

## Original Research Article

# ***In vitro* antibiotic susceptibility profiles and multiple antibiotic resistance indices of *Staphylococcus aureus* isolates from healthy personnel and students at the University of Nigeria, Nsukka**

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## **Abstract**

**Purpose:** The susceptibility studies of *Staphylococcus aureus* (*S. aureus*) isolated from healthy personnel and students at the University of Nigeria, Nsukka, were investigated.

**Methods:** *S. aureus* was isolated by collecting swab specimens from the skin, hand, and nose of healthy personnel and students from various Departments and Faculties within the University of Nigeria, Nsukka. The colonies were isolated and identified using standard biochemical tests. Antibiotic susceptibility was tested using Kirby-Bauer disk diffusion method with standard Oxoid antibiotic disks and their inhibition zone diameters (IZDs) were measured. The IZD<sub>50</sub> and IZD<sub>95</sub> were calculated statistically. Minimum Inhibitory Concentration (MIC) of selected antibiotics against isolates was determined by agar dilution method.

**Results:** A total of 84 *Staphylococcal* isolates were obtained. Fifty isolates (59.5%) were *S. aureus*, and the remaining 40.5 % were other *Staphylococcal* species. The percentage of *S. aureus* isolated from skin, hand, and nose samples was 54, 32, and 14 %, respectively. The IZD<sub>50</sub> and IZD<sub>95</sub> ranged from 0.00 - 29.25 and 14.50 - 37.60 mm, respectively. The multiple antibiotic resistance (MAR) index ranged from 0.0 - 0.7, with 90 % of isolates exhibiting MAR  $\geq$  0.2. The MIC values of selected antibiotics (amoxicillin, ciprofloxacin and ceftriaxone) against *S. aureus* isolates ranged from 0.780 - 50.000, 0.195 - 100.000, and 1.563 - 25.000  $\mu$ g/mL, respectively.

**Conclusion:** Isolates collected from skin, hands and nose of healthy personnel at the University of Nigeria, Enugu, harbour pathogenic *S. aureus* in substantial amounts, which are multidrug resistant to some available antibiotics. The results underscore the importance of maintaining continuous surveillance, adhering to proper hygiene, and utilizing antibiotics rationally.

**Keywords:** *Staphylococcus aureus*, Multidrug resistance, Antibiotics, Resistance

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## INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is a resident normal microflora that colonizes the skin and mucous membranes of the human body. However, some strains of *Staphylococcus* are pathogenic, and they cause a variety of infections in humans, including acne, suppuration, abscess formation, and other pyogenic infections [1,2]. The organism is a ubiquitous microbe that is found on clothing, bed linens and on other fomites in the environment, and they have been implicated in several food-borne diseases because it is toxigenic in nature [3]. As a ubiquitous bacterium constantly found in the nasal tract, respiratory, and gastrointestinal tract (GIT); *S. aureus* is responsible for a handful of nosocomial infections due to the ease with which they can be passed on from one person to another especially from patients to physicians, nurses, and laboratory scientists during treatment, diagnosis or care and vice versa [4,5]. Notably, some hospital personnel harbour resistant *S. aureus* strains, and these individuals could serve as both reservoirs for the pathogen and a route via which antibiotic-resistant bacteria can be transmitted to susceptible human hosts in either the hospital environment or community setting. Besides this, human contact with contaminated surfaces (such as door handles in hospitals), cuts or wounds on the skin, living in crowded conditions and poor personal hygiene are some of the risk factors that predispose both patients and non-patients to the acquisition of *S. aureus* infection [6,7].

*Staphylococcus aureus* is a toxigenic bacterium in that it produces toxins, and it is also known to produce some enzymes such as catalase and coagulase, which help to distinguish pathogenic *S. aureus* (that produce them) from non-pathogenic *S. aureus* (that do not produce any of these enzymes). Toxic shock syndrome toxin-1 (TSST-1), *Staphylococcal* Scalded Skin Syndrome (SSSS), pneumonia, meningitis, empyema, endocarditis, osteomyelitis and methicillin-resistant *S. aureus* (MRSA) infection are some of the diseases caused by pathogenic *S. aureus* [2,3]. Most areas of highest risk for severe *S. aureus* infections in the hospital environment are usually the newborns ward, intensive care units (ICU), operating rooms, out-patients departments (OPD) and other high-dependency wards, thus it is paramount that health personnel, patients and visitors alike adhere and imbibe strict personal hygiene around the hospital environment by washing hands and observing other protective protocols to diminish *S. aureus* infections both in the community and in the hospital [8].

Currently, some strains of *S. aureus* have emerged that are resistant to some commonly available antibiotics, including methicillin, penicillin, and vancomycin, amongst others [9-11]. Antibiotic susceptibility testing is a vital step in diagnostic microbiology because it helps to diagnose the recommended therapeutic pattern or approach. It equally helps to predict the outcome of treatment with the antimicrobial agents tested. Furthermore, antibiotic susceptibility testing aims to guide the clinician in the selection of the most appropriate agent for a particular clinical challenge [12]. Considering these, this study was undertaken to evaluate the antimicrobial susceptibility profiles and multiple antibiotic resistance indices of *S. aureus* isolates from healthy staff and students at the University community from various departments and faculties within the University of Nigeria, Nsukka (6.8571°N, 7.3958°E), Enugu State, Nigeria.

## EXPERIMENTAL

### Isolation and characterization of *S. aureus*

The isolation of *S. aureus* was carried out using the streak plate method, by obtaining swab specimens from the skin, hand, and nose of healthy personnel and students within the University of Nigeria, Nsukka community (6.8571°N, 7.3958°E), Enugu State, Nigeria over 4 months which were subsequently cultured on Mannitol salt agar (MSA) and incubated at 37 °C for 18 – 24 h. The resultant, yellowish-raised staphylococcal colonies on MSA were sub-cultured on nutrient agar slant and incubated at 37 °C for 24 h. The resultant cultures were stored in a refrigerator set at 4 °C before characterization. The isolates were standardized to bring the cell population to McFarland's 0.5 turbidity standard before every microbiological assay. Thereafter, the isolates were subjected to a step-by-step characterization test described by Cowan and Steel's manual for identification of medical bacteria [13] and District Laboratory Practice in Tropical Countries [14], namely Gram stain, catalase, coagulase, and mannitol fermentation.

### Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing was performed following the Kirby-Bauer disk diffusion method [15]. Exactly, 100 µL of standardized inoculum of the test organisms (adjusted to 0.5 McFarland turbidity standards) was seeded into 20 ml sterile molten Mueller-Hinton agar (MHA) plates at 45 °C, and thoroughly mixed. Antibiotic disk of oxacillin (1 µg), cefaclor (30 µg),

ampicillin/sulbactam (20 µg), vancomycin (5 µg), amoxicillin (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), and cefuroxime (30 µg; Oxoid, UK) were aseptically placed on the surfaces of MHA plates. All plates were incubated at 37 °C for 18 - 24 h. Inhibition zone diameter (IZD) was measured, and the mean IZD was thereafter calculated. The results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) interpretative values of 2008 [16].

### Minimum inhibitory concentration (MIC)

The MIC of some selected antibiotics (amoxicillin, ciprofloxacin, and ceftriaxone; based on their high order of activity according to IZD<sub>95</sub>, i.e., the 95th percentile of the sum of the IZDs of the various antibiotics against 95 % of the isolates) was determined using the agar dilution method described by Esimone *et al* [17]. Stock concentration of 1000 µg/mL of the antibiotics was prepared with suitable solvents, namely sterile distilled water (used to dissolve amoxicillin and ciprofloxacin) and Dimethyl sulfoxide (used for dissolution of Ceftriaxone). Serial dilutions of the stock concentrations of the antibiotics were carried out, starting with 1000 µg/mL down to 0.0049 µg/mL, with 1 mL of each subsequently seeded into empty sterile Petri dishes. Thereafter, 19 mL of sterile molten Mueller-Hinton agar at 45 °C was poured into each of the plates containing the different dilutions of the test antibiotics and thoroughly mixed. The plates were allowed to set and later dried in an oven at 30 °C for five minutes. Later, standardized suspension of all isolates was streaked in triplicate aseptically on the surface of the dried Mueller-Hinton agar. The plates were allowed to stand for at least 15 min at room temperature, after which they were incubated at 37 °C for 24 h. The MIC for each of the *S. aureus* isolates was determined as the lowest concentration or dilution of the test drug showing no visible bacterial growth. The interpretation of the MIC results was done as per the CLSI criteria of 2008 [16].

### Multiple Antibiotic Resistance (MAR) indices of *S. aureus*

Multiple antibiotic resistance (MAR) index, which is a helpful tool for analysing health risk and resistance, was determined for each isolate of the *S. aureus* using Eq 1 [18].

$$\text{MAR} = a/b \dots\dots\dots (1)$$

where “a” represents the number of antibiotics to which the test isolate was resistant to and “b” is the total number of antibiotics to which the test isolate was exposed.

### Statistical analysis

Data was analyzed using IBM SPSS version 25. Descriptive statistics (mean ± SD) were calculated, with experiments done in triplicate. One-way ANOVA (Tukey's post-hoc test) was used to compare inhibition zones among antibiotics, with  $p < 0.05$  considered statistically significant.

## RESULTS

A total of 84 Staphylococcal isolates were obtained. Among the eighty-four (84) Staphylococcal isolates, 50 isolates (59.5 %) were *S. aureus*, and the rest (40.5 %) were other Staphylococcal species. The number and percentage of *S. aureus* isolated from skin, hand and nose samples were 27 (54 %), 16 (32 %), and 7 (14 %), respectively. The number and percentage frequency of the *S. aureus* isolates that originated from the various sites of isolation are shown in Table 1.

**Table 1:** Number and percentage of *Staphylococcus aureus* isolated from the nose, hand, and skin

| Organism         | Specimens           |                      |                     |
|------------------|---------------------|----------------------|---------------------|
|                  | Nose isolate, n (%) | Skin isolates, n (%) | Hand isolate, n (%) |
| <i>S. aureus</i> | 7 (14)              | 27 (54)              | 16 (32)             |

The results of our antimicrobial susceptibility studies, as shown in Table 2 and Figure 1, show that the *S. aureus* isolates showed varying rates of susceptibility and resistance to the tested antibiotics. However, ampicillin/sulbactam (a beta-lactamase inhibitor), cefuroxime and ciprofloxacin were the most effective antibiotics against test isolates. Cefaclor, ceftazidime, oxacillin, vancomycin and erythromycin were the least active agents. Mean IZDs differed significantly among antibiotics ( $p < 0.05$ ), with ciprofloxacin having significantly higher inhibition zones than cefaclor and ceftazidime. The inhibitory zone diameter (IZD) of the selected antibiotics against 95 % of *Staph aureus* isolates (IZD<sub>95</sub>) ranged from 14.50 - 37.60 mm (Table 1). Activity was in the order of AS > CP > EY or CR > CX > AM > VA > CD > OX > CL.

The results of the IZD<sub>95</sub> show that 95 % of the isolates were most sensitive to AS (a beta-lactamase inhibitor) and least to CL, as indicated in the order of activity of antibiotics above.

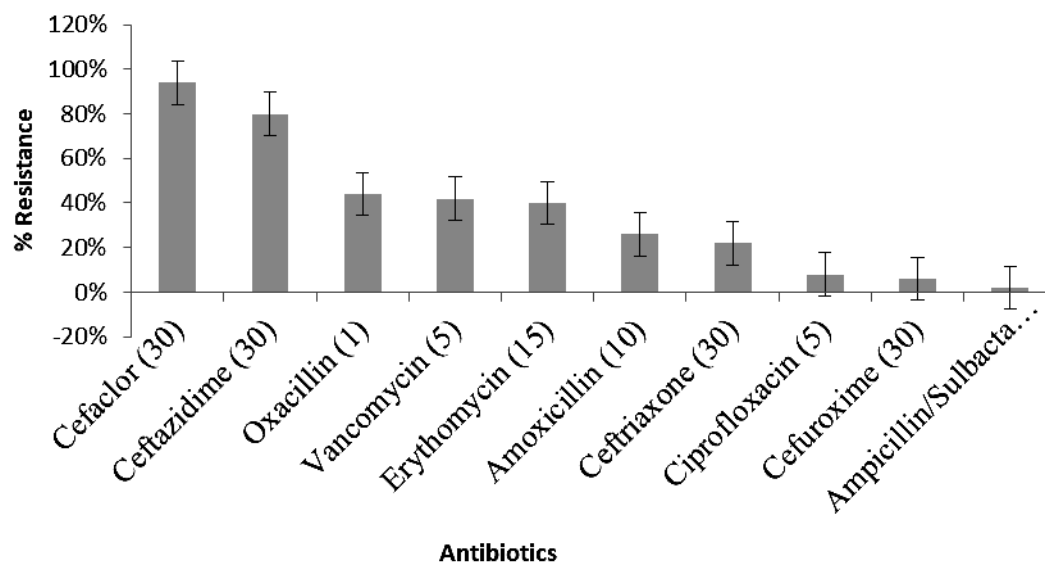
**Table 2:** Antibiogram of *Staphylococcus aureus* isolates, and the mean Inhibitory zone diameter produced by antibiotic disc (mm)

| Isolate no.       | OX    | CL    | AS    | VA    | AM    | CD    | CX    | EY     | CP    | CR    |
|-------------------|-------|-------|-------|-------|-------|-------|-------|--------|-------|-------|
| 1                 | 13.50 | 0.00  | 21.50 | 14.50 | 9.00  | 10.50 | 11.50 | 0.00   | 14.50 | 20.50 |
| 2                 | 0.00  | 0.00  | 26.50 | 14.50 | 10.50 | 10.50 | 15.50 | 29.50  | 22.50 | 23.50 |
| 3                 | 12.50 | 11.00 | 29.50 | 17.50 | 20.50 | 12.50 | 23.00 | 15.50  | 35.50 | 32.50 |
| 4                 | 11.50 | 14.50 | 34.50 | 16.50 | 24.50 | 10.50 | 20.50 | 0.00   | 39.50 | 32.50 |
| 5                 | 11.50 | 0.00  | 34.50 | 16.50 | 24.50 | 9.50  | 20.50 | 0.00   | 35.50 | 30.50 |
| 6                 | 13.50 | 11.50 | 31.00 | 15.50 | 21.50 | 0.00  | 20.50 | 21.00  | 31.50 | 25.00 |
| 7                 | 14.00 | 10.50 | 36.50 | 13.50 | 22.50 | 10.50 | 17.50 | 31.00  | 29.50 | 10.50 |
| 8                 | 12.50 | 0.00  | 33.00 | 13.50 | 19.50 | 0.00  | 15.50 | 30.50  | 29.00 | 25.50 |
| 9                 | 29.50 | 24.50 | 41.00 | 25.50 | 39.50 | 14.50 | 25.50 | 31.00  | 29.00 | 37.00 |
| 10                | 10.00 | 0.00  | 30.50 | 14.50 | 10.50 | 10.50 | 15.50 | 34.00  | 28.50 | 23.50 |
| 11                | 17.00 | 0.00  | 16.00 | 12.00 | 11.50 | 13.00 | 25.50 | 0.00   | 22.50 | 26.00 |
| 12                | 0.00  | 0.00  | 21.50 | 14.50 | 11.50 | 0.00  | 15.50 | 0.00   | 25.50 | 22.50 |
| 13                | 0.00  | 10.50 | 21.50 | 12.50 | 13.50 | 0.00  | 12.50 | 0.00   | 28.50 | 20.50 |
| 14                | 19.50 | 0.00  | 25.50 | 15.50 | 14.50 | 12.00 | 18.50 | 30.50  | 31.00 | 30.50 |
| 15                | 11.00 | 0.00  | 32.50 | 12.50 | 17.50 | 11.50 | 16.50 | 25.00  | 27.00 | 25.50 |
| 16                | 9.50  | 0.00  | 25.50 | 12.50 | 15.50 | 11.50 | 17.50 | 0.00   | 29.50 | 27.00 |
| 17                | 14.00 | 10.50 | 34.50 | 11.50 | 22.50 | 9.50  | 20.50 | 30.50  | 31.00 | 29.50 |
| 18                | 12.00 | 9.50  | 26.00 | 12.50 | 14.50 | 7.50  | 16.50 | 0.00   | 25.50 | 26.00 |
| 19                | 15.50 | 0.00  | 15.50 | 13.50 | 10.50 | 14.50 | 20.50 | 0.00   | 30.50 | 31.00 |
| 20                | 18.00 | 11.50 | 36.50 | 19.00 | 26.50 | 10.50 | 25.50 | 34.50  | 29.00 | 34.50 |
| 21                | 14.50 | 0.00  | 21.50 | 14.00 | 0.00  | 12.50 | 12.50 | 0.00   | 12.50 | 20.50 |
| 22                | 15.50 | 0.00  | 19.50 | 13.50 | 9.50  | 9.00  | 14.50 | 0.00   | 20.50 | 19.50 |
| 23                | 12.50 | 0.00  | 31.50 | 12.50 | 17.50 | 10.50 | 18.50 | 24.00  | 23.50 | 25.50 |
| 24                | 0.00  | 0.00  | 20.50 | 13.50 | 0.00  | 13.00 | 0.00  | 19.50  | 21.50 | 13.00 |
| 25                | 9.50  | 0.00  | 27.50 | 13.00 | 14.50 | 10.50 | 15.50 | 11.50  | 28.00 | 25.50 |
| 26                | 18.00 | 9.50  | 31.00 | 15.50 | 20.50 | 23.50 | 29.50 | 41.00  | 31.50 | 35.50 |
| 27                | 14.50 | 0.00  | 39.50 | 20.50 | 25.50 | 21.00 | 21.00 | 34.00  | 34.50 | 31.00 |
| 28                | 9.50  | 12.50 | 25.50 | 16.00 | 12.50 | 9.00  | 15.50 | 0.00   | 30.50 | 23.50 |
| 29                | 0.00  | 0.00  | 25.50 | 13.00 | 12.50 | 11.50 | 11.50 | 23.50  | 26.50 | 17.50 |
| 30                | 13.50 | 9.00  | 29.50 | 12.50 | 18.50 | 0.00  | 13.50 | 24.50  | 28.50 | 25.50 |
| 31                | 0.00  | 0.00  | 19.50 | 12.50 | 8.50  | 0.00  | 16.00 | 26.00  | 12.50 | 21.50 |
| 32                | 15.50 | 11.00 | 27.50 | 13.50 | 17.50 | 0.00  | 17.50 | 0.00   | 31.50 | 27.00 |
| 33                | 7.50  | 0.00  | 34.00 | 15.50 | 23.00 | 9.50  | 14.50 | 20.00  | 29.50 | 19.00 |
| 34                | 0.00  | 0.00  | 29.50 | 16.00 | 12.50 | 9.50  | 14.00 | 32.00  | 31.50 | 14.00 |
| 35                | 9.50  | 0.00  | 25.50 | 15.50 | 11.50 | 17.50 | 17.00 | 25.50  | 30.50 | 29.00 |
| 36                | 0.00  | 0.00  | 19.50 | 23.00 | 0.00  | 0.00  | 0.00  | 0.00   | 9.50  | 16.00 |
| 37                | 0.00  | 0.00  | 24.50 | 15.00 | 11.00 | 17.00 | 14.50 | 23.50  | 17.50 | 19.50 |
| 38                | 0.00  | 14.50 | 38.50 | 20.50 | 26.50 | 0.00  | 29.50 | 39.50  | 28.50 | 31.00 |
| 39                | 11.50 | 10.50 | 32.50 | 15.50 | 21.00 | 14.50 | 18.50 | 29.50  | 28.50 | 30.50 |
| 40                | 10.50 | 13.50 | 25.50 | 15.50 | 13.50 | 15.00 | 19.50 | 9.50   | 30.50 | 26.00 |
| 41                | 9.50  | 0.00  | 28.50 | 14.50 | 15.50 | 7.00  | 16.00 | 12.50  | 30.50 | 26.00 |
| 42                | 13.00 | 10.50 | 32.50 | 15.50 | 24.50 | 11.00 | 16.00 | 32.50  | 30.50 | 31.50 |
| 43                | 9.50  | 0.00  | 28.50 | 14.50 | 9.50  | 0.00  | 19.50 | 10.50  | 29.00 | 28.50 |
| 44                | 9.50  | 9.50  | 31.50 | 12.50 | 18.50 | 10.50 | 16.00 | 30.50  | 34.50 | 37.50 |
| 45                | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 25.50 | 27.50 | 12.50  | 35.50 | 15.50 |
| 46                | 17.00 | 0.00  | 35.00 | 16.00 | 25.50 | 0.00  | 22.00 | 35.50  | 31.00 | 26.50 |
| 47                | 0.00  | 0.00  | 21.50 | 13.00 | 12.00 | 0.00  | 9.50  | 30.50  | 28.50 | 14.50 |
| 48                | 0.00  | 0.00  | 19.50 | 14.50 | 0.00  | 13.00 | 14.00 | 0.00   | 34.00 | 25.50 |
| 49                | 11.50 | 0.00  | 25.50 | 14.50 | 15.00 | 0.00  | 12.50 | 29.50  | 36.50 | 21.50 |
| 50                | 16.50 | 0.00  | 24.50 | 15.50 | 12.00 | 17.00 | 14.50 | 31.00  | 31.00 | 23.50 |
| IZD <sub>95</sub> | 18    | 14.05 | 37.6  | 20.5  | 26.05 | 19.43 | 26.6  | 35.05  | 35.5  | 35.05 |
| IZD <sub>50</sub> | 11.5  | 0     | 27.5  | 14.5  | 14.5  | 10.5  | 16.25 | 23.5   | 29.25 | 25.5  |
| SD                | 6.833 | 6.142 | 7.292 | 3.461 | 8.014 | 6.597 | 5.761 | 13.986 | 6.290 | 6.273 |

OX = Oxacillin, CL = Cefaclor, AS = Ampicillin /Sulbactam, VA = Vancomycin, AM = Amoxicillin, CD = Ceftazidime, CX = Ceftriaxone, EY = Erythromycin, CP = Ciprofloxacin, CR = Cefuroxime, 0 = No inhibition, SD = Standard Deviation. All assays were performed in triplicate (n = 3), and data are expressed as mean  $\pm$  SD

The corresponding IZD<sub>50</sub> ranged from 0.00 - 29.25 mm (Table 2), and the order of activity was CP > AS > CR > EY > CX > VA or AM > OX > CD > CL. Again 50 % of the isolates were most sensitive to CP (a fluoroquinolone) and least to

CL, as indicated in the order of activity above. The highest activity for IZD<sub>95</sub> and IZD<sub>50</sub> was recorded for the following antibiotics: AS<sub>95</sub> and CP<sub>50</sub>. However, CL recorded the lowest activity for IZD<sub>95</sub> and IZD<sub>50</sub>.



**Figure 1:** Antibiotic susceptibility rates among the *S. aureus* isolates

**Table 3:** Percentage resistance among isolates

| Antibiotics (µg)          | Standard level for resistance (mm) | Number of resistant isolates | Percentage resistance (%) |
|---------------------------|------------------------------------|------------------------------|---------------------------|
| Oxacillin (1)             | ≤10                                | 22                           | 44                        |
| Cefaclor (30)             | ≤14                                | 47                           | 94                        |
| Ampicillin/Sulbactam (20) | ≤11                                | 1                            | 2                         |
| Vancomycin (5)            | ≤14                                | 21                           | 42                        |
| Amoxicillin (10)          | ≤11                                | 13                           | 26                        |
| Ceftazidime (30)          | ≤14                                | 40                           | 80                        |
| Ceftriaxone (30)          | ≤14                                | 11                           | 22                        |
| Erythromycin (15)         | ≤13                                | 20                           | 40                        |
| Ciprofloxacin (5)         | ≤15                                | 4                            | 8                         |
| Cefuroxime (30)           | ≤14                                | 3                            | 6                         |

Furthermore, the antibiotic percentage resistance for the isolates ranged from 2 – 94 % (Table 3). Resistance was in the order of CL>CD>OX>VA>EY>AM>CX>CP>CR>AS.

Higher percentages of resistance were recorded for cefaclor (94%), ceftazidime (80%), oxacillin (44 %), vancomycin (42 %) and erythromycin (40 %). The highest percentage resistance was recorded by cefaclor (94 %), whereas ampicillin/sulbactam recorded the lowest percentage resistance of 2 %.

Below is the result of the Antibiotic resistance patterns among isolates of *S. aureus*. The predominant antibiotic resistance patterns are CL-CD (12 %), CL (8 %), CL-VA-CD (8 %), CL-VA-CD-EY (6 %), and OX-CL-CD-EY (6 %).

The results of the MAR indices of *S. aureus* isolates are shown in Table 5 below. The multiple antibiotic resistance indices of *S. aureus* were in the range of 0.0 - 0.6, and 90 % of the isolates

had a MAR index of 0.2 and above, while 10 % of the isolates had a MAR index below 0.2.

The percentage of *S. aureus* isolates with MAR is presented in Table 6 below, and it ranged from 4 – 24 %. Resistance to four (4) antibiotics recorded the highest percentage of 24 %, followed by resistance to two (2) antibiotics (22 %). The lowest percentage of isolates showing MAR (4 %) was recorded by resistance to seven antibiotics.

Furthermore, the result of the percentage frequency of the MAR index among the *S. aureus* isolates is presented in Table 7 below. The MAR index of 0.4 recorded the highest percentage frequency (24 %). followed by MAR indices of 0.2 (22 %), 0.3 (16 %), and 0.5 (14 %), respectively.

The result of the minimum inhibitory concentration (MIC) of the selected antibiotics against the *S. aureus* isolates is shown in Table

8 below. Notably, the MICs fall in the ranges of 0.195 – 100.000, 0.780 – 50.000 and 1.563 – 25.000 µg/mL for ciprofloxacin, amoxicillin, and ceftriaxone, respectively, against *S. aureus* isolates.

## DISCUSSION

*S. aureus* is a common coloniser of both human and animal skin, and it is also associated with nosocomial and community-acquired infections in recent times [1,10]. Apart from colonising the skin, *S. aureus* is also notorious for colonising mucosal surfaces, where it attaches firmly to cause some pathological changes in its host. *S. aureus* strains that show multiple resistance to available antibiotics continue to be a major concern for human healthcare providers worldwide, and these have contributed to the increasing rate of morbidity and mortality caused by these organisms. Antimicrobial resistance caused by pathogenic *S. aureus* strains has been well documented [5,9,10].

This study was aimed at determining the susceptibility profiles of *S. aureus* isolates

obtained from both healthy personnel and students at the University of Nigeria, Nsukka (UNN).

The results of this present study show that both hospital personnel and students at the University of Nigeria, Nsukka (UNN) harbour *S. aureus* isolates that are resistant to some commonly available antibiotics. Among all antibiotics, ciprofloxacin demonstrated the highest mean inhibitory activity, corroborating its strong efficacy against *S. aureus* isolates observed in this study. Cefaclor and ceftazidime showed the least form of antibacterial activity against all the test isolates of *S. aureus* used in this study. This was followed by oxacillin, amoxicillin, vancomycin, erythromycin and ceftriaxone. Reduced susceptibility of *S. aureus* isolates to some available antimicrobial agents, as obtained in this study, has also been previously reported [1,4,9]. However, cefuroxime, ciprofloxacin and ampicillin-sulbactam showed an appreciable level of antimicrobial activity against the test *S. aureus* isolates, as these were the most active antibiotics tested.

**Table 4:** Antibiotic resistance patterns among isolates of *Staph aureus*

| Isolate no. | Resistance pattern   | No of isolates | Percentage of isolates showing pattern (%) |
|-------------|----------------------|----------------|--------------------------------------------|
| 1           | No Resistance        | 1              | 2                                          |
| 2           | CL                   | 4              | 8                                          |
| 3           | CL-CD                | 6              | 12                                         |
| 4           | CD-EY                | 1              | 2                                          |
| 5           | OX-CL                | 1              | 2                                          |
| 6           | OX-CD                | 1              | 2                                          |
| 7           | CL-EY                | 1              | 2                                          |
| 8           | CL-CD-EY             | 1              | 2                                          |
| 9           | CL-VA-CD             | 4              | 8                                          |
| 10          | OX-CL-CD             | 1              | 2                                          |
| 11          | OX-CL-AM             | 1              | 2                                          |
| 12          | CL-CD-CX             | 1              | 2                                          |
| 13          | OX-CL-AM-CD          | 2              | 4                                          |
| 14          | CL-VA-CD-CR          | 1              | 2                                          |
| 15          | CL-VA-CD-EY          | 3              | 6                                          |
| 16          | OX-CL-CD-EY          | 3              | 6                                          |
| 17          | CL-VA-AM-EY          | 1              | 2                                          |
| 18          | CL-VA-CD-CX          | 1              | 2                                          |
| 19          | OX-CL-VA-CD          | 1              | 2                                          |
| 20          | OX-CL-VA-CD-EY       | 2              | 4                                          |
| 21          | CL-VA-AM-CD-EY       | 1              | 2                                          |
| 22          | OX-CL-VA-CD-CX       | 2              | 4                                          |
| 23          | OX-CL-CD-CX-CR       | 1              | 2                                          |
| 24          | OX-CL-AM-CD-EY       | 1              | 2                                          |
| 25          | CL-AM-CD-CX-EY-CP    | 1              | 2                                          |
| 26          | OX-CL-VA-CD-CX-EY    | 1              | 2                                          |
| 27          | OX-CL-VA-AM-CD-CP    | 1              | 2                                          |
| 28          | OX-CL-AS-VA-AM-EY    | 1              | 2                                          |
| 29          | OX-CL-AM-CD-CX-EY    | 1              | 2                                          |
| 30          | CL-VA-AM-CD-CX-EY-CP | 1              | 2                                          |
| 31          | OX-CL-VA-AM-CD-CX-CR | 1              | 2                                          |
| 32          | OX-CL-AM-CD-CX-EY-CP | 1              | 2                                          |

**Table 5:** Results of multiple antibiotic resistance (MAR) indices

| Isolate no. | Antibiotic resistance count per isolate | Multiple antibiotic resistant (MAR) index |
|-------------|-----------------------------------------|-------------------------------------------|
| 1           | 6.00                                    | 0.60                                      |
| 2           | 4.00                                    | 0.40                                      |
| 3           | 2.00                                    | 0.20                                      |
| 4           | 2.00                                    | 0.20                                      |
| 5           | 3.00                                    | 0.30                                      |
| 6           | 2.00                                    | 0.20                                      |
| 7           | 4.00                                    | 0.40                                      |
| 8           | 3.00                                    | 0.30                                      |
| 9           | 0.00                                    | 0.00                                      |
| 10          | 4.00                                    | 0.40                                      |
| 11          | 4.00                                    | 0.40                                      |
| 12          | 4.00                                    | 0.40                                      |
| 13          | 2.00                                    | 0.20                                      |
| 14          | 2.00                                    | 0.20                                      |
| 15          | 3.00                                    | 0.30                                      |
| 16          | 5.00                                    | 0.50                                      |
| 17          | 3.00                                    | 0.30                                      |
| 18          | 4.00                                    | 0.40                                      |
| 19          | 4.00                                    | 0.40                                      |
| 20          | 2.00                                    | 0.20                                      |
| 21          | 7.00                                    | 0.70                                      |
| 22          | 5.00                                    | 0.50                                      |
| 23          | 3.00                                    | 0.30                                      |
| 24          | 7.00                                    | 0.70                                      |
| 25          | 5.00                                    | 0.50                                      |
| 26          | 1.00                                    | 0.10                                      |
| 27          | 1.00                                    | 0.10                                      |
| 28          | 4.00                                    | 0.40                                      |
| 29          | 5.00                                    | 0.50                                      |
| 30          | 4.00                                    | 0.40                                      |
| 31          | 4.00                                    | 0.40                                      |
| 32          | 4.00                                    | 0.40                                      |
| 33          | 3.00                                    | 0.30                                      |
| 34          | 5.00                                    | 0.50                                      |
| 35          | 2.00                                    | 0.20                                      |
| 36          | 7.00                                    | 0.70                                      |
| 37          | 3.00                                    | 0.30                                      |
| 38          | 2.00                                    | 0.20                                      |
| 39          | 1.00                                    | 0.10                                      |
| 40          | 2.00                                    | 0.20                                      |
| 41          | 4.00                                    | 0.40                                      |
| 42          | 2.00                                    | 0.20                                      |
| 43          | 5.00                                    | 0.50                                      |
| 44          | 4.00                                    | 0.40                                      |
| 45          | 6.00                                    | 0.60                                      |
| 46          | 2.00                                    | 0.20                                      |
| 47          | 5.00                                    | 0.50                                      |
| 48          | 6.00                                    | 0.60                                      |
| 49          | 3.00                                    | 0.30                                      |
| 50          | 1.00                                    | 0.10                                      |

**Table 6:** Percentage of Isolates with Multiple Antibiotic Resistance

| Number of antibiotics | Number of resistant isolates | Resistant isolates (%) |
|-----------------------|------------------------------|------------------------|
| 2                     | 11                           | 22                     |
| 3                     | 8                            | 16                     |
| 4                     | 12                           | 24                     |
| 5                     | 7                            | 14                     |
| 6                     | 4                            | 8                      |
| 7                     | 2                            | 4                      |

**Table 7:** Percentage frequency of MAR Index (%)

| MAR index | No of isolates | Percentage frequency of MAR index (%) |
|-----------|----------------|---------------------------------------|
| 0.00      | 1              | 2                                     |
| 0.10      | 4              | 8                                     |
| 0.20      | 11             | 22                                    |
| 0.30      | 8              | 16                                    |
| 0.40      | 13             | 26                                    |
| 0.50      | 7              | 14                                    |
| 0.60      | 3              | 6                                     |
| 0.70      | 3              | 6                                     |

It is interesting to note that while very high resistance was displayed for cefaclor (a second generation) and ceftazidime (a third generation), cephalosporins, some of the least resistance (highest susceptibility) were displayed by ceftriaxone (third generation), and Cefuroxime (a second generation), which are equally cephalosporins. Like penicillin, the cephalosporins exert their antimicrobial effect by inhibition of the synthesis of peptidoglycan, which is a heteropolymer essential in the cell wall formation and provides a rigid mechanical stability by virtue of its highly cross-linked lattice wall structure. The result of the inhibition is loss of bacterial cell rigidity and subsequent rupture or lysis of the bacterial cells [19,20].

Also worthy of note is the activity of Ampicillin/Sulbactam, which displayed the highest level of susceptibility (98 %). This is a beta-lactamase inhibitor. Sulbactam present in the Ampicillin/Sulbactam complex is meant to afford protection to the beta-lactam nucleus present in Ampicillin, and this protection has been reported to enhance the activity of Ampicillin [21]. This explains the favourable observed activity demonstrated on the isolates over the Amoxicillin (another penicillin sensitive to the activities of the  $\beta$ -lactamase enzymes). The minimum inhibitory concentrations (MICs) of amoxicillin, ceftriaxone and ciprofloxacin against the test isolates were 50, 25 and 100  $\mu$ g/mL, respectively. The highest MIC value observed in this present-day study was 100  $\mu$ g/mL. The MIC results of antimicrobial agents are usually a useful representative pre-clinical quantitative

analytical parameter that finds prospective application in both pre-clinical and clinical settings. Apart from the practical utility of MIC values as a means of cutoff points between microbial species and strains based on the antimicrobial susceptibility profile, the possession of lower MICs by an antimicrobial agent is suggestive of a higher inherent antimicrobial property [22]. It is thus expected that lower MIC values would enhance clinical outcome as well as help to limit the occurrence of unwanted drug side effects since smaller but effective doses of the antibiotics could then be administered to patients in line with pre-determined frequencies. Thus, considering the MIC results generated (Table 8), Ceftriaxone appears to be the agent of choice for an empiric/nonspecific clinical treatment of infections caused by the isolated *S. aureus* strains within the studied community.

The result of the multiple antibiotic resistance index (MARI) showed that 90 % the tested isolates of *S. aureus* had an MAR index of 0.2 and above, which indicates that these Staphylococcal isolates originated from potentially dangerous sources where antibiotics are regularly used and overused [18]. The high prevalence of multiple antibiotic resistance as obtained in this study indicates a serious need for a continuous antibiotics surveillance program. Secondly, such a result does not portend well for the available antimicrobial resources in clinical practice because resistance amongst pathogenic bacteria, including *S. aureus*, limits therapeutic options and worsens the condition of the patients due to therapeutic failure. *S. aureus* isolates from hospital origin and even from the community are multidrug resistant to some commonly available antibiotics such as vancomycin, penicillin, erythromycin, and gentamicin, amongst others [1,4].

*S. aureus* isolates, especially those that are of nosocomial origin and are multidrug-resistant, are capable of initiating hospital-acquired infection within a hospital setting, and these pathogens can spread from patients to patients and from hospital personnel to patients and even to the community and vice versa. It is, therefore, paramount to check the resistance profile of *S. aureus* isolates in the hospital via routine and proper antimicrobial susceptibility studies to prevent multiple resistant strains of the pathogen from emerging and spreading to the community. Though antimicrobial susceptibility testing or antibiogram may not be sufficient to detect drug-resistant pathogens in a hospital setting, routine susceptibility testing in the hospital environment, especially for bacterial pathogens, including *S. aureus*, is crucial.

**Table 8:** Mean MIC values ( $\mu\text{g/mL}$ ) of selected antibiotics against the *Staphylococcus aureus* isolates

| Isolate no. | Ciprofloxacin ( $\mu\text{g/mL}$ ) | Amoxicillin ( $\mu\text{g/mL}$ ) | Ceftriaxone ( $\mu\text{g/mL}$ ) |
|-------------|------------------------------------|----------------------------------|----------------------------------|
| 1           | 50.000                             | 6.250                            | 12.500                           |
| 2           | 3.125                              | 12.500                           | 25.000                           |
| 3           | 0.195                              | 0.780                            | 6.250                            |
| 4           | 0.195                              | 0.780                            | 12.500                           |
| 5           | 0.390                              | 3.125                            | 12.500                           |
| 6           | 0.390                              | 1.563                            | 6.250                            |
| 7           | 0.390                              | 0.780                            | 12.500                           |
| 8           | 0.780                              | 6.250                            | 12.500                           |
| 9           | 0.780                              | 0.390                            | 3.125                            |
| 10          | 0.390                              | 6.250                            | 12.500                           |
| 11          | 3.125                              | 3.250                            | 12.500                           |
| 12          | 1.563                              | 6.250                            | 12.500                           |
| 13          | 0.390                              | 1.563                            | 25.000                           |
| 14          | 0.195                              | 6.250                            | 6.250                            |
| 15          | 0.390                              | 0.780                            | 6.250                            |
| 16          | 0.390                              | 6.250                            | 12.500                           |
| 17          | 0.390                              | 12.500                           | 12.500                           |
| 18          | 1.563                              | 6.250                            | 12.500                           |
| 19          | 25.000                             | 6.250                            | 12.500                           |
| 20          | 0.780                              | 0.780                            | 6.250                            |
| 21          | 25.000                             | 6.250                            | 25.000                           |
| 22          | 100.000                            | 6.250                            | 12.500                           |
| 23          | 0.390                              | 3.125                            | 12.500                           |
| 24          | 25.000                             | 12.500                           | 25.000                           |
| 25          | 0.780                              | 1.563                            | 12.500                           |
| 26          | 0.780                              | 1.563                            | 12.500                           |
| 27          | 0.390                              | 1.563                            | 3.125                            |
| 28          | 0.195                              | 1.563                            | 3.125                            |
| 29          | 0.390                              | 0.780                            | 25.000                           |
| 30          | 0.390                              | 6.250                            | 25.000                           |
| 31          | 25.000                             | 1.560                            | 25.000                           |
| 32          | 25.000                             | 3.125                            | 6.250                            |
| 33          | 0.780                              | 1.563                            | 3.125                            |
| 34          | 0.390                              | 1.563                            | 25.000                           |
| 35          | 0.780                              | 6.250                            | 3.125                            |
| 36          | 100.000                            | 12.500                           | 3.125                            |
| 37          | 25.000                             | 6.250                            | 25.000                           |
| 38          | 0.780                              | 3.125                            | 1.563                            |
| 39          | 0.390                              | 3.125                            | 3.125                            |
| 40          | 0.195                              | 12.500                           | 6.250                            |
| 41          | 0.390                              | 6.250                            | 3.125                            |
| 42          | 0.390                              | 1.563                            | 3.125                            |
| 43          | 0.195                              | 3.125                            | 25.000                           |
| 44          | 0.390                              | 1.563                            | 6.250                            |
| 45          | 0.195                              | 50.000                           | 6.250                            |
| 46          | 0.780                              | 3.125                            | 3.125                            |
| 47          | 0.390                              | 6.250                            | 3.125                            |
| 48          | 0.780                              | 6.250                            | 6.250                            |
| 49          | 0.390                              | 6.250                            | 3.125                            |
| 50          | 0.195                              | 6.250                            | 25.000                           |

This is because it will go a long way in helping clinicians as well as laboratory scientists to monitor the changing breakpoints of clinically important bacterial pathogens. The multiple antibiotic resistance (MAR) index has been useful in differentiating bacteria from different

sources using antibiotics that are commonly used for human therapy. When compared to other methods of bacteria source tracking, such as genotypic characterisation, the MAR indexing method is cost-effective, rapid and easy to perform. It is equally simple and does not require

specialised training and expensive equipment [18].

Summarily, this study shows that healthy personnel and students at the University of Nigeria, Nsukka (UNN), Enugu, Nigeria harbour pathogenic *S. aureus* in substantial amounts, which are multidrug resistant to some commonly available antibiotics. Thus, the personnel as well as students should imbibe and practice good personal hygiene and in addition to abiding by all protective measures. There should also be sustained surveillance and monitoring of antibiotic resistance in commensal *Staphylococcus aureus* from humans in the studied community. Such studies would form a basis for the implementation of sound antibiotic-use policies.

## CONCLUSION

The findings of this study show that *S. aureus* is common among healthy staff and students at the University of Nigeria, Nsukka, with the skin being the most frequent site of colonization. The isolates displayed varied responses to antibiotics, with ciprofloxacin exhibiting the highest activity, while cefaclor was the least effective.

The observed multiple antibiotic resistance patterns emphasize the potential risks of community- and hospital-acquired infections. Ongoing surveillance, proper hygiene practices, and rational antibiotic use are therefore vital to curb the spread of resistant *S. aureus* strains.

## DECLARATIONS

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None.

### Ethical approval

None provided.

### Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Conflict of interest

No conflict of interest is associated with this work.

## Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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