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# Characterization of methicillin resistant *Staphylococcus aureus* (MRSA) isolates using oxacillin-cefoxitin disk diffusion test (OCDDT)

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### ABSTRACT

**Introduction:** Methicillin-resistant *Staphylococcus aureus* (MRSA) still remains an important nosocomial and community-acquired pathogen because of its multidrug resistant nature which gives them the innate/acquired ability to evade the onslaught of antibiotics. MRSA infection now occurs globally; and it is important to be on the lookout for these resistant pathogens in clinical samples in order to effectively guide therapy for patients.

**Objective:** This study was aimed at evaluating the frequency of MRSA strains from urine samples of out-going patients in a tertiary hospital using oxacillin-cefoxitin disk diffusion test (OCDDT).

**Materials and methods:** In this study, a total of thirty nine (39) non-duplicate isolates of *S. aureus* from urine samples of out-going patients who attended a tertiary hospital in Abakaliki, Nigeria for medical attention was bacteriologically investigated for methicillin resistance. All the *S. aureus* isolates was re-characterized using standard microbiology techniques. The modified Kirby-Bauer disk diffusion technique was used to evaluate the antibiogram of the *S. aureus* clinical isolates while MRSA positive isolates was phenotypically confirmed using the oxacillin-cefoxitin disk diffusion technique (OCDDT). Multiple antibiotic resistance index (MARI) was used to calculate the multidrug resistant nature of the MRSA positive *S. aureus* isolates.

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**Results:** Our result shows that the *S. aureus* isolates showed varying rates of susceptibility and resistance to the tested antibiotics which are usually used in hospitals for treating infections caused by the organism. The *S. aureus* isolates was highly resistant or intermediately resistant to cefoxitin (56.4 %), bacitracin (89.7 %), oxacillin (89.7 %), and mupirocin (71.7 %). Clindamycin which is usually used for the treatment of *S. aureus* infections had no inhibitory activity on the *S. aureus* isolates evaluated in this study. Out of the 39 isolates of *S. aureus*, the detection of MRSA positive isolates was recorded at 35.8 % (n=14). All the MRSA positive isolates had MAR of 0.5 on average; and this indicates the multiple antibiotic resistance nature of the MRSA positive isolates recovered in this study. Conclusively, this study has presumptively shown that *S. aureus* isolates of clinical origin in this region are methicillin resistant. Further studies are required to characterize the genetic factors of the MRSA isolates. The worldwide problem of antibiotic resistance especially those caused by MRSA isolates warrants the need for accurate and prompt detection of MRSA from clinically important samples in order to ensure proper antibiotic therapy in infected individuals as well as to stop any disease outbreak that may be due to them.

**Keywords:** MRSA, *Staphylococcus aureus*, Antimicrobial Resistance, Gram positive bacteria, Nigeria

## Introduction

The resistance of bacterial pathogens (from the community and hospital settings) to readily available antibiotics is a global health menace, and it has contributed to the health burdens of patients across the world with an increasing frequency of morbidity and mortality. *Staphylococcus aureus* is a Gram positive, asporogenous bacterium ubiquitously found on the skin, nasal passages and mucous membranes of humans and animals. However, pathogenic strains of staphylococci have been implicated in a number of community- and hospital-acquired infections, which is why this Gram positive organism is important in clinical medicine [1,2,3,4]. According to recent reports, *S. aureus* is an important cause of hospital-acquired pneumonia (HAP) and community-acquired pneumonia (CAP); and methicillin resistant *S. aureus* community-acquired pneumonia (MRSA-CAP) together with post-influenza staphylococcal pneumonia have been well noted [1,3,5]. Methicillin was the first  $\beta$ -lactamase-resistant and semi-synthetic penicillin, and it is a  $\beta$ -lactam antibiotic that is

stable to  $\beta$ -lactamase enzymes produced by *S. aureus* and other Gram negative bacteria [6]. Though not administered orally but parenterally, methicillin was introduced into clinical medicine in the early 1960's [7] as a substitute to penicillin, which was before then made less-efficacious by  $\beta$ -lactamase enzymes produced by pathogenic bacteria such as *S. aureus*. Nowadays, strains of *S. aureus* that are resistant to methicillin (a potent bacterial cell wall inhibitor) are now found in both the hospital and community settings [3,8,9]. Methicillin resistant *Staphylococcus aureus* (MRSA) are strains of *S. aureus* that are resistant to methicillin [8]. MRSA started making rounds in the health sector and became a global public health issue in the early 1960s when the first strain of *S. aureus* that resisted the actions of methicillin was reported in the UK [7,10]. This first report of MRSA in England in the early 1960s followed the introduction of methicillin into clinical medicine for the treatment of bacterial infections that defied the antimicrobial actions of penicillin. MRSA strains have since emerged and spread as a serious worry in

human medicine because of their multiple antibiotic resistant nature. MRSA according to Brook *et al.* [11] harbour genes (known as *mecA*) that render pathogenic *S. aureus* insensitive to methicillin, a once potent class of antibiotic. The acquisition of *mecA* gene by *S. aureus* strains confers on the organism total resistance to all  $\beta$ -lactam antibiotics including penicillin, clindamycin, erythromycin, tetracycline and the cephalosporins – which are used clinically for the treatment of infections caused by the pathogen [7,11,12]. The gene, *mecA* codes for a special type of penicillin-binding-protein known as PBP2a [13] - which confers on MRSA strains the ability to be multiply resistant. The emergence and spread of MRSA strains should be kept under check through proper monitoring and evaluation of antibiotic policy/usage; and by prompt and accurate detection of resistant bacteria from clinical samples. In this study, the occurrence of MRSA isolates from urine samples of out-going patients who attended a tertiary hospital for medical attention in Abakaliki, Nigeria was phenotypically characterized using disk method.

## Materials and methods

**Collection and re-characterization of bacterial isolates:** Non-duplicate bacterial isolates (n=39) of *Staphylococcus aureus* were obtained from the culture collection unit of a Federal Teaching Hospital in Abakaliki, Ebonyi State, Nigeria for this study. All the *S. aureus* isolates were re-identified and confirmed as *S. aureus* isolates using standard microbiology techniques including growth characteristics on culture media, coagulase test, catalase test and Gram staining technique [14].

**Antimicrobial susceptibility test:** Antimicrobial susceptibility test (AST) was performed on the *S. aureus* isolates using the modified Kirby-Bauer disc diffusion method on unsupplemented Mueller-Hinton (MH) agar plates aseptically swabbed with the standardized test isolates. The already swabbed plates were allowed to stand for 10 -

15 min; and antibiotic impregnated discs namely: clindamycin (2  $\mu$ g) erythromycin (15  $\mu$ g), cefoxitin (30  $\mu$ g), cloxacillin (5  $\mu$ g), mupirocin (5  $\mu$ g), bacitracin (10  $\mu$ g), oxacillin (1  $\mu$ g) and gentamicin (10  $\mu$ g) [Oxoid, UK] were placed on the MH agar plates using sterile forceps. The MH agar plates laddened with the test *S. aureus* isolates and antibiotic disks were incubated at 37°C overnight, and the zones of inhibition around each disc were measured, recorded and interpreted using standard zone size (breakpoints) as recommended by Clinical and Laboratory Standard Institute, CLSI [9,15,16].

**Oxacillin-cefoxitin disk diffusion test (OCDDT):** MRSA isolates was phenotypically detected using the oxacillin-cefoxitin disk diffusion technique (OCDDT) as described by Ike *et al.* [9], and with little modification. The test was performed using oxacillin disk (1  $\mu$ g) and cefoxitin disk (30  $\mu$ g). Oxacillin (1  $\mu$ g) and cefoxitin disk (30  $\mu$ ) was aseptically placed at a distance of 20 mm apart on MH agar plates previously swabbed with *S. aureus* isolates. All MH agar plates were incubated at 37°C for 18-24 hours. The zone of inhibition produced was measured, recorded and interpreted as per the guideline of Clinical and Laboratory Standard Institute, CLSI [16]. *S. aureus* isolates whose inhibition zone diameter (IZD) was  $\leq$  19 mm for oxacillin and  $\leq$  21 mm for cefoxitin were phenotypically confirmed MRSA isolates [9]

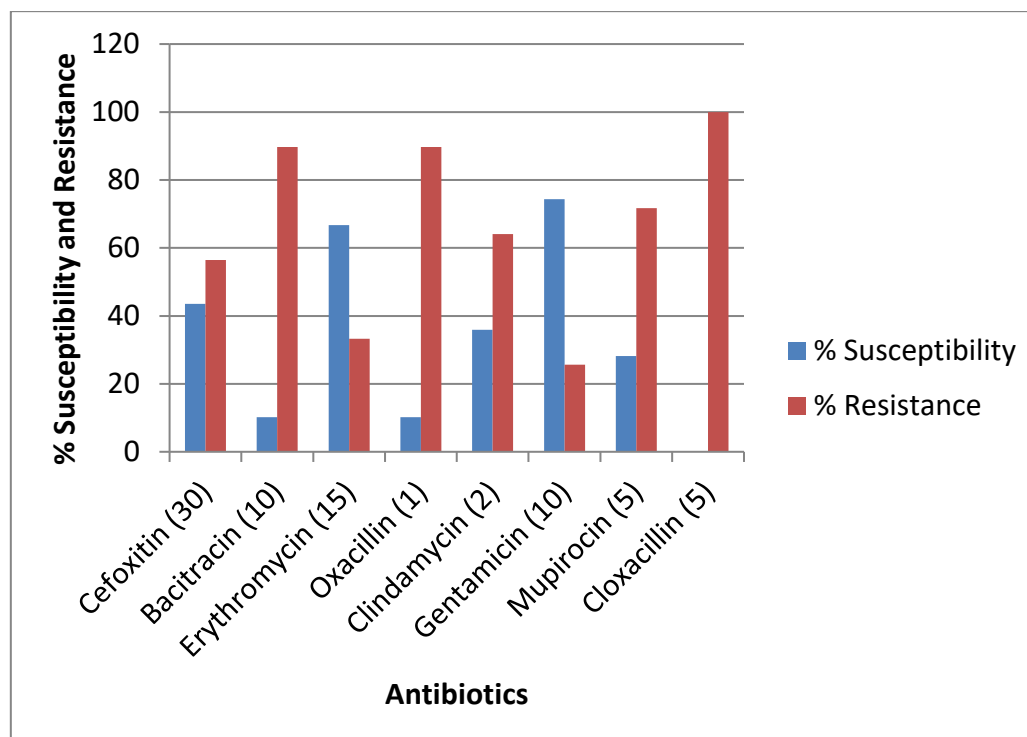
**Multiple antibiotic resistance index (MARI):** Multiple antibiotic resistance was calculated for only MRSA positive isolates [15]. This was done using the MARI formula as follows:  $MARI = a/b$ , where “a” is the number of antibiotics to which the resistant isolate was resistant to, and “b” is the total number of antibiotics to which the resistant isolate has been evaluated for.

## Results

Thirty nine (39) non-duplicate isolates of *Staphylococcus aureus* were collected from the culture collection unit of a tertiary hospital in

Abakaliki, Ebonyi State, Nigeria. The *S. aureus* isolates were from the urine samples of out-going patients who attended the hospital for medical attention; and they were collected in transport swabs and transported to the

microbiology laboratory of Ebonyi State Univeristy, Abakaliki for further bacteriological analysis within one hour of collection. The result of the antimicrobial susceptibility profile of the *S. aureus* isolates is shown in Figure 1.



**Figure 1. Percentage susceptibility and resistance of the 39 *S. aureus* isolates**

The *S. aureus* isolates was highly resistant or intermediately resistant to cefoxitin (56.4 %), bacitracin (89.7 %), oxacillin (89.7 %), and mupirocin (71.7 %). All the *S. aureus* isolates were completely resistant to the antimicrobial action of cloxacillin (Figure 1). However, the *S. aureus* isolates showed some level of susceptibility to gentamicin (74.3 %), erythromycin (25.6 %) and cefoxitin (43.5 %). The result of the prevalence of MRSA positive isolates is shown in Table 1. Out of the 39

isolates of *S. aureus* evaluated for methicillin resistance in this study, only 14 isolates of *S. aureus* were phenotypically confirmed to be MRSA positive isolates (Table 1). Table 2 shows the result of the multiple antibiotic resistance index (MARI) calculated for only MRSA positive isolates. The MRSA positive isolates had MARI that ranged between 0.3-0.7, showing the multiple antibiotic resistance profile of the MRSA positive isolates.

**Table 1. Detection of MRSA isolates**

Isolate	MRSA positive (n %)	MRSA negative (n %)
<i>S. aureus</i>	14 (35.8)	25 (64.1)

**Table 2. Result of MARI**

Isolates	MARI	Antibiotics
B11	0.6	FOX, B, X, DA, MUP and OB
B2	0.4	B, OX, MUP and OB
B10	0.7	FOX, B, E, OX, DA, MUP and OB
B4	0.5	E, OX, DA, MUP and OB
B15	0.5	E, OX, DA, MUP and OB
B6	0.3	B, OX and OB
B7	0.6	FOX, B, OX, DA, MUP and OB
B8	0.2	B and OX
B3	0.4	B, OX, MUP and OB
B10	0.6	E, B, OX, DA, MUP and OB
B18	0.7	FOX, B, OX, DA, CN, MUP and OB
B26	0.6	E, B, OX, DA, MUP and OB
B1	0.6	E, B, OX, DA, MUP and OB
B14	0.6	FOX, B, OX, DA, MUP and OB

**KEY:** FOX = Cefoxitin, B = Bacitracin, E = Erythromycin, OX = Oxacillin, DA = Clindamycin, CN = Gentamicin, MUP = Mupirocin and OB = Cloxacillin.

## Discussion

Antibiotic-resistant bacteria pathogens (including those mediated by MRSA) cause serious therapeutic challenge to clinical medicine - owing to the difficulty encountered in their treatment and accurate detection in the hospital laboratory. *Staphylococcus aureus* accounts for many hospital- and community-acquired infections including skin infections, bacteraemia, and pneumonia; and most strains of this organism are fast becoming resistant to some commonly available antibiotics. We presumptively investigated the prevalence of MRSA isolates from clinical samples (urine) of out-going patients who attended a tertiary hospital for medical services in a Nigerian city. The *S. aureus* isolates evaluated in this study showed varying levels of resistance and susceptibility to the tested antibiotics. Surprisingly, all the *S. aureus* isolates were highly resistant to cloxacillin (100 %), which is clinically used to treat infections caused by *S. aureus* strains (Swenson *et al.*, 2005). The *S. aureus* isolates also showed least susceptibility

to oxacillin (89.7 %), clindamycin (64.1 %), mupirocin (71.7 %), cefoxitin (56.4 %) and bacitracin (89.7 %). The high level of resistance of *S. aureus* isolates reported in this study have been previously documented in Awka, Southeast Nigeria – where it was reported that *S. aureus* isolates are becoming resistant to some commonly available antibiotics especially the penicillinase-stable penicillins (PSPs) such as methicillin and cloxacillin which are used clinically to treat infections caused by *S. aureus* [5,8,9,17,18]. In our study, only 14 isolates of *S. aureus* out of the 39 isolates evaluated in this study for methicillin resistance was phenotypically confirmed to be MRSA positive isolates. This result suggest that the *S. aureus* isolates in this region are methicillin resistant; and this could negatively affect antimicrobial therapy of infected patients if proper susceptibility studies is not undertaken prior to treatment. The frequency of MRSA positive isolates we recorded in this study is in accordance with previous studies that also reported that MRSA is common in hospital

environments [2,3,8]. Previous studies conducted in Taiwan, Europe, and the United States of America have also reported the increasing frequency of MRSA isolates from clinical samples in the hospital or healthcare environment [12,19,20]. On average, the MRSA positive isolates had a median MARI of 0.5; and this implies that the MRSA positive isolates detected in this study were resistant to 5 antibiotics out of the 8 antibiotics that we used in this study. In conclusion, our study provides further evidence that some *S. aureus* isolates from clinical samples of patients in this region are methicillin resistant and at the same time multiply resistant to some commonly available antibiotics. Further molecular characterization of the genetic factors responsible for methicillin resistance in the *S. aureus* isolates is therefore necessitated. However, we therefore recommend the introduction or use of the oxacillin-cefoxitin disk diffusion technique (OCDT) in our hospital's laboratories for the phenotypic detection of MRSA isolates in order to forestall any disease outbreak due to these organisms since the routine antimicrobial susceptibility test is insufficient to detect MRSA isolates without modification.

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