Antibiogram and Virulence Characteristics of Multi-drug Resistant *Staphylococcus aureus* from Nasal Cavity of Healthy Students of Niger Delta University, Amassoma, Bayelsa State, Nigeria

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ABSTRACT

Introduction: Nasal carriage of *Staphylococcus aureus (S.aureus)* in healthy population has been reported to be a significant risk factor for the development of severe infections with limited clinical treatment options. This is due to the increasing emergence of Multidrug resistance (MDR) which is posing a serious health problem in the society.

Aim: To determine the antibiogram and virulent characteristics of nasal *S. aureus* by accessing its antibiotic resistance profile and pathogenic potentials among healthy students of Niger Delta University, Bayelsa State, Nigeria.

Materials and Methods: Four-hundred healthy University students were screened for nasal carriage of *S. aureus* from April to October 2016 using conventional standard techniques. The suspected *S. aureus* isolates were screened for the presence of *nuc* and *mecA* genes for confirmation of *S. aureus* and Methicillin-resistant *S. aureus* (MRSA) respectively using Polymerase Chain Reaction (PCR). Antimicrobial susceptibility patterns of confirmed isolates were determined using Kirby-Bauer diffusion technique. Phenotypic expressions of haemolysin and biofilm production among the isolates were determined using Blood-Agar and Congo Red Agar tests.

Results: The prevalence of nasal carriage of *S. aureus* was 47 (11.8%). Thirty-seven (37/47; 78.7%) and 30 (30/47; 63.8%) of the isolates were haemolysin and biofilm producers respectively. The biofilm producing *S. aureus* isolates were found to be significantly higher in the males than the females (Odds ratio=2.394; χ^2=5.474; p=0.019). No isolate possessed *mecA* gene but 31 (66%) *S. aureus* were phenotypic MRSA. The *S. aureus* isolates exhibited high rates of resistance to erythromycin (87.2%), co-trimoxazole (85.1%), tetracycline (83%), ciprofloxacin (74.5%), amoxicillin/clavulanic acid (51.1%) and low rates to gentamycin (36.2%), and levofloxacin (6.4%). The prevalence of MDR among the isolates was 41 (87.2%) and the most prominent phenotypic antibiogram combinations among the isolates were "E, SXT, TE" and "CIP, SXT, TE".

Conclusion: The high prevalence of virulent and MDR *S. aureus* among these healthy individuals may lead to their potential spread in the community thereby constituting a serious health concern.

Keywords: Antibiotic resistance, Asymptomatic, Biofilm, Haemolysin, Nasal carriers

INTRODUCTION

Staphylococcus aureus (S. aureus) is one of the human microflora that commonly inhabit the nasal cavity, skin, oral cavity, gastrointestinal tract and other anatomical locales but a large proportion of its carriage is through the anterior nares of the nasal cavity [1]. *S. aureus* persistently colonises the anterior nares of approximately 20-30% of normal healthy human population but its presence does not always/necessarily indicate infection [2-4].

However, nasal *S. aureus* colonisation may result in infections through a combination of its invasiveness on the host innate immunity and a weakened or defective host immunity thereby resulting into community and hospital associated infections such as impetigo, boils, abscesses, bactaeremia, meningitis, pneumonia, osteomyelitis and endocarditis [5]. Studies have however linked an increased risk of *S. aureus* diverse infections to the bacterium's nasal colonisation and this has serious public health implications [2,4].

Biofilm producing *S. aureus* have been associated with chronic infections of the host tissues like bone and heart halves causing osteomyelitis and endocarditis respectively [6]. Biofilm matrix in *S. aureus* infections provides a defence against several clearance mechanisms by inhibiting the access of certain immune defences like macrophages and hence prevents phagocytosis [6,7]. Additionally, biofilm cells display increased tolerance to antibiotics by blocking agents' access to actively growing bacterial cells within the biofilm and hence decreasing the antibiotic diffusion rates leading to the bacterial antibiotic resistance [8].

Staphylococcus aureus is a well-known pathogen with an increasing level of developing resistance to most available antimicrobial agents and most of its infections particularly those caused by methicillin resistant strains have been reported to have very limited treatment options [9,10]. MRSA strains are known to possess *mecA* gene which encodes for an altered Penicillin Binding Protein (PBP2a) called transpeptidase that has a lower affinity for β-lactams (penicillins, cephalosporins, and carbapenems) thereby conferring resistance to methicillin and all other β-lactam antibiotics [11]. These strains have also been reported to develop an increasing resistance to other classes of antimicrobial agents limiting the treatment options of its infections in both community and hospital settings [10,12].

Nasal carriage of MRSA by apparently healthy populations represents a major risk factor for subsequent infection and its transmission to vulnerable individuals with weakened immune systems in a community. Hence, this study determined the antibiogram and virulent characteristics of *Staphylococcus aureus* isolated from the nasal cavity of healthy students of Niger Delta University, Amassoma, Bayelsa State, Nigeria as means of adopting effective measures for

the treatment and prevention of its infections transmission to the University community and the neighbouring environments.

MATERIALS AND METHODS

Study Population
The apparently healthy undergraduate students of College of Health Sciences in Niger Delta University, Amassoma, Bayelsa State, who had not taken a course of antimicrobial therapy nor admitted in a hospital within a period of three months prior to the commencement of the survey were recruited into this study. Niger Delta University is one of the fast growing State Universities in Nigeria, with its location in the Southern part of Nigeria and majority of its students are from the Southern region of the country. The volunteers in this cross-sectional study were randomly selected from the University lecture rooms and they gave written informed consent before the collection of nasal specimens. The basic demographic data such as age and sex (excluding names) were obtained from the volunteers and the study was approved by the ethical committee of the institution before the commencement of the study which spanned through April to October 2016. The sample size for this study was determined using Cochran's formula as described by Bartlett JE et al., [13].

Collection of Samples and Bacteriology
Nasal specimens of 400 healthy volunteers were collected with sterile swab sticks moistened with sterile normal saline solution (0.9%w/v Sodium chloride) and transferred immediately to the Department of Pharmaceutical Microbiology and Biotechnology laboratory for inoculation and streaking on the already prepared sterilised Nutrient Agar plates. The discrete colonies obtained from the inoculated Nutrient agar (Oxoids, UK) plates after incubation at 37°C for 24 hour were sub-cultured on Mannitol Salt Agar (Oxoids, UK) plates and then incubated at 35°C for 24-48 hour. The characteristic colonies on the Mannitol Salt agar plates were identified using standard established microbiological methods, which include colonial morphology, Gram's stain reaction and biochemical characteristics. The isolates that were Gram-positive grape-like clustered cocci, positive to catalase test, mannitol fermentation test, DNase test and slide coagulase test were subjected to PCR analysis for the presence of *nuc* gene as a confirmation for *S. aureus* [14,15].

Extraction of DNA from Biochemically Confirmed *S. aureus*
A single colony of each of the biochemically confirmed *S. aureus* isolates was cultured in sterilised Luria-Bertani broth (Oxoids) and the bacterial cells were recovered after centrifugation. The cells were emulsified in 0.5 ml of sterile DNase-free biological grade water (Inqaba Biotec), boiled at 100°C for 10 minutes in a thermal block and rapidly cooled in a freezer for 30 minutes. The mixture was centrifuged at 10,000 rpm for 10 minutes, and supernatant containing DNA collected, quantified using Nano drop 1000 and stored at 4°C until further investigation [16].

Screening for *S. aureus nuc* and *mecA* Genes by PCR Amplification
PCR was used to separately amplify *S. aureus* specific *nuc* gene and methicillin resistant *mecA* gene using the primers described by Ali R et al., [15] and Nakagawa S et al., [17]. The *S. aureus* specific *nuc* gene was amplified using a 21-nucleotide forward primer 5'-GCGATTGATGGTGATACGGTT-3' and 25-nucleotide reverse primer 5'-AGCCAAGCCTTGAACGAACTAAAGC-3' with a product size of 270 bp while the *mecA* gene was amplified using a 20-nucleotide of both primers forward 5'-CGGTAACATTGATCGCAACG-3'and reverse 5'-TTTGCCAACCTTTACCATCG-3' with a product size of 154 bp. The PCR was performed in a 20 µL reaction mixture containing 1X taq master mix (Taq polymerase, dNTPs, MgCl$_2$

buffer), the primers (forward and reverse) at a concentration of 0.4M, water and the extracted DNA as template. The *nuc* gene was amplified at DNA denaturation at 94°C for 5 minutes, 94°C for 60 seconds, primers annealing at 55°C for 30 seconds, extension at 72°C for 60 seconds, 72°C for 7 minutes for 37 cycles while the *MecA* gene was amplified at DNA denaturation at 95°C for 15 minutes, 95°C for 30 seconds, primers annealing at 55°C for 40 seconds, extension at 72°C for 50 seconds, 72°C for 20 minutes for 35 cycles. The amplified PCR products were analysed on a 2% agarose gel (Biogene, UK) stained with ethidium bromide (Sigma, UK) and electrophoresis was performed in 1X TBE buffer at 120 V for 30 minutes with a 100 bp molecular marker. The gels were subsequently visualised using UV illuminator and photographed.

Antimicrobial Susceptibility Testing
Antimicrobial susceptibility of all *S. aureus* strains was determined for amoxicillin/clavulanic acid (AMC 20/10 µg), erythromycin (E 15 µg), co-trimoxazole (trimethoprim/sulfamethoxazole) (SXT 1.25/23.75 µg), tetracycline (TE 30 µg), gentamycin (CN 10 µg), cefoxitin (FOX 30 µg), ciprofloxacin (CIP 5 µg) and levofloxacin (LEV 5 µg) (Qxoids, UK) using the modified Kirby-Bauer disc diffusion technique in accordance with the Clinical and Laboratory Standard Institute guidelines [18]. The *S. aureus* isolates that were resistant to at least one agent in three or more of the eight classes of antimicrobial agent used in this study were defined as having MDR [19].

Screening for Haemolysin Production in the *S. aureus* Isolates
Haemolytic property of the *S. aureus* isolates was determined by inoculating the isolates on freshly prepared sterile 5%v/v Blood Agar (consisting 5 mL of human blood in 100 mL of Nutrient Agar) plates using straight wire loop and incubated at 37°C for 24 hours. Thereafter, plates were observed for green to dark colouration of the agar (partial lysis of red blood cells-alpha haemolysis) and clear zones (complete lysis of red blood cells-beta haemolysis) around inoculated organisms, indicating the production of haemolysin.

Screening for Biofilm Production in the *S. aureus* Isolates
Biofilm production in *S. aureus* isolates was performed using Congo Red Agar medium which was prepared using the combination of brain heart infusion agar 52 g/L, sucrose 50 g/L and Congo red indicator 8 g/L as described by Mathur T et al., [20]. The Congo red was prepared as concentrated aqueous solution separately from other medium constituents and then sterilised in different containers before adding them together when the agar had cooled to 55°C before distributing to sterile plates to solidify. The plates were then inoculated with the test organisms and incubated at 37°C for 24 hours before examining for black colonies with a dry crystalline consistency indicating biofilm production.

STATISTICAL ANALYSIS
The groups differences were tested using the Chi-square test (or Fisher's-exact test when expected frequencies were too low), with the assumed level of statistical significance at a p-value of <0.05. Data analysis was performed with SPSS version 15.0 for Windows (SPSS Inc, USA).

RESULTS

The Prevalence, Distribution and Confirmation of *S. aureus* Isolates
The 400 nasal specimens from females 276 (69%) and males 124 (31%) volunteers of ages 15-35 years, yielded 183 (45.8%) significant growth of Staphylococci. Only 47 (11.8%) *S. aureus* isolates were recovered through the biochemical tests and the

PCR targeted *S. aureus* specific *nuc* gene [Table/Fig-1]. The age distribution of the volunteers with the *S. aureus* carriage is shown in [Table/Fig-2]. The gender distribution of the volunteers with *S. aureus* isolates was females 25 (9.1%; 25/276) and males 22 (17.7%; 22/124). The nasal carriage of *S. aureus* isolates was found to be significantly higher in the males than the females (Odds ratio=2.165; χ^2=5.413; p=0.013) [Table/Fig-3].
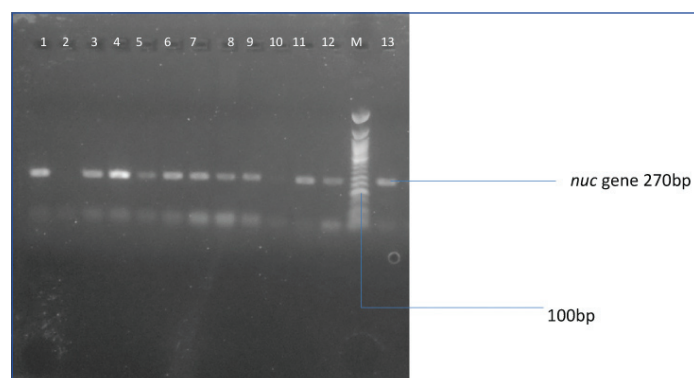
[Table/Fig-1]: Agarose gel showing the amplified *nuc* gene of *S. aureus* isolates. Lanes 1, 3, 4, 5, 6, 7-9 and 11-13 represents the positive bands while Lane M represents the 100bp Quick-Load Molecular ladder respectively.

Age (yrs)	No. of sample	*S. aureus* (%)
15-19	48	5 (10.4)
20-24	243	27 (11.1)
25-30	84	12 (14.3)
31-35	25	3 (12)
Total	400	47 (11.8)

[Table/Fig-2]: The age distribution of the volunteers with nasal *S. aureus*.

Gender	No. of sample	Detection of *S. aureus* (%)		Chi-square	p-value
		Yes	No		
Females	276	25 (9.1)	251 (90.9)	5.413	0.013*
Males	124	22 (17.7)	102 (82.3)		
Total	400	47 (11.8)	353 (88.2)		

[Table/Fig-3]: The gender distribution of the volunteers with nasal *S. aureus*.
*Statistically significant (p<0.05)

Prevalence of Virulence among the *S. aureus* Isolates

A total of 37 (78.7%) *S. aureus* isolates phenotypically expressed haemolysin (alpha toxin) production by lysing human blood while 30 (63.8%) phenotypically expressed biofilm production. The difference in the gender distribution of the volunteers with haemolysin producing *S. aureus* isolates was not statistically significant (Odds ratio=1.799; χ^2=2.857; p=0.091). However, biofilm producing *S. aureus* isolates were significantly higher in the males than the females (Odds ratio=2.394; χ^2=5.474; p=0.019) [Table/Fig-4].

| Virulent factor	Detection	No. of sample	Females	Males	Chi-square	p-value
Haemolysin	Yes	37	21	16	1.799	0.091
	No	363	255	108		
Biofilm	Yes	30	15	15	5.474	0.019*
	No	370	261	109		

[Table/Fig-4]: The gender distribution of volunteers with haemolysin and biofilm producing nasal *S. aureus*.
*Statistically significant (p<0.05)

Antimicrobial Resistant Patterns of *S. aureus* and MRSA Isolates

The antimicrobial susceptibility testing of the *S. aureus* isolates revealed that 31 (66%) of them phenotypically expressed methicillin resistance being moderately resistant to cefoxitin (66%), an agent approved by CLSI for the phenotypic screening for methicillin resistant *S. aureus* (MRSA). Hence, 31 (66%; 31/47) of the isolates

were MRSA while the overall nasal carriage rate of MRSA among all the healthy volunteers was 31 (7.8%; 31/400). No *mecA* gene was detected in all the 47 *S. aureus* isolates screened by PCR analysis. The isolates also exhibited moderate resistance to amoxicillin/clavulanic acid (51.1%) and gentamycin (36.2%) but high resistance to erythromycin (87.2%), co-trimoxazole (85.1%), tetracycline (83%) and ciprofloxacin (74.5%). However, very low resistance was exhibited to levofloxacin (6.4%). The biofilm producers in this study were found to be resistant to at least two antimicrobial agents. The antimicrobial resistance profiles of all the 47 *S. aureus* and 31 MRSA isolates are shown in [Table/Fig-5,6] respectively.
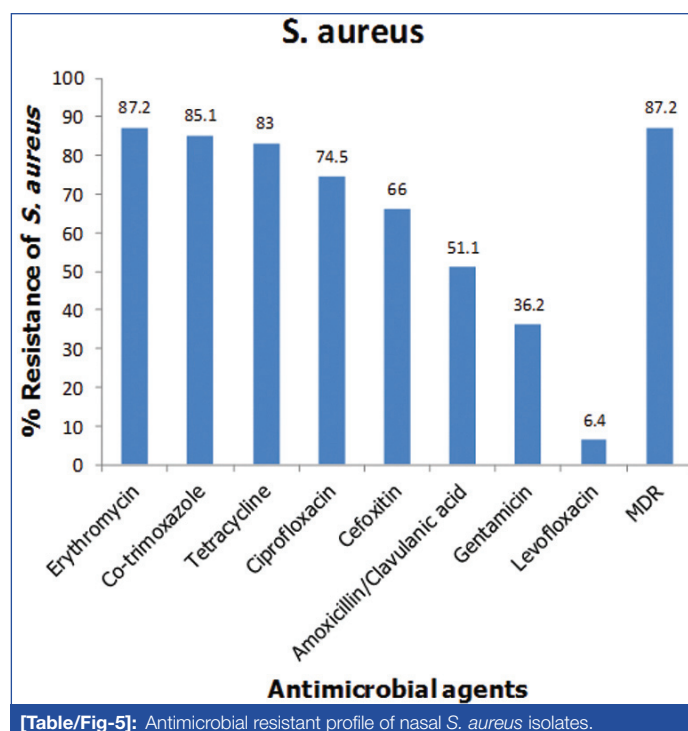
[Table/Fig-5]: Antimicrobial resistant profile of nasal *S. aureus* isolates.
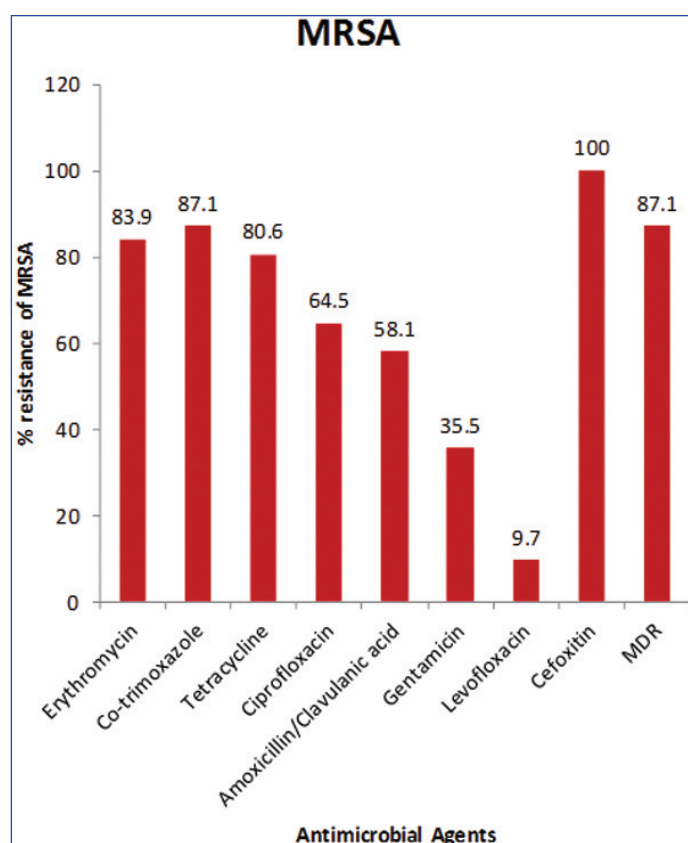
[Table/Fig-6]: Antimicrobial resistant profile of nasal MRSA isolates.

Prevalence and Antibiogram of MDR

The prevalence of multidrug resistance among the isolates was 41 (87.2%) and 27 (65.9%) of them were MRSA. A total of 16 types

of antibiogram of the antimicrobial agents were observed among the multidrug resistant *S. aureus* isolates and the most predominant antibiotic combinations among the multi-drug resistant isolates were "E, SXT, TE" (35, 85.4%), CIP, SXT, TE" (31, 75.6%), "CIP, E, SXT, TE" (28, 68.3%) and "CN, CIP, E, SXT, TE" (14, 34.1%) as shown in [Table/Fig-7]. Thirty-three (33/41; 80.5%) and 26 (26/41; 63.4%) of the MDR isolates were haemolysin and biofilm producers respectively.

Combination of antimicrobial agents to which the isolates exhibited multidrug resistant	Number of resistant isolates
AMC, E, TE	1
FOX, CIP, SXT	1
AMC, CIP, SXT, TE	1
FOX, E, SXT, TE	2
FOX, CIP, SXT, TE	2
CIP, E, SXT, TE	4
AMC, FOX, E, SXT, TE	3
FOX, CN, CIP, E, SXT	1
FOX, CIP, E, SXT, TE	2
CN, CIP, E, SXT, TE	4
AMC, CIP, E, SXT, TE	2
AMC, FOX, CIP, E, SXT, TE	6
AMC, CN, FOX, E, SXT, TE	2
AMC, CN, CIP, E, SXT, TE	2
FOX, CN, CIP, E, SXT, TE	1
AMC, FOX, CN, CIP, E, SXT, TE	7
Total	41 (87.2%)

[Table/Fig-7]: The Antibiogram of multi-drug resistant Staphylococcus aureus.
AMC: Amoxicillin/Clavulanic acid; CIP: Ciprofloxacin; CN: Gentamycin; E: Erythromycin; FOX: Cefoxitin; SXT: Co-trimoxazole; TE: Tetracycline

DISCUSSION

Nasal carriage of *S. aureus* has been reported to be a significant risk factor for the development of nosocomial and community-acquired infection in a variety of populations [2,21,22]. The rapid emergence of multidrug resistant strains among this organism especially those that possess *mecA* gene which encodes for methicillin resistance has made the treatment of *S. aureus* infections difficult because of limited treatment options [23,24]. Hence, regular surveillance of the pathogenic and resistance potentials of this organism in this environment where there is little or no control on the use of antibiotics is needed for adoption of strategies for healthy society.

The asymptomatic nasal carriage rate of *S. aureus* has been widely reported in relevant studies to be around 20-30% [2-4]. The nasal carriage rate in this study (11.8%) was however smaller than the general range but it is similar to the recent reports of Omuse G et al., in Kenya (18.3%), Ben-Slama K et al., in Tunisia (13%), and Tigabu A et al., in Ethiopia (13.3%) [10,25,26]. In contrast to our results are the findings from Gabon (29%) [27], Netherlands (25.2%) [28] and Norway (27.6%) [29]. The nasal carriage rate of *S. aureus* in healthy population has been observed to depend on the geographical location, age of the subjects and the screening techniques [21,30]. Thus, differences across the various study centres might be due to these observations. Also, the observed significantly lower prevalence of nasal carriage of *S. aureus* among the female students (p=0.013) might be due to their higher consciousness of hygiene practices than the males which help to reduce the transfer of this organism from other parts of the body to the nostrils.

The investigation into the capacity of the studied *S. aureus* isolates to phenotypically expression haemolysin production as a characteristic virulence trait, revealed 37 (78.7%) isolates to have secreted alpha-haemolytic toxin having lysed the red blood cells. *Staphylococcus aureus* α-haemolysin is known to possess *Hla* gene on its chromosome which encodes for the formation of a complete beta-barrel pore-forming cytotoxins responsible for the development of pores in the cellular membrane that eventually cause cell death [5,31]. An increased *Hla* gene expression in *S. aureus* alpha-toxin can cause a significant disease burden in healthy humans intoxicating multiple human cell types ranging from epithelial cells, endothelial cells, and an array of other haematopoietic-lineage cells [31]. Hence, the high prevalence of these strains among the asymptomatic carriers of nasal *S. aureus* in this study can lead to increase morbidity and mortality as well as healthcare costs due to opportunistic infections and its transmission to the entire society [32]. The difference in the prevalence of the haemolysin producing *S. aureus* among the genders was however not significant. This therefore suggests that no activity of the males predisposes them more to haemolysin production than the females.

A prevalence of 30 (63.8%) isolates was found to phenotypically expressed biofilm production in this study. This result is similar with the findings in Nepal (61%) by Devapriya F et al., and in Pakistan (54.8%) by Wroblewska J et al., from healthy volunteers using the same conventional screening method [33,34]. Similar screening for biofilm formation among nasal *S. aureus* in healthy populations is very scarce in Nigeria and other Africa countries. This observed high prevalence of biofilm producing nasal *S. aureus* in this study is a major public health problem since biofilm has been known to exhibit protective strategies against the host defense mechanism and restrict the penetration of some antimicrobial agents causing recurrent infections that are irresponsive to treatments [6,8,21,33]. The prevalence of biofilm producing *S. aureus* isolates was however significantly higher in the males than the females (p=0.019) and this might be due the relatively lower practices of personal hygiene among the male students than the females. This study also revealed a high proportion of 22 (59.5%) biofilm producers among the isolates that expressed the secretion of alpha-haemolytic toxin and this supports the finding of Anderson MJ et al., which reported that alpha-toxin promote the formation of *S. aureus* mucosal biofilm [35]. It was therefore suggested that strategies to neutralise alpha-toxin in *S. aureus* could be a potent therapeutic target for some of its infections that are difficult to treat [35].

The antimicrobial susceptibility screening showed the isolates to be highly resistant to erythromycin, co-trimoxazole, tetracycline and amoxicillin/clavulanic acid in this study. Many recent studies have reported similar findings among nasal *S.aureus* isolates from healthy adults in Nigeria and other developing countries [23,36-38]. The heavy and uncontrollable usage of these agents in both human and agricultural activities in the community is likely to be responsible for the organism's high resistance to them [38]. A comparison of this present result with the findings of Onanuga A et al., in a similar study in the same environment five years ago, in which nasal *S. aureus* from healthy volunteers exhibited lower resistance to amoxicillin/clavulanic acid (5%), cotrimoxazole (32.5%) and erythromycin (35%), shows the rapid increasing rate (100-350%) at which this organism develops resistance within the same environment [39]. This therefore suggests the increasing antibiotic pressure in the environment which favours the observed increased antibiotic resistance over the short periods of time [40].

The prevalence of MRSA, a pathogenic strain of *S. aureus* that is known to cause difficult to treat infections in both hospital and community settings worldwide, has been widely reported in nasal cavity of healthy volunteers. Our study detected 31 (66%) of the isolates to be cefoxitin resistant, a measure of the phenotypic methicillin resistant (MRSA). Thus, the overall nasal carriage rate of MRSA among all the healthy volunteers was 31 (7.8%). Higher MRSA prevalence among isolates from healthy volunteers has been reported by Owolabi JB et al., in Ota, Nigeria (100%) and El Aila NA et al., in Gaza-Palestine, Israel (82.3%) [38,41]. However, lower prevalence has also been reported among isolates from similar subjects by Shinde RV et al., in India (34.6%), Akerele JO et al., in

Benin-City, Nigeria (22%), Ugwu MC et al., in Agbor, Nigeria (25.7%), and Shibabaw A et al., in Northeast Ethiopia (44.1%) [24,42-44]. The observed differences might be due to the different populations and their exposure to antibiotics and other risk factors.

The cefoxitin-resistant *S. aureus* isolates in this study did not possess *mecA* gene that code for MRSA. This observed absence of *mecA* gene in phenotypic MRSA has been reported by El Aila NA et al., in Gaza-Palestine, Israel, Olayinka BO et al., in Zaria, Nigeria, and Elhassan MM et al., in Sudan [41,45,46]. There have been reports of a recently recognised form of MRSA encoding a divergent *mec* gene called *mecC* MRSA in European countries, which colonise and cause disease in humans and a wide range of other host species [47]. However, the screening for *mecC* gene among the isolates was not within the scope of this study. Hence, the observed resistance in these isolates might be due to the possession of *mecC* which was not investigated in this study. Furthermore, the types of isolates that do not have an altered PBP2a, encoded by the *mecA* or *mecC* gene have also been described as Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) and their resistance has been typically associated with hyperproduction of beta-lactamases or point mutations in PBP genes [48,49]. The clinical presentation and epidemiology of these BORSA infections are similar to those of MRSA and are found in both hospital and community settings [45,48]. Hence, the increasing emergence of *mecC* MRSA and BORSA in the phenotypic MRSA that lack *mecA* gene demands their frequent surveillance in our environments.

The *S. aureus* and MRSA isolates in this study exhibited very high susceptibility to levofloxacin and gentamycin but high resistance was observed to ciprofloxacin. This findings have been reported in many recent studies [38,42,50]. The higher ciprofloxacin resistance (74.5%) among these isolates in this environment where 10% were resistant among similar subjects five years ago is alarming, since ciprofloxacin in the last decade was an alternative for the treatment MRSA infections. This observed increased resistance to ciprofloxacin relative to levofloxacin might be due to its over-prescription for virtually all bacterial infections without recourse to culture and susceptibility testing of the causative agent which leads to the proliferation of its different brands with cheaper prices in the market compared to levofloxacin which is not often prescribed. Thus, this might lead to selective pressure on the organism due to the vast inappropriate and uncontrollable usage in the community. Gentamycin still maintains its high activity on the organism (though with low increase in resistance) possibly because it is an injectable which is dislike by many individuals.

The prevalence of MDR among the isolates was 41 (87.2%) and 27 (65.9%) of them were MRSA. Also, 33 (33/41; 80.5%) and 26 (26/41; 63.4%) of the MDR isolates were haemolysin and biofilm producers respectively suggesting higher virulence capability of the MDR strains. Prominent among the MDR isolates are the E, SXT, TE (85.4%) and CIP, SXT, TE (75.6%) antibiogram which suggest that the isolates have been majorly exposed to erythromycin, co-trimoxazole, tetracycline and ciprofloxacin in the community which therefore indicates that these agents have been grossly misused by the study subjects in this environment thus increasingly limiting the therapeutic options for infections caused by these *S. aureus* strains. This situation calls for urgent strict antibiotic control and stewardship in the community.

LIMITATION
This study has some limitations which are: the phenotypic detection of MRSA isolates were carried out only with cefoxitin disc and the non investigation of *mecC* gene among the phenotypic MRSA isolates that lack *mecA* gene. Thus, the use of both cefoxitin and oxacillin discs in the detection of phenotypic MRSA and the screening for *mecC* gene among *S. aureus* isolates are recommended for future studies since there is an increasing emergence of the *mecC* form of MRSA in the world.

CONCLUSION
This study revealed a high prevalence of MDR isolates with high proportions of haemolysin and biofilm producers among the healthy University students. This therefore calls for prompt implementation of proper personal hygiene strategies through regular daily hand washing in order to prevent the spread of these virulent MDR strains and strict antibiotic stewardship for the control of MDR in the community.

ACKNOWLEDGEMENTS

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