

Original Research Article

ISOLATION AND ANTIBIOGRAM OF *Staphylococcus aureus* FROM PATIENTS ADMITTED AT SPECIALIST HOSPITAL OKITIPUPA, ONDO STATE, NIGERIA

ABSTRACT

Aims: To isolate and determine the antimicrobial resistance and susceptibilities of *Staphylococcus aureus* from patients hospitalized at State Specialist Hospital, Okitipupa, Ondo State, Nigeria.

Study design: Prospective cross sectional.

Place and Duration of Study: Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, between March and April 2015.

Methodology: Forty three (43) samples were obtained from nasal cavity, skin, wound, or umbilical cord of 20 patients aged 4 days to 80 years. Isolates were identified by cultural characteristics on Mannitol Salt Agar, Nutrient agar and biochemical tests. Antibiotic susceptibility testing was performed by the disk diffusion method (Kirby-Bauer). Multiple antibiotic resistance (MAR) index for each *S. aureus* was determined.

Results: Seventeen *S. aureus* isolates were identified. Nine *S. aureus* strains recovered from 18 samples from male patients was higher than 8 strains recovered from 25 samples from females. 21-30 age-group yielded the highest number of *S. aureus* isolates (7 strains), followed by 31-40 age group (5 strains). Eleven (64.7%) strains showed multiple resistances ranging from 55.6% to 88.8%, four (23.5%) strains showed multiple resistances ranging from 33.3% to 44.4%, one (5.9%) strain (N₁) showed 100% resistance, while one (5.9%) strain (O₁) showed 100% sensitivity. 70.6% of the isolates had a MAR index above 0.5 indicating that they probably originated from an environment where antibiotics are frequently used or abused. The highest resistance was offered to the β -lactam antibiotics, including oxacillin (88.2%), cloxacillin (82.4%) and amoxicillin/clavulanic acid (76.5%); and the cephalosporins: cefuroxime (70.6%) and ceftazidime (64.7). Resistance to ceftriaxone (cefuxitin) was the lowest (23.5%). Resistance

to erythromycin was high (70.6%), but gentamicin and ofloxacin were offered relatively low resistance (41.2%) respectively.

Conclusion: The study revealed multiple antibiotic resistances in *S. aureus* isolated from hospitalized patients. The preponderance of multiple antibiotic *S. aureus* in the hospital environment continues to present challenges to the health sector. The high rates of resistance to the β -lactams – oxacillin, cloxacillin, amoxicillin-clavulanic acid and cephalosporins highlight the decreasing importance of β -lactams in the therapy of *S. aureus* infections.

Keywords: *Staphylococcus aureus*, antibiogram, MAR Index, hospital environment, Nigeria

1. Introduction

Staphylococcus aureus is a Gram-positive, facultative anaerobic, unencapsulated cocci which occur in clusters. The bacterium occurs as a commensal on the human anterior nares and on the skin (and less commonly in other locations) of the human body [1,2]. It is a normal inhabitant of the healthy lower reproductive tract of women [3,4]. *S. aureus* colonizes approximately 20% to 30% of the human population [5], and is reported to be found in 25% to 27% of health workers [6]. The presence of *S. aureus* does not always indicate infection. *S. aureus* can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain [7].

Though the bacterium occurs as normal flora, it can infect tissues when the skin or mucosal barriers have been breached. This can lead to many different types of infections ranging from furuncles and carbuncles to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis [8,9]. *S. aureus* is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections [10]. Each year, some 500,000 patients in United States' hospitals contract a staphylococcal infection [11]. The control of infections caused by *S. aureus* relies heavily on intensive use of antibiotic drugs. However, there is increasing evidence of drug resistance by this microorganism [12].

27 The treatment of choice for *S. aureus* infection is penicillin, a β -lactam. Penicillin is bactericidal by
28 inhibiting the formation of peptidoglycan cross-linkages that provide the rigidity and strength in a bacterial
29 cell wall. In most countries, but penicillin resistance is extremely common. This is because many
30 members of the genus *Staphylococcus* produce penicillinase (a β -lactamase), which inactivate
31 penicillins, making them naturally resistant to these antibiotics [13]. Following the emergence of penicillin
32 resistance, penicillinase-resistant β -lactam antibiotics such as oxacillin or flucloxacillin were developed
33 and used as first-line therapy. However resistance to penicillinase-resistant β -lactams soon emerged in
34 many bacterial strains and has become a global health problem [14].

35 *Staphylococcus aureus* continues to offer challenges to medical science in the area of resistance to
36 chemotherapeutic agents leading to treatment failures using common antibiotics [15]. Adejuwon *et al.*,
37 (2010) reported the presence of *S. aureus* in pus from acne which was sensitive to gentamicin,
38 tetracycline, amoxicillin, amoxicillin/clavulanic acid, chloramphenicol and sulphamethoxazole but resistant
39 to ampicillin, erythromycin, cloxacillin, cotrimoxazole, streptomycin and penicillin [16].

40 Attempts to control diseases caused by *S. aureus* through the use of antibiotics have resulted in
41 increased prevalence of resistant strains of the organism [17,18]. Therefore, in order to effectively treat
42 infections caused by *S. aureus*, culture and antibiotic sensitivity tests must first be performed. Once
43 culture and sensitivity results confirm the type of bacterial infection and sensitivity pattern, treatment may
44 be modified [19].

45 An antibiogram is the result of an in vitro sensitivity test of an isolated bacterial strain to different
46 antibiotics. The antibiogram shows the profile of the antimicrobial resistance and susceptibility of a
47 particular microorganism. The correlation of in vitro to in vivo is often high enough for the test to be
48 clinically useful. In the hospital, antibiograms are commonly used to help guide empirical antimicrobial
49 treatment and are an important component of detecting and monitoring trends in antimicrobial resistance
50 [20].

51 Strategies for monitoring antibiotic susceptibility patterns should include periodic isolation and testing of
52 isolates for antibiotic susceptibility in all localities, using a state-owned hospital as a sampling center for a

53 cross sectional study. Results of cross sectional studies usually provide a good overview of situations in
54 the entire community.

55 The aims of the present study were to isolate and determine the profile of antimicrobial resistance and
56 susceptibilities of ~~Staphylococcus~~ *S. aureus* isolated from patients hospitalized at State Specialist
57 Hospital, Okitipupa, Ondo State, Western Nigeria. No previous study of this nature has been carried out
58 in Okitipupa L.G.A in Ondo South senatorial district. This study seeks to provide data on the resistance
59 pattern for *S. aureus* isolated from the hospital in Okitipupa.

60 2. Materials and methods

61 2.1 Samples collection, Handling, Culture and Identification

62 Samples were collected from State Specialist Hospital, Okitipupa from March to April 2015. Sterile cotton
63 swabs moistened in sterile distilled water were used to collect samples from the skin, nostrils, wound
64 exudates or umbilical cord excision sites of hospitalized patients. The swabs sticks were inserted into
65 their tubes and transported immediately into the Microbiology laboratory for analysis. Prior to sample
66 collection, permission was obtained from the Hospital Authorities through a written application. The
67 patients also had to give their consent before samples were collected from them.

68 All swab samples were directly inoculated onto Nutrient agar and Mannitol salt agar (MSA) plates. The
69 plates were incubated at 37°C for 24 h, and then examined for colony characteristics. Colonies that were
70 white, round, smooth, substantial and opaque on Nutrient agar, and colonies producing a golden-yellow
71 pigment on MSA were presumptively considered *S. aureus* and subsequently sub-cultured on nutrient
72 agar to obtain pure, discrete colonies. Seventeen Ppure colonies (named A1...V2) that yielded Gram
73 positive clustered cocci, and reacted positively to coagulase and catalase tests were identified as *S.*
74 *aureus* [21,22].

Comment [A1]: Mention the name of all isolates here

75 2.2 Antimicrobial susceptibility testing

76 The isolates were screened using the disc diffusion method [23]. Gram positive antibiotic discs
77 (Rapidflex) included ceftazidime 30µg, cefuroxime 30µg, ceftriaxone 30µg, cloxacillin 5µg,
78 amoxicillin/clavulanic acid 30µg, gentamicin 10µg, erythromycin 30µg, ofloxacin 5µg. Oxacillin (1µg)
79 obtained from Oxoid Ltd Britain was also screened.

Antimicrobial susceptibility tests were performed for all isolates according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) [24]. Bacterial suspensions were prepared, adjusted to the 0.5 McFarland Standards, and inoculated onto Mueller-Hinton agar (Oxoid) by surface swabbing. Using sterile forceps, the antibiotic-containing discs were placed aseptically on the inoculated plates and left on the table for 1 hour for proper diffusion to occur. The plates were incubated in an inverted position, at 35°C for 16-18 hours and thereafter examined for clear zones of inhibition. Inhibition zone diameters (IZD) around each antibiotic disk (if any), were measured using a transparent ruler, and recorded in millimeters (mm). A standardized table was used to determine if the bacterium was "Resistant", "Intermediate" or "Sensitive".

2.3 Determination of multiple antibiotic-resistance (MAR) index

Multiple antibiotic-resistance (MAR) index of each *S. aureus* isolate was calculated using the formula: MAR Index = a/b, where a is number of antibiotics the isolate is resistant to; and b is number of antibiotics tested.

3. Results

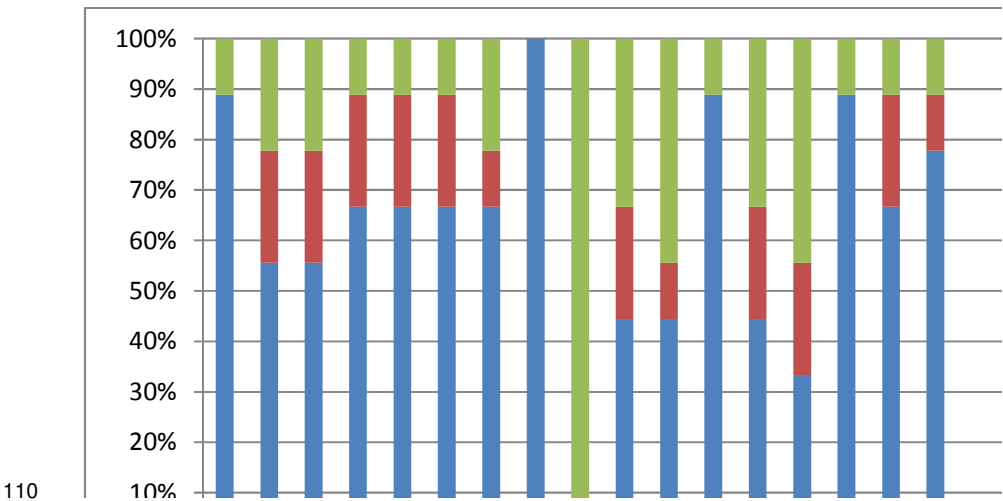
3.1 Isolation of *S. aureus*

A total of 43 samples were collected from skin, nares, wound or umbilical cord scar of twenty patients and analyzed. Seventeen (17) *S. aureus* isolates were recovered from the samples. The skin samples yielded 8 (47.06%) of the *S. aureus* isolates, nasal samples yielded an equal number of *S. aureus* isolates: 8 (47.06%), while a traumatic wound yielded 1 (5.88%). Sex-based analysis revealed that for male patients, nine *S. aureus* isolates were recovered from eighteen samples (9/18), while for females, eight *S. aureus* isolates were recovered from twenty-five samples (8/25). Age-based analysis showed rates of *S. aureus* isolation among the age groups were: 21-30 years (7 isolates), 31-40 years (5 isolates), 41-50 years (2 isolates), 51-60 years (1 isolate), and 71-80 years (2 isolates). The presence of *S. aureus* from the skin of 4 day old infants and not from their umbilical cord wounds implies that skin of infants as young as 4 days can be colonized by *S. aureus* in consonance with previous views [2].

3.2 ~~Results of antibiotic~~ Antibiotic susceptibility testing of *S. aureus* isolates

3.2.1 Percentage of resistances, intermediates and sensitivities of *S. aureus* isolates

107 The seventeen (17) *S. aureus* isolates showed varying degrees of susceptibilities and resistances to the
108 antibiotics. The percentage resistances, intermediates and sensitivities of each *S. aureus* isolate to the
109 antibiotics are represented by a stacked column chart (Figure 1).



111 | Figure 1: Percentage resistances (R), intermediates (I) and sensitivities (S) shown by *S. aureus* isolates (A₁ –V₂) to the antibiotics.
112 Axis-Y: percentage of susceptibility. Axis-X: isolate number.
113 The Figure shows the overall susceptibility of each isolate to nine antibiotics. E.g isolate B₁ was resistant to 5 (55.6%) antibiotics,
114 sensitive to 2 (22.2%) and intermediate to 2 (22.2%).

115 The chart in Figure 1 clearly depicts the degrees of resistances, intermediates and sensitivities shown by
116 each *S. aureus* isolate to the antibiotics. Strain N₁ showed resistance to all the antibiotics used (100%
117 resistance), and strain O₁ showed sensitivity to all the antibiotics used (100% sensitivity). Eleven (64.7%)
118 isolates showed multiple resistances ranging from 55.6% to 88.8%. Four (23.5%) isolates showed
119 multiple resistances ranging from 33.3% to 44.4%.

120 3.2.2. Multiple antibiotic-resistance (MAR) indices *S. aureus* isolates

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121 Multiple antibiotic-resistance (MAR) index was calculated using the formula: MAR Index = a/b, where a is
122 number of antibiotics the isolate is resistant to; and b is number of antibiotics tested. MAR Indices of the
123 isolates are presented in Table 1.

124 **Table 1: Multiple antibiotic resistance (MAR) Indices of *S. aureus* isolates**

<i>S. aureus</i> isolate (Code number)	MAR index
A ₁	0.89
B ₁	0.56
B ₂	0.56
C ₂	0.67
G ₂	0.67
H ₂	0.67
I ₃	0.67
N ₁	1.00
O ₁	0.00
O ₂	0.44
P ₁	0.44
P ₂	0.89
Q ₁	0.44
Q ₂	0.33
T ₂	0.89
V ₁	0.67
V ₂	0.78

Comment [A2]: Be consistent in writing the isolate name in all Tables and Figs and through the MS, such as A1 or A₁

125 MAR index shows the level of multiple antibiotic-resistance attained by a particular bacterial strain. From
 126 data presented in Table 1, MAR Indices of the isolates varied from 0.00 (not resistant to any antibiotic
 127 tested) to 1 (resistant to all antibiotics tested). 70.6% of the isolates had a high MAR index above 0.5.

128 **3.2.3. Resistance profile of *S. aureus* isolates**

129 The number and percentage of *S. aureus* isolates resistant to each antibiotic used is presented on Table
 130 2.

131 **Table 2: Number (Percentage) of *S. aureus* isolates resistant to each antibiotic**

Antibiotics	Number (%) of resistant strains
CAZ	11 (64.7)

CRX	12 (70.6)
CTR	4 (23.5)
OX	15 (88.2)
CXC	14 (82.4)
AUG	13 (76.5)
GEN	7 (41.2)
ERY	12 (70.6)
OFL	7 (41.2)

132 **Key:** CAZ- ceftazidime, CRX- cefuroxime, CTR- ceftriaxone, OX- oxacillin, CXC- cloxacillin, AUG-
133 amoxicillin/clavulanic acid, GEN- gentamicin, ERY- erythromycin, OFL- ofloxacin.

134 Table 2 shows how each antibiotic fared with the isolates. For instance, to ceftazidime (CAZ) 64.7% of all
135 the isolates were resistant.

136 The highest resistance was offered to the β -lactam antibiotics, including oxacillin (88.2%), cloxacillin
137 (82.4%) and amoxicillin/clavulanic acid (76.5%); and the cephalosporins: cefuroxime (70.6%) and
138 ceftazidime (64.7). However, resistance to ceftriaxone (cefuxitin) was the lowest (23.5%). Resistance to
139 erythromycin was high (70.6%), but gentamicin and ofloxacin were offered relatively low resistance
140 (41.2%).

141 **4. Discussion**

142 The recovery of *S. aureus* from patients varied among the sexes and age groups. Sex-based analysis
143 revealed that for male patients, nine *S. aureus* isolates were recovered from eighteen samples (9/18),
144 while for females, eight *S. aureus* isolates were recovered from twenty-five samples (8/25). Age-based
145 analysis showed rates of *S. aureus* isolation among the age groups were: 21-30 years (7 isolates), 31-40
146 years (5 isolates), 41-50 years (2 isolates), 51-60 years (2 isolates), and 61-70 years (1 isolate)

147 Results from the present study revealed multiple antibiotic resistance (MAR) in *S. aureus* strains isolated
148 from Specialist Hospital, Okitipupa. 70.6% of the isolates had a MAR index above 0.5 indicating that they

149 probably originated from an environment where antibiotics are frequently used or abused. Multiple
150 resistance in *S. aureus* seems to be a longstanding problem yet to be solved, since a similar finding was
151 reported from Zaria, Nigeria in 2003 [25].

152 The resistance profile showed a high rate of resistance to β -lactam antibiotics including oxacillin (88.2%),
153 cloxacillin (82.4%), amoxicillin/clavulanic acid (76.5%), and the cephalosporins: cefuroxime (70.6%) and
154 ceftazidime (64.7). However, resistance to ceftriaxone (cefuxitin) was the lowest (23.5%). Resistance to
155 β -lactam antibiotics in this study may be due to hyper-production of β -lactamase enzymes which
156 inactivate this class of antibiotics [13,14]. Resistance to erythromycin was high (70.6%), but gentamicin
157 and ofloxacin were offered relatively low resistance (41.2%). Findings in this study agree with previous
158 workers who reported high rates of antibiotic resistance in *S. aureus* [15,16, 26]. However, the findings
159 differ from reports from another part of Nigeria where phenotypic resistance to oxacillin was 40.3%,
160 cefuxitin 46.5%, gentamicin 11.5% and erythromycin 21.2% [27].

161 The findings of this study are in consonance with reports that resistant microbes abound in the hospital
162 environments because of antibiotic treatment regimes administered to hospital patients. In hospitals,
163 inappropriate use of antimicrobial agents tends to create a selective pressure that promotes the
164 emergence of resistant strains and predisposes patients to colonization with such organisms. The
165 carriage of *S. aureus* on the bodies, especially hands of 25%-27% of health workers reported by previous
166 workers [6] is an important factor in transferring resistant microbes to patients hospitalized in the wards.

167 The dominance of antibiotic resistant pathogens in the hospital environment is a phenomenon that must
168 be approached in a more proactive manner. More emphasis should be placed on proper and regular hand
169 washing by health workers, sterilization/disinfection of instruments, materials and surfaces, as well as
170 maintenance of sanitary conditions in the wards. All these will greatly reduce the occurrence and transfer
171 of resistant microbes in the hospitals. Regular washing of bed clothes with soaps and disinfectants, and
172 use of sterile hand gloves when handling patients will ensure that antibiotic resistant microbes do not
173 survive and are not transferred from patient to patient.

174 Research efforts to develop alternative treatments for resistant bacterial infections should attract more
175 funds from the government and stakeholders on the private sector. Special attention should be focused
176 on medicinal plant extracts which have been used as antimicrobials for centuries with no development of
177 resistance. Vaccine development is another area that should not be undermined in the search for solution
178 for antimicrobial resistance in bacterial pathogens. More research effort is needed in the area of DNA
179 vaccines, which is reported to be effective, with no adverse effects in experimental animals [28,29].

180 **5. Conclusions**

182 High MAR indices above 0.5 shown by 70.6% of *S. aureus* strains isolated from Specialist Hospital,
183 Okitipupa, reveals the challenges in the area empirical therapy for diseases caused by *S. aureus*. The
184 high rates of resistance to the β -lactam antibiotics including oxacillin (88.2%), cloxacillin (82.4%),
185 amoxicillin/clavulanic acid (76.5%), and the cephalosporins: cefuroxime (70.6%) and ceftazidime (64.7)
186 highlights the decreasing importance of these drugs in antimicrobial therapy for *S. aureus* infections.
187 Antibiotic resistance is highest where a majority of the population is poor and ignorant of appropriate use
188 of antibiotics as is the case with the study area in this investigation. The authorities in the health sector
189 should mount public enlightenment programs to educate the masses on the dangers of abusing
190 antimicrobials, as well as the appropriate ways to use them. Development of and strict adherence to
191 sound antibiotic policies in the health sector will help slow the emergence of drug resistance.

192 **Competing Interests**

193 Author has declared that no competing interests exist.

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