Induction of Abscess by Non-abscess Producer Staphylococci Using Staphylococcal Fatty Acid Modifying Enzyme (FAME)

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Abstract *Staphylococcus epidermidis* (*S. epidermidis*), *S. warneri* and *S. aureus subsp. anaerobius* were isolated from sheep abscess. Crude FAME was extracted from supernatant fluid of a 24hrs culture of *S. aureus subsp. anaerobius* grown in Trypticase Soy Broth at 37°C with continuous agitation. Fourteen heads of sheep were used. Three of them were inoculated with *S. epidermidis* + FAME and other three were inoculated with *S. warneri* + FAME. Eight animals were divided into four groups, two animals in each, used as control and inoculated with *S. epidermidis*, *S. warneri*, *S. aureus subsp. anaerobius* and FAME. All animals were inoculated subcutaneously at the neck region. After fourteen to eighteen days, sites inoculated with *S. epidermidis* + FAME, *S. warneri* + FAME and *S. aureus subsp. anaerobius* showed abscesses grossly then confirmed bacteriologically and histopathologically, while those inoculated with *S. epidermidis* alone, *S. warneri* alone and FAME showed no abscess.

Keywords: *Staphylococcus*, abscess, fatty acid modifying enzyme

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1. Introduction

Abscess disease in sheep has a great economical importance in Sudan. It is caused by *S. aureus subsp. anaerobius* and characterized by superficial lymph nodes enlargement. For the organism to survive and multiply in the host tissue and causes abscess it must produce an esterifying enzyme known FAME which inactivates the bacteriocidal fatty acids [4,5,7]. In natural abscess disease, many *Staphylococcus spp.* have been isolated. Some of them were known to produce FAME but unable to form abscess; from these *S. epidermidis* and *S. warneri* (coagulase-negative Staphylococci) are examples [6]. FAME activity was detected in 54% of CNS and 80% *S. aureus* strains surveyed but none in *E. coli* or *S. uberis*. While some CNS strains produced FAME activity comparable to the lab strain of *S. aureus*, the pattern of FAME activity varied among strains and across species of staphylococci [9].

S. aureus FAME has a pH optimum between 5.5-6.0 and a temperature optimum of about 40°C. Expression of FAME is controlled genetically by the global regulators Agr and Sar [2]. FAME has an extracellular activator which can increase FAME expression and activity when added to the FAME-producer *Staphylococcus* culture [3].

2. Materials and Methods

2.1. Preparation of Organisms

Abscesses were collected from sheep for isolation of *S. epidermidis*, *S. warneri* and *S. aureus subsp. anaerobius*. The isolates were identified according to [1,8]. *S. aureus subsp. anaerobius* was grown microaerophilically in ten ml nutrient broth at 37°C overnight while, *S. epidermidis* and *S. warneri* were grown aerobically in 10 ml nutrient broth at 37°C overnight. Serial ten-fold dilution from 1 ml of each culture was made and inocula from dilutions 10⁻¹ (containing about 10⁴ bacterial cells) and 10⁻³ (containing about 10³ bacterial cells) were used.

2.2. Preparation of Crude FAME

Crude FAME was prepared according to the method of Long [6]. *S. aureus subsp. anaerobius* was grown microaerophilically in 100 ml of Trypticase Soy Broth (TSB) at 37°C for 18 hrs. with continuous shaking. The culture was centrifuged at 3000 r.p.m. for 30 minutes using cold centrifuge and the supernatant fluid was sterilized by filtration using 0.22 µm. filter membrane. The culture filtrate was concentrated to half volume at 4°C using dialysis tubes and carbowax.

2.3. Inoculation of Experimental Animals (Sheep)

Fourteen healthy fattened sheep were clipped and divided into groups then labeled as follows:

Group (1): Three animals were inoculated with 0.5ml of dilution 10^{-1} of *S. epidermidis* + 0.5ml of FAME and 0.5ml of dilution 10^{-1} of *S. epidermidis* + 1.0ml of FAME at each site of the neck region.

Group (2): Three animals were inoculated with 0.5ml of dilution 10^{-1} of *S. warneri* + 0.5ml of FAME and 0.5ml of dilution 10^{-1} of *S. warneri* + 1.0ml of FAME at each site of the neck region.

Group (3): Eight animals were divided into four equal subgroups (3a-3d) which were inoculated with: 0.5 ml of dilution 10^{-1} of *S. epidermidis*, 0.5 ml of dilution 10^{-1} of *S. warneri*, 0.5 ml of FAME and 0.5 ml of dilution 10^{-3} of *S. aureus subsp. anaerobius*, respectively and kept as control.

All animals were inoculated S/C at the neck region, followed up daily and any clinical observations were recorded. After fourteen to eighteen days they were slaughtered and samples were collected for bacteriological examination. Sections from lesions were fixed in 10% neutral formalin for histopathological examination.

3. Results

Inoculation of sheep with treated bacteria resulted in abscess formation (Figure 1 and Figure 2). Transient increase in body temperature and mild tissue reaction at the inoculation sites were observed in the first three days of inoculation. At day six some swellings developed into abscesses which ruptured by day eight (Figure 3 and Figure 4). Other swellings remained closed until incised at postmortem. The control sites which were injected with *S. epidermidis*, *S. warneri* and FAME revealed in no swelling or caseation (Table 1).

Figure 1. Subcutaneous abscess in sheep experimentally infected with *S. epidermidis* plus FAME

Figure 2. Subcutaneous abscess in sheep experimentally infected with *S. warneri* plus FAME

Table 1. show the comparison of abscess sizes (cm.) obtained from addition of FAME to *S. epidermidis* and *S. warneri* with their control positives

Animal No. group (1 and 2)	0.5ml of FAME + 0.5ml of <i>S. epidermidis</i>	0.5ml of FAME+ 0.5ml of <i>S. warneri</i>	0.5ml of <i>S. epidermidis</i> Alone	0.5ml of <i>S. warneri</i> Alone
1	2,1	0.6	≥ 0.1	≥ 0.1
2	1.2	1.0	≥ 0.1	≥ 0.1
3	1.2	0.8	-	-

Figure 3. Skin: Image (73,4) shows abscess formation at the site of *S. epidermidis* plus FAME injection And image (73,5) shows Injection site of *S. epidermidis* alone

Figure 4. Abscess formation at the injection site of *S. warneri* plus FAME

Figure 5. Skin: Subcutaneous tissue showing a pyogranuloma (H and E x 100) (section from site injected with *S. epidermidis* plus FAME)

Figure 6. Skin: Subcutaneous tissue showing multiple abscess formation (H and E x 100). (Section from site injected with *S. warneri* plus FAME)

Figure 7. Section showing dermal abscesses (H and E x 100). (section from site injected with *S. epidermidis* plus FAME)

Figure 8. Skin section showing slight dermal edema (H and E x 100) (section from site injected with FAME alone)

Results were confirmed histopathologically by the presence of abscess in sites inoculated with *S. epidermidis* plus FAME and *S. warneri* plus FAME. A chronic pyogranulomatous reaction was seen; composed of dense collection of mononuclear, lymphocytes and

plasma cells with neutrophils in the centre. Most sections showed dermal edema, fibrosis, mononuclear infiltration (observed also perivascularly), subcutaneous muscle degeneration and necrosis with widened and fibrosed intermuscular connective tissues. Pyogranulomas and

wide suppuration were seen in deep dermis and subcutaneous tissues. In some sections the granulomatous reaction was not severe and other one showed dermal haemorrhage (Figure 5, Figure 6 and Figure 7). FAME injected sites showed no significant gross change and histopathologically there was dermal edema. Subcutaneous muscles stained pale and some muscle fibers showed intense eosinophilia (Figure 8).

4. Discussion

Previous studies about abscess formation showed that, organism to form abscess and causes the disease must possess two enzymes: FAME and lipase. Those studies explained the role of FAME in abscess formation in vitro.

Studies in Sudan were conducted on different aspects of the disease but there is no work being published on bacterial enzymes specially FAME; the main factor of abscess formation.

As shown in this study, abscess could be generated in animals (sheep) experimentally by non-abscess producer *Staphylococcus* when FAME was added. These results confirmed the findings reported by [4,5,7], who assured that, FAME is an important factor in the process of abscess formation. Other work conducted on extracellular activator of FAME explained that addition of *S. aureus* activator to *S. epidermidis* cultures increased FAME production but there was no increase or inhibition in FAME production when *S. epidermidis* activator was added to *S. aureus* cultures [3]. Also other report showed that, some CNS strains produced FAME activity comparable to the lab strain of *S. aureus*, while the pattern of FAME activity varied among strains and across species of staphylococci [9]. In Sudan also found that injection of *S. epidermidis* alone (isolated from Sheep abscess) could not produce abscess but when inoculated with other bacteria (*S. aureus subsp. anaerobious*) abscess was produced (Unpublished data). Three later observations emphasized the present result.

The results of the present study suggest that, *S. epidermidis* FAME is non-functional and abscess formed due to *S. aureus subsp. anaerobious* FAME or it was activated by *S. aureus* activator.

5. Conclusion and Recommendation

We concluded that FAME is an important factor in abscess formation, and non-abscess producer *Staphylococcus* FAME can be activated, so as to get rid of the disease must inactivate the FAME. Further researches must be conducted for more information.

Competing Interests

The authors declare that they have no competing interests.

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