



Prevalence of Multidrug Resistant *Staphylococcus aureus* Isolated from Nasal Cavities of MOUAU Students

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Authors' contributions

This work was carried out in collaboration among all authors. Author OJO designed the study, wrote the protocol. Authors CGU, OJO and EKA wrote the first draft of the manuscript. Authors BCU and IMO helped with the analyses of the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the prevalence of multidrug-resistant *Staphylococcus aureus* isolated from nasal cavity of MOUAU students.

Methods: The nasal specimens were evaluated using standard microbiological techniques and methicillin resistance test was checked for all isolates of *Staphylococcus* species by the disc diffusion method.

Results: A total of Eleven (11) *Staphylococcus aureus* isolates were obtained from forty (40) nasal samples of MOUAU students i.e. four (4) from female and seven (7) from male. This study also showed that the highest number and percentage of *Staphylococcus aureus* isolates was observed in the male nasal samples 7(30.4%), while the lowest isolate was recorded in female nasal samples 4(23.5%). About 21(52.5%) of the 40 samples showed no traces of *Staphylococcus* specie. However, the drug susceptibility profile of bacterial isolate from nasal samples reveals varying percentage of sensitivity and resistance to the antibiotics. From this study, Ofloxacin (5

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mcg) and Gentamicin (10 mcg) exhibited high percentage of sensitivity against the *Staphylococcus* isolates at 10(90.9%) each. Cefuroxime (30 mcg) and Cefotaxime (30 mcg) showed high level of resistance against the *Staphylococcus aureus* isolates at 11(100%). No resistance to Ofloxacin (5 mcg) was noted. This study also revealed that 72.7% of *Staphylococcus aureus* isolates from the nasal cavity of healthy male and female students of Michael Okpara University of Agriculture, Umudike (MOUUAU), showed multiple resistance to the antibiotics used.

Conclusion: This study highlights the need to discourage the misuse of antibiotics and to implement strategies that could help eliminate of nasal carriage of *Staphylococcus aureus*. This will help to prevent severe *Staphylococcus aureus* infections in our environments. Also, it was report that Ofloxacin (5 mcg) and Gentamicin (10 mcg) antibiotics could be an alternative choice to use and to control MRSA infection as an effective antibacterial agent.

Keywords: Nasal; MRSA; *Staphylococcus aureus*; prevalence; multi-drug.

1. INTRODUCTION

Staphylococcus aureus is a gram-positive spherical bacterium, mostly found in the nasal passages, intestine, vagina, throat, gastrointestinal tract and skin of human body [1]. The nasal cavity is considered to be the most important habitat of *Staphylococcus aureus* in human, although some parts of body can harbor this bacterium [2]. *Staphylococcus aureus* strains are notably human pathogens and are ready to contaminate any human body tissue, resulting to life-threatening sicknesses. *Staphylococcus aureus* is both a human commensal and a major cause of clinically important infections [2]. The ecological niches of *Staphylococcus aureus* strains are the anterior nares [3].

Staphylococcus aureus is opportunistic pathogen that has the ability to adapt, strive and multiply in a wide range of environments. This could cause a wide spectrum of diseases in both humans and animals [4]. Most nosocomial and community-acquired infections cases in humans are caused by *Staphylococcus aureus*. These infections include skin and wound infections, toxic shock syndrome, arthritis, endocarditis, osteomyelitis and food poisoning [5]. In animals, staphylococcal infections cause substantial economic losses in livestock industry worldwide [6]. Healthy individuals can host *Staphylococcus aureus* in the body surface, nasopharynx and vagina [7]. It is reported that 80% of infections caused by *Staphylococcus aureus* strains are endogenous [8]. Risk factors that could promote the colonization of this organism include; young age, male sex, sharing a carrier's household, smoking, having a history of hospitalization, and recent contact with animals [9]. It has been also estimated that about 35% of apparently healthy individuals harbours this organism in the nasal vestibule and its carriage varies between

different ethnic and age groups [10]. Also, its prevalence has been severally reported in healthy individuals which includes; 36% in nares of Japanese adults and 32.4% in nasal cavity of adults in the USA [11].

The diseases caused by *Staphylococcus aureus* can be categorized into three sorts in general which include; shallow sores, (for example, wound contaminations), systemic undermining factors, (for example, osteomyelitis, endocarditis, pneumonia, mind abscesses / wounds, bacteraemia and meningitis), then toxinoses, (for example, poisonous stun disorder, sustenance harming and singed skin disorder [12]. Notably, most boils that contain whitish substance which is made up of dead neutrophils, dead and living microbial cells, tissue (necrotic), and lysed host substance are as a result of staphylococcal infection.

Solberg [13] in his study stated that the first reported the association between *Staphylococcus aureus* nasal carriage and staphylococcal disease was reported by Danbolt in 1931 in a study on furunculosis. There is need to for a better understanding of the pathogenesis of staphylococcal disease. This will help to reduce the rise in the incidence rate of penicillin-resistant *Staphylococcus aureus* hospital infections which could be dated back in 1947. Subsequently, numerous studies confirmed Danbolt's finding. A review conducted by Heimann et al. [14], on the role of nasal carriage in *Staphylococcus aureus* infections, stated that a causal relation exists between *Staphylococcus aureus* nasal carriage and infection. This is supported by the fact that the nasal *Staphylococcus aureus* strain and the infecting strain share the same genetic makeup [15].

Furthermore, Kluytmans and Wertheim [16] stated that there is a temporal decolonisation of the nose and other body sites, which prevents infection as a result of nasal application of an anti-staphylococcal drug. Our knowledge of the mechanisms, risks, and treatment of *Staphylococcus aureus* nasal carriage has greatly expanded over the past decade. This calls for effective studies to further sustain this knowledge. Colonization of *Staphylococcus aureus* in humans is a critical prerequisite of subsequent clinical infection of the skin, blood, lung, heart, and other deep tissues, and also sepsis [17]. This accounts for the growing health problems worldwide, with mortality rates ranging from 6% to 40% [17].

Methicillin-resistant *Staphylococcus aureus* has been known which have been known in recent time to be a major cause of hospital and community acquired infections worldwide. These staphylococcal strains are categorized as Hospital-acquired MRSA and community-acquired MRSA. Hospital-acquired are contracted in health care and medical settings such as hospitals and clinics. Community acquired MRSA is associated with infections that are transmitted through person-person contact or through direct contact with infected surfaces. This type of MRSA infection may also develop as a result of poor hygiene [18]. *Staphylococcus aureus* has become resistant to various antimicrobial agents including the commonly used penicillin-related antibiotics [1]. However, there has been report of increased multi-drug resistant strains of *Staphylococcus aureus* worldwide [1]. Moreover, its burden has increased recently due to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in the community [9]. This has increased the importance of screening to avoid epidemic spread of MRSA [19].

There has been rise in cost treating diseases, high mortality and period of hospitalization due to increased antibiotics resistance [20]. The increase in the number of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains, which are now most often caused by multi-resistant therapy, has become problematic. In order to reduce the increased number of multidrug-resistant isolates from the major routes of entry in healthy individuals, it is pertinent to identify the major carriers of *Staphylococcus aureus*. Therefore, this study was to determine the prevalence of multidrug

resistant *Staphylococcus aureus* isolated from nasal cavity of MOUAU students.

2. MATERIALS AND METHODS

2.1 Sample Collection

Prior to sample collection, participants were asked to complete a questionnaire regarding gender, age, status, hospitalization history, and antimicrobial drug use, in the preceding six months. All the participants in this study gave written informed consent following exchange of information about the study background and procedures with preservation of their autonomy, privacy, and confidentiality. A total of forty (40) nasal samples were collected aseptically using a sterile swab sticks moistened with physiological saline according to the method described by Aliyu et al. [21]. This is to ensure that microorganisms in the nasal passage adhere to the swab sticks. The swab sticks were placed back into the various containers to avoid contamination, labeled and taken to the laboratory for microbiological analysis [21].

2.2 Preparation of Media for Isolation and Identification of *Staphylococcus* species

All the glass wares used were sterilized in a hot air oven 121°C for 30 minutes. The media used were Mannitol Salt agar, and Mueller Hinton agar (for sensitivity). Appropriate quantities of each powdered media were weighed and prepared according to the manufacturer's specification. They were dissolved and sterilized at 121°C for 15 minutes in an autoclave. The media were then allowed to cool to 45°C before pouring into Petri dishes.

2.3 Inoculation and Isolation

The nasal specimens were respectively streaked on freshly prepared Mannitol Salt Agar plates. The plates were incubated at 37°C for 24hrs. Upon establishment of growth, the colonies were subculture on nutrient agar plates and again incubated at 37°C for 24hrs. The resulting pure cultures were used for biochemical characterization, and antibiotics testing.

2.4 Identification of the Isolates

Isolates were analyzed based on morphological features, Gram staining and biochemical

characterization which includes; catalase and coagulase tests of the isolates were carried out to verify the identity of the organisms. The bacterial isolates were identified and confirmatory identities of bacteria were made using Bergey's manual of determinate bacteriology [22].

2.4.1 Gram staining techniques

A smear of the isolate was made and air dried on clean sterilized glass slides. Crystal violet dyes were added for 60 seconds. The dye stain was washed off with distilled water and the smear was covered with Gram's Iodine for 60 seconds, and rapidly decolorized with acetone and washed immediately with distilled water. The slide was counter-stained with Safranin for one minutes and then washed with distilled water. The back of the slide were wiped off and placed in draining rack for the smear to air dry. The slides were observed microscopically under oil immersion and x100 objective for the morphology of the bacteria [23]. Gram positive organisms appeared purple while Gram negative organisms appeared red [24].

2.4.2 Biochemical test for identification of *Staphylococcus* species

2.4.2.1 Catalase test

This biochemical test detects the presence of catalase enzymes that catalyzes the release of oxygen from hydrogen peroxide. It is used to differentiate catalase producing organisms such as *Staphylococci* from non-catalase producing organisms such as *Streptococci*. Here, 2ml of 3% freshly prepared hydrogen peroxide solution was placed in a test tube; a sterile applicator stick was used to pick colonies of the isolate and placed inside the test tube containing the hydrogen peroxide solution. Vigorous effervescence indicates positive test [23].

2.4.2.2 Coagulase test

This test detects the presence of the enzyme coagulase produced by *Staphylococcus aureus* which differentiates the organism from *Staphylococcus saprophyticus* and *S. epidermidis* which do not produce the enzyme coagulase. A drop of physiological saline was placed on a clean, grease free slide; sterile wire loop was used to pick the bacterial suspension and rotated gently. Presence of clumping within 5-10 seconds indicates positive test [23].

2.5 Determination of Methicillin Resistance among the *Staphylococcus aureus* Isolates

Methicillin resistance test was determined for all *Staphylococcus aureus* isolates by the disc diffusion method of the Clinical and Laboratory Standards Institute (CLSI) [25] using Oxacillin discs (5µg) on Mueller-Hinton agar. The diameter zone of inhibitions were measured and recorded after 24h incubation at 37°C. The results were categorized as sensitive (≥ 13 mm), intermediate (11-12 mm), or resistant (≤ 10 mm) [26]. All isolates resistant to Oxacillin (5µg) were methicillin-resistance *Staphylococcus aureus*.

2.6 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolates was tested against the following antibiotics CTR Ceftiaxone (30 mcg), GEN Gentamicin (10 mcg), OFL Ofloxacin (5 mcg), CXC Cloxacillin (5 mcg), AUG Amoxicillin (30 mcg), CRX Cefuroxime (30 mcg), ERY Erythromycin (30 mcg), CAZ Ceftazidime (30 mcg) using Kirby Bauer's antibiotics disk method in consultation with the Clinical and Laboratory Standards Institute (CLSI) [25]. A colony of the test organism was picked with sterile wire loop and immersed in peptone water. The suspension of the organism was streaked on the entire plate of Mueller Hinton agar plate and antibiotic disk was placed on the plate using forceps. The diameter of the zone of inhibition was measured using (CLSI) [25] standard guidelines.

3. RESULTS

Table 1 shows the Prevalence of *Staphylococcus* species in Nasal Samples. A total of eleven (11) *Staphylococcus aureus* were isolated from forty (40) nasal samples i.e. four (4) from female and (7) from male. The highest number and percentage of *Staphylococcus aureus* isolates was observed in the male nasal samples 7(30.4%), while the lowest isolate was recorded in female nasal samples 4(23.5%). A total of 21(52.5%) samples without *Staphylococcus aureus* isolates were observed in this study.

Table 2 shows colonial morphology and biochemical characteristics of the bacterial isolates from the nasal samples. These nasal samples were identified by their morphological characteristics and biochemical test. Coagulase test differentiated Isolate 1 from Isolate 2 which

reveals the major bacterial isolates to *Staphylococcus aureus* and Coagulase Negative *Staphylococcus aureus* respectively.

showed high level of resistance against the *Staphylococcus* isolates at 11(100%). No resistance to Ofloxacin (5 mcg) was noted.

Table 3 shows the susceptibility profile of bacterial isolate from nasal samples with varying percentage of sensitivity and resistance to the antibiotics. Ofloxacin (5 mcg) and Gentamicin (10 mcg) exhibited high percentage of sensitivity against the *Staphylococcus* isolates at 10(90.9%). Cefuroxime (30 mcg) and Ceftazidime (30 mcg)

Table 4 shows the multidrug resistant profile of the *Staphylococcus aureus* isolates from the nasal samples. This revealed that 1(9.0%) out of 11 *Staphylococcus aureus* isolates was resistant to the antibiotics tested, while a total of 8(72.7%) isolates were resistant to three (3) or more antibiotics tested.

Table 1. Prevalence of *Staphylococcus aureus* isolates among the students of MOUAU

Samples (N=40)	No. (%) of samples with isolates	No. (%) of samples without isolates
Male (23)	7 (30.4)	16 (69.5)
Female (17)	4 (23.5)	13 (76.4)
Total	11 (27.5)	21 (52.5)

Table 2. Colonial Morphology and Biochemical Characteristics of the bacterial Isolates

	Isolate 1	Isolate 2
Colonial features	Colonies appeared with bright yellow colour on MSA plates	Milkfish-yellow colonies on MSA Plates
Gram reactions	+	+
Cell arrangement	Cocci in Clusters	Cocci in Clusters
Catalase	+	+
Coagulase	+	-
DNase	+	+
Organisms	<i>Staphylococcus aureus</i>	Coagulase Negative <i>Staphylococcus</i>

+ = present, - = absent

Table 3. Drug Susceptibility Profile of the bacterial Isolates from the Nasal Samples

Antibiotics	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistant
CTR	2 (18.1)	3 (27.2)	5 (45.4)
OFL	10 (90.9)	1 (9.0)	0 (0.0)
AUG	0 (0.0)	2 (18.1)	9 (81.8)
GEN	10 (90.9)	0 (0.0)	1 (9.0)
CXC	0 (0.0)	4 (36.3)	7 (63.6)
CRX	0 (0.0)	0 (0.0)	11 (100)
ERY	4 (36.3)	1 (9.0)	6 (54.5)
CAZ	0 (0.0)	0 (0.0)	11 (100)

Key: CTR = Ceftioxone (30 mcg), GEN = Gentamicin (10 mcg), OFL = Ofloxacin (5 mcg), CXC = Cloxacillin (5 mcg), AUG = Amoxicillin (30 mcg), CRX = Cefuroxime (30 mcg), ERY = Erythromycin (30 mcg), CAZ = Ceftazidime (30 mcg)

Table 4. Multidrug Resistant Profile of the *Staphylococcus aureus* isolates from the Nasal Samples

No. of antibiotics tested	No of resistant isolates	Percentage (%)
1	0	0.0
2	1	9.0
3	2	18.1
4	2	18.1
5	4	36.3
> 3	8	72.7

4. DISCUSSION

The findings of this study reveal that the prevalence of multidrug resistant nasal carriage *Staphylococcus aureus* isolated from nasal cavities of male and female students in MOUAU was 11(27.5%). This is in contrast and disagreement with studies carried out by Osinupebi et al. [27], in Abeokuta and Udobi et al. [28] in Zaria, Nigeria, which recorded a prevalence rate of 41% and 85.25% respectively. This study also showed lower prevalence rate when compared with the works of Jean-marie et al. [29], who carried out a study on surgical site infection in Kinshasa and recorded a prevalence of 63.5%. This study also revealed much more lower prevalence rate than Barkin et al. [30], who reported prevalence of 53.4% from Florida, and the work of Indian researchers (INSAR) where they reported 42% in 2008 and 40% in 2009. Also, the prevalence rate recorded in this study was lower when compared with study by Chigbu and Ezeronye [31] in Abia state of Nigeria, who reported 50% nasal colonization in both hospital and non-hospital patients, but is in agreement with studies by Adesida et al. [10] who reported a much lower (14.0%) nasal colonization in medical students in Lagos, Nigeria. These variations in the prevalence may be due to varying study area and the population under study.

However, George et al. [32] reported prevalence of 0.9% from his study which is lower than the result obtained from this study. The result of this study however, is in concordance with a report of the study carried out by Geoffrey et al. [33] from Tanzania, and Islam et al. [34] from Bangladesh who reported 13.9% and 25% respectively. The underlying attributes of the population under study may contribute to variations in the prevalence rates. Other factors that can cause variations may be sampling and culture techniques [32]. It is also important to note that persons on antibiotics at the point of sampling would produce much lower prevalence. Hospital environment can also be a predisposing factor to high prevalence rate due to higher infectious patients [11].

This study also showed the overall prevalence of *Staphylococcus aureus* to be 11(27.5%) in healthy young female and male students of MOUAU. This supports several studies which has shown the prevalence of *Staphylococcus aureus* on healthy adults in different countries including Malaysia (23.4%), Nigeria (33.3%), Iran

(26.5%), and Jordan (40%), [35,1,36,37]. Furthermore, most studies have found 10-35% of healthy adults to persistently carry *Staphylococcus aureus* in their nares while 20-75% of the adults have been reported to be intermittent carriers [38]. In contrast, 5-70% of healthy adults does not carry the organism [39]. The presence MRSA among the study population as recoded in this study could further support the fact that nasal cavities remain a principal reservoir of this organism. There is need to eliminate the virulent strains of this organism because they contribute to most severe cases of hospital and community associated *Staphylococcus aureus* infections in colonized individuals [13,16].

From this study, the drug susceptibility profile of bacterial isolate from nasal samples reveals varying percentage of sensitivity and resistance to the antibiotics. Ofloxacin (5 mcg) and Gentamicin (10 mcg) exhibited high percentage of sensitivity against the *Staphylococcus* isolates at 10(90.9%) each. Cefuroxime (30 mcg) and Ceftazidime (30 mcg) showed high level of resistance against the *Staphylococcus* isolates at 11(100%). No resistance to Ofloxacin (5 mcg) was noted. The observed resistance to Cefuroxime (30 mcg) and Ceftazidime (30 mcg) is higher than the reports of Gorwitz et al. (2008) 1.5% in U.S.A, Chen and Huang, (2005) 13.6% in Taiwan and Olonitola et al. [40] 14.85% in Zaria, Nigeria, from anterior nares of healthy population, adults and school pupils respectively. However, Rijal et al. [41] reported a much higher 56.1% resistance of *Staphylococcus aureus* from nasal colonization in healthy school children in Nepal. The isolated strains from this study were also resistant to Amoxicillin 9(81.9%), and Cloxacillin (63.6%). This result is similar with the result obtained by Osinupebi et al. [27], which indicated that the MRSA isolates were resistance to almost all antibiotics tested.

From this present study, about 2(18.1%), 10(90.9%), and 4(36.3%) of MRSA isolates were sensitive to Ceftiaxone, Ofloxacin, Erythromycin and Gentamicin respectively. The lower resistance to these antibiotics is higher than the report of Olonitola et al. [40] 2.97%. This may be due to the classes of subjects involved and as result of no cross-resistance between penicillinase resistant penicillin (e.g. Oxacillin) and other classes of antibiotics [29]. In Nigeria, there is a high degree of self-medication associated with an inadequate administration of antibiotic dose and little or no care availability to

customers with or without a prescription. Also, the indiscriminate use of antibiotics leaves them entirely useless in the treatment of multiple infections [21]

There have also been widely reported cases of susceptibility of *Staphylococcus aureus* from various sites of healthy individuals and nosocomial infections against these antibiotics [42]. This is due to the presence of active enzymes such as the beta-lactamases produced by *Staphylococcus aureus*. The insistent availability of these antibiotics in every drug vendors has led to frequent use and misuse of these drugs. This has contributed a greater selection pressure for the resistant strains thereby making these agents ineffective for treatment of staphylococcal infections [43].

Notably, asymptomatic colonization of this organism can persist for months to years [44]. In addition, the rise in resistant cases of this pathogen to various antibiotics tested would complicate the treatment of *Staphylococcus aureus* associated infections. Therefore, there is need to implement effective health care policies to effectively manage and prevent *Staphylococcus aureus* infections [45]. Pertinently, additional factors that could naturally protect individuals from *Staphylococcus aureus* colonization are also needed. This may lead to novel strategies for preventing infections [38].

The multi-resistant character of MRSA observed in the present study limits the choice of antibiotics for treatment. In hospitals, Ofloxacin (5 mcg) and Gentamicin (10 mcg) remains the suitable antibiotic for the treatment of MRSA infections. This study reported high percentage (72.7%) of multi-drug resistant *Staphylococcus aureus* isolates from nasal cavity of healthy male and female students of MOUUAU. This finding correlate with the work of Osinupebi et al. [27], Igbinosa et al. [46] and George et al. [32] which gave high multi-drug resistant *S. aureus* isolates against the antibiotics used. This findings call for great concern because it has been shown by Kluytmans et al. [16] in a study, that carriage of *Staphylococcus aureus* in the nose appears to play a key role in the pathogenesis of infection. This also calls for strategies for elimination of nasal carriage of *S. aureus* so as to reduce the incidence of *Staphylococcus aureus* infections.

5. CONCLUSION

This study highlights the need to discourage the misuse of antibiotics and to implement strategies

that could help eliminate of nasal carriage of *Staphylococcus aureus*. This will help to prevent severe *Staphylococcus aureus* infections in our environments. It was also observed in this study that prevalence of *Staphylococcus aureus* tends to occur more in the nasal cavities of male individuals than the female counterparts from MOUUAU. Consequently, further studies are required to accurately assess the epidemiology of *Staphylococcus aureus* present in the nasal cavities of individuals in various geographical locations. Also, cost effective measures needs to be put in place to effectively screen the population at increased risk of colonization by this organism. It is also report that Ofloxacin (5 mcg) and Gentamicin (10 mcg) antibiotics could be an alternative choice to use and to control MRSA infection as an effective antibacterial agent.

CONSENT AND ETHICAL APPROVAL

The authors declare that all experiments have been examined and approved by the appropriate ethics committee. Informed consents were obtained from all relevant authority.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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