Journal of Animal and Veterinary Advances 4 (12): 962-964, 2005

SDS-Polyacrylamide Gel Electrophoresis for Comparison of *S. aureus subsp. anaerobius* Local Sudanese Isolates and the Reference Strain ATCC 35844

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Abstract: Six local Sudanese isolates of *S. aureus subsp. anaerobius* which were isolated from sheep abscesses were compared with the reference strain of *S. aureus subsp. anaerobius* ATCC 35844 DSM (Deutsche Sammlung von Microorganismen und Zelkulturen, Braunschweig, Germany) No. 20714 in their protein profiles to choose the candidate local isolate for vaccine production.Vertical electrophoresis was used to separate protein of cell lysates and supernatants of the local Sudanese isolates and the reference strain of *S. aureus subsp. anaerobius*. It was found that the electrophoretic protein pattern for all local isolates and the reference strain resembles each others to a greater extent and many bands are common in all strains. Cell lysates of all local isolates (except isolate 5) and the reference strain had protein bands ranged between 19-24 with molecular mass ranging from 53.35-113.84 KDa. Isolate 5 have 17 protein bands with molecular mass ranging from 53.62-74.89 KDa. All local isolates and the reference strain had no protein bands in their supernatants, except isolate 2 which has 5 protein bands in its supernatant. Most of the local isolates are the same as the reference strain, two local isolates have minor differences compared to the reference strain.

Key words: Electrophorsis, protein, local sudanese isolation, strain

INTRODUCTION

Staphylococcus aureus subsp. anaerobius was recently reported by[1] as respiratory deficient *S. aureus* that has a cell wall typical to *S. aureus* ATCC 12600, also DNA –DNA hybridization indicated that the organism was very closely related to *S. aureus* at the species level. However, because of biochemical distinctiveness (Catalase and benidizine negative and negative aerobic growth) and the aetiological importance of this organism, they classified it as *S. aureus subsp. anaerobius*. This name was also adopted in Bergy's manual of determinative bacteriology ninth edition[2].

Karamalla, 1997[3] reported that among eleven staphylococcal species only *S. aureus subsp. anaerobius* had 31 protein bands. It had eleven protein bands overlapping and ranging between 45-67 KDa. These bands are characteristic of *S. aureus subsp. anaerobius*, indicating that, these cell wall proteins may have significant role in pathogenicity of the organism.

MATERIALS AND METHODS

Buffers and reagents for polyacrylamide gel electrophoresis were prepared according to[4].

Preparation of the strains: Seven strains of *Staphylococcus aureus subsp. anaerobius* were used; six were local Sudanese isolates and the seventh one is the reference strain. The strains were cultivated in tubes containing 7 mL brain heart broth, incubated at 37°C under 5% CO_2 for 48 h after which the tubes were centrifuged at 500 g for 15 min and the cell pellets were used.

Preparation of cell lysates: Lysis of *S. aureus subsp. anerobius* cells was done by lysostaphin. Bacterial lysates were prepared according to[5]. The cell pellets were resuspended in 3 mL extraction buffer, which consists of 50 mM Tris/HCL, pH 8.0, 0.5mM EDTA, 50 μg Sodium azide per mL and 0.2 mg lysostaphin per mL. Suspensions were incubated at 37°C for 15 min, then centrifuged at 5000 g for 1 h. The supernatant was retained and stored at –20°C for further use.

Preparation of bacterial supernatants: Cultures of *S. aureus subsp. anaerobius* were centrifuged at 500 g for 10 min; the supernatants were stored at –20°C for further use.

Casting of the gel: The gel cassette consists of a glass plate, a notched aluminium oxide plate, two spacers and a comb (BIO-RAD). The glasses were prepared, fixed on the stand by clamps and assured that at the down side the two glasses are at the same level. The separating gel mixture was prepared after fixing the glass slides and was

immediately and slowly poured from one end until the cavity was about ¾ full. The gel mixture was left for 30 min to polymerize, the surface of the gel was covered with 96% ethanol to avoid drying, which was poured off before casting the stacking gel.

Immediately after preparing the stacking gel, the comb was fixed over the separating gel, pulled off at one end, then the stacking gel poured slowly over the polymerized separating gel until the cavity is filled and left for 15 min to polymerize, then the comb was carefully removed and the formed wells were covered with electrophoretic (running) buffer to prevent drying.

Electrophoresis of samples: The gel cassette was transferred to the electrophoresis chamber, which was filled with the electrophoretic (running) buffer, the samples which were mixed with the stain by equal volumes were poured slowly in the wells in amounts of 16-20 µl per well. In the first well the BIO-RAD SDS-PAGE standard protein was poured in an amount of 1.0 µl. Samples were electrophoresed using BIO-RAD POWER/PAC 3000 device adjusted to program 1 (100/5 min., then 200/40 min. and in the final stage 100/5 min.).

Silver staining protocol for proteins: Silver stain was used to visualize the protein bands. Electrophoresed gels were released from the cassette and transferred to the Hoeffer Automated Gel Stainer (PHARMACIA BIOTECH), which was connected to flasks containing different solutions of silver stain protocol. The protocol comprised the steps, fixation (30 min.), sensitization (30 min.), washing (3x5 min.), staining (20 min.), washing (2x1 min.), developing (2-5 min.), washing (3x5 min.) and preserving (2x30 min.). Stained gels were photographed and kept as a reference. The molecular masses of separated proteins were determined with a PC utilizing a software RFLPscan Plus 3.0.

RESULTS

The electrophoretic protein patterns of the different *S. aureus subsp. anaerobius* isolates are presented in (Fig. 1). The analysis revealed indistinguishable protein patterns with 19-24 bands in the molecular mass range between 53.35-113.84 KDa. Isolate (5) shows 17 protein bands with molecular weights ranging from 53.62-74.89 KDa.

No protein bands were detected in the supernatants of the reference strain and all local isolates except in isolate (2) which shows 5 protein bands, having low molecular weights ranging from 21.27 – 51.89 KDa.

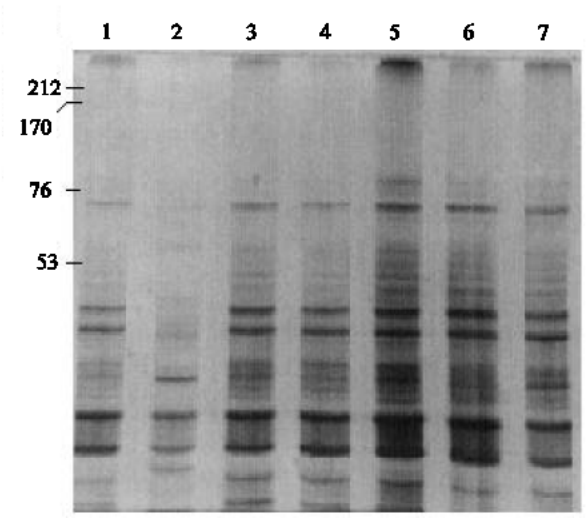

Fig. 1: Elecrophoretic patterns of protein of S. *aureus subsp. anaerobius* isolates. Lane 1 (isolate no.1), lane 2 (isolate no. 2), lane 3 (isolate no. 3), lane 4 (isolate no. 4), lane 5 (isolate no. 5), lane 6 (reference strain), lane 7 (isolate no.11). Molecular mass markers are indicated.

DISSCUSSION

The study done concering *S. aureus subsp. anaerobius* was very little and there was no study in the determination of its proteins, only the study done by[3], in which she compared protein bands of *S. aureus subsp. anaerobius* with other Staphylococcus species causing abscesses.

This study revealed that the electrophoretic protein patterns of all local isolates and the reference strain are mostly the same with few differences such as in isolate 5 which have lesser protein bands with low molecular weight and isolate 2 which has 5 protein bands in its supernatant.

The protein profile of these local isolates resembled that was found by[3].

From the above any local isolate can be used as a candidate for vaccine production, with special reference to isolate 2 which has protein bands in its supernatant,hence developing toxin.

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