



## **Antibiotics Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) In Enugu State, South-East Region of Nigeria**

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### **Authors' contributions**

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

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### **ABSTRACT**

Development of antimicrobial resistance by bacteria is now a worldwide health issue, as infection is one of the leading causes of death in the world today. The aim of this study was to evaluate the prevalence and antimicrobials susceptibility pattern of Methicillin-Resistant *Staphylococcus aureus* in 3 different hospitals in South-East geopolitical region of Nigeria. The identification and confirmation of the *S. aureus* were done using selective and differential medium (Mannitol salt agar) for *S. aureus* and by coagulase/staphylase test using Oxoid<sup>®</sup> reagents kits (DR0595A). The method used for antibiotics susceptibility pattern of the characterised *S. aureus* isolates was discs diffusion method, as recommended by the Clinical Laboratory Standards Institute (CLSI), discs containing oxacillin (5 µg/disc), vancomycin (30 µg/disc), cephalexin (30 µg/disc), levofloxacin (5 µg/disc), ciprofloxacin (5 µg/disc), tetracycline (30 µg/disc), cotrimoxazole (25 µg/disc), gentamicin

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(30 µg/disc), clindamycin (2 µg/disc) and rifampicin (5 µg/disc). MRSA confirmation was done using Oxoid® DR0900 penicillin binding protein (pbp2) latex agglutination test kits. The results showed that out of 218 characterized clinical isolates, 39 of it were confirmed MRSA with varying percentages of resistance to various antibiotics thus: oxacillin (62.07%), vancomycin (60.35%), cephalexin (55.18%), levofloxacin (56.90%), ciprofloxacin (65.52%), tetracycline (68.97%), cotrimoxazole (67.25%), gentamicin (62.07%), clindamycin (63.79%) and rifampicin (62.07%). The *S. aureus* are more sensitive to Levofloxacin and less sensitive to tetracycline, clindamycin and rifampicin. Latex agglutination test confirmed 39 strains of the clinical isolates to be MRSA. The results shows open wound as a source with highest prevalence and sputum with lowest prevalence of the MRSA with no significant change ( $P > 0.05$ ).

**Keywords:** Antimicrobials susceptibility; Methicillin-resistant *Staphylococcus aureus*; South-East; Nigeria.

## 1. INTRODUCTION

### 1.1 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

*Staphylococcus aureus* is responsible for a broad range of clinical infections, most notable of which are cases of bacteremia and endocarditis [1]. *Staphylococcus aureus* is an important cause of serious infections in both hospitals and the community. Methicillin-resistant *Staphylococcus aureus* (MRSA) were first reported in 1961 and the first hospital outbreak of MRSA was reported in 1963 [2]. When MRSA strains first appeared, they occurred predominantly in the healthcare setting. Cases of community-associated MRSA (CA-MRSA) infections were first reported in the late 1980s and early 1990s [3]. Health care-associated MRSA (HA-MRSA) is particularly efficient at developing resistance to antimicrobial agents. Methicillin resistance among *staphylococci* has steadily increased worldwide, especially among cases acquired in hospitals. It is associated with longer hospital stay and more infections in intensive care units and leads to more antibiotic administration [1]. Asymptomatically colonized patients and health care workers are the major sources of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in the hospital environment [3].

MRSA-infected patients in burns units are particularly problematic because the big surface area of denuded skin can produce a large inoculum of organisms that can be easily transmitted to other patients via the hands of health care workers. Extensive skin lesions also result in heavy shedders of MRSA [2]. The commonest site of MRSA carriage is the anterior nares. A significant risk factor for acquisition of MRSA is the duration of hospital stay. Prolonged

stay in the hospital is likely with patients in orthopedics and dermatology wards, which may result in high rates of carriage observed in these patients [3].

HACO (Health care associated MRSA with a community onset) refers to community onset for a person associated with a hospital environment, e.g., a person living in a residential home, a health care worker, a dialysis patient, or an individual with a history of hospitalization within the previous year [4,5]. Risk factors associated with MRSA bacteremia include the following: residence in an extended care facility, prior antibiotic exposure, insulin dependent diabetes, prolonged hospitalization, urinary catheterization, nasogastric tube placement, prior surgery, and having an underlying disease [6]. The elderly population ( $\geq 65$  years old) is at a significantly higher risk of death due to MRSA bacteremia than are younger populations.

The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*. Strains that possess *mecA* gene are either heterogeneous or homogeneous in their expression of resistance [7,8].

The study aims to evaluate the prevalence and antimicrobials susceptibility pattern of Methicillin-Resistant *Staphylococcus aureus* in 3 different hospitals in South-East geopolitical region of Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Specimens Collection

This study was conducted from September 2012 to June 2013 at 3 different hospitals in the South-

East region of Nigeria. The hospitals are Bishop Shanahan hospital, Nsukka, University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu State and Federal Medical Centre, Abakaliki, Ebonyi State. A sample size of 2,372 hospitalized patients with skin, throat, open wound, ear/nasal infections and abscess were enrolled for this study. Samples were taken from sputum, open wound, abscess, ear and nasal swab from male and female wards in the orthopedic and intensive care departments of the hospitals. 1,230 samples were taken from University of Nigeria teaching hospital, 200 samples from Bishop Shanahan hospital and 942 samples from Federal medical centre, Ebonyi. The samples were distributed according to their clinical specimens as sputum (376), Skin Swab (327), abscess swab (466), open wound swab (762) and ear/nasal swab (441).

## 2.2 Characterisation of Clinical Isolates

The characterisation of the 2,372 isolates was done in sequence to eliminate the unwanted isolates and to confirm the *S. aureus* isolates. The viable clinical isolates used for this research was 218 isolates. The clinical isolates were characterised as cocci and non-cocci isolates by cultural and microscopic characters with gram staining reaction.

The catalase reaction test, mannitol salt agar test and slide coagulase test confirmed 58 (26.6%) clinical isolates to be *S. aureus*. The isolates were distributed as follows, 7 from sputum, 10 from skin swab, 11 from abscess, 19 from open wound and 11 from ear/nasal swab.

## 2.3 Antimicrobials Susceptibility Testing

A standard disc diffusion technique for antimicrobial susceptibility testing of *S. aureus* of each isolate was performed as recommended by the Clinical Laboratory Standards Institute (CLSI) [9] on Mueller Hinton agar. Standard antibiotics disks (Oxoid, Ltd) set were oxacillin (5 µg), vancomycin (30 µg), cephalixin (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), tetracycline (30 µg), cotrimoxazole (25 µg), gentamycin (30 µg), clindamycin (2 µg) and rifampicin (5 µg).

A direct colony method by CLSI [9] was used, pure isolated colonies were taken from Mueller Hinton agar into sterile broth tubes and a suspension equivalent to a 0.5 McFarland standard was prepared and used to inoculate the

test media. The discs were applied to the plate within 15 minutes of inoculation and incubated for 24 hours at 35°C.

## 2.4 Penicilin-binding Protein (PBP2<sup>+</sup>) Latex Agglutination Test for MRSA Confirmation

The PBP2<sup>+</sup> test was performed only on *Staphylococcus* species (Gram positive cocci). A coagulase test confirming the isolates used for this test to be *S. aureus* was done prior to the PBP2<sup>+</sup> test. A pure clinical isolates of *S. aureus* were used for this test according to the manufacturer's procedures Oxoid U.K. MRSA strain.

ATCC<sup>®</sup> 43300 (Oxoid Culti – Loops C9022) was used as positive control and ATCC<sup>®</sup> 25923 (Oxoid Culti – Loops<sup>®</sup> CL7010) was used as negative control.

Statistical analysis was done with ANOVA followed by Duncan post Hoc test using SPSS v 17 software.

## 3. RESULTS

### 3.1 Antimicrobials Susceptibility Test

Table 1 shows the antibiotic sensitivity and resistance pattern of *Staphylococcus aureus* to various antibiotics, 67.24% (39/58) of the *S. aureus* isolates shows resistance in varying degree to the antibiotics (MRSA), while 32.75% (19/58) of the isolates were sensitive to all the antibiotics (MSSA). The highest frequency of sensitivity of the isolates to the antibiotics was observed with cephalixin 44.82% (26/58) followed by levofloxacin 43.10% (25/58). The least was observed with tetracycline 31% (18/58). However, there was no significant difference ( $p > 0.05$ ) in susceptibility between cephalixin and levofloxacin and also in the resistance pattern. There was significant difference ( $p < 0.05$ ) among the various antimicrobials resistance, with tetracycline and cotrimoxazole as the most resisted antibiotics by the *S. aureus* isolates.

Vancomycin has been the drug of choice for MRSA infections until the first *S. aureus* isolate with reduced sensitivity to Vancomycin (Vancomycin intermediate *Staphylococcus aureus* (VISA)) was reported in Japan in 1997 [2].

### 3.2 Penicillin-binding Protein (PBP2<sup>1</sup>) Latex Agglutination Test Results

The 39 clinical isolates of *S. aureus* with varying percentages of resistance to the 10 antibiotics used for the susceptibility study were subjected to PBP2<sup>1</sup> latex agglutination test and result gave different types of resistance strains as indicated by latex agglutination test into VISA and MRSA. The results confirmed 39 strains of the isolates as MRSA, while some are suspected to be VISA as shown in Table 2.

### 3.3 Specimen Prevalence of Clinical Isolates of MSSA and MRSA

The prevalence of the *S. aureus* isolates of the different specimen are presented in Table 3. The

table shows the number of staphylase positive isolates, MSSA and MRSA isolates out of the 58 *S. aureus* from different specimen. The prevalence is as follow: Open wound (coagulase positive 19: MSSA 5, MRSA 14), greater than abscess (coagulase positive 11: MSSA 3, MRSA 8), greater than ear/nasal swab (coagulase positive 11: MSSA 4, MRSA 7), greater than skin swab (coagulase positive 10: MSSA 4, MRSA 6), greater than sputum (coagulase positive 7: MSSA 3, MRSA 4). The results shows open wound as a source with highest prevalence and sputum with lowest prevalence of the *S.aureus* and the MRSA.

Table 1. Antibiotics susceptibility test

S/N	Antimicrobials	Conc. µg/disc	MSSA	% (MSSA)	MRSA	% (MRSA)
1	Oxacilin	5	22.3±0.5	37.93	36.0±0.5	62.07
2	Vancomycin	30	23.0±1.0	39.65	35.6±1.1	60.35
3	Cephalexin	30	26.0±1.0	44.82	32.3±0.5	55.18
4	Levofloxacin	5	25.3±0.5	43.10	32.6±0.5	56.90
5	Ciprofloxacin	5	21.0±0.5	34.48	37.6±0.5	65.52
6	Tetracycline	30	18.3±0.5	31.03	39.6±0.5	68.97
7	Cotrimoxazole	25	20.0±0.5	32.75	39.3±0.5	67.25
8	Gentamycin	30	21.3±1.0	37.93	36.3±0.5	62.07
9	Clindamycin	2	21.3±0.5	36.20	37.3±0.5	63.79
10	Rifampicin	5	21.3±0.5	37.93	36.3±0.5	62.07

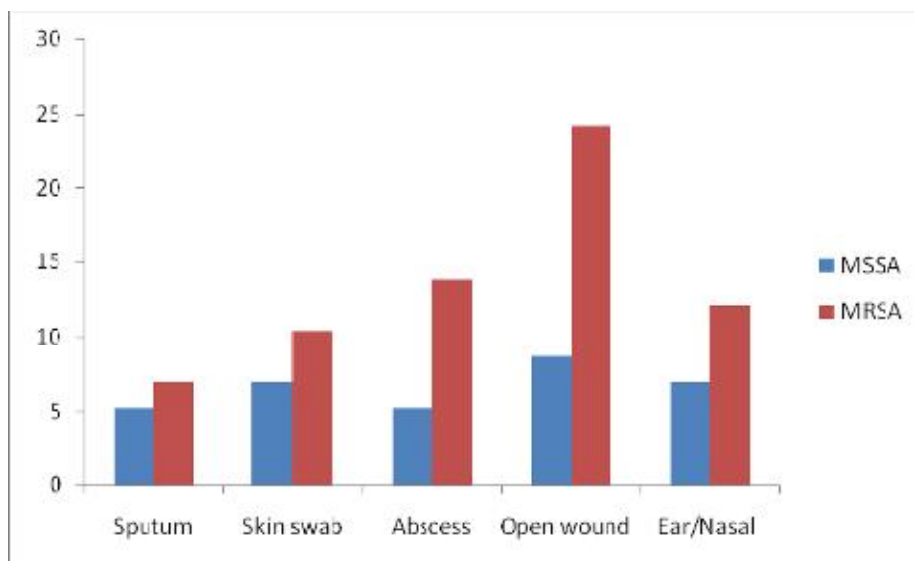


Fig. 1. Specimen prevalence of clinical isolates of MSSA and MRSA

**Table 2. Penicillin binding protein (PBP2<sup>1</sup>) latex agglutination test**

S/N	Sputum	L T	Inf.	Skin swab	L T	Inf.	Abscess	LT	Inf.	Open wound	LT	Inf.	Ear/ Nasal	LT	Inf.
1	SP4	+	MRSA	SS8	+	MRSA	AB5	-	MSSA	OW12	-	MSSA	EN4	-	MSSA
2	SP22	+	MRSA	SS10	-	MSSA	AB20	+	MRSA	OW30	+	MRSA	EN8	-	MSSA
3	SP35	-	MSSA	SS19	-	MSSA	AB31	-	MSSA	OW36	+	MRSA	EN35	+	MRSA
4	SP46	-	MSSA	SS27	-	MSSA	AB53	-	MSSA	OW53	+	MRSA	EN38	+	MRSA
5	SP651	+	VISA	SS33	+	MRSA	AB61	+	MRSA	OW66	-	MRSA	EN62	+	MRSA
6	SP720	-	MSSA	SS42	+	MRSA	AB187	+	MRSA	OW101	-	MSSA	EN127	+	VISA
7	SP1172	+	MRSA	SS46	-	MSSA	AB570	+	MRSA	OW123	+	VISA	EN208	+	VISA
8				SS57	+	MRSA	AB600	+	VISA	OW154	+	VISA	EN390	+	VISA
9				SS235	+	MRSA	AB841	+	MRSA	OW238	-	MSSA	EN504	-	MSSA
10				SS310	+	MRSA	AB1009	+	MRSA	OW270	-	MSSA	EN551	-	MSSA
11							AB1956	+	MRSA	OW417	+	MRSA	EN831	+	MRSA
12										OW578	+	MRSA			
13										OW620	+	MRSA			
14										OW819	+	MRSA			
15										OW940	+	MRSA			
16										OW947	+	MRSA			
17										OW1104	+	MRSA			
18										OW1420	+	MRSA			
19										OW1827	+	VISA			
T T	7 (58)			10 (58)			11 (58)			19 (58)			11 (58)		

Key: LT: Latex Test Inf.: Inference; +: Agglutination; -: No agglutination; T T: Total.;

**Positive control:** A known MRSA strain was used following the method given in the test procedure, agglutination occurs within 3 minutes.

**Negative control:** A know Methicillin – Sensitive Staphylococcus aureus was used following the method given in the test procedure, there was no agglutination within 3 minutes

**Table 3. Specimen prevalence rate of clinical isolates of MSSA and MRSA**

Samples	Staphylase positive <i>S. aureus</i>	MSSA	% (MSSA)	Latex test	MRSA	% (MRSA)
Sputum	7	3	5.17	4 PBP2 <sup>1</sup> +ve	4	6.89
Skin swab	10	4	6.89	6 PBP2 <sup>1</sup> +ve	6	10.34
Abscess	11	3	5.17	8 PBP2 <sup>1</sup> +ve	8	13.79
Open wound	19	5	8.62	14 PBP2 <sup>1</sup> +ve	14	24.13
Ear/Nasal	11	4	6.89	7 PBP2 <sup>1</sup> +ve	7	12.06
Total	58	19	32.74	39 PBP2 <sup>1</sup> +ve	39	67.21

#### 4. DISCUSSION

A study at Ilorin [8], Nigeria reported wound infections of 38 % as the highest frequency of *S. aureus* isolates. This agrees with the result of the present study where the order of the Coagulase positive *S. aureus*, MSSA and MRSA prevalence is as follow: Open wound 19 (32.75%), greater than abscess 11 (18.96%), greater than Ear/Nasal swab 11 (18.96%), greater than Skin swab 10 (17.24%), greater than Sputum 7 (12.06%) except the little difference between the abscess and ear/nasal in the staphylase positive *S. aureus* and MRSA prevalence.

The association of MRSA with therapeutic challenges, complications, deaths and cost related to longer hospital stay compared with MSSA has been widely documented [10,11]. More importantly, the multidrug resistant hospital-acquired MRSA (HA-MRSA) strains and their intrinsic resistance to beta-lactam antibiotics made treatment difficult using the most available and less costly antibiotics in developing countries [11]. In agreement with the previous study by (Olowo O. A) [12] open wound has the highest prevalence of *S.aureus* and MRSA.

##### 4.1 Antibiotics Susceptibility Pattern of the Clinical Isolates

The susceptibility and resistance pattern of the 58 clinical isolates *S. aureus* were as seen in the Table 1. Susceptibility test profile revealed a high level of resistance amongst the *S. aureus* (39 isolates) to all the commonly used antimicrobials, ciprofloxacin, gentamicin, clindamycin, levfloxacin, cephalaxcin, tetracycline, rifampicin cotrimoxazole, oxacillin and vancomycin. The results were comparable to those in a previous study carried out by Nwankwo and Nasiru [13], but not as high as in this study. Vancomycin was the drug of choice for MRSA infections for many years, until the first *S. aureus* isolate with

reduced sensitivity to vancomycin (vancomycin intermediate *Staphylococcus aureus* (VISA) was reported in Japan in 1997 [14].

The susceptibility and resistance pattern of these clinical isolates in oxacillin and vancomycin was not different, almost no significance difference ( $P > 0.05$ ). Hence, most of the MRSA among these isolates were also VISA and VIRA as it is revealed in Table 2. It was discouraging to note that vancomycin resistance was highly observed among the isolates.

In this study levofloxacin and cephalaxcin are the least resisted antibiotics, while tetracycline and cotrimoxazole are the highest resisted antibiotics [12,13].

#### 5. CONCLUSION

In this study the highest prevalence of *S. aureus* isolates and MRSA strains were detected in wound samples. The *S. aureus* isolates were resistant to tetracycline (68.97%) and cotrimoxazole (67.25%), and sensitive to levofloxacin (43.10%) and cephalaxcin (44.82%).” The MRSA strains resisted all the antimicrobials in varying percentages including vancomycin (60.35%), levofloxacin (56.90%) and cephalaxcin (55.18%) respectively. This shows that the MRSA isolates are multiple drug resistant strains and a threat to public health and healthcare workers [15].

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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