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Pulsed-field Gel Elecrophoresis for Comparison of *Staphylococcus aureus* SSP. Anaerobius Local Sudanese Isolates

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Abstract: Local Sudanese isolates of *S. aureus ssp. anaerobius* (which were isolated from sheep abscesses in the qurantene and slaughter house for exported sheep) were compared with the reference strain of *S. aureus* ssp. *anaerobius* on the genetic level by detection of the Restriction Fragment Length Polymorphism (RFLP) by Pulsed-Field Gel Electrophoresis (PFGE). PFGE involves embedding organisms in agarose, lysing the restriction endonucleases that cleave infrequently. Slices of agarose containing DNA fragments are inserted into the wells of an agarose gel and the restriction fragments are resolved into a pattern of discrete bands in the gel by an apparatus that switches the direction of current according to a predetermined pattern. The DNA restriction patterns of the isolates are then compared with one another to determine their relation. PFGE of *S. aureus* ssp. *anaerobius* local Sudanese isolates determined that all local isolates were identical, regarding the RFLP, whereas the reference strain have two bands at different locations, but it considered as same strain.

Key words: Gel electrophoresis, *Staphylococcus aureus*, Sudan

INTRODUCTION

Abscess (Morel's) disease is a problem which threatens Sudan economy, because the disease affects young sheep used for exportation. The disease caused by *S. aureus subsp. anaerobius* which is a respiratory deficient *Staphylococcus aureus*, Fuente and Suarez[1]. However Fuente *et al.*[2] reported a respiratory deficient *S. aureus* that has a cell wall typical to *S. aureus* ATCC 12600 and DNA-DNA hybridization indicated that the organism was very closely related to *S. aureus* at the species level.

Because of the failure of this organism to grow aerobically and to produce catalase and benzidine and for the aetiological importance of this organism, they classified it as *S. aureus* sp. *anaerobius*. This name was also adopted in Bergys manual of determinative bacteriology ninth edition[3].

MATERIALS AND METHODS

Bacteria: Six local Sudanese isolates of *S. aureus* sp. *anaerbius*, isolated from sheep abscesses were used in this study, with a reference strain ATCC 35844 DSM (Deutsche SammLung von Microorganismen und Zelkulturen, Braunschweig, Germany) No. 20714[2].

Preparation of chromosomal DNA: The protocol for the preparation of chromosomal DNA was done according to Bannerman *et al.*[4] who used a modified protocol from that described by Goering and Winters[5].

The cultures were grown in 5 mL brain heart broth for 48 h at 37.0°C on 5% Co_2 0.7 mL of these cultures were harvested by centrifugation (2000 g for 2 min) in a microcentrifuge tubes. Cells were washed once in 1 mL of autoclaved TEN buffer (0.1 M Tris Cl, 0.15 M NaCl, 0.1 M EDTA) and centrifuged again. The washed cells were resuspended in 0.3 mL of autoclaved EC buffer (6 mM Tris Cl, 1 M NaCl, 0.1 M EDTA, 0.5% Brij 58, 0.2% deoxycholate, 0.5% Lauryl sarosine).

Two microliter of a 1 mg mL^{-1} solution of lysostaphin dissolved in 20 mM sodium acetate was added to the cell suspension and the mixture was subsequently vortexed. Three hundreds microliter of 2% Sea Plaque agarose dissolved in EC buffer was added to the lysostaphin-cell suspension. The suspension was briefly vortexed and quickly pipetted into a plug molds.

The plugs were allowed to solidify at room temperature for 10 min. After solidification, the plugs were placed in a tube containing 3 mL of EC buffer and the cells in the plugs were lysed for 1 h at 37.0°C without shaking. After that the EC buffer was removed and replaced with 3 mL of autoclaved TE buffer (10 mM Tris Cl, 5 mM EDTA) and the tubes were incubated for 1 h at 55.0°C without shaking. The plugs were then transferred to 3 mL fresh TE buffer and stored at 4.0°C for further use.

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Electrophoresis of samples: For electrophoresis one plug was placed in 125 µL total restriction enzyme mixture (restriction buffer+sterile distilled water) containing 20 U of SmaI. After overnight incubation at 25.0°C without shaking, chromosomal restriction fragment patterns were analysed by loading the plugs into a well of a 1% SeaKem agarose running gel. The running gel was prepared in 10x TBE buffer (890 mM Tris Cl, 20 mM EDTA, 890 mM Boric acid). The wells containing the plugs were sealed with the running gel.

Electrophoresis was performed with the SHEF-DR III system electrophoresis cell (Bio-Rad). Bacteriophage lambda DNA concatemer (Bio-Rad) was used as size standard and served as a control for the running parameters.

The running parameters were as follows: initial pulse 5 seconds final pulse 40 seconds, voltage 200 or 6V cm, time 20 hours and temperature 12-14°C. The gels were stained with ethidium bromide and photographed.

Interpretation of PFGE banding patterns: The interpretation of the banding patterns was done visually according to the following guidelines: (i) banding patterns from the majority of epidemiologically related isolates that appeared identical in size and number of bands were considered to represent the same strain (Modal pattern), which was designated by strain A (ii) isolates banding patterns that differed from the main pattern because of one or two genetic events were considered as subtypes within the main group (e.g. A_1, A_2, A_3, etc) and (iii) isolates banding patterns that differed from the main pattern by four or more bands which could not be explained by at most two genetic events were considered as different strains (Fig. 1).

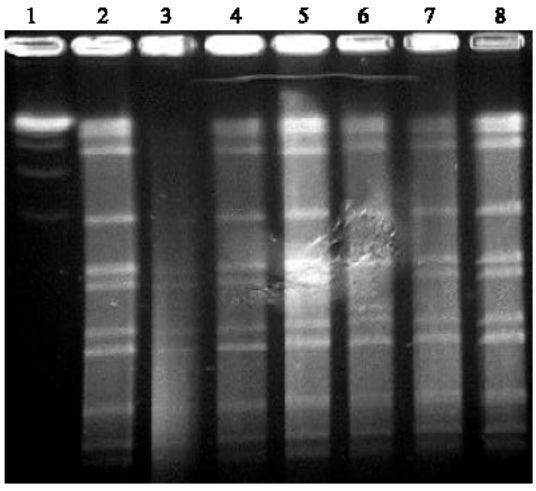

Fig. 1: PFGE patterns from different isolates of *S. aureus* sp. *anaerobius*. Molecular size standard (lambda oligomer) is in lane 1. Lane 2 contain strain 1, lane 3 contain strain 2, lane 4 contain strain 3, lane 5 contain strain 5, lane 6 contain reference strain, lane 7 contain strain 4 and lane 8 contain strain 11

RESULTS

Pulsed Field Gel Electrophoresis (PFGE) was used to compare (RFLP) of the local isolates with the reference strain. Generally no restriction fragments length polymorphism (RFLP) was detected comparing the local isolates. However, the reference strain shows two bands at different locations (Fig. 1), but all are considered in one group according to Bannerman *et al*.[4].

DISCUSSION

Comparing the DNA restriction patterns of the different local isolates and the reference strain of *S. aureus* sp. *anaerobius* by (PFGE), it was found that the restriction patterns were similar in all local isolates, in regard to reference strain it had the same pattern, but showed two bands at different locations, this indicates, that they are all involved in one strain, but the Sudanese isolates are genetically identical. Present result gives the possibility that all isolates from the same locality can be genetically identical.

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