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Characterization of Some *Staphylococcus aureus* Subspecies Anaerobius Isolates by *spa* and *coa* Genes

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Abstract: Twenty one clinical and sub-clinical sheep abscess disease isolates of *Staphylococcus aureus* subsp. anaerobius in addition to two reference strains were analysed for polymorphism of X region of protein A (*spa*) and staphylocoagulase (*coa*) coding genes. Two genotypes were obtained for *spa* gene: all of the 21 Sudanese isolates in addition one reference strain being located in one genotype. With *coa* gene, three genotypes were obtained: one genotype contained all of the Sudanese isolates; each of the two reference strains stood alone in its own genotype.

Key words: *S. aureus* subsp. anaerobius, *spa* gene, *coa* gene, sheep abscess disease, genotype

INTRODUCTION

Staphylococcus aureus subspecies anaerobius (*S. aureus* anaerobius) bacteria are the cause of abscess disease or Morel's disease of sheep (Bajmocy *et al*., 1984; de la Fuente *et al*., 1985; Hamad *et al*., 1992) and goats (El-Sanousi *et al*., 1989; Alhendi *et al*., 1993). This organism represents an important animal pathogen in the Sudan. The disease caused by this organism accounted for losses of some millions of US dollars during the nineteenths of the last century because of rejection of whole shipments of animals from Saudi Arabia due to appearance of abscesses in some animals. A vaccine developed against this disease using a local strain could prevent abscess formation in experimentally challenged lambs (Rodwan *et al*., 2004; Musa, 2009), but protection was incomplete in field trials.

One of the assumptions made to interpret this failure was the presence of more than one local strain of *S. aureus* anaerobius. To test this hypothesis, we sought to find possible genetic variations between local isolates of *S. aureus* anaerobius using some marker genes. We report here that all local isolates of *S. aureus* anaerobius seem to refer to one genotype with regard to *spa* and *coa* genes.

MATERIALS AND METHODS

Bacterial strains: Twenty one isolates of *S. aureus* anaerobius were included in this study. Among these isolates, 14 were randomly selected from over 100 tons obtained from superficial lymph node abscesses of sheep at meat inspection in two abattoirs located at different areas of Khartoum State of the Sudan over a period of time of about one year. The remaining seven isolates were obtained from animals during outbreak of Morel's disease in a flock of sheep, as has previously been reported (Musa *et al*., 2007). All isolates were recovered from pus in pure cultures and were identified by standard biochemical methods. For comparison, two reference strains were included: *S. aureus* subsp. anaerobius DSM No. 20714/ ATCC35844 and *S. aureus* subsp. anaerobius No. 9199/2628 (Strains Collection of IBT, University of Gottingen, Germany).

DNA extraction: Genomic DNA was extracted using Axy Prep Bacterial Genomic DNA Miniprep Kit of Axygen (Bioron, Ludwigshafen, Germany) with some modifications of the manufacturer's protocol. In brief, 3-5 colonies from 48 h blood agar culture were suspended in 150 μL of the recommended buffer. Lysis of the cells was

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Table 1: Oligonucleotides used in this study

Primer	Sequence	Gene	Reference
Nuc F	5´GCGATTGATGGTGATACGGTT 3´	Thermonuclease	Brakstad *et al.* (1992)
Nuc R	5´AGCCAAGCCTTGACGAACTAAAGC 3´	Thermonuclease	Brakstad *et al.* (1992)
*Spa*1	5´CACCAGGTTTAACGACAT 3´	X region of protein A	Zschock *et al.* (2000)
*Spa*2	5´CAAGCACCAAAAGAGGAA 3´	X region of protein A	Zschock *et al.* (2000)
*Coa*1	5´CGAGACCAAGATTCAACAAG 3´	Coalgulase gene	Zschock *et al.* (2000)
*Coa*2	5´AAAGAAAACCACTCACATCA 3´	Coalgulase gene	Zschock *et al.* (2000)

achieved by treatment with 10 μL of 1% lysostaphin (Sigma, Taufkirchen, Germany) for 1 h at 37°C followed by addition of 2 μL of 10% Proteinase K (Bioron) at 56°C for 2 h. Other steps were carried out according to the manufacturer's protocol.

PCR reaction mixture: For all PCR procedures, 50 μL PCR reaction mixture contained 3 μL DNA template, 100 pMol of each primer, 45 μL of 1× SuperHOT Mastermix (Bioron, Ludwigshafen, Germany).

***Nuc* gene:** For confirmation of the biochemical identification of the isolates, a conserved region of the thermonuclease gene (*nuc* gene) of *S. aureus* was amplified by PCR using 8 primers (Table 1) and conditions described by Brakstad *et al.* (1992). In brief, PCR mixtures were subjected to initial heating at 94°C for 5 min followed by 37 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 0.5 min, extension at 72°C for 1.5 min and a final step at 72°C for 3.5 min.

***Spa* and *coa* genes:** Primers for the X region of protein A (*spa*) and coagulase (*coa*) variable number of tandem repeats loci of *S. aureus* genome (Table 1) were amplified as described by Zschock *et al.* (2000). Amplification conditions composed of 40 cycles of denaturation at 94°C for 0.5 min, annealing at 55°C for 2 min, extension at 72°C for 4 min and a final step at 72°C for 5 min.

PCR products were subjected electrophoresis in 2% agarose and visualized under UV light and photographed.

RESULTS AND DISCUSSION

This study was conducted to compare between local isolates *Staphylococcus aureus* subsp. anaerobius by molecular methods. Molecular methods are now recognized as more effective than the traditional methods of phenotyping for typing different organisms (Pereira *et al.*, 2002). Isolates used in this study were identified as *Staphylococcus aureus* based on many biochemical tests and were affiliated to the subspecies anaerobius because of negative catalase activity and inability of aerobic growth. With PCR amplification of *nuc* gene, this biochemical identification could be confirmed to the species level only, i.e. as *Staphylococcus aureus*. To the best of the knowledge, differentiation between *S. aureus* subsp. aureus and *S. aureus* subsp. anaerobius at the molecular level can only be made by sequencing of the catalase gene. Complete or partial sequences of the catalase gene of ten isolates used in this study including the reference strain *S. aureus* subsp. anaerobius DSM No. 20714/ ATCC35844 have shown that all of these isolates were *S. aureus* subsp. anaerobius bacteria (Musa, Eltom, Gessler, Bohnel, Babiker and El Sanousi, GenBank No. EU281993, FJ935782-FJ935790, unpublished data). So, all isolates in this study were considered *S. aureus* anaerobius isolates and were subsequently analysed for polymorphism of *spa* and *coa* genes.

Spa and *coa* genes have various numbers of degenerate repeats which are clearly polymorphic in both number and sequence (van Belkum *et al.*, 1998). Both genes have been used for analysis of polymorphism and genetic relationship of *S. aureus* strains in many epidemiological studies. To use these marker genes for typing of the local isolates of *S. aureus* anaerobius, 14 isolates were randomly selected from among >100 tons obtained from lymph node abscesses of sheep at meat inspection. As animals are brought to slaughter houses in Khartoum State from different areas of the Sudan and the isolates were obtained over an extended period of time, at least some of them are likely to represent the whole country. Amplification with primers for *spa* gene yielded two patterns: all of the local strains in addition to one reference strain (*S. aureus* subsp. anaerobius DSM No. 20714) yielded amplicons of ~100 bp, while the other reference strain yielded an amplicon of ~300 bp (Fig. 1). This means that *spa* gene typing could not differentiate between the local isolates and the reference strain *S. aureus anaerobius* DSM No. 207147/ ATCC35844. But, this strain was found distinct from the local isolates with regard of the catalase gene sequence (GenBank accession No. FJ935790) and in RAPD-PCR (Musa, Eltom, Gessler, Bohnel, Babiker and El Sanousi, unpublished data).

Typing with *coa* gene resulted in three different genotypes (Fig. 2): all local isolates being located in one genotype (yielded amplicons of ~550 bp) and each of the two European reference strains (DSM No. 20714 and IBT No. 9199/2628) represented a distinct genotype (yielded amplicons of ~600 and ~800 bp, respectively). These

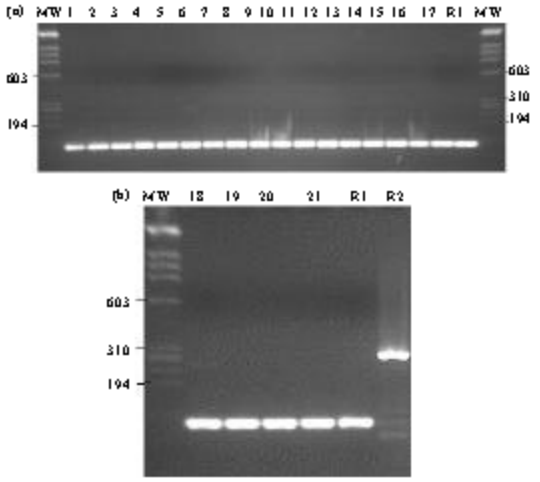

Fig. 1a-b: Two percent agarose gel electrophoresis of PCR products using primers for the *spa* gene of local and reference strains of *Staphylococcus aureus* subspecies anaerobius. MW: Molecular mass marker, λ DNA-Hind III/ΦXHaeIII (Finnzymes, Espoo, Finland). 1-21: Sudan local isolates; R1: *S. aureus* subsp. *anaerobius* from IBT-Gottingen strain collection No. 9199/2628; R2: *S. aureus* subsp. anaerobius DSM No. 20714/ ATCC35844

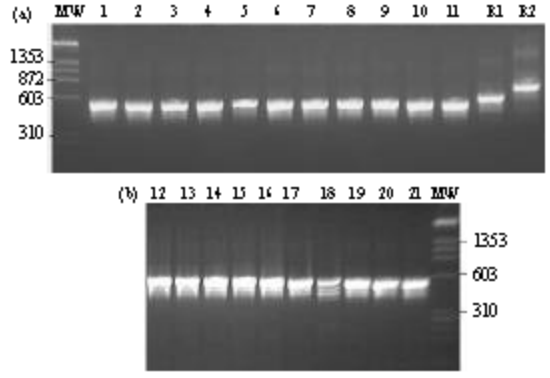

Fig. 2a-b: Two percent agarose gel electrophoresis of PCR products using primers for the *coa* gene of local and reference strains of *Staphylococcus aureus* subspecies *anaerobius*. MW: Molecular mass marker, λ DNA-Hind III/ΦXHaeIII (Finnzymes, Espoo, Finland). 1-21: Sudan local isolates; R1: *S. aureus* subsp. anaerobius from IBT-Gottingen strain collection No. 9199/2628; R2: *S. aureus* subsp. anaerobius DSM No. 20714/ ATCC35844

results show that Sudan local strains of S. aureus anaerobius are more likely to refer to one genotype. Elhaj and El-Sanousi (2005) reported the same observation when they analysed a smaller number of local *S. aureus* anaerobius isolates in PFGE. Importance of these results arises when selecting of a vaccine strain for Morel's disease.

CONCLUSION

Results of this study besides confirming previous investigations that Sudan local isolates of *S. aureus* anaerobius are genetically identical, they suggest the presence of at least three genotypes of *S. aureus* anaerobius with regard of the coagulase gene.

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