Methicillin Resistance and Beta-Lactamase Production in *Staphylococcus aureus* Isolated from Different Clinical Samples in Abeokuta, Nigeria

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ABSTRACT: The Medical Importance of *Staphylococcus aureus* cannot be overemphasized both as a commensal and a pathogen, MRSA has been a major source of serious community and hospital acquired infections world wide. Recent studies suggest an increase in the incidence of MRSA infections in Nigeria. We have therefore evaluated the current status of MRSA in isolates of *Staphylococcus aureus* in Abeokuta, Nigeria. Fifty isolates of *Staphylococcus aureus*, recovered from clinical samples were analyzed for Methicillin resistance and Beta lactamase production using acidodometric and standard disk diffusion methods. Minimum Inhibitory concentration (MIC) to Cloxacillin was also determined by standard broth microdilution method. About 38(76%) of isolates tested positive to β-lactase production, 20(40.0%) of isolates were methicillin resistant (MRSA) by disk diffusion. Females had a higher MRSA distribution than males while the peadiatric age group had the highest MRSA distribution with 9(45.0%), whiles age group 16-30 had the lowest occurrence 5(25.0%). Distribution of Beta-lactamase production in MRSA isolates was 16(32.0%) and 22(68.0%) was recorded by MSSA isolates. Cloxacillin MIC_{50} value for all isolates tested was 62.25µg/ml, while MIC_{90} value was 250µg/ml. Antibiotic susceptibility distribution of MRSA isolates revealed that Gentamycin was the most active antibiotic with 75.0% sensitivity, amoxicillin and augumentin displayed poor sensitivity in both MRSA and MSSA isolates. Generally resistance was high to Erythromycin and tetracycline. We report a high incidence of MRSA in recent *S. aureus* isolates; however a major limitation to this study was our inability to identify PVL positive isolates, as this would have revealed the level of potential public health risk *Staphylococcus* infection. It is recommend that public health authorities should enforce better surveillance programs to include molecular epidemiology studies as well as routine MRSA screening in our health institutions, and improve on preventive health planning strategies.

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1. INTRODUCTION

Staphylococcus aureus is a major bacteria pathogen capable of causing serious community and hospital acquired infectious diseases. It has been reported that about 20% of the human population are long term carriers of S. aureus (Kluytmans et al., 1997). Humans are a natural reserviour for *S. aureus* and asymptomatic colonization is more common than actual infection. The nasopharynx, skin and perineum are common sites of active S. aureus colonization particularly if the cutaneous barrier has been previous disrupted or damaged (Payne et al., 1966). Methicillin resistant staphylococcus aureus (MRSA) have been implicated in serious often life threatening infections and nosocomial outbreaks. MRSA strains have shown high level of resistance to a wide range of antibiotics, thus limiting the treatment options to very few agents such as vancomycin and teicoplanin (Baron, 1992). MRSA, together with Vancomycin resistant S. aureus, extended spectrum beta lactamase (ESBL) are classified as Multi-drug resistant organisms (MDRO) (Siegel et al., 2006). Pathogenicity of S. aureus is enhanced by the possession of several virulence factors chief of which are Panton valentine leucocidin (PVL) which is capable of lytic destruction of leucocytes, exfoliative toxin(ETA) which is responsible for scalding skin syndrome , coagulase, heamolysin and toxic shock syndrome protein (Alli et al., 2011).

Resistance to methicillin a penicillin derivative is mediated in stapylococcuas aureus by the acquisition of a large DNA fragment known as the stapylococal chromosome cassette mec (SCCmec), which contains the mecA gene, that

codes for an altered penicillin binding protein (PBP2a or PBP2') which has a lower affinity for binding β-lactams (Penicillins, cephalosporins and carbapenems). This allows for resistance to all β-lactam antibiotics and obviates their clinical efficacy during MRSA infections (Shittu et al., 2011). The global epidemiology of MRSA infections has shown a steady increase in its incidence in most countries. In Nigeria several reports have demonstrated this increase in incidence of this pathogen (Esan et al., 2009; Shittu et al., 2011; Alli et al., 2011). An illustration of this fact is shown in a study done at Ilorin, that reported an MRSA prevalence of 34.7% in 2004 (Taiwo et al., 2004) and in 2010 a repeat study done reported 52.5% MRSA prevalence (Fadeyi, 2010).With the afore mentioned rates and other factors such as the importance of MRSA infections in hospital communities and its risk for rapid nosocomial spread, we therefore carried out this study to detect β-lactamase production and methicillin resistance in randomly selected *S. aureus* isolates in Abeokuta using simple but reliable laboratory techniques to determine the current status of MRSA in Abeokuta, Nigeria.

2. MATERIALS AND METHODS

Bacteria Isolates: Fifty isolates of *Staphylococcus aureus* were randomly selected, after isolation from various clinical specimen submitted for routine processing at the Microbiology laboratory of the Federal Medical center, Abeokuta. The isolates were identified by gram reaction, biochemical test following standard procedure (Cheesbrough, 2006); tube coagulase was done on all isolates. Isolates were store in Mueller-Hinton agar slopes at 40c until they were ready for use.

2.1. Antibiotic susceptibility testing and MRSA screening:

Antibiotic susceptibility testing was done on Muller Hinton agar plates following standard disk diffusion technique of Kirby and Bauer (Cheesbrough, 2006) using gram-positive multi disk (Abteck) containing, Erythromycin(15µg), Amoxicillin (20µg), Cloxacillin (1µg), tetracycline (10µg) augmentin (30µg), Gentamycin(µg). Zones of inhibition were read and interpreted using NCCLS breakpoints for disk diffusion. MRSA screening was done on all selected isolated by inoculating dried Mueller-Hinton plates containing 5% NaCl inoculated with 0.5 Mac Farland standard and single disks of Oxacillin (Oxoid U.K) and Cefoxitn (Oxoid U.K) placed on the inoculated plates and incubated at 370c for 24hrs, the zones of inhibition were measured with a ruler and results were interpreted following NCCLS guidelines (NCCLS, 2006).

2.2. Beta-Lactamase testing:

Beta-lactamase production in isolates was tested using the acidometric method using starch iodide paper as described by Odugbemi et al. (1977).

2.3. Minimum Inhibitory Concentration (MIC):

MIC was carried out on 40 of the selected isolates positive for beta-lactamase production. Ten dilutions of varying concentrations of test antibiotic Cloxacillin were made ranging 0.625µg/ml to 500µg/ml in doubling dilutions. Dilutions were done according to standard broth microdilution technique. Inocula were standardized to obtain 0.5 Mac Farland standard of each organism. Three controls were set up along with the tested isolates, positive control (containing glucose broth and *S. aureus* ATCC 25923 strain), sterility control (Glucose broth only) and negative control (Glucose broth and antibiotic only). Microtitre plates were incubated aerobically at 37^0C for 24hrs.

3. RESULTS ANALYSIS

A total of 50 Staphylococcal isolates were randomly selected from various clinical sites consisting of 46 isolates of coagulase positive *S. aureus*. Average age of tested patients' isolates was 20 years with a gender distribution of 35(70.0%) female and 15(30.0%) male. Beta-lactase production in isolates was 38(76.0%), 20(40.0%) of isolated tested positive for methicillin resistance by disk diffusion. Distribution of MRSA by gender and age group is shown in Table 1. Females had a higher MRSA distribution than males while the peadiatric age group had the highest MRSA distribution with 9(45.0%), whiles age group 16-30 had the lowest occurrence 5(25.0%) as shown in Table 1.

Table 1: Gender and age range distribution of MRSA isolates and beta-lactamase positivity

Characteristic		
Gender	**MRSA(N[%])**	**β lactamase Positive (N[%])**
Male	6(30.0)	5(29.4)
Female	14(70.0)	12(70.6)
Age group (Years)		
1-15	9(45.0)	8(47.1)
16-30	5(25.0)	5(29.4)
31 and above	6(30.0)	4(23.5)
Total	**20(100.0)**	**17(100.0)**

Table 2 shows the distribution of MRSA isolates by sample site. MRSA isolates was most predominant in blood samples (40.0%) and least predominant in urine and genital samples (10.0%). β-lactamase positive isolates were most predominant in blood samples (47.4%) and least predominant in ear samples (7.9%) as shown in Table 2.

Table 2: Distribution of MRSA isolates by sample site

Sample site	MRSA (N[%])	β-lactamase Positive (N[%])
Blood	8(40.0)	18(47.4)
Urine	2(10.0)	5(13.2)
Wound	4(20.0)	7(18.5)
Genital	2(10.0)	5(13.2)
Ear	4(20.0)	3(7.9)
Total	**20(100.0)**	**38(100.0)**

Table 3 shows antibiotic susceptibility pattern of Methicillin resistant *Staphylococcus aureus* (MRSA) isolates. Distribution of Beta-lactamase production in MRSA isolates was 16 (32.0%) and 22 (68.0%) was recorded by MSSA isolates. Cloxacillin MIC_{50} value for all isolates tested was 62.25µg/ml, while MIC90 value was 250µg/ml. Antibiotic susceptibility distribution of MRSA isolates to six commonly prescribed antibiotics revealed that Gentamycin was the most sensitive antibiotic with 16 (80.0%) sensitivity followed by Amoxicillin with 7 (35.0%) sensitive and 2 (10.0%) intermediate, the highest level of resistance was recorded by tetracycline with 17 (85.0%). Amongst MSSA isolates the antibiotic susceptibility pattern revealed that Gentamycin displayed the highest antibacterial activity with sensitivity rate of 14 (46.7%) and intermediate susceptibility of 7 (23.3%) as shown in Table 3.

Table 3: Antibiotic susceptibility pattern of Methicillin resistant *Staphylococcus aureus* (MRSA) isolates

Antibiotic	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Amoxicillin	7(35.0)	2(10.0)	11(55.0)
Tetracycline	0(0.0)	3(15.0)	17(85.0)
Erythromycin	0(0.0)	5(25.0)	15(75.0)
Gentamycin	16(80.0)	0(0.0)	4(20.0)
Chloramphenicol	3(15.0)	5(25.0)	12(60.0)
Augmentin	5(25.0)	6(30.0)	9(45.0)
Ceftrazone	6(30.0)	3(15.0)	11(55.0)

Table 4 shows antibiotic susceptibility pattern of Methicillin susceptible *Staphylococcus aureus* (MSSA) isolates. Augmentin also displayed a fairly good sensitivity pattern with 9 (30.0%) sensitive and 11 (36.7%) intermediate susceptibility, highest resistance was recorded by Tetracycline with 24 (86.7%) resistance (Table 4).

Table 4: Antibiotic susceptibility pattern of Methicillin susceptible *Staphylococcus aureus* (MSSA) isolates

Antibiotic	Sensitive N (%)	Intermediate N (%)	Resistance N (%)
Amoxicillin	8(26.7)	6(20.0)	16(53.3)
Tetracycline	0(0.0)	4(13.3)	24(86.7)
Erythromycin	5(16.7)	9(30.0)	16(53.3)
Gentamycin	14(46.7)	7(23.3)	9(30.0)
Chloramphenicol	12(40.0)	7(23.3)	11(36.7)
Augumentin	9(30.0)	11(36.7)	10(33.3)
Ceftrazone	5(16.7)	5(16.7)	20(66.6)

Figure 1: MRSA colonies on Mannitol Salt Agar

4. DISCUSSION

The medical importance of Staphylococcal infections cannot be overemphasized world-wide. Antibiotic resistance to staphylococcus aureus, has been reported to be on the increase globally (Shittu et al., 2011), methicillin resistance is the most prominent resistance property acquirable by s. aureus with its attendant community and institutional spread, first isolated in 1962 (Jevons, 1961), its increasing incidence has posed a major in infectious disease medicine world wide. Our study was aimed at determining the current status of MRSA infections in Abeokuta, as well as determining any other resistance properties co-acquired by MRSA isolates in hospital subjects in Abeokuta. Our study reveals an overall prevalence rate of 20 (40.0%), and a gender distribution of 6(30.0%) male and 14(70%) female. The result of

our study is in agreement with a recent done within the same region of Nigeria that reported a rate of 40.0% MRSA isolations from 2 tertiary hospitals (Alli et al., 2011), our report is also similar to a report form Oshogbo, Nigeria that recorded a prevalence rate of 47.7% (Olowe et al., 2007).

Gender distribution is indicative of a higher occurrence rate of MRSA infections to male although this is still subject to further investigation and debate because our sampling method was not representative of the entire population of the area under investigation. Beta-lactamase, production was detectable in 38(76.0%) of all isolates tested, this repot is in concordance with several other reports of s. aureus with rates as high as 100.0% in MRSA isolates (Alli et al., 2011). The high rate of beta-lactamase production in isolates is an indication of the possession of beta lactamase resistance which could include the dreaded Extended spectrum beta-lactamase enzyme (ESBL), this shows the possibility of indiscriminate drug use and over the counter prescriptions of antibiotics in our community subjecting various bacteria including *S. aureus* to drug pressure and possible clonal dissemination of beta-lactamase resistance genes as earlier reported in a recent study in Abeokuta (Akinduti et al, 2011).

Peadiatric age group recorded the highest MRSA isolations, followed by the Adolescent age group, this could be attributed to the high risk of acquisition of community and nosocomial infections with common bacteria pathogens by children, coupled with unregulated administration of various oral antibiotics such as ampicillin by mothers for mild conditions not requiring antibiotic treatment such as common colds, allowing for rapid acquisition of resistance properties by often non pathogenic colonizing staphylococcal bacteria in children. In our current study, blood culture isolates recorded the highest level of MRSA isolations, this is quite worrisome because, possession of other virulent factors particularly the Panton Valentine Leucocidin (PVL) by these could become very problematic for patients with systemic infections, because of the possibility of development of severe complications resulting from the action of PVL isolates in infections such as septicemia. This calls for regular surveillance and possible investigation into PVL *S. aureus* isolates in our environment. Our inability to investigate PVL possession of our study isolates is however regretted.

Our detection method of disk-diffusion using oxacillin and cefoxitin disks has been reported to be sufficiently reliable enough for MRSA detection (Islam et al., 2008), some investigators however have recommended the routine usage of more sensitive techniques such mecA detection by PCR because of the heterogeneity displayed by *Staph. aureus* (Alli et al. 2011). MIC_{50} to cloxacillin was 62.25µg/ml and MIC_{90} was 250µg/ml in all isolates tested this result is confirmatory of significant number of MRSA isolations, judging from cut off values for methicillin resistance using cloxacillin MIC≥4µg/ml (NCCLS, 2006).

Antibiotic susceptibility pattern of MRSA isolates displayed fairly average activity for Amoxicillin with 35.0% sensitivity and 10.0% intermediate sensitivity. Gentamycin displayed the highest activity with 85% sensitivity, very high resistance was recorded by erythromycin and tetracycline, Augmentin also displayed a poor susceptibility with 25.0% sensitivity and 45.0% resistance, the resistance pattern of our result is not in agreement with similar large scale studies on MRSA which recorded better susceptibility profiles (Olowe et al., 2007; Shittu et al., 2011; Gracia Avarez et al., 2011). MSSA isolates also displayed a high level of resistance even to very potent antibiotics such as ceftazidime and augmentin, this is an indication of emergence of multi- resistance MRSA isolates with possibly co acquisition of novel antibiotic resistant genes such as Macrolide-streptogramin lincosamide allele(a). This call for better and more detailed molecular characterization of MRSA isolates from particularly endemic regions with poor hygiene and institutional infection control practices as recorded in developing countries like Nigeria.

In conclusion, we report a high incidence of MRSA in recent *Staph. aureus* isolates with a high level of resistance even in MSSA isolates, however a major limitation to this study was our inability to identify PVL positive isolates, as this would have revealed the level of potential public health risk *Staph. aureus* posses to the general population of Abeokuta, Nigeria. We hereby recommend to the public health authorities of the region concerned to enforce better surveillance programs to include molecular epidemiology studies as well as routine MRSA screening in our health institutions, to improve on our preventive health policy planning strategies.

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