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Antibiogram of Methicillin Resistant *Staphyloccocus aureus* solated from Nasal Carriage of Some University Students of Rivers State University, Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Staphylococcus aureus is ubiquitous, and highly adaptive pathogen that colonizes the skin and mucous membrane of the anterior nares, MRSA most often causes skin infections. In some cases, it causes pneumonia (lung infection) and other infections. If left untreated, MRSA infections can become severe and cause sepsis—the body's extreme response to an infection.Therefore, this study was aimed to evaluate the antibiogram of Methicillin Resistant*Staphylococcus aureus* from nasal carriage of Students in Rivers State University, Port Harcourt. Fifty (50) Nasal swab samples were collected using standard method, from the anterior nares of 25 male and 25 female students of Rivers State University using sterile cotton swabs and examined for *Staphylococcus aureus* using standard bacteriological methods. *Staphylococcus aureus* isolated were screened for methicillin resistance and antibiotic sensitivity pattern of Methicillin resistance *Staphylococcus* using 8 different antibiotics such as Augmentin (30 µg), Ceftriaxone (30 µg), Gentamicin (10 µg),

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Cefuroxime (5 µg, Ofloxacin (5 µg), Erythromycin (5 µg), Cloxacillin (30 µg), Ceptazidime (30 µg were carried out using Kirby–Bauer disk diffusion technique, Data obtained were analysed using SPSS version 20. The results of the prevalence of *Staphylococcus aureus,* among students showed that out the 50 samples 48 (96%) were positive of *S. aureus,* with male having the 25 (52%) while female had 23 (48%). The responses of *Staphylococcus aureus* isolated showed that methicillin susceptible *S. aureus* (75.44%) were significantly ($p<0.05$ higher than ethicillin resistant *S. aureus* (MRSA) which were14.03% and methicillin intermediate *S. aureus* (MISA) which were 10.53% The results of me a gene screening revealed that all the (8) methicillin resistant *S. aureus* isolated posses mec A gene. Five Out of the eight (8) oral antibiotics tested against the methicillin resistant *S. aureus* showed some degree of susceptibility in the range of Ceftriaxone (37.5%), Erythromycin (50%), Cefuroxime (50%) < Ofloxacin (62.5%). The high prevalence of *S. aureus* (96%) isolated from nose of students between the age of 17 to 30 in this study indicated serious public health risk among students and the percentage of resistance of Methicillin Resistance *S aureus* to commonly uses antibiotics calls for continuous surveillance, therefore screening of target population, and decolonization of carriers should be conducted to decrease the spread and burden of drug resistant *S. aureus* and MRSA in schools and the community at large.

Keywords: Methicillin; resistant; staphylococcus nasal; carriage and antibiogram.

1. INTRODUCTION

Staphylococcus aureus is a ubiquitous, and highly adaptive pathogen that colonizes the skin and mucous membrane of the anterior nares, gastro- intestinal tracts, perineum, the genitourinary tracts and pharynx [1]. It is the causative agent of a wide range of infections in humans and animals with a significant impact on public health [2]. Clinically, *S. aureus* is the most pathogenic member of the genus staphylococci and the etiologic agent of a wide variety of diseases that ranges from superficial skin abscess, food poisoning and life threatening diseases such bacteremia, necrotic pneumonia in children and endocarditis [3]. The anterior nares of nose are the primary reservoir for replication and spread to other body sites. The organism is in carrier state in the anterior nares and can remain so without causing infections for weeks or months. The colonization proceeds to infection under certain predisposing factors such as prolonged hospitalization, immune suppression, surgeries, use of invasive medical devices and chronic metabolic diseases. Localized skin abscess develop when the organism is inoculated into the skin from a site of carriage. According to Odu and Okonko [4], there is increasing evidence that community acquired *Staphylococcus aureus* infections are spreading among healthy children.

First reports of *S. aureus* strains that were resistant to penicillin appeared after a year of its clinical use [5]. Such penicillin-resistant isolates carried a plasmid gene, blaZ which encoded a beta-lactamase enzyme, referred to as penicillinase [6]. The enzyme is capable of cleaving the beta-lactam ring of penicillin resulting inactivation of the antibiotic. The emergence and spread of penicillinase-mediated resistance in *S. aureus* is referred to as first wave of resistance which was countered by the discovery of methicillin, penicillinase-stable semi synthetic penicillin. The drug was introduced into clinics in 1961 and subsequently strains showing methicillin resistance (MRSA) was reported in the same year. After the initial report, MRSA clones spread rapidly across the world but restricted to nosocomial settings. This is referred to as second wave of beta-lactam resistance in *S. aureus* [7]. Methicillin resistance was mediated by the presence of mecA gene. The therapeutic outcome of MRSA infections was worse than methicillin sensitive *S. aureus* (MSSA) due to the underlying co morbid factors such as old age, immune suppression and, importantly, lack of effective antibiotics to treat MRSA, which were often multi drug resistant. The rise in MRSA infections in hospitals resulted in high morbidity and mortality and increase in cost of healthcare [8]. The third wave of beta-lactam resistance in *S. aureus* began with reports of MRSA infections in community in early 1990s, strains were phenotypically and genetically distinct from MRSA isolates from hospitalized patients, resulting in definitions of HA-MRSA and CA-MRSA. In the last decade, community MRSA strains invaded the hospital settings and the difference between HA and CA MRSA is now blurred [8]. There is increasing evidence that community acquired methicillin-resistant *S. aureus* (CA-MRSA) is spreading among healthy individuals, especially students [9,10,4].

MRSA is resistant to a large group of antibiotics called the beta lactams. Methicillin is a β-lactam antibiotic produced to treat penicillin resistant *S. aureus.* Methicillin resistant *S. aureus* is a pathogenic strain responsible for difficult to treat infections in human [11]. MRSA resistance to antibiotics encoded by the mobile genetic element Staphylococcal chromosomal cassette (SCC) which carry the mecA gene and these elements vary in size and genetic content. The mecA gene encodes an altered penicillin binding protein (PBP2a) which permits the bacteria *S. aureus* to grow in the presence of methicillin and other β-lactam antibiotics [12]. PBP2ais located in the bacterial cell wall and has a low binding affinity for β-lactam antibiotics [13].

The association between the nasal carriage of *S. aureus* and subsequent infection has been comprehensively established. It has been shown that nasal carriers of *S. aureus* have an increased risk of acquiring an infection with this pathogen. The nose is the main ecological niche where *S. aureus* resides in human beings, but the determinants of the carrier state are incompletely understood [14]. The anterior nares of the nose have been shown to be the main reservoir of *S. aureus* in both adults and children [10]. The *S. aureus* is transmitted to nares by contaminated hands and from surfaces where it can survive for months [4,10]. Nasal carriage of *S. aureus* acts as endogenous reservoir for clinical infections in the colonized individual but also as a source of cross-colonization for community spread [10]. Healthy individuals have a small risk of contracting an invasive infection caused by *S aureus*, but they can be carriers of the organism [15]. There is a paucity of information on the antibiotic profile of MRSA among university students especially of Rivers State University, Port Harcourt, Nigeria. Therefore, this study was aimed to assess and fill the information gap of the current nasal carriage rate and antibiotic susceptibility pattern, of MRSA *S. aureus* among the year one and two students of Rivers State University, Port Harcourt.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the Department of Microbiology of Rivers State University; It is located at Nkpolu-Oroworukwo in Port Harcourt, the capital of Rivers State, Nigeria. It is the first Technological University in Nigeria and the first state owned State University in the Niger Delta region of Nigeria.

2.2 Study Design and Periods

A cross-sectional campus-point survey was conducted while taking the Nasal Swap from 50 students carefully corrected from their nostril with a sterile swab strick, in December, 2020. Year one and two students of Microbiology Department who were healthy or highly suspicious of having nasal cavity infection (carter) based on their responses to questionnaire and symptom observed at the time of data collection were recruited in the study using simple random sampling echnique. Then, questionnaires designed with both open-ended questions such as age and closed ended questions with nominal categorical values such as gender were administered. Data including age, gender and level of education, were also collected [4].

2.3 Sample Collection

Nasal swabs were collected from the anterior nares of 50 students using sterile cotton swabs (Improswab, Guangzhou, China) moistene with normal saline. The sample size was were determined based on the design of the study Both nostrils were sampled one at a time using the same swab by rotating gently against the inner surface. The used swabs were placed in Stuart's transport media (in-house made) (Oxoid, Basingstoke, UK) and transported to the Microbiology Laboratory at Rivers State University for processing within eight hours of collection [4].

2.4 Isolation and Identification

Each Nasal swab sample was inoculated onto Mannitol salt agar plates (Oxoid, Basingstoke, UK) for *S. aureus* isolation. The plates were incubated at 37°C and examined for growth after 24–48 hours. *S. aureus* were initially screened based on the presence of golden yellowish colonies on Mannitol salt agar and sub cultured on same media. The isolates were primarily identified biochemically by Gram's staining reaction, Oxidase and catalase test reaction and confirmed phenotypically by coagulase and Mannitol salt agar fermentation tests [4].

2.5 *mecA* gene amplification

2.5.1 DNA extraction (Using ZR bacterial DNA miniprep)

The bacterial DNA from two milliliter (2 ml) of bacterial cells in Luria Bertani broth (LB) were extracted using ZR bacterial DNA Mini prep Manufactured by Zymo research cat number: D6005 following the Manufacturer's analytical procedures [16].

2.5.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The sotfware of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestral; the upper pedestral was brought down to contact the extracted DNA on the lower pedestral. The DNA concentration was measured by clicking on the "measure button and concentration of the extracted genomic DNA was displayed on a computer screen [16].

2.5.3 Cycling Conditions for mec A gene

Initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 secs and elongation at 72˚C for 45 sec. Followed by a final elongation step at 72˚C for 7 minutes. The product was resolved on a 1% agarose gel at 120 V for 20 minutes and visualized on a blue light transilluminator [16].

2.6 Screening for methicillin Resistance *Staphylococcus aureus*

Staphylococcus aureus isolates were screened for methicillin resistance using the disk diffusion method of the Clinical Laboratory Standards Institute [17] as described by Odu and Okonko [4]. Overnight cultures from *S. aureus* were plated on Mueller-Hinton agar, and a 1-µg oxacillin disk was placed on the inoculated plate. Zone diameters were measured and recorded after a 24-hour incubation at 37°C; the results were classified as susceptible (≥ 13 mm), intermediate (11–12 mm), or resistant (≥ 10 mm).

2.7 Antibiotic Susceptibility Testing

A suspension of pure colony from each confirmed methicillin resistance *Staphylococcus aureus* was done in sterile normal saline and incubated at 37C for at least 15 minutes. The suspension was adjusted at 0.5% MacFarland standard and inoculated on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England). Standardized Kirby–Bauer disk diffusion technique was employed to test antibiotic sensitivity pattern of methicillin resistant *Staphylococcus aureus* using different antibiotics such as Augmentin (30 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Cefuroxime 30 ug (5 µg), Ofloxacin (5 µg), Erythromycin (5 µg), Cloxacillin (30 µg), Ceptazidime 30 µg) which were commercially prepared on foreign antibiotic dics. The inoculated plates of Mueller–Hinton agar (MHA) supplemented with 2% NaCl containing the different antibiotics were incubated at 37°C for 24 hours. An inhibition zone in millimeter of each antibiotics were measured and interpreted as resistance (R), intermediate (I), and susceptibility (S) according to CLSI [17] guideline, after the incubation period.

2.8 Calculation of Multiple Antibiotic Resistances Index (MAR)

Multiple antibiotic resistance **(**MAR) index was calculated using the formula, a/b (where, a=number of antibiotics to which the organism was resistant and b = total number of antibiotics to which the organism was tested [18].

2.9 Statistical Analysis

Frequencies were obtained and percentages were calculated for study variables. Demographic characteristics were compared with the use of Chi-square and Fisher's exact test (two tailed), with the SPSS statistical program (version 20). Results with P. value of less than or equal to 0.05 was considered to have significant difference ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Prevalence of *Staphylococcus aureus,*

To total number of fifty samples were screened for the prevalence of *Staphylococcus aureus,* among students, out the 50 samples revealed that 48(96%) were positive of *S. aureus,* with male having the 25(52%) while female had

23(48%). The results of the prevalence of *Staphylococcus aureus* based on sexes showed that there was no significant difference between sex for carriage rate of *S. aureus* among the students at p>0.05 Figs. 1 and 2 respectively.

3.2 Response of *Staphylococcus aureus* to methicillin

The response of *Staphylococcus aureus* isolated from both healthy and students that showed sign of respiratory tract infection is presented in Table 1. It showed that methicillin susceptible *S. aureus* (MSSA) (75.44%) was significantly (P<0.05), higher than methicillin resistant *S. aureus* (MRSA) which was 14.03% and methicillin intermediate *S. aureus* (MISA) 10.53%.

The gel image showing Mec A gene revealed that all the (8) methicillin resistance *S. aureus* isolates that screened posses Mec A gene (Plate 1)

Table 1. Sensitivity of *Staphylococcus aureus* to methicilin

Antibiotic response	Rate (%)
Susceptibility	75.44
Intermediate	10.53
Resistance	14.03

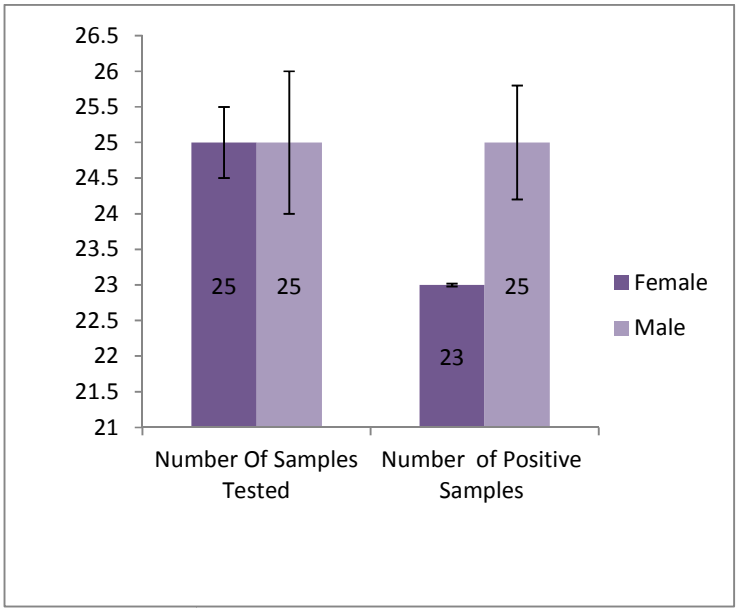


Fig. 1. Prevalence of *S. aureus* among volunteer students

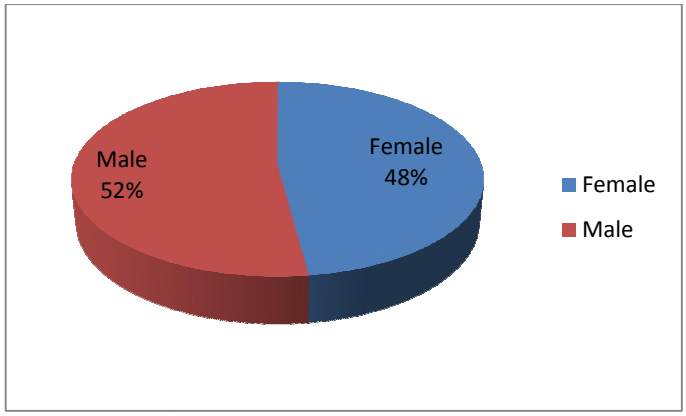
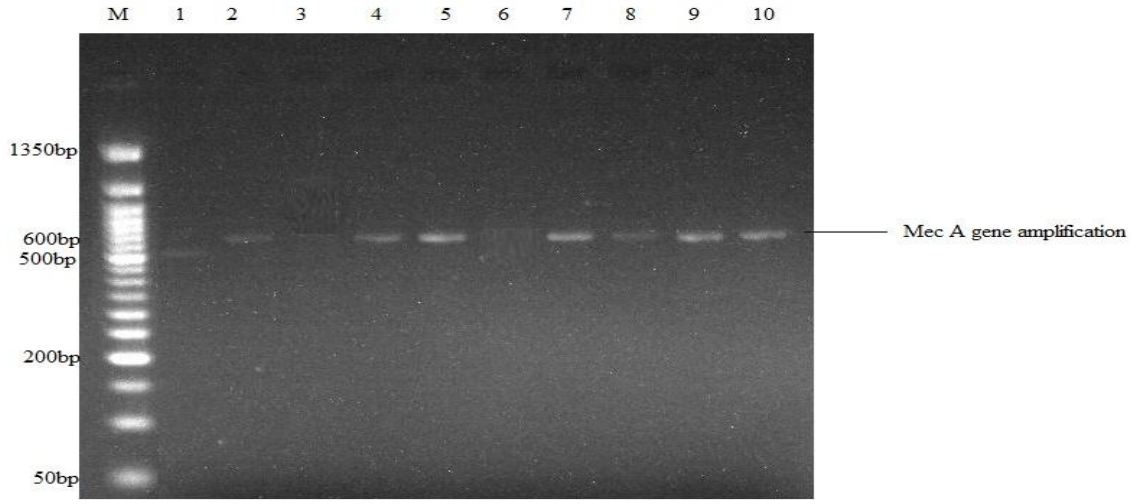


Fig. 2. Percentage of *Staphylococcus aureus,* occurrence among the students

3.3 Antibiogram of Methicilin Resistance *Staphylococcus aureus*

Staphylococcus aureus that showed Resistance to Methilicin were further tested against eight difference antibiotics. Table 2 revealed the in-vitro antibiotic sensitivity pattern of methicillin resistance *S. aureus* (MRSA) to most commonly used oral antibiotics, such as Augmentin (30 µg), Ceftriaxone (30 µg), Gentamicine (10 µg), Cefuroxine 30 ug (5 µg), Ofloxacin (5 µg), Erythromycin 5 µg), Cloxacillin (30 µg), Ceptazidine (30 µg). The percentage of resistance of eight (8) isolates of methicillin resistance *S. aureus* to the commonly used oral antibiotics ranged from Ofloxacin (25%), Gentamicin (25%), Erythromycin (37.5%), Cefuroxine (37.5%), Ceftriaxone (37.5%), Augmentin (78.5%), Cloxacillin (100%), Ceptazidine (100%). Five Out of the eight (8) oral antibiotics tested against the methicillin resistance *S. aureus* showed some degree of susceptibility in the range of Ceftriaxone (37.5%) < %), Erythromycin (50%), Cefuroxine (50%) < Ofloxacin (62.5%).



Gel image showing Mec A gene amplification ranging from 500bp to about 600bp.
Lane M is a 50bp ladder from NEB.
Lane 1-5 shows amplification of Mec A at the genomic DNA lavel while Lane 6-10 shows amplification of Mec A gene at the Plasmide level. The gene is almost absent in lane 3 and 6 while present in other lanes.

Plate 1. Antibiotics profile of the methicillin resistance *S. aureus* isolates

Table 2. Antibiotics profile of the methicillin resistance *S. aureus* isolates

		RATE (%)			MAR INDEX
		R	I	S	
1	**AUG**	7(78.5)	1(12.5)	0 (0)	
2	**CAZ**	8(100)	0(0)	0 (0)	
3	**CRX**	3(37.5)	1(12.5)	4(50)	
4	**GEN**	2(25)	0 (0)	6(75)	
5	**CTR**	3(37.5)	2(25)	3(37.5)	
6	**ERY**	3(37.5)	1(12.5)	4(50)	
7	**CXC**	8(100)	0 (0)	0 (0)	
8	**OFL**	2(25)	1(12.5)	5(62.5)	
					0.7

key: AUG=Augmentin (30 µg), CAZ = Ceptazidine (30 µg), CRX= Cefuroxine (30 µg),GEN = Gentamycine, (10 µg), CTR = Ceftriaxone (30 µg), ERY= Erythromycin (5 µg), CXC= Cloxacillin (5 µg), OFL= Ofloxacin (5 µg), R=Resistance, I =Intermediate, S =Susceptibility, MAR = Multi antibiotics Resistance The MAR index is a good tool for health risk assessment which identifies if isolates are from a region of high or low antibiotic use. A MAR indexw 0.2 indicates a 'high-risk' source of contamination. The result of MAR indexw obtained showed 0.7 which indicated high level of commonly use antibiotics among students Table 2

3.4 Discussion

In the present study, a total of Fifty (50) swap samples (25 male and 25 female), were screened for the prevalence of *S.aureus* in the nostrils of the volunteers students in year one and two of the Department of Microbiology, Rivers state University. Out the 50 samples 48(96%) were positive of *S. aureus,* with male having the 25(52%) while female had 23(48%), The results of this study showed an overall prevalence of 96% of *S. aureus* in the nostrils of the volunteer students. The prevalence rate observed is significantly higher than the previous reports of 80% isolation of *S. aureus* by Chigbu and Ezeronye [19], and 32.4% and 39% reported by Kuehnert (2006) and Abdulhadi [20] respectively. A higher carriage rate was observed in male students which is in line with the study that reported a significant difference between sex for carriage rate [21], but no significant difference at p=0.05 between the male and female was observed in this study. Nevertheless, Odu and Okonko, [4], report higher carriage rate in female, this further support the report of Adesida et al*.,* (2007) that gender has no significant effect on carriage rate. These variations in carriage rates *S. aureus* in the male and female students may be attributed to the characteristics of the population under study. This study's high prevalence of nasal carriage of *S. aureus* further supports the fact that anterior nares remains a principal reservoir of this organism and there is need to eliminate its virulent strains because of their involvement in most severe community and hospital *S. aureus* infection in colonized individual.

The study revealed that 96% of year one and two students of Rivers State University, Port Harcourt, Nigeria between the age 17 to 30years of age had *S. aureus* out of which 14.03% of the *S. aureus* were MRSA. Ramana et al. [22] reported a prevalence of 16.0% for *S. aureus*, of which 19.0% were MRSA among school going children. It showed that methicillin susceptible *S. aureus* (MSSA) (75.44%) was significantly ($p<0.05$), higher than methicillin resistant *S. aureus* (MRSA) which was 14.03% and methicillin intermediate *S. aureus* (MISA) 10.53%. Previous studies had proven that Nasal carriage of MRSA or MSSA varies in different geographical areas [23,24,15]. Study of nasal carriage of MRSA is important to the community since carriage plays a key role in the epidemiology and pathogenesis of community associated disease [4]. The prevalence of methicillin resistance *S. aureus* obtained in study could attributed the presence of the mecA gene in all isolate which enhance the encodes an altered penicillin binding protein (PBP2a) and permits the bacteria *S. aureus* to grow in the presence of methicillin and other β-lactam antibiotics according to Srinivasan et al. (2010).

The percentage of resistance of methicillin resistance *S. aureus* to commonly use antibiotic Ofloxacin (25%), Gentamycine (25%), Erythromycin (37.5%), Cefuroxine (37.5%), Ceftriaxone (37.5%), Augmentin (78.5%), Cloxacillin (100%), Ceptazidine (100%) obtained in this study could be as a results of overuse and frequent misuse of antibiotics in developing countries which have resulted in changing antibiotic resistance profiles of microorganisms amongst bacterial populations [25]. The observation of MRSA isolates, resistance was seen to antibiotics that are important for empirically treating severe infections was also reported by Odu and Okonko, [4] who carried out a research on the Nasal carriage and antibiotics susceptibility of *Staphylococcus aureus* in healthy students of University of Port Harcourt, Rivers State, Nigeria. The high low resistance observed to observed in this study may be largely due to their mode of action which is the antibiotics.

4. CONCLUSION AND RECOMMENDATIONS

The high prevalence of *S. aureus* (96%) isolated from nose of students between the age of 17 to 30 in this study confirmed that The anterior nares of the nose is the main reservoir of *S. aureus* in both adults and children, this revealed that there serious public health risk among students specially the spread of Staphylococci infection. The findings in this study confirm that prevalence of *S. aureus* nasal colonization is not dependent on sexes; however, it also shows that the rate of MRSA carriage remains high. Few demographic or clinical characteristics are related to either S. aureus carriage or, more specifically, MRSA carriage. Since Staphylococcus aureus is quickly spread by nose picking (hand contamination) continuous surveillance and improve personal hygiene standards among students should be encouraged. The results emphasize the need to discourage antibiotics' abuse, due to the effect observed on antibiotics activities on this organism in this environment which may also be due to the fact that they are so easily abuse among students, Therefore these agents may no

longer be very good for the treatment of methicilin resistance *S. aureus* infections. Screening of target population, and decolonization of carriers should be conducted to decrease the spread and burden of drug resistant *S. aureus* and MRSA in the schools and the community at large.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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