Antibiotic Resistance Pattern of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Mubi, Adamawa State, Nigeria

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ABSTRACT

This study draws attention to the increasing prevalence of multi-drug resistant (MDR) MRSA in Mubi especially those mediated by mecA. The use of Cefoxitin disc diffusion test as a suitable substitute procedure for the detection of mecA-mediated Methicillin resistance has been widely reported and accepted. A standard procedure was used for both the isolation and identification of Staphylococcus aureus and the phenotypic screening of Methicillin-resistant strains. Most of the MRSA was isolated from semen, wound swab and high vaginal swab. Of the 34 isolated S. aureus, 32(94.1%) and 22(64.7%) were found to be Methicillin-resistant (MRSA) by Oxacillin and Cefoxitin disc diffusion test respectively. Out of the 12 S. aureus susceptible to Cefoxitin, two were also susceptible to Oxacillin while 10 were resistant to Oxacillin. The resistance pattern of MRSA revealed that MRSA was mostly resistant to Norfloxacin 18(81.8%), followed by Ampiclox 16(72.7%), Amoxicillin 15(68.2%) and the least resistance was recorded on Levofloxacin and Ciprofloxacin 6(27.3%) each. Most of the MRSA also exhibited multi-drug resistant (MDR) phenotype. Oxacillin disc diffusion test overestimated MRSA when compared with Cefoxitin disc diffusion test. More so, the high prevalence of MRSA coupled with MDR phenotype in this study is worrisome.

Keywords: MRSA, Resistance, S. aureus, mecA, Mubi

INTRODUCTION

Staphylococcus aureus is a member of the normal flora of the human body, but the ability of some strains to produce some virulent factors makes them virulent.[1] Many strains of *Staphylococcus aureus are resistant to a wide spectrum of antibiotics by intrinsic mechanisms and their ability* to acquire resistance genes through mobile genetic elements.[2] Methicillin-resistant *Staphylococcus aureus* (MRSA) is any strain of *S.aureus* that has developed resistance to β-lactam antibiotics, including the Cephalosporins. Strains unable to resist these antibiotics are classified as Methicillin-sensitive *S.aureus* (MSSA).[3,4,5] Resistance to Methicillin is due to the presence of the *mecA* gene, which encodes a modified Penicillin-binding protein 2 (PBP2' or PBP2a) with low affinity for Methicillin and all β-lactam antibiotics.[6]Bacteria expressing this protein are not only resistant to β-lactam antibiotics but also aminoglycosides and quinolones.[7,8]

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In addition to the cefoxitin disc diffusion test method, other phenotypic tests used in the detection of MRSA from clinical samples include Oxacillin screen agar and disc diffusion methods and MIC breakpoint (by both agar and broth dilution) method. Oxacillin disc has been used in a routine test for the determination of MRSA; nonetheless, several studies have accounted for low specificity and sensitivity of Oxacillin when related with Cefoxitin for the screening of MRSA.[9]

Several studies have reported that Cefoxitin disc diffusion test is a good alternative method for the detection of *mecA*-gene-mediated Methicillin resistance.[10,11,12] This is because Cefoxitin is a powerfully persuasive activator of the *mecA* regulatory system.[12] The study was aimed to detect and subject *S. aureus* for Methicillin resistance using Oxacillin and Cefoxitin disc diffusion methods and to determine their resistance pattern to various antibiotics.

MATERIALS AND METHODS

Study Area

This study was carried out in Mubi metropolis located in Adamawa state which is a bordered State in Nigeria with Cameroun Republic. Mubi is situated in the northern part of Nigeria between latitude 90°26 and 10° 10N

and between longitude 73° 1 and 13° 44E, the area has a landmass of 566.4km^3 with a population of 759,045 at a density of 160.5 per square kilometre.[13]

Sample Collection

Clinical samples were collected from patients attending Mubi General Hospital and New Medical Centre Mubi between May-August, 2018. The clinical samples include high vaginal swab (HVS), semen, sputum, wound swab and urine. Samples were subsequently transported to the Microbiology Laboratory of the Department of Biological Science Technology, Federal Polytechnic Mubi for further processing.

Identification of *S. aureus*

Standard procedure was used to identify Staphylococci isolates based on growth and fermentation on mannitol salt agar (MSA), morphological characteristics, Gram staining and reactions to catalase and coagulase test.[14]

Phenotypic Detection of Methicillin-resistant *Staphylococcus aureus*

Methicillin resistance among *Staphylococcus aureus* isolated from clinical samples was detected phenotypically using 1µg oxacillin disc CT0159B (Oxoid, UK) and 30µg cefoxitin disc (oxoid, UK). Each isolate was introduced into sterile normal saline and compared with a 0.5 McFarland Standard. The suspension was carefully swabbed on the surface of Mueller-Hinton Agar (MHA) plates. Commercially available oxacillin and cefoxitin discs were placed separately on the plate of Mueller-Hinton agar and incubated aerobically at 35-37°C for 24 hrs. Zone of inhibition ≤12mm was interpreted as methicillin-resistant, while inhibitory zone ≥13mm was interpreted as methicillin-sensitive for oxacillin disc.[15] For cefoxitin disc, the diameter zones of inhibition were measured and interpreted according to CLSI standard; that is ≤21mm is *mecA* positive while ≥22 is *mecA* negative.[16]

Determination of antibacterial resistance profile of Methicillin-resistant *S. aureus*

Pure isolates of identified *S. aureus* were subjected to antibacterial susceptibility testing using the disc diffusion method based on Clinical Laboratory Standard Institute (CLSI), using the following antibiotic discs; Erythromycin (E) 30µg, Ciprofloxacin **(**CIP**)** 10µg, Ampiclox (APX) 20µg, Gentamicin (CN) 10µg, Rifampicin (RD) 20µg, Amoxicillin (AMX) 20µg, Streptomycin (S) 30µg, Norfloxacin (NB) 10µg, Chloramphenicol (CN) 30µg, and Levofloxacin (LEV) 20µg. Isolates were inoculated into Mueller-Hinton agar medium. The antibiotic discs were placed on the agar plates with the aid of a sterile pointed tip forceps and incubated at 37°C for 24hrs. The presences of a clear zone around the antibiotic discs were measured and interpreted according to CLSI standard.[17]

Statistical analysis

Non-parametric Mann-Whitney statistics were used to determine the level of significance in resistant phenotype between methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA). All statistical analyses were carried out using the SPSS 17.0 window based program. Statistical significance difference was set at $p < 0.05$.

RESULTS

Thirty-four (34) *Staphylococcus aureus* was identified from 47 clinical samples. Of the 34 isolated *S. aureus*, 32(94.1%) and 22(64.7%) were found to be methicillin-resistant (MRSA) by oxacillin and cefoxitin disc diffusion test respectively (Table 1). Most of the MRSA was isolated from semen, wound swab and a high vaginal swab. The resistance pattern of MRSA based on Cefoxitin disc diffusion test (Table 2) revealed that MRSA was mostly resistant to Norfloxacin 18(81.8%), followed by Ampiclox 16(72.7%) and Amoxicillin 15(68.2%). The least resistance was recorded on Levofloxacin and Ciprofloxacin - 6(27.3%) each. Most of the MRSA also exhibited multi-drug resistant (MDR) phenotype. There was no statistical difference in resistance phenotype between MRSA and MSSA (P=0.940).

Table 1: Distribution of MRSA among clinical samples

Samples	Frequency	No of *S.aureus*	Cefoxitin disc (30µg)		Oxacillin disc (1µg)	
			MRSA	MSSA	MRSA	MSSA
HVS	9	7(77.87)	6(85.7)	1(14.3)	7(100)	0
Semen	4	4(100)	3(75.0)	1(25.0)	4(100)	0
Sputum	4	4(100)	0	4(100)	3(75)	1(25)
Wound swab	7	4(57.1)	3(75.0)	1(25.0)	4(100)	0
Urine	23	15(65.2)	10(66.7)	5(33.3)	14(93.3)	1(6.7)
Total	47	34(72.3)	22(64.7)	12(35.3)	32(94.1)	2(5.6)

Legend: MRSA=Methicillin-resistant *Staphylococcus aureus*, MSSA = Methicillin sensitive *Staphylococcus aureus*

Table 2: antibiotic resistance pattern of MRSA (Based on Cefoxitin disc diffusion test)

Antibiotics	MRSA (n=22) (%)[a]	MSSA (n=12) (%)[a]
Gentamycin (CN)	13(59.1)	5(41.7)
Ampiclox (APX)	16(72.7)	7(58.3)
Rifampicin (RD)	8(36.4)	6(50.0)
Amoxicillin (AMX)	15(68.2)	9(75.0)
Streptomycin (S)	8(36.4)	4(33.3)
Norfloxacin (NB)	18(81.8)	12(100)
Chloramphenicol (CH)	9(40.9)	4(33.3)
Ciprofloxacin (CIP)	6(27.3)	4(33.3)
Erythromycin (E)	9(40.9)	5(41.7)
Levofloxacin (LEV)	6(27.3)	2(16.7)

a= not statistically different (P=0.940)

DISCUSSION

Staphylococcus aureus is a well known human pathogen that is primarily associated with infection in a human causing a variety of diseases with increasing resistance to β-lactam antibiotics. Increased resistance of Methicillin-resistant *Staphylococcus aureus* (MRSA) to antibiotics often used to treat staphylococcal infection have become a major cause of concern worldwide.[18]

In this study, a 64.7% prevalence rate was observed for MRSA using cefoxitin disc diffusion test. The prevalence rate in this study was higher than previous reports in Nigeria, [19,20] India [21] and Iran.[22] However, higher prevalence rate comparable to that of our findings has also been reported in Nigeria.[23,24] The discordances in the prevalence rate could be attributed to the type of media used, the method used (phenotypic or molecular), type of antibiotic disc used, the density of inoculums, period and temperature of incubation and the presence or absence of some limiting factors in the growth medium. Other studies reported the use of phenotypic methods such as Oxacillin screen agar test, MIC breakpoint for both oxacillin and cefoxitin, E-test, agglutination test (Masterlex MRSA) and molecular method [25] which were not used in our study. The high prevalence rate of MRSA in this study area could best be explained by overused and misused of β-lactams to treat various infections especially those involving Staphylococci spp. This is because this class of antibiotics are relatively inexpensive, readily available and accessible.

The highest frequencies of MRSA isolates were from high vaginal swab (HVS), semen and wound swab. This was contrary to

previous reports which showed a higher frequency of MRSA from pus, urine and ear swab.[21,26] However, a previous study reported a higher frequency of MRSA from wound specimen.[27]

Studies have shown that detection of *mecA* or its product is a gold standard for MRSA confirmation.[10,11,26] Recent studies indicate the superiority of Cefoxitin over other recommended phenotypic methods in detecting surrogate *mecA* gene as a marker for MRSA and is now an accepted method for the detection of MRSA by many reference groups including CLSI.[3,22,25]

In this study, 22 (64.7%) of the *S. aureus* isolates were resistant to Cefoxitin which is often used as a surrogate marker for *mecA* gene. This implies that resistance by this strain may have been mediated by *mecA* gene. Several studies have confirmed and reported the reliability of Cefoxitin disc over other phenotypic methods in detecting Methicillin resistance, especially those mediated by *mecA* gene or its products. This is because Cefoxitin is more specific, sensitive and a potent inducer of *mecA* gene or Penicillin-binding protein 2a (PBP2a).[9,25] Some previous studies had shown 100% specificity and sensitivity in the use of Cefoxitin to phenotypically detect *mecA* gene in comparison to *mecA* gene detection by PCR method.[10,25,28] Thus, recommended the Cefoxitin disc diffusion method for MRSA detection and PCR alternative for detection of *mecA*-mediated MRSA in areas where resources are scarce or limited.[22,28]

The results also showed that 10 isolates were sensitive to Cefoxitin but resistant to Oxacillin. This could be due to hyper production of beta-lactamase which may lead to the phenotypic expression of Oxacillin resistance as has been shown by Swenson and colleagues.[11] One study reported that isolates that were resistant to Oxacillin but sensitive to Cefoxitin were negative for *mecA* gene.[22]

In this study, there was no significant difference in antibiotic resistance between MRSA and MSSA. This is contrary to a previous study which revealed that MRSA resistance to different antibiotics was more than MSSA strains.[22] This suggests that MSSA in our study area may possess resistance factors other than *mecA* which enable them to express resistance phenotype similar to that of MRSA. More so, lack of significant difference between MRSA and MSSA in terms of resistance phenotype implies that *S. aureus* from our study area may constitute a nuisance to antibiotic therapy which consequently constitute a risk to clinical and public health. Also, MRSA in this study was more resistant to Norfloxacin, Ampilox and Amoxicillin. This can be correlated to previous studies in Nepal.[29]

Similarly, most of the MRSA in this study exhibited multi-drug resistant phenotype. This observation is in correlation with previous researchers.[22,30] Findings from previous studies revealed an association between MRSA and resistance to other antibiotics.[31] This was collaborated in our study as MRSA were highly resistant to Norfloxacin and Gentamycin. Relatively the same kind of resistance to β-lactams (Amoxicillin and Ampiclox) used in this study was similar to that of previous studies.[31] This may be attributed to the expression of β-lactamase enzymes which is common among MRSA.

High resistance to antibiotics by MRSA could be attributed to the use of these drugs in the treatment of diseases involving both humans and animals. Resistant bacteria may transfer-resistant genes to other bacteria and become important in the spread of antibiotic resistance. Indiscriminate use of antimicrobial agents and antibiotic sale behaviour (for example, sale of antibiotics without prescription, sale of under dose and substituting brands) enhances the development of drug resistance.[32] The multidrug resistance of MRSA in this study area constitutes a major health risk not only to the community but to the country at large. Although the routine phenotypic test for the detection of MRSA is rarely carried out in health facilities in our environment probably because the epidemiological trend is usually obscure or not monitored and research has shown that MRSA is responsible for most of the hospital-acquired infections.[3] The presence of MDR MRSA may also limit the

treatment option for infections caused by this organism. This emphasizes the need for rapid and early detection of MRSA for appropriate therapy and management.

One limitation in this study was that we were unable to use molecular studies to establish the presence of *mecA* gene which is a gold standard for methicillin resistance. Also, the number of samples used limited our ability to demonstrate strong associations.

CONCLUSION

The result of this study showed that Oxacillin disc diffusion test may overestimate MRSA in this environment when compared with Cefoxitin disc diffusion test. The high prevalence of MRSA coupled with MDR phenotype in this study is worrisome. This is because therapeutic options may be limited especially to infections caused by these organisms. A routine check for Methicillin resistance in *S.aureus* should be encouraged in all tiers of health centres in Nigeria as this will help in prompt, appropriate management of MRSA.

AUTHORS' CONTRIBUTION

Author MYT conceived and designed the study and wrote the first draft of the manuscript. Authors IO help in manuscript editing and review including literature search. Author FJ facilitated sample collections in the field and analyses in the laboratory. All authors read and approved the final version of the manuscript.

COMPETING INTEREST

All authors' have declared no competing interest

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