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Research article

Distribution of *mecA* gene amongst *Staphylococcus aureus* isolates from Southwestern Nigeria

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ABSTRACT: *Staphylococcus aureus* (*S. aureus*) is an important pathogen in human infections and is implicated in a wide variety of infections, from mild skin infections to more serious and invasive infections. Methicillin resistant *S. aureus* (MRSA) is one of the major causes of nosocomial infection in hospital setting. The study was aimed at determining the distribution of *mecA* gene among the *S. aureus* isolates from south western tertiary hospitals, Nigeria. In this study, a total of 194 isolates of *S. aureus* were collected from the specimen submitted to diagnostic section of Medical Microbiology Department, University College Hospital (UCH) Ibadan, Obafemi Awolowo University Teaching Hospital, Ile-Ife, and LAUTECH Teaching Hospital (LTH) Osogbo, in the South Western Nigeria. Antibiotics susceptibility testing including methicillin sensitivity testing, beta lactamase testing, PCR for detection of *mecA* gene, and minimum inhibitory concentrations to methicillin were carried out on all the 194 isolates of *S. aureus*. Among the 194 strains, 40 (20.6%) were MRSA using 10 µg methicillin disc. PCR analysis showed that *mecA* gene was present only in 43 (22.2%) of 194 *S. aureus* isolates. Minimum inhibitory concentration (MIC) was also carried out to determine the degree of resistant of the isolates showed that the MIC_{50} and MIC_{90} for MRSA or *mecA*$^+$ were > 128 µg/ml while the MIC_{50} and MIC_{90} for *mecA*$^-$ were 0.5 and 2 µg/ml, respectively. Furthermore, 124 (64 %) of the 194 *S. aureus* isolates were β-lactamase producers. The study found that there were strong associations between isolation site of specimens / nature of specimens (X^2= 16.74; $p < 0.05$), beta lactamase producing *S. aureus* strains (X^2= 29.21; $p < 0.05$) and no association was found between the hospitals, gender, age and the prevalence of MRSA in this study ($p > 0.05$). The study concludes that the prevalence of *mecA* gene in *S. aureus* in South Western, Nigeria is 22% and *mecA* gene detection is a good predictor of methicillin resistance in *S. aureus* in Nigeria, hence use as a method of detection of MRSA

Keywords: *Staphylococcus aureus, mecA gene,* MRSA, antibiotic resistance, Nigeria.

INTRODUCTION

Staphylococcus aureus is an important pathogen in hospital and community settings. Methicillin resistant *Staphylococcus aureus* (MRSA) was first isolated soon after introduction of methicillin into clinical use in 1960 (Barber, 1961; Jevons, 1961). MRSA is a strain of *S. aureus* that is resistant to methicillin or beta lactamase resistant group of penicillin and usually these strains of *S. aureus* are usually resistant to more than one antibiotic and because of this, the infections due to this strain of MRSA are very difficult to treat. The pathogenicity of *S. aureus* infections is associated with various bacterial surface components (e.g., capsular polysaccharide and protein A), including those recognizing adhesive matrix molecules (e.g., clumping factor and fibronectin binding protein), and to extracellular proteins (e.g., coagulase, hemolysins, enterotoxins, toxic-shock syndrome (TSS) toxin, exfoliatins, and Panton-Valentine leukocidin (PVL) (Archer, 1998), and MRSA strains being group of *S. aureus* are likely to have one or more of these pathogenicity traits.

The incidence of MRSA has been on the increase in the world since it first reported. Subsequently, the occurrence of MRSA particularly in hospitalized patients has increased steadily and nosocomial infections caused by such strains have become a serious problem worldwide. Methicillin resistant strains of *S. aureus* produce a new penicillin binding protein (PBP),

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otherwise known as PBP2' or PBP2a (Hartman and Tomasz, 1984, Utsui and Yokota, 1985), with a low affinity for β-lactam antibiotics. This protein is encoded by *mecA* gene (Ubukata *et al.,* 1985).

In the clinical microbiology laboratory, MRSA can be detected using disc diffusion and broth and agar dilution tests. The detection of MRSA is not a clear cut test when compare to other antibiotics susceptibility test as a result of the heterogeneity of the strain as most of the population of organisms are susceptible to low concentrations of β-lactam antibiotics, which complicates the detection of strains resistant to low levels of antibiotic. The problem of detection of MRSA is still persistent because of the reason given above. It is in view of this that *mecA* gene detection has been used as an alternative way of detecting or confirming MRSA either by use of DNA probe, commercially available fluorescence test (Ieven *et al.,* 1995), latex agglutination test (Cavassini *et al.,* 1999), and PCR (Rohrer *et al*., 2001). More so, the aforementioned tests have also found to be accurate and provide results more quickly than standard susceptibility tests. PCR has been used for detection of MRSA by amplifying *mecA* gene in *S. aureus* in many laboratories especially in developed countries like USA, UK. It is in view of this, this study was aimed at determining the distribution of *mecA* gene in *S. aureus* isolates from South Western region of Nigeria by polymerase chain reaction (PCR) so as to determine the diagnostic value of using this technique for detection or confirmation of MRSA. The study found that the prevalence of *mecA* gene is 22% in *S. aureus* isolates from South Western part of Nigeria and this correlates with high level of methicillin resistance.

MATERIALS AND METHODS

Bacterial isolates

A total number of 194 strains of *S. aureus* isolated from the specimens submitted to diagnostic section of Medical Microbiology and Parasitology Department of University College Hospital (UCH), Ibadan; Obafemi Awolowo University Teaching Hospital, Ile-Ife; and LAUTECH Teaching Hospital (LTH), Osogbo; in the South Western Nigeria. The strains were isolated from different clinical specimens such as urine, blood; wound swab, ear swab, high vaginal swab and endocervical swab. The isolates were identified to be *S. aureus* following Gram stain that showed Gram positive cocci and biochemical tests showed positive results for catalase and tube coagulase as described in standard Medical Microbiology laboratory manual (Cheesbrough, 2000). All *S. aureus* isolates were stored at 4^{o}C on Mueller Hinton agar slope until they were ready to use.

Antibiotics susceptibility testing

The antibiotic susceptibility testing was performed using disc diffusion method on Mueller Hinton (MH) agar. Gram positive multiple antibiotic disc containing erythromycin (5 µg), chlorampehnicol (10 µg), tetracycline (10 µg), streptomycin (10 µg), penicillin (1 µg) and gentamicin(10 µg), was used. Also single antibiotic disc such as vancomycin (30µg), pefloxacin (30 µg), and ceftriazone (30 µg) were used to determine the susceptibility pattern of the isolates at 37^{o}C overnight as previously described. Briefly, inocula of bacteria were prepared to 0.5 McFarland standards and tested against all the aforementioned antibiotics discs. Sterile swab stick was dipped into the bacteria suspension and used to streak the MH agar, after which the antibiotic disc was placed on the surface of MH agar plate and incubated at 37^{o}C for 24 hrs. After which the zone of inhibition was measured with ruler, which was now compared with the standard value of zone of inhibition of each antibiotic. Methicillin susceptibility was determined as described before on blood agar (15% v/v) with a heavy inoculum obtained from a blood agar plate (Alli *et al.,* 2007). The test was incubated at 30^{o}C overnight with 10 µg/disc of methicillin. The isolates were considered sensitive, intermediate and resistant based on the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2001).

Beta-lactamase test

Detection of β-lactamase was carried out using nitrocefin solution (a chromogen) on all the *S. aureus* isolates. Broth culture of MRSA strains as well as an ampicillin resistant strains (wild type *E. coli* 1048 with plasmid *PUC* 18) a positive control were incubated overnight, 200 µl of the overnight cultures were transferred into wells of a microtitre tray; broth without inoculum was also included to serve as a negative control. Ten microlitre of nitrocefin solution was prepared according to the manufacturer's instruction and was added to each well using a multipoint pipette. Nitrocefin is a chromogenic cephalosporin that changes colour from yellow to red on hydrolysis. β-lactamase production was inferred when the broth turned red within 30 min on addition of nitrocefin as directed by the manufacturer (Fisher Scientific, UK).

DNA Extraction and PCR for detection of *mecA* gene

DNA was extracted from 500 µl overnight Muller Hinton broth using lysostaphin and lysozyme to digest

the cell wall as described before (Alli *et al.*, 2007). PCR was carried out on all the strains using forward primer designated mecA_F1 (AGTTCTGCAGTACCGGATTG) and backward primer designated mecA_B1 (AAAATCGATGGTAAGGTTCGC) in a 30 µl reaction containing 50 ng of DNA, *Taq* polymerase and dNTP using the cycling parameter - denaturation temperature at 94^{o}C for 30 sec, annealing temperature at 55^{o}C for 30 sec, followed by extension at 72^{o}C for 1 min for 40 cycles. Positive control (MRSA DNA) and negative control DNA from NCTC 6571 (Oxford *S. aureus*) were included in each batch of PCR run. A successful amplification of *mecA* gene would be indicated by 533 bp. Sequencing of the PCR products to confirm 533 bp *mecA* gene amplification was carried out on few of the positive PCR products.

Minimum Inhibitory Concentration

MIC was carried out on the selected *mecA* gene positive strains, *mecA* gene negative strains of MRSA and methicillin sensitive strains of *S. aureus*. About 12 dilutions were made using concentration covering 0.125 and 256 µg/ml of methicillin, using the original concentration as 250 µg/ml and using two fold dilution methods. Suspension of the isolates was made to obtain 0.5 MacFarland standard of the organism and 2-fold of the organism was pipette into each of the dilution to make two fold dilutions. Three controls were set up: positive control (containing MH broth and NCTC 6571 (Oxford *S. aureus* strain), negative control (containing MH broth and the antibiotic), and sterility control (containing only MH broth). The test was incubated aerobically at 37ºC for 24 hrs.

Statistical Analysis

Data were analysed using statistical package within the Microsoft Excel and Epi-info software for Disease control and prevention, USA. Chi square was used to determine the effect of sex, age, hospital location, type of specimens, and beta lactamase production on the data obtained. The p value less than 0.05 was considered to be significant.

RESULTS

Among the 194 isolates of *S. aureus* obtained from different clinical specimens from 194 patients with average age of 35 years old, 45 isolates were collected from UCH, Ibadan, 58 isolates from OAUTHC, Ife, and 91 isolates from LTH, Osogbo, South Western region of Nigeria. Antimicrobial susceptibility pattern revealed that the isolates showed varying degree of resistant to various types of antibiotics tested in this study (Table 1). Highest degree of antibiotic resistant was recorded for penicillin about 186 (95.8%) out of 194 isolates of *S. aureus* whereas no resistant was found in vancomycin. The methicilin disc susceptibility testing showed that 40 (20.6%) out of 194 isolates of *S. aureus* were resistant to methicillin.

We decided to screen for *mecA* gene by PCR in all the *S. aureus* isolates irrespective of the result of methicillin susceptibility testing – a gene that has been shown to confer methicillin resistance in *S. aureus* in order to confirm the phenotypic detection of MRSA. The result of PCR (Fig. 1) showed that 43 (22.2%) out of the 194 isolates of *S. aureus* were found to contain *mecA* gene as indicated by the amplification of 533 bp expected product.

Table 1.
Antibiotics susceptibility pattern of *S. aureus* isolates from South Western, Nigeria.

Antibiotic	Number of Sensitive (%)	Number of Intermediate (%)	Number of Resistant (%)
Methicillin	154 (79.4%)	0 (0.0%)	40 (20.6%)
Ampicillin	0(0.0%)	4 (2.2%)	190 (97.8%)
Penicillin	0(0.0%)	8 (4.1%)	186 (95.9%)
Streptomycin	20 (9.9%)	62 (31.9%)	102 (58.2%)
Tetracycline	10 (5.15%)	10 (5.15%)	174 (89.7%)
Erythromycin	60 (30.9%)	16 (8.2%)	110 (56.7%)
Gentamicin	34 (17.5%)	28 (14.5%)	132 (68.0%)
Chloramphenicol	42 (21.2%)	22 (11.7%)	130 (67.2%)
Ceftriazone	28 (14.8%)	42 (21.3%)	124 (63.9%)
Perfloxacin	48 (25.0%)	18 (9.4%)	128 (65.9%)
Vancomycin	194 (100.0%)	0 (0.0%)	0(0.0%)

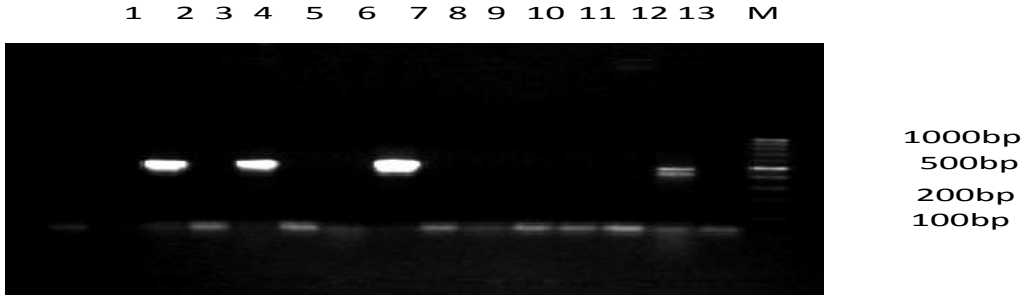

Fig 1
PCR for detection of *mecA* gene among the *S. aureus* isolates. Representative agarose gel electrophoresis of PCR products. Lanes 1, 3, 6 are positive for *mecA* as indicated by 533 bp PCR product, Lane 2, 4, 5, 7-11 are negative for mecA, Lane 12: positive control; Lane 13: negative control; Lane M: molecular weight size marker.

Representatives of the positive PCR products sent for DNA sequencing confirmed the product to be part of *mecA* gene as revealed by 100% identity to the *mecA* gene (data not shown). PCR repeated for detection of *mecA* gene at higher and lower concentrations of DNA in order to rule out the effect of DNA concentration on PCR showed similar result to what obtained before. The result of the PCR confirmed phenotypic detection of MRSA with additional 3 MRSA strains detected that were not detected with disc diffusion test.

Among the 91 isolates collected from LTH, Osogbo, 20 (22%) isolates were confirmed as MRSA by detection of *mecA* gene, and of the 58 isolates collected from OAUTHC, Ife, 18 (31%) isolates were confirmed as MRSA by detection of *mecA* gene (Fig.2). There was no association between frequency distribution of MRSA and the hospitals where the isolates came from ($X^2 = 1.76$; $P > 0.05$).

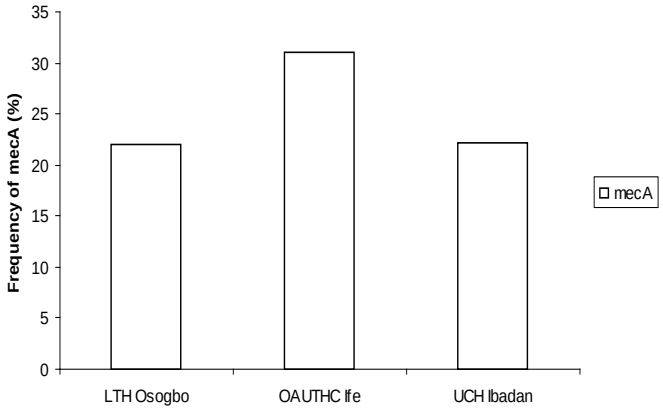

Fig .2
Distribution of *mecA* gene amongst the *S. aureus* isolates from the three teaching hospitals in South Western Nigeria.

Twenty six (22.4%) of the 116 males had MRSA while 17 (21.8%) of the 78 females had MRSA. There was no association between gender and the frequency distribution of MRSA ($X^2 = 0.04$; $P > 0.05$). Age group 21-30 recorded the lowest frequency distribution (10.5%) of MRSA while the highest frequency distribution of MRSA was recorded in 51-60 age groups (Fig.3). No association was found between age group and prevalence of MRSA in South Western, Nigeria ($X^2 = 9.23$; $P > 0.05$).

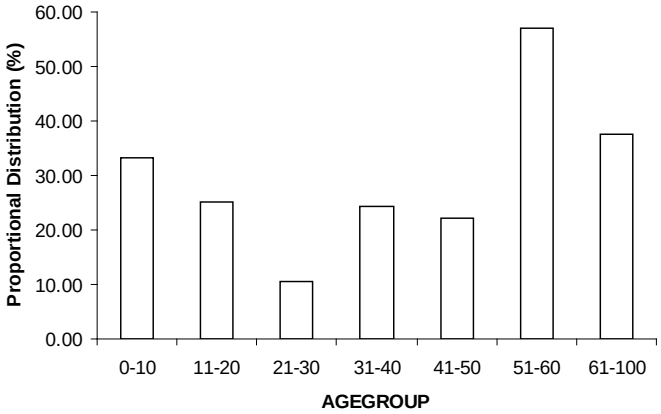

Fig. 3.
Distribution of *mecA* gene in *S. aureus* according to different age groups.

The distribution of MRSA according to the specimen type / site of isolation showed that indwelling catheters and endocervical swabs accounted for 100% proportional distribution of MRSA while the lowest proportional distribution were noted in blood and nasal swabs (Table 2). There was strong association between specimen type / site of isolation of the organism and prevalence of MRSA ($X^2 = 16.74$; $P < 0.05$).

In order to determine the degree of methicilin resistance by the *S. aureus* isolates under study, the *S. aureus* isolates were divided into two groups – *mecA* positive isolate (*mecA*$^+$) and *mecA* negative isolate

(*mecA*$^-$) and minimum inhibitory concentration to methicillin was determined. The MIC_{50} and MIC_{90} to methicillin for representative isolates of *mecA*$^+$ were found to be 128 and 256 µg/ml, respectively while the MIC_{50} and MIC_{90} to methicillin for *mecA*$^-$ were found to be 0.125 and 2 µg/ml, respectively, indicating low degree of resistance to methicillin in strains without *mecA* gene and high degree of resistance to methicillin for strains with *mecA* gene.

β –lactamase test was also carried out on the *S. aureus* isolates to detect whether the organism would be able to produce the enzyme β-lactamase – an enzyme that inactivates β- lactam antibiotics. The test was carried out on the 194 isolates, and 124 (64%) of the isolates were able to produce β-lactamase enzyme by changing colour from yellow to red on addition of nitrocefin solution. Among the 91 LTH isolates, 46 (50.6%) were able to produce β- lactamase, while 37 (64%) of 58 *S. aureus* OAUTHC isolates and 34 (75.5%) of 45 *S. aureus* UCH isolates were also able to produce the enzyme β-lactamase. All the 43 (100%) *mecA*$^+$ strains showed beta lactamase activity while 81 (53.6%) of the 151 *mecA*$^-$ strains showed beta lactamase activity. There was strong association between the presence of *mecA* gene and beta lactamase activity (X^2= 29.21; $P < 0.05$).

Nasal carriage of MRSA amongst the hospital staff was also determined in order to determine the role hospital staff might play in the epidemiology of MRSA. A total of 120 samples were analysed, *S. aureus* was detected in 49 (40.4%) of the workers. Interestingly, all the *S. aureus* isolates was found to be sensitive to 10 µg methicillin disc. Furthermore, high levels of antibiotic resistant were detected among the *S. aureus* isolates from staff of the hospital.

DISCUSSION

Methicillin resistance detection in staphylococci can be problematic in the clinical microbiology laboratory because of the heterogeneity of the bacterium under test. The detection of resistance in these isolates has been troubled due to variability in standard techniques used in determining methicillin resistance. Resistant to methicillin is determined by *mecA* gene which is part of a mobile genetic element called the staphylococcal cassette chromosomes (SCC) *mec* (Wu *et al., 1996)*, and this could be a good predictor of methicillin resistant when molecular technique such as PCR are used for screening methicillin resistant gene in *S. aureus*. In this present study, methicillin resistance was detected with disc susceptibility testing, minimum inhibitory concentration using broth macro-dilution method, and screening for the presence of *mecA* gene by PCR in all the *S. aureus* isolates from South Western Nigeria during the period of study. PCR for detection of *mecA* gene showed that 22.6% of the isolates carried the *mecA* gene. There was a strong correlation between high level of methicillin resistance as determined by MIC and presence of *mecA* gene. *S. aureus* isolate was considered susceptible if the MIC was less than or equal to 8 µg/ml while ≥16 µg/ml as resistance, was used in interpretation of the results according to the NCCLS (Jorgensen and Turnidge, 2003).

The *mecA*$^-$ strains of *S. aureus* were found to be sensitive to methicillin as indicated by MIC_{50} and MIC_{90} of 0.5 µg/ml and 2 µg/ml, respectively while the *mecA*$^+$ strains of *S. aureus* were 128 µg/ml and 256 µg/ml, respectively indicating high level of resistance to methicillin being coded for by *mecA* gene.

Table 2.
Distribution of *mecA*$^+$ S. aureus according to type of specimens

Specimen type	Number of mecA+	Number of mecA-	Total	Proportional Distribution (%)
Blood	0	6	6	0.00
Catheter tip	4	0	4	100.00
Ear swab	6	8	14	42.86
Eye swab	6	20	26	23.08
Endocervical swab	2	0	2	100.00
High vaginal swab	6	12	18	33.33
Nasal swab	5	65	70	7.14
Wound swab	8	20	28	28.57
Urine	6	20	26	23.08
Total	43	151	194	22.16

This result indicates the acquisition of *mecA* gene is responsible for methicillin resistance; confirming other Furthermore, in order to develop high degree of resistance to methicillin, *mecA* is required. Studies have shown that most of the MRSA isolates contains *mecA* gene, while *mecA* is also absent in some strains of MRSA (Aires de Sousa *et al.,* 1998). Studies have also shown that apart from *mecA* gene, PBP4 and *ica* gene cluster can also encode resistant in MRSA (Cramton *et al.,* 1999, Memmi *et al.,* 2008). In our study, out of the 194 isolates, 43 (22.2%) of the isolates were confirmed as MRSA by the detection of *mecA* gene. In support of our observation, Martin-Lopez *et al.* (2002) recorded 96.5% of MRSA by detection of *mecA* gene, which is slightly lower than the 100% detection of *mecA* gene in MRSA from South Western, Nigeria. A recent multi-centre study in South Western Nigeria carried out by Adesida *et al.* (2005) confirmed resistance to methicillin by the detection of the *mecA* gene using PCR and reported a prevalence of 1.4 %, which is far lower to the prevalence in this study.

There is one inference that can be drawn from our study and that is *mecA* gene is responsible for phenotypic behaviour of methicillin resistance in this part of the world. Low level resistance to methicillin has been reported to be conferred by penicillin binding protein 4 (PBP4) ((Memmi *et al.,* 2008). We are currently looking at this possibility among our strains of MRSA. It is also interesting to know that the MRSA nasal carriage rate was very low among the hospital workers with the non-detection of *mecA* gene in the entire *S. aureus* isolates from the hospital workers suggesting lesser role of hospital workers in dissemination of MRSA in these hospitals. More needs to be done on typing the circulating strains as previously done before in other environments using molecular epidemiological techniques (Taiwo *et al.,* 2005, Alli *et al.,* 2007, Okon *et al.,* 2009) in order to monitor the epidemiology of MRSA in Nigeria. The prevalence of MRSA in this study was 22%, which is low compared to similar study carried in people living with HIV in South Africa where 77% was reported (Cotton *et al.,* 2008), and other study in Nigeria where 34.7% was reported (Taiwo *et al.,* 2005). Beta lactamase production by *S. aureus* has been identified in this study to be a risk factor for the prevalence of MRSA in South Western, Nigeria. In this study, out of the 194 isolates of *S. aureus,* 124 (64 %) of the isolates produce the enzyme β–lactamase, which is different from the previous study carried out by Olowe *et al.* (2007) in which all the isolates were found to produce β-lactamase using starch paper method. It did not come to our surprise the link between MRSA and beta lactamase production in *S. aureus* since the reason for previous studies on the role of *mecA* gene in methicillin resistance (Hartman and Tomasz, 1984). the use of methicilllin (beta lactamase resistant penicillin) is to combat the beta lactamase producing *S. aureus* infections. This further confirmed previous study (Alli, 1988) where majority of beta lactamase producers were methicillin resistant.

The indiscriminative use of antibiotics and because of the easy availability of antibiotic without prescription has enhanced the emergency of resistant strains. Many investigators have reported an increase in the incidence of MRSA (Kim *et al.,* 2004, Adebayo *et al.,* 2006). Furthermore, the prevalence of MRSA in this study by the detection of *mecA* gene (22%) is similar to prevalence of MRSA obtained by Kesah *et al.* (2003) from Nigeria, which range from 21% -30%. Hospitals' location was not recognised as a risk factor in this study because no association was found between hospitals location and the prevalence of MRSA ($p > 0.05$). The proportional distribution of *mecA* gene in MRSA in this study was higher in OAU isolates (31%) compared to that of LTH isolates (22%) and UCH isolates (23%). UCH, Ibadan has been shown to be the breeding ground for multi-drug resistant bacteria as recently demonstrated by Ogbolu *et al.* (2011) where high degree of resistance was recorded for Gram negative enteric bacilli with no single mechanism could be used to explain the molecular basis of resistance of these isolates. Other variables such as gender and age were found not to be risk factors in MRSA infections in this study. This is in sharp contrast to a study carried out in Taiwan where age has been found to be a risk factor for *S. aureus* colonization (Lu *et al.,* 2005). Interestingly, site of isolation of MRSA / specimen type has been found to be associated with prevalence of MRSA in this study; hence consider being a risk factor. High rate of isolation of MRSA had been found in indwelling devices and endocervical swabs, while low rate of isolation of MRSA had been found in blood and nasal swabs. This is in agreement with a previous study (Carnicer-Pont *et al.,* 2006) where MRSA was found to be associated with catheter or indwelling devices.

Antimicrobial disc susceptibility pattern of the *S. aureus* isolates was determined in order to determine the multiple antibiotics resistant nature of *S. aureus* isolated in this study. Some of the *S. aureus* isolates were susceptible to ceftriazone, streptomycin, and perfloxacin. High level of resistance was recorded for penicillin despite the fact that the hospitals antibiotic usage policy in South Western, Nigeria has put a stop to the use of penicillin in treatment of infection. Although this can not rule the possibility of patients obtaining it as over the counter medicine in pharmacy shops, hence, the selective pressure on *S. aureus*

strains. Multiple antibiotic resistant has been the major property of MRSA. Antimicrobial resistance among nosocomial pathogens is a significant problem in clinical settings that adds to the cost of medical care and the morbidity and mortality of patients (Bouchillon *et al.*, 2004). Resistance to vancomycin was found to be absent in this study. Adebayo *et al* (2006), recorded a 0 % resistance of vancomycin in clinical isolates of *S. aureus* collected from South Western of Nigeria, our present study corroborates that vacomycin resistant *S. aureus* is a rare event in Nigeria. This is good news, knowing fully well that vancomycin is the antibiotic of choice for treatment of MRSA.

In conclusion, the prevalence of MRSA as detected by *mecA* gene in South Western Nigeria is high enough to warrant the need for surveillance of this strain of *S. aureus* and screening of *S. aureus* for mecA gene is a good predictor of MRSA in Nigeria.

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REFERENCES

Adebayo, S., Johnson, L. and Deboye, K. (2006): Staphylococcus aureus characterization of MRSA in Southwestern Nigeria. *Sidebars,* **18**.

Adesida, S., Boelens, H., Babajide, B., Kehinde, A., Snijders, S., van Leeuwen, W., Coker, A., Verbrugh, H. and van Belkum, A. (2005): Major epidemic clones of Staphylococcus aureus in Nigeria*Microb Drug Resist,* **11,** 115-21.

Aires de Sousa, M., Sanches, I. S., Ferro, M. L., Vaz, M. J., Saraiva, Z., Tendeiro, T., Serra, J. and de Lencastre, H. (1998): Intercontinental spread of a multidrug-resistant methicillin-resistant Staphylococcus aureus clone *J Clin Microbiol,* **36,** 2590-6.

Alli, O. (1988): Incidence of Methicillin resistant Staphylococcus aureus at University College Hospital, Ibadan. In *Medical Microbiology and Parasitology,* School of Medical Laboratory Sciences, University College Hospital, Ibadan, Nigeria. 1-40.

Alli, O., Akinloye, O., Rowley, D. and Butcher, P. (2007)*:* A comparative assessment of ribosomal DNA polymorphisms in methicillin resistant Staphylococcus aureus (MRSA) epidemiology. *African Journal of Biomedical Research,* **10,** 117-125.

Archer, G. (1998): Staphylococcus aureus: a well-armed pathogen. *Clinical Infectious Disease,* **26,** 1179-1181.

Barber, M. (1961): Methicillin-resistant staphylococci.*J Clin Pathol,* **14,** 385-93.

Bouchillon, S. K., Johnson, B. M., Hoban, D. J., Johnson, J. L., Dowzicky, M. J., Wu, D. H., Visalli, M. A. and Bradford, P. A. (2004): Determining incidence of extended spectrum beta-lactamase producing Enterobacteriaceae, vancomycin-resistant Enterococcus faecium and methicillin-resistant Staphylococcus aureus in 38 centres from 17 countries: the PEARLS study 2001-2002. *Int J Antimicrob Agents,* **24,** 119-24.

Carnicer-Pont, D., Bailey, K. A., Mason, B. W., Walker, A. M., Evans, M. R. and Salmon, R. L. (2006): Risk factors for hospital-acquired methicillin-resistant Staphylococcus aureus bacteraemia: a case-control study.*Epidemiol Infect,* **134,** 1167-73.

Cavassini, M., Wenger, A., Jaton, K., Blanc, D. S. and Bille, J. (1999): Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in Staphylococcus aureus. *J Clin Microbiol,* **37,** 1591-4.

Cheesbrough, M. (2000): *District Laboratory Practice in Tropical Countries Part 2,* Cambridge University Press, Cambridge.

Cotton, M. F., Wasserman, E., Smit, J., Whitelaw, A. and Zar, H. J. (2008): High incidence of antimicrobial resistant organisms including extended spectrum beta-lactamase producing Enterobacteriaceae and methicillin-resistant Staphylococcus aureus in nasopharyngeal and blood isolates of HIV-infected children from Cape Town, South Africa. *BMC Infect Dis,* **8,** 40.

Cramton, S. E., Gerke, C., Schnell, N. F., Nichols, W. W. and Gotz, F. (1999): The intercellular adhesion (ica) locus is present in Staphylococcus aureus and is required for biofilm formation. *Infect Immun,* **67,** 5427-33.

Hartman, B. J. and Tomasz, A. (1984): Low-affinity penicillin-binding protein associated with beta-lactam resistance in Staphylococcus aureus**.** *J Bacteriol,* **158,** 513-6.

Ieven, M., Jansens, H., Ursi, D., Verhoeven, J. and Goossens, H. (1995): Rapid detection of methicillin resistance in coagulase-negative staphylococci by commercially available fluorescence test. *J Clin Microbiol,* **33,** 2183-5.

Jevons, M. (1961): Celbenin resistant staphylococci. *British Medical Journal,* **1,** 124-125.

Jorgensen, J. and Turnidge, J. (2003): Susceptibility test methods: Dilution and Disk diffusion methods. In *Manual of Clinical Microbiology,* Vol. 1 (Eds, Murray, P., Baron, E., Jorgensen, J., Pfaller, M. and Yolken, R.) ASM, Washington DC, pp. 1108-1127.

Kesah, C., Ben Redjeb, S., Odugbemi, T. O., Boye, C. S., Dosso, M., Ndinya Achola, J. O., Koulla-Shiro, S., Benbachir, M., Rahal, K. and Borg, M. (2003): Prevalence of methicillin-resistant Staphylococcus aureus in eight African hospitals and Malta. *Clin Microbiol Infect,* **9,** 153-6.

Kim, H. B., Jang, H. C., Nam, H. J., Lee, Y. S., Kim, B. S., Park, W. B., Lee, K. D., Choi, Y. J., Park, S. W., Oh, M. D., Kim, E. C. and Choe, K. W. (2004): In vitro activities of 28 antimicrobial agents against Staphylococcus aureus isolates from tertiary-care hospitals in Korea: a

nationwide survey. *Antimicrob Agents Chemother,* **48,** 1124-7.

Lu, P. L., Chin, L. C., Peng, C. F., Chiang, Y. H., Chen, T. P., Ma, L. and Siu, L. K. (2005): Risk factors and molecular analysis of community methicillin-resistant Staphylococcus aureus carriage *J Clin Microbiol,* **43,** 132-9.

Martin-Lopez, J. V., Perez-Roth, E., Claverie-Martin, F., Diez Gil, O., Batista, N., Morales, M. and Mendez-Alvarez, S. (2002): Detection of Staphylococcus aureus Clinical Isolates Harboring the ica Gene Cluster Needed for Biofilm Establishment. *J Clin Microbiol,* **40,** 1569-70.

Memmi, G., Filipe, S. R., Pinho, M. G., Fu, Z. and Cheung, A. (2008): Staphylococcus aureus PBP4 is essential for beta-lactam resistance in community-acquired methicillin-resistant strains. *Antimicrob Agents Chemother,* **52,** 3955-66.

Ogbolu, D. O., Daini, O. A., Ogunledun, A., Alli, A. O. and Webber, M. A. (2011): High levels of multidrug resistance in clinical isolates of Gram-negative pathogens from Nigeria. *Int J Antimicrob Agents,* **37,** 62-6.

Okon, K. O., Basset, P., Uba, A., Lin, J., Oyawoye, B., Shittu, A. O. and Blanc, D. S. (2009): Cooccurrence of predominant Panton-Valentine leukocidin-positive sequence type (ST) 152 and multidrug-resistant ST 241 Staphylococcus aureus clones in Nigerian hospitals. *J Clin Microbiol,* **47,** 3000-3.

Olowe, O., Eniola, K., Olowe, R. and Olayemi, A. (2007): Antimicrobial susceptibility and beta- lactamase detection of MRSA in Osogbo. South West of Nigeria. *Nature and Science,* **5,** 44-48.

Rohrer, S., Tschierske, M., Zbinden, R. and Berger-Bachi, B. (2001): Improved methods for detection of methicillin-resistant Staphylococcus aureus. *Eur J Clin Microbiol Infect Dis,* **20,** 267-70.

Taiwo, S. S., Bamidele, M., Omonigbehin, E. A., Akinsinde, K. A., Smith, S. I., Onile, B. A. and Olowe, A. O. (2005): Molecular epidemiology of methicillin-resistant Staphylococcus aureus in Ilorin, Nigeria. *West Afr J Med,* **24,** 100-6.

Ubukata, K., Yamashita, N. and Konno, M. (1985): Occurrence of a beta-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci *Antimicrob Agents Chemother,* **27,** 851-7..