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Comparative Evaluation of Methicillin-resistant *Staphylococcus aureus* (MRSA) Isolates from Hospital and Community Settings in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ENM, CIM and UOE designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors ENM, CIM, CFU and UOE anchored the field study, managed the analyses of the study while authors ENM, UEG, IT and UOE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The ability of *Staphylococcus aureus* to resist cefoxitin amongst other antibiotics has made it a significant public health problem in hospital and community settings. In this study, the occurrence of cefoxitin (methicillin) resistant *S. aureus* (MRSA) in the University of Calabar Medical Center and community was evaluated after obtaining informed consent and ethical approval. A total of 150 clinical samples collected from participants seen at the Medical Center and community settings

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were analyzed. Isolates were identified and characterized following standard microbiological procedures while antimicrobial sensitivity was carried out using the disc diffusion method. A total of 42 *S. aureus* strains were isolated, out of which 27(64.3%) were from the Medical Center and 15(35.7%) were from the community samples. Antimicrobial susceptibility testing of test isolates showed high resistance to the test antibiotics with cefoxitin being the highest (60%). Out of the 60% MRSA recovered, 74.1% (20/27) were from University of Calabar Medical Center while 33.3% (5/15) were from the Community. In addition, MRSA isolates from both locations also showed resistance to other antibiotics including amoxicillin, ampicillin+cloxacillin, levofloxacin, norfloxacin and erythromycin. This study revealed a high occurrence of Hospital-setting methicillin-resistant *S. aureus* (HA-MRSA) strains compared to Community-setting methicillin-resistant *S. aureus* (CA-MRSA) strains. This study further revealed that MRSA were multi-drug resistant. Thus, good infection control practices including identifying and treating MRSA carriers, moderate use of antibiotics and hand washing could reduce the burden associated with MRSA-related infections. To further establish and characterize multidrug resistant *S. aureus* strains, genotypic studies may be employed.

Keywords: Cefoxitin; frequency; MRSA; resistance; Staphylococcus aureus.

1. INTRODUCTION

Staphylococcus aureus is the most important human pathogen among the *Staphylococci* that has been associated with high morbidity and mortality rates; making it a major public health challenge globally [1]. *S. aureus* is commonly reported as a commensal in the external nares of 20 – 40% of adults, as well as in the intertriginous skin folds, perineum, the axillae and the vagina [2]. It is a versatile human pathogen that have been implicated in infections ranging from relatively mild skin and soft tissues to life threatening sepsis and pneumonia including toxic shock syndrome [2]. In addition, it has been implicated in the majority of hospital and community-acquired infections reported [3,4]. Its disease-causing potential is influenced by the ability of the organism to secrete numerous cell surface virulence factors, as well as the propensity to develop resistance to multiple antibiotics [1].

Originally, penicillin was the drug of choice for the treatment of *S. aureus* infections but this only lasted for a few years due to the development of resistance [5]. Studies have revealed that the development of resistance is due to the ability of the organism to synthesize β-lactamases which hydrolyze the β-lactam ring in Penicillin [3,5]. Equally implicated in this phenomenon is the acquisition of extra chromosomal elements that codes for *mecA* gene [6]. In 1961, a few years after methicillin was introduced into clinical practice, methicillin resistant *S. aureus* was observed [3]. *S. aureus* strains resistant to methicillin are also reported to be resistant to cefoxitin [7,8]. This may be due to the fact that methicillin and cefoxitin are both β-lactam antibiotics [8].

Cefoxitin (methicillin) resistant *S. aureus* (MRSA) could be hospital-acquired cefoxitin-resistant *S. aureus* (HA-MRSA) and community-acquired cefoxitin resistant *Staphylococcus aureus* (CA-MRSA). HA-MRSA is one of the common pathogen associated with surgical wound infections and infections related to the use of implanted devices such as catheters within hospital settings [9] whereas CA-MRSA is generally accepted as the major cause of skin infections such as pimples or boils, and is classified. CA-MRSA is commonly reported among healthy individuals [10].

Studies revealed that Community-acquired strains differ significantly from Hospital-acquired strains in their possession of *Staphylococcus* cassette chromosome *mec* (SSCmec) types IV-V and in their ability to synthesize the bio-competent cytotoxin Panton-Valentine Leukocidin (PVL) [11,8]. In addition, they have the potential to resist particularly the β-lactam antibiotics [12].

In Nigeria, several studies have shown that the prevalence of resistance to antibiotics as a result of factors like poverty, self-medication, administration of sub-optimal dose, availability of counterfeit drugs, and so on, have led to significant increase in healthcare cost, morbidity and mortality rates [13]. A number of studies exist on antibiotic resistance in *S. aureus* [14,15], however, data on their resistance in settings with respect to cefoxitin is limited. Furthermore, in Calabar, there is little or no data on cefoxitin

resistance in both hospital and community settings. Given the public health implications of MRSA infections and their changing patterns of resistance, periodic surveillance may be considered an important monitoring tool. This study was therefore, aimed at comparatively evaluating the resistance of *S. aureus* to cefoxitin in hospital and community settings.

2. MATERIALS AND METHODS

2.1 Study Site

Calabar is the capital of Cross River State in Southern Nigeria. The major dwellers are the Efiks, Efuts, Quas, Ejagams and Ibibios. Others include the Igbos, Yorubas, Hausas/Fulanis, other ethnicities and nationalities. It lies on latitude $50°32^1$ and $40°22^1$ and longitude $70°50^1$ and $90°28^1$, and about 481 sqkm and had a population of 371,022 as at the 2006 census [16]. The city is currently experiencing population explosion with its attendant high crime rates, poverty, malnutrition, high cost of living and health complication together with high morbidity and mortality [17]. The University of Calabar Medical centre is a service department of the University saddled with the responsibility of providing appropriate healthcare for staff, students and others engaging in downstream activities within the University of Calabar community and environs.

2.2 Sources of Sample

A total of 150 swab specimens were collected with 75 from the nose of people within the University of Calabar community while the remaining 75 samples were collected from patients in the University of Calabar Medical Centre. Informed consents were obtained from all the participants before their inclusion in the study. In addition, ethical approval with ref number (RP/REC/2017/428) granted by the Ethical board of the University of Calabar was obtained prior to this study. All participants were between the ages ranging from 20-69 in both the community and the hospital settings.

2.3 Antibiotics Used in this Study

In addition to cefoxitin, the following antibiotics were also evaluated. They include; ciprofloxacin (10 μg), norfloxacin (10 μg), gentamycin (10 μg), amoxicillin (20 μg), streptomycin (30 μg), erythromycin (30 μg), chloramphenicol (30 μg), ampicillin+cloxacillin (20 μg), levofloxacin (20 μg), and rifampicin (20 μg).

2.4 Collection and Processing of Samples

This was done following the procedures described by Murray et al. [18]. Briefly, swab samples including bronchial aspirates, urinogenital, oropharygeal, pus from wounds, naris, axilla and groin were collected from patients at the University of Calabar Medical Centre, aseptically packaged and immediately transported to the laboratory in Microbiology Department for microbiological analysis. Similarly, nasal swab samples were collected from people living within the University of Calabar community and immediately transported to Microbiology laboratory. These samples were inoculated onto Nutrient agar and Blood agar (T.M. Media, Nigeria) and incubated at 37°C for 24 hrs. Following incubation, discrete colonies were sub-cultured onto Mannitol Salt Agar (T. M. Media, Nigeria) and incubated at 37°C for 24 hrs. A series of microbiological tests including Gram's reaction, catalase and coagulase tests were employed to characterize and identify the test organisms. Following characterization and identification, test isolates were rejuvenated in Nutrient agar prior to antimicrobial sensitivity testing.

2.5 Antibiotics Susceptibility Testing

Test isolates were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion method on Muller Hinton agar (Oxoid, UK) plates following the Clinical and Laboratory Standards Institute, (CLSI) [19]. The discs used were commercially procured. Briefly, 3-5 colonies of the test organism were selected using a sterile inoculating loop and suspended in saline after which the inoculum was adjusted to a turbidity equivalent of a 0.5 McFarland standard (corresponds to approximately 1.5×10^8 CFU/ml). The suspension was then vortexed to make sure it was well-mixed. Then, a fresh sterile cotton-tipped swab was dipped into the suspension, the excess liquid from the swab removed by pressing it against the side of the tube. Subsequently, the swab was inoculated unto a plate containing freshly prepared Muller Hinton Agar (MHA) starting at the top; the surface was inoculated with the swab covering the entire plate by spreading back and forth from edge to edge, rotating the plate approximately 60° and repeating the swabbing procedure thrice, ensuring that the entire surface was properly covered. Then, the discs containing the antimicrobial agents were applied using a sterile pair of forceps within 15 minutes of inoculating

the MHA plate and pressed down firmly to ensure firm, leveled contact with the agar. The plate was inverted and incubated in ambient air at 35°C for 16-18 hours. Following incubation, the clear zone around each disc was measured and referred to as sensitive, intermediate or resistant following CLSI [19] guidelines. This procedure was carried out on all test isolates.

2.6 Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations (MIC and MBC)

Exactly 30 µg of cefoxitin was used to prepare the following concentrations 7.50, 3.75, 1.875, 0.94, 0.47 and 0.23 g/ml and dispensed into separate tubes. Then, 0.5ml of the standardized *S. aureus* inoculums were added to each of the tubes and incubated overnight. The MIC was then reported as the lowest concentration of antimicrobial required to prevent visible growth. The MBC was determined by sub-culturing tubes which showed no growth (turbidity) during the MIC test into plates containing freshly prepared nutrient agar. A loopful from each test tube was sub-cultured onto plates containing freshly prepared nutrient agar and incubated at 37°C for 24 hours. The least concentration in the MIC test which showed no growth in the sub-culture plate was recorded as MBC. This was carried out on all test isolates according to CLSI guidelines [19].

2.7 Statistical Analysis

All data obtained in this study were analyzed using SPSS version 21 for windows to evaluate the association between *S. aureus* isolates from hospital and community settings. Significance level was set at 95% (0.05) and p values less than 0.05 were considered significant.

3. RESULTS

A total of 42 (28%) *S. aureus* isolates were recovered in this study out of which 27(64.3%) isolates were from hospital settings and 15(35.7%) were from the community. Consistently, 25(60%) of the recovered isolates were identified to be MRSA out of which 20(74.0%) were from hospital settings and 5(33.0%) were from community setting as shown in Table 1. Pus samples recorded the highest level of *S. aureus* strains as presented in Table 2. Table 3 shows the age distribution of the participants employed in this study. A total of 150 individuals participated in this study out of which 69% were females and 31% were males. The age of the participants ranged from 20-69 with a mean age of 44.5. Exactly, 68% (51/75) of the participants in the community setting were females while 32% (24/75) were males. Similarly, 70.6% (53/75) of participants in the hospital setting were women while 29.3% (22/75) were males. *S. aureus* strains were more frequent among the age groups 20-29 and 30-39 years in the hospital setting (42.9% and 28.6%, respectively). Similarly, *S. aureus* strains in the community setting were mostly recovered from participants within the age range of 40-49 years (40%). Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) from the hospital setting were more frequent among participants within the age range of 20-29 and 30-39 with frequencies of 50% and 20%, respectively meanwhile, in the community setting, MRSA was frequently isolated from the age groups; 20-29years (20%), 30-39 years (20%) and 40-49years (60%). In addition, 60% of MRSA from hospital setting were recovered from females while 40% were from males. Similarly, 20% of MRSA from community setting were recovered from females while 80% were from males as presented in Fig. 1.

Table 1. Prevalence of MRSA and MSSA isolates

Type of isolates	Number isolated (%)
MSSA (17/42)	17 (40)
MRSA (25/42)	25 (60)
HAMRSA (20/27)	20 (74)
CAMRSA (5/15)	5 (33)

Key: MSSA: Methicillin-sensitive S. aureus, MRSA: Methicillin-resistant S. aureus, HAMRSA: Hospital-settings methicillin-resistant S. aureus, CAMRSA: Community-settings methicillin-resistant S. aureus

The susceptibility pattern of *S. aureus* isolated from the hospital and community settings are presented in Fig. 1. Hospital-setting isolates showed the greatest level of resistance to cefoxitin (74.1%), followed by amoxicillin (67%), ampicillin+cloxacillin (63%) and levofloxacin (63%). Similarly, highest level of resistance of 33.3% was observed against cefoxitin and norfloxacin in the antibiotic susceptibility test of *S. aureus* isolates from the community setting. Test isolates from the community also exhibited moderate resistance to ciprofloxacin (27%), erythromycin (27%), ampicillin+cloxacillin (27%) and amoxicillin (20%). Comparatively, isolates from the two sample sites showed highest susceptibility to streptomycin followed by Chloramphenicol 80%. Gentamycin showed an activity of 74% and 80% while rifampicin showed

an activity of 80% and 81% in hospital-setting and community-setting isolates, respectively. Consistently, HAMRSA and CAMRSA exhibited minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against cefoxitin. HAMRSA and CAMRSA exhibited MICs of 0.94 mg/ml and 0.47mg/ml, respectively. Similarly, the MBCs were 1.875 mg/ml for HAMRSA and 0.47 mg/ml for CAMRSA.

Table 2. Association of methicillin-resistant *S. aureus* to different clinical samples

Type of specimen	HA-MRSA	HA-MSSA	CA-MRSA	CA-MSSA	Total
Pus	10(50.0)	2(28.6)	0(0.0)	0(0.0)	12(44.4)
Nasal swab	0(0.0)	0(0.0)	5(100)	10(100)	15(100)
Throat swab	2(10.0)	1(14.3)	0(0.0)	0(0.0)	3(11.1)
Urine	1(5.0)	1(14.3)	0(0.0)	0(0.0)	2(7.4)
bronchial aspirate	2(10.0)	0(0.0)	0(0.0)	0(0.0)	2(7.4)
Body fluid	2(10.0)	3(42.9)	0(0.0)	0(0.0)	5(18.5)
Sputum	3(15.0)	0(0.0)	0(0.0)	0(0.0)	3(11.1)
Upper & lower boundaries for x-variables	HA-MRSA vs HA-MSSA	-1.8819, 4.1319	CA-MRSA vs CA-MSSA	-0.4040, 1.2123	
Upper & lower boundaries for y-variables	HA-MRSA vs HA-MSSA	-2.6696, 6.1338	CA-MRSA vs CA-MSSA	-2.4238, 2.9718	
P-value for x	HA-MRSA vs HA-MSSA	0.3803	CA-MRSA vs CA-MSSA	0.001	
P-value for y	HA-MRSA vs HA-MSSA	0.3581	CA-MRSA vs CA-MSSA	0.4914	
Pearson correlation	HA-MRSA vs HA-MSSA	0.40	CA-MRSA vs CA-MSSA	1.00	

Key: HA-MRSA- Hospital-settings methicillin-resistant S. aureus, HA-MSSA- Hospital-settings methicillin-susceptible S. aureus, CA-MRSA- Community-settings methicillin-resistant S. aureus, CA-MSSA- Community-settings methicillin-susceptible S. aureus

Table 3. Association of methicillin-resistant pattern of S. aureus in study participants with gender and age group

Variable		HA-MRSA	HA-MSSA	CA-MRSA	CA-MSSA
Sex	**Male**	8(40.0)	3(42.9)	4(80.0)	4(40.0)
	Female	12(60.0)	4(57.1)	1(20.0)	6 (60.0)
Age group	20-29	10(50.0)	3(42.9)	1(20.0)	2(20.0)
	30-39	4(20.0)	2(28.6)	1 (20.0)	1(10.0)
	40-49	3 (15.0)	1 (14.3	3 (60.0)	4 (40.0)
	50-59	1 (5.0)	1(14.3)	0(0.0)	1(10.0)
	60-69	2(10.0)	0(0.0)	0(0.0)	2(20.00)
Total		20(100)	7 (100)	5 (100)	10(100)
Upper &lower boundaries for x-variables	HA-MRSA vs HA-MSSA	1.6324, 4.0342		CA-MRSA vs CA-MSSA	-0.4040, 1.2123
Upper & lower boundaries for y-variables	HA-MRSA vs HA-MSSA	-2.823, 2.9184		CA-MRSA vs CA-MSSA	-2.4238, 2.9718
P-value for x	HA-MRSA vs HA-MSSA	0.0018		CA-MRSA vs CA-MSSA	0.2550
P-value for y	HA-MRSA vs HA-MSSA	0.96		CA-MRSA vs CA-MSSA	0.8044
Pearson correlation	HA-MRSA vs HA-MSSA	0.94		CA-MRSA vs CA-MSSA	0.50

Key: HA-MRSA- Hospital-settings methicillin-resistant S. aureus, HA-MSSA- Hospital-settings methicillin-susceptible S. aureus, CA-MRSA- Community-settings methicillin-resistant S. aureus, CA-MSSA- Community-settings methicillin-susceptible S. aureus

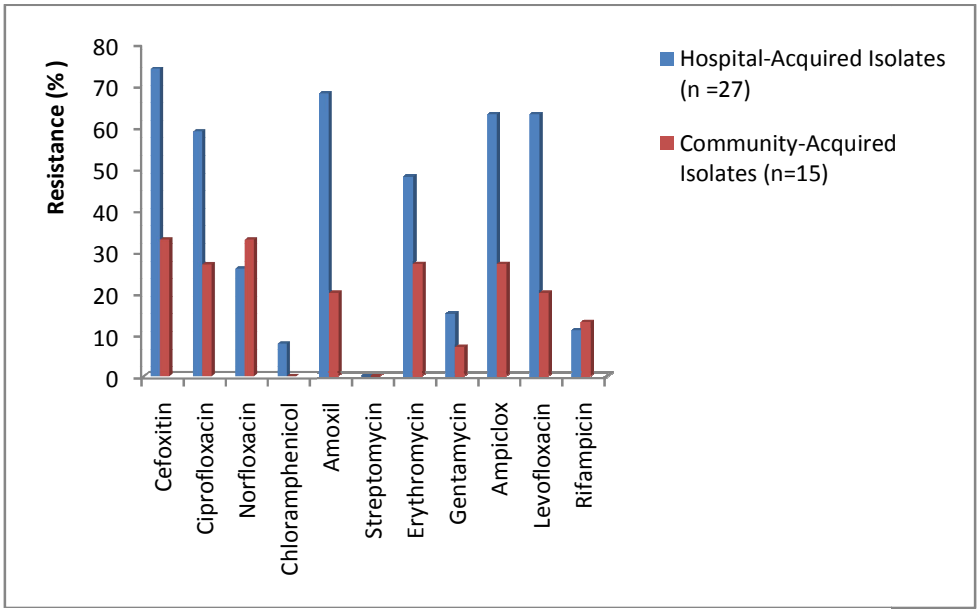

Fig. 1. Resistant pattern of hospital-setting and community-setting *Staphylococcus aureus*

4. DISCUSSION

The frequency of *S. aureus* in this study was 28% (42/150). The isolation of *S. aureus* varied according to age and gender. Frequency of *S. aureus* isolation in the hospital setting ranged from 7.4% among the age groups 50-59 and 60-69 years to 48.1% among the age groups 20-29 years whereas in the community setting, highest frequency of isolation was among the age groups of 40-49 years (46.7%). The finding of higher frequency of *S. aureus* isolation among the age groups 20-29 years in the hospital setting is consistent with report of Dilnessa and Bitew, [20] who recorded same among the age groups 14-24. Similarly, this study revealed that females had a higher isolation rate of *S. aureus* compared to males. This is contrary to report of Dilnessa and Bitew, [20] who observed a higher frequency among the male participants. This may be due to the fact that majority (69%) of the participants employed in this study were females.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as a major public health challenge globally, due to its association with several nosocomial and community-acquired infections [1]. Consistently, the frequency of MRSA observed in this study was 60.0% out of which 20(74.0%) were from hospital-setting and 5(33.0%) were community-setting. Community-setting MRSA detection in healthy individuals as reported by Iyer et al. [21] is necessary because these individuals act as carriers, serving as potential source of microorganisms which are important for the epidemiology and pathogenesis of hospital infections. However, the 60% frequency observed in this study is somewhat higher than 12.5% reported by Okon et al. [13] in tertiary hospitals in North-Eastern Nigeria and the 21% and 21.4% reported by Omuse et al. [22] in two private hospitals in Nairobi, Kenya and Al-Abdli and Baiu, [23] among health care workers in Benghazi hospitals, Libya. As previously reported by Orrett [24] and Diekema et al. [25], over 70% of MRSA have been recorded in Japan and Hong Kong, 61% in Singapore and Taiwan, 50% in Portugal and Italy and 25% in England, Greece and France, respectively. According to Akpaka et al. [26], the variations in prevalence of MRSA observed in studies may largely be due to length of study period, sample size, number of study sites, sample type and laboratory procedures employed. These factors may have influenced the outcome of results in this study. The frequency of MRSA isolates from the Hospital-setting patients were 74.1% and that from community-setting 33.3% recorded in this study is similar to the reports of Chadha et al. [9] where they observed that HA-MRSA and CA-MRSA had a prevalence of 79.4% and 20.6%, respectively. Furthermore, MRSA rate in this study did not vary significantly by gender and age group. This observation is consistent with report of Dilnessa and Bitew, [20] who revealed

that age and gender are not risk factors for the colonization of MRSA.

Though, there was no statistical association between the isolation rates of MSSA and MRSA with any clinical sample ($p>0.05$), the highest frequency of isolation of MSSA and MRSA was recorded with pus samples in this study. This may be due to the fact that most of the pus samples came from surgical and burnt wards. This observation is consistent with reports of Akpaka et al. [26] and Orrett and Land, [27].

Resistance of microorganisms to antimicrobial agents have been reported to be markedly influenced by factors including; the practice of self-medication, availability of counterfeit drugs, the use of sub-optimal dosage of antibiotics, use of broad spectrum antibiotics, proximity to patients with MRSA and the acquisition of new genetic materials called plasmids [13,21]. In this study, the highest level of resistance to cefoxitin was 74% (20/27) and was observed in the hospital setting while a comparatively lower percentage (5/153, 33%) was recorded in the community. The high level of resistance to cefoxitin observed in this study may be due in part to their ability to secrete extracellular enzymes that deactivate the drug, rendering it ineffective or in whole to the acquisition of *MecA* gene responsible for the development of resistance to cefoxitin and all other antibiotics [21]. This probably explains the observed marked resistance exhibited by these organisms to cefoxitin and other antibiotics including, amoxicillin (67% and 20%), levofloxacin (63% and 20%), ampicillin+cloxacillin (63% and 27%) and Erythromycin (45% and 23%) in the hospital and community settings, respectively. This observation is similar to reports from [20,28].

The marked resistance of MRSA from the hospital setting could be due to the fact that these isolates were recovered from environments where antibiotics are often used. This is because as reported by Byarugaba, [29], microbial isolates from the hospital settings are often pressured to develop resistance mechanisms towards antibiotics with corresponding multidrug resistance indices (MAR) to ensure their survival.

The high resistance exhibited by isolates from the hospital setting was further confirmed by the observable MICs and MBCs recorded by these organisms. While MRSA isolates from the community-setting recorded MIC and MBC of 0.47 and 0.94mg/ml, respectively, those from the hospital-setting recorded MIC and MBC of 0.94 and 1.875 mg/ml, respectively. The low MIC and MBC recorded by isolates from the community setting in this study is consistent with the observation of Sobhy et al. [28] who stated that isolates from the community-setting have a narrow spectrum of resistance.

Similarly, Community-setting MRSA strains in this study exhibited higher susceptibility to beta-lactam and non beta-lactam antibiotics, compared to Hospital strains. This is a contrary to reports from [9,28] where they observed that the susceptibility patterns of MRSA from both the community and hospital settings were not different. Consistently, MRSA isolates from the community and hospital settings exhibited 100% susceptibility against Streptomycin followed by Chloramphenicol (80% and 82%), rifampicin (89% and 87%) and gentamycin (80% and 81%). The reason for this level of activity might not be unconnected with the mechanisms of action of these drugs. Gentamycin and streptomycin are aminoglycosides which bind to the 30S ribosomal sub-unit and cause a misreading of the genetic code, leading to the interruption of normal bacterial protein synthesis. Chloramphenicols on the other hand, inhibit protein synthesis while rifampicin inhibits nucleic acid synthesis [30]. However, the susceptibility of *Staphylococcus aureus* isolates to rifampicin, gentamycin and chloramphenicol as observed in this study was slightly lower than 100% previously reported [31,32]. This could be due to drug abuse, purchase of cheap and counterfeit drugs among other factors. Thus, effective infection control practices could halt the spread of MRSA and reduce the rate of morbidity and mortality associated with MRSA infections.

5. CONCLUSION

The frequency of MSSA and MRSA varied considerably according to the type of sample. Pus was the main source of hospital-setting methicillin-resistant *Staphylococcus aureus* (HA-MRSA) and methicillin-susceptible *Staphylococcus aureus* (HA-MSSA). This study further revealed a high frequency of HA-MRSA strains compared to Community-setting methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains. The rate of MRSA isolates obtained in this study was high when compared with rates recorded from previous studies conducted in Nigeria but however, considerably lower when compared to other similar studies conducted elsewhere. This study further revealed

that MRSA were multi-drug resistant. Thus, good infection control practices including identifying and treating MRSA carriers, moderate use of antibiotics and hand washing could reduce the burden associated with MRSA-related infections. To further establish and characterize multidrug resistant *S. aureus* strains, genotypic studies may be employed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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