Association of virulence genes with meca gene in Staphylococcus aureus isolates from Tertiary Hospitals in Nigeria

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ABSTRACT

Introduction: Staphylococcus aureus is the etiological agent for a wide range of human infections, and its pathogenicity largely depends on various virulence factors associated with adherence, evasion of the immune system and damage of the host. This study determined the prevalence of methicillin-resistant S. aureus (MRSA) and some selected virulence genes in clinical isolates of S. aureus from South-Western Nigeria. Materials and Methods: The antibiotic susceptibility of 156 S. aureus isolates to various antibiotics was determined. Moreover, polymerase chain reaction detection of the meca gene was performed including SCCmec typing, and the isolates were screened for selected genes (alpha hemolysin [hla], intracellular adhesion A [icaA], Panton-Valentine leukocidin [PVL], fibronectin binding protein A [fnbA], bone sialoprotein binding protein [bbp], exfoliative toxin A [eta], exfoliative toxin B [etb], and collagen binding adhesion [cna]) associated with virulence. Results: The prevalence of meca gene was 42.3% (66 out of 156 S. aureus), and SCCmec typing showed that 24 (36.4%) carried the SCCmec II element, 4 (6.1%) with type III, 10 (15.2%) with SCCmec IV, and 28 (42.4%) harbored type V. The proportion of S. aureus with the following genes was ascertained: Hla (55.1%), icaA (42.3%), PVL (34.6%), fnbA (8.3%), bbp (4.5%), and eta (3.8%). All the isolates were etb and cna negative. The prevalence of the PVL gene in methicillin susceptible Staphylococcus aureus (MSSA) was 53.3% compared with 9.1% of MRSA. An association between virulence genes (eta and icaA) and meca positive S. aureus; and significant difference in the distribution of virulence genes in in-patients and out-patients were found. The MRSA strains in South-Western Nigeria were dominated by SCCmec II and SCCmec V. Conclusion: The study concluded that there is a high prevalence of MRSA in Nigeria with association of eta and icaA genes with meca gene in S. aureus isolates.

KEY WORDS: meca, Staphylococcus aureus, virulence genes

INTRODUCTION

Staphylococcus aureus has been well-documented as a human opportunistic pathogen.[1] This organism is implicated in various disease conditions such as furuncles or boils, cellulitis, impetigo, and postoperative wound infections of various sites. It has also been associated with serious and life-threatening infections such as bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cerebritis, and meningitis. Moreover, it is linked with toxin-related diseases.[2] The ability of S. aureus to cause various infections is linked mainly with various virulence genes located on the plasmid or bacterial genome. There are about 40 virulence genes/factors that are known to be involved in almost all processes from colonization of the host to nutrition and dissemination.[3]

The most remarkable feature of S. aureus is that the virulence genes are expressed at various periods during colonization.
and infection. *S. aureus* infection is treatable; however, the evolution and clonal expansion of methicillin-resistant *S. aureus* (MRSA) has continued to be a major challenge in healthcare and community-acquired infections worldwide.[4,5] Moreover, the Panton-Valentine leukocidin (PVL) otherwise known as lukF-PV/lukS-PV gene (a virulence gene) has been associated with community-acquired MRSA (CA-MRSA) in many parts of the world.[6,7] A number of investigations have provided evidence that the prevalence of the PVL gene is high among methicillin-susceptible *S. aureus* (MSSA) in Nigeria,[4,5,8] and only one PVL-positive MRSA has been reported.[9] However, there is paucity of data on the epidemiology of CA-MRSA in Nigeria. Moreover, the distribution of various virulence genes among clinical *S. aureus* isolates has not been investigated. Therefore, this study aimed to determine the prevalence of mecA and virulence genes (bone sialoprotein binding protein [bbp], collagen binding adhesion [cna], fibronectin binding protein A [fnbA], alpha hemolysin [hla], intracellular adhesion A [icaA], exfoliative toxin A [eta], exfoliative toxin B [etb], and PVL) in *S. aureus* isolates from four teaching hospitals in South-Western Nigeria along with SCCmec typing of the MRSA isolates.

**MATERIALS AND METHODS**

**Bacterial strains**

A total of 156 nonduplicate *S. aureus* isolates obtained from clinical specimens submitted to four diagnostic laboratories of tertiary health care institutions in Nigeria for routine laboratory diagnosis between March and June, 2013 were investigated in a cross-sectional/observational study. Demographic data such as sex, age, and site of isolation of specimen were obtained from the laboratory request forms forwarded to the diagnostic microbiology laboratories. Confirmation as *S. aureus* was based on the identification profile of the API 20 Staph kit (Biomerieux, France) and tube coagulate test. All the isolates were stored at 4°C on Mueller Hinton (MH) agar slope until ready for use. All the organisms analyzed in this study were culture collections from various hospitals with adequate histories and no active solicitation of clinical samples from patients was involved. This study had no bearing on clinical diagnosis and treatment of patients.

**Antibiotic susceptibility testing**

The isolates were sub-cultured onto mannitol salt agar and incubated at 35°C for 24 h to ensure that the isolates were pure isolates of *S. aureus*. The antimicrobial disk diffusion susceptibility testing was performed on MH agar. The following antibiotics: Penicillin G (10 units), cefoxitin (30 µg), erythromycin (15 µg), tetracycline (30 µg), gentamicin (10 µg), fusidic acid (10 µg), and trimethoprim-sulfamethaxazole (1.25/23.75 µg) were used to determine the susceptibility pattern of the isolates according to the guidelines of CLSI.[10]

**Minimum inhibitory concentration**

The minimum inhibitory concentration (MIC) of the isolates to cefoxitin and vancomycin was performed with cation-adjusted MH broth (CAMHB) as previously described.[10] Briefly, about 12 dilutions were made using concentration covering 0.128 and 256 µg/ml of cefoxitin, using the original concentration as 256 µg/ml and using two-fold dilution methods. Suspension of the isolates was made to obtain 0.5 MacFarland standard of the organism and 0.05 ml of the suspension was added to each dilution. Three control experiments were set up; positive control (containing CAMHB and ATCC 25913 strain of *S. aureus*), negative control (containing CAMHB and the antibiotic), and sterility control (containing only CAMHB).

**Beta-lactamase testing**

Detection of β-lactamase was carried out on all the isolates using nitrocefin solution (a chromogen) as previously stated.[11] Briefly, 200 µl of the overnight broth cultures were transferred into wells of a microtiter tray; broth without inoculum was also included to serve as a negative control. Ten microliter 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 color 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**

The suspension of *S. aureus* was lysed to release DNA from a centrifuged deposit of 5 ml overnight MH broth culture of the organism in 0.5 ml of TES (Tris HCl pH 8.0, 1 mM EDTA and 0.1 M NaCl) containing lysostaphin (1 mg/ml) (Sigma, UK) incubated at 37°C for 15 min, after which the DNA was extracted with phenol-chloroform, purified, and precipitated with ethanol as previously described.[12]

**Polymerase chain reaction for detection of the mecA gene**

Polymerase chain reaction (PCR) detection of the mecA gene was carried out on all the isolates as previously described using PCR kit from New England Biolab (NEB, USA).[13] Sequencing of the PCR products and BLAST analysis were performed on representative positive isolates to confirm the identity of the mecA gene. In this study, MRSA will be referred as mecA positive, while MSSA will be regarded as mecA negative.

**Polymerase chain reaction for detection of virulence genes**

PCR was carried out to detect eight different virulence genes: PVL, eta, etb, icaA, bbp, hla, fnbA, and cna genes using the primers and cycling parameters are listed in Table 1a. The PCR was carried out for each gene singly. Sequencing was done on representative isolates with positive PCR products to confirm the identity of the virulence genes.

**SCCmec typing**

SCCmec typing was performed on the mecA positive isolates as described previously using the following sets of primers as shown in Table 1b.[14] In addition to SCCmec typing, the MRSA isolates were screened by PCR for the presence of CA-MRSA as...
previously described by Memmi et al. with the following PCR conditions: Initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 30 s, followed by extension at 72°C for 1 min. Each primer had a concentration of 1 µM while the Taq mix made up of the following: 10 mM of MgCl₂, 0.2 mM of dNTP mix, and 1 U of Taq polymerase (NEB, USA).

Statistical analysis
Data were analyzed using statistical package within the Microsoft Excel and Epi-info software from Centre for Disease Control and Prevention, USA. The Chi-square was used to determine if there was association between various sociodemographic data (sex, age, hospital and specimen type/clinical diagnosis) and virulence and mecA genes. Analysis of variance (ANOVA) and Student’s t-test were calculated for selected data using Microsoft Excel software, The P < 0.05 was considered to be statistically significant.

RESULTS
Distribution of sample population and *Staphylococcus aureus* isolates from clinical samples
Of the 156 *S. aureus* obtained from various clinical specimens, 38 isolates each were from Obafeimi Awolowo University Teaching Hospitals Complex (OAUTHC), Ille-Ife, and the University College Hospital, Ibadan while, 30 and 50 isolates were obtained from LAUTECH Teaching Hospital (LTH), Osogbo, and University of Ilorin Teaching Hospital (UITH), Ilorin, respectively. A total of 96 (61.5%) of the isolates were from in-patients and 60 (38.5%) from out-patients. Eighty-four (53.8%) isolates were female and 72 (46.2%) were male. The mean age of the patients was 27.14 years old with a median of 23 years old, and standard deviation of 23.99 years old. A total of 50 (32.1%) of the isolates were isolated from wound samples, 24 (15.4%) from other sites (catheter tips, aspirates, high vaginal swab etc.) [Table 2].

Antibiotic susceptibility testing
The susceptibility of the isolates to the antibiotics showed that more than 80% of the isolates were resistant to penicillin, tetracycline and trimethoprim-sulfamethoxazole, and 42.3% were identified (phenotypically) as MRSA (based on resistance to cefoxitin) [Table 3]. Also, 49.4% (66 of 135 isolates) were resistant to erythromycin while 153 (98.1%) of the isolates were susceptible to fusidic acid. Seventy percent of the MRSA isolates (46 of 66 isolates) were resistant to gentamicin while 50% of MSSA isolates (45 of 90 isolates) were resistant to gentamicin. The resistance to gentamicin was significantly higher in MRSA.
Table 3: Antibiotic susceptibility patterns of Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>Antibiotic (µg)</th>
<th>Resistant (%)</th>
<th>Sensitive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline (30)</td>
<td>140 (89.7)</td>
<td>16 (10.3)</td>
</tr>
<tr>
<td>Penicillin G* (10)</td>
<td>140 (89.7)</td>
<td>16 (10.3)</td>
</tr>
<tr>
<td>Fusidic acid (10)</td>
<td>3 (1.9)</td>
<td>153 (98.1)</td>
</tr>
<tr>
<td>Gentamicin (10)</td>
<td>85 (54.5)</td>
<td>71 (45.5)</td>
</tr>
<tr>
<td>Clindamycin (2)</td>
<td>39 (25)</td>
<td>117 (75)</td>
</tr>
<tr>
<td>Erythromycin (15)</td>
<td>77 (49.4)</td>
<td>79 (50.6)</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole (1.25/23.75)</td>
<td>143 (91.7)</td>
<td>13 (8.3)</td>
</tr>
<tr>
<td>Cefoxitin (30)</td>
<td>66 (42.3)</td>
<td>90 (57.7)</td>
</tr>
</tbody>
</table>

*The unit is different from other antibiotics and expressed as "Units".*

than MSSA ($\chi^2 = 5.29; P = 0.021$). No significant difference ($t$-test; $P = 0.052$) in resistance to other antibiotics such as clindamycin, erythromycin, trimethoprim-sulfamethoxazole, and tetracycline between MRSA and MSSA was observed.

All the isolates were susceptible to vancomycin (MIC values: Between 0.2 and 1 µg/ml). MIC to cefoxitin showed that the MIC₉₀ and MIC₉₀ of the MRSA strains were >256 µg/ml (indicating high-level resistance to methicillin) while MIC₅₀ and MIC₉₀ values for MSSA were 2 and 4 µg/ml, respectively.

### β-lactamase production

β-lactamase test revealed that 126 (80.8%) isolates were β-lactamase producers. All the MRSA strains were β-lactamase positive while 76 (84.4%) of the 90 MSSA strains were β-lactamase positive.

### Polymerase chain reaction detection of mecaA gene

All the 156 isolates were screened for mecaA gene by PCR. The result showed that 66 (42.3%) isolates were mecaA MRSA positive showing 100% correlation with the phenotypic cefoxitin susceptibility test results. The distribution of the MRSA isolates in clinical samples and various health care institutions in South-Western Nigeria are shown in Tables 2 and 4.

### Detection of virulence genes

The prevalence of six virulence genes (PVL, icaA, bbp, hla, fnbA, and eta) in all the 156 S. aureus examined is described in Tables 2, 4 and Figure 1a, b. Figure 1a and b show the distribution of the six virulence genes along with mecaA gene amongst different age groups. The etb and cua genes were not detected in all the age groups. There was varying distribution of the virulence genes among the S. aureus isolates investigated, with hla gene accounting for the highest proportional distribution (56%) followed by the icaA gene (42%) while the eta gene was only detected in 3.9% of the isolates.

Moreover, the eta and bbp positive S. aureus isolates were detected only in UITH [Table 4] while bbp, fnbA and eta positive isolates were identified only from in-patients [Figure 2a]. It is noteworthy all the eta positive strains were observed in MRSA strains alone while none of the isolates analyzed in this study possessed etb gene [Figure 2b]. There was a strong association...
between methicillin-resistance and possession of the \(\text{ eta}\) and \(\text{ icaA}\) genes \(\chi^2 = 86.53; P = 0.00001\) [Figure 2b], and between specimen type and \(\text{ hla}\)-positive isolates [Table 3]. A total of 54 (34.6%) isolates were \(\text{ PVL}\) positive and of these 11.1% (6 of 54) were \(\text{ mecA}\) positive while 48 (88.8%) were \(\text{ mecA}\) negative \(\text{ S. aureus}\) isolates. \(\text{ PVL}\) positive \(\text{ S. aureus}\) isolates were identified in all the four health care institutions in South-West Nigeria, and more than 40% were identified from wound samples [Table 2]. \(\text{ PVL}\) positive MRSA strains were limited to two teaching hospitals (4 at LTH and 2 at UITH).

SCC\(\text{ mec}\) typing showed that SCC\(\text{ mec}\) I was not detected in this study, but 24 (36.4%) MRSA possessed SCC\(\text{ mec}\) II element, 4 (6.1%) with SCC\(\text{ mec}\) III, 10 (15.2%) with SCC\(\text{ mec}\) IV and 28 (42.4%) carried the SCC\(\text{ mec}\) V element [Table 5]. Twenty-two (33%) of the 66 MRSA strains were nontypeable. Furthermore, the virulence genes were not uniformly distributed among different SCC\(\text{ mec}\) types [Table 6]. Hence, there was a significant difference in the distribution of virulence genes amongst various SCC\(\text{ mec}\) types MRSA isolates from this study (ANOVA; \(P = 0.045\)). Two of the \(\text{ PVL}\) positive MRSA strains were SCC\(\text{ mec V}\) while the remaining four were nontypeable.

Based on SCC\(\text{ mec}\) typing, 38 (10 SCC\(\text{ mec}\) IV + 28 SCC\(\text{ mec}\) V) (57.6%) isolates were classified as CA-MRSA positive while 28 (24 SCC\(\text{ mec}\) II + 4 SCC\(\text{ mec}\) III) (42.4%) were grouped as hospital-acquired MRSA (HA-MRSA). Subtyping of SCC\(\text{ mec}\) IV showed that only SCC\(\text{ mec}\) IVa and SCC\(\text{ mec}\) IVd were identified in this study and type IVd was only noted in OAUTHC. Furthermore, SCC\(\text{ mec}\) III was only detected in UITH, and more diversity of SCC\(\text{ mec}\) elements were observed among the MRSA isolates in this health care institution compared with other hospitals [Table 5]. We identified the emergence of 2 \(\text{ PVL}\) positive MRSA strains that carried the SCC\(\text{ mec V}\) in LTH and 4 \(\text{ PVL}\) positive MRSA strains that were nontypeable from two different hospitals (LTH and UITH). The study also identified two MRSA strains that were tetracycline resistant that possessed three virulence genes (\(\text{ bbp}, \text{ hla}\) and \(\text{ icaA}\)), with SCC\(\text{ mec}\) type II and they were both isolated from blood samples of the patients from in-patients diagnosed for sepsis. This could represent a virulent strain amongst the MRSA strains. On the other hand, we identified 2 MSSA strains that had three virulence genes (\(\text{ bbp}, \text{ fabA}\), and \(\text{ hla}\)) from blood samples of in-patient diagnosed with sepsis; the clonality of these strains could not be determined. Of interest was that the four virulent strains of \(\text{ S. aureus}\) were from the same hospital, and they were all susceptible to most of the antibiotics tested with the exception of penicillin, trimethoprim/sulfamethoxazole, and tetracycline.

**DISCUSSION**

The antibiotic susceptibility testing showed that all the \(\text{ S. aureus}\) isolates were susceptible to vancomycin and majority to fusidic acid, which is similar to the results obtained from previous studies in South-West Nigeria.\(^{6,20}\) This indicates that vancomycin and fusidic acid are effective agents for the treatment of MRSA infections in Nigeria. However, in order to minimize the emergence of vancomycin resistance strains, it is advisable that monotherapy with vancomycin should be discouraged, and a combination with another antistaphylococcal agent should be recommended based on susceptibility testing results. This
Table 6: Distribution of virulence genes amongst different SCCmec types

<table>
<thead>
<tr>
<th>Virulence gene</th>
<th>SCCmec type</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
<th>Type V</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL</td>
<td></td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>hla</td>
<td></td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>ica</td>
<td></td>
<td>0</td>
<td>14</td>
<td>4</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>bbp</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>fnb</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>etb</td>
<td></td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

ANOVA: P = 0.045; PVL: Panton-Valentine leukocidin; hla: Alpha hemolysin; ica: Interacellular adhesion; bbp: Bone sialoprotein binding protein; etb: Exfoliative toxin A gene.

ANOVA: Analysis of variance

study showed that the prevalence of MRSA was 42.3% that is similar to the report (41.1%) of a study recently conducted in South-West Nigeria. It is suffice to say that there has been an increase of 1.2% over a year. However, this is higher than the 20.23% prevalence rate reported in 2009 by Gbehremedhin et al., suggesting that MRSA is still on the increase in this region. This calls for urgent intervention to curb the spread of MRSA and antimicrobial resistance at large in Nigeria.

In determining the association between the various SCCmec types with clinical history, the distribution of SCCmec II and SCCmec V observed in the community (out-patients) and in the hospital settings (in-patients) strongly indicate that the distinction between HA-MRSA and CA-MRSA is blurred in this region, hence an overlap between CA-MRSA and HA-MRSA. However, we found the usefulness of this technique in differentiating two important strains that were found to be associated with sepsis but were found to be different from each other because one was found to be SCCmec II and the other nontypeable as a result of being MSSA; although, the possibility of acquiring the SCCmec II by the MSSA cannot be ruled out. The virulence of these strains could be explained with the presence of hla, bbc and fbp, or ica genes. In this particular situation, SCCmec typing has provided a snapshot for monitoring and understanding the evolution and clonal dissemination of MRSA clones in South-Western Nigeria. The clones in circulation in MRSA under this study were dominated by SCCmec II and V with highest distribution (42.4%) recorded for SCCmec V. SCCmec II, III, IV, and V MRSA strains have been previously identified in this area, which is in line with our finding.[4,8,9] A major drawback is the inability of linking the MRSA to MSSA. Future research should be geared toward a technique that is cost saving that can be incorporated into routine investigation. Other powerful techniques such as multilocus sequence typing, pulse field gel electrophoresis and whole genome sequencing can be exploited baring the expense. More research is needed in SCCmec typing in order to reduce the number of MRSA strains that are nontypeable.

The microbial adherence to cells and extracellular matrix is considered as an essential first step in the process of colonization and infection.[1] A well-characterized family of staphyloccocal surface adhesins, called microbial surface components recognizing adhesive matrix molecules are known to mediate adherence to host extracellular matrix components, such as fibrinogen, fibronectin and collagen. Binding to fibronectin is mediated by two closely related proteins, fnbA and fnbB, encoded by the genes fnbA and fnbB.[21] In this study, 13 of the 156 (8.3%) isolates possessed the fnbA gene. The isolates were obtained only from blood and wound samples and four fnbA isolates from wound were diagnosed with osteomyelitis. These observations indicate that fnbA gene could be important in invasive diseases such as endocarditis and osteomyelitis as reported by Peacock et al.[22] Although, there are limited data on the prevalence of fnbA gene in S. aureus, several studies have shown that fnbA gene is significantly common in invasive isolates and that it contributes independently to virulence.[22,23]

Alpha toxin (hla) is known to play a role in the pathogenesis of staphylococcal disease, as S. aureus mutants lacking hla display reduced virulence in invasive disease models.[24] In this study, 86 (55.1%) isolates were hla gene positive. Furthermore, it was detected in MRSA (33.3%) and MSSA (24.4%) strains indicating that the gene is relatively conserved. Moreover, no association between hla gene and MSSA (χ² = 1.49; P = 0.22). Overall, the high prevalence of this gene suggests that it plays a significant role in the pathogenesis of S. aureus infection. The hla gene was found mainly in wound and blood samples suggesting that the hla gene is important for the survival of S. aureus at these sites. Staphylococcal pathogenicity is not due to a single virulence factor as infections occur in a stepwise manner, each step involving one or several virulence factors.[1] We observed that all the fnbA positive isolates harbored the hla gene and were predominantly MSSA.

All the etb positive isolates in this investigation were MRSA which is in contrast with the study carried out in Japan by Nakaminami et al.[23] Moreover, none of the isolates studied possessed etb gene. The low rate of etb positive isolates (3.8%) is in accordance with previous reports in South-West Nigeria (3.1%) and in Cote d’Ivoire (3.6%).[26,27] Interestingly, all the etb positive isolates were from
age group of <10 years. Age was found to be associated with \textit{eta} positive \textit{S. aureus}, hence it could be considered as a risk factor.

\textit{PVL} gene had been reported to cause necrotizing pneumonia in infants and in immune-compromised individuals and its role in skin and soft tissue infections have been controversial.\cite{1}

The \textit{PVL} gene has also been used as an additional marker alongside SCCmec IV and V to classify MRSA into CA-MRSA, though not all CA-MRSA carry the \textit{PVL} genes. This study revealed that 53.3% and 9.1% of all MSSA and MRSA respectively were \textit{PVL} positive. This is in line with other recent reports on the high prevalence of \textit{PVL} positive MSSA in Nigeria.\cite{2-3} \textit{PVL}-positive MRSA is of serious concern, and only one report is available in the South-West Nigeria,\cite{4} and few in other regions in sub-Saharan Africa.\cite{5-6} Our result clearly showed an association between MSSA and \textit{PVL} gene ($\chi^2 = 32.93; P = 0.000001$). \textit{PVL} positive MRSA cannot happen by chance. Our observation about \textit{PVL} positive isolates is in agreement with the hypothesis that \textit{PVL} gene is an indigenous gene located on prophage of bacteria,\cite{7} which can be lost through the acquisition of \textit{mecA}. The major limitation is the inability to compartmentalize the virulence genes detected into chromosome or plasmid; future research will look into this.

CONCLUSION

The prevalence of \textit{S. aureus} with the following genes was ascertained: \textit{mecA} (42.3%) \textit{hla} (55.1%), \textit{icaA} (42.3%), \textit{PVL} (34.6%), \textit{fnbA} (8.3%), \textit{bbp} (4.5%), and \textit{eta} (3.8%). There was a significant difference in the distribution of virulence genes between in-patients and out-patients ($t$-test; $P = 0.00022$); and on the other hand, there was an association between virulence (\textit{eta} and \textit{icaA}) genes and \textit{mecA} positive isolates ($\chi^2 = 86.53; P = 0.000001$). All the \textit{eta} positive isolates in this study were from \textit{mecA} positive \textit{S. aureus} strains. The study also reaffirmed the prevalence of MRSA and the emergence of virulent MRSA strains in Nigeria. Therefore, there is the need for the establishment of more effective infection control measures in our hospitals in Nigeria. Finally, more research is needed in identifying molecular epidemiological marker that is less expensive and not labor intensive that can be used in low resource countries.

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Conflicts of interest

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REFERENCES


