

Full Length Research Paper

Epidemiology and antibiotic susceptibility pattern of methicillin-resistant *staphylococcus aureus* recovered from tertiary hospitals in north-eastern 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 two of the 6 hospital (11 from UMTH and 1 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 percent 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 chemotherapeutic option like vancomycin. The non-definition of SCCmec types affirmed divergent element of staphylococcal flora.

Keywords: Antibiotic susceptibility pattern, *S.aureus*, north-eastern 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 (Kleven, 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 (Kleven, 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 (Kleven, 2007).

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The unique characteristic of MRSA strains is the multidrug resistance pattern to β -lactam and other classes, due to acquisition of *mecA* gene, key genetic determinant located on the staphylococcal cassette chromosome (SCCmec) (Hiramatsu et al., 2001, Ito et al., 2001). The *mecA* gene, encodes the PBP2a a inducible 75kb PBP responsible for low-affinity to β -lactam and other drugs (Enright et al., 2003, 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., 2000).

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 (Dikema et al., 2001). In Africa, MRSA prevalence varies with different countries, high in some and low in others (Bell and Turrige, 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 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, Shittuet al., 2005, Grehdemedin *et al.*, 2009). To the knowledge of the authors, no similar work 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 dissemination within the hospital environment. We examined the epidemiological characteristic of MRSA isolates isolated from clinical specimens in northeastern Nigeria

MATERIAL 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 was 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, penicillin(10IU), oxacillin (1 μ g), cefoxitin (30 μ g), gentamycin (10 μ g), erythromycin (15 μ g), clindamycin (2 μ g), ciprofloxacin (5 μ g), cotrimoxazole (25 μ g), rifampicin (30 μ g) vancomycin (30 μ g), fusidic acid (10 μ g), and mupirocin (5 μ g). The determination of sensitive, immediate or resistant isolates depend on the zone of growth inhibition diameter of CSLI break point. *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 cefoxitin discs. The D-test for inducible and constitutive phenotype was determined according to method described by Freibekorn et al (2003), in which the erythromycin and clindamycin discs are placed at 12-14mm 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 hours. A urea's-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 *mecA* 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 our results with Kondo et al 2007 PCR assay. The MRSA isolates were further analysed with PCR assay method described by Oliveria et al (2007). *SCCmec* type controls (i.e. I to VI) were included in each test run. Chromosomal DNA extraction method was as described by Ito et al (2003), using lysostaphin. The PCR conditions for both methods was as follows, initial denaturation step at 94°C for 2minutes, denaturation step at 94°C for 2minutes, annealing at 57°C for 1minute 30seconds, extension at 72°C for 2minutes and final elongation step at 72°C for 2 minutes 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 presented in tables 1 and 2 respectively.

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

	Antibiotics											
	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 = ceftiofloxacin, GM = gentamicin, E = erythromycin, CC = clindamycin, SXT = cotrimoxazole, CIP = ciprofloxacin, RA = rifampicin, VA = vancomycin, FA = fusidic acid, MUP = mupirocin.

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

Antibiotic	Inpatient			Outpatient			p value	
	S	R	Resistance (%)	S	R	Resistance(%)		
Penicillin	2	35	94.6	5	54	91.5	.448	
Gentamicin		29	8	21.6	51	8	13.6	.307
Erythromycin		27	10	27.0	50	9	15.3	.162
Clindamycin		32	5	13.5	53	6	10.2	.621
Co-trimoxazole 28	9	24.3	48	11	18.6		.338	
Ciprofloxacin		26	11	29.7	46	13	22.0	.271
Rifampicin		37			56	3	5.1	.228
Vancomycin		37						
Fusidic acid		37						
Mupirocin		37						

RESULT

Of the 96 *S.aureus* isolates, 38.5%(n=37) were recovered from wounds specimens, 19.8% (n=19) HVS/endo-cervical 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. Seventy-three percent(n=71) of the *S.aureus* 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 outpatients. Eighty (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) showed 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, fusidic acid and mupirocin. Beta-lactamase production showed similar pattern with susceptibility pattern. Comparison of antibiotic susceptibility between *S.aureus* isolates from inpatient and outpatient showed slight difference (table 3).

Twelve putative MRSA isolates identified by ceftiofloxacin 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 multi-resistant pattern, with 57(59.4%) MSSA isolates were resistant to only penicillin and 4(4.8%) susceptible to penicillin. 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 figures 1 and 2, depicting uncharacterized SCCmec element. Interestingly, three MSSA isolates amplified the SCCmec without the presence of *mecA* gene. Using the MPCR-5 of Kondo method, the MRSA isolates amplified only cadmium gene.

DISCUSSION

Paucity of epidemiological data on MRSA strains in most sub-Saharan African countries, cast shadow on the

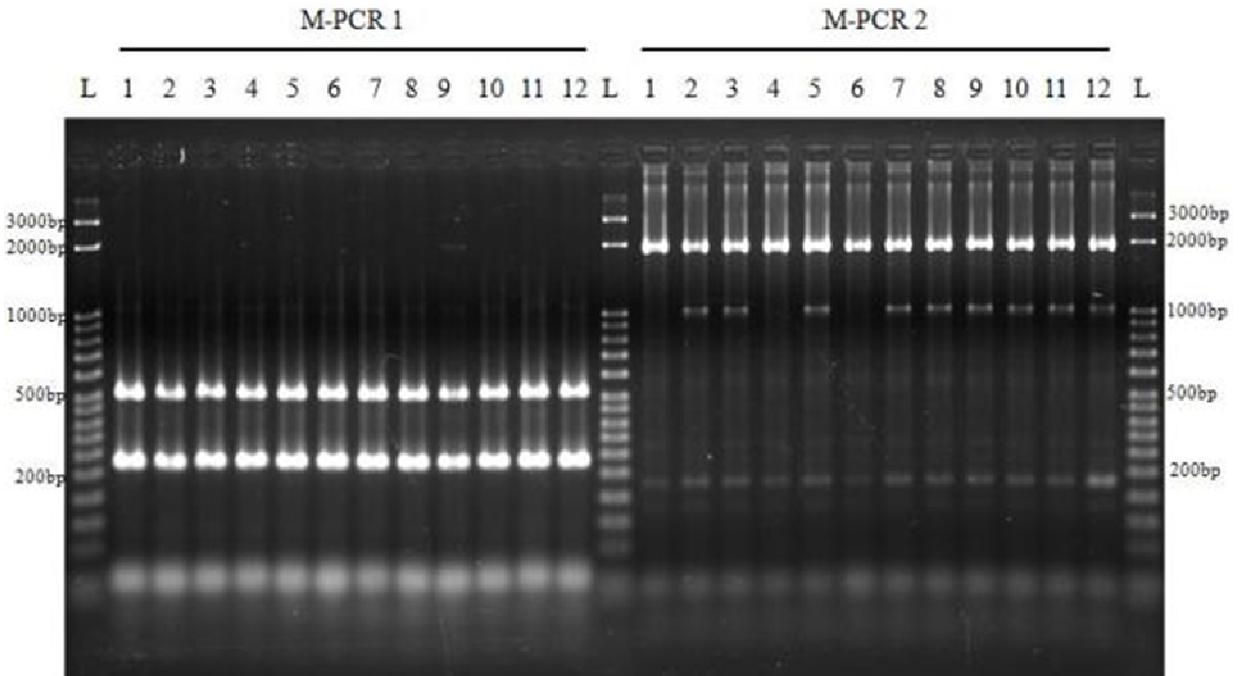


Figure 1-Figure 6. M-PCR 1 and M-PCR 2(Kondo method), detection of *mecA* , *ccr* and SCCmec gene respectively. Lane 1-12 are the MRSA strain, 1-11 from UMTH, 12-Gombe,the Ladder, is 50bp scale.*mecA* and *ccr* gene amplified at 209bp, 518 bp, and SCCmec at 1798 bp.

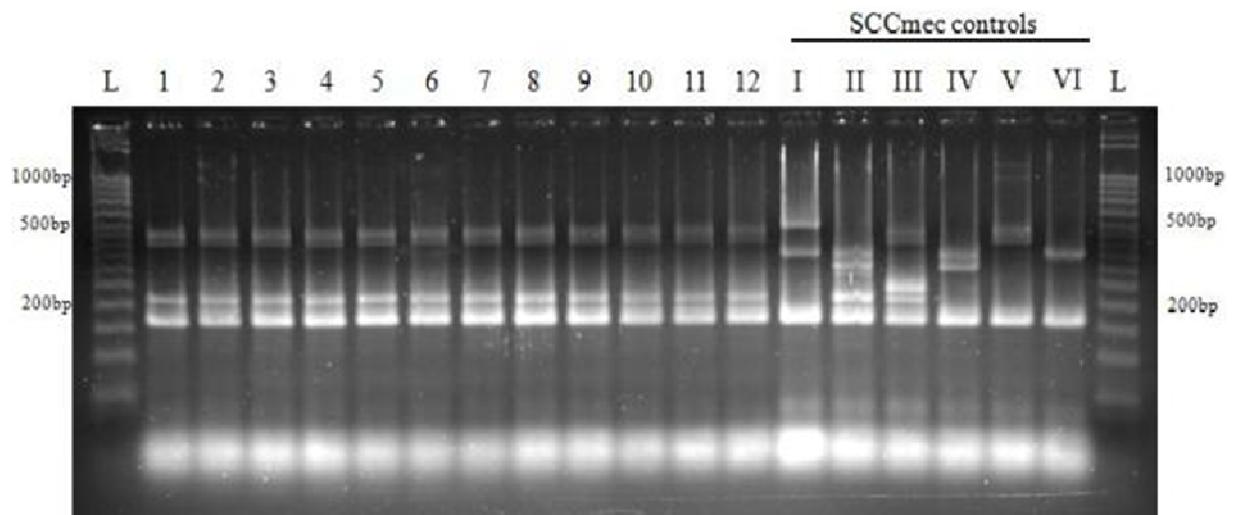


Figure 2. Multiplex PCR assay(Oliveria method).shows the 12 MRSA strains and SCCmec types(I-VI).The *mecA* gene amplified at 162 bp, SCCmec at 243bp and *ccr* at 449bp.The SCCmec differ in number of amplified PCR products band, type I, 3bands, II 4, III 4bands, VI 3 bands, V 2 bands and VI 2 bands.

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 geographical zone. Although, MRSA prevalence are

known to vary with geographical location, type of health institution, studied population and method of detection employed.. However, our MRSA prevalence may be considered moderate, when compared with similar study in Ibadan, Southwestern Nigeria with a prevalence level of 20.3% (Grehenedhemin 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 less than 2% (Shittu et al., 2005, 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 (Simor et al., 2001; Becker et al., 2002). 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% (Rotimi et al. Ako-Nai et al) and in north central Nigeria of 34%(Taiwo et al., 2006).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, 1997). In this study, six putative MRSA isolates with oxacillin was misidentified with PCR assay in contrast to cefoxitin test that compared favorably with PCR assay, this finding simply affirmed the cefoxitin as good marker (Skov et al., 2003).

Antibiotic susceptibility pattern of *S.aureus* isolates showed that 12 MRSA and 4 MSSA isolates exhibited multi-resistant 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 penicillin, is consistent with other studies elsewhere (Ontego et al., 2004; Kesah et al., 1998; Rotimi et al., 1995). 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 (Acar and Goldstein 1997). 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; Shittu et al., 2005). The reason for such pattern in our study is unclear as unrestricted usage and administration of cotrimoxazole for variety of diseases conditions, is a common in our locality. The susceptibility pattern exhibited by rifampicin 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; Orretta and Land, 2006; shittu

and Lin, 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 less than 250 beds is approximately 400km 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(200beds)(Schmitz et al 1997). The remaining one MRSA isolates was recovered another tertiary hospital that is approximately 400km 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 health care workers could facilitate the spread.

Staphylococcal chromosome cassette *SCCmec* typing, is used as epidemiological marker of isolates into either of noscomial or community-associated infections(Ma et al 2000). Based on the results of the two *SCCmec* typing methods employed, revealed the presence of an un characterized *SCCmec* element MRSA strains. Using the first two multiplex PCRs of Kondo et al (2007), we 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., 2006; Chongtrakool et al., 2006). However, the combination of the *mec* class A with a single *ccr* type 5 has to our 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; Feil et al., 2003; Fitzgerald et al., 2001). Nevertheless, further investigations 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; Shittu et al., 2005; Ghredemedin 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 (Nübel 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* 9CAMRSA), and their prevalence is increasing world-wide. 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

In conclusion, the MRSA prevalence of 12.5% may be considered to be high that warrant urgent infection awareness, considering isolation from two tertiary hospitals in the zone. Considering the common practise, of unregulated sale of antimicrobial agent, sub-standard/fake drugs and movement of people and livestock 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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