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Characterization of *Staphylococcus aureus* isolated from the anterior nares of students in a tertiary institution, Abia state, south-eastern, Nigeria.

*Nwankwo, E.O[1], Ezuruonye, C.N[1] and Onusiriuka[1], K.N.
Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State. Nigeria.
**Corresponding Author's Email: emmaonwubiko@yahoo.com*

Abstract
Carriage of *S. aureus* in the anterior nares is widely believed to play a key role in the epidemiology and pathogenesis of the infection. This study characterized *Staphylococcus aureus* obtained from the anterior nares of University students in Abia State, South-eastern Nigeria. Nasal swabs of 200 students (90 male and 110 female) were randomly collected, cultured on Blood and Mannitol Salt agar plates and incubated aerobically at 37^{o}C for 24hours. The isolation and identification of *S. aureus* was carried out by standard microbiological techniques. Out of the 200 samples screened, 139(69.5%) harboured *S. aureus* from which 96(69.1%) were positive for biofilm forming potentials. The rate of *S. aureus* carriage was higher in females (58.3%) than in males (41.7%).Production of beta-lactamase by *S. aureus* was higher amongst biofilm formers (71%) than non-biofilm formers (28.7%). Antibiotic sensitivity testing of the isolates revealed a pattern of high susceptibility to Streptomycin and Ceftriaxone and a moderate level of resistance to some commonly available antibiotics such as Ampicillin, Chloramphenicol and Amoxicillin. Test for methicilin resistance showed a low level of resistance to oxacillin (6.5%). The study revealed that there was a high prevalence of nasal carriage among the population examined most of which had the potential to form biofilm and also produce beta-lactamase.

Keywords: *S. aureus*, anterior nares, university students

1. Introduction

Staphylococcus aureus is a microorganism that colonizes the skin and mucosal surfaces like the anterior nares of both healthy individuals and individuals with underlying disease; it is also one of the most common causes of community-acquired and hospital infections. The spread of colonization occur especially in close contact areas like schools, pre-schools or households (Peacock *et al.,* 2003).

Nasal carriage of *S. aureus* has been identified by several authors as a major risk factor for subsequent infections as carriers act as reservoirs for the pathogen, assisting its spread in the community (Sollid *et al*., 2013; Olayemi *et al*., 2018). Rates of nasal colonization with *S. aureus* have been reported to range from 6.3 to 17.8% and 18.2 to 43.8% in the general population and healthcare workers, respectively (Arucha *et al*., 2013).It is transmitted to the

nares by contaminated hands and from surfaces where it can survive for months (Kluytmans, Van BelkumandVerbrugh, 1997; Pathak *et al*., 2010).

Nasal carriage of invasive human pathogens like *S. aureus* has been associated with increased incidences of infection and morbidity. A study of the risks and outcomes of nosocomial *S. aureus* bacteremia in nasal carriers concluded that at least 80% of *S. aureus* bacteremia was identical to a nasal carrier strain revealed as endogenous by genotyping. Nasal colonization of *S. aureus* was also relevant in the occurrence of impetigo in children respectively (Arucha *et al*., 2013). In addition, the ability to form biofilms and increasing resistance of *S. aureus* to various antibiotics has been known to complicate the treatment of diseases caused by it (Olayemi *et al*., 2018). *Staphyloccocus aureus* biofilm-associated infections are difficult to treat with antibiotics (Jones *et al*., 2001). Biofilm formation by *S. aureus* has been identified to play an important role in the pathogenesis of staphylococcal infections. The biofilm causes bacteria to survive in the stress conditions such as UV damage, metal toxicity, anaerobic conditions, acid exposure, salinity, pH gradients, desiccation, bacteriophages, and amoebae and to resist antibiotics, antimicrobials, and host immune defense (Archer *et al*., 2011; Hall-Stoodley *et al*., 2004). The purpose of this study was to characterize *S. aureus* isolated from the anterior nares of apparently healthy University students.

2. Materials and Methods

2.1. Study location

This study was conducted in Michael Okpara University of Agriculture, Umudike, Umuahia in Abia State, South-eastern Nigeria.

2.2. Study design

Cross-sectional survey.

2.3. Study population

The study population included 90 males and 110 females aged between 18 and 25 years.

2.4. Sample collection

A total of 200 nasal swabs were obtained from the students (90 male and 110 female).The isolation, culture and sensitivity testing was then carried out at the Microbiology laboratory after collection of the samples.

To minimize the mild irritation that may happen by dry swabs, swabs were moistened with sterile phosphate buffered saline (PBS). Collection was made by carefully inserting a sterile cotton-tipped swab into the anterior nares and rotating it mildly. It was then taken to the laboratory immediately for culture and isolation. Informed consent of each student was obtained before sample collection. Nasal swabs were taken from both nostrils of the students.

2.5. Inoculation of the nasal sample

The swab specimen was inoculated on Blood agar and Mannitol Salt Agar by the streak method. The plates were incubated aerobically at 37ºc for 18-24 hours. After incubation, the bacterial isolates were examined using colony size, colour, cultural and morphological characteristics.

2.6. Identification of isolates

Isolates were identified by standard microbiological methods (Cheesebrough, 2006) on the basis of their morphological, Gram and biochemical characteristics (catalase, slide and tube coagulase tests).

2.7. Antibiotic susceptibility test

The Kirby Bauer disc diffusion technique was used. Colonies of *Staphylococcus aureus* from each of the culture plates were compared with 0.5 McFarland standard in saline. Swabs were streaked uniformly on Nutrient agar plates to obtain uniform growth. Sensitivity discs (Streptomycin 10µg, Levofloxacin 5µg, Gentamicin 10µg, Chloramphenicol 30µg, Norfloxacin 10µg, Amoxicilin 20µg, Rifampicin 5µg, Erythromycin 15µg, Ampicillin 10µg, Ciprofloxacin 5µg, Ceftriazone 30µg) were placed on the surface of the media using sterile forceps. A little pressure was used to ensure firm contact with agar plate. The plates were inverted and incubated at 37ºc for 24 hours. The plates were examined for zone of inhibition by measuring the diameter using a ruler from the back of the plate. The zone sizes of each plate were interpreted using the guidelines by Clinical Laboratory Standards Institute (CLSI, 2011).

2.8. Detection of Methicillin resistance.

Staphylococcus aureus isolates were screened for methicillin resistance by the disk diffusion method of the Clinical and Laboratory standards institute (CLSI, 2006). *S. aureus* isolates were inoculated onto Mueller Hinton agar supplemented with 4% sodium chloride, from a 0.5 McFarland equivalent standard suspension. Afterwards1µgoxacillin disk was applied firmly on the surface of the agar plate. The plates were incubated at 35°C for 24 hours as recommended by the Clinical Laboratory Standards Institute (CLSI, 2006). Zone diameters were interpreted as resistant (≤10 mm), intermediate (11-12 mm) and susceptible (≥13 mm) respectively.

2.9. Detection of Biofilm forming potential

Detection of Biofilm forming capacity was conducted by using Congo Red Agar method. Black colonies with a dry crystalline consistency indicated ability to form biofilm, while non-biofilm forming isolates retained the colour of the culture media.

2.10. Detection of Beta-lactamse production:

This was done using the rapid acidometric filter paper technique as described by (Cheesebrough, 2006). A strip of Whatman No. 1 filter paper was placed in the bottom of a petri dish. Few drops of buffered crystalline bromocresol purple solution was added until the paper was almost saturated, using a sterilized wireloop. Few colonies were transferred from the Nutrient agar plate to the filter paper to cover an area of 5mm in diameter. The plate was incubated at 35°C for 30 minutes. Positive results for beta-lactamase production were confirmed by the appearance of a yellow colour after about 20-60minutes of incubation which indicates the production of penicilloic acid from the breakdown of penicillin by beta-lactamase producing isolates. Non-appearance of yellow colour after about 60minutesof incubation was considered as negative for beta-lactamase production.

3. Results

The incidence of occurrence of *S. aureus* among the sampled population is being highlighted in Table 1. Out of the 200 respondents sampled, 139 (69.5%) harboured *S. aureus* in their

anterior nares. The rate of *S. aureus* carriage was higher in females (58.3%) than in males (41.7%). However it was not statistically significant, P> 0.05.

The relative frequency of *S. aureus* isolates with biofilm forming potentials is being highlighted in Table 2. Out of the 139 *S. aureus* isolated, 96(69.1%) were positive for biofilm forming potentials.

Results of the antimicrobial susceptibility profile of biofilm forming and non-biofilm forming *Staphylococcus aureus* isolates is shown in tables 3 and 4. Antibiotic susceptibility for biofilm forming *S. aureus* revealed that 7 out of 11 drugs tested showed more than 70% sensitive while 8 out of 11 drugs tested for non biofilm forming *S. aureus* showed above 70% sensitive.

The number and percentage of isolates that were methicillin-resistant and those that could produce beta-lactamases among the *Staphylococcus aureus* isolates is being highlighted in Table 5 and 6. In total, 6.5% of the isolates were found to be methicilin resistant. While 52.5% of isolates were found to be beta-lactamase producing.

Table 1: Prevalence of *S. aureus* among the sampled population

Sex	No. Sampled	No. positive for *S. aureus*
Male	90(45%)	58(41.7%)
Female	110(55%)	81(58.3%)
Total	200	139(69.5%)

χ^2=1.60, df = 1, p > 0.05

Table 2: Prevalence of *S. aureus* with biofilm forming potentials among the sampled population

Sex	No. Sampled	No. positive for biofilm formation
Male	58(41.7%)	39(40.6%)
Female	81(58.3%)	57(59.4%)
Total	139	96(69.1%)

χ^2=0.1549, df = 1, p > 0.05

Table 3: Antimicrobial susceptibility profile of biofilm forming *Staphylococcus aureus* from the nasal passages of students

Antimicrobial agent	Code	No. of sensitive isolates (%) N= 96	No. of resistant isolates (%) N= 96
Amoxicillin	AMX	22(22.9)	74(77)
Ampicillin	AMP	20(20.8)	76(79.2)
Ceftriaxone	CRO	87(90.5)	9(9.4)
Streptomycin	STR	92(95.8)	4(4.2)
Gentamycin	GEN	83(86.5)	13(13.5)
Rifampin	RIF	96(100)	0(0)
Chloramphenicol	CHL	77(80.2)	19(19.8)
Norfloxacin	NOR	81(84.4)	15(15.6)
Ciprofloxacin	CPX	76(65.5)	20(20.8)
Levofloxacin	LVX	78(81.3)	18(18.7)
Erythromycin	ERY	54(56.3)	42(43.7)

Table 4: Antimicrobial susceptibility profile of non-biofilm forming *Staphylococcus aureus* from the nasal passages of students

Antimicrobial agent	Code	No. of sensitive isolates (%) N= 43	No. of resistant isolates (%) N= 43
Amoxicillin	AMX	12(27.9)	31(72.1)
Ampicillin	AMP	10(23.3)	33(76.7)
Ceftriaxone	CRO	40(93.1)	3(6.9)
Streptomycin	STR	43(100)	0(0)
Gentamicin	GEN	38(88.4)	5(11.6)
Rifampin	RIF	43(100)	0(0)
Chloramphenicol	CHL	35(81.4)	8(18.6)
Norfloxacin	NOR	32(74.4)	11(25.5)
Ciprofloxacin	CPX	34(79.1)	9(20.9)
Levofloxacin	LVX	37(86.1)	6(13.9)
Erythromycin	ERY	27(62.8)	16(37.2)

Table 5: Percentage of isolates resistant to Oxacillin

Category	No. tested	No sensitive to Oxacillin (%)	No resistant to Oxacillin(%)
Biofilm forming	96	89(68.4)	7(77.7)
Non-biofilm forming	43	41(31.5)	2(22.2)
Total	139	130(93.5)	9(6.5)

χ^2=0.3419, df = 1, p > 0.05

Table 6: Production of beta-lactamase by *Staphylococcus aureus*

Category	No. Tested	No. positive for beta-lactamase (%)	No. negative for beta-lactamase (%)
Biofilm forming	96	52(71.2)	44(66.6)
Non-biofilm forming	43	21(28.7)	22(33.3)
TOTAL	139	73(52.5)	66(47.4)

χ^2=0.3383, df = 1, p > 0.05

4. Discussion

Out of the 200 samples analyzed, *Staphylococcus aureus* was isolated in 139(69.5%) of them. This is not surprising as *S. aureus* is a normal flora of humans (Makoni, 2002) with the nostrils as the main reservoir of *S. aureus* in both adults and children of both gender (Pathak *et al*., 2010). In their own study Olayemi *et al*., (2018) reported an overall prevalence of 56.7% of nasal carriage of *S. aureus* among University students in South-western Nigeria.

The prevalence of oxacillin resistance *S. aureus* in the study is 6.5%. This is in agreement with the studies by Demirel *et al.,* (2014) who isolated 9% MRSA from university students in Turkey and that of Nwankwo and Nasiru (2011) who reported 10.7% MRSA in a tertiary health institution in Kano, Nigeria; and those of Habeeb *et al.,* (2014) and Chen *et al.,* (2012) who reported low MRSA incidence of 2.04% and 2.2% respectively in their independent studies. Also, the prevalence of MRSA in some countries is still low. In the Netherlands for example, it is as low as 1.0% (Lytkainen *et al*., 2004; El-Jalil *et al*., 2018).Some studies have shown that carriage of Methicillin resistant *S. aureus* (MRSA) or

Methicillin sensitive *S. aureus* (MSSA) varies in different geographical areas (El-Jalil *et al*., 2018; Sa-Lea0 *et al*., 2001).

However, reports of the present findings in the same institution disagrees with the findings of Edward *et al.,* (2012) who isolated MRSA from 59.3% of students in their previous study on the Prevalence of methicillin resistant *Staphylococcus aureus* amongst the student community of Michael Okpara University of Agriculture, Umudike, Nigeria. This shows a very significant reduction in the prevalence of MRSA among the students apparently due to improved sanitary conditions and standards of the hostels and classrooms within the past years. Erection of new lecture halls and well spaced hostels could have equally aided in reducing the chances of spreading the organism through overcrowding. This also supports studies by Jernigan *et al*., (1996) who cited prevention of overcrowding as an effective control measure of CA-MRSA.

High susceptibility of *S. aureus* to Rifampicin(100%) and Streptomycin(100%) is an indication that these drugs have not been widely abused in this environment. Susceptibility of *S. aureus* to Rifampicin compares favorably with results from Nwankwo and Nasiru, (2011).

The moderate level of resistance to commonly available antibiotics such as Ampicillin, Chloramphenicol and Amoxicillin could be associated with earlier exposure of these drugs to isolates which could have enhanced development of resistance. Other researchers (Nwankwo and Nasiru, 2011) made the same observations in their various centres.

The highest rates of resistance were recorded with Amoxicillin and Ampicillin. This could be due to the beta-lactamase production of *S. aureus* and the rate at which unprescribed and incomplete dosage of antibiotics are taken (Odugbemi, 1981).

Also, 69.1%and 52.5% of the isolates were found to be biofilm forming and beta-lactamase producing respectively. This agrees with the findings of Devapriya *et al.,*(2014) who reported 61% prevalence biofilm producing S. *aureus* from nasal and throat swabs *out* of which 46.3% were beta-lactamase positive. Biofilm formation has been suggested by several authors to have implications on *S. aureus* colonization of surfaces and internal body parts such as the throat and nasal passage (Sollid *et al*., 2013).Comparatively, the high incidence of biofilm production showed little effect on the antimicrobial susceptibility patterns of the isolates in vitro, especially for Methicilin resistance and beta-lactamase production.

5. Conclusion

The results of this study show a high prevalence of *S. aureus* nasal carriage in our environment. Methicillin resistance *S. aureus* showed a prevalence of 6.5%. It has become necessary to advice regular screening of students in view of the fact that it can be easily transferred to others by the hands. Application of mupirocin cream will reduce this prevalence.

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