World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

MOLECULAR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN CLINICAL ISOLATES FROM MAITAMA DISTRICT HOSPITAL, ABUJA, NIGERIA

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Article Received on 08/04/2017 **Article Revised on 27/04/2017** **Article Accepted on 18/05/2017**

ABSTRACT

Methicillin-resistant *S. aureus* (MRSA) is recognized globally as an important pathogen causing difficult- to treat infections in hospital and community setting due to its multidrug resistance to β-lactam antibiotics, posing a significant limitation on the treatment of diseases caused by *S. aureus* strain. A total of 118 non-duplicate suspected *S. aureus* isolates were collected from Maitama District Hospital, Abuja, Nigeria. Using conventional biochemical analysis, 79(66.9%) of the isolates were observed to ferment Mannitol salt agar, of which 60(76.9%) were Coagulase-positive and 19(31.7%) were Coagulase-negative. Further evaluation using Microgene Staph. Identification kit 56(93.3%) were confirmed as *S. aureus*, 3(5%) as *S. xylosus* and 1(1.7%) as *S. hyicus*. The antibiogram showed that the *S. aureus* isolates were most susceptible to gentamicin, vancomycin, ciprofloxacin, erythromycin and linezolid. However, they were highly resistance to cefoxitin 46(82.1%). The *S. aureus* isolates also showed reduced susceptibility to tigercycline 40(71.4%), clindamycin 37(66.1), chloramphenicol 27(48.2%), amoxicillin-clavulanic acid 30(53.6%) and sulphamethoxazole-trimethoprim 27(48.2%). Twenty six (46.4%) of the coagulase-positive *S. aureus* were observed to be methicillin-resistance *S. aureus* (MRSA) while 30(53.6%) were methicillin-sensitive *S. aureus* (MSSA), identified phenotypically using Cefoxitin disc diffusion and PBP2[a] agglutination kit. Molecular analysis showed that 14(73.7%) harbored *mecA* gene while 16(84.2%) harbored *femB*. These MDR characteristics found in this isolates might be a contributing factor to the antibiotic susceptibility pattern of the *mecA* and *femB S. aureus* strain isolated in this environment, suggesting that most of the drugs might not be effective in the treatment of staphylococcal infection due to MRSA.

KEYWORDS: *Staphylococcus aureus*, *mecA* and *femB* genes, MRSA, Antibiotics resistance.

INTRODUCTION

Staphylococcus aureus is a dangerous pathogen, responsible for a multitude of human infections around the world.[17] Many *S. aureus* infections present as moderately severe infections of the skin or respiratory tract, but *S. aureus* may also cause more dramatic forms of disease that may be life-threatening, such as necrotizing fasciitis or necrotizing pneumonia. Considerable efforts have been undertaken to decipher the importance that specific molecular determinants have in defining *S. aureus* virulence and interaction with the host. From a clinical point of view, a major problem that physicians have to face when treating *S. aureus* infections is antibiotic resistance. Resistance to the first antibiotic, penicillin emerged in the 1940s.[3] In 1942, penicillin resistant *S. aureus* was detected.

Mechanistically, resistance to penicillin is due to an enzyme called penicillinase, which was found even before the introduction of penicillin into clinical use.[1] Penicillinase cleaves the β-lactam ring that is characteristic of β-lactam antibiotics such as penicillin and its derivatives. Already in the 1950s, penicillinase-containing strains of *S. aureus* were pandemic in hospitals and the community.[25] Nowadays, most infectious *S. aureus* isolates are resistant to penicillin.

To overcome the problem with penicillin-resistant *S. aureus*, the semi synthetic antibiotic methicillin was

developed, which is derived from penicillin, but resistant to β-lactamase inactivation. Methicillin was introduced by Beecham in 1959; but already about 1 year later, methicillin-resistant *S. aureus* was detected in the UK.[12] Unlike in the case of resistance to penicillin, the mechanism underlying methicillin resistance protects the bacteria from the entire class of β-lactam antibiotics including penicillins, cephalosporins and carbapenems. *Staphylococcus aureus* epidemics occur in waves of antibiotic resistance.[5] The first epidemic penicillin-resistant strains were replaced by the so-called 'archaic' methicillin-resistant *S. aureus* (MRSA) strains first found in the UK. This epidemic was largely restricted to Europe. Starting in the 1980s, novel lineages of MRSA emerged, leading to a worldwide pandemic of MRSA that is still ongoing. Nowadays, many industrialized countries report that methicillin-resistant strains account for at least 25–50% of infectious *S. aureus* isolates in hospitals.[8] In contrast, some countries such as The Netherlands and the Scandinavian countries historically have low MRSA infection rates (often < 1%), most likely owing to rigid search-and-destroy and surveillance policies, as well as restraint in antibiotic prescription. In fact, a recent Japanese study indicates that high antibiotic consumption rates lead to increased MRSA burden over time.[19] While for a long time MRSA infections were limited to hospitalized patients, the most recent epidemic MRSA wave, beginning in the mid- to late 1990s, is characterized by the emergence of community associated MRSA (CA-MRSA) with the capacity to infect otherwise healthy individuals.

MATERIALS AND METHODS

Collection of isolates

Total of 118 consecutive, non-duplicate suspected *S. aureus* isolates were collected from Maitama General Hospital, Abuja, between November 2014 to April 2015 after the ethical clearance has been obtained from the FCTA Hospital Management Board. Epidemiological information collected with bacterial isolates includes, age, sex of the patient, type and source of clinical specimens. The sources of clinical specimen were classified into inpatient, of the patients on admission on the wards and outpatient, of those patients seen on outpatient basis.

Purification and Identification of Isolates

To purify the *S. aureus* isolates, each of the collected isolates was inoculated on nutrient broth overnight at 37^{o}C overnight; and a loopful of the turbid culture was streaked on Nutrient agar plates (Oxoid, UK) and incubated at 37^{o}C for 18-24 hrs. Identification of the bacterial isolates was carried out using standard microbiological procedures as was previously described including catalase test, coagulase test, Gram staining, colony morphology and fermentation of the mannitol salt.[16]

Antibiogram

Antibiotic susceptibility testing of the *S. aureus* was determined by the Kirby-Bauer disk diffusion method. According to EUCAST, 2015 guidelines, using the following antibiotics, Gentamycin(CN) (10µg), Ciprofloxacin(CIP) (5µg), Cefoxitin(FOX) (30µg), Vancomycin(VAN) (30µg), Erythromycin(E) (15µg), Tigercycline(TGC) (15µg), Clindamycin(DA) (2µg), Trimethoprim/Sulphamedazol(SXT) (25µg), Chloramphenicol(C) (30µg), Linezolid(LZD) (10µg), Amoxicillin/Clavulanic acid(AMC) (20µg /10µg).

PCR detection of *mecA* and *femB* genes

Methicillin resistance of *S. aureus* isolates was determined by using EUCAST, 2015 breakpoint of Cefoxitin discs and altered penicillin binding protein using PBP2^{a} agglutination kit. PCR assay amplification was used to amplify *mecA* and *femB* genes. Screening of *mecA* and *femB* genes was determined by PCR assay according to method described by Baba T, *et al*, 2002. The primer sequence is summarized in Table 1. The PCR reaction mixture was made up of (25 µl) which contained 100 pmol (NORGEN, Germany) each primer, Taq polymerase (2.5 U), Mg^{2+} (2.5mM), 2.5 µl PCR buffer and 3 µl template DNA. The PCR programme included an Initial denaturation 3 min at 94 ^{o}C; followed by 30 cycles of a 30 s denaturation step at 94 ^{o}C, a 30 s annealing step at 50.45 ^{o}C and 52.95 ^{o}C for *mecA* and *femB* respectively and a 1 min extension at 72 ^{o}C; and a final 5 min extension step at 72 ^{o}C. The amplified products was at 651 bp and 500bp sequences, which were resolved on 1.5 % agarose gel electrophoresis with (0.5 µg ml^{-}1) chromogene staining and was viewed under the blue light.

Table 1: Oligonucleotide primer sequences and PCR conditions used for detection of *mecA* and *femB* genes.

Gene	Primers and Probes Description	Sequence	Amplicon Size (bp)	PCR and Real Time PCR Conditions Cycling
mecA	Forward	5'- GGG-ATC-ATA-GCG-TCA-TTA-TTC 3'	500	95°C 10 min; 40 × (95°C 15 s, 52.9°C 60 s, 72°C 60 s); 72°C 5 min.
	Reverse	5'- AAC-GAT-TGT-GAC-ACG-ATA-GCC'3'		
femB	Forward	5´ TTA CAG AGT TAA CTG TTA CC 3´	651	94°C 5 min; 35 × (94°C 60 s, 550.5°C 60 s, 72°C 60 s); 72°C 5 min.
	Reverse	5´ TTA CAG AGT TAA CTG TTA CC 3´		

RESULTS

This study evaluated the occurrence of Methicillin Resistance *S. aureus* (MRSA) in clinical isolates of suspected *Staphylococcus aureus* (n=118) clinical isolates from Maitama District Hospital Abuja, Nigeria. Out of the 118 clinical isolates of *S.aureus* employed for this study, only 56 isolates were biochemically confirmed as pathogenic *S. aureus* , and these 56 isolates were tested for their antimicrobial susceptibility. Figure 1 shows the percentage distribution of the *S. aureus* isolates according to the specimen they were isolated from. Most of the isolated *S. aureus* came from abscess (29%), wound (11%), sputum (25%), urine (18%), swab (5%), blood (5%) and semen (2%). The highest number of *S. aureus* isolates was from abscess samples (29%) while the semen samples produced the lowest number of *S. aureus* isolstes (2%). The antimicrobial susceptibility patterns of the isolated *S. aureus* are shown in Table 2.

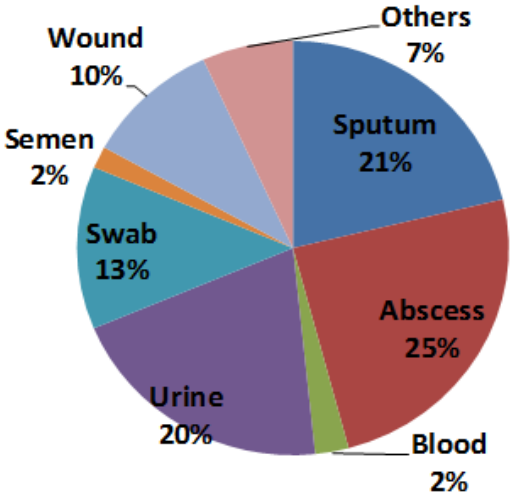

Figure 1: Percentage distribution of confirmed *S. aureus* isolates by source.

Table 2: Percentage Distribution of antibiotic resistant pattern (n=56).

Antibiotics	Percentage
FOX	58(74.4%)
CN	13(16.7%)
LZD	29(37.2%)
VAN	15(19.2%)
AMC	29(37.2%)
TGC	63(80.8%)
C	40(52.3%)
CIP	35(44.2%)
E	33(42.2)
DA	51(65.4%)
SXT	52(66.7%)

Table 2.1: Antibiotic Susceptibility Profile of Resistant PBP2a +ve Isolates (n=56).

Lab Code	Resistant pattern	MARI	No. Resistant	Resistant Category
M92 & 78	FOX, CN, LZD, AMC, TGC, C, CIP, E, DA, TRI/SUL	0.9	10	MDR
M49	FOX, CN, LZD, TGC, C, CIP, E, DA, TRI/SUL	0.8	9	MDR
M14	FOX, LZD, VAN, AMC, TGC, E, DA, TRI/SUL	0,8	9	MDR
M107	FOX, LZD, VAN, TGC, E, DA, TRI/SUL	0.6	7	MDR
M116	FOX, TGC, CIP, E, DA, TRI/SUL	0.5	6	MDR
M82	FOX,CN, LZD, CIP, DA, TRI/SUL	0.5	6	MDR
M108	FOX, LZD, VAN, E, DA, TRI/SUL	0.5	6	MDR
M95	FOX, C, CIP, E, DA, TRI/SUL	0.5	6	MDR
M35	FOX, TGC, C, CIP, E, TRI/SUL	0.5	6	MDR
M89	FOX, CN, C, CIP, TRI/SUL	0.5	5	MDR
M113	FOX, TGC, C, CIP, TRI/SUL	0.5	5	MDR
M13	LZD, TGC, C, DA, TRI/SUL	0.5	5	MDR
M15	FOX, LZD, AMC, TGC, E	0.5	5	MDR
M25	FOX, AMC, TGC, DA, TRI/SUL	0.5	5	MDR
M50	FOX, AMC, TGC, C, E	0.5	5	MDR
M94	FOX, TGC, C, CIP, TRI/SUL	0.5	5	MDR
M97	FOX, TGC, CIP, DA, TRI/SUL	0.5	5	MDR
M89	FOX, CN, C, CIP, TRI/SUL	0.5	5	MDR
M8	FOX, AMC, TGC, TRI/SUL	0.4	4	MDR
M31	FOX, LZD, E, DA	0.4	4	MDR
M37	FOX, TGC, C	0.3	3	DR
M3	FOX, AMC, TGC	0.3	3	DR
M1 & M4	FOX, TGC	0.2	2	DR

The antibiogram showed that the *S. aureus* isolates were most susceptible to gentamycin (85.7%) and this was followed by vancomycin (80.4%), ciprofloxacin(62.5%), erythromycin (53.6%) and linezolid (62.5%). However, the *S. aureus* isolates still showed reduced susceptibility to some of the tested antibiotics- which are also vital for the treatment of infections caused by pathogenic *S. aureus*. The highest level of antibiotic resistance was observed against cefoxitin 46 (82.1%), a cephamycin antibiotic with broad-spectrum of antimicrobial activity. The *S. aureus* isolates also showed reduced susceptibility to tigercycline 40 (71.4%), clindamycin 37 (66.1), chloramphenicol 27 (48.2%), amoxicillin-clavulanic acid 30 (53.6%) and sulphamethoxazol-trimethoprim 27 (48.2%). The result of the PCR amplification of Methicillin Resistance *S. aureus* (MRSA) and *femB* genes in the isolated pathogenic S. aureus isolates. Figure 2 showed the amplicon of the *mecA* and *femB* genes

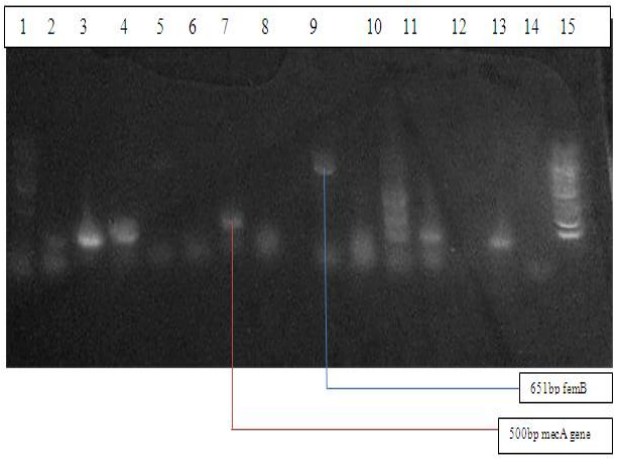

Figure 2: Amplicons of the *mecA* and *femB* genes of S. aureus with a molecular size of 500 and 651 bp. Lane 12 and 15 is 1kb molecular marker as a size marker, lane 14 is positive control , lane 2 – 11 and 13-14 are different isolates of the the pathogenic *S. aureus*, and lane 1 is negative control

Twenty six (46.4%) of the *S. aureus* were observed to be methicillin-resistance *S. aureus* (MRSA) while 30 (53.6%) were methicillin-sensitive *S. aureus* (MSSA), identified phenotypically using Cefoxitin disc diffusion and PBP2[a] agglutination kit. Molecular analysis showed that 14 (73.7%) harboured *mecA* gene and 16 (84.2%) harboured *femB* genes. Figure 2 showed the amplicon of the *mecA* and *femB* which might be responsible for the *mecA* mediated antimicrobial resistance and the virulence nature of pathogenic *S. aureus* isolates.

DISCUSSION

Staphylococcus. aureus has long been recognized as an important pathogen in human disease and is the most common cause of both community and hospital acquired infections.[13] The prevalence of *Staphylococcus aureus* 56 (47.5%) observed among the suspected *S. aureus* isolates from hospital samples in this study shows the versatility of this organism amongst other staphylococci pathogen makes in clinical settings,[6,20,11] The highest prevalence of *S. aureus* was in found in abscess (25%) and sputum (25%), a finding that is consistent with other reports[23] and is in contrast with the observation of Orji *et al.*, 2012. The high prevalence of the isolates in abscess and sputum could be attributed to poor personal hygiene and exposure of the wounds, which might have made it more prone to contamination and infection.[22]

The treatments of infections caused by *S. aureus* have always been limited nowadays due to the alarming rate of resistance to the current conventional antibiotics. These organisms are capable of producing or acquiring some resistant gene that code for enzymes that are able to destroy or inactivate some antibiotics. Greater than 40% resistance was observed to Cefoxitin, Amoxicillin/clauvulanic acid, Tigercyclin, Clindamycin, Chloramphenicol, Erythromycin and Trimethroprime/ Sulphamedazol among the *S. aureus* isolates in this study. The relative susceptibility to Vancomycin has been commonly noted among the *S. aureus* isolated at different hospitals worldwide.[26,15]

This study provides important data on current antimicrobial resistance, including methicillin resistance, from a recent collection of clinical isolates of *S. aureus* from Maitama District Hospital, Abuja. A total 82.1% prevalence rate of methicillin (Cefoxitin) resistance was observed among the isolates this compares with prevalence rates of 54% in Japan,[16] 43% from a study in the USA[14] and 30% in European countries.[27] This observed difference could be due in part to the increased antimicrobial resistance associated with district hospitals, possibly due to increased selective pressure arising from widespread antimicrobial use, as well as high density patient population in contact with healthcare staff and the attendant risk of cross infection.[10]

The pattern of antibiotic susceptibility of *S. aureus* differed significantly between MSSA and MRSA isolates. In contrast, in the case of MRSA, multiple-drug resistance was common and only few antibiotics were active against these isolates. Chloramphenicol did not show good activity against MRSA isolates, which is in contrast with the disappointing results of clinical trials that used chloramphenicol for treatment of MRSA infections.[4] Gentamicin was active against most MSSA and MRSA isolates. There is debate on the efficacy of gentamicin as an alternative agent for treatment of infections caused by *S. aureus*, due to problems with toxicity and reports of emergence of resistance during therapy.[20] There are few clinical reports on the use of ciprofloxacin for treatment of infections caused by MRSA.[18] In this study, we found 37.5% of the MRSA and MSSA isolates, were resistant to ciprofloxacin. This is of concern because this broad-spectrum antibiotic has only recently entered clinical use. However, it is one of the few antibiotics with clinically documented efficacy for treatment of infections caused by susceptible MRSA

isolates.[19] Methicillin resistance is associated with multi-drug resistance in *S. aureus*, it is likely that consistent use of Amoxillin/Clauvulanic acid as the first-line drug could be the reason for the high prevalence of MRSA isolates among hospital patients in the study area. Against this background, methicillin resistance might be useful as an index for the selection of appropriate antimicrobial agents for the treatment of infections caused by *S. aureus* due to the difference in drug resistance patterns of hospital and community MRSA organisms.

In Third world countries like Nigeria it is a common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public, thus contributing to the emergence and spread of antimicrobial resistance. Other causal factors could be poor hospital hygienic conditions, accounting for the spread of resistant bacteria and inadequate surveillance, i.e. lack of information from routine antimicrobial susceptibility testing of bacterial isolates. The Surveillance of antimicrobial susceptibility testing of common bacteria pathogen is crucial to good clinical practice and for rational policies against antibiotic resistance.[7]

CONCLUSION

Overall antibiotic susceptibility pattern of *S. aureus* isolates revealed relatively high degree of resistance of *mecA*-positive strains to the drugs tested, suggesting that most of the drugs may no longer be effective in the treatment of staphylococcal infection due to acquisition of *mecA* genes.

ACKNOWLEDGMENTS

The molecular works were all carried out in the Molecular Biology Laboratory, Department of Chemical Sciences, Fountain University, Oke-osun, Oshogbo, Nigeria. We would like to acknowledge Mr Patrick Oko and the Medical Laboratory Scientists at the Department of Microbiology, Laboratory Unit Maitama District Hospital, Abuja, Nigeria.

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