MATERIALS AND METHODS

Sample collection, Isolates and culture

Thirty (30) S. aureus isolates from the pig section of an abattoir were analysed in this study. In brief, sterile swabs were used to collect nasal samples from 100 pigs for a period of 2 months. Sampled animals originated from farms, and all pigs sampled were adult pigs. All samples were placed on ice following collection and processed within 24 h. The samples were inoculated into 5 ml Brain-heart infusion broth (Oxoid) containing 6.5% NaCl for enrichment and incubated for 24 h at 37°C. Preliminary verification of S. aureus was based on colony characteristics on Baird Parker agar (Oxoid) supplemented with egg yolk tellurite, Sheep blood agar, and positive results for catalase, coagulase and DNase tests. The isolates were further confirmed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and antibiotic sensitivity to cefoxitin (30 µg, Oxoid) and oxacillin (1 µg, Oxoid) discs were performed using the Kirby Bauer disc diffusion method (Bauer et al., 1966) on Mueller Hinton media.
**Microarray Procedures**

*S. aureus* genomic DNA was extracted from an 18-24 hour old culture on sheep blood agar using lysis buffer and lysis enhancer (StaphyType Kit, Alere Technologies GmbH, Jena, Germany). DNA microarray analysis was carried out as described by the manufacturer. The microarray kit covers 334 target sequences including *S. aureus* species markers, resistance associated genes, genes encoding SEs and enterotoxin-like proteins, accessory gene regulator, capsule and biofilm-associated markers, and a variety of other genes. Resulting DNA microarray profiles were grouped to various clonal complexes (CCs) by the imaging software Iconoclast based on comparison of hybridization profiles to a collection of reference strains previously characterized by multilocus sequence testing (MLST).

**RESULTS**

**Antibiotic Resistance-associated and enterotoxin genes**

All of the 30 isolates were phenotypically susceptible to cefoxitin (inhibition zone ≥22 mm) and oxacillin (inhibition zone ≥13 mm) and the mecA gene was also not detected hence there were no MRSA among the tested isolates. Amongst all the resistance-associated genes tested, the beta-lactamase operon (*blaZ/I/R*) and the tetracycline genes (*tetK*) were detected among twenty (66.66%) isolates respectively. Furthermore, macrolide genes (*ermA/msrA*) were present in ten (33.33%) isolates while only one of the 30 isolates (3.33%) carried the chloramphenicol gene (*cfr*) while seven isolates (23.31%) carried the *sdrM* (a multidrug efflux pump). Based on the genes encoding staphylococcal enterotoxins (SE) and enterotoxin-like proteins, twenty (66.66%) isolates harboured the *sea, sreb* and the enterotoxin gene cluster *egc* (seg, sei, selm, selo, selu).

**Representation of Clonal complex**

The 30 isolates were assigned to 4 Clonal Complexes (CC). Of the 30 isolates, 16 (53.33%) belonged to CC15, 8 (26.66%) isolates belonged to CC152 and while 6 (20%) isolates three each belonged to CC1 and CC5, respectively. All the isolates belonging to CC15 were positive for *srb* whereas isolates CC152 and CC5 harboured the enterotoxin cluster *egc* and enterotoxin A alleles (*sea, sea-N315*) respectively. Isolates belonging to CC5 and CC15 carried the *sasG, sdbC sdrD* genes. These isolates also showed positive hybridization to *agr* group II and harboured both capsule type 5 and capsule type 8.

The second most common clonal complex was CC152 (8; 26.66%). These isolates carried the *cra, sdrD*, capsule type 5, *cra* but lacked *saG* gene. The Panton-Valentine leukocidin genes (*lukF-PV+lukS-PV*) were present in two CC152 isolates. Isolates CC5, CC15 and CC152 all harboured genes for intracellular adhesion proteins (*icaA, icaC, icaD*).

Finally, all CC-groups were found to be positive for the beta lactamase operon and *tetK* except for CC5 that only harboured *tetK*. Two isolates of CC1 and five isolates of CC15 harboured the *sdrM* (multidrug efflux pump gene) respectively. Also, all isolates except isolates belonging to CC1 carried *clfA, clfB* as well as *icaA, icaC, icaD*. CCl did not harbour any capsular and enterotoxin genes.

**Table 1** Analysis of 30 *S. aureus* isolates recovered from pigs at slaughter: assigned clonal complexes, spa types, presence of genes encoding antibiotic resistance, staphylococcal enterotoxins and enterotoxin-like proteins, capsule and biofilm-associated markers

<table>
<thead>
<tr>
<th>No of Isolates</th>
<th>Clonal Complexes</th>
<th>Antibiotic genes</th>
<th>Resistant-associated genes and virulence</th>
<th>Genes encoding enterotoxins and virulence</th>
<th>Capsule and Biofilm-associated accessory gene regulator and adhesion genes</th>
</tr>
</thead>
</table>

**DISCUSSION**

This study gives a first insight into the population structure and the presence of resistance and virulence-associated genes of *S. aureus* isolates of pig origin. In total, there were no MRSA among the tested 30 isolates. This finding corroborates the fact that there are very scanty reports on MRSA in animals in Nigeria and Africa at large. The presence of *blaZ/I/R* and *tetK* genes is not surprising considering the intensive use of these antimicrobials in livestock production as they are relatively cheap drugs and readily available over the counter for purchase without prescription (Momoh et al., 2018; Adesokan et al., 2015). The presence of *ermA/msrA* (macrolide resistance) genes among these isolates is worrisome as these genes are reported to be widely distributed in staphylococci of human origin and they are plasmid borne (Shaker et al., 2014). This finding is of public health importance due to their capability of horizontal gene transfer between species and genera (Svarara and Rankin, 2011).

Interestingly, CC5 has so far been reported as the predominant CC-group observed among pigs (Monecke et al., 2011; He et al., 2013; Frana et al., 2013; Smith et al., 2013) while CC15 and CC152 have been reported to be the prevailing CC among pigs in Dakar while Song et al. (2015) reported CC5 among raw and processed food in Shanghai. Among the isolates, CC15 and CC152 were the most predominant in this study. Similar results have been reported by Breurec et al. (2011) and Shittu et al. (2012) from other studies on the molecular structure of African MSSA.

Genes associated with enterotoxins are very important and little is known about enterotoxigenic *S. aureus* from pigs. The presence of genes encoding classical SEs in most of the *S. aureus* isolates in this study is interesting considering the fact that these SEs reported in this study are known to induce emetic reactions which imply their role in staphylococcal poisoning and they have also been reported among other animals and humans. In most of the pig isolates in this study, genotype-enterotoxin association was similar to that known from human *S. aureus* isolates. This report is in tandem with the report of Bryston et al. (2015) who reported *S. aureus* isolates harbouring genes encoding emetic SEs in pork and pigs in Poland.
The accessory gene regulator (agr) and capsule typing methods are important tools for the characterization of S. aureus (Goerke et al. 2005). All the isolates showed positive hybridization to agrII. The higher incidence of agrII may be associated with the virulence potential of S. aureus (Cheung et al., 2011). This observation is in agreement with the report of Song et al. (2015) who reported that agr-type identified is important among S. aureus strains as this locus regulates the synthesis of virulence determinants. DNA microarray analysis showed that CC5, CC15 groups harboured both cap5 and cap8 while CC152 harboured only cap5 and CC1 did not harbour any capsular gene. Also, 16 isolates harboured genes for intracellular adhesion proteins (icaA, icaC, icaD) which play a significant role in biofilm formation (Arciola et al., 2001) and are frequently identified among clinical isolates. All isolates except isolates belonging to CC1 showed harbour capfA and capB (clumping factor A/B). These are genes that allow S. aureus to sustain and survive in the anterior nares (Sivaraman et al., 2009).

Another interesting finding is the presence of sak (staphylokinase gene), sak was present in 28.6% of the pig isolates from this study. SAK plays a role in the establishment of infections in humans (Nguyen and Vogel, 2016) and 70%–90% of sak has been reported in humans (Luedicke et al., 2010; Monecke et al., 2007a; 2007b) and in 48.89% of camel isolates (Monecke et al., 2011). The presence of sak in this study contradicts the report that S. aureus strains from veterinary sources commonly lack SAK production (Katayama et al., 2013; Resch et al., 2013). However, the presence of sak, lukD+lukE and scn among some of the isolates suggests a possible human-pig transmission.

Among other genes, agrII, capsule type 5, clfA and clfB were most common. The higher incidence of agrII may be associated with the virulence potential of S. aureus (Cheung et al., 2011). This observation is in agreement with the report of Song et al. (2015) who reported that agr-type identified is important among S. aureus strains as this locus regulates the synthesis of virulence determinants. DNA microarray analysis showed that CC5, CC15 groups harboured both cap5 and cap8 while CC152 harboured only cap5 and CC1 did not harbour any capsular gene (Table 1). Also, 16 isolates harboured genes for intracellular adhesion proteins (icaA, icaC, icaD) which play a significant role in biofilm formation (Arciola et al., 2001) and are frequently identified among clinical isolates. Furthermore, the presence of sdrC and sdrD in clinical isolates is in tadem with the report of Liu and Yu, (2015) who reported that these genes are associated with MSSA isolates. These genes are known to promote both bacterial adherence to surfaces and biofilm formation (Liu and Yu, 2015). All isolates except isolates belonging to CC1 showed harbour clfA and clfB (clumping factor A/B). These are genes that allow S. aureus to sustain and survive in the anterior nares (Sivaraman et al., 2009).

CONCLUSION

The use of microarray in this study provides first insight into the population structure, virulence factor profiles and carriage of antibiotic resistance genes among S. aureus from pigs at slaughter. Despite the few number of S. aureus isolates used in this study, it was still difficult to compare our data with other reports due to limited reports on the use of microarray to analyse the gene content of isolates from pigs and other animals within Africa. They understand host specificity of some of these virulence factors of S. aureus. Also, the DNA microarray assay provided rapid assessment of the virulence potential of the S. aureus strains. An increased attention should be allotted to research involving food animals so as to have insight into the population structure of S. aureus isolates in animals in Africa.

Acknowledgement: We thank the abattoir personnel for their cooperation during the collection of samples. Our utmost thanks goes to Dr A.R Larosen of the National Center for Antimicrobial and Infection Control, Statens Serum Institut, Copenhagen, Denmark for providing the laboratory space and Christiana Bleis for her technical support in the course of the laboratory analysis.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES


