Emergence of a Community-Associated Methicillin-Resistant Staphylococcus aureus Strain with a Unique Resistance Profile in Southwest Nigeria

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Phenotypic, genotypic, and toxin gene analyses have not yet been done all in one for the Nigerian Staphylococcus aureus population. This study provides a comprehensive overview of the molecular epidemiology and genetic diversity of S. aureus strains at the largest university clinic in Ibadan, Nigeria. From 1,300 patients’ clinical samples collected at the University Teaching Hospital in Ibadan, Nigeria, during a 1-year-surveillance in 2007, 346 nonduplicate S. aureus isolates were obtained. All isolates underwent antibiotic susceptibility testing, toxin gene analysis, multilocus sequence typing, agr group typing, and spa typing. For methicillin (meticillin)-resistant S. aureus (MRSA), staphylococcal cassette chromosome mec (SCCmec) typing was also performed. Of the 346 isolates, 20.23% were methicillin resistant. Thirty-three patients’ isolates (47.15%) fulfilled the definition criteria for community-associated MRSA (CA-MRSA) according to a review of the medical charts. The majority of MRSA strains analyzed were isolated from surgical or pediatric patients. The commonest types of MRSA infection identified were surgical-site infections (>70%), whereas those for CA-MRSA were conjunctivitis and otitis (19 patients [57.6%]) and accidental skin and subcutaneous tissue infections (14 patients [42.4%]). The methicillin-susceptible S. aureus strains (ST1, ST5, ST15, ST7, ST8, ST25, ST30, ST72, ST80, ST121, and ST508) were heterogeneous by phenotypic and genotypic analyses. The first report of a Panton-Valentine leukocidin-positive ST88 strain (agr III, SCCmec IV) in Nigeria, as well as genetic analyses of this strain, is presented in this study. The ST88 strain was resistant to trimethoprim-sulfamethoxazole as well as to penicillin and oxacillin. CA-MRSA infections are increasing rapidly among young patients with ophthalmologic and auricular infections. Urban regions with populations of lower socioeconomic status and evidence of overcrowding appear to be at high risk for the emergence of this clone.

Staphylococcus aureus is an important human pathogen and is implicated in a wide variety of infections (13, 19, 28). In Nigeria, S. aureus causes significant epidemiologic and therapeutic problems. Nigeria is the most densely populated African country, and Ibadan is the capital of the Southwest province Oyo, which has a population of 3.6 million and is the largest geographical area. Over the past 20 years, the incidences of community-associated (CA) and hospital-acquired (HA) S. aureus infections have increased, while antibiotic treatment is increasingly hampered by the spread of S. aureus strains that are resistant to multiple antibiotics, including methicillin (meticillin) (10, 11, 19, 32).

The African data on S. aureus, particularly on antibiotic susceptibility, are extremely limited (3, 27), although methicillin-resistant S. aureus (MRSA) has disseminated in African countries. Between 1996 and 1997, the prevalences of MRSA, determined in eight African countries, were relatively high in Nigeria, Kenya, and Cameroon (21 to 30%) and were below 10% in Tunisia and Algeria, although in Algeria this rate increased to 14% (16, 26). All MRSA isolates were sensitive to vancomycin.

The isolates were also highly sensitive to ciprofloxacin, except in Kenya, Morocco, and Tunisia, where relative resistance to this drug has been reported (16). Moreover, the results of 4 years of studies from a number of hospitals in Kenya have shown that 90% of patients admitted to burn units were colonized or infected with MRSA (20). The increasing prevalence of MRSA infections among nonhospitalized patients due to the emergence of unique community-associated S. aureus strains has become a Nigerian problem as well as a global problem. Because the genetic analysis of indigenous S. aureus strains is limited in Nigeria, we aimed to study the genetics, prevalence, and dissemination of such strains in Ibadan, where one of the largest university hospitals in Nigeria is located (2, 23).

The objectives of this study were (i) to determine the antibiotic susceptibility profiles, genotypes, and toxin profiles of methicillin-susceptible S. aureus (MSSA) and MRSA strains from two hospitals in Ibadan, Nigeria, (ii) to determine the prevalence of MRSA, and (iii) to characterize the genetic determinants of CA-MRSA strains upon hospital admission.

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MATERIALS AND METHODS

Study duration and population. From 2006 to 2007, out of 1,300 clinical samples, 346 nonduplicate S. aureus isolates identified in patients upon hospital
Antimicrobial susceptibility testing. Identification and testing of susceptibility (to penicillin, oxacillin, trimethoprim-sulfamethoxazole [SXT], tetracycline, erythromycin, clindamycin, ciprofloxacin, moxifloxacin, gentamicin, vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, fosfomycin, fusidic acid, nitrofurantoin, norfloxacin, levofloxacin, rifampin [rifampicin], and tobramycin) were performed by the automated Vitek 2 system (bioMérieux, Marcy l’Étoile, France). The results were interpreted in accordance with the current guidelines of the Clinical and Laboratory Standards Institute (4): breakpoints for oxacillin susceptibility were used, with MICs of 2 mg/liter indicating susceptibility and MICs of 4 mg/liter indicating resistance. Details have been described previously (7).

DNA extraction. Chromosomal DNA was isolated from overnight cultures grown on blood agar at 37°C. Genomic DNA was extracted by using the Qiagen DNA extraction kit according to the manufacturer’s suggestions (Qiagen, Hilden, Germany) with the modification that 20 μl of lysostaphin (1 mg/ml; Sigma) and 20 μl of lysosome (100 mg/ml; Qiagen) were added at the cell lysis step. The concentration of the DNA was assayed by a spectrophotometer (7).

agr group-specific multiplex PCR and detection of the Panton-Valentine leukocidin (PVL) and toxin genes. Extracted genomic DNA was used as a template to amplify specific agr alleles. For multiplex PCR, one primer set was prepared to amplify the four specific S. aureus agr alleles using the primers described by Lima et al. (18). Details have been given previously (7).

The sea–seg–h, eta, hlgA, hlgCB, lukE-lukD, lukS-PV, and lukF-PV genes were detected by PCR as described previously (7). The presence of specific staphylococcal virulence genes and detected sequences specific for staphylococcal enterotoxin genes (sea–seg–seg, seh, sea–sej, and seh) were confirmed by sequencing of their PCR products.

PCR for analysis of the SCCmec type. Staphylococcal cassette chromosome mec (SCCmec) types were determined by use of a multiplex PCR strategy that generated a specific amplification pattern for each SCCmec structural type, according to the method described by Oliveira and de Lencastre (25). The SCCmec type was analyzed according to previously described procedures (25).

spa gene typing. The polymorphic X region of the protein A gene (spa) was amplified from all S. aureus isolates as described previously by Harmsen et al. (12): the sequencing reactions were carried out with an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA). The spa type was assigned by using Ridom StaphType software (version 1.4; Ridom GmbH, Würzburg, Germany).

MLST. Multilocus sequence typing (MLST) was performed according to previously published protocols. (5). Briefly, standard DNA amplification and sequencing of the seven housekeeping genes (arcC, arne, glpF, gmk,pta,spi, and vqI) were performed on all S. aureus isolates. Nucleotide sequences were determined for both strands by using published primers and were compared to existing sequences in the MLST database (http://www.mlst.net) for the assignment of allelic numbers. The isolates were assigned sequence type (ST) numbers according to their allelic profiles. Clonal complexes (CC) were defined as isolates that were identical at five or more alleles. Phylogenetic relationships among the MSSA and MRSA strains were then assessed by cluster analysis, the unweighted-pair method using average linkages, and application of the minimal spanning tree (MST) algorithm of the BioNumerics software to the MLST sequence data. The sequences for the variable sites from the seven gene fragments were concatenated into a single sequence.

RESULTS

Antibiotic resistances of S. aureus isolates. A total of 346 S. aureus isolates were investigated during the study period; of these, 70 were methicillin resistant, giving an MRSA prevalence rate of 20.23% among the entire subject population. The youngest patient with MRSA in the study population was 2 years old, while the oldest was 57 years old. The age range for patients with HA-MRSA was 2 to 57 years, whereas the age range for CA-MRSA patients was 6 to 20 years. All S. aureus isolates studied were uniformly sensitive to vancomycin, teicoplanin, and fusidic acid. We found different resistance phenotypes (Table 1) by using a panel of antibiotics (penicillin, oxacillin, SXT, tetracycline, erythromycin, clindamycin, gentamicin, vancomycin, teicoplanin, linezolid, moxifloxacin, ciprofloxacin, and rifampin).

The overall patterns of susceptibility of the CA-MRSA strains to antibiotics were as follows: 3% were susceptible to SXT, and 100% were susceptible to ciprofloxacin, erythromycin, fusidic acid, gentamicin, rifampin, tetracycline, linezolid, teicoplanin, and vancomycin. The higher prevalence of resistance to SXT in this environment could be due to widespread, indiscriminate use of these antibiotics.

MLST and relatedness of the clonal clusters. Among the 14 STs identified, ST88 (n = 32), ST241 (n = 7), and ST250 (n = 30) were representative of MRSA strains. An MST was constructed by applying BioNumerics software. Figure 1 depicts the clustering of the STs detected in Nigeria together with representatives of international S. aureus clones, indicating the relatedness between MSSA strains in this study and pandemic MRSA clones (Fig. 1). MSSA ST8, MRSA or MSSA ST250, and MRSA ST241 strains were closely related and formed the clonal cluster CC8.

Clinical significance of MSSA and MRSA strains. Among the MRSA strains, clone ST88-MRSA-IV—a community-associated clone—was detected in clinical samples from outpatients at the University Hospital (n = 26) and the Oyeyewo Hospital (n = 7) in Ibadan with orthopaedic and otic infections and no health care history in the previous 1.5 years. These CA-MRSA ST88 isolates were detected within 48 h of admission to the hospital for patients who had not had a permanent indwelling catheter or percutaneous medical device (e.g., a tracheostomy tube or another catheter) within 1 year before MRSA detection. Additional criteria were no known positive culture for MRSA and no history of hospitalization, surgery, dialysis, or residence in a long-term care facility. The patients with ST88 isolates presented predominantly with conjunctivitis, catarracts, otitis, pyomyositis, and posttraumatic wound infections, whereas MRSA ST241 strains were common among hospitalized burn patients. MRSA ST250 and MSSA ST508 isolates were detected in samples from diabetic patients and those with urinary tract infections. MSSA ST88 isolates were mostly associated with cellulitis and posttraumatic infections, whereas MSSA ST30 isolates were obtained from patients with different diagnoses: traumatic head injury, osteomyelitis, cata- ract, posttraumatic wound infections, and accidental skin infections. MSSA ST5, ST7, and ST8 strains were isolated from patients with diabetic foot problems, urinary tract infections, gunshot injuries, multiple fractures, or posttraumatic wounds and from polytraumatized patients.

Toxin gene detection and agr groups. None of the S. aureus isolates were positive for the sea gene, whereas 32 out of 276 MSSA isolates were sea positive. Forty-four of 276 MSSA isolates (ST1, ST121, ST15, ST25, ST30, and ST5) were positive for seb, as were 6 MRSA
isolates (ST250). All the MSSA isolates that were typed as ST508 (n/H11005 9) and a few others (ST5 n/H11005 2, ST5 n/H11005 2, and ST7 n/H11005 2) were positive for sec, whereas none of the MRSA isolates expressed this toxin gene. MSSA isolates from skin lesions or from eye or wound infections were more likely to produce toxins (81% or 68%, respectively) than nasal isolates (11%). Strains belonging to agr group III were preferentially positive for PVL expression, especially the CA-MRSA ST88 (n/H11005 33) and MSSA ST30 (n/H11005 30) isolates. MSSA isolates typed as ST1 (22 out of 22 isolates), ST121 (21 out of 38 isolates), and ST80 (8 out of 8 isolates) were also PVL positive. Forty-two (52.85%) of the 70 MRSA isolates were HA. The 33 MRSA ST88 isolates (47.15%) were acquired in the community (see Table 1 for details on toxin gene expression), and the patients with those isolates fulfilled the criteria for CA-MRSA according to a review of the medical charts.

TABLE 1. Antibiotic resistance patterns and genotypic analysis (MLST, spa types) of Staphylococcus aureus isolates in Ibadan, Nigeria, 2006 to 2007

<table>
<thead>
<tr>
<th>ST (no. of isolates)</th>
<th>agr type(s) (no. of isolates)</th>
<th>spa type(s)</th>
<th>Antibiotic resistance pattern; agr type (no. of isolates)</th>
<th>Toxin genes</th>
</tr>
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</table>
| ST5 (76)             | I (39), II (37)               | t311        | PEN, SXT, TET; agr I and II (37)                         | sea, seb, sec, seg, tst, lukED, luk-PV, 
|                      |                               |             | PEN, SXT, TET; agr I (16)                                | hlgA        |
| ST7 (45)             | I (33), II (12)               | t091        | PEN, SXT, TET; agr I (18)                                | sea, seg, lukED, luk-PV, hlgA |
| ST121 (38)           | IV                             | t159, t314  | PEN, SXT (12)                                          | seb, seg, lukED, luk-PV, hlgA |
| ST250b (35)          | (I, III)                       | t194, t292  | PEN, OXA, TET, CIP; agr I (23)                           | seb, tst, lukED, hlgA |
| ST88 (33)            | III (33)                      | t186        | PEN, OXA; agr IV (5)                                    | seg, luk-PV, hlgA, hlgB |
| ST30 (30)            | III                            | t318        | PEN, SXT (22)                                          | seg, tst, lukED, luk-PV, hlgA, hlgB |
| ST8 (25)             | I                               | t064, t068  | PEN, SXT, TET, CIP, GEN (12)                            | sea, seb, seg, tst, lukED, luk-PV, hlgA |
| ST1 (22)             | III                            | t273        | PEN (20)                                               | tst, luk-PV, hlgA |
| ST15 (10)            | II                             | t084, t085  | PEN, SXT, TET (8)                                      | lukED, hlgA |
| ST508 (9)            | I                              | NTc         | Sensitive (9)                                          | seb, sec, tst, hlgA, hlgB |
| ST80 (8)             | III                            | t359        | PEN, SXT, TET (8)                                      | tst, lukED, luk-PV, hlgA |
| ST241 (7)            | I                              | t037        | PEN, OXA, TET, CIP, GEN, ERY, CLI (7)                   | lukED, hlgA |
| ST25 (5)             | I                              | t355        | PEN, SXT (5)                                           | sec, seg, lukED |
| ST72 (3)             | I                              | t357        | PEN, TET (3)                                           | seg, lukED, hlgA |

*The major frequent antibiotic resistance patterns are given for each sequence type (PEN, penicillin; OXA, oxacillin; TET, tetracycline; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; CLI, clindamycin).

b ST250 represents both MRSA and MSSA isolates.

c NT, not typeable.

d Detection of this gene in this strain was rare.

FIG. 1. The MST based on MLST indicates the estimated relationships of the 14 STs, including representatives of the clonal complexes CC1, CC5, CC7, CC8, CC30, CC88, based on sequence alignments and analysis of the ST allelic profiles. ST88, ST241, and ST250 represent MRSA strains (although some MSSA strains also belong to ST250). Each ST is represented by a circle whose size is proportional to the number of isolates belonging to the ST. Filled circles, MRSA; shaded circles, MSSA. The relationships between the strains are indicated by the connections between the isolates and the lengths of the branches linking them.
MRSA strains analyzed were isolated from surgical and pediatric patients. The commonest types of infections for which MRSA isolates were identified during the period of study were surgical-site infections (70%), whereas for CA-MRSA isolates, the commonest infections were conjunctivitis and otitis (for 19 patients [57.6%]) and accidental skin and subcutaneous tissue infections (for 14 patients [42.4%]). Expression of the \(\text{tst} \) gene was detected in 8 of the 70 MRSA isolates (5 ST250 isolates, 2 ST88 isolates, and 1 ST8 isolate) and in 54 of the 276 MSSA isolates (including all the ST1 and ST308 isolates, 4 ST30 isolates, and 8 ST5 isolates).

**SCCmec typing of MRSA isolates.** Sequence types MRSA ST88 (n = 33) and MRSA ST241 (n = 7) were grouped in SCCmec type IV, whereas 30 MRSA ST250 isolates were typed as SCCmec type I and designated HA-MRSA.

**DISCUSSION**

Knowledge of epidemiology of bacterial infections is very important for appropriate decision-making in the treatment of infections, such as septicemia, wound infections, and post-surgical infections. Iroha et al. (15) investigated conjunctivitis cases among neonates in Lagos, Nigeria, in a prospective study. The incidence of conjunctivitis in newborns was 18 per 1,000 live births. \(S. aureus\) (37.4%) was the predominant etiologic pathogen among the bacteria (others were coagulase-negative staphylococci [12.3%], \(Klebsiella pneumonieae\) [12.9%], and \(Pseudomonas aeruginosa\) [8.2%]). The incidence of \(S. aureus\)-caused eye infection is consistent with the findings of our study, in particular for outpatients with CA-MRSA-positive conjunctivitis. This might be an indication that antibiotics, especially broad-spectrum antibiotics, are now used extensively in the community as well as in hospital settings. \(S. aureus\) (61.2%) was the dominant cause of septicemia and mortality among neonates according to Udo et al. (30). Another study investigated the bacteriology of nonsurgical wound infections in Ibadan. \(S. aureus\) (38%) was the predominant pathogen, followed by gram-negative bacteria (7 to 19% each). High rates of antibiotic resistance were recorded among these isolates (24). Thanni et al. (29) determined the prevalence of bacterial pathogens in wounds for patients from various units of the orthopedics and traumatology department of a Nigerian tertiary hospital. In this retrospective study from 1995 to 2001, \(Pseudomonas aeruginosa\) was the most common pathogen among the inpatients, whereas \(S. aureus\) was more common among outpatients. The rate of isolation of gram-positive bacteria in general decreased, while that of \(S. aureus\) in particular increased, as stated by Thanni et al. (29).

The prevalence of MRSA in our study was moderate (20.23%) compared to those in previous studies in Southwestern Nigeria, which ranged from 1.4% to 50% (1–3, 16, 23, 27). However, it should be considered that the presence of the \(mecA\) gene, which is the “gold standard” for determining methicillin resistance, was not investigated in some of these previous studies. A recent multicenter study in Southwestern Nigeria confirmed resistance to methicillin by the detection of the \(mecA\) gene by PCR and reported a lower prevalence rate of 1.4% (1). Despite the low MRSA rate in our study, it is evident that multiresistant MSSA strains occurred frequently in Southwestern Nigeria (Table 1). However, the MRSA isolates were predominantly associated with infections (87%), as observed elsewhere (27). Nevertheless, the prevalence of CA-MRSA (47%) was higher in our study than that (29%) in the study of Taiwo et al. (27) The prevalence of MRSA among the \(S. aureus\) isolates at the University College Hospital, Ibadan, Nigeria, in 1999 was generally set at 27%, which was higher than the 1972 prevalence of 1%. Forty-one percent of the MRSA isolates were from inpatients, while 59% were from outpatients. The high incidence of MRSA among outpatients was unusual at that time (23). A survey of MRSA strains at a teaching hospital in Ilorin, Nigeria, suggested a similar MRSA prevalence rate of 34.7% (27). Many African countries have a strong tradition of herbal or traditional medicine (21). The low cost, high acceptability, and ease of access to such traditional “therapies” make them the most common form of African alternative medicine. Therefore, antibiotics do not play a major role in the community, and hence the level of resistance to oxacillin or methicillin is rather low. In the hospital environment, the acquisition of the SCCmec (in its various forms) by multiresistant MSSA strains could make infection control measures extremely difficult and could have serious consequences. The levels of resistance to sulfonamides and tetracycline were remarkable among MRSA clones; sulfonamides have been recommended and administered to treat MRSA infections in Nigeria (27). Hence, these antibacterial agents should no longer be considered first-line drugs for the treatment of MRSA infections in Nigeria.

**MLST of \(S. aureus\) strains in Nigeria** indicated that certain major MSSA clones are extremely successful in Nigeria (1). These include ST25, ST30, and ST120/ST121 clones, which have been recognized as internationally well disseminated clones (6), along with the MSSA ST8 clone, which appeared to possess some epidemic potential and had acquired the \(mecA\) gene (1). These STs were also detected in our study. MSSA isolates are often more genetically variable than MRSA isolates and have commonly been the subject more of general surveillance studies than of molecular typing studies. Goering et al. (8) reported ST121 as the most common PVL-positive MSSA clone (pulsed-field type USA1200), which was found primarily in South Africa and the Russian Federation. In our study, the ST121 clone was also prevalent and was positive for the \(luk-PV\) gene. Another MSSA clone in our study belonging to CC1 was ST1, which was detected predominantly in PVL-negative isolates originating in India, South Africa, the United States, and Germany, with the difference that the Nigerian clone expressed the \(luk-PV\) and \(tst\) genes. MSSA ST5 (CC5) and ST30 (CC30) clones were identified in South Africa, the United States, and Germany as well, according to Goering et al. (8). In comparison to those of our study isolates, Adesida et al. (2) identified different lineages for their MSSA isolates from Lagos, Nigeria. For their 17 MSSA isolates, spa typing revealed nine different types, among which spa types 1007 and 1454 were predominant. Considering the global and dynamic nature of MRSA in HA and CA infections, continued surveillance is important for a clearer understanding of the epidemiology of these organisms.

To date, most CA-MRSA SCCmec IV isolates have had MLST allelic profiles that are not found in studies of HA-MRSA isolates. However, CA-MRSA has the potential to move into the hospital setting and cause outbreaks. This study
reiterates that the detection of CA-MRSA SS
cme type IV was associated with PVL production.

In contrast to MRSA strains in Algeria (26), our MRSA isolates were not resistant to gentamicin. In the Algerian study, the treatment options for multiple-antibiotic-resistant MRSA strains included cotrimoxazole (SXT) for minor infections and glycopeptides for severe infections. In contrast, SXT is no longer recommended for Nigerian MRSA strains in Ibadan, because the rate of resistance to SXT is approximately 53%. Nearly all the MRSA ST88 and MRSA ST241 isolates, but none of the MRSA ST250 isolates, were SXT resistant. Grim et al. (9) stated that clonal outbreaks of MRSA resistant to SXT have been reported; of these, the Brazilian clone has more often been resistant to SXT than the Iberian clone. Rates of SXT resistance were particularly high in institutions serving large numbers of patients infected with human immunodeficiency virus, due to increased exposure to *Pneumocystis jirovecii* prophylaxis.

Vandenesch et al. (31) described continent-specific PVL-positive CA-MRSA clones—mainly on an *agr* group III background—and characterized them by their STs. The main European CA-MRSA clone, ST88, was detected in France, Switzerland, The Netherlands, England, Belgium, and Germany, but also in northern Europe (e.g., Denmark), where MRSA strains are rare in hospitals. MRSA clone ST80 is usually resistant to tetracycline (resistance mediated by the *tetK* gene), intermediate to fusidic acid (the *far1* gene), and resistant to kanamycin (the *aph3*-III gene). In Nigeria, we observed the prevalence of MSSA ST80 isolates that were tetracycline resistant as well but susceptible to fusidic acid. One of the most prevalent PVL-positive CA-MRSA clones in the United States (USA300) belongs to ST8; other U.S. clones include USA400 (ST1), USA1000 (ST59), and USA1100 (ST30). ST30 is also a major clone in Asia and Oceania and is referred to as the South West Pacific MRSA clone. This ST was prevalent in Nigeria as an MSSA strain. In Singapore, an international travel hub, several clones belonging to ST80, ST30, and ST59 have been reported. The prevalence of PVL-positive CA-MRSA differs considerably from continent to continent. In the United States, MRSA strains were isolated from 50% of patients with skin and soft-tissue infections seen in the emergency departments of 11 cities (97% of the isolates belonged to clone USA300). In Europe, the prevalence of CA-MRSA is lower, at ~1 to 3%, and in Africa the prevalence of CA-MRSA needs more investigational study.

In our study, the MRSA strains were less “toxigenic” than the MSSA strains (ST5, ST7, and ST30), which were more frequently positive for PVL and *tst*. In contrast to our findings, a Japanese study found that both MRSA and MSSA isolates carried a number of superantigenic toxin genes but that the MRSA isolates harbored more superantigenic toxin genes than the MSSA isolates (14). Hu et al. (14) compared the prevalences of superantigenic toxin genes in MRSA and MSSA isolates and concluded that some of their MRSA isolates were *sec*, *seg*, and *tst* positive. In our study, none of the MRSA isolates carried these genes together, and only one MSSA isolate, of ST508, was positive for *sec*, *seg*, and *tst* together. This notably higher prevalence among Japanese MRSA isolates indicated that possession of the *sec* and *tst* genes in particular appeared to be a habitual feature of Japanese MRSA strains (14), in contrast to Nigerian MRSA strains.

This study provides a comprehensive overview of the molecular epidemiology and genetic diversity of the *S. aureus* population at the largest university clinic in Nigeria. First, it shows a high prevalence of PVL-positive MSSA isolates. Second, it demonstrates high heterogeneity of the MSSA strains, with broader resistance profiles than those of the MRSA strains (ST250 and ST88), which are homogenous. ST88 was resistant to SXT in addition to penicillin and oxacillin, whereas ST250, as HA-MRSA, was additionally resistant to tetracycline, ciprofloxacin, and gentamicin (Table 1). SXT and tetracycline are listed among antibacterial agents that have been rendered ineffective or for which there are serious concerns regarding bacterial resistance in Nigeria (22). Therefore, the formulation and implementation of national drug policies by governments are fundamental to ensure rational drug use. Control of CA *S. aureus* will remain a challenge for some regions in Nigeria, since transmission is linked to migration and tourism.

Two types of SS*Mc* were found in CC8, for the ST241-MRSA-IV strain and the ST250-MRSA-I strain (Fig. 1). MSSA ST250 is most probably the putative ancestor of MRSA ST250 by the insertion of SS*Mc*. MSSA ST250 itself might have derived from a putative MSSA ST8 ancestor, common to all strains belonging to CC8 (14).

In the present study, we showed that in most of the clinical departments, the less toxigenic MRSA strains circulate together with the more toxigenic MSSA strains, e.g., ST30 and ST1 strains. This might benefit toxin gene transfer, which could occur among MSSA and MRSA strains (17).

In conclusion, the application of the different typing methods to our Nigerian strains provided important information on their clonal relationships and might also strengthen our understanding of the *S. aureus* population circulating in Nigeria. Moreover, this study summarizes comprehensive epidemiologic data and uses genetic markers to investigate outbreaks in health care settings.

To the best of our knowledge, this is the first report of the detection and genetic characterization of an ST88 clone in Nigeria. An ST88 clone was also isolated in Asia (11), with the significant difference that the Nigerian clone expresses PVL and is sensitive to tetracycline and fusidic acid, in contrast to European CA-MRSA clones (32). Therefore, epidemiological studies of the clonal relationships of MRSA strains in Nigeria with worldwide clones would be useful and important for understanding the global dissemination of such clones.

The limitations of our study include the limited number of patients. Thus, the prevalence of MRSA in general and CA-MRSA in particular might be underestimated. Future prospective studies may further elucidate possible epidemiologic risk factors associated with the acquisition of CA-MRSA infections.

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