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Investigation of Toxin Genes of *Staphylococcus aureus* Strains Isolated from Gangrenous Mastitis in Ewes

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Summary

In this study, it was aimed to determine staphylococcal enterotoxin (SE) genes, toxic shock syndrome toxin (TSST) gene and exfoliative toxin (ET) genes in 110 *Staphylococcus aureus* strains from gangrenous mastitis cases in seven ewe flocks in Sanliurfa, Turkey. Among investigated isolates, only sec and tst toxin genes were detected. The results of this study showed that the rates of enterotoxin production were high (100%) in *S. aureus* strains isolated from ovine gangrenous mastitis. Moreover, it was demonstrated that *S. aureus* causing gangrenous mastitis harboured sec and tst genes suggesting that these genes may have a role in ovine mastitis pathogenesis.

Keywords: Gangrenous mastitis, Ewe, *Staphylococcus aureus*, Toxin genes

Koyunlarda Gangrenli Mastitislerden İzole Edilen *Staphylococcus aureus* Suşlarının Toksin Genlerinin Araştırılması

Özet

Bu çalışmada, Şanlıurfa yöresinde, yedi koyun sürüsündeki gangrenli mastitis vakalarından izole edilen 110 *Staphylococcus aureus* suşlarında stafilokokal enterotoksin, toksik şok sendrom toksin ve eksfoliyatif toksin genleri bakımından araştırılması amaçlandı. İncelenen suşlarda yalnızca sec ve tst toksin genleri saptandı. Bu çalışmanın sonucunda koyunlarda gangrenöz mastitislerden izole edilen *S. aureus* suşlarında enterotoksin üretiminin yüksek (%100) olduğu, izole edilen *S. aureus* suşlarının sec ve tst genlerini içerdiği ve bu genlerinde koyunlarda mastitisin patogenezisinde rol oynayabileceği kanısına varıldı.

Anahtar sözcükler: Gangrenöz mastitis, Koyun, *Staphylococcus aureus*, Toksin genleri

INTRODUCTION

Mastitis is an important disease with serious economic consequences, such as reduced milk yield leading to decreased growth of lambs, cost of treatment associated with clinical mastitis and culling due to permanent udder damage in ewes ¹. In severe cases, when gangrene developed in the mammary gland, infection may result in death of ewe. Therefore, mastitis has a major impact on both economy and animal welfare in sheep industry ².

Infectious mastitis outbreaks of small ruminants are usually caused by Gram positive bacteria, mostly

Staphylococcus spp. ^{1,3}. *S. aureus* is frequently isolated from clinical mastitis cases. *S. aureus* can produce several virulence factors contributing to its pathogenicity ⁴. Staphylococcal enterotoxins (SEs) play an important role in staphylococcal diseases, also pose risk for public health due to their ability to cause food poisoning. Toxic shock syndrome toxin-1 (TSST-1) is another toxin produced by *S. aureus*, which cause toxic shock syndrome in humans ⁵. Also, some *S. aureus* strains that produce one or both of two immunologically distinct exfoliative toxins (ETs), ETA and ETB, have been associated with a series of



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impetiginous staphylococcal diseases in humans, referred to as staphylococcal scalded skin syndrome ⁶.

In Turkey, there is no study investigating toxin genes in *S. aureus* isolated from ewe gangrenous mastitis cases. Thus, we aimed to determine the presence of the SAg genes by polymerase chain reaction (PCR) in *S. aureus* strains isolated from gangrenous mastitis cases in ewes.

MATERIAL and METHODS

The study was carried out in 2009 and 2010. Udder secretions were collected from 163 ewes with gangrenous mastitis, belonging to 7 flocks in Sanliurfa, Turkey. Each flocks located at a distance of 15-150 km from each other and consisted of about 4.000 dairy Awassi sheep. All flocks were grazed, with some additional concentrate during spring, summer and fall. Ewes were mostly housed during winter and fed wheat straw, barley grain and wheat bran. The ages of the ewes ranged from three to four years and mean milk yield of the ewes were 150- 225 kg per lactation period of 150 days. All ewes lambed between December and January, and the lambs were kept with their dams for 6-8 weeks. Clinical examinations were performed during full lactation between December and April. Gangrenous mastitis was diagnosed with clinical examination by observation of cold and blue skin of udder and teat as well as presence of blood in the milk sample.

A minimum of 5 mL milk from one udder lobe was collected in a sterile container, after cleaning the teat with denatured 90% ethanol and discarding the first drops of the secretion. Samples were refrigerated and subjected to microbiological examination within 24 h after sample collection. For microbiological examination, 10 µL of sample were inoculated in 5% sheep blood agar and incubated at 37°C for 24-48 h. Identification of *S. aureus* was performed by inspection of colonies for presence of haemolysis in 5% blood agar, growth and fermentation in Mannitol Salt Agar (MSA), production of free coagulase (BBL Coagulase Plasma Rabbit with EDTA, Becton Dickinson, Heidelberg, Germany) and presence of clumping factor (Staphylase Test, Oxoid, Cambridge, UK) ⁷. Isolates of *S. aureus* were examined for toxin genes.

DNA Isolation and PCR Amplification of Toxin Genes

Bacterial DNA was extracted from *S. aureus* isolates as reported previously by Sambrook et al.⁸. Amplification of toxin genes *sea-see* and *eta-etb*, *tst* were performed by using primers reported by Johnson et al.⁶, and those of *seg-sei* reported by Monday and Bohach ⁹. The strains for which specific PCR products of expected size were obtained were considered positive. The PCR products were separated by electrophoresis on 2% agarose gel, visualized under ultraviolet light after staining with ethidium bromide.

RESULTS

Of the toxin genes investigated, *sec* was detected in all *S. aureus* isolates, while 97.3% of the isolates harboured *tst* (Fig. 1). None of the isolates harboured other toxin genes.

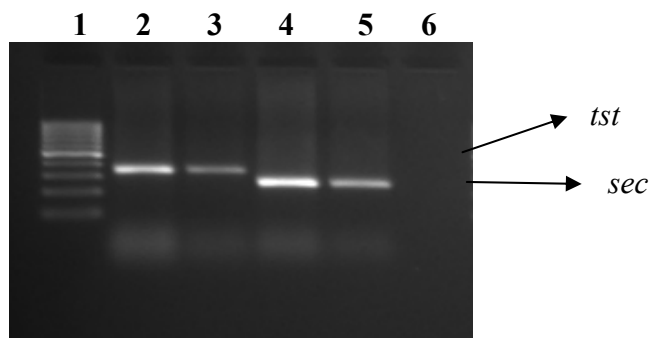


Fig 1. Agarose gel electrophoresis of *sec* and *tst* genes

Lane 1: 100 bp DNA ladder, Lane 2-3: *tst* (350 bp); Lane 4-5: *sec* (257 bp); Lane 6: Negative control

Şekil 1. *sec* ve *tst* genlerinin agaroz jel elektroforezi

Sütun 1: 100 bp DNA ladder, Sütun 2-3: *tst* (350 bp), Sütun 4-5: *sec* (257 bp). Sütun 6: negatif kontrol

DISCUSSION

SEC and TSST-1 belong to the SAg family, and are responsible for a variety of human and animal diseases. Although many types of toxin genes have been reported in *S. aureus* isolated from ewe mastitis cases, *sec* was the most encountered one in those studies ^{10,11}. In agreement with the findings of those authors, *sec* was detected in all isolates in this study. *tst* gene was detected together with *sec* gene in 107 (97.3%) isolates in this study. A positive correlation between *sec-tst* genes has been reported in previous studies ^{11,12}. Co-existence of these genes could be explained by presence of *sec* gene on a pathogenicity island together with *tst* ¹³. In current study, all *S. aureus* strains isolated from ovine gangrenous mastitis harboured both *sec* and *tst* genes. Approximately 70% of the isolates from milk of mastitic cows in Japan possessed these toxin genes ¹⁴. Therefore the results indicated that superantigenic toxins of *S. aureus* might play an important role in ovine gangrenous mastitis. The role of these toxins in the udder pathogenicity remains unclear. However Ferens et al.¹⁵ have reported that the superantigens of *S. aureus* possibly inhibit the immune response in cows. The pathogens can escape or efficiently inhibit the immune response during the infection and continue to survive in the host.

Many authors studied the presence of exfoliative toxin genes among *S. aureus* isolates from bovine mastitis and have reported a rare prevalence of these toxins ^{16,17}. Absence of these genes in the *S. aureus* isolates in the

present study confirms that these genes are rare in *S. aureus* isolates from ewe mastitis cases.

In conclusion, this study indicated that *S. aureus* strains isolated from ovine gangrenous mastitis carried *sec* and, to large extent, *tst* toxin genes. Further studies are needed to clarify possible role of these toxin genes in the pathogenesis of gangrenous mastitis in ewes.

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