain (ATCC 43300) were subjected to Kirby Bauer Disc Diffusion method using Cefoxitin (FOX 30 µg) discs [8]. Using a sterile loop, a part of the colony was inoculated in a sterile normal saline test tube 0.45% to the equivalent of a 0.5 McFarland turbidity standard using biomerieux densichek calibration standard. A sterile swab was immersed in the standardized inoculum and excess inoculum squeezed by pressing the swab on the sides of

the test tube and streaked on Mueller Hinton agar plate in three directions on one half of the plate so as to get a uniform confluent growth. The other half of the plate was used for another swab. Care was taken during streaking so that the two inoculates on the same plate don't mix. Using sterile forceps, FOX disc was loaded on streaked agar surface, and then pressed down to ensure complete contact with the agar surface. Two discs were distributed evenly and distantly on the agar surface to avoid overlap of disc zones, and then plates were incubated at 35- 37°C for 18-24 hours.

Interpretation of results was done after incubation by measurement of diameter of inhibition zones around antibiotic discs as per CLSI M100S Guidelines that indicate to zone of Inhibition of FOX  $\leq 21\text{mm}$ =Methicillin-Resistant *Staphylococcus aureus* (MRSA) while, zone of Inhibition of FOX  $\geq 22\text{mm}$ =Methicillin-Sensitive *Staphylococcus aureus* (MSSA) [9].

### Statistical Analysis

Statistical analyses were done by using Microsoft Office Excel 2007. Discrete variables were expressed as percentages and proportions were compared using the Chi-square test. Value of  $P \leq 0.05$  was considered statistically significant.

### Results

Of the 120 school children included in this study 50.8% (61/120) were males children, while 49.2% (59/120) were females children and 37.5% (45/120) were aged between 5-10 years, and 62.5% (75/120) were aged between 11-15 years, whereas 41.7% (50/120) were from AlAahed AlJadeed school followed by AlAwael school 37.5% (45/120) and AlKmeshat school 20.8% (25/120,) (Table 1). 240 specimens were collected from 120 children (120 nasal swabs and 120 fingerprints).

### Frequency of Isolation of *S. aureus*

From a total of 240 nasal swabs and fingerprints specimens, the overall frequency of isolation of *S. aureus* was 20% (48/240). As shown in table 2, with respect to sources of specimens, the isolation rate of *S. aureus* at nares and hands were 18.3% (22/120) and 21.7% (26/120), respectively. *S. aureus* carriage rate was the highest among males 22.1% (27/122) in both their nares 21.3% (13/61) and hands 23% (14/61), while among females was 17.8% (21/118), where their nares 15.3% (9/59) and their hands 20.3% (12/59) with no significantly different between types of gender ( $P=0.401$ ).

Schools	Age of children (years) (N=120)						Gender of children (N=120)					
	5-10		11-15		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Alkmeshat	0	0	25	100	25	20.8	12	48	13	52	25	20.8
Alaahed Aljadeed	20	40	30	60	50	41.7	25	50	25	50	50	41.7
Alawaeal	25	55.6	20	44.4	45	37.5	24	53.3	21	46.7	45	37.5
<b>Total</b>	45	37.5	75	62.5	120	100	61	50.8	59	49.2	120	100

Table 1: Age and gender distribution of school children participated in the study in each school, Gharyan city, Libya. N= total number of children participated in the study.

School	Source of specimens	No. (%) of isolate							
		Gender				Age (years)			
		Specimens No. (Male+Female)	Male N(%)	Female N(%)	Total N(%)	Specimens No. (5-10+11-15)	5-10 N(%)	11-15 N(%)	Total N(%)
Alkmeshat	Nares	25(12+13)	4(33.3)	2(15.4)	6(24)	25(0+25)	0(0)	6(24)	6(24)
	Hand	25(12+13)	2(16.7)	4(30.8)	6(24)	25(0+25)	0(0)	6(24)	6(24)
	Total	50(24+26)	6(25)	6(23.1)	12(24)	50(0+50)	0(0)	12(24)	12(24)
Aljadeed Alaahed	Nares	50(25+25)	6(24)	4(16)	10(20)	50(20+30)	5(25)	5(16.7)	10(20)
	Hand	50(25+25)	5(20)	5(20)	10(20)	50(20+30)	6(30)	4(13.3)	10(20)
	Total	100(50+50)	11(22)	9(18)	20(20)	100(40+60)	11(27.5)	9(15)	20(20)
Alawaeal	Nares	45(24+21)	3(12.5)	3(14.3)	6(13.3)	45(25+20)	4(16)	2(10)	6(13.3)
	Hand	45(24+21)	7(29.2)	3(14.3)	10(22.2)	45(25+20)	9(36)	1(5)	10(22.2)

	Total	90(48+42)	10(20.8)	6(14.3)	16(17.8)	90(50+40)	13(26)	3(7.5)	16(17.8)
Total	Nares	120(61+59)	13(21.3)	9(15.3)	22(18.3)	120(45+75)	9(20)	13(17.3)	22(18.3)
	Hand	120(61+59)	14(23)	12(20.3)	26(21.7)	120(45+75)	15(33.3)	11(14.7)	26(21.7)
	Total	240(122+118)	27(22.1)	21(17.8)	48(20)	240(90+150)	24(26.7)	24(16)	48(20)

Table 2: Frequency of isolation of *S. aureus* from nares and hands of school children according to gender and age in Gharyan city, Libya.

N= number of isolated *S.aureus*

*S. aureus* nasal and hand colonization prevalence was 24 isolates from each age group, but its carriage rate was higher among children aged from 5-10 years, 26.7% (24/90) than 11-15 years, 16% (24/150). (P=0.046).

A high rate of isolation of *S. aureus* was observed in Alkmeshat school, 24% (12/50) followed by Alaahed Aljadeded school, 20% (20/100) then Al-awaeal school 17.8% (16/90).

### Prevalence of Methicillin-Resistant *S. aureus* (MRSA)

Of the total 48 *S. aureus* isolates 39.6% (19/48) were found resistant to Cefoxitin (FOX) 30µg. Table 3 shows,

more than a half of *S. aureus* isolates at nares were MRSA 54.5% (12/22), while at the hands isolates were 26.9% (7/26). MRSA carriage rate was higher among males 40.7% (11/27) than females 38.1% (8/21), with no significantly different between types of gender (P=0.853). Children aged from 11-15 years show MRSA nasal and hand carriage rate 58.3% (14/24) higher than children aged from 5-10 years 20.8% (5/24) (P=008).

Of the three schools, the rate of isolation of MRSA was the highest among Alkmeshat school children 66.7% (8/12), followed by Alawael school children 31.3% (5/16) then Alaahed Aljadeded school children 30% (6/20).

School	Source of specimens	No. (%) of isolates							
		Gender			Age (years)				
		Specimens No. (Male+Female)	Male N(%)	Female N(%)	Total N(%)	Specimens No (5-10+11-15)	5-10 N(%)	11-15 N(%)	Total N(%)
Alkmeshat	Nares	6(4+2)	3(75)	2(100)	5(83.3)	6(0+6)	0(0)	5(83.3)	5(83.3)
	Hand	6(2+4)	0(0)	3(75)	3(50)	6(0+6)	0(0)	3(50)	3(50)
	Total	12(6+6)	3(50)	5(83.3)	8(66.7)	12(0+12)	0(0)	8(66.7)	8(66.7)
Alaahed Aljadeded	Nares	10(6+4)	3(50)	1(25)	4(40)	10(5+5)	1(20)	3(60)	4(40)
	Hand	10(5+5)	2(40)	0(0)	2(20)	10(6+4)	0(0)	2(50)	2(20)
	Total	20(11+9)	5(45.5)	1(11.1)	6(30)	20(11+9)	1(9.1)	5(55.6)	6(30)
Alawaeal	Nares	6(3+3)	2(66.7)	1(33.3)	3(50)	6(4+2)	2(50)	1(50)	3(50)
	Hand	10(7+3)	1(14.3)	1(33.3)	2(20)	10(9+1)	2(22.2)	0(0)	2(20)
	Total	16(10+6)	3(30)	2(33.3)	5(31.3)	16(13+3)	4(30.8)	1(33.3)	5(31.3)
Total	Nares	22(13+9)	8(61.5)	4(44.4)	12(54.5)	22(9+13)	3(33.3)	9(69.2)	12(54.5)
	Hand	26(14+12)	3(21.4)	4(33.3)	7(26.9)	26(15+11)	2(13.3)	5(45.5)	7(26.9)
	Total	48(27+21)	11(40.7)	8(38.1)	19(39.6)	48(24+24)	5(20.8)	14(58.3)	19(39.6)

Table 3: Prevalence of MRSA from nares and hands of school children according to gender and age in Gharyan city, Libya. N= number of isolated MRSA.

### Discussion

Because of nasal and hand carriage of *S. aureus* has been demonstrated to be a significant risk factor for nosocomial and community-acquired infection in a variety of populations [9], and MRSA has become an important issue in public health, mostly due to

community-associated MRSA (CA-MRSA) isolates [10], this study was conducted to determine nasal and hand carriage of *S. aureus* and MRSA prevalence among school children in Gharyan city, Libya.

The overall nasal and hand carriage of *S. aureus* in this study population was 20% (48/240), 21.7% (26/120)

were of fingerprints, while 18.3% (22/120) were of nasal swabs. In a related study, Maureen, *et al.* 2012 in Nigeria also reported an isolation rate of 18.3% (22/120) from nasal swabs of primary school children [11]. According to Rijal, *et al.* in 2008 the rate of isolation of *S. aureus* from school children in Pokhara city in western Nepal was 31% (57/184) [12]. Our finding differs from Marina Dinic *et al* 2013 who found 2.6% (1328/2040) nasal carriage in Serbia [13]. Such differences may due to variations in sample size, socioeconomic status and community nature of enrolled children.

Gender distribution in this study was 50.8% males and the rest 49.2% females. Though the higher proportions of males 22.1% (27/122) of the subjects were found to be the carriers of *S. aureus* than females 17.8% (21/118). Similarly, Reta, *et al.* 2015, reported higher prevalence of *S. aureus* colonization in males children than females children 46.4% (71/153) and 35.4% (52/147) respectively [14].

Children included in this study aged from five to fifteen years. 26.7% (24/90) of *S. aureus* nasal and hand carriage was at age group 5-10 years, while age group 11-15 years was 16% (24/150). In the study conducted in 2008 in school children of Pokhara city in western Nepal, *S. aureus* nasal carriage in age group 6-10 years was 32.5% (29/89), however, in age group ranged from 11-15 years was 27.3% (15/55) [12]. Hands and nares of Alkmeshat school children were contained the highest isolation rate of *S. aureus* 24% (12/50) followed by Alaahed Aljaded school children 20% (20/100) and then Alawaeal school children 17.8% (16/90).

MRSA was detected using Cefoxitin (30µg) disc which has high efficiency to detect MRSA and done by standard disc diffusion method according to CLSI-2016 recommendations [15].

The prevalence of MRSA among *S. aureus* isolates was 39.6% (19/48), the most MRSA carriages 54.5% (12/22) were at *S. aureus* isolates of nares. A recent report from Ujjain, India in 2013 showed the prevalence of MRSA among preschools 29% (102/351) [16]. Prevalence of MRSA among primary school children according to study in Southwest Ethiopia was 23.1% (39/169) [17]. In another study conducted in 2014 by Suliman, *et al*, at Omdurman City, Sudan, the prevalence of MRSA was reported to be 16.3% (7/43) [18]. These variable results in the prevalence of MRSA were reflections of the local endemicity, sanitary standard, environmental conditions,

timing and seasonal differences in the design of the work and personal hygiene.

The current study shown that MRSA nasal and hand carriage was higher among males than females, 40.7% (11/27) VS 38.1% (8/21). Similarly, Reta, *et al.* reported higher prevalence of MRSA in males children than females children, 16.9% (12/71) VS 9.6% (5/52) [14].

In this study, 58.3% (14/24) of MRSA strains were isolated from age group ranged from 11-15 years, however 20.8% (5/24) were isolated from children aged from 5-10 years old, these differences may due to lack of children aged from 5-10 from Alkmeshat school that contained the highest isolation rate of *S. aureus*. In 2014, Hamed M, *et al.* in Jordan, suggested to prevalence of MRSA among children aged from 9-12 years old higher than among age group 6-9 years old [19].

This study showed 66.7% (8/12) MRSA nasal and hand carriage among Alkmeshat school children, while MRSA nasal and hand carriage was closely converged among Alaahed Aljaded school children and Alawaeal school children 30% (6/20) and 31.3% (5/16), respectively.

Hand colonization with MRSA has been considered by many to be a relatively unimportant, transient phenomenon. However, it may represent a means of person-to-person MRSA transmission that is amenable to intervention, particularly in the community in settings in which hand washing is not frequent.

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