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PREVALENCE OF MULTIDRUG-RESISTANT AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE AMONG ASYMPTOMATIC INDIVIDUALS IN A TERTIARY INSTITUTION

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ABSTRACT: ***Introduction:*** *Nasal carriage of Staphylococcus aureus plays a major role in its spread to other parts of the body or to other individuals, which usually results in hospital and community-associated diseases. This study was aimed at investigating the prevalence of multidrug-resistant and methicillin-resistant strains of Staphylococcus aureus in asymptomatic individuals.*

Methods: *This cross-sectional study investigated the prevalence of nasal carriage of multidrug-resistant and methicillin-resistant Staphylococcus aureus among asymptomatic university students. In this study, samples were collected from the nasal cavity of 250 asymptomatic individuals after seeking and obtaining their individual verbal and signed written consent. The swabs were streaked on selective agar, and bacterial colonies were subjected to biochemical tests. Antibiograms of the isolates were determined using multi-disc antibiotics and agar dilution methods, and the Multiple Antibiotic Resistance index (MAR_{index}) of each isolate was calculated.*

Results: *From the study, 152(60.8%) out of the 250 samples collected were identified as Staphylococcus aureus. Seventy-two (72, 47.37%) isolates were identified as multidrug-resistant S. aureus. Eighty-five (55.9%) isolates were resistant to cloxacillin, while 94 (61.8%) and 100 (65.8%) isolates demonstrated resistance to oxacillin at concentrations of 2 µg/mL and 4 µg/mL, respectively, indicating a high prevalence of phenotypically methicillin-resistant Staphylococcus aureus. The MAR_{index} showed that 51.3% of the isolates had $MAR_{index} \leq 0.2$, while 48.7% of the isolates had $MAR_{index} > 0.2$.*

Conclusion: *The high prevalence of multidrug-resistant and phenotypically methicillin-resistant Staphylococcus aureus among asymptomatic individuals highlights the potential role of healthy carriers as reservoirs of antimicrobial-resistant bacteria within the community. Enhanced surveillance and antimicrobial stewardship measures are recommended.*

KEYWORDS: Infection, Multidrug resistance, Multiple Antibiotic Resistance index, Phenotypic methicillin resistance, *Staphylococcus aureus*, Susceptibility.

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INTRODUCTION

The human body provides various ecological niches for the bacterium *Staphylococcus aureus*. The skin and nose, especially the latter, are the major sites of carriage or colonization in healthy individuals (Hamdan-Partida et al., 2010). While about 20-50% of healthy adults are asymptomatic, persistent or transient carriers of the organism (Yan et al., 2015; Sakr et al., 2018; Bush, 2019), its ability to colonize is usually dependent on various factors which include bacterial surface components such as wall teichoic acids or clumping factor B, presence of suitable host ligands and the composition and interaction of the nasal microbiota (Hanssen et al., 2017). Furthermore, while nasal carriage of *Staphylococcus aureus* plays a major role in its spread to other parts of the body or to other individuals which usually results in hospital and community associated diseases (Sharma et al., 2014), respiratory infections aid in its dissemination into the air in the form of aerosols even as hands play important roles in its transfer from nose to other surfaces and from other surfaces (fomites) into the nose (Sakr et al., 2018). Old people, newborns, breastfeeding mothers, and persons with chronic illnesses are at a higher risk of contracting diseases caused by *S. aureus* (Bush, 2019). The resulting diseases, ranging from minor to life-threatening cases, may include skin infections, meningitis, pneumonia, endocarditis, bacteremia, osteomyelitis, and septicemia (Ansari, 2016).

The major problem associated with *Staphylococcus aureus* is the treatment of its resulting ailments. In 1944, treatment of Staphylococci was easily achieved with beta-lactam antibiotics such as penicillin G. However, the production of beta-lactamase by strains of *S. aureus* led to a surge in resistance to beta-lactam drugs and rendered this form of treatment ineffective. Over time, the development of beta-lactamase-inhibiting drugs provided some sort of relief, but this was also short-lived due to the emergence of strains that showed resistance to this new form of treatment (Abimana et al., 2019). Finally, a new derivative of penicillin, isoxazolyl penicillin, including the likes of methicillin, oxacillin, nafcillin, and cloxacillin, was developed. Resistance to the prototype of this group (methicillin) was, however, observed in some *S. aureus* strains barely a year after the drug was introduced. These strains, known as methicillin-resistant *Staphylococcus aureus* (MRSA), are characterized by resistance to most β-lactam antibiotics (Harkins et al., 2017), through the acquisition of the *mecA* gene encoding the altered penicillin-binding protein PBP2a. Today, there is a high prevalence rate of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage even in healthy individuals. While Hassoun et al. (2017) reported a worldwide prevalence level of 60%, Siddiqui & Koirala (2019) indicated that 60% of the *S. aureus* in Europe were methicillin-resistant. In the US, over 150,000 individuals were reportedly infected with MRSA, leading to about 19,000 deaths (Garoy et al., 2019). In Africa, prevalence rates ranging between 42 and 80% were recorded due to the lack of efficient surveillance systems, as this information may be regarded as inconsistent and variable (Wangai et al., 2019). Due to its significant public health burden and increasing antimicrobial resistance, methicillin-resistant *Staphylococcus aureus* remains an important pathogen of global concern requiring continuous surveillance and effective infection control measures; doctors have been forced to turn to alternative drugs or a combination of drugs for the treatment of its infections, even though this may further increase the burden of drug resistance. Asymptomatic nasal carriers serve as important reservoirs for the transmission of *Staphylococcus aureus*, including antimicrobial-resistant strains; assessing nasal carriage patterns is essential for understanding potential community transmission dynamics. Therefore, this study

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aimed to determine the prevalence of nasal carriage of multidrug-resistant and phenotypically methicillin-resistant *Staphylococcus aureus* among asymptomatic university students in Ogun State, Nigeria.

MATERIALS AND METHODS

Study Design

This institution-based cross-sectional study investigated the prevalence of nasal carriage of multidrug-resistant and methicillin-resistant *Staphylococcus aureus* among asymptomatic undergraduate students at Babcock University, Ogun State, Nigeria.

Sample collection

After providing written informed consent approved by the Babcock University Research and Ethics Committee (BUREC), samples were collected from 250 asymptomatic individuals in Babcock University, Ogun State, Nigeria. Individuals who had been treated with any antibiotic in the last 4 weeks were excluded. Nasal specimens were collected by gently rotating sterile swab sticks moistened with sterile peptone water within both anterior nares of 250 apparently healthy undergraduate students. The swabs were tightly sealed and immediately transported to the laboratory for sample analysis. The collected nasal swab sticks were streaked on mannitol salt agar (OXOID CM0085 – Oxoid Ltd. Wade Road, Basingstoke, Hants, RG24 8PW, UK (MSA)) and nutrient agar (LAB M -620 Lesher Place, Lansing, MI 48912 USA) before incubating at 37°C for 24 – 48 h (Forbes et al., 2007). Presumptive *Staphylococcus aureus* isolates were identified based on colony morphology, Gram-staining characteristics, catalase production, tube coagulase activity, and DNase production using standard microbiological procedures according to standard protocols (Holt et al*.,* 1994; Cheesbrough, 2002; 2009).

Antibiogram Study of Isolates from the Nasal Swabs Using Multi-Disc Antibiotics

The standard disc diffusion test was performed according to the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2019). Four separate colonies of each of the isolates from nutrient agar were homogenized with an inoculating loop in 2 mL sterile normal saline and vortexed to obtain uniform bacterial suspensions. Each strain's suspension was adjusted to 0.5 McFarland Standards by adding more organisms when the suspensions were too light or by diluting further with sterile saline when the suspensions were too heavy to give a resultant concentration of 1×10^7 cfu/ml. The antibacterial activity was determined according to the modified Kirby–Bauer disk diffusion technique (Bauer et al*.,* 1966). Mueller-Hinton agar (MHA) (OXOID CM0085 – Oxoid Ltd. Wade Road, Basingstoke, Hants, RG24 8PW, UK) plates were swabbed with the resultant adjusted culture of each of the test isolates. With sterile forceps, commercial antibiotic discs (Abtek) containing Augmentin (30 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Cloxacillin (5 µg), Erythromycin (5 µg), Cotrimoxazole (25 µg), Chloramphenicol (10 µg), Gentamicin (10 µg), Ofloxacin (5 µg), Streptomycin (10 µg), Tetracycline (10 µg), and Cefoxitin (30 µg) were aseptically placed on the inoculated agar and

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incubated at 37°C for 24 h. After 24 h of incubation, the plates were examined for inhibition zones. The diameter of the inhibition zones produced by each antibiotic disk was measured to the nearest millimeter, recorded, and interpreted using the Clinical and Laboratory Standards Institute Zone Diameter Interpretive Standards, and Methicillin resistance was determined using cefoxitin (30 µg) disk diffusion according to CLSI guidelines. Isolates with inhibition zone diameters ≤21 mm were classified as methicillin-resistant *Staphylococcus aureus* (MRSA), while isolates with diameters ≥22 mm were considered methicillin-susceptible *Staphylococcus aureus* (MSSA).

Multiple Antibiotic Resistance Index (MAR Index) of *Staphylococcus aureus* Isolates

The Multiple Antibiotic Resistance index (MAR_{index}) of each isolate was calculated as the number of antibiotics to which the isolate was resistant divided by the total number of antibiotics to which it was exposed, as indicated by Krumperman (1983). According to Adeleke and Omafuvbe (2011), isolates with a MAR index ≤0.2 are considered to originate from environments with low antibiotic-use pressure, whereas isolates with a MAR index >0.2 are considered to originate from high-risk environments with frequent antibiotic exposure.

Susceptibility Test of Samples to Oxacillin

The susceptibility of the isolates was further determined by the agar dilution method according to the guidelines of CLSI (CLSI, 2019). The isolates were subjected to susceptibility testing with 2 µg/ml and 4 µg/ml concentrations of oxacillin prepared by dissolving 0.4 mg and 0.8 mg of oxacillin into two different conical flasks, each containing 200 mL of sterilized Mueller-Hinton agar maintained at a temperature of 50°C. The overnight broth culture of each isolate was adjusted to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, before inoculation and incubation at 37°C for 24 h.

Data Analysis

Data were analyzed using IBM SPSS Statistics version 2020. Frequencies and percentages were used to summarize prevalence, antimicrobial resistance patterns, and MAR index distribution.

RESULTS

A total of 152 (60.8%) out of the 250 samples collected from the nasal cavity of asymptomatic individuals in the University were identified as *Staphylococcus aureus*. The isolates were identified based on their morphology and reactions to various biochemical tests. Of the 152 *Staphylococcus aureus* isolates recovered from the nasal cavities of asymptomatic individuals, 72 (47.4%) were classified as multidrug-resistant (MDR) isolates. Among the MDR isolates, 43 (59.7%) were obtained from males and 29 (40.3%) from females (Table 1).

Table 1: Distribution of *S. aureus* and MRSA in the nasal cavity of asymptomatic individuals

Sex	No of isolates (n=152)	N%	MRSA (n=72)	N%
M	87	57.24	43	59.72
F	65	42.76	29	40.28

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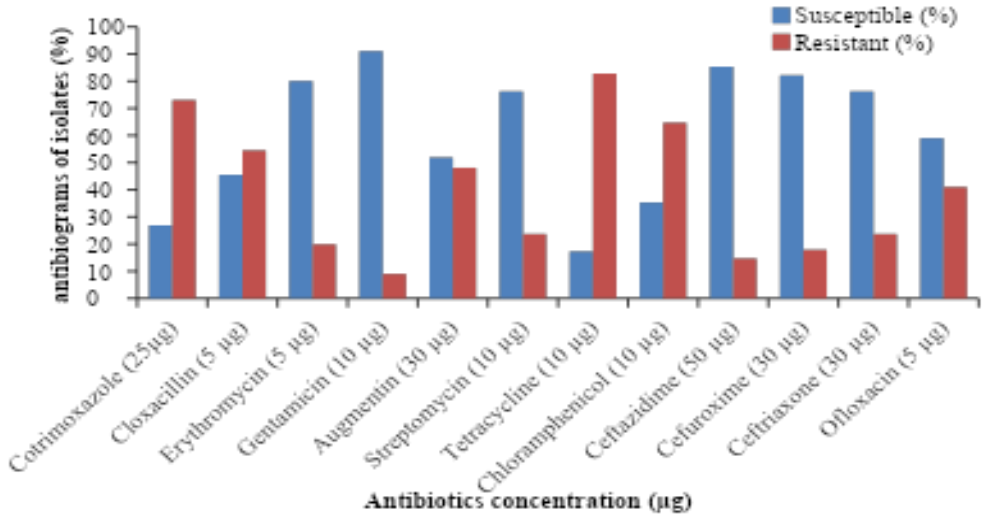
Among the 152 *S. aureus* isolates, 85 (55.9%) were resistant to cloxacillin, whereas gentamicin demonstrated the highest activity, with 91.0% of isolates remaining susceptible. High susceptibility rates were observed for ceftazidime [133 (87.5%)], cefuroxime [128 (84.2%)], ceftriaxone [119 (78.3%)], and streptomycin [119 (78.3%)]. Susceptibility to the remaining antibiotics decreased in the following order: erythromycin [125 (82.2%)], ofloxacin [92 (60.5%)], augmentin [81 (53.3%)], cloxacillin [71 (46.7%)], chloramphenicol [55 (36.2%)], cotrimoxazole [42 (27.6%)], and tetracycline [27 (17.8%)] (Figure 1).

Figure 1: Susceptibility pattern of *Staphylococcus aureus* isolates from the nasal cavity of asymptomatic individuals



The MAR index analysis showed that 78 (51.3%) isolates had MAR index values ≤0.2, whereas 74 (48.7%) had MAR index values >0.2 (Table 2). Isolates with MAR index values >0.2 suggest exposure to environments where antibiotics are frequently used, while values ≤0.2 indicate lower antibiotic selection pressure.

Table 2: Multidrug Antibiotic Resistance Pattern of the Isolates

S/N	Number of Isolates	MAR_{index}	Percentage distribution %	No. of antibiotics
1	24	0	15.8	0
2	54	0.125	35.5	1
3	12	0.25	7.9	2
4	22	0.375	14.5	3
5	14	0.5	9.2	4
6	15	0.625	9.8	5
7	10	0.75	6.6	6
8	1	1	0.7	8

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To further assess β-lactam susceptibility, the isolates were subjected to oxacillin susceptibility testing at concentrations of 2 µg/mL and 4 µg/mL. At 2 µg/mL, 94 (61.8%) isolates were resistant, and 58 (38.2%) were susceptible. At 4 µg/mL, 100 (65.8%) isolates were resistant, and 52 (34.2%) were susceptible (Figure 2).

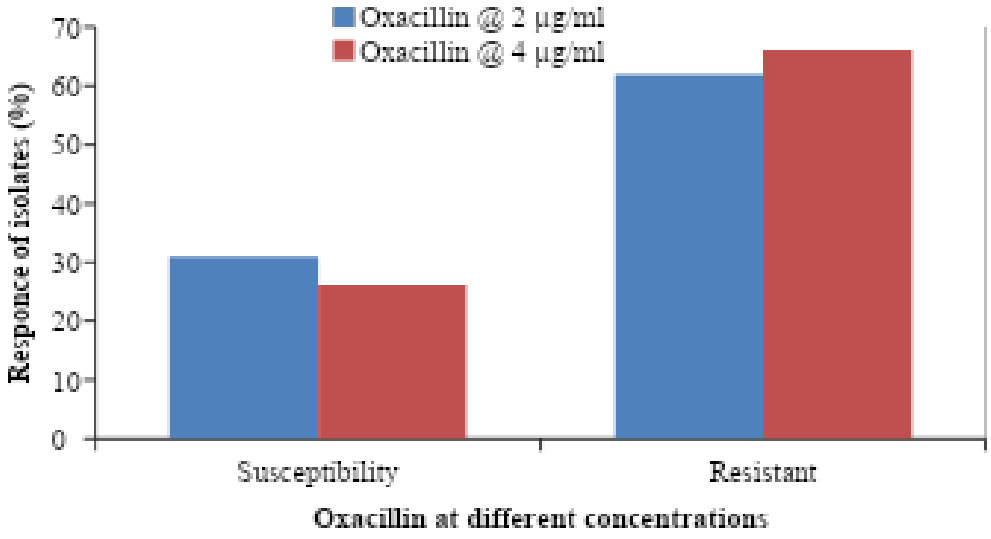

Figure 2: Susceptibility of the isolated *S. aureus* to different concentrations of oxacillin

DISCUSSION

The prevalence of *Staphylococcus aureus* in the nasal cavity of asymptomatic individuals observed in this study was in agreement with reports of Emaneini et al. (2017), Joachim et al. (2018), Oudri (2018), & Abimana et al. (2019) that indicated a prevalence of *Staphylococcus aureus* ranging between 20 and 50%. However, the report is in contrast to the works of Dinic et al. (2013), Sharma et al. (2014), Ansari et al. (2016), and Onanuga et al. (2019), where low prevalence levels ranging between 8 and 15% were recorded. On the other hand, Ugwu et al. (2016) reported a higher prevalence level of 72.7%, which was also in contrast with the findings of this study. While gender was identified as a factor affecting the level of carriage by Sollid et al. (2014), this study, in agreement with the works of Onanuga & Temedie (2011), Ansari et al. (2016) and Ahmadi et al. (2019), indicated that males had a higher carriage level compared to women.

Furthermore, having a higher percentage of the resistant isolates being from individuals not frequently using antibiotics and all the MRSA from individuals not on any antibiotics for quite some time are strong indications pointing to the potential health challenges that may possibly arise from the spread of multi-drug resistant and MRSA from asymptomatic carriers and individuals. The presence of the opportunistic pathogen in the nasal cavity of healthy individuals signified an increased risk of developing nosocomial *S. aureus* infections like skin and soft tissue infections and other diseases in non-hospitalized individuals (Sakr et al., 2018). Wertheim et al. (2005) reported that 80% of invasive nosocomial infections caused by the bacterium are usually of

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endogenous origin in nasal carriers, validating the potential of the commensal bacterium to cause opportunistic diseases. Nasal carriage of *S. aureus* represents a recognized reservoir for transmission and may contribute to the spread of the organism through close human contact and contaminated environments (Bush, 2019).

Additionally, treating the resulting ailments may be difficult, as seventy-two (72; 47.37%) isolates were identified as multidrug-resistant *S. aureus,* with more isolates being highly resistant to cotrimoxazole, cloxacillin, Augmentin, tetracycline, and chloramphenicol. In previous studies (Ansari et al., 2016; Ike et al., 2016; Onanuga et al.,2019 & Wangai et al., 2019), all isolates were highly resistant to the test antibiotics used. Sharma et al. (2014), Oudri (2018), and Joachim et al. (2018), however, recorded a high level of resistance to Gentamicin, which is in contrast with the obtained value in this study. The MAR_{index} ≥0.2, equal to 48.7%, showed that the isolates were multidrug-resistant strains, and this result is contrary to that of Ungureanu et al. (2017), who reported that >50% of the *S. aureus* isolates exhibited drug resistance to more than three drugs. The 48.7% of the MAR_{index} ≥0.2 reported in this study indicated heavy use and misuse of these antibiotics by the individuals sampled, probably due to the availability, affordability, and previous efficacy of the drugs prior to the commencement of the study.

A total of 85 (55.9%) isolates were resistant to cloxacillin, while 94 (61.8%) and 100 (65.8%) isolates demonstrated resistance to oxacillin at concentrations of 2 µg/mL and 4 µg/mL, respectively. These findings suggest a high prevalence of phenotypically methicillin-resistant *Staphylococcus aureus* (MRSA) among the isolates studied. This level of resistance is disturbing, and this is in line with the works of Onanuga & Temedie (2011), Sharma et al. (2014), Oudri (2018), Abimana et al. (2019), Onanuga et al. (2019), and Vaez & Ghalehnoo (2019), in which the prevalence levels ranged between 42 and 66% among primary and tertiary school students. This is, however, in contrast with the works of Chen (2012), Dinic et al. (2013), and Chatterjee et al. (2009) that recorded a lower percentage ranging between 2 and 10% of MRSA from community samples in India, Taiwan, and Serbia. The percentage of MRSA level in this study signifies that there could be an increase in the risk of developing hospital associated-MRSA infections (HA-MRSA) and complications due to difficulty in its treatment as indicated by Davis et al. (2004) and Clarke (2014) who reported that over 25% of patients with MRSA nasal carriage before hospitalization developed infections difficult to treat compared to patients without MRSA nasal carriage. However, this is in contrast with the works of Prates et al. (2010) and Ugwu et al. (2016), indicating that community-acquired methicillin-resistant *S. aureus* (CA-MRSA) can be treated with other classes of drugs apart from beta-lactam drugs because they usually do not exhibit resistance to these drugs. This study, however, indicated that MRSA infections might be treatable, as high percentages of the isolates were very susceptible to Cefuroxime, Ceftazidime, and Ceftriaxone use in this study.

LIMITATION

This study was limited by its cross-sectional design, which precludes causal inference; its single-institution sampling that may limit generalizability; and the absence of molecular confirmation

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(e.g., *mecA* or *mecC* gene detection) to verify phenotypic MRSA identification. In addition, risk factors associated with nasal carriage and antimicrobial resistance were not investigated.

CONCLUSION

This study demonstrated a high prevalence of nasal carriage of *Staphylococcus aureus*, including multidrug-resistant and phenotypically methicillin-resistant isolates, among asymptomatic university students. The findings highlight the potential role of healthy individuals as reservoirs of antimicrobial-resistant bacteria within the community. Strengthened antimicrobial stewardship, surveillance programs, and molecular characterization of resistant isolates are recommended to better understand the epidemiology and public health significance of MRSA carriage in this population.

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Not applicable

Competing interests

The authors declare that they have no competing interests.

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