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Antibiotic Resistance Profile of Methicillin-Resistant *Staphylococcus aureus* in Abeokuta, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author JAO designed the study and carried out the practical work. Author OAO wrote the protocol. Author PAA managed the statistical analyses of the study. Authors OE and SOM managed the literature searches. Authors NOS and EOO wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A considerable increase in infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has caused a high rate of morbidity among the populace with huge economic loss and severe debility. Therefore, this study examines the pattern of antibiotic resistance of MRSA in clinical samples of patients in Abeokuta, Nigeria using standard recommended procedures. Coagulase test, β-lactamase production, and mannitol fermentation were performed using standard methods. Antibiotic susceptibility was determined using disc diffusion assay and minimum inhibitory concentration by micro-broth dilution method. Vancomycin and Azithromycin resistance profile was performed using colorimetric micro-broth dilution assay. Multi-resistant antibiotic index of MRSA was also determined.

A total of 338 clinical specimens of Pus, Aspirate, Ear, and Wound swabs were collected from three major health facilities in Abeokuta, Nigeria. Each sample was cultured for bacteria isolates and

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examined for colonial and cellular morphology while biochemical characterization was performed. Of the 161 *Staphylococcus aureus* (32.2%) isolated; there was no significant disparity found in relation to the study sites (χ^2=7.145, p-value = 0.308). 60.2% of the *S. aureus* analyzed were beta-lactamase producers while only 39.8% were β-lactamase negative. More than 65% of MRSA showed resistance to Cotrimoxazole, Tetracycline, Cefoxitin, and Erythromycin while 96.9% and 98.5% were resistant to Vancomycin and Azithromycin at MIC≥16ug/mL respectively. All the MRSA showed MBC>64ug/ml to both Vancomycin and Azithromycin. Multi-antibiotic resistance index rate of more than 0.2 was shown by 97.0% MRSA to 12 different antibiotics.
The present study indicates a high prevalence rate of MRSA that require empirical and urgent intervention to prevent staphylococcal infection among the hospital patients and its outbreak.

Keywords: Staphylococcus aureus; methicillin; resistance.

1. INTRODUCTION

The name "Staphylococcus" was first described in the 1880s by Sir Alexander Ogston, a Scottish surgeon who identified this organism in pus from a surgical abscess in a knee joint. This name was later amended to *Staphylococcus aureus* by Friedrich Julius Rosenbach, who was credited by the official system of nomenclature at the time. *Staphylococcus* in Greek refers to "Grape-cluster berry" while *aureus* in Latin means "golden" [1].

Staphylococcus aureus is usually a harmless colonizer of about one-third of healthy humans and is most likely found in the nares. Nasal carriage of *S. aureus* has been closely associated with staphylococcal disease [2]. Colonization increases the risk of subsequent infection since those with *S. aureus* infections are usually infected with their colonizing strain [3]. Infection may occur when there is a breach of the skin or mucosal barrier that allows the organism access to adjoining tissues or the blood stream [4].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to methicillin. Usually, these strains of *Staphylococcus aureus* are resistant to more than one antibiotic hence, the infections due to this strain of MRSA are very difficult to treat. The organisms produce catalase and coagulase, often used for their identification [5]. Resistance in MRSA is related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin-binding protein called PBP2a or PBP21. Penicillin-binding proteins are membrane-bound enzymes, which targets for all β-lactam antibiotics. PBP2a has a decreased affinity for binding β-lactam antibiotics resulting in resistance not only to methicillin but also to all β-lactams including Penicillins and Cephalosporins [6]. The *mecA* gene complex also contains insertion sites for plasmids and transposons that facilitate the acquisition of resistance to other antibiotics. Thus, cross-resistance to non-β-lactam antibiotics such as Erythromycin, Gentamicin, Co-trimoxazole, and Ciprofloxacin is common [7].

Methicillin-resistant *Staphylococcus aureus* has been severally shown to cause a variety of diseases ranging from mild, superficial dermatological diseases to severe and potentially fatal systemic debilitations [5]. In spite of the availability of considerable number of effective antimicrobial chemotherapeutic agents, MRSA still remains an increasing cause of post-surgical wound infections [8,9] and some invasive infections such as nosocomial sepsis, acute endocarditis and osteomyelitis, pneumonia and other soft tissue infections [10].

The increasing prevalence of MRSA multiple–drug-resistant strains which limit the therapeutic options available for the management of MRSA associated infections has become a worrisome issue worldwide [11] and has posed a serious therapeutic challenge. Many studies have characterized *S. aureus* and methicillin-resistant *Staphylococcus aureus* isolates from individual hospitals [12,13] in Nigeria, but there is still a paucity of information on the resistance trends of *S. aureus* and MRSA in health-care settings in Abeokuta. Therefore, there is a need to determine the antibiotic resistance patterns of the isolates obtained from different clinical specimens to commonly prescribed antibiotics in these localities.

2. MATERIALS AND METHODS

2.1 Ethical Approval

Ethical approval was sought from the ethical committee of Federal Medical Centre, Abeokuta

with REGISTRATION NUMBER: NHREC/08/10-2015.

2.2 Collection of Samples

A total of 338 clinical specimens consisting of wound swab, ear swab, aspirate, and pus were collected from Medical Microbiology unit of Federal Medical Centre, Idi-Aba, Sacred Heart Hospital, Lantoro and Ogun State Management Board, Ijaiye, all in Abeokuta, Ogun State for the period of 6 months. The swab samples were collected using commercially prepared sterile swab sticks (Oxoid U.K.). They were kept refrigerated at 4°C until delivery, to the laboratory. All the samples were cultured immediately on appropriate media, within 12 hours of collection.

2.3 Isolation and Identification

The sterile culture media plates of Mannitol Salt Agar (MSA) and Blood agar (BA) were dried in the oven to remove water of condensation in the plates as well as on the surface of the culture media. The swab samples were rubbed over one-quarter of each of the different agar plates (i.e. MSA and BA); the rest parts of the plates were streaked with a sterile wire loop to obtain discrete colonies. The inoculated culture media were incubated at 37°C in an incubator for 24-48 hours. Suspected discrete colonies of *Staphylococcus aureus* were sub-cultured on Nutrient agar plates to obtain pure culture and for further analyses.

Each organism was identified according to Cowan and Steel [14] method of bacteria identification, by their colonial appearance such as size, shape, consistency, color, elevation and Gram staining was done to further identify the isolates alongside with biochemical identification. Isolates that were Gram-positive cocci, catalase positive, coagulase positive and mannitol positive were considered as *S. aureus* in this study.

2.4 Beta-Lactamase Test

This is a modification of the technique by Odugbemi et al. [15]. Pieces of Whatman No.1 filter paper, each measuring approximately 5 x 6cm, were immersed in 1% soluble starch and dried. When needed, the starch paper was placed in a Petri dish and soaked with a solution containing 60,000µg/ml benzyl penicillin in phosphate-buffered saline, pH 7.3 (Oxoid). With a bacteriological loop, several colonies from a culture were spread over an area approximately 5mm in diameter. Several different strains of Staphylococci were tested on the same paper, separated from each by approximately 1cm. The paper was incubated at 37°C for 30 minutes with the Petri dish cover on. After incubation, the paper was soaked with a solution of iodine 1 g, Potassium iodine 2 g in distilled water 200 ml. The Petri dish was held at an angle of 30° to allow the iodine solution to drain to the bottom. The starch paper turned black within seconds. Colonies with decolorized zones are positive for Beta-lactamase, but colonies with black background are Beta-lactamase negative.

2.5 Antimicrobial Sensitivity Testing

The susceptibility of isolates to various antibiotics was determined according to Bauer et al. [16] and Clinical Laboratory Standard Institute [17] modified disc diffusion technique [18]. The inoculum was prepared by touching a colony of the test organism with a sterile wire loop and the growth transferred into a Bijou bottle containing Mueller Hinton Broth. The cell suspensions were incubated for 2 hours and the bacterial suspension adjusted to 0.5 McFarland's standard using Mueller Hinton Broth. Mueller Hinton agar plates were dried in the hot-air oven by inverting Petri dishes containing the media to remove surface moisture. Plates were then inoculated within 15 minutes of preparation of the suspension. A sterile cotton swab was dipped into the suspension of isolate and excess fluid was removed by rotation of the swab against the side of the bottles above the fluid level. The media were then inoculated by even streaking of the swab over the entire surface of the plate in three directions at 60° to each other. The plate was left for 10 to 15 minutes on the laboratory bench before single antibiotic discs were aseptically placed using the Antibiotic disc dispenser. Plates were incubated at 37°C for 18 to 24 hours. Growth inhibition was shown as a circular zone of no growth around the disc. The diameter of the zone of inhibition was measured using a graduated ruler and result interpreted according Clinical Laboratory Standard Institute [17].

2.6 Methicillin-Resistance Screening

Isolates were screened for methicillin resistance using the method of Markowitz et al. [19]. *Staphylococcus aureus* was inoculated into Mueller Hinton Broth (MHB) and was incubated at 37°C overnight. A suspension of the inoculum

which is equivalent to 0.5 McFarland Standard was streaked on Mueller Hinton agar (MHA) with 4% NaCl. Cefoxitin (30 µg) was aseptically placed on the inoculated plate and the plate was incubated for 18 hours at 35°C. The isolate with an inhibition zone of ≤21mm was considered Methicillin-resistant *Staphylococcus aureus* (MRSA) Strain. *Staphylococcus aureus* ATCC 25923 was used as a control.

3. RESULTS

Out of the 500 bacterial isolates obtained from 338 clinical specimens from Federal Medical Centre, Idi-Aba, Sacred Heart Hospital, Lantoro and Ogun State Hospital, Ijaiye; 161 (32.2%) were identified by biochemical reactions to be *S. aureus* as shown in Fig. 1. Out of these samples, 14.9% were recovered from Aspirate, 20.5% Pus, 29.8% and 34.8% from Ear and wound swabs respectively (Table 1).

Also, out of the 161 *Staphylococcus aureus*, 81 (50.3%) were males while 80 (49.7%) females were observed in the study. The distribution of β-lactamase production among the *S. aureus* isolates reveals that 97 (60.2%) were β-lactamase positive and 64 (39.8%) were β-lactamase negative as observed in Fig. 2 with no statistical association existing between β-lactamase production and age groups in Table 2 (χ^2 = 6.234, p-value = 0.513).

Table 3 shows that the relationship between the β-lactamase reactions in *Staphylococcus aureus* to the major hospitals sampled in Abeokuta is statistically significant (χ^2=6.257, P-value = 0.044).

The susceptibility pattern of *S. aureus* to different antibiotics is shown in Table 4. Majority of the Methicillin-resistant *Staphylococcus aureus* (MRSA) were resistant to other antibiotics such as Cotrimoxazole (93.9%), Tetracycline (89.4%), Augmentin (72.7%), Erythromycin (71.2%), etc. as explicitly revealed in Fig. 4.

The result of the Minimum inhibitory concentration implies that 96.9% and 98.5% were resistant to Vancomycin and Azithromycin at MIC≥16µg/mL respectively as observed in Table 5. All the MRSA showed MBC>64µg/mL to both Vancomycin and Azithromycin.

Multi-antibiotic resistance index rate of more than 0.2 was shown by 97.0% MRSA to 12 different antibiotics as displayed in Fig. 3. Percentage distribution of resistant *Staphylococcus aureus* clearly reveals that more than 50% were found to be MRSA as observed in Fig. 5.

Table 1. Frequency of *Staphylococcus aureus* in clinical specimens obtained from different health facilities in Abeokuta

Clinical specimens	Number (%)
Ear swab	48 (29.8)
Wound swab	56 (34.8)
Pus	33 (20.5)
Aspirate	24 (14.9)
Total	161 (100)

Table 2. Distribution of beta-lactamase reactions in *Staphylococcus aureus* according to age group of the subjects

Age groups	β-Lactamase Positive	β-Lactamase negative	χ^2	P-value
	Number (%)	Number (%)		
0-9	35 (36.1)	14 (21.9)		
10-19	9 (9.3)	10 (15.6)		
20-29	17 (17.5)	11 (17.2)		
30-39	8 (8.2)	5 (7.8)		
40-49	9 (9.3)	10 (15.6)	6.234	0.513
50-59	10 (10.3)	9 (14.1)		
60-69	7 (7.2)	3 (4.7)		
70-79	2 (2.1)	2 (3.1)		
Total	97 (100)	64 (100)		

Table 3. Distribution of beta-lactamase reactions in *Staphylococcus aureus* (n=161) in relation to study sites

	Health facilities (%)	χ^2	P-value
β-lactamase –ve	64 (39.8)	6.257	0.044
B-Lactamase +ve	97 (60.2)		

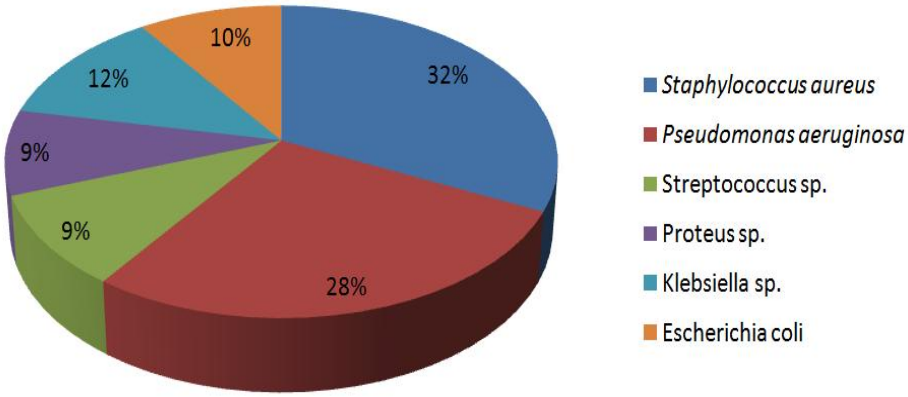

Fig. 1. Distribution of bacterial isolates from clinical samples in Abeokuta

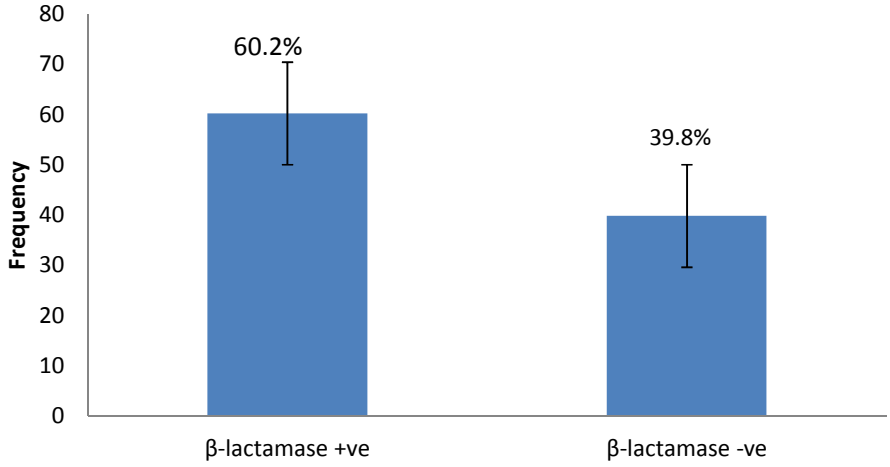

Fig. 2. Distribution of Beta-lactamase production among the *Staphylococcus aureus* isolates

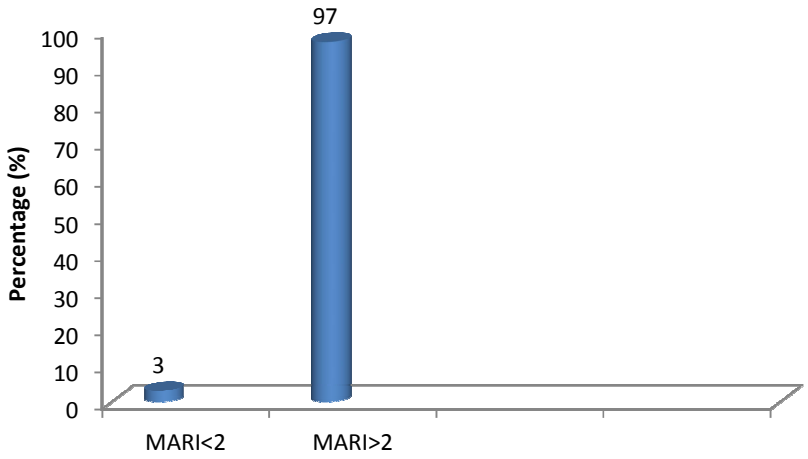

Fig. 3. Multi-antibiotic resistance indices (MARI) rate of Methicillin resistant *Staphylococcus aureus*

Table 4. Susceptibility Patterns of *Staphylococcus aureus* isolated from Clinical Specimens in Abeokuta

Antibiotics	Concentrations (µg)	Susceptible number (%)	Intermediate number (%)	Resistant number (%)
Tetracycline	30	39 (24.2)	13 (8.1)	109 (67.7)
Ciprofloxacin	5	76 (47.2)	44 (27.3)	41 (25.5)
Gentamicin	30	82 (50.9)	38 (23.6)	41 (25.5)
Augumentin	30	92 (57.1)	0 (0.0)	69 (42.9)
Cotrimoxazole	25	21 (13.0)	14 (8.7)	126 (78.3)
Ofloxacin	5	73 (45.3)	38 (23.6)	50 (31.1)
Cefoxitin	30	95 (59.0)	0 (0.0)	66 (41.0)
Erythromycin	15	83 (51.6)	19 (11.8)	59 (36.6)
Ceftazidime	30	73 (45.3)	44 (27.3)	44 (27.3)
Ceftriaxone	30	71 (44.1)	51 (31.7)	39 (24.2)
Vancomycin	30	98 (60.9)	37 (23.0)	26 (16.1)
Linezolid	30	125 (77.6)	0 (0.0)	36 (22.4)

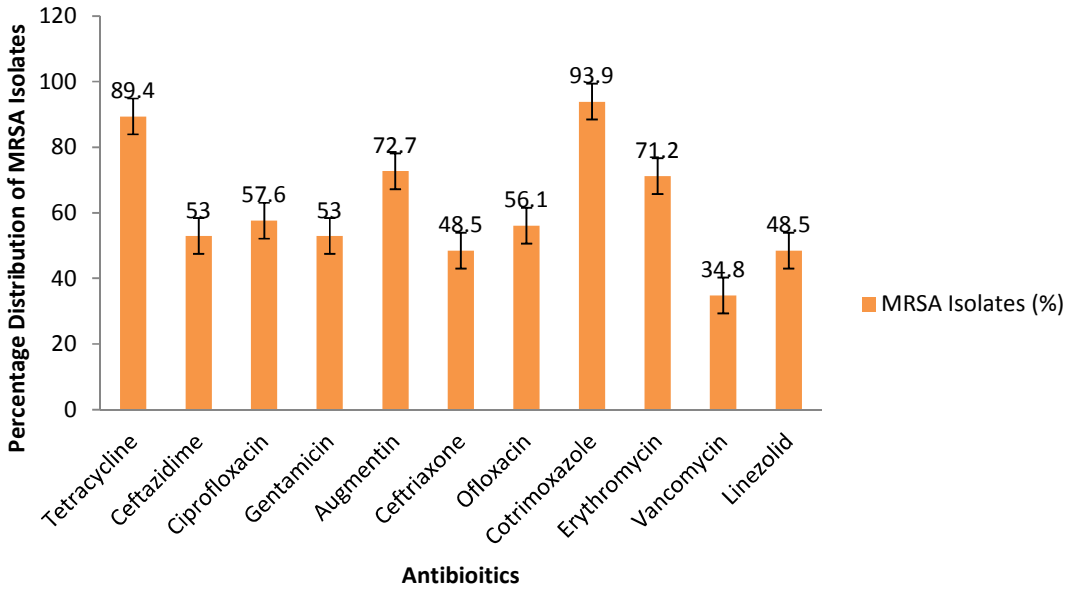

Fig. 4. Antibiotic Resistance Pattern of identified MRSA isolates

4. DISCUSSION

The medical importance of Staphylococcal infections cannot be overemphasized worldwide. Antibiotic resistance to *Staphylococcus aureus* has been reported to be on the increase globally [20]. Methicillin resistance is the most prominent resistance property acquirable by *S. aureus* with its attendant community and institutional spread, first isolated in 1962 [21], its increasing incidence has posed a major threat in infectious disease medicine worldwide; due to the ability of Staphylococci to change over time, the MRSA will continue to be a problem in the future, as it has been in the past and still is, at present. Despite intensive efforts to control resistant organisms with aggressive infections control methods antibiotic resistant Staphylococci, especially MRSA has become the most common cause of hospital-acquired infections worldwide [22].

The antimicrobial susceptibility pattern of MRSA isolates also varies with place and time. In most of the studies conducted over the years, there was a clear indication of the progressive

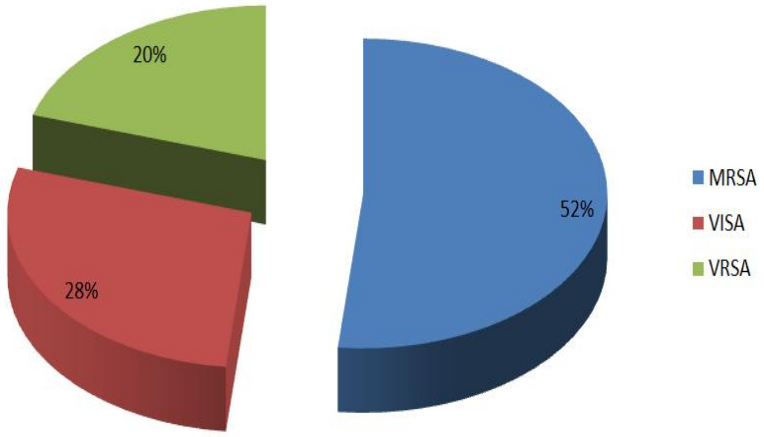

Fig. 5. Percentage distribution of resistant *Staphylococcus aureus* isolates

Table 5. Minimum inhibitory concentration of MRSA with vancomycin and azithromycin

MIC (µg/ml)	Vancomycin number (%)	Azithromycin number (%)
512	48 (72.7)	43 (65.2)
256	16 (24.2)	21 (31.8)
128	0 (0)	1 (1.5)
64	0 (0)	0 (0)
32	0 (0)	0 (0)
16	0 (0)	0 (0)
8	0 (0)	0 (0)
4	0 (0)	0 (0)
2	0 (0)	0 (0)
1	2 (3.1)	1 (1.5)
	66 (100)	66 (100)

development of antimicrobial resistance to several antibiotics. In some of the studies, high resistance to Augmentin and Cotrimoxazole was reported in MRSA isolates ranging from 46 to 99% [23,24,25] whereas in our study this was only 72.7% and 93.9% respectively and less resistance rate was observed against Ceftazidime (53.0%), Gentamicin (53.0%), Ceftriaxone (48.5%), Linezolid (48.5%) and Vancomycin (34.8%).

In this study, wound swab isolates recorded the highest level of MRSA isolations with 40.9%. More than 70% MRSA isolated showed high resistance to Augmentin, which supports the findings that MRSA strains are equally resistant to all β-lactam antibiotics [6] which may be due to the presence of chromosomal *mecA* gene. 96.9% and 98.5% were resistant to Vancomycin and Azithromycin at MIC≥16 µg/mL respectively. All the MRSA showed MBC>64 µg/ml to both Vancomycin and Azithromycin.

The prevalence of MRSA multi-drug resistance strain limit the therapeutic option available for management of MRSA, associated infection and this has become worrisome. The wide use of antibiotics has been reported to increase antibiotic resistance [26].

About 34.8% and 48.5% of the MRSA isolated in this study were resistant to both Vancomycin and Linezolid respectively. This is contrary to the work done in Sidery Hospital in Saudi Arabia in which 100 *Staphylococcus aureus* screened for MRSA were all sensitive to Vancomycin [27].

In addition, multi-drug resistance in this study is defined as resistance to three or more classes of the antibiotics tested. Thus, 64 (97%) of the MRSA isolates showed multi-drug resistance and none was totally susceptible to all the tested antibiotics.

5. CONCLUSION

In conclusion, the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region. The use of antibiotics inevitably requires the need for *in vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories. Our study is a preamble to enable epidemiologists to understand the nature of MRSA isolates in this part of Nigeria.

Abused and injudicious use of antibiotics will lead to the development of drug-resistance. Timely

detection of methicillin-resistant strain will help in the prevention of hospital-acquired infections. Control of MRSA infections is essential and it can be achieved by proper implementation of hospital control measures and regular surveillance activity for proper documentation and control measures aimed at combating spread and control.

6. RECOMMENDATIONS

There should be an effective infection control committee to coordinate implementation of its policies especially regular hand washing and strict ward antisepsis to reduce nosocomial infections. Although vancomycin-resistant MRSA is not yet common in this part of the world, the rate of spread of this pathogen and its unique ability to acquire and transfer antibiotic resistance calls for urgent and well-coordinated surveillance programme to combat this situation.

Therefore, there should also be strict antibiotic prescription policies enforced by the appropriate authorities to contain the abuse of antibiotics and reduce acquisition of resistance by pathogens. Educational awareness should be encouraged to update health care workers with new intervention strategies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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