



Incidence of Methicillin Resistant *Staphylococcus aureus* Isolated from Nostrils of Mouau Students

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Authors' contributions

This work was carried out in collaboration among all authors. Author MIO designed the study, wrote the protocol, author PCO wrote the first draft of the manuscript. Authors EKA and JKP performed the microbial analysis. Author CVN helped with the analyses of the work especially in the area of antimicrobial susceptibility testing and authors EOA and JOO helped generally in the collection of samples. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study is carried out to determine the prevalence of nasal carriage of *staphylococcus aureus* among Michael Okpara University of Agriculture Umudike (MOUAU) students and determine the antibiotics susceptibility pattern.

Methods: Nasal swab specimens collected from the anterior nares were cultured on mannitol salt agar. *S.aureus* isolates were identified by mannitol fermentation, coagulase positivity and catalase positivity. Antimicrobial susceptibility test was performed on Mueller-Hinton Agar (MHA) by modified Kirby-Bauer disc diffusion method.

Result: Out of the 100 swabs collected, *S.aureus* was isolated from 60% of the samples. Among colonized students, 63.3% (n=38) were from male students whereas 36.7% (n=22) were from female. The antibiotic susceptibility pattern of the *Staphylococcus aureus* isolates indicates that *Staphylococcus aureus* isolates showed high rate of sensitivity towards antibiotics as follows; Gentamycin (81.67%), followed by Ciprofloxacin(80%), Levofloxacin (76.67%), Ceftriaxone (58.33%), and resistance towards antibiotics Imipenem (100%) followed by Cefotaxime

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(90%), Cefexime (83.33%), Cefoxitin (65%), azithromycin (55%) and cefuroxime (50%).

Conclusion: This study showed a high rate of nasal carriage of *Staphylococcus aureus* among MOUAU students and a high incidence of antibiotics resistant *Staphylococcus aureus* carriage.

Keywords: Antibacterial; *staphylococcus aureus*; methicillin resistant; antibiotics; antimicrobial; nasal swab.

1. INTRODUCTION

Staphylococcus aureus causes a variety of suppurative infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furuncles; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of super-antigens into the blood stream [1].

Although methicillin-resistant *Saureus* (MRSA) has been entrenched in hospital settings for several decades, methicillin-resistant *S. aureus* MRSA strains have recently emerged outside the hospital becoming known as community associated- methicillin- resistant *S. aureus* (CA-MRSA) or superbug strains of the organism, which now account for the majority of staphylococcal infections seen in the clinic [2].

The primary reservoir of staphylococci is the nares, with colonization also occurring in the axillae, vulva, pharynx, and other skin surfaces. Nasal carriage in patient admitted to the hospital is common because close contact among patients and hospital personnel is not unusual; transfer of organisms often takes place. Increased colonization in patients and hospital workers frequently occurs in hospitals. Both hospital and community-acquired infections caused by drug resistant *S. aureus* have increased in the past 20 years [3].

S. aureus is both a human commensal and a frequent cause of clinically important infections. It is frequently found on the human respiratory tract and on the skin. Strains that are associated with disease often result in infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine. *S.*

aureus screening, today, is mainly done to identify methicillin-resistant *S. aureus* (MRSA) carriers. The prevalence of methicillin-resistant *S. aureus* (MRSA) is still quite low in some parts of the world, such as Northern European countries, but there is a worldwide increase in the number of infections caused by methicillin-resistant *S. aureus* (MRSA) [2]. Almost 25% of the Health Care Workers are stable nasal carriers, and 30% to 50% of them also possess the bacteria on their hands. Health Care Workers that carry *S. aureus* in their nares can occasionally cause outbreaks of surgical-site infections [4]. Most of the invasive *S. aureus* infections are assumed to arise from nasal carriage [5].

Staphylococcus aureus is one of the most important pathogens worldwide and has emerged as a prominent organism infecting critically ill persons; the impact of *S. aureus* infection on human health has dramatically increased as a result of its remarkable ability to become resistant to antimicrobials. Because of its primary habitat is moist squamous epithelium of the anterior nares, most invasive *S. aureus* infections are assumed to arise from nasal carriage [6]. The difference between methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* is resistance to β -lactam antibiotics; this is often associated with resistance to multiple other antibiotics, which limits the therapeutic options [7].

National estimates in the United States from 2000-2002 suggested that the prevalence of *S. aureus* and methicillin resistant *S. aureus* (MRSA) colonization ratios were 31.6% and 0.84%, respectively. And about 7% or more of patients admitted to the hospital are colonized with MRSA. Although asymptomatic nasal colonization with *S. aureus* is common, it appears to be an important factor in the development of most infections due to this organism [8].

S. aureus is the most clinically significant species of staphylococci; *Saureus*

characteristics gave the reason for their pathogenicity; which takes many forms. They grow comparatively well under conditions of high osmotic pressure and low moisture, which partially explains why they can grow and survive in nasal secretions and on the skin. *S. aureus* has been recognized as an important cause of disease around the world and it has become a major pathogen associated with both hospital and community acquired infections [9].

Antimicrobial resistance developed by pathogenic organisms is a global menace and has escalated over the years by the emergence of multi-drug resistant strains among these pathogens [10]. Development of resistance to antimicrobial agents by pathogens is a fitness trait acquired to survive in whatever environment they find themselves. This evolution trait (survival of the fittest) has accounted for the unparalleled success of microbial existence in any part of the earth irrespective of the extreme conditions [9]. Bacteria may possess intrinsic resistance that protect them from a particular antibiotic; or acquire resistance through chromosomal mutation or acquisition of genetic materials from other bacteria either through vertical or horizontal transfer of genes. This has led to some strains being called superbugs due to acquisition of resistant genes to different classes of antibiotics, making their treatment highly problematic for both the clinicians and patients. Antimicrobial resistant infections can be acquired in health care facilities, in the community or through food supply [9]. Globalization also makes possible the easy spread of these pathogenic organisms from one country to other countries. Examples of clinically important pathogens that are increasingly becoming multi-drug resistance to antibiotics in use are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. The treatment of infectious diseases caused by microorganisms that have become resistant to commonly used antibiotics has become a major global health care problem in the 21st century [11]. Microbial resistance to drugs was recorded early in the history of chemotherapy and post introduction of penicillin into medical practice; resistant bacteria emerged rendering the "magic drug" ineffective, a pattern the microbes maintained for many years. However, over the past decades microbes have proved themselves to be adapted at becoming resistant to each new antimicrobial agent produced by man [12].

2. METHODS

2.1 Collection of Specimens

Hundred nasal samples were collected from the students (males and females) of Michael Okpara University of Agriculture Umudike for the purpose of this work. Samples were collected aseptically from the anterior nares, using sterile swab sticks moistened with physiological saline for both nostrils. This is to ensure that microorganisms in the nostrils 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.

2.2 Preparation of Culture Media

The media used were Mannitol Salt agar, Nutrient agar and Mueller Hinton agar. They were prepared according to the manufacturer's instruction of each medium. The required amount of the powdered medium was weighed following manufacturer's specification and dissolved in distilled water in a conical flask. The dissolved media were autoclaved at 121°C for 15 minutes.

2.3 Inoculation and Isolation

All specimens collected were transported to Microbiology laboratory immediately and inoculated using the streak plate method on Mannitol salt agar and incubated for 24 hours at 37°C for bacterial growth.

2.4 Purification of Isolates

The resulting colonies from the Mannitol salt agar plates were purified by sub-culturing on freshly prepared nutrient agar plates. The plates were incubated at 37°C for 24 hours. After overnight incubation, the resulting discrete colonies were stored in agar slant for further use.

2.5 Identification of the Isolates

Isolates were characterized based on colonial morphology, Gram staining reaction and biochemical tests which include; catalase and coagulase tests of the isolates were carried out to verify the identity of the organisms.

2.5.1 Gram staining

An inoculum of the test organism was emulsified in a drop of physiological saline on a sterial

clean and grease free slide to obtain a thin film. It was passed over a gentle flame to fix the organism on the slide. Crystal violet was added to the fixed smear and allowed to stand for 60 seconds and rinsed with distilled water. Iodine (mordant) was added, allowed for 1 minute and then rinsed with distilled water. It was decolorized by addition of acetone-alcohol and rinsed after 30 seconds with distilled water. It was counter stained with safranin red for 60 seconds, rinsed with distilled water and allowed to air dry. Those that retained the crystal violet dye (primary dye) after decolorization with acetone-alcohol (decolorizer) are referred to as the Gram positive bacteria (violet colour), while those that were decolorized by the decolorizer and took up safranin red (secondary dye) are known as the Gram negative bacteria which appears red in color.

2.5.2 Biochemical tests

Catalase test: Using a small sterile applicator stick, a small amount of colonies of the test organism was immersed in a drop of freshly prepared 3% H₂O₂ solution on a clean glass slide. Immediate bubble production indicated a positive test and no bubbling indicated a negative test.

Coagulase test: A drop of sterile distilled water was placed on a clean glass slide. A colony of the test organism was emulsified with a drop of distilled water to make a thick suspension. A loopful of human plasma was stirred into the suspension on the slide. Clumping visible to the eye within 10 seconds indicates positive result.

2.6 Antibiotic Susceptibility Testing

For systemic infections, adequate and immediate detection of *S. aureus* strains and their sensitivity to different antibiotics will be of fundamental importance, for the implementation of appropriate treatment and the initiation of relevant control measures [13].

The antibiotic susceptibility of the isolates then, was tested against the following Gram- negative antibiotics. Cefoxitin (30mcg), Ceftriaxone (45mcg), Cefexime (5mcg), Lefofloxacin (5mcg), Gentamicin (10mcg), Ciprofloxacin (5mcg), Imipenem (10mcg), Cefuroxime (30mcg), Azithromycin (15mcg) and Cefotaxime (25mcg), using Kirby Bauer antibiotics disk method. A colony of the test organism was picked with sterile wire loop and immersed in peptone water.

The turbidity of the suspension was compared against a reference 0.5 Mcferland tube. 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.

3. RESULTS

Table 1 shows the distribution of *S. aureus* from the nasal swab samples of Michael Okpara University of Agriculture Umudike (MOUUAU) students. Out of 100 nasal samples studied, 59 were obtained from the male students and 41 were from the female students. *S. aureus* could be isolated from 60 samples. Among the isolates, 38 (63.3%) were from male whereas 22 (36.7%) were from female. There was no significant sex difference in colonization of *S. aureus*. The study showed that the highest colonization of *S. aureus* was found in the age group 19-21 (63.9%), followed by 16-18 (58.6%) and 22-24 (57.1%), respectively.

Table 2 shows the Antibiotic Susceptibility pattern of the *Staphylococcus aureus* Isolates. The *Staphylococcus aureus* isolates showed high rate of sensitivity towards antibiotics Gentamycin (58.33%), followed by Lefofloxacin (56.67%), Ciprofloxacin (56.67%), Cefoxitin ((35%) and resistance towards antibiotics Imipenem (100%) followed by Cefotaxime (90%), Cefexime (83.33%), Cefoxitin (65%), Azithromycin (55%), Cefuroxime (50%), and Ceftriaxone (41.67%).

Table 3 shows the Methicillin resistance among *Staphylococcus aureus* isolates obtained from the nose of MOUUAU students. In our study the rate of MRSA isolation was found to be 65%. The MRSA carriage rate was found to be 73.9% in the age group 19-21years, 60% in the age group 22-24years and 58.8% in the age group 16-18years.

4. DISCUSSION

S. aureus is an important pathogen colonizing humans and animals with an alarmingly increasing level of developing resistance to most available antimicrobial agents. *S. aureus* remains a versatile and potent pathogen in humans, since it is one of the most common causes of nosocomial and community acquired infections. Nasal carriage of *S. aureus* has been demonstrated to be a significant risk factor for nosocomial and community acquired infection in

Table 1. Distribution of *S. aureus* from the nasal swab samples of MOUAU students

Age Group (years)	Male			Female			Total		
	Number of Samples	Number of <i>S. aureus</i> Isolates	% of <i>S. aureus</i> Isolates	Number of Samples	Number of <i>S. aureus</i> Isolates	% of <i>S. aureus</i> Isolates	Number of Samples	Number of <i>S. aureus</i> Isolates	% of <i>S. aureus</i> Isolates
16-18	19	11	57.9	10	6	60	29	17	58.6
19-21	20	14	70	16	9	56.3	36	23	63.9
22-23	20	13	65	15	7	46.7	35	20	57.1
Total	59	38	64.4	41	22	53.7	100	60	60

Table 2. Antibiotic Susceptibility pattern of the *Staphylococcus aureus* Isolates

Antimicrobial agents	Code	Disc potency	Number of sensitive isolate %	Number of intermediate isolate %	Number of resistant isolate %
Azithromycin	AXN	15µg	10(16.67)	17(28.33)	33(55)
Cefexime	ZEM	5 µg	0(0)	10(16.67)	50(83.33)
Cefotaxime	CTX	25 µg	0(0)	6(10)	54(90)
Ceftriaxone	CRD	45 µg	10(16.67)	25(41.67)	25(41.67)
Cefuroxime	CXM	30 µg	10(16.67)	20(33.33)	30(50)
Ciprofloxacin	CIP	5 µg	34(56.67)	15(25)	11(18.33)
Gentamycin	GN	10 µg	35(58.33)	14(23.33)	11(18.33)
Imipenem	IMP	10 µg	0(0)	0(0)	60(100)
Lefloxacin	KBC	5 µg	34(56.67)	15(25)	11(18.33)
Cefoxitin	FOX	30 µg	21(35)	0(0)	39(65)

Table 3. Methicillin resistance among *Staphylococcus aureus* isolates obtained from the nose of MOUAU students

Age Group (years)	Male			Female			Total		
	No. of <i>S. aureus</i> Isolates	No. of MRSA Isolates	% of MRSA Isolates	No. of <i>S. aureus</i> Isolates	No. of MRSA Isolates	% of MRSA Isolates	No. of <i>S. aureus</i> Isolates	No. of MRSA Isolates	% of MRSA Isolates
16-18	11	7	63.6	6	3	50	17	10	58.8
19-21	14	11	78.6	9	6	66.7	23	17	73.9
22-24	13	8	61.5	7	4	57.1	20	12	60
Total	38	26	69.4	22	13	59.1	60	39	65

variety of population. This study evaluated the nasal carriage of *Staphylococcus aureus* among MOUAU students. A total of 60 isolates of *Staphylococcus aureus* were isolated from the 100 nasal swab samples. *S. aureus* carriage has been demonstrated to be highly variable and age dependent and little is known of the factors that make one person to be a chronic carrier or a transient carrier.

In present study, *Staphylococcus aureus* isolates showed high rate of sensitivity towards antibiotics Gentamycin (58.33%), followed by Lefloxacin (56.67%), Ciprofloxacin (56.67%), Cefoxitin ((35%) and resistance towards antibiotics Imipenem (100%) followed by Cefotaxime (90%), Cefexime (83.33%), Cefoxitin (65%), Azithromycin (55%), Cefuroxime (50%), and Ceftriaxone (41.67%). This is comparable to the study of Rai et al. [14] who reported rate of resistant of *S. aureus* towards antibiotics ampicillin (38.1%), erythromycin (33.3%), coxacillin (14.3%), gentamycin (9.5%) and methicillin (9.5%) respectively [14]. Ugwu et al., [15], reported rate of resistant of *S. aureus* towards antibiotics Amoxicillin 30 (54%), Streptomycin 25 (45%), Amoxicillin-clavulanic acid 14 (25%), Erythromycin 13 (23%), Chloramphenicol 12 (21%), Co- trimoxazole 10 (18%), Ofloxacin 8 (13%), Ciprofloxacin (16%) and Gentamicin 5 (9%). The MRSA isolates recorded a high resistance to Amoxicillin and Streptomycin among all the tested antibiotics. The highest level of resistance was observed to the amoxicillin [15].

In our study the rate of MRSA isolation was found to be 65%. The MRSA carriage rate was found to be 73.9% in the age group 19-21years, 60% in the age group 22-24years and 58.8% in the age group 16-18years. Similarly, Ugwu et al., [15] reported (72.7%) of *S. aureus* in Healthy Students in Agbor, Delta State, Nigeria. Nsofor et al., [16] which reported 62.9% carriage in school children in Elele, Rivers State Nigeria. In contrary, Onanuga and Temedie, [17] reported a lower *S. aureus* nasal colonization rate (33.3%) in healthy inhabitants of Amassoma in Niger delta region of Nigeria. Chijioke et al., [18,19] reported 56.3% of *S. aureus* in Apparently Healthy School Children in Owerri Metropolis, Nigeria. It is worthy to note that Imipenem is a broad spectrum antibiotic widely prescribed in hospital and it is still considered as a better choice against fatal infections. Soon enough, studies showed the continuous resistance of antimicrobials to this particular antibiotic.

Although, the emergence of antimicrobial resistance against effective antibiotics is a global issue. The invitro antimicrobial activity of imipenem was carried out by disc diffusion method (Kirby-Bauer test) during one of the research of Batayal et. al. 29.5% of the isolates were shown to be imipenem resistant *Staphylococcus aureus*. This concluded that clinical isolates have started developing resistance against imipenem [18]. In comparison to other studies, the rate of isolation of MRSA and resistant rate towards different antibiotics from our study was slightly high. It may be due to certain limitations of our study. The first limitation of this study included the lack of knowledge of some risk factors associated

with incidence and prevalence of MRSA infections in the community. Second, due to limited time and resources, we did not take higher number of samples for study. *S. aureus* is an important pathogen colonizing humans and animals with an alarmingly increasing level of developing resistance to most available antimicrobial agents. *S. aureus* remains a versatile and potent pathogen in humans, since it is one of the most common causes of nosocomial and community acquired infections. Third and final limitation is that only the phenotypic identification of the specimens was performed.

5. CONCLUSION

S. aureus remains a versatile and potent pathogen in humans, since it is one of the most common causes of nosocomial and community acquired infections. Hence identification of infection caused by staphylococci might help modify the antibiotic therapy and prevent infection. In conclusion, antibiotic use is one of

the most important determinants of antibiotic resistance, thus antibiotic stewardship programs that promote judicious use of antibiotic are urgently needed and could prove to be more cost effective than targeted screening based on risk factors, isolation of the carriers and decolonization. Simple hygiene measures like hand washing are effective in preventing spread of resistant organisms in the community. The importance of hygiene is exemplified in an intervention program in Swedish day care centers, which introduced alcohol-based hand washing for children. Finally there is need to implement strategies for elimination of nasal carriage of methicillin resistant *Staphylococcus aureus* (MRSA), so as to prevent severe multi-

drug resistant *S. aureus* infections in our environments.

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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