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Epidemiology and Characteristic Pattern of Methicillin-Resistant *Staphylococcus aureus* Recovered from Tertiary Hospitals in Northeastern, Nigeria

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Abstract: Ninety six consecutive, non-duplicate *S. aureus* isolates from clinical specimens were collected between January to December 2007 from six tertiary hospitals in Northeastern Nigeria analysed by phenotypic and molecular methods. Of the 96 *S. aureus* isolates, 12 (12.5%) MRSA isolates were identified by disc diffusion and confirmed by PCR assay, recovered from 2 of the 6 hospital (11 from UMTH and I from Gombe). Twelve MRSA and 4MSSA isolates exhibited multiresistant pattern to the commonly used antibiotics and 3 of the 12 MRSA were sensitive to clindamycin while all the *S. aureus* (MRSA and MSSA) isolates were susceptible to vancomycin, mupirocin and fusidic acid. Overall antibiotic susceptibility pattern demonstrated high level resistance with penicillin (92.1%), moderate level with gentamicin (14.6%), erythromycin (15.6%), cotrimoxazole (19.8%), ciprofloxacin (15.6) while low-level with clindamycin (9.4%) and rifapicin (2.1%). The SCCmec typing of the MRSA isolates by two standard typing methods revealed presence of novel SCCmec element that have not be documented in literature. The MRSA prevalence of 12.5% may be considered to be high in an environment without previous surveillance studies. The multiresistant pattern of the pathogens to frontline antibiotics posed serious public health problem because of cost and unavailability of alternate chemothrauptic option like vancomycin. The definition of SCCmec types affirmed divergent element of staphylococcal flora.

Key words: Antibiotic susceptibility pattern, *S. aureus*, literature, penicillin, pathogens, Northeastern Nigeria

INTRODUCTION

Staphylococcus aureus, a versatile human pathogens responsible for nosocomial and community-associated infections is associated with high morbidity and mortality rate. However, emerging reports revealed that increase rate of hospital-acquired infections are mostly due to antibiotic-resistant pathogens (Klevens *et al*., 2007). Of the resistant pathogens that had attracted public health interest worldwide is Methicillin-Resistant *S. aureus* (MRSA). It is major cause of nosocomial infection and colonization, resulting in morbidity and mortality.

Consequential effect of MRSA infection had resulted in prolonged hospitalization, increased in medical expenses and difficulty in patient treatment and management. In US hospitals, MRSA accounts for most of invasive *S. aureus* infections with high fatality rate (Klevens *et al*., 2007). The financial burden of hospital acquired infection due to MRSA is higher in term of treatment that could cost approximately $25,000) compared to non-MRSA hospital-acquired infection of $13,973 (Klevens *et al*., 2007).

The unique characteristic of MRSA strains is the multidrug resistance pattern to β-lactam and other classes due to acquisition of *mec A* gene, key genetic determinant located on the Staphylococcal Cassette Chromosome (SCCmec) (Hiramatsu *et al*., 2001; Ito *et al*., 2001). The *mec A* gene, encodes the PBP2a a inducible 75 kb PBP responsible for low-affnity to β-lactam and other drugs (Enright *et al*., 2002; Lowy, 1998). Six SCCmec type have been described, SCCmec I-VI which is used in defined MRSA strain source as SCCmec type I-III are known to be of nosocomial origin while SCCmec type IV of community origin (Ma *et al*., 2002).

MRSA prevalence varies greatly with geographical location, type of hospital and studied population. High prevalence have been recorded in tertiary hospitals in US, Southern European countries, Asia and South America (Diekema *et al*., 2001). In Africa, MRSA prevalence varies with different countries, high in some and low in others (Bell *et al*., 2002). Despite this epidemiological data on MRSA in some African countries, available data are still relatively limited when compared to information from developed countries which may be attributable to

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high level of awareness of MRSA infections and its clinical and societal consequences. Although, in Nigeria, few studies on phenotypic and molecular characterization of *S. aureus* have been conducted, particularly in southwestern zone (Adesida *et al*., 2005; Shittu and Lin, 2006; Ghebremedhin *et al*., 2009). To the knowledge of the researchers, no similar research have been carried in this geographical zone that may highlight the epidemiological characteristic pattern of MRSA isolates. The peculiarity of the study area boarded by three republics involved massive movement of peoples and animals witnessed in interboard trading, unregulated sales of antimicrobials agents, all these are known predisposing factors for emergence of resistant strains. Epidemiological knowledge of MRSA in the geographical zone would provides valuable information particularly on antibiotic usage and infection control strategy for for dissemination within the hospital environment. The researchers examined the epidemiological characteristic of MRSA isolates isolated from clinical specimens in Northeastern Nigeria.

MATERIALS AND METHODS

The study area comprises of six administrative states, of Borno, Adamawa, Bauchi, Gombe, Jalingo and Yobe with a tertiary hospital located in each state. The tertiary hospitals used in the recovery of *S. aureus* isolates are multidisciplinary and varies in beds size capacity. The University of Maiduguri Teaching hospital is a major referred centre with bed size capacity of 500 while other tertiary hospital bed size ranged between 100-250. The 96 consecutive non-duplicate *S. aureus* isolates were confirmed by both tube coagulase and DNase test. Demographic information collected includes, age, sex and type of clinical specimens. For this study, bacterial isolates were classified as inpatient recovered from clinical specimens of patient on admission while outpatient were those seen at the general outpatient clinic.

Antibiotic susceptibility testing was determined by disc-diffusion method in accordance to CSLI guideline using the following antibiotic discs, pencillin (10 IU), oxacillin (1 μg), cefoxitin (30 μg), gentamycin (10 μg), erythromycin (15 μg), clindamycin (2 μg), ciprofloxacin (5 μg), cotrimoxazole (25 μg), rifamipicin (30 μg) vancomycin (30 μg), fusidic acid (10 μg) and mupirocin (5 μg). The determination of sensitive, immediate or resistant isolates depend on the zone of growth inhibition diameter of CSLI breakpoint. Staphylococcus aureus ATCC25932, standard strain was included in each batch analysis as control strain. Methicillin resistance expression was determined by disc diffusion method using both oxacillin and cefoxitn discs. The D-test for inducible and constitutive phenotype was determined according to method described by Fiebelkorn *et al*. (2003) in which the erythromycin and clinidamycin discs are placed at 12-14 mm apart. Beta-lactamase production was determined by the iodometric method as described by Odugbemi *et al*. (1977). Urease production was determined by inoculation of *S. aureus* isolates on Christensen urea slant and incubated at 37°C for 24 h. A urease-positive result is indicated by change of colour from orange to pink.

The SCCmec typing of MRSA isolates was performed as described by Kondo *et al*. (2007) using two multiplex PCR assay. The first PCR (M-PCR-1) identifies the presence of *mec A* gene as well as the ccr types and the PCR (M-PCR-2) identifies the mec class. The combination of both ccr type and mec class determines the SCCmec type. In confirmation of the results with Kondo *et al*. (2007) PCR assay. The MRSA isolates were further analysed with PCR assay method described by Oliveria *et al*. (2006). SCCmec type controls (I-VI) were included in each test run. Chromosomal DNA extraction method was as described by Ito *et al*. (2001) using lysostaphin. The PCR conditions for both methods was as follows initial denaturation step at 94°C for 2 min, denaturation step at 94°C for 2 min, annealing at 57°C for 1 min 30 sec, extension at 72°C for 2 min and final elongation step at 72°C for 2 min at the end for a total of 30 cycles. The PCR-product was removed from the thermocycler at the end of the process, resolved and visualized in 1.5% agarose. The primers sequences of both PCR assay is shown in Table 1 and 2, respectively.

RESULTS AND DISCUSSION

Of the 96 *S. aureus* isolates, 38.5% (n = 37) were recovered from wounds specimens, 19.8% (n = 19) HVS/endocervical swab, 17.7% (n = 17) urine, 9.4% (n = 9) ear, 4.2% (n = 4) pus, urethral 3.1% (n = 3) each from urethral and eye swab, 2.1% (n = 2) semen and 1.0% (n = 1) from sputum and blood culture, respectively. The 73% (n = 71) of the *S. auresu* isolates were recovered from UMTH (n = 71) and the remaining 26% (n = 25) from the five tertiary hospitals. The mean age of the patients with *S. aureus* infection was 27.7 (+15.5, CI 1-80) years with even gender distribution (male and female) of 48 (50%). Thirty seven *S. aureus* isolates were from inpatient and 59 from outparients. About 80 (83.3%) were scored positive for urease production and 65 (67.7%) for beta-lactamase production. Overall antibiotic susceptibility pattern of *S. aureus* isolates (Table 1) shows high level resistance with penicillin (92%), moderate level with gentamicin (14.6%), erythromycin (15.6%), cotrimoxazole (19.8%), ciprofloxacin (15.6%) and low-level with clindamycin (9.4%) and rifampicin (2.1%). All the isolates were susceptible vancomycin, fusdic acid and mupirocin. Beta-lactamase production showed similar

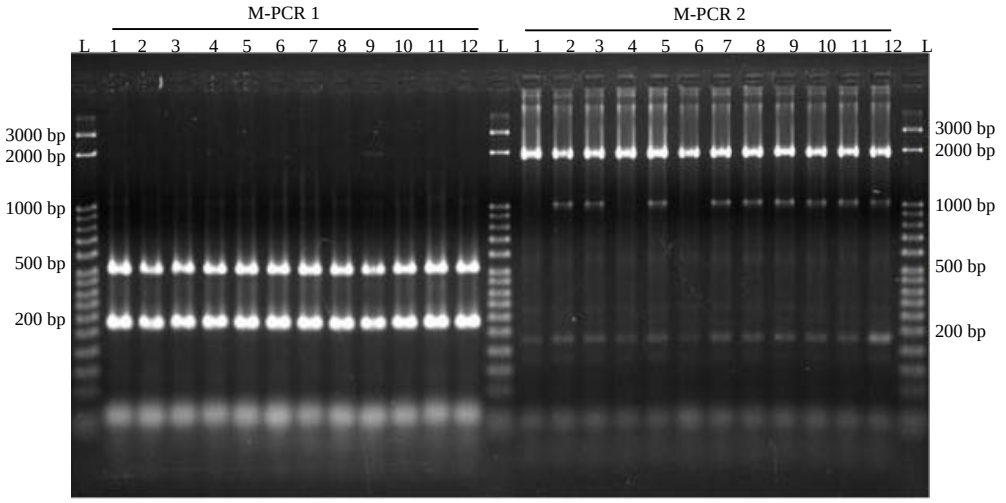

Fig. 1: M-PCR 1 and M-PCR 2 (Kondo method), detection of *mec A*, *ccr* and *SCCmec* genes, respectively. Lane 1-12 are the MRSA strain, 1-11 from UMTH, 12-Gombe, the Ladder is 50 bp scale. *mec A* and *ccr* gene amplified at 209, 518 bp and SCCmec at 1798 bp

Table 1: Frequency of resistance of *S. aureus* (MRSA and MSSA) strains to common antibiotics

	Antibiotics											
Strains	P	OX	FOX	GM	E	CC	SXT	CIP	RA	VA	FA	MUP
MSSA	91.6	0.0	0.0	2.4	3.6	0.0	8.3	3.6	2.4	0.0	0.0	0.0
MRSA	100.0	100.0	100.0	100.0	100.0	75.0	100.0	100.0	0.0	0.0	0.0	0.0
Total	92.7	12.5	12.5	14.6	15.6	9.4	19.8	15.6	2.1	0.0	0.0	0.0

[a]P = Penicillin, OX = Oxacillin, FOX = Cefoxitin, GM = Gentamycin, E = Erythromycin, CC = Clindamycin, SXT = Co-trimoxazole, CIP = Ciprofloxacin, RA = Rifampicin, VA = Vancomycin, FA = Fusidic Acid, MUP = Mupirocin

Table 2: Antibiotic resistance of *S. aureus* isolates based on patient classification

	Inpatient			Outpatient			
			Resistance			Resistance	
Antibiotic	S	R	(%)	S	R	(%)	p-value
Penicillin	2	35	94.6	5.0	54.000	91.5	0.448
Gentamicin	29	8	21.6	51.0	8.000	13.6	0.307
Erythromycin	27	10	27.0	50.0	9.000	15.3	0.162
Clindamycin	32	5	13.5	53.0	6.000	10.2	0.621
Co-trimoxazole	28	9	24.3	48.0	11.000	18.6	0.338
Ciprofloxacin	26	11	29.7	46.0	13.000	22.0	0.271
Rifampicin	37			56.0	3.000	5.10	0.228
Vancomycin	37			59.0			
Fusidic acid	37			59.0			
Mupirocin	37			59.0			

pattern with susceptibility pattern. Comparsion of antibiotic susceptibility between *S. aureus* isolates from inpatient and outpatient showed slight difference (Table 2).

Twelve putative MRSA isolates identified by cefoxitin disc diffusion test were confirmed by PCR assay for detection of *mecA* gene in contrast to 6 putative MRSA detected by oxacillin showed discordant result with PCR assay. Of the 12 MRSA isolates identified, 9 recovered from wounds specimens, 2 from high vaginal/endocervical swabs and 1 from eye swab. In addition, 11 MRSA were recovered from UMTH and 1 from Gombe. Of the 12 MRSA isolates, 8 were positive for urease production and recovered from outpatient. The 12 MRSA isolates and 4 MSSA isolates exhibited multiresistant pattern with 57 (59.4%) MSSA isolates were resistant to only penicillin and 4 (4.8%) susceptible to pencillin. Of the 12 MRSA strains identified, 3 were clindamycin-susceptible and one demonstrated the inducible phenotype. The SCCmec typing of the 12 MRSA isolates in Fig. 1 and 2 depicting uncharacterized SCCmec element. Interestingly, three MSSA isolates amplified the SCCmec without the presence of *mec A* gene. Using the MPCR-5 of Kondo method, the MRSA isolates amplified only cadmium gene.

Paucity of epidemiological data on MRSA strains in most sub-Saharan African countries, cast shadow on the public health implication and awareness of the organism. Epidemiological information from this study intends to provides the baseline data for eventual appreciation on the need for cautious approach and intervention measures.

In this study, the MRSA prevalence of 12.5% may be considered to be high, reason being that there has not been any previous epidemiological data on MRSA in

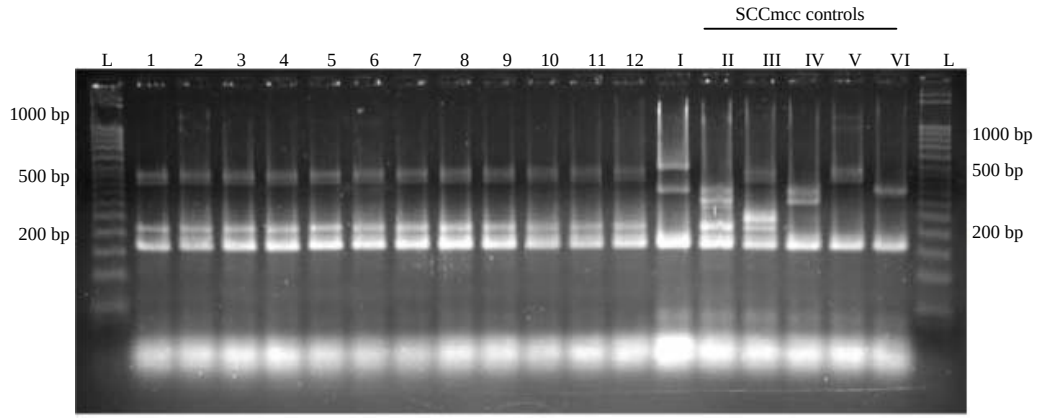

Fig. 2: Multiplex PCR assay (Oliveria method) shows the 12 MRSA strains and SCCmec types (I-VI). The *mec A* gene amplified at 162 bp, SCCmec at 243 bp and ccr at 449 bp. The SCCmec differ in number of amplified PCR products band, type I, 3 bands, II 4, III 4, VI 3, V 2 and VI 2 bands

geographical zone. Although, MRSA prevalence are known to vary with geographical location, type of health instution, studied population and method of detection employed. However, the MRSA prevalence may be considered moderate when compared with similar study in Ibadan, Southwestern Nigeria with a prevalence level of 20, 3% (Ghebremedhin *et al*., 2009) as both phenotypic and molecular methods were employed. Previous studies on MRSA in the southwestern Nigeria with same detection methods had reported prevalence of <2% (Adesida *et al*., 2005).

Oxacillin disc diffusion method was the earliest method of detection for methicillin resistance expression. Because of low specificity and sensitivity, cefoxitin disc diffusion was introduced by CSLI that is known as a good surrogate marker of *mecA* gene detection. Most earlier MRSA prevalence reported from Nigeria were based on phenotypic methods using oxacillin disc diffusion method had reported high prevalence level. Studies conducted in eight African and Malta hospital reported prevalence ranged between 20-30% (Kesah *et al*., 2003) in Southwestern Nigeria 9-50% and in north central Nigeria of 34%. The disc diffusion method is known to be influenced by both extrinsic and intrinsic factors which includes temperature, pH, size of inoculums, concentration of sodium chloride and cell population (Hartman and Tomasz, 1984). In this study, six putative MRSA isolates with oxacillin was misidentified with PCR assay in contrast to cefoxitin test that compared favourably with PCR assay, this study simply affirmed th cefoxitin as good marker (Skov *et al*., 2003), Antibiotic susceptibility pattern of *S. aureus* isolates showed that 12 MRSA and 4 MSSA isolates exhibited multresistant pattern to the commonly prescribed and administered frontline antibiotics in the tertiary hospital in the study area. This pattern therefore, signals a public health problem as these agent are relatively affordable and readily available for administration in both hospital and community setting. The high level resistance pattern observed with pencillin is consistent with other studies elsewhere (Ontengo *et al*., 2004; Kesah *et al*., 1997). However, of interest is the moderate to low-level resistance observed with ciprofloxacin, gentamicin, erythromycin, cotrimoxazole and rifampicin among MSSA strains. This pattern underscores the need by physician to be prudent and cautious in their prescription while such pattern is maintained or further stemmed down against possible emergence of resistance strains. However, other studies have reported resistance with ciprofloxacin of *S. aureus* isolates recovered from hospital and community setting. In this study, cotrimoxazole resistance level was low which is in contrast to high level resistance reported in studies conducted in Southwestern Nigeria (Kesah *et al*., 2003). The reason for such pattern in the study is unclear as unrestricted usage and administration of cotrimoxazole for variety of diseases condtions is a common in the locality. The susceptibility pattern exhibited by rifamipcin in this study, simply revealed the use of this agent primarily in the treatment of *Mycobacterium tuberculosis* infection. Significant proportion of *S. aureus* isolates (9 MRSA) were recovered wounds specimens, similar to other reported studies (Akpaka *et al*., 2007; Orrett and Land, 2006).

MRSA isolates are commonly associated with nosocomial infection with high isolation rate recorded in tertiary hospitals. In this study, 11 of the 12 MRSA isolates were recovered from UMTH (>500 beds), a major tertiary hospital and also doubles as major referred centre

to other hospital and the remaining one from hospital with <250 beds is approximately 400 km apart, this finding is consistent with reports of other investigators (Panlilo *et al*., 1992; Schmitz *et al*., 1997). In US, high incidence of MRSA (7.8%) was reported in a 1500 beds capacity hospital compared to low incidence of 0.5% in small hospital (200 beds) (Schmitz *et al*., 1997). The remaining one MRSA isolates was recovered another tertiary hospital that is approximately 400 km apart. The possible scenario for such level might that the level in this hospital might be assumed to very low and also the transfer of infected/or colonized patient or through heath care workers could facilitate the spread.

Staphylococcal chromosome cassette SCCmec typing is used as epidemiological marker of isolates into either of noscomial or community-assciated infections (Ma *et al*., 2002). Based on the results of the two SCCmec typing methods employed, revealed the presence of an uncharacterized SCCmec element MRSA strains. Using the first two multiplex PCRs of Kondo *et al*. (2007), the researchers found that these MRSA carry the ccr type 5 and the mec class A. So, far the combination of these two elements had only been observed in strains carrying simultaneously two SCCmec elements, a type III SCCmec and an SCCmercury (Kondo *et al*., 2007; Chongtrakool *et al*., 2006). However, the combination of the mec class A with a single ccr type 5 has to the knowledge never been observed. Recombination between different SCCmec types and/or local acquisition may explain the emergence of a new resistance elements (Branger *et al*., 2003; Fiebelkorn *et al*., 2003; Fitzgerald *et al*., 2001). Nevertheless, further reseaches are needed to address this hypothesis and to characterize the new cassette from Nigeria. In Nigeria, SCCmec element I and IV have been reported in Southwestern Nigeria (Adesida *et al*., 2005; Ghebremedhin *et al*., 2009). The presence of untypable reaffirmed the possibility of several new SCCmec element that could be linked to local emergence of some different clones. However, recent data indicate that the local acquisition of SCCmec elements is a frequent phenomenon (Nubel *et al*., 2008), highlighting the need to compare the molecular epidemiology of both MSSA and MRSA.

Apart from the hospital environment, MRSA isolates have been detected in community setting termed as Community-associated methicillin-resistance *S. aureus* CAMRSA) and their prevalence is increasing worldwide. CAMRSA isolates can be identified by high susceptibility to variety of antimicrobial agent, urease positivity, PVL-positive and SCCmec type IV (Deurenberg and Stobberingh, 2008).

Demographically, 4 of the 12 MRSA were recovered from outpatients, urease-positive and untypable SCCmec. Based on this pattern, these MRSA isolates cannot be classified as community-associated methicillin-resistant *S. aureus* isolates.

CONCLUSION

This study shows that the MRSA prevalence of 12.5% may be considered to be high that warrant urgent infection awareness, considering isolation from two tertiary hospital in the zone. Considering the common practise, of unregulated sale of antimicrobial agent, sub-standard/fake drugs and movement of people and livestocks may be agent necessary for rapid dissemination. Therefore, MRSA surveillance studies be instituted in these tertiary hospital, to provide necessary epidemiology update on MRSA.

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