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Antimicrobial Susceptibility Profiles of *Staphylococcus aureus* Strains from Ear, Nose and Wound Swabs of Patients Attending Health Care Facilities, Ebonyi Sate, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VUO and VON designed and wrote the protocol and first draft of the manuscript. Author VUO managed the literature search and analysis of the study. Authors MCK, MOO, NUO and IO revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study assessed age group related level of infection and antimicrobial susceptibility profiles of *Staphylococcus aureus* strains from a clinical specimen.
Study Design: This is a prospective cross-sectional study conducted among in-patients and out-

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patients suspected of having a bacterial infection to determine the age group related level of infection and antimicrobial susceptibility profile of *Staphylococcus aureus* strains.
Place and Duration of Study: The study was conducted between May, 2015 and June, 2016 at the Microbiology Laboratory of Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.
Methodology: A total of 723 clinical specimens including ear, nose and wound swabs were analysed for the presence of *S. aureus.* Standard microbiology laboratory tests were used to isolate and identify the strains. Antibiotic susceptibility testing of the strains was determined by the disc diffusion test (Kirby-Bauer method). Multidrug resistance was defined in this study as resistance to at least three different classes' of antibiotics.
Results: A total of 215 (29.74%) *S. aureus* strains were obtained. The prevalence rate of *S. aureus* concerning types of specimen was 85 (51.52%) for wound swabs, 66 (33.33%) for nose swabs and 64 (17.78%) for ear swabs. There was no significant difference (P = 2.50) observed in the number of nose, ear and wound swabs that yielded *S. aureus.* Age group basis observation of *S. aureus* strains shows that the age group 21 – 30 years yielded the highest number of *S. aureus* strains representing 81 (37.67%). In the tertiary hospital (MMH), almost all the strains showed high sensitivity to ofloxacin 101 (89.38%), followed by gentamycin 95 (84.07%). Antibiotic resistance was highest with cloxacillin 70 (61.95%) followed by cefuroxime 68 (60.18%). Also, in the teaching hospital (FETHA), a high level of sensitivity to ofloxacin 100 (98.04 %) and gentamycin 94 (92.16%) was recorded. Here, cloxacillin 61 (59.80%) followed by ceftazidime 57 (55.88%) showed the highest antibiotic resistance. A total of 76 (35.35%) of *S. aureus* strains were multidrug resistant (MDR). Out of this total number of MDR *S. aureus* strains, 36 (42.35%), 24 (36.36%) and 16 (25.00%) were from wound, nose and ear swabs respectively. Constant antibiotic resistance monitoring is required in order guide physicians in choosing antibiotics against *S. aureus*.

Keywords: Prevalence; clinical strains; antibiotics; resistance.

1. INTRODUCTION

There is an increasing trend in antibiotic resistance now recognized as a worldwide problem. Many antibiotics which were once thought to be the first choice in the treatment of bacterial infections are no longer effective. The reason for the high resistance rates could be traced to underuse or overuse of antibiotics due to poverty and ignorance, self-prescription and easy accessibility of drugs in local pharmacies or open-air markets, inappropriate prescription by physicians due to lack of effective antibiotic policies in our hospitals, among other factors [1].

The alarming rate of resistance in bacteria pathogens raises concern for the effectiveness of antibiotic therapy [1]. Reports of high morbidity and mortality rates due to antibiotic resistance continue to dominate the pages of health journals [1-3].

The emergence of antimicrobial resistance, especially the multidrug resistance further complicates the treatment of bacterial infections. This is because the resistant organisms fail to respond to first-line treatment, hence, leading to high cost of treatment, prolonged illness and high risk of death with its concomitant financial burden and loss in man-hour to families and societies [4].

Increasing rates of infections caused by multidrug resistant (MDR) bacteria as well as the emergence of new antibiotic resistant pathogens in clinical environments have been reported by the World Health Organization (WHO), the USA Centre for Disease Control (CDC), and several research laboratories [5,6].

The spread of multidrug resistant pathogens is a real threat to public health and a major concern for infection control practitioners globally [4]. This spread has paved the way for the re-emergence of previously controlled diseases and a high frequency of opportunistic and chronic infection cases in developing countries like Nigeria [4].

S. aureus, has been noted as a major risk for the development of infection in patients undergoing haemodialysis, continuous ambulatory peritoneal dialysis, surgical patients, and patients with intravascular devices [7]. Researchers from different parts of Nigeria have isolated *S. aureus* from several clinical specimens [8-10]. According to Onewu and Ophori [10], *S. aureus* is important in human infections ranging from minor skin infections to serious, life-threatening infections that may include endocarditis, deep-seated abscesses, septicaemia, catheter-associated bacteraemia, ventilator-associated pneumonia, foodborne-illness and toxic shock syndrome (TSS) among other infections. *S. aureus* ability to

acquire antimicrobial resistance mechanisms and its advantageous pathogenic determinants has contributed to the emergence of infections in both nosocomial and community settings [7]. Antimicrobial resistance among nosocomial pathogens is a significant problem in many countries, and, it is with severe consequences including increased medical costs, morbidity and mortality of patients [7].

This study aims at assessing age group related level of infection and antimicrobial susceptibility profiles of *Staphylococcus aureus* strains from clinical specimens.

2. MATERIALS AND METHODS

2.1 Study Area

Clinical specimen for this study was obtained from two major hospitals [one tertiary hospital (MMH) and one teaching hospital (FETHA)] in Ebonyi State, Nigeria. These hospitals were selected because they receive a large number of patients seeking medical attention and also serve as a referral centre for Ebonyi state and neighbouring states.

2.2 Sample Collection

The clinical specimens used in this study was collected from in-patients and out-patients who were suspected of having a bacterial infection and were requested to undergo medical diagnosis at the microbiology laboratories of these hospitals. A total of 723 specimens comprising of ear swabs, nose swabs and wound swabs were collected from patients aged 11 years and above. No specimen was got from the age group 0 -10 years. Each of all the swabs collected was inoculated into 10 ml cooked meat broth immediately, labelled appropriately, and incubated at 37ºC for 48 hours [11].

2.3 Isolation of Bacteria

After turbidity was observed, a loopful of the organisms were inoculated on Mannitol salt agar plates using the streaking technique. The inoculated Petri dishes were inverted and incubated at 37ºC for 24 hours. Suspected discrete colonies of *S. aureus* that appeared golden yellow on mannitol salt agar were inoculated into the nutrient broth and nutrient agar slants and incubated at 37ºC for 24 hours. The strains were confirmed by adopting a standard microbiological procedure which includes: Gram stain reaction, catalase, DNase and coagulase tests [12,13].

2.4 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of the strains was carried out using the disc diffusion test (Kirby-Bauer method) as recommended by the Clinical and Laboratory Standard Institute (CLSI) [14]. The antibiotic disc used in this study is the product of Abtek Biologicals Ltd, Liverpool, U.K. They include: Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamycin (10 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Ofloxacin (5 µg), and Amoxicillin/clavulanate (30 µg). A sterile Pasteur pipette was used to drop 0.2 ml of the standardized inoculum equivalent to 0.5 McFarland turbidity standards (1.0×10^8 cfu/ml) on the surface of the dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. The agar was left for about 10 minutes for the inoculum to dry and after that, antibiotic discs were aseptically placed on the surface of the inoculated Mueller-Hinton agar plate using heat-sterilized forceps. They were incubated at 37ºC for 18-24 hours. The diameter of zones of inhibition for each antibiotic was measured in millimetre and compared with values provided by the CLSI [14].

2.5 Statistical Analysis

Statistical analysis was carried using the statistical package for social sciences (SPSS) version 15. $P < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

In this study, a total of 215 (29.74 %) *S. aureus* strains were recovered from wound, nose and ear swabs. The prevalence rate of *S. aureus* concerning type the of specimen in this study were 85 (51.52%) for wound swabs, 66 (33.33%) for nose swabs and 64 (17.78%) for ear swabs (Table 1). However, there was no significant difference ($P = 2.50$) observed in the number of nose, ear and wound swabs that yielded *S. aureus.*

Age group basis observation of the *S. aureus* strains (Fig. 1) shows that the age group 21 – 30 years yielded the highest number of *S. aureus* strains representing 81 (37.67%). Within this age group, males 46 (44.23%) accounted for the highest *S. aureus* infection than females 35 (31.53%).

Table 1. Distribution of *S. aureus* strains from clinical specimens

Clinical specimen screened	Total clinical specimen	Number of bacterial strains obtained	Percentage (%) bacterial strains
Wound swab	165	85	51.52
Nose swab	198	66	33.33
Ear swab	360	64	17.78
Total	723	215	29.74

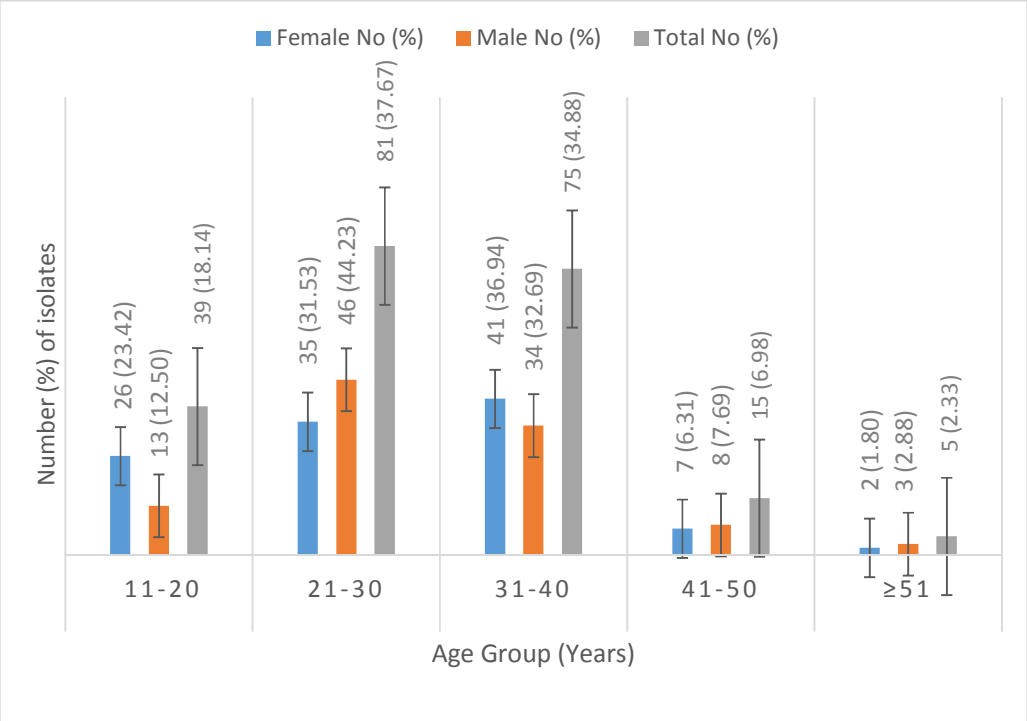

Fig. 1. Mean age group and gender distribution of *S. aureus* strain from wound, nose and ear swabs.

Key: % = Percentage in bracket

The susceptibility profile of *S. aureus* strains (n=113) from wound, nose and ear swabs from MMH against eight different antimicrobial agents are presented in Table 2. The microorganisms were classified as being sensitive, intermediate and resistant to antibiotics depending on the size of inhibition zone diameters when compared to the standard provided by CLSI [14]. Almost all the *S. aureus* strains showed high sensitivity to ofloxacin 101 (89.38%), followed by gentamycin 95 (84.07%) and ceftriaxone 63 (55.75%). The rate of antibiotic resistance was highest with cloxacillin 70 (61.95%) followed by cefuroxime 68 (60.18%) and ceftazidime 65 (57.52%).

The antibiotic susceptibility profile of *S. aureus* strains (n=102) from wound, nose and ear swabs from FETHA are presented in Table 3. Ofloxacin 100 (98.04%) was found to be the most effective drug against the *S. aureus* strains, followed by gentamycin 94 (92.16%). Resistance of the strains to cloxacillin was 61 (59.80%), ceftazidime 57 (55.88%) and cefuroxime 56 (54.90%).

Multidrug resistance was defined in this study as resistance to at least three different classes' of antibiotics (≥ 3 different classes of antibiotics) [15]. Out of 215 *S. aureus* strains from the two hospitals, a total of 76 (35.35%) were multidrug resistant (Table 4). Multidrug resistance among the strains did not show much variation in the two hospitals. However, *S. aureus* strains from MMH had 37.17% while those from FETHA had 33.33 %. Out of the total number of MDR *S. aureus* strains, 36 (42.35%), 24 (36.36%) and 16 (25.00%) were from wound, nose and ear swabs respectively.

Table 2. Antibiotic Susceptibility Profile of *S. aureus* Strains from MMH.

Antibiotic	Disc content	Number of sensitive strains (%)	Number of intermediate strains (%)	Number of resistant strains (%)
Ceftazidime	30 µg	48 (42.48)	0 (0.00)	65 (57.52)
Cefuroxime	30 µg	21 (18.58)	24 (21.24)	68 (60.18)
Gentamycin	10 µg	95 (84.07)	10 (8.85)	8 (7.08)
Ceftriaxone	30 µg	63 (55.75)	22 (19.47)	28 (24.78)
Erythromycin	5 µg	49 (43.36)	8 (7.08)	56 (49.56)
Cloxacillin	5 µg	35 (30.97)	8 (7.08)	70 (61.95)
Ofloxacin	5 µg	101 (89.38)	6 (5.31)	6 (5.31)
Amoxicillin/ Clavulanate	30 µg	38 (33.63)	7 (6.19)	68 (60.18)

Key: % = Percentage in bracket; MMH = Code of tertiary hospital used

Table 3. Antibiotic susceptibility profile of *S. aureus* strains from FETHA.

Antibiotic	Disc content	Number of sensitive strains (%)	Number of intermediate strains (%)	Number of resistant strains (%)
Ceftazidime	30 µg	39 (38.24)	6 (5.88)	57 (55.88)
Cefuroxime	30 µg	46 (45.10)	0 (0.00)	56 (54.90)
Gentamycin	10 µg	94 (92.16)	2 (1.96)	6 (5.88)
Ceftriaxone	30 µg	59 (57.84)	1 (0.98)	42 (41.18)
Erythromycin	5 µg	52 (50.98)	2 (1.96)	48 (47.06)
Cloxacillin	5 µg	40 (39.22)	1 (0.98)	61 (59.80)
Ofloxacin	5 µg	100 (98.04)	0 (0.00)	2 (1.96)
Amoxicillin/ Clavulanate	30 µg	60 (58.82)	1 (0.98)	41 (40.20)

Key: % = Percentage in bracket; FETHA = Code of teaching hospital used

Table 4. Prevalence of MDR strains of *S. aureus* from Wound, Nose and Ear Swabs.

Hospital	Total no. of strains	Total no. (%) of MDR strains	No. of strains from			Number (%) of MDR strains from		
			Wound	Nose	Ear	Wound	Nose	Ear
MMH	113	42 (37.17)	53	32	28	20 (37.74)	14 (43.75)	8 (28.57)
FETHA	102	34 (33.33)	32	34	36	16 (50.00)	10 (29.41)	8 (22.22)
Total	215	76 (35.35)	85	66	64	36 (42.35)	24 (36.36)	16 (25.00)

Key: MMH and FETHA = Code of hospitals used, MDR = Multidrug resistance

The overall prevalence of *S. aureus* obtained in this study is similar to the reports of Ikeagwu et al. [16] who documented overall yield of 29.2% from clinical specimens whereas it was higher than the reports of Gizachew et al. [17] who recorded 7.2% of *S. aureus* strains from wound swabs. In a similar study by Zinnat et al. [18], 271(34.9%) *S. aureus* was isolated from 776 specimens analyzed. Nwoire *et al*. [19] reported a higher prevalence of *Staphylococcus aureus* 87/144 (60.4%) from wound swabs, ear swabs and urine specimens from Federal Teaching Hospital Abakaliki. This shows a comparative reduction in *S. aureus* infection in the area under study. Onewu and Ophori [10] also reported a comparatively higher incidence (37.2%) of *S. aureus* from a clinical specimen in Benin, Nigeria.

This result of the specimen specific prevalence of *S. aureus* from wound in this study is higher than the reports of Daniyan and Sani [17] who reported the prevalence rate of 37.5% for wound swabs whereas; they reported similar result, 20.0% for ear swabs. The prevalence rate of *S. aureus* from nasal swabs in this study agrees with the report of Onanuga and Temedie [20] who recorded a prevalence rate of 33.3% in nares specimens from students in Bayelsa State, Nigeria. Nwankwo and Nasiru [21] recorded a lower prevalence rate of 30.7% from wound swabs; however, their prevalence rate for ear swab (21.3%) was slightly higher than that of the present study. Saana et al. [11] recorded a lower (35.3%) prevalence rate of *S. aureus* in wound swabs and a higher (37.3%) prevalence rate in nasal swabs compared to the present study. Earlier, in Federal Teaching Hospital Abakaliki, Nwoire et al. [19] reported a lower prevalence of *S. aureus* (31.6%) in wounds while the prevalence of *S. aureus* in ear swab (17.9%) was similar to that of the present study. Likewise, Mama et al. [22] isolated 47 (32.4%) *S. aureus* from infected wounds. Zinnat et al. [18] found a

lower prevalence rate of *S. aureus* from wound and ear swabs in their study. In a similar study by Eke et al. [23], nose and ear swabs yielded 66.70% and 44.40% *S. aureus* from apparently healthy students in Edo State, Nigeria. This result is quite higher than the present finding. However, there was no significant difference (P = 2.50) observed in the number of wound, nose and ear swabs that yielded *S. aureus* in this study.

According to Prescott et al. [24], nostrils serve as a habitat for *S. aureus*; this may be responsible for the level of *S. aureus* strains from nasal swabs in this study. Their presence in nostrils could serve as a source of infection [24]. *S. aureus* from the anterior nares have been reported to be linked to most community and hospital-associated infections, and a study also showed that nasal carriage is a source of *S. aureus* bacteremia [20]. According to Zinnat et al. [18], the nose is the main site of colonization of *S. aureus,* and approximately 30% of people are colonized at any one time.

S. aureus is one of the most common causes of surgical-wound infections [18]. The isolation of *S. aureus* from wound swabs could be attributed to the level of contamination arising from the hands of healthcare workers or contamination arising from the habit of some patients who treat their wounds before seeking for appropriate medical attention [25,26]. Furthermore, some patients make use of quacks in medicine stores before seeking for appropriate medical attention. According to Daniyan and Sani [25], the significance of *S. aureus* strains from ear swabs could be due to the colonization of the organism as a result of infection. The best way to prevent the spread of infections in hospitals is to improve upon hygiene and to screen healthcare workers for the presence of these organisms.

Age group basis observation of the total *S. aureus* strains corroborated an earlier report of Saana et al. [11] who found the age group 20 – 29 as having the highest *S. aureus* infection rate. In contrast, Nwankwo and Nasiru [21] and Gizachew et al. [17] isolated most of their *S. aureus* from the age group 0 – 10 years and < 5 years which accounted for 47.3% and 8.5% *S. aureus* respectively. According to Agwu et al. [27], the variation in the number of infection within age groups could be due to the level of contamination arising from the habit of patients, low personal hygiene and poor education in many African countries. The age group 21 – 30 is the most active age group that comes in contact with various objects that may serve as a source of infection with *S. aureus.*

In this study, cloxacillin had the highest level of resistance to *S. aureus* in the two hospitals representing 70 (61.95%) and 61 (59.80%) for MMH and FETHA respectively. The high level of resistance to cloxacillin in this study confirms the earlier report of Nwankwo and Nasiru [21]. The resistance of *S. aureus* to erythromycin (47.06% in FETHA and 49.56% in MMH) in the present study is higher than the 38.5% reported by Rajeevan *et al.* [28]. Whereas Rajeevan *et al.* [28] found 43.6% resistance to gentamycin, this study recorded a comparatively low resistance of 5.88 % in FETHA and 7.08% in MMH. Nmema [1] reported 100% resistance to amoxicillin/clavulanate as against 68 (60.18%) and 41 (40.20%) resistance recorded in MMH and FETHA, respectively. In comparison to the present study, Onewu and Ophori [10] registered low resistance to amoxicillin/clavulanate (28.6%), similar resistance to ceftazidime (54.3%), high resistance to cloxacillin (79.4%), ofloxacin (42.4%), gentamycin (52.9%) and erythromycin (68.0%) in Benin City, Nigeria.

The highest number of multidrug-resistant *S. aureus* strains was observed in wound swab followed by nose swabs. The level of multidrug-resistant *S. aureus* in this study is slightly higher than the finding of Saana et al. [11] who reported an overall multidrug resistance of 32.1% in Ghana. Earlier, Newman et al. [29] found a multidrug resistance rate of 42.5% among *S. aureus* strains in Ghana. Khan et al. [30] found that 26 out of 38 strains of *S. aureus* from clinical specimens were multidrug resistant. In Bayelsa State, Nigeria, Onanuga and Temedie [20] registered a higher level (52.5%) of multidrug-resistant *S. aureus* strains from nasal swabs. In a study carried out by Adegoke and Komolafe [31] in Ile-Ife, Nigeria using various clinical specimens, they reported that 21 (19.6%) *S. aureus* strains were resistant to 7 antibiotics together and another 19.6% were resistant to 5 antibiotics. Previously, Onanuga et al. [32] reported 43 (71.7%) MDR *S. aureus* among women in Zaria, Nigeria.

S. aureus has been reported as being among the primary bacterial species showing a high prevalence rate of multidrug resistance patterns because of its intrinsic ability to develop resistance to many antimicrobials [33]. Multidrug resistance in *S. aureus* is a serious public health

issue because of the ubiquity of the organism in hospital infections. Multidrug resistance among *S. aureus* strains have been attributed to self-medication [34], which prevents early reporting of patients to hospitals at the onset of disease symptoms, except where complications had occurred [35], unnecessary prescription and substandard antibiotics [11,36,37].

4. CONCLUSION

In this study, 215 (29.74%) strains of *S. aureus* were obtained from wound swabs, nose swabs and ear swabs. Age group basis observation of *S. aureus* strains showed that the age group 21 – 30 years yielded the highest number of strains. Bacteria resistance to commonly used antibiotics is now considered a global emergency. In the two hospitals studied, the strains showed highest sensitivity to ofloxacin. Antibiotic resistance was highest with cloxacillin. Exactly, 35.35% *S. aureus* strains were multidrug resistant. Constant antibiotic resistance monitoring is required in order guide physicians in choosing antibiotics against *S. aureus*.

CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this case report.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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