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An assessment on DNA microarray and sequence-based methods for the characterization of methicillin-susceptible \textit{Staphylococcus aureus} from Nigeria

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\textit{Staphylococcus aureus} is an important human pathogen causing nosocomial and community-acquired infections worldwide. In the characterization of this opportunistic pathogen, DNA microarray hybridization technique is used as an alternative to sequence based genotyping to obtain a comprehensive assessment on the virulence, resistance determinants, and population structure. The objective of this study was to characterize a defined collection of \textit{S. aureus} isolates from Nigeria using the microarray technique, and to assess the extent that it correlates with sequence-based genotyping methods. The clonal diversity and genomic content of 52 methicillin-susceptible \textit{Staphylococcus aureus} (MSSA) were investigated by \textit{spa} typing, MLST and DNA microarray hybridization. More than half (55.8\%) of these isolates were associated with clonal complexes (CCs) typically associated with methicillin-resistant \textit{S. aureus} (MRSA) clones i.e., CC1, CC5, CC8, CC30, and CC45. Certain genes linked with virulence (\textit{hlgA} and \textit{clfA}) and adherence (\textit{ebpS}, \textit{fnbA}, \textit{sspA}, \textit{sspB}, and \textit{sspP}) were detected in all isolates. A number of genes or gene clusters were associated with distinct clonal types. The enterotoxin gene cluster (\textit{egc}) was linked with CC5, CC25, CC30, CC45, and CC121, enterotoxin H gene (\textit{seh}) with CC1, exfoliative toxin D gene (\textit{etd}) with CC25 and CC80, and the epidermal cell differentiation inhibitor B gene (\textit{edinB}) with CC25, CC80, and CC152. The excellent agreement between data from DNA microarray and MLST in the delineation of Nigerian MSSA isolates indicates that the microarray technique is a useful tool to provide information on antibiotic resistance, clonal diversity and virulence factors associated with infection and disease.

Keywords: \textit{Staphylococcus aureus}, microarray, MLST, genotyping, Nigeria

Abbreviations: Agr, accessory gene regulator; CC, Clonal complex; CLSI, Clinical Laboratory Standards Institute; MSSA, Methicillin susceptible \textit{Staphylococcus aureus}; MLST, Multilocus sequence typing; PVL, Panton-Valentine Leukocidin; \textit{S.aureus}, \textit{Staphylococcus aureus}; SCC\textit{mec}, Staphylococcal chromosome cassette \textit{mec}; \textit{spa}, \textit{Staphylococcus aureus} protein A; ST, Sequence Type.
INTRODUCTION

Staphylococcus aureus is implicated in a variety of human infections with high rates of morbidity and mortality (Lowy, 1998; Corey, 2009). In infection, S. aureus exhibits a coordinated and regulated expression for a wide variety of cell and surface-associated virulence factors (Foster and Höök, 1998; Novick, 2006). These factors mediate adherence to host cells and damaged tissue, facilitate tissue destruction and spreading, promote iron uptake and evasion of host immune system, as well as tissue damage (Skaar and Schneewind, 2004; Grumann et al., 2014). Recent studies in Cameroon (Kihla et al., 2014), Egypt (Ahmed et al., 2014), Gabon (Alabi et al., 2013), Nigeria (Jido and Garba, 2012; Oladeinde et al., 2013), South Africa (Groome et al., 2012; Naidoo et al., 2013), and Tanzania (Kayange et al., 2010; Mhada et al., 2012) have identified S. aureus as the main etiological agent for various infections in Africa. Moreover, this species has been recognized as one major cause of community-acquired neonatal sepsis in Africa (Waters et al., 2011). These studies clearly establish the important role of this major human pathogen in tropical Africa.

In many health care institutions in sub-Saharan Africa, the lack of skilled laboratoy manpower and resources is a major constraint in the identification of bacterial pathogens from clinical samples. If such analysis can be provided at all, identification of S. aureus typically relies on phenotypic methods precluding in-depth strain characterization. Molecular analysis of clonal attribution and presence of single genes contained in S. aureus isolates have emerged in pilot studies from select African centers, areas and populations (Ateba Ngoa et al., 2012; Shittu et al., 2012; Seni et al., 2013; Aiken et al., 2014; Egyir et al., 2014; Oosthuysen et al., 2014; Conceição et al., 2015; De Boeck et al., 2015; Kraef et al., 2015; Schaumburg et al., 2015). Nevertheless, in view of the impact of S. aureus disease in sub-Saharan Africa, the clonal characterization in concert with a comprehensive analysis of the hitherto ill-described virulence factor armamentarium of S. aureus isolates from this region is urgently warranted. Such analyses should target a broad spectrum of variable staphylococcal factors such as genes or gene clusters conferring antibiotic resistance, toxins, virulence, adhesion or immune evasion factors. These analyses have not been performed on a collection of S. aureus isolates in Nigeria, and reports from African countries are limited and only addressed a limited and select analytical spectrum (Raji et al., 2013; Aiken et al., 2014; Rovira et al., 2015).

The DNA microarray used for this analysis is a unique and comprehensive genotyping technique based on the analysis of 334 target sequences corresponding to approximately 170 distinct genes and their allelic variants. It enables the simultaneous identification of various gene classes including species markers, genes encoding resistance and virulence properties, exotoxin and adhesion factors, accessory gene regulator (agr), capsule, and SCCmec types (Monecke et al., 2011). Based on the observation of a high level of genetic diversity from previous investigations on methicillin-susceptible S. aureus (MSSA) in Nigeria (Shittu et al., 2011, 2012; Kolawole et al., 2013), we studied MSSA isolates obtained from various clinical sources in Nigeria using this comprehensive, array-based approach to provide an insight on the major factors associated with infection and disease.

MATERIALS AND METHODS

Identification and Antibiotic Susceptibility Testing of S. aureus Isolates

The isolates (n = 52) were obtained from samples processed as part of surveillance activities in the microbiology laboratories of six health care institutions located in Ado-Ekiti, Ile-Ife, Osogbo, Lagos, and Ibadan in South-West Nigeria, and Maiduguri in North-East Nigeria. The duration of collection of isolates was from March 2009 to April, 2010. Only the isolates were analyzed in this study. Preliminary verification as S. aureus was based on colony characteristics on blood agar, positive results for catalase, coagulase and DNase tests. Twelve isolates from a previous study (Shittu et al., 2011) were also included in this investigation. Identification was confirmed by Matrix-Assisted Laser Desorption/Ionization-Time Of Flight analysis (MALDI-TOF). Susceptibility testing to penicillin (10 units), cefoxitin (30 µg), doxycyline (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), chloramphenicol (30 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg) were determined using the disk diffusion method according to the Clinical Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute (CLSI), 2009).

DNA Extraction

S. aureus genomic DNA was extracted from an 18–24 h old culture on sheep blood agar using lysis buffer and lysis enhancer (StaphyType Kit, Alere Technologies GmbH, Jena, Germany) and processed using a DNeasy tissue kit (Qiagen, Hilden, Germany).

Molecular Typing of the Isolates

Typing of S. aureus was based on sequencing of the hypervariable region of the protein A gene (spa). The spa types were determined using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany, version 2.1.1) (Harmsen et al., 2003). Multilocus sequence typing (MLST) was performed for one isolate of each spa type (Enright et al., 2000), as a spa type usually belongs to one sequence type (ST) with few exceptions due to homoplasies (Basset et al., 2009, 2012). The allelic profiles and STs were assigned using the MLST S. aureus database (www.mlst.net), and the sequence types of the remaining isolates were inferred from the derived MLST data.

DNA Microarray Hybridization

The DNA microarray of the StaphyType™ kit (Alere Technologies GmbH, Jena, Germany) was used in this study according to previously established protocols (Monecke et al., 2008). The isolates were grouped with various clonal complexes (CCs) by the imaging software Iconoclust based on comparison of hybridization profiles to a collection of reference strains previously characterized by MLST.
Splits Graph Construction

The SplitsTree algorithm (Huson and Bryant, 2006) and software was used to analyze the similarities between hybridization patterns, and network tree construction was performed using SplitsTree 4.10 on default settings (characters transformation, uncorrected P; distance transformation, Neighbor-Net; and variance, ordinary least squares).

RESULTS

Identification of S. aureus Isolates

A total of 52 MSSA (3 and 49 isolates from nasal and clinical sources, respectively) were analyzed (Table 1). The clinical isolates were obtained from wounds and associated infections (n = 29; 59.2%), urinary tract infections (n = 6; 12.2%), semen/infertility diagnosis (n = 4; 8.2%), ocular infections (n = 3; 6.1%), and pneumonia (n = 2; 4.1%). One isolate each was from otitis media, and blood related infections, while information on three isolates was not available. The clinical isolates were obtained from health care institutions located in Ile-Ife (n = 26; 53.1%), Osogbo (n = 11; 22.4%), Maiduguri (n = 5; 10.2%), Lagos (n = 4; 8.2%), Ibadan, and Ado-Ekiti (n = 2 isolates each: 4.1%).

Antibiotic Susceptibility Testing

All the isolates were susceptible to cefoxitin and 98.1% (n = 51) were resistant to penicillin. Only two isolates each exhibited resistance to chloramphenicol and gentamicin, and four to doxycycline. Intermediate susceptibility to clindamycin and erythromycin were identified in six and 21 isolates, respectively. The predominant antibiotic was resistance only to penicillin (n = 23; 44.2%), and resistance to penicillin with intermediate susceptibility to erythromycin (n = 10; 19.2%) (Table 1).

Sequence based Typing (spa and MLST)

A total of 26 spa types were identified among the 52 MSSA isolates and the most common were t318 (n = 7), t311 (n = 5), t084, t127, and t2304 (n = 4 each). Based on MLST, the MSSA were classified into 13 sequence types (STs) (Table 1).

DNA Microarray Analysis

The assay confirmed the identity of the isolates (S. aureus) by positive results for specific markers including rndD1 (domain 1 of 23S rRNA), protein A (spa), glyceraldehyde 3-phosphate dehydrogenase (gapA), catalase A (katA), thermostable nuclease (nuc), and staphylococcal accessory regulator A (sarA) (Supplementary Materials 1, 2). The hybridization profiles revealed that the 52 MSSA isolates clustered in 12 different CCs. More than half (55.8%) of the CCs were associated with the genetic background common to the major methicillin-resistant S. aureus (MRSA) clones i.e., CC1 (n = 6 isolates), CC5 (n = 9), CC8 (n = 4), CC30 (n = 8), and CC45 (n = 2). The rest were assigned with CC7 (n = 1), CC15 (n = 7), CC25 (n = 2), CC80 (n = 1), CC97 (n = 1), CC121 (n = 8), and CC152 (n = 3).

Antibiotic Resistance Genes

A total of 69.2% (n = 36) of the isolates yielded a hybridization signal for the beta-lactamase gene (blaZ) and only 10 and three isolates were positive for the tetracycline resistance genes (tetK and tetM), respectively. The two MSSA in CC8 which exhibited phenotypic resistance to chloramphenicol and gentamicin possessed the corresponding resistance genes (cat and aacA-aphD). In addition, the single CC80 isolate was positive for the lincosamide resistance gene (lnuA).

Accessory Gene Regulator and Capsular Typing

The distribution of agr/CCs/capsule types for the MSSA is indicated in Figure 1. Overall, 13 (25%) isolates assigned to different clonal lineages (CC7, CC8, CC25, CC45, CC97, and CC152) were associated with agr group I, 16 (30.2%; CC5 and CC15) with group II, and 15 (28.8%; CC1, CC30 and CC80) with group III. CC121 was the only representative for agr group IV (n = 8; 15.4%) (Table 1). The capsule type 8 was the most frequent and detected in 33 (63.5%) isolates affiliated with CC1, CC7, CC15, CC30, CC45, CC80, and CC121. The remaining isolates (20; 38.5%) belonged to capsule type 5 (assigned with CC5, CC8, CC25, CC97, and CC152).

Enterotoxin Genes

PVL-positive isolates (n = 27) belonged to CC1, CC5, CC15, CC30, CC80, CC121, and CC152 (Supplementary Material 1). Moreover, the lukF gene (haemolysin gamma; component B) was universally detected in all the CCs and the lukE genes was a common feature except with MSSA isolates in CC30, CC45, and CC152 (Supplementary Material 3). With respect to the carriage of superantigen genes, only three MSSA (one isolate in CC1 and two in CC45) tested positive for the toxic shock syndrome toxin gene (tst-1) (Supplementary Material 1). All the isolates in this study lacked a hybridization signal for the enterotoxin E gene (see Supplementary Material 2) and the enterotoxin genes were not detected in MSSA assigned with CC80, CC97, and CC152. In the haemolysin family, almost all (98.1%) the isolates in the various CCs possessed the haemolysin alpha and delta genes (hla, hld), while the haemolysin beta gene (hbb) was identified in the various CCs except in CC15, CC45, and CC152.

Microbial Surface Components Recognizing Adhesive Matrix Molecule (MSCRAMM) Genes

All the isolates were negative for the surface protein involved in biofilm production (bap), but possessed the genes for the intercellular adhesion protein (icaA/C/D) (CC152 isolates were icaC negative). The genes for clumping factor A (clfA), cell surface elastin binding protein (ebpS), fibronectin-binding protein A (fnbA) and proteases (sspA, sspB, and sspP) were detected in all the isolates (Supplementary Materials 1, 3).

Splits Tree Analysis

The analysis identified four main clusters (CC5/CC25; CC8/CC97; CC1/CC7/CC80; and CC30/CC45) indicating the phylogenetic relationship between the isolates (Figure 2).
### TABLE 1 | Characterization of the methicillin-susceptible *S. aureus* (MSSA) from Nigeria based on antibiotyping, microarray analysis, spa typing, and MLST.

<table>
<thead>
<tr>
<th>Isolate Number</th>
<th>Location</th>
<th>Sample/Clinical diagnosis</th>
<th>Antibigram</th>
<th>Score (%) (Alere)</th>
<th>agr/Clonal complex (Alere)</th>
<th>spa type</th>
<th>MLST</th>
</tr>
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<td>11486_24</td>
<td>Ile-Ife</td>
<td>Wound Infection</td>
<td>PEN</td>
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<td>UTI</td>
<td>PEN, ERY(i)</td>
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<td>ST1</td>
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<td>Semen</td>
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<td>Not available</td>
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<td>94.3</td>
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<td>t127</td>
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<td>Wound infection</td>
<td>PEN, ERY(i), CC(i)</td>
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<td>t321</td>
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<td>6056_34</td>
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<td>Urine</td>
<td>PEN</td>
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<td>Urine</td>
<td>PEN, ERY(i), SXT(i)</td>
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<td>ST5</td>
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<td>Pneumonia</td>
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<td>ST5</td>
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<td>PEN, DO(i), GM, CHL, SXT</td>
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<td>Lagos</td>
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<td>Blood</td>
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<td>93.9</td>
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<td>susceptible to all antibiotics tested</td>
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<td>Nasal swab/screening</td>
<td>PEN, ERY(i), CC(i)</td>
<td>91.8</td>
<td>agr_III/CC45</td>
<td>t109</td>
<td>ST508</td>
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<tr>
<td>3950_33</td>
<td>Osogbo</td>
<td>Urine</td>
<td>PEN</td>
<td>91.5</td>
<td>agr_III/CC45</td>
<td>t110433</td>
<td>ST508</td>
</tr>
<tr>
<td>GDC_35</td>
<td>Osogbo</td>
<td>Semen</td>
<td>PEN</td>
<td>94.9</td>
<td>agr_III/CC80</td>
<td>t193</td>
<td>ST80</td>
</tr>
<tr>
<td>MD14_2*</td>
<td>Maiduguri</td>
<td>Wound infection</td>
<td>PEN, DO(i)</td>
<td>92.9</td>
<td>agr_J/CC97</td>
<td>t145</td>
<td>ST79</td>
</tr>
<tr>
<td>2U_26</td>
<td>Ile-Ife</td>
<td>Unavailable</td>
<td>PEN, ERY(i)</td>
<td>89.3</td>
<td>agr_J/CC121</td>
<td>t1159</td>
<td>ST121</td>
</tr>
<tr>
<td>UC47_38</td>
<td>Ibadan</td>
<td>Eye swab</td>
<td>PEN, DO, ERY(i), CC(i)</td>
<td>92.1</td>
<td>agr_J/CC121</td>
<td>t1159</td>
<td>ST121</td>
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<td>Wound infection</td>
<td>PEN, ERY(i)</td>
<td>91.8</td>
<td>agr_J/CC121</td>
<td>t131</td>
<td>ST121</td>
</tr>
<tr>
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<td>Wound infection</td>
<td>PEN, ERY(i), CC(i)</td>
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<td>agr_J/CC121</td>
<td>t131</td>
<td>ST121</td>
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<tr>
<td>6376_3</td>
<td>Ile-Ife</td>
<td>Abscess</td>
<td>PEN, DO(i)</td>
<td>93.1</td>
<td>agr_J/CC121</td>
<td>t12304</td>
<td>ST121</td>
</tr>
<tr>
<td>6540_10</td>
<td>Ile-Ife</td>
<td>Bone Marrow Infection</td>
<td>PEN</td>
<td>93.5</td>
<td>agr_J/CC121</td>
<td>t12304</td>
<td>ST121</td>
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<tr>
<td>NS2986_20</td>
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<td>Nasal swab/screening</td>
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<td>agr_J/CC121</td>
<td>t12304</td>
<td>ST121</td>
</tr>
<tr>
<td>3920_31</td>
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<td>Aspirate</td>
<td>PEN</td>
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<td>agr_J/CC121</td>
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<td>ST121</td>
</tr>
<tr>
<td>D3_12</td>
<td>Ile-Ife</td>
<td>Cervical cancer</td>
<td>PEN, ERY(i)</td>
<td>94.6</td>
<td>agr_J/ST152</td>
<td>t355</td>
<td>ST152</td>
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</tbody>
</table>

(Continued)
**TABLE 1 | Continued**

<table>
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<tr>
<th>Isolate Number</th>
<th>Location</th>
<th>Sample/Clinical diagnosis</th>
<th>Antibiogram</th>
<th>Score (%) (Alere)</th>
<th>agr/Clonal complex (Alere)</th>
<th>spa type</th>
<th>MLST</th>
</tr>
</thead>
<tbody>
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<td>D12_13</td>
<td>Ile-Ife</td>
<td>Ocular infection</td>
<td>PEN</td>
<td>94.6</td>
<td>agr_I/ST152</td>
<td>t356</td>
<td>ST152</td>
</tr>
<tr>
<td>W7_2_4*</td>
<td>Ile-Ife</td>
<td>Wound infection</td>
<td>PEN</td>
<td>96.3</td>
<td>agr_I/ST152</td>
<td>t356</td>
<td>ST152</td>
</tr>
</tbody>
</table>

* S. aureus analyzed in a previous study; †: intermediate susceptibility; agr, accessory gene regulator; PEN, Penicillin; DO, Doxycycline; ERY, Erythromycin; CJI, Clindamycin; GEN, Gentamicin; CHL, Chloramphenicol; SXT, trimethoprim/sulfamethoxazole; CC, Clonal Complex; ST, Sequence type.

†spa types selected for Multilocus sequence typing (MLST); Sequence types (STs) of the remaining isolates were inferred from the derived MLST data.

**FIGURE 1 | Distribution of agr/CCs/capsule type of MSSA in Nigeria.**

**DISCUSSION**

We observed a complete agreement between DNA microarray analysis and MLST in the delineation of the isolates (Table 1), showing that the hybridization profile could be used to predict the lineages. Furthermore, the heterogeneous and divergent nature of the isolates observed in this study provided evidence on the overall higher diversity of MSSA compared with MRSA (Deurenberg and Stobberingh, 2008; Goering et al., 2008; Ghasemzadeh-Moghaddam et al., 2011; Rufing et al., 2012; Blomfeldt et al., 2013; Rasmussen et al., 2013, 2014). In Nigeria, many diagnostic microbiology laboratories rely on the disc diffusion technique for antibiotic susceptibility testing, but this protocol does not provide information on the nature of resistance genes. The antibiotic susceptibility results observed in this study were in accordance with the corresponding resistance gene profiles by DNA microarray. MSSA isolates that exhibited full resistance to trimethoprim-sulfamethoxazole clustered with CC8 and CC25, but were dfrS1 negative indicating that a different mechanism could be attributed to resistance. A recent study (Nurjadi et al., 2014) has provided strong evidence that the dfrG gene is the predominant trimethoprim resistance determinant on *S. aureus* in Africa. Overall, resistant determinants for antibiotics, heavy metal and quaternary ammonium compounds were observed more often in CC8 than other CCs (Supplementary Materials 1, 3).

The accessory gene regulator (agr) and capsule typing methods are useful front-line tools for the characterization of *S. aureus* (Goerke et al., 2005). Hybridization signals for agr type I and IV were observed for one, three, and four isolates grouped with CC25, CC152, and CC121, respectively (Supplementary Materials 1, 2). This could be attributed to possible cross-hybridization as the alleles for the two agr types are closely related (Monecke et al., 2010). Our observations on CCs and agr groups were similar to previous reports on MSSA in five major African towns (Breurec et al., 2010), Gabon (Ateba Ngoa et al., 2012), and Nigeria (Ghebremedhin et al., 2009; Kolawole et al., 2013). In addition, our study also support the view (Wright et al., 2005; Holtfreter et al., 2007; Rasmussen et al., 2014) that an agr type may be detected in isolates which are assigned to genetically diverse CCs, whereas, it is also associated with specific CCs. The dominance of capsule type 8 in MSSA is consistent with data from Gabon (Schaumburg et al., 2011), Norway (Blomfeldt et al., 2013), and Sweden (Rasmussen et al., 2013, 2014).

Staphylococcal enterotoxins are typically encoded by genes located on mobile genetic elements (Baba et al., 2002). The egc cluster (seg+sei+sem+seo+seu) is located on the genomic island vSA$^+$ and reported to be associated with specific clonal types regardless of the geographical strain distribution (Lindsay and Holden, 2006). In this investigation, the egc-enterotoxin gene cluster was a unique feature for CC5, CC25, CC30, CC45, and CC121. Previous studies have indicated that the cluster is predominantly present in MSSA assigned with CC5, CC25, CC30, and CC45 (Van Trijp et al., 2010; Rasmussen et al., 2013). The seh gene is linked to the staphylococcal cassette chromosome mec (SCC$mec$ elements) and reported to be restricted to the CC1 genomic background (Baba et al., 2002). Moreover, the seh gene has also been reported mainly in MSSA-CC30 (Blomfeldt et al., 2013). Nevertheless, our observation on seh-positive MSSA-CC1 is in agreement with previous reports (Chen et al., 2013; Rasmussen et al., 2013).

The genes associated with staphylococcal complement inhibitor (scn) and staphylokinase (sak) were also widely distributed across the CCs but CC15 isolates were sak gene negative. Virulence associated with the exfoliative toxins has been identified to cause epidermal cleavage in staphylococcal scalded skin syndrome (SSSS) and bullous impetigo (Ladhani et al., 1999). The exfoliative toxin D (ETD) is a 27-kDa protein which causes epidermal blisters in newborn mice (Yamasaki et al., 2006). The epidermal cell differentiation factors (EDIN) target and inhibit the small host protein RhoA, a master regulator of the host cell actin cytoskeleton (Inoue et al., 1991; Jaffe and Hall, 2005; Aktories, 2011). Furthermore, the edin-isofom (edinB) and etd genes are located in tandem in a *S. aureus* etd pathogenicity island in a chromosome of etd-positive *S. aureus* strains (Yamaguchi et al., 2002). A strong association of the etd gene with invasive CC25 *S. aureus* isolates has also been
reported. In this study, all the isolates assigned with CC25 and CC80 were etd-positive, which is in agreement with a previous study in Nigeria (Shittu et al., 2011). Moreover, MSSA grouped with CC25, CC80, and CC152 were edinB positive but CC152 isolates were etd negative. Our observations were similar to a study on the distribution of the edin gene in S. aureus from diabetic foot ulcers (Messad et al., 2013). A study in MSSA bacteremia isolates in Sweden showed that the collagen binding protein (Cna) was detected in CC1, CC30, and CC45. Our report identified the gene in isolates assigned with CC1, CC30, CC45, CC121, and CC152.

Our study has a number of limitations. Although all isolates were of human origin, and the large majority was obtained from clinical samples, a clear distinction between commensal and clinical strains could not be made based on the available information. An association of isolates within the context of endemicity i.e., nosocomial vs. community associated infections, is also not clear. Furthermore, whereas the microarray analytical database is exhaustive, well-characterized, and validated with isolates from all continents, the attribution of CCs is based on the hybridization reactions and resulting microarray profile rather than gene sequencing, and a positive signal does not necessarily imply the presence of gene product (e.g., protein). In addition, the microarray method was unable to separate ST8 from ST2427.

This might be due to the close phylogenetic relation of both STs as they are single locus variants (ST8: 3−3−1−1−4−4−3 and ST2427: 3−3−297−1−4−4−3). Finally, with a collection of 52 isolates studied, and a large number of genes and genetic profile ascertained by microarray, the potential for individual statistical comparisons is limited. Yet, with this comprehensive genetic-analytical approach performed on a clinical isolate collection obtained from patients of various medical institutions in a sub-Saharan African country, Nigeria, a number of important observations could be made which clearly characterize and demarcate the clonal distribution as well as the virulence gene equipment.

More than one half (55.8%; n = 29) of these MSSA isolates were associated with a genetic background which is attributable to classic methicillin-resistant S. aureus (MRSA) clones. PVL-positive isolates were identified in seven of the 12 CCs. Moreover, toxin genes were observed to be distributed mainly with certain clonal types, and in agreement with previous investigations (Holtfreter et al., 2007; Monecke et al., 2008). Antibiotic resistance gene profiles of the isolates by the DNA microarray demonstrated concordant results with data on antibiotic susceptibility testing. The array-based, comprehensive approach has been shown to yield such diverse CC and gene specific results on an isolate collection from sub-Saharan Africa.
Overall, microarray analysis proved to be a useful tool to provide useful information on antibiotic resistance, population structure and various virulence factor profiles associated with infection and disease. It is assumed that these findings might be useful for a better understanding of clinical staphylococcal disease presentation, patient care and for assistance in outbreak investigation in health care institutions in a country such as Nigeria. Moreover, our study also underlines the need for further trials employing well-controlled, prospectively collected clinical isolates to delineate the genetic pathogen profile in conjunction with the clinical disease presentation in sub-Saharan Africa.

AUTHOR CONTRIBUTIONS

AS, UR, GP, FS, LM, and MH conceived the study, OO, KO, AR conducted the sample collection and preliminary identification of the isolates. AS performed the microarray technique, AS and UR analyzed the microarray data, and AS wrote the manuscript (with input from all authors). All authors read and approved the final version of the manuscript.

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REFERENCES


SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2015.01160

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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