Originally published as:

Ghebremedhin, B., Olugbosi, M.O., Raji, A.M., Layer, F., Bakare, R.A., König, B., König, W.
Emergence of a community-associated methicillin-resistant Staphylococcus aureus strain with a unique resistance profile in Southwest Nigeria
(2009) Journal of Clinical Microbiology, 47 (9), pp. 2975-2980.

DOI: 10.1128/JCM.00648-09

Emergence of a community-associated methicillin-resistant *Staphylococcus aureus* with unique resistance profile in Southwest of Nigeria

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J. Clin. Microbiol. doi:10.1128/JCM.00648-09
JCM Accepts, published online ahead of print on 1 July 2009

Abstract

Pheno-, genotypic and toxin gene analysis has not been done yet all in one for Nigerian *Staphylococcus aureus* population. This study provides a comprehensive overview on the molecular epidemiology and genetic diversity of *S. aureus* at the largest university clinic in Ibadan, Nigeria**.** At the University Teaching Hospital in Ibadan, Nigeria, out of 1300 patients' clinical samples 346 non-duplicate *S. aureus* isolates were obtained during the 1-year surveillance in 2007. All isolates underwent antibiotic susceptibility testing, toxin gene analysis, MLST (multilocus sequence typing), *agr* group and *spa* typing. And for MRSA SCC*mec* typing was performed. 20.23% of the 346 isolates were methicillin-resistant. 33 patients' isolates (47.15%) fulfilled the definition criteria for CA-MRSA according to the review of the medical charts. The majority of MRSA strains analyzed were isolated from surgical and paediatric patients. The commonest types of infection identified with MRSA were surgical site infections (>70%) whereas for CA-MRSA were conjunctivitis and otitis in 19 patients (57.6%) and accidental skin and subcutaneous tissue infection in 14 patients (42.4 %). The MSSA strains (ST1, ST5, ST15, ST7, ST8, ST25, ST30, ST72, ST80, ST121 and ST508) were heterogeneous by pheno- and genotypic analysis. The first report and genetic analyses of PVL-positive ST88 strain (*agr* III, SCC*mec* IV) in Nigeria is shown in this study. ST88 was resistant to trimethoprim-sulfamethoxazole besides to penicillin and oxacillin. CA-MRSA infections are rapidly increasing among young patients with ophthalmologic and auricular infections. Urban regions of lower socioeconomic status and evidence of overcrowding appear to be at higher risk for the emergence of this clone.

Introduction

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

The African data on *S. aureus*, particularly antibiotic susceptibilities, are extremely limited (3, 27), although methicillin-resistant *S. aureus* (MRSA) has disseminated in African countries as well. Between 1996 and 1997 the prevalence of MRSA was determined in eight African countries

and was relatively high in Nigeria, Kenya, and Cameroon (21 to 30%) and below 10% in Tunisia and Algeria, although in Algeria this rate creased 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 described (16). Moreover, the results of four years' studies from a number of hospitals in Kenya have shown that 90% of patients admitted in burn units were colonized or infected with MRSA (20). The increasing prevalence of MRSA infections in non-hospitalized patients due to the emergence of unique community-associated *S. aureus* strains became also a Nigerian problem as it is global. Due to the fact that 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, one of the biggest university hospitals in Nigeria (2,23).

The objectives of this study were (1) to determine antibiotic susceptibility profiles, genotypes and toxin profiles of methicillin-susceptible *S. aureus* and MRSA from two hospitals in Ibadan, Nigeria; (2) to determine the prevalence and (3) to characterize the genetic determinants of CA MRSA strains on hospital admission.

Materials and Methods

Study duration and population

From 2006 to 2007, out of 1300 clinical samples 346 non-duplicate *S. aureus* isolates identified in patients on hospital admission were obtained from two hospitals in Southwestern Nigeria, Oluyewo Hospital and University Teaching Hospital, both in Ibadan.

Among the 1300 patients 65% (845) were adults at the departments of surgical units, 20% (260) at the department of paediatrics and neonatology, and the remaining 195 patients were at the medical department. Most of the isolates (90%) were obtained from the University Teaching Hospital and the remaining number of isolates was from the Oluyewo Hospital, both in Ibadan. More than 70% of the total number of isolates was recovered from wound samples, 72 (21%) from corneal, conjunctival and auricular swabs, 15 (4.3%) from genital swabs, and 16 (4.6%) from nasal swabs.

Antimicrobial susceptibility testing

The identification and susceptibility testing (penicillin, oxacillin, trimethoprim/sulfamethoxazole, tetracycline, erythromycin, clindamycin, ciprofloxacin, moxifloxacin, gentamicin, vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin, fosfomycin, fusidic acid, nitrofurantoin, orfloxacin, levofloxacin, rifampicin, tobramycin) were performed by the automated VITEK 2® system (*bio*Mérieux, Marcy-l'Etoile, France). The results were interpreted in accordance to the current Clinical Laboratory Standards Institute (4) guidelines: breakpoints for oxacillin susceptibility were used: MICs of 2 mg/l indicated susceptibility and MICs of 4 mg/l indicated resistance. Details have been previously described (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 manufacturers suggestions (Qiagen, Hilden, Germany) with the modification that 20µl of lysostaphin (Sigma; 1mg/ml) and 20µl lysozyme (Qiagen; 100mg/ml) were added at the cell lysis step. The concentration of the DNA was assessed by a spectrophotometer (7).

agr group-specific multiplex PCR, PVL gene and toxin gene detection

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 as described by Lina *et al.* (18). Details were given previously (7).

Genes for *sea-e*, *seg-h, tsst-1*, *eta*, *etb, hlgA, hlgCB*, *lukE-lukD, lukS-lukF-PV*, were detected by PCR as described previously (7). We determined the presence of specific staphylococcal virulence genes and detected sequences specific for staphylococcal enterotoxin genes (*sea-e, seg, seh, sei, sej*), as
well as the toxic-shock syndrome toxin gene (*tst*), PVL genes (*luk*S-PV–*luk*F-PV), LukE–lukD leukocidin
genes (*luk*E–*luk*D), and hemolysin genes (*hlg*). The positivity of the genes *pvl* and *tst* was confirmed by sequencing of their PCR products.

PCR for analysis of SCCmec type

SCC*mec* types were determined by use of a multiplex PCR strategy that generated a specific amplification pattern for each SCC*mec* structural type, according to the method described by Oliveira and de Lencastre. The analysis of the SCC*mec* type was performed 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). All 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)..

Multilocus-Sequence-Typing (MLST)

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

Results

Antibiotic resistance of S. aureus isolates

A total of 346 *S. aureus* isolates were investigated during the study period, out of which 70 were methicillin-resistant, giving an MRSA prevalence rate of 20.23% among the entire subject population. The youngest patient with MRSA was 2 years old while the oldest was 57 years in the study population. The age range for patients with HA-MRSA is 2-57 years whereas the age range in the CA-MRSA was 6-20 years. All studied *S. aureus* isolates were uniformly sensitive to vancomycin, teicoplanin, and fusidic acid. We found different resistance phenotypes (Table1) using a panel of antibiotics (penicillin [PEN], oxacillin [OXA], trimethoprim/sulfamethoxazole [SXT], tetracyline [TET], erythromycin [ERY], clindamycin [CLI], gentamycin [GEN], vancomycin [VAN], teicoplanin [TEI], linezolid [LIN], fusidic acid [FUS], ciprofloxacin [CIP], rifampicin [RIF]).

The overall susceptibility patterns of the CA-MRSA to the following antibiotics were as follows: 9.5% for SXT, 66.7% for TET, GEN, CIP and ERY, and 100% for FUS, LIN, RIF, TEI and VAN. The higher prevalence of resistance to trimethoprim-sulfamethoxazole in this environment

could be due to widespread, indiscriminate use of these antibiotics.

MLST and relatedness of the clonal clusters

Among the 14 identified STs ST88 (*n*=32), ST241 (*n*=7) and ST250 (*n*=30) were representatives for MRSA strains. A minimum spanning tree was constructed by applying the software Bionumerics. Figure 1 depicts the clustering of the STs detected in Nigeria together with representatives of the international *S. aureus* clones which indicate the relatedness between MSSA in this study and MRSA pandemic clones (Fig. 1). The sequence types ST8-MSSA, ST250-MRSA/MSSA and ST241-MRSA strains were closely related and formed the clonal cluster CC8.

MSSA and MRSA strains and clinical significance

Among the MRSA strains ST88-MRSA-IV – as community-associated clone - was detected in outpatients from the University Hospital (*n*=26) and Oluyewo Hospital (*n*=7) in Ibadan with respective diseases and no healthcare history in previous 1.5 years. These were detected within within 48 hours of admission to the hospital without permanent indwelling catheter and percutaneous medical device (e.g. tracheostomy tube and other catheter) within one year of MRSA detection. Additional criteria were no known positive culture for MRSA and no history of hospitalization, surgery, dialysis, or long-term care facility. The patients were presented predominantly conjunctivitis, cataract, otitis, pyomyositis and posttraumatic wound infections, whereas ST241-MRSA strains were common in hospitalized burn patients. ST250-MRSA and ST508-MSSA isolates were detected in diabetic patients and those with urinary tract infection (UTI). ST88-MSSA was mostly associated with cellulitis and postsurgical infections whereas ST30-MSSA isolates were obtained from different diagnosed patients with traumatic head injury, osteomyelitis, cataract, postsurgical wound infections and accidental skin infections. ST5-, ST7- and ST8-MSSA strains were isolated from patients with diabetic foot, UTI, gun-shot injury, multiple fractures, postsurgical wounds and polytraumatized patients.

Toxin gene detection and agr groups

None of the *S. aureus* isolates were positive for the toxin genes *sed*, *see*, and *seh*. Furthermore, none of the MRSA isolates were positive for *sea* gene whereas 32 out of 276 MSSA isolates were *sea* positive. 44 out 276 MSSA isolates (ST1, ST121, ST15, ST25, ST30, ST5) were positive for *seb* and 6 MRSA isolates (ST250) were positive for *seb*. All the MSSA isolates which were typed as ST508 (*n*=9) and few others (ST5 (*n*=2), ST5 (*n*=2), ST7 (*n*=2)) were positive for *sec* whereas none of the MRSA isolates expressed this toxin gene. MSSA isolates from skin lesions or eye and wound infections were more likely to produce toxins (81% and 68% respectively) than nasal isolates (11%). Those belonging to *agr* III were preferentially positive for *pvl* expression, especially the CA-MRSA ST88 (*n*=33), besides the MSSA ST30 (*n*=30). PVL positive were also the MSSA isolates typed as ST1 (*n*=22 out of 22), ST121 (*n*=21 out of 38), and ST80 (*n*= 8 out of 8). Forty-two (52.85%) of the 70 MRSA isolates were hospital-acquired (HA). And the 33 patients' isolates (47.15%) were acquired in the community (CA) (see Table 1 for details on toxoin gene expression) and these patients fulfilled the definition of having CA-MRSA according to the review of the medical charts. The majority of MRSA strains analyzed were isolated from surgical and paediatric patients. The commonest types of infection identified with MRSA isolates during the period of study were surgical site infections (>70%) whereas for CA-MRSA were conjunctivitis and otitis in 19 patients (57.6%) and accidental skin and subcutaneous tissue infection in 14 patients (42.4 %). The expression of the *tst* gene was detected in 8 of the 70 MRSA isolates (five ST250, two ST88 and one ST8) and in 54 of the 276 MSSA isolates (all ST1 and ST508, four ST30 and eight ST5).

SCC*mec* typing of MRSA isolates

The sequence types MRSA-ST88 (*n*=33) and MRSA-ST241 (*n*=7) were grouped in SCC*mec* type

IV whereas MRSA-ST250 (*n*=30) was typed as SCC*mec* type I attributing to hospital-associated MRSA.

Discussion

Knowledge of epidemiology of bacterial infections is very important for appropriate decision making in the treatment of infections, such as septicaemia, wound and postsurgical infections. Iroha *et al*. (15) investigated in Lagos conjunctivitis cases of neonates in a prospective study. The incidence of conjuctivitis in the newborn was 18 per 1000 live births. *S. aureus* (37.4%) was the most predominant aetiologic pathogens among the other bacteria (coagulase negative staphylococci (12.3%), *Klebsiella pneumoniae* (12.9%) and *Pseudomonas aeruginosa* (8.2%). The incidence of S. aureus-caused eye infection is consistent with our study, in particular in out patients with CA-MRSA-positive conjunctivitis. This might be an indication that antibiotics are now more extensively used in the community besides hospital-settings, especially broad spectrum antibiotics. *S. aureus* (61.2%) was the dominant cause of septicaemia and mortality in neonates according to Udo *et al*. (30). Another study investigated the bacteriology of non surgical wound infections in Ibadan. *S. aureus* (38%) was the predominant pathogen followed by the Gram-negative bacteria (each 7-19%). High rates of antibiotic resistance were recorded among these isolates (24). Thanni *et al*. (29) determined the prevalence of bacterial pathogens in wounds from various units of a Nigerian tertiary hospital orthopedics and traumatology department. In this retrospective study from 1995 to 2001 *Pseudomonas aeroginosa* 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 MRSA prevalence in our study was moderate (20.23%) as compared to previous studies in Southwestern Nigeria, which ranged from 1.4% to 50% (1-3, 16, 23, 27). However, it should be considered that the detection 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 multi-resistant MSSA occurred frequently in Southwestern Nigeria (Table 1). However, the MRSA isolates were predominantly associated with infections (87%) as elsewhere observed (27). Nevertheless, the prevalence of community associated MRSA (47%) was higher in our study as compared to that (29%) 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 1972 value of 1%. Forty-one percent of the MRSA isolates were from inpatients while 59% were from outpatients. The high incidence of MRSA in outpatients was unusual at that time (23). A survey of MRSA at a teaching hospital in Ilorin, Nigeria suggested a similar MRSA prevalence rate at 34.7% (27). Many African countries have an extraordinary tradition of herbal or traditional medicine (21). Thus, the low cost and high acceptance and ease of access to such traditional ‘therapies’ make them the most common form of African alternative medicine. Therefore, the antibiotics in the community do not play the major role and hence the resistance problem is rather low for oxacillin or methicillin. In the hospital environment, the acquisition of the SCC*mec* (in its various forms) by multi-resistant MSSA could make infection control measures extremely difficult and could have serious consequences. The resistances to sulfonamides and tetracycline were remarkable in MRSA clones and sulfonamides were recommended and administered to treat MRSA infections in Nigeria (27). Hence, these antibacterial agents should not be considered anymore as first-line drugs for treatment of MRSA infections in Nigeria.

MLST of *S. aureus* strains in Nigeria indicated that certain major clones of MSSA are extremely successful in Nigeria (1). They include ST25, ST30, and ST120/121 which have been recognized as internationally well-disseminated clones (6) along with the ST8 MSSA clone, which appeared to possess some epidemic potentials and had acquired the *mecA* gene (1). These STs were also detected in our study. MSSA isolates are often more genetically variable and have commonly been the subject of more general surveillance studies, but relatively few studies with molecular typing. 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 ST121 clone was also prevalent and was positive *luk-PV* gene. Another

MSSA clone in our study belonging to the clonal complex CC1 was ST1 which was detected in predominantly PVL-negative isolates originating in India, South Africa, the United States, and Germany, with the difference that the Nigerian clone expressed *luk-PV* and *tst* genes. The MSSA clone ST5 (CC5) and ST30 (CC30) were identified South Africa, the United States, and Germany as well according to Goering *et al*. (8). In comparison to our study isolates Adesida *et al*. (2) identified in their MSSA isolates from Lagos different lineages. Among their 17 MSSA isolates the *spa* typing revealed nine different types with predominance of *spa* t007 and t454. Considering the global and dynamic nature of MRSA in HA and CA infections, continued surveillance is important for clearer understanding of the epidemiology of these organisms.
To date, most SCC*mec* IV CA-MRSA isolates have had MLST allelic profiles that are not found in studies of HA-MRSA. However, CA-MRSA has the potential to move into the hospital setting and cause outbreaks. This study reiterates that the detection of SSC*mec* type IV CA-MRSA was associated with the PVL production.

In contrast to the MRSA strains in Algeria (26) our MRSA isolates were not resistant to gentamicin. In the Algerian study the treatment options in the case of multiple-antibiotic-resistant MRSA strains included cotrimoxazole (SXT) for minor infections and glycopeptides for severe infections whereas SXT is not recommended for the Nigerian MRSA strains in Ibadan anymore as the resistance rate to SXT is approx. 53%, nearly all ST88-MRSA isolates and ST241-MRSA isolates, but none of the ST250-MRSA isolates were SXT resistant. Grim *et al.* (9) stated that clonal outbreaks of MRSA resistant to SXT have been described; 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 by the human immunodeficiency virus, due to increased exposure for *Pneumocystis* prophylaxis.

Vandenesch *et al*. (31) described continent-specific PVL-positive CA-MRSA clones - mainly on an *agr* group 3 background - and characterized them by their sequence type (ST). The main European CA-MRSA clone, ST80, 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. ST80-MRSA clone is usually resistant to tetracycline (mediated by the *tetK* gene), intermediate to fusidic acid (*far1* gene), and resistant to kanamycin (*aph3´-III* gene). We observed the prevalence of ST80-MSSA isolates in Nigeria which were tetracycline resistant as well, but susceptible towards fusidic acid. One of the most prevalent PVL-positive CA-MRSA clones in the United States (USA300) belongs to ST8; other US 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 sequence type was prevalent in Nigeria as a MSSA strain. In Singapore, as an international travel hub, several clones belonging to ST80, ST30, and ST59 have been reported. The prevalence of PVL-positive CA MRSA varies considerably from continent to continent. In the United States, MRSA were isolated from 50% of patients with skin and soft-tissue infections seen in emergency departments of 11 cities (97% of isolates belonged to clone USA300). In Europe, the prevalence of CA MRSA is lower, at ≈1–3% and in Africa this needs more investigational study.
In our study the MRSA strains were less “toxigenic” as compared to the MSSA strains (ST5, ST7 and ST30) which were more positive for PVL and *tst*. In contrast to our finding the MRSA isolates in a Japanese study evaluated that both MRSA and MSSA isolates carried a number of superantigenic toxin genes, but the MRSA isolates harboured more superantigenic toxin genes than the MSSA isolates (14). Hu *et al*. (14) compared the prevalence of superantigenic toxin genes in MRSA and MSSA and concluded that some of their MRSA isolates were *sec*, *seg* and *tst* positive. In our study none of the MRSA isolates shared these genes together, only one MSSA isolate typed ST508 was positive for *sec*, *seg* and *tst* together. This notable higher prevalence in Japanese MRSA isolates indicated that possession of the *sec* and *tst* genes in particular appeared to be a habitual feature of MRSA (14), unlike to the Nigerian MRSA.

This study provides a comprehensive overview on the molecular epidemiology and genetic diversity of *S. aureus* at the largest university clinic in Nigeria. First, it shows high prevalence of PVL-positive MSSA. Second, high heterogeneity of MSSA with broader resistance profiles was seen as compared to the MRSA strains (ST250 and ST88) which are homogenous. ST88 was resistant to trimethoprim-sulfamethoxazole besides to penicillin and oxacillin whereas ST250 as hospital MRSA was additionally resistant to tetracycline, ciprofloxacin and gentamycin (Table 1). Trimethoprim-sulfamethoxazole 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 a national drug

policy by governments are fundamental to ensure rational drug use. Control of community acquired *S. aureus* will still remain a challenge for some regions in Nigeria, since the transmission is linked to migration and touristic reasons.

Two types of SCC*mec* were found with CC8 complex, the ST241-MRSA-IV strain and ST250-MRSA-I (Fig. 1). ST250-MSSA is most probably putative ancestor of ST250-MRSA by the insertion of the SCC*mec* I. ST250-MSSA itself might have derived from putative ST8-MSSA ancestor, common to all strains belonging to CC8 (14).
In our present study we showed that in most of the clinical departments the less toxigenic MRSA strains circulate together with the more toxigenic MSSAs, e.g. ST30 and ST1. This might be benefiting for the toxin gene transfer which could occur among the methicillin-susceptible and -resistant strains (17).
In conclusion, the application of the different typing methods to our Nigerian strains provided important information on their clonal relationship and might also strengthen the representation of the population of *S. aureus* circulating in Nigeria. Moreover, this study summarizes comprehensive epidemiologic data and use of other genetic marker to investigate outbreak situations in such healthcare setting.
To the best of our knowledge, this is the first report of detection and genetic characterization of ST88 strain in Nigeria. ST88 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 the European CA-MRSA clones (32). Therefore, epidemiological studies on the clonal relationship of MRSA strains in Nigeria with worldwide clones would be useful and important in understanding the global dissemination of such clones.

Limitations to our study include the limited number of patients. Thus, the prevalence of the MRSA in general and CA-MRSA might be underestimated. Future prospective studies may further elucidate possible epidemiologic risk factors associated with acquiring CA-MRSA infection.

Acknowledgments

This study was supported by the German Ministry for Economical Cooperation and Development (DAAD scholarship). Part of this work was presented at the 47th ICAAC Meeting in Chicago, September 2007. We have no potential conflicts of interest and no commercial or other associations.

References

1. Adesida, S.A., H. Boelens, B. Babajide, A. Kehinde, S. Snijders, W. van Leeuwen,A. Coker, H. Verbrugh, and A. van Belkum. 2005. Major epidemic clones of *Staphylococcus aureus* in Nigeria. Microb Drug Resist.; **11** (2):115–121.
2. Adesida, S.A., Y. Likhoshvay, W. Eisner, A. O. Coker, O.A. Abioye, F.T. Ogunsola, and B.N. Kreiswirth. 2006. Repeats in the 3' region of the protein A gene is unique in a strain of *Staphylococcus aureus* recovered from wound infections in Lagos, Nigeria African J Biotechnol; **5** (20): 1858-1863.
3. Ako-Nai, A.K., A.D. Ogunniyi, A. Lamikanra, and S.E. Torimiro. 1991. The characterisation of clinical isolates of *Staphylococcus aureus* in Ile-Ife, Nigeria. J Med Microbiol.; **34** (2):109-12.
4. Clinical and Laboratory Standards Institute (CLSI). 2005. Performance standards for antimicrobial susceptibility testing; 15th informational supplement M100–S15. Wayne (PA): The Institute.
5. Enright, M.C., N.P. Day, C.E. Davies, S.J. Peacock, and B.G. Spratt. 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol.; **38**: 1008–15.
6. Enright, M.C., D.A. Robinson, G. Randle, E.J. Feil, H. Grundmann, and B.G. Spratt. 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci USA.; **99** (11):7687–7692.
7. Ghebremedhin, B., W. König, W. Witte, K.J. Kardy, P.M. Hawkey, and B. König. 2007. Subtyping of ST22-MRSA-IV (Barnim epidemic MRSA strain) at a university clinic in Germany from 2002 to 2005. J Med Microbiol.; **56** (Pt 3): 365-75.

8. Goering, R.V., R.M. Shawar, N.E. Scangarella,F.P. O'Hara,H. Amrine-Madsen,J.M. West, M. Dalessandro, J.A. Becker, S.L. Walsh, L.A. Miller, S.F. van Horn, E.S. Thomas, and M.E. Twynholm. 2008. Molecular Epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from global clinical trials. J Clin Microbiol.; **46** (9): 2842-2847.
9. Grim, S.A., R.P. Rapp, C.A. Martin, and M.E. Evans. 2005. Trimethoprim sulfamethoxazole as a viable treatment option for infections caused by methicillin resistant *Staphylococcus aureus*. Pharmacother; **25** (2):253-64.
10. Hallin, M., O. Denis, A. Deplano, R. De Ryck, S. Crèvecoeur, S. Rottiers, R. de Mendonça, and M.J. Struelens. 2008. Evolutionary relationships between sporadic and epidemic strains of healthcare-associated methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect.; **14** (7):659-69.
11. Harbarth, S., P. François, J. Schrenzel, C. Fankhauser-Rodriquez, S. Hugonnet, T. Koessler, A. Huyghe, and D. Pittet. 2005. Community-associated methicillin-resistant *Staphylococcus aureus*, Switzerland. Emerg Infect Dis.; **11**, 962-65.
12. Harmsen, D., H. Claus, W. Witte, J. Rothganger, H. Claus, D. Turnwald, and U. Vogel.2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol; **41** (12):5442-8.
13. Holmes, A., M. Ganner, S. McGuane, T.L. Pitt, B.D. Cookson, and A.M. Kearns. 2005. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J Clin Microbiol.; **43** (5):2384–2390.
14. Hu, D.L., K. Omoe, F. Inoue, T. Kasai, M. Yasujima, K. Shinagawa, and A. Nakane. 2008. Comparative prevalence of superantigenic toxin genes in meticillin-resistant and meticillin-susceptible *Staphylococcus aureus* isolates. J Med Microbiol.; **57** (Pt 9):1106-12.
15. Iroha, E.O., C.N. Kesah, M.T. Egri-Okwaji, and T.O 424 . Odugbemi. 1998. Bacterial eye infection in neonates, a prospective study in a neonatal unit. West Afr J Med.; **17** (3):168-72.
16. Kesah, C., S. Ben Redjeb, T.O. Odugbemi, C.S. Boye, M. Dosso, J.O. Ndinya Achola, S. Koulla-Shiro, M. Benbachir, K. Rahal, and M. Borg. 2003. Prevalence of methicillin430 resistant *Staphylococcus aureus* in eight African hospitals and Malta. Clin Microbiol Infect.; **9** (2):153–156.
17. Layer, F., B. Ghebremedhin, W. König, and B. König.2006. Heterogeneity of methicillin susceptible *Staphylococcus aureus* strains at a German University Hospital implicates the circulating-strain pool as a potential source of emerging methicillin-resistant *S. aureus* clones. J Clin Microbiol.; **44** (6):2179-85.
18. Lina, G., F. Boutite, A. Tristan, M. Bes, J. Etienne, and F. Vandenesch. 2003. Bacterial competition for human nasal cavity colonization: role of staphylococcal *agr* alleles. Appl Environ Microbiol.; **69** (1):18-23.
19. Lowy, F.D. 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest.; 111(9):1265–1273.
20. Muthotho, J.N., P.G. Waiyaki, M. Mbalu, A. Wairugu, B. Mwanthi, and B. Odongo. 1995. Control of spread of Methicillin Resistant Staphylococcus aureus (MRSA) in Burns Units. Afr J Health Sci.; **2** (1):232-235.
21. Ogunshe, A.A.O., T.R. Fasola, and A. Egunyomi. 2006. Bacterial profiles and consumer preference of some indigenous orally consumed herbal medications in Nigeria. *J Rural Trop Publ Health*; **5:** 27-33.
22. Okeke, I.N. 2003. Factors contributing to the emergence of resistance. *In*: Knobler SL, Lemon SM, Najafi M, Burroughs T, eds. The Resistance Phenomenon in Microbes and Infectious Disease Vectors: Implications for Human Health and Strategies for Containment—Workshop Summary. Washington, DC: The National 456 Academies Press; 132–139.
23. Okesola, A.O., A.A. Oni, and R.A. Bakare. 1999. Prevalence and antibiotic sensitivity pattern of methicillin-resistant *Staphylococcus aureus* in Ibadan, Nigeria. J Hosp Infect.; **41** (1):74–75.
24. Okesola, A.O., and A.O. Kehinde. 2008. Bacteriology of non-surgical wound infections in Ibadan, Nigeria. Afr J Med Med Sci.; **37** (3):261-4.
25. Oliveira, D.C., and H. de Lencastre. 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother; **46** (7):2155-61.

26. Ramdani-Bouguessa, N., M. Bes, H. Meugnier, F. Forey, M.-E. Reverdy, G. Lina, F. Vandenesch, M. Tazir, and J. Etienne. 2006. Detection of methicillin-resistant *Staphylococcus aureus* strains resistant to multiple antibiotics and carrying the Panton-Valentine Leukocidin genes in an Algiers Hospital. Antimicrob Agents Chemother.; **50** (3): 1083–1085.
27. Taiwo, S.S., M. Bamidele, E.A. Omonigbehin, K.A. Akinsinde, S.I. Smith, B.A. Onile, and A.O. Olowe. 2005. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Ilorin, Nigeria. West Afr J Med.; **24** (2):100-6.
28. Tenover, F.C., and R.P. Gaynes. 2000. The epidemiology of *Staphylococcus* infections. In: Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, Rood JI (eds.) Gram-Positive Pathogens. Washington, DC: American Society for Microbiology, 414–421.
29. Thanni, L.O, O.A. Osinupebi, and M. Deji-Agboola. 2003. Prevalence of bacterial pathogens in infected wounds in a tertiary hospital, 1995-2001: any change in trend? J Natl Med Assoc.; **95** (12): 1189–1195.
30. Udo JJ, Anah MU, Ochigbo SO, Etuk IS, Ekanem 488 AD. 2008. Neonatal morbidity and mortality in Calabar, Nigeria: a hospital-based study. Niger J Clin Pract.; **11** (3):285-9.
31. Vandenesch, F., T. Naimi, M.C. Enright, G. Lina, G.R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M.E. Reverdy, and J. Etienne. 2003. Community acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis.; **9** (8):978-84.
32. Witte, W., B. Strommenger, C. Cuny, D. Heuck, and U. Nuebel. 2007. Methicillin resistant *Staphylococcus aureus* containing the Panton-Valentine leucocidin gene in Germany in 2005 and 2006. J Antimicrob Chemother.; **60** (6):1258-63.

Table 1
Antibiotic resistance patterns and genotypic analysis (MLST, *spa* types) of the *Staphylococcus aureus* isolates, in Ibadan, Nigeria, 2006–2007

Table 1

ST (*n*)	*agr* type (*n*)	*spa* type	antibiotic resistance pattern (*n*)[a]	toxin gene(s)
ST5 (76)	I (39),	t311	PEN-SXT-TET (37; *agr* I and II)	*sea, seb, sec, seg,*
	II (37)		PEN-SXT-TET-CIP (16; *agr* I)	*tst, lukED, (luk-PV),*
			PEN-SXT-TET-ERY (19; *agr* II)	*hlgA*
ST7 (45)	I (33),	t091	PEN (10; *agr* I and II)	*sea, seg, lukED,*
	II (12)		PEN-SXT (18; *agr* I)	*luk-PV, hlgA*
			PEN-SXT-TET (16; *agr* I)	
ST121 (38)	IV	t159, t314	PEN-SXT (12)	*seb, seg, lukED,*
			PEN-SXT-TET (23)	*luk-PV,hlgA*
ST250[#] (35)	I (30),	t194, t292	PEN-OXA-TET-CIP-GEN (23; *agr* I)	*seb, tst, lukED, hlgA*
	IV (5)		PEN-OXA (5; *agr* IV)	
ST88 (33)	III (33)	t186	PEN-OXA-SXT (32)	*seg, luk-PV, hlgA,*
			PEN-OXA (1)	*hlgB*
ST30 (30)	III	t318	PEN-SXT (22)	*seg, tst, lukED, luk-*
			PEN-SXT-TET (4)	*PV, hlgA, hlgB*
ST8 (25)	I	t064, t068	PEN-SXT-TET-CIP-GEN (12)	*sea, seb, seg, tst,*
			PEN-SXT-TET (6)	*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	NT	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	t353	PEN-SXT (5)	*sec, seg, lukED*
ST72 (3)	I	t537	PEN-TET (3)	*seg, lukED, hlgA*

[a] the major frequent antibiotic resistance patterns are given for each sequence type [PEN, penicillin; OXA, oxacillin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamycin; CLI, clindamycin]; NT = not-typable; [#] ST250 represents MRSA and MSSA as well

Figure 1
Minimum spanning tree based on MLST indicates the estimated relationships of the 14 STs based on sequence alignments and analysis of the ST allelic profiles, including representatives of the clonal complexes (CC). ST88, ST241 and ST250 represent MRSA. Each ST is represented by a circle and the size of circle is proportional to the isolate size belonging to the respective ST. Relationships between the strains were depicted as connections between isolates and the lengths of the branches linking them (MRSA are black- and MSSA grey-colored).