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# Antibiotic Sensitivity Profile of *Staphylococcus* Species from Anatomical and Environmental Sites in the Federal University of Technology, Akure, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author AOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author FOE managed the analyses of the study. Author AOO managed the literature searches. Both authors read and approved the final manuscript.*

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## ABSTRACT

This study reveals the methicillin sensitivity pattern of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The two species of Staphylococci were isolated from polluted and unpolluted soil and water; anatomical sites such as nose, ear, skin, hand and throat; wastes from dustbin, roof, poultry, postgraduate hostel's bathroom and toilet in the Federal University of Technology Akure, Ondo State. Clinical isolates and typed culture were also collected from the Microbiology Laboratory Obafemi Awolowo University and Medical Microbiology Laboratory, Ibadan, Nigeria respectively. Isolation, characterisation and identification were done according to standard microbiological methods. The occurrence of *S. aureus* was more prevalent with 66.67% while *S. epidermidis* was 33.33%. Staphylococci isolated, clinical isolates from hospital and typed culture (ATCC-25923)

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were all resistant to tested antibiotics. Ninety percent (90%) of *S. aureus* and sixty percent (60%) of *S. epidermidis* from the samples showed resistance to Methicillin. More regulations should be encouraged on the use of antibiotics and formation of antibiotic policy guidelines is highly recommended.

*Keywords: Antibiotic resistance; Staphylococcus; vancomycin; methicillin resistance.*

## 1. INTRODUCTION

Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 µm and characterised by individual cocci, which divide in more than one plane to form grape-like clusters. They are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation [1]. There are 32 species and eight sub-species in the genus *Staphylococcus*, many of which preferentially colonise the human body [2], However, *S. aureus* and *S. epidermidis* are the two most characterised and studied strains. *S. aureus* is a major pathogen of increasing importance due to the rise in antibiotic resistance [3]. It is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora [4]. Resistance to antibiotics is one of the biggest problems that face public health [5,6]. This problem is a natural consequence of the adaption of infectious pathogens to antimicrobials used in several areas, including medicine, food animals, crop production and disinfectants in farms, hospital and households [7]. Bacteria have developed resistance to all known antibiotics and, as so, the economic burden associated with these multidrug-resistant bacteria is high.

After the emergence of MRSA as a nosocomial pathogen in the early 1960s [8], an increasing number of outbreaks due to MRSA infections in hospitals have been reported from many countries, ranging from abscesses to life-threatening sepsis, endocarditis, and osteomyelitis [9]. Currently, therapeutic options for MRSA infections are limited to a very few expensive and potentially toxic drugs like teicoplanin, vancomycin, linezolid, daptomycin and streptogramins. Thus, control of MRSA is essential to curtail the introduction and spread of infection [10]. Early detection of MRSA and formulation of an effective antibiotic policy, along with infection control in tertiary care hospitals is of paramount importance from an epidemiological viewpoint. The purposes of this retrospective study were to determine the antibiotic susceptible pattern of *S. aureus* and *S. epidermidis* isolated from environmental, exposed plates and anatomical sites.

## 2. MATERIALS AND METHODS

### 2.1 Sampling

Simple random sampling technique was employed to collect samples from apparently healthy humans, air, water, soil, cow dung within the Federal University of Technology Akure. A total of 75 samples were aseptically collected using a sterile swab sticks from the skin, hand, ear, throat and nasal mucosa of humans, and immediately brought to microbiology laboratory of the Federal University of Technology Akure for bacteriological assay. Soil samples were collected using a soil auger at surface depth (0-15 cm) from a virgin fallow land in the school farm area of The Federal University of Technology Akure, having no pollution history and devoid of hydrocarbon contamination, while polluted soil was taken from The Federal University of Technology Akure, power house and stored in the dark at ambient temperature (±20℃) throughout the study.

### 2.2 Bacteriology

On arrival at the laboratory, each swab stick, exposed plates and serial diluted samples were immediately inoculated onto mannitol salt agar plates and incubated at 37ºC for 24 h. The characteristic isolates were aseptically isolated and characterized using established microbiological methods that include colonial morphology, Gram stain characteristics and catalase and coagulase tests [11]. Isolates that were Gram-positive cocci, catalase positive and coagulated human plasma were considered as *S. aureus* and *S. epidermidis* in addition to other standard biochemical test [12].

### 2.3 Determination of Methicillin Susceptibility for Staphylococci

All confirmed Staphylococci isolates were used to screen for methicillin resistance by inoculation on Mueller Hinton agar supplemented with 4% NaCl. The isolates were similarly inoculated onto the surfaces of plain Mueller-Hinton agar plates and tested against different antibiotics such as

gentamicin (CN, 10 µg), amoxycillin (AM, 30 µg), streptomycin (S, 30 µg), ciprofloxacin (CPX, 10 µg), erythromycin (E, 10 µg), pefloxacin (PEF, 30 µg), septrin (SXT, 30 µg), tetracycline (TE, 30 µg), ampiclox (APX, 30 µg), rocephin (R, 25 µg), zinnacef (Z, 25 µg), vancomycin (VA, 4 µg), flucoxacillin (FL, 4 µg) discs purchased from maxicare medical laboratory were placed and incubated at 37ºC for 24 hrs. The zones of inhibition were measured and compared with national committee for clinical laboratory standards (CLSI) guidelines [13]. The isolates which were resistant to flucoxacillin (<14 mm) were termed methicillin resistant staphylococci while those with zone of inhibition as (≥14 mm) were termed susceptible.

## 2.4 Statistical Analysis of Data

Data obtained were subjected to one way analysis of variance (ANOVA) and Duncan's New Multiple Range Test at 95% confidence level using SPSS 17.0 version. Differences were considered significant at P≤0.05.

## 3. RESULTS

Of the 75 strains, fifty were identified as *S. aureus*, twenty-five as *S. epidermidis*. Among all strains the 33.33% were from exposed plates, 33.33% of anatomical and 33.33% from the environment. In particular ten of the *S. epidermidis* were isolated from exposed plates, five from environmental site and ten from anatomical site. Among *S. aureus*, fifteen strains were isolated from exposed plates, twenty from environmental site and fifteen from anatomical site. The range of values obtained for environmental samples were within 2.1 to 5.4 $x10^6$ cfu /g. The exposed plates have *Staphylococcus* counts of 0.6 to 3.8 $x10^6$ while the *Staphylococcus* counts for anatomical samples ranged from 0.8 to 9.6 $x10^6$. Highest mean count was obtained from examined hands with the value of 9.6 $x10^6$ as shown in Tables 1a-1c. The antibiotic susceptibility testing data are shown in Table 3a, 3b and 3c of the seventeen *S. aureus* and *Staphylococcus epidermidis* screened for susceptibility to the 13 antibiotics the results showed that 5 (6.67%), 25 (33.33%), 75 (100%), and 25 (33.33%) of *S. aureus* and *S. epidermidis* were sensitive to erythromycin, tetracyclin, vancomycin and flucoxacillin respectively. A total of 75 (100%) of *S. aureus* and *S. epidermidis* were resistant to pefloxacin, gentamycin, ampiclox, zinnacef, amoxicillin, rocephin, ciprofloxacin, streptomycin and septrin while 33.33% and 80% were resistant to tetracycline and flucoxacillin and 20% showed intermediate susceptibility. The result showed that 66.67% of *S. aureus* and 33.33% of *S. epidermidis* were resistant to ten antibiotics. Seven and two resistance patterns were shown by *S. aureus* and *S. epidermidis* respectively. The most common resistance pattern for *S. aureus* and *S. epidermidis* was PEF, CN, APX, Z, AM, R, CPX, S, SXT, TE, FL and PEF, CN, APX, Z, AM, R, CPX, S, SXT, E. All *S. aureus* and *S. epidermidis* isolates showed multiple antibiotic resistance. Such that, five isolates were resistant to ten types of antibiotics (8.33%), five isolate were resistant to eleven types of antibiotics (8.33%), ten isolates were resistant to eleven types of antibiotics (16.67%), fifteen isolate was resistant to twelve types of antibiotics (25%), Ten isolates were resistant to twelve types of antibiotics (16.67%), one isolates were resistant to twelve types of antibiotics (8.33%), Two isolates were resistance to thirteen types of antibiotics (16.67%) while for *S. epidermidis,* fifteen isolate was resistant to ten types of antibiotics (60%), Ten isolates were resistant to twelve types of antibiotics (16.67%), five isolates were resistant to eleven types of antibiotics (40%) as shown in Tables 4a and 4b.

**Table 1a. Enumeration of *Staphylococcus* species from exposed plates**

| Source of sample | Bacterial counts |
|---|---|
| Toilet air | $0.6\pm2.00^a$ |
| Waste dustbin air | $0.7\pm2.08^a$ |
| Bathroom air | $1.2\pm2.08^a$ |
| Roof air | $2.6\pm2.65^b$ |
| Poultry air | $3.8\pm1.53^c$ |

**Table 1b. Enumeration of *Staphylococcus* species from environmental sites**

| Source of sample | Bacterial counts ($x10^6$ cfu/g) |
|---|---|
| Polluted soil | $2.1\pm1.53^b$ |
| cow dung | $2.4\pm2.00^b$ |
| Unpolluted water | $3.5\pm3.00^c$ |
| Polluted water | $3.6\pm4.00^c$ |
| Unpolluted soil | $5.4\pm4.73^d$ |

**Table 1c. Enumeration of *Staphylococcus* species from anatomical sites**

| Source of sample | Bacterial counts |
|---|---|
| Ear | $1.2\pm2.00^a$ |
| Nose | $0.8\pm1.52^a$ |
| Throat | $2.1\pm1.00^b$ |
| Skin | $6.3\pm1.53^e$ |
| Hand | $9.6\pm15.23^f$ |

*Significantly difference at P≤0.05*

**Table 2. *Staphylococci* source and the types of *Staphylococcus* isolated**

| *Staphylococcus* sources | *Staphylococcus* type | Percentage distribution (%) |
|---|---|---|
| Unpolluted soil, Nose, Waste dustbin air, Toilet air, Ear. | *Staphylococcus epidermidis* | 33.33% |
| Throat, cow dung, Bathroom air, Polluted water, Polluted soil, Hand, Roof air, unpolluted water, skin, poultry air. | *Staphylococcus aureus* | 66.67% |

**Table 3a. Antimicrobial susceptibility patterns of *S. aureus* isolates from anatomical site**

| Antibiotic | Drug concentration (µg) | Susceptibility (%) | Resistant (%) | Intermidate (%) |
|---|---|---|---|---|
| PEF | 30 | 0 | 100 | 0 |
| CN | 10 | 0 | 100 | 0 |
| APX | 30 | 0 | 100 | 0 |
| Z | 25 | 0 | 100 | 0 |
| AM | 30 | 0 | 100 | 0 |
| R | 25 | 0 | 100 | 0 |
| CPX | 10 | 0 | 100 | 0 |
| S | 30 | 0 | 100 | 0 |
| SXT | 30 | 0 | 100 | 0 |
| E | 10 | 33.33 | 66.67 | 0 |
| TE | 30 | 33.33 | 33.33 | 33.33 |
| VA | 30 | 100 | 0 | 0 |
| FL | 30 | 50 | 50 | 0 |

**Table 3ai. Antimicrobial susceptibility patterns of *S. epidermidis* isolates from anatomical site**

| Antibiotic | Drug concentration (µg) | Susceptibility (%) | Resistant (%) | Intermidate (%) |
|---|---|---|---|---|
| PEF | 30 | 0 | 100 | 0 |
| CN | 10 | 0 | 100 | 0 |
| APX | 30 | 0 | 100 | 0 |
| Z | 25 | 0 | 100 | 0 |
| AM | 30 | 0 | 100 | 0 |
| R | 25 | 0 | 100 | 0 |
| CPX | 10 | 0 | 100 | 0 |
| S | 30 | 0 | 100 | 0 |
| SXT | 30 | 0 | 100 | 0 |
| E | 10 | 0 | 0 | 0 |
| TE | 30 | 100 | 0 | 0 |
| VA | 30 | 100 | 0 | 0 |
| FL | 30 | 50 | 50 | 0 |

*Keys: PEF, pefloxacin; CN, gentamycin; APX, ampiclox; Z, zinnacef; AM, amoxicillin; R, rocephin; CPX, ciprofloxacin; S, streptomycin; SXT, septrin; E, erythromycin; TE, tetracyclin; VA, vancomycin; FL, flucoxacillin; PR, percentage resistance (%)*

**Table 3b. Antimicrobial susceptibility patterns of *S. aureus* isolates from exposed plates**

| Antibiotic | Drug concentration (µg) | Susceptibility (%) | Resistant (%) | Intermidate (%) |
|---|---|---|---|---|
| PEF | 30 | 0 | 100 | 0 |
| CN | 10 | 0 | 100 | 0 |
| APX | 30 | 0 | 100 | 0 |
| Z | 25 | 0 | 100 | 0 |
| AM | 30 | 0 | 100 | 0 |
| R | 25 | 0 | 100 | 0 |
| CPX | 10 | 0 | 100 | 0 |
| S | 30 | 0 | 100 | 0 |
| SXT | 30 | 0 | 100 | 0 |
| E | 10 | 0 | 0 | 0 |
| TE | 30 | 66.67 | 33.33 | 0 |
| VA | 30 | 100 | 0 | 0 |
| FL | 30 | 33.33 | 66.67 | 0 |

**Table 3bi. Antimicrobial susceptibility patterns of *S. epidermidis* isolates from exposed plates**

| Antibiotic | Drug concentration (µg) | Susceptibility (%) | Resistant (%) | Intermidate (%) |
|---|---|---|---|---|
| PEF | 30 | 0 | 100 | 0 |
| CN | 10 | 0 | 100 | 0 |
| APX | 30 | 0 | 100 | 0 |
| Z | 25 | 0 | 100 | 0 |
| AM | 30 | 0 | 100 | 0 |
| R | 25 | 0 | 100 | 0 |
| CPX | 10 | 0 | 100 | 0 |
| S | 30 | 0 | 100 | 0 |
| SXT | 30 | 0 | 100 | 0 |
| E | 10 | 0 | 0 | 0 |
| TE | 30 | 100 | 0 | 0 |
| VA | 30 | 100 | 0 | 0 |
| FL | 30 | 50 | 50 | 0 |

*Keys: PEF, pefloxacin; CN, gentamycin; APX, ampiclox; Z, zinnacef; AM, amoxicillin; R, rocephin; CPX, ciprofloxacin; S, streptomycin; SXT, septrin; E, erythromycin; TE, tetracyclin; VA, vancomycin; FL, flucoxacillin; PR, percentage resistance (%)*

**Table 3c. Antimicrobial susceptibility patterns of *S. aureus* isolates from environmental samples**

| Antibiotic | Drug concentration (µg) | Susceptibility (%) | Resistant (%) | Intermidate (%) |
|---|---|---|---|---|
| PEF | 30 | 0 | 100 | 0 |
| CN | 10 | 0 | 100 | 0 |
| APX | 30 | 0 | 100 | 0 |
| Z | 25 | 0 | 100 | 0 |
| AM | 30 | 0 | 100 | 0 |
| R | 25 | 0 | 100 | 0 |
| CPX | 10 | 0 | 100 | 0 |
| S | 30 | 0 | 100 | 0 |
| SXT | 30 | 0 | 100 | 0 |
| E | 10 | 0 | 0 | 0 |
| TE | 30 | 25 | 25 | 50 |
| VA | 30 | 100 | 0 | 0 |
| FL | 30 | 25 | 0 | 0 |

**Table 3ci. Antimicrobial susceptibility patterns of *S. epidermidis* isolates from environmental samples**

| Antibiotic | Drug concentration (µg) | Susceptibility (%) | Resistant (%) | Intermidate (%) |
|---|---|---|---|---|
| PEF | 30 | 0 | 100 | 0 |
| CN | 10 | 0 | 100 | 0 |
| APX | 30 | 0 | 100 | 0 |
| Z | 25 | 0 | 100 | 0 |
| AM | 30 | 0 | 100 | 0 |
| R | 25 | 0 | 100 | 0 |
| CPX | 10 | 0 | 100 | 0 |
| S | 30 | 0 | 100 | 0 |
| SXT | 30 | 0 | 100 | 0 |
| E | 10 | 0 | 0 | 0 |
| TE | 30 | 100 | 0 | 0 |
| VA | 30 | 100 | 0 | 0 |
| FL | 30 | 100 | 0 | 0 |

*Keys: PEF, pefloxacin; CN, gentamycin; APX, ampiclox; Z, zinnacef; AM, amoxicillin; R, rocephin; CPX, ciprofloxacin; S, streptomycin; SXT, septrin; E, erythromycin; TE, tetracyclin; VA, vancomycin; FL, flucoxacillin; PR, percentage resistance (%)*

**Table 4a. Resistant pattern of *Staphylococcus aureus* antibiotics**

| No of antibiotics | No of isolates | Resistant pattern | Percentage (%) |
|---|---|---|---|
| 10 | 1 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E | 8.33 |
| 11 | 1 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E | 8.33 |
| 11 | 2 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E, FL | 16.67 |
| 12 | 3 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, TE, FL | 25 |
| 12 | 2 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E, TE, FL | 16.67 |
| 12 | 1 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E, FL | 8.33 |
| 13 | 2 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E, TE, FL | 16.67 |

**Table 4b. Resistant pattern of *Staphylococcus epidermidis* antibiotics**

| No of antibiotics | No of isolates | Resistant pattern | Percentage (%) |
|---|---|---|---|
| 10 | 3 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E | 60 |
| 11 | 2 | PEF, CN, APX, Z, AM, R, CPX, S, SXT, E, FL | 40 |

*Keys: PEF, pefloxacin; CN, gentamycin; APX, ampiclox; Z, zinnacef; AM, amoxicillin; R, rocephin; CPX, ciprofloxacin; S, streptomycin; SXT, septrin; E, erythromycin; TE, tetracyclin; VA, vancomycin; FL, flucoxacillin; PR, percentage resistance (%)*

## 4. DISCUSSION

Staphylococci are gram-positive, non-motile and ubiquitous bacteria that include different pathogenic species known to be responsible for human and animal infections. These groups of microorganism colonize the skin, hair, nose, foods, animals and throat of people. *S. aureus* is a common pathogen associated with multiple disease processes; an important nosocomial and community-acquired pathogen and one of the major bacterial agents causing foodborne diseases in humans [2]. The staphylococci loads monitored from the different environments were significantly different at p≤0.5. The range of count obtained for staphylococci conformed to the findings of Boboye et al. [14] who reported the index of $10^4$ to $10^6$ cfu/g for the isolated organisms from unpolluted soil and water in Ilaje, Nigeria. High Staphylococcal loads were observed from *Staphylococcus aureus* from hand, *Staphylococcus aureus* from skin, *Staphylococcus epidermidis* from unpolluted water and *Staphylococcus aureus* from typed culture. The presence of Staphylococci in exposed plated, anatomical and environmental samples conformed to Songer and Post [15] who isolated *S. aureus* from water, dust, air, mucosa of nasopharynx, skin of humans and animals. Balaban and Rasooly [16] had also stated that *S. aureus* is an important agent of food poisoning all over the world. The presence of *S. aureus* in soil samples could be attributed to the shedding of animal wastes into the soil. The occurrence of *Staphylococcus aureus* and *S. epidermidis* in polluted water has been reported by Al-Zubeiry [17]. Staphylococci displayed varying degree of resistance to commonly used antibiotics. The isolates from this study were resistant to the amoxicillin, which is in agreement with previous report by Ateba et al. [18]. The susceptibility of the isolates to some antibiotics such as tetracyclin, vancomycin and flucoxacillin have been reported in Nigeria [10] and Italy [19] but deviated from what had been reported for tested staphylococci in United States [20]. Also, Daka et al.[21] discovered that *S. aureus* was resistant to ampiclox and pefloxacin which is in agreement with this study. The release of cross-contaminated water into environment has been the several means of increasing the number of multiple resistant staphylococci and has been a serious health problem [22]. Gundogan et al. [23] had associated the resistant pattern of Staphylococci to the ability to produce an exo-polysaccharide, which limits the action of drugs. Methicillin was indicated for treatment of staphylococcal infections due to penicillinase producing Staphylococci. Methicillin resistant strains gradually evolved during last three decades which accounted for less than 0.1% of *S. aureus* in 1960s. The resistance to gentamycin, zinnacef, amoxicillin, rocephin, ciprofloxacin, streptomycin, septrin, erythromycin may not be unconnected with the production of β-lactamase or cephalosporinase enzymes by *S. aureus* and *S. epidermidis* which destroy the antimicrobial agents. Another observation is that most isolates of *S. aureus* and *S. epidermidis* were resistant to a large number of commonly prescribed antibiotics as described by Olukoya et al. [24]. MRSA strains isolated in the current study have shown a high level of resistance to antibiotics, this is higher than levels of resistance described in earlier studies in India [25] and Iran [26]. Infections caused by methicillin-resistant strains may be more difficult to manage or more expensive to treat, because vancomycin is

inherently less efficacious. The increasing prevalence of MRSA will inevitably increase vancomycin use, adding further to the problem of antibiotic-resistant gram-positive bacteria [27]. The activities of antibiotics against *S. aureus* and coagulase negative *S. epidermidis* isolated from the natural environment, exposed plates and anatomical sites and the human body showed the varied levels of multiple antibiotics resistance.

## 5. CONCLUSION

From the result of the study, it is therefore recommended that tetracyclin, vancomycin and flucoxacillin may be the best choice of antimicrobials for treating infections caused by *S. aureus* and *S. epidermidis*. Antimicrobial susceptibility patterns are important for clinicians in selecting empiric antimicrobial therapy. Nationwide survey should be undertaken for rational formulation of public healthcare policies and providing useful information on the global surveillance of this pathogen.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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