MICROARRAY-BASED ANALYSIS OF STAPHYLOCOCCUS AUREUS ISOLATES FROM NON-CLINICAL SOURCE

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ABSTRACT

This study was aimed to genotype virulence, toxin and resistance genes of 30 S. aureus isolates from pigs using DNA microarray. The 30 isolates analyzed in this study all belonged to clonal complexes (CC) CC1 (3; 10%), CC5 (3; 10%), CC15 (16; 53.33%) and CC152 (8; 26.66%), respectively. Of antibiotic resistance-associated genes reported: Twenty out of 30 isolates (66.66%) carried the beta-lactamase operon (blaZ/I/R) and the tetracycline resistance gene (tetK), respectively. Also, the macrolide resistance gene (ermA/msrA) were detected in ten isolates (33.33%); the multidrug efflux pump resistance gene (sdrM) was detected in seven isolates (23.33%) and one isolates (3.33%) harboured the chloramphenicol resistance gene (cat). None of the isolates harboured genes conferring methicillin resistance. In terms of genes encoding enterotoxins; seb was detected among all isolates of CC15 while the enterotoxin gene cluster egc and a variant of the enterotoxin A (sea-N315) were detected among isolates of CC5 and CC152, respectively. Two CC152 isolate was positive for genes encoding the Panton-Valentine leukocidin (lukF-PV+lukS-PV). In addition, all the isolates harboured the genes encoding for intracellular adhesion proteins (icaA/C/D) and the biofilm-associated genes (clfA and clfB) except for CC1 isolates. The capsular genes cap5 and cap8 were detected in isolates of CC5 and CC15 while only cap5 was present in CC152 isolates. The study provides detailed genotyping data on the population structure, presence of toxins and antibiotic resistance markers of S. aureus isolates and indicates the importance of the microarray technique in analyzing multiple gene contents of bacteria.

Keyword: Staphylococcus aureus; Animals; Pigs; DNA-Microarray; Virulence genes

INTRODUCTION

S. aureus is a well-known colonizer of human population as well as livestock and domestic animals worldwide (Eko et al., 2015). In humans, it causes a wide range of infections ranging from mild to life threatening diseases (Bazzi et al., 2015). Some animal strains of S. aureus have been reported to be zoonotic (Monecke et al., 2011), as a result of its association with pigs and people who work in close contact with animals. It is however not well understood which factors are responsible for host specific behaviour in S. aureus (Gordon et al., 2008). In humans, the success of S. aureus as a pathogen is partly due to its ability to express a variety of virulence factors that mediate host colonization, tissue invasion and dissemination (Sabat et al., 2013).

There is paucity of data on the host specific virulence factor for animals. Many animal species are known to be colonized or infected by S. aureus but of all livestock, pigs appear to be the most implicated. However, due to lack of typing data, it is not known whether isolates of animal origin harbour their own host specific strains or promiscuous strains with zoonotic potentials (Monecke et al., 2011). Hence, it is of great interest to systematically genotype the S. aureus isolates from pigs considering that they are important food animals with considerable contact with humans especially those occupationally exposed.

In Nigeria and sub-Saharan Africa at large, identification of bacterial pathogens is still limited to phenotypic methods which lacks the ability to give an in-depth analysis of the pathogens. The identification of bacterial clones with enhanced virulence or increased ability to spread is therefore important. Hence, DNA based micro-array analysis used in this study is a rapid method for analyzing the gene contents of a pathogen. It can simultaneously analyse the antimicrobial resistance and virulence gene content of S. aureus (Sabat et al., 2013) and even other pathogens.

In order to obtain an insight into the population structure of S. aureus in pigs at slaughter, a collection of isolates originating from an abattoir were genotyped using microarray.

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 2months. 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 5ml Brain-heart infusion broth (Oxoid) containing 6.5% NaCl for enrichment and incubated for 24 h at 37℃. 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 Iconocluist 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/mrsA*) were present in ten (33.33%) isolates while only one of the 30 isolates (3.33%) carried the chloramphenicol gene (*cat*) 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*, *seb* and the enterotoxin gene cluster *egc* (egc, sei, selm, sele, 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 *seb* 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*, *sdrC, 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 *cna, sdrD*, capsule type 5, *cna* but lacked *soG* 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*. CC1 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

| No of Isolates | Clonal Complexes | Antibiotic genes | Resistant-associated and virulence genes | Capsule and Biofilm-associated accessory gene regulator and adhesion genes |
|----------------|------------------|------------------|----------------------------------------|--------------------------------------------------------------------------------|
| 16             | CC15             | *blaZ/I/R, tetK, fosB, sdrM* (5) | *seb* | *SasG, sdrC, sdrD, agrII, cap5, cap8, icaA/C/D, clfA, clfB* |
| 8              | CC152            | *blaZ/I/R, tetK, ermA/mrsA* (2) | (egc) *seg/sei/selm/seh/selu, sea*-N315, *lukD+lukF* (2), *scn, cna* | cap5, *sdrD, icaA/C/D, clfA, clfB* |
| 3              | CC1              | *blaZ/I/R, tetK, sdrM* (2) | *N.D* | *sasG, sdrC, sdrD, agrII, cap5, cap8, icaA/C/D, clfA, clfB* |
| 3              | CC5              | *blaZ/I/R, tetK, icaA (1) | (egc) *seg/sei/selm/seh/selu, sea*-N315 | |

Key: *blaZ/I/R* - penicillin, *ermA/mrsA* - macrolides, *tetK* - tetracycline, *cat* - chloramphenicol, *fosB* - metallothiol transferase, N.D - No gene detected

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/mrsA* (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 (Svara and Rankin, 2011).

Interestingly, CC5 has so far been reported as the predominant CC-group observed among pigs (Momcke 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-group among clinical isolates associated with bacteremia in Ghana and Nigeria, respectively (Egyir et al., 2013; Shittu et al., 2012) and in China (Frana et al., 2013). Also, Fall et al. (2012) reported the presence of CC15 and CC152 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 (Aricola 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; 2009), 10% of cattle isolates (Monecke et al., 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 (Aricola 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 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).

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 analyze the gene content of isolates from pigs and other animals within Africa. To understand host specificity of some of these virulence factors of *S. aureus*, 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.

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

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