Original Article

Investigation into toxin and slime genes in staphylococci isolated from goat milk and goat cheese in southern Turkey

Sevda Pehlivanlar Onen[1] Zafer Cantekin[2*] Faiza Mebkhout[3] Osman Aygun[4] Yasar Ergun[5]

Abstract

The aim of this study was to investigate the presence of *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) isolated from goat milk and cheese, as well as their toxin genes and slime genes. *S. aureus* and CNS isolates were subjected to polymerase chain reaction (PCR) analyses to determine the prevalence of enterotoxin (*sea*, *seb*, *sec*, *sed*, *see*), toxic shock syndrome (TSS) toxin (*tst*), exfoliative toxin (*eta* and *etb*) and slime genes (*icaA* and *icaD*). *sec*, *sed* and *tst* genes together were detected in 4 (33.3%), *sec* + *tst* genes in 2 (16.7%) and *tst* gene in 1 (8.3%) of the 12 *S. aureus* isolates. However, *see* gene was detected in 6 (9.7%), *sed* gene and *tst* gene were detected in 1 (1.6%) and 7 (11.3%) of the 62 CNS isolates, respectively. No *sea*, *seb*, *see*, *eta* or *etb* genes were detected in the *S. aureus* isolates, whereas no *sea*, *seb*, *sec*, *eta* or *etb* genes were detected in the CNS isolates. At least one *ica* gene was present in all *S. aureus* isolates and in 36 of the 62 CNS isolates. The presence of *icaA* and *icaD* genes in the *S. aureus* isolates was clearly higher than those in the CNS isolates. In conclusion, toxin and slime genes were detected in the *S. aureus* and CNS isolates from goat milk and goat cheese. The potential risk of enterotoxigenic CNS should not be ignored as well as *S. aureus* in food safety and public health.

Keywords: *Staphylococcus aureus*, staphylococci, toxin genes, goat milk, goat cheese

[1]*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Hatay, Turkey*
[2]*Department of Microbiology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Hatay, Turkey*
[3]*École Nationale Supérieure Vétérinaire d'Alger, Algiers, Algeria*
[4]*Program of Food Technology, Keban Vocational School, Firat University, Elazig, Turkey*
[5]*Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Hatay, Turkey*
***Correspondence**: zcantekin@hotmail.com*

Introduction

Staphylococci are the most commonly isolated microorganisms in food poisoning in the world (Podkowik et al., 2013). Food contamination with staphylococci is associated with improper handling and protection of foods under conditions that allow growth of staphylococci and production of enterotoxins. Staphylococcal food poisoning results from the consumption of sufficient amounts of foods containing staphylococcal enterotoxins produced by *S. aureus* (Le Loir et al., 2003; Argudin et al., 2010). Meat, fish and poultry products, milk and dairy products, egg products, salads and cream-filled pastries and cakes are generally considered to be among the foods implicated with staphylococcal food poisoning (Balaban and Rasooly, 2000; Argudin et al., 2010). However, some coagulase-negative staphylococci (CNS), such as *S. xylosus* and *S. carnosus*, are considered as food-grade in some fermented meat and milk products (Even et al., 2010). Their presence or use in foods is a source of concern for human health because they are opportunistic pathogens in certain clinical situations in humans and animals (Kloos and Bannerman, 1994; Irlinger, 2008). There are no clear results regarding the involvement of CNS in staphylococcal food poisoning, however, the ubiquitous prevalence of CNS in ruminants and/or in milk raises a number of concerns (Podkowik et al., 2013).

On the other hand, staphylococci are the most commonly isolated microorganisms from goat milk from subclinical mastitis cases (Doğruer et al., 2016; Cantekin et al., 2016). It has also been stated that goats are reservoirs for staphylococci (Valle et al., 1990). The pathogenicity of CNS differs from that of *S. aureus*; however, both survive in a range of environments, such as foodstuffs, medical equipment and clinical samples (Coton et al., 2010).

Staphylococcal enterotoxins (SE) are divided into two groups: major enterotoxins (A, B, C, D and E) and minor enterotoxins (G, H, I, J, K, L, M, N, O, P, Q, R and U). Major enterotoxins are responsible for 95% of food poisoning cases (Letertre et al., 2003). Superantigen toxins, such as toxic shock syndrome (TSS) toxin-1 and staphylococcal enterotoxins B and C, produced by methicillin-resistant *S. aureus*, have been implicated in staphylococcal-induced TSS (McCormick et al., 2001). Toxic shock syndrome, which is initially characterized by fever, hypotension and rash, can cause multiple organ failure and lethal shock. Exfoliative (epidermolytic) toxins (ET) are also produced by *S. aureus* and can lead to staphylococcal scalded skin syndrome, a type of blistering skin disease (Ladhani et al., 1999). Slime production is an important virulence factor in staphylococcal infections (Vasudevan et al., 2003; Namvar et al., 2013). It is responsible for adherence of microorganisms, resistance of isolates to antibiotics and resistance of microorganisms to the host's immune defence system. Slime production is encoded by intercellular adhesion (*ica*) genes, which are detected by the presence of *icaA*- and *icaD*-specific primers (Namvar et al., 2013).

The economic importance of local products such as goat milk and cheese produced in southern Turkey is increasing (Kamber, 2007; Yasan Ataseven and Gülaç, 2014). In this region, milking of goats is still done by hand, and local cheese producers utilize bulk tank milk in the production of goat cheese. Cheese production from goat milk is mostly done using traditional methods, and goat cheeses are generally sold at local markets or outdoor bazaars (Yasan Ataseven and Gülaç 2014; TUIK, 2017). Hence, there is always the risk of using mastitic milk in the manufacture of dairy products. Although *S. aureus* and *CNS* have long been considered important agents in udder health, *CNS* were not thought to pose a threat to food safety (Bergdoll, 1983; Marrack and Kappler, 1990). Therefore, there is a need to detect enterotoxin genes in *S. aureus* and *CNS* in goat dairy products in terms of consumer health. The aims of this study were to detect and compare enterotoxins, (*sea*, *seb*, *sec*, *sed* and *see*), TSS toxin (*tst*), exfoliative toxins (*eta* and *etb*) and slime genes (*icaA* and *icaD*) in *S. aureus* and *CNS* isolates from goat milk and goat cheese.

Materials and Methods

Isolation and identification: Staphylococci were isolated from goat bulk milk samples and goat cheese samples by classical microbiological methods and identified by using VITEK 2, as described in previous study (Pehlivanlar Onen and Aygun, 2017). Briefly, 10 g of sample was taken in aseptic conditions and homogenized with 90 mL sterile peptone water for approximately 2 min. Prepared decimal dilutions of samples were plated on the Baird Parker Agar and incubated for 18-24 h at 37 °C. All typical black grey, bright, convex colonies were transferred to Brain Heart Infusion Broth and incubated for 18-24 h at 37 °C. *S. aureus* and CNS were identified by using Gram positive (GP) identification card (BioMerieux GP 21342, France) in VITEK 2 automated system after Gram staining, catalase, oxidase and coagulase tests. The staphylococcal isolates examined in the present study were randomly selected from isolates obtained in the above mentioned study (Anonymous, 1999).

DNA extraction: In total, 74 staphylococci isolates consisting of 12 *S. aureus* and 62 CNS isolates were examined by PCR analysis to determine the prevalence of enterotoxin (*sea*, *seb*, *sec*, *sed* and *see*), TSS toxin (*tst*), exfoliative toxin (*eta* and *etb*) and slime genes (*icaA* and *icaD*) (Table 1). The 12 *S. aureus* isolates were from two milk (2 isolates) and five cheese (10 isolates) samples. The 62 CNS isolates were from 34 milk (34 isolates) and 28 cheese (28 isolates) samples. The following strains were used as positive controls: ATCC 25923 (*sea*), NCTC 10654 (*seb*), NCTC 10655 (*sec*), NCTC 10652 (*sed*) and FRI913 (*see* and *tst*). Field isolates (*eta*, *etb*, *icaA* and *icaD*) were taken from Rachid Achek (High National Veterinary School, Issad Abbes Avenue, Qued Smar, Algiers, ALGERIA) and were used as positive controls.

Table 1 Number and distribution of *S. aureus* and CNS isolates

Isolate	Number	Isolate	Number	Isolate	Number
S. aureus	12	*S. equorum*	3	*S. simulans*	2
S. caprae	15	*S. haemoliticus*	3	*S. capitis*	1
S. saprophyticus	15	*S. cornosus*	2	*S. cohnii*	1
S. xylosus	8	*S. hominis spp. hominis*	2	*S. epidermidis*	1
S. chromogenes	6	*S. sciuri*	2	*S. warnei*	1

The isolates were stored at -20 °C in Tripticase soy broth with 20% glycerol and activated in Baird-Parker agar and mannitol salt agar. A loop full of pure colonies was re-suspended in 500 µl of Tris-EDTA buffer (10 mM Tris chloride, pH 7.5; 1 mM EDTA, pH 8.0). Each isolate was treated with lysozyme (20 mg/ml) and lysostaphin (40 mg/ml) at 37 °C for 30-60 min for total bacterial DNA extraction. The phenol/chloroform extraction method was then used to extract DNA. The extracted DNA was stored at -20 °C until PCR analyses (Sambrook and Russel, 2001).

Detection of toxin genes: Two sets of multiplex PCR were applied for the detection of enterotoxin, TSS toxin, exfoliative toxin genes and also *femA* primers used for detection of *S. aureus* according to Mehrotra et al. (2000). Properties of the primers used in this study are shown in Table 2.

Detection of slime genes: Detection of *icaA* and *icaD* genes was done using primers recommended by Vasudevan et al. (2003). PCR amplification products were analysed by 2% agarose gel electrophoresis and stained with RedSafe (Intron, Korea). The bands were visualized under ultraviolet light. Properties of the primers are shown in Table 2.

Table 2 Characteristics of the primers used for PCR analysis

Gene	primer	Oligonucleotide sequence (5'-3')	Size of amplified product (bp)	Reference
sea	GSEAR-1 GSEAR-2	5′-GGTTATCAATGTGCGGGTGG-3′ 5′-CGGCACTTTTTTCTCTTCGG-3′	102	Mehrotra et al. (2000)
seb	GSEBR-1 GSEBR-2	5′-GTATGGTGGTGTAACTGAGC-3′ 5′-CCAAATAGTGACGAGTTAGG-3′	164	Mehrotra et al. (2000)
sec	GSECR-1 GSECR-2	5′-AGATGAAGTAGTTGATGTGTATGG-3′ 5′-CACACTTTTAGAATCAACCG-3′	451	Mehrotra et al. (2000)
sed	GSEDR-1 GSEDR-2	5′-CCAATAATAGGAGAAAATAAAAG-3′ 5′-ATTGGTATTTTTTTTCGTTC-3′	278	Mehrotra et al. (2000)
see	GSEER-1 GSEER-2	5′-AGGTTTTTTCACAGGTCATCC-3′ 5′-CTTTTTTTTCTTCGGTCAATC-3′	209	Mehrotra et al. (2000)
femA	GFEMAR-1 GFEMAR-2	5′-AAAAAAGCACATAACAAGCG-3′ 5′-GATAAAGAAGAAACCAGCAG-3′	132	Mehrotra et al. (2000)
eta	GETAR-1 GETAR-2	5′-GCAGGTGTTGATTTAGCATT-3′ 5′-AGATGTCCCTATTTTTGCTG-3′	93	Mehrotra et al. (2000)
etb	GETBR-1 GETBR-2	5′-ACAAGCAAAAGAATACAGCG-3′ 5′-GTTTTTGGCTGCTTCTCTTG-3′	226	Mehrotra et al. (2000)
tst	GTSSTR-1 GTSSTR-2	5′-ACCCCTGTTCCCTTATCATC-3′ 5′-TTTTCAGTATTTGTAACGCC-3′	326	Mehrotra et al. (2000)
icaA	ICAAF ICAAR	5′-CCTAACTAACGAAAGGTAG-3′ 5′- AAGATATAGCGATAAGTGC-3′	1315	Vasudevan et al. (2003)
icaD	ICADF ICADR	5′-AAACGTAAGAGAGGTGG-3′ 5′-GGCAATATGATCAAGATAC-3′	381	Vasudevan et al. (2003)

Results

As shown by the results of PCR analyses, *icaA* was detected in 7 (58.3%) of the 12 *S. aureus* isolates. *icaD* was detected in 12 (100%) of the *S. aureus* isolates, and both *icaA* and *icaD* were detected in 7 (58.3%). Prevalence of individual toxin genes found as *tst* 7 (58.3%), *sec* 6 (50%) and *sed* 4 (33.3%) and virulence gene profiles were detected as s*ec* + *sed* + *tst* 4 (33.3%), *sec* + *tst* 2 (16.7%) and *tst* 1 (8.3%) of the 12 *S. aureus* isolates. No *sea*, s*eb*, s*ee*, *eta* or *etb* genes were detected in these isolates. *icaA* gene was detected in 15 (24.2%), *icaD* in 8 (12.9%) and *icaA* + *icaD* in 13 (21.0%) of the 62 CNS isolates. *see* gene was detected in 6 (9.7%), *sed* in 1 (1.6%) and *tst* in 7 (11.3%) of the 62 CNS isolates. No *sea*, *seb*, *sec*, *eta* or *etb* genes were detected in these isolates (Table 3).

The distribution of toxin genes and slime genes in CNS isolates according to the types of samples is shown in Table 4. Toxin genes were detected in 12 of the 34 (35.3%) CNS isolates isolated from bulk tank milk. However, these genes were found in only 2 of the 28 (7.1%) CNS isolates isolated from cheese samples (Table 4).

Discussion

In general, *S. aureus* isolates were clearly more virulent than CNS virulence factors in this study. However, the virulence factor of CNS isolates cannot be ignored. Taponen and Pyorala (2009) stated that CNS could cause persistent infections, resulting in increased milk somatic cell count (SCC), which affects milk quality and may be related to decreased milk

production. In this study, toxin genes were detected in 7 (58.3%) of the 12 *S. aureus* isolates and in 14 of the 62 (22.6%) CNS isolates. These results showed that high numbers of *S. aureus* isolates contained toxin genes which could lead to public health risks. Additionally, the presence of toxin genes in CNS is also remarkable, but their prevalence was not as high as *S. aureus*. Some studies (Lyra et al., 2013; Xing et al., 2016) have stated that the virulence genes of *S. aureus* are more prevalent than those of CNS. Lyra et al. (2013) reported that enterotoxin genes were present in 23.3% of coagulase-positive staphylococci and in 4.5% of CNS isolates from raw goat milk. They emphasized that enterotoxigenic CNS posed a potential risk to consumer health and this should not be ignored. Zell et al. (2008) reported that enterotoxin production was found in 18 (51.4%) of 35 CNS isolates from food and starter cultures. Furthermore, Veras et al. (2008) reported that CNS capable of producing enterotoxin were also isolated from cases of staphylococcal food poisoning.

Table 3 Distribution of the studied genes in staphylococci isolates

Isolate	**Number**	***icaA***	***icaD***	***sea***	***seb***	***sec***	***sed***	***see***	***eta***	***etb***	***tst***
Bulk milk isolates											
S. capitis	1	-	-	-	-	-	-	-	-	-	-
S. caprae	14	8	8	-	-	-	-	6	-	-	-
S. chromogenes	6	4	3	-	-	-	-	-	-	-	6
S. cohnii	1	1	-	-	-	-	-	-	-	-	-
S. epidermidis	1	1	-	-	-	-	-	-	-	-	-
S. haemolyticus	1	-	-	-	-	-	-	-	-	-	-
S. hominis spp. hominis	2	-	1	-	-	-	-	-	-	-	-
S. saprophyticus	1	-	-	-	-	-	-	-	-	-	-
S. sciuri	1	-	1	-	-	-	-	-	-	-	-
S. simulans	1	-	-	-	-	-	-	-	-	-	-
S. warnei	1	1	1	-	-	-	-	-	-	-	-
S. xylosus	4	1	1	-	-	-	-	-	-	-	-
Total	34	16	15	-	-	-	-	6	-	-	6
Cheese isolates	Number	*icaA*	*icaD*	*sea*	*seb*	*sec*	*sed*	*see*	*eta*	*etb*	*tst*
S. caprae	1	1	-	-	-	-	-	-	-	-	-
S. chromogenes	2	-	-	-	-	-	-	-	-	-	-
S. equorum	3	2	-	-	-	-	-	-	-	-	-
S. haemolyticus	2	-	-	-	-	-	-	-	-	-	-
S. saprophyticus	14	7	3	-	-	-	1	-	-	-	-
S. sciuri	1	-	-	-	-	-	-	-	-	-	-
S. simulans	1	1	-	-	-	-	-	-	-	-	-
S. xylosus	4	1	2	-	-	-	-	-	-	-	1
Total	28	12	5	-	-	-	1	-	-	-	1
Total CNS	62	28 (45.2%)	21 (33.9%)	-	-	-	1 (1.6%)	6 (9.7%)	-	-	7 (11.3%)
Total *S. aureus*	12	7 (58.3%)	12 (100%)	-	-	6 (50%)	4 (33.3%)	-	-	-	7 (58.3%)
Total staphylococci	74	35 (47.3%)	33 (44.6%)	-	-	6 (8.1%)	5 (6.8%)	6 (8.1%)	-	-	14 (18.9%)

In this study, s*ec* + *sed* + *tst* were detected in 4 (33.3%) of the 12 *S. aureus* isolates. In addition, *sec* + *tst* were detected in 2 (16.7%) and *tst* in 1 (8.3%) of the 12 *S. aureus* isolates. The most frequently detected toxin genes were *tst* (58.3%), *sec* (50.0%) and *sed* (33.3%) in *S. aureus* (Table 2). These findings are in accord with those of earlier studies (Chu et al., 2012; Lyra et al., 2013; Xing et al., 2016) which found that *sec* and *tst* genes were common toxin genes in *S. aureus* isolated from milk and dairy products. Other studies also detected *tst* and *sec* genes in *S. aureus* isolated from goat milk and cheese (Akineden et al., 2008; Rosengren et al., 2010; Lyra et al., 2013; Chu et al., 2017), and some researchers also reported that *sec* and *tst* were present

together (McLauchlin et al., 2000; Akineden et al., 2008, Xing et al., 2016). In the present study, *see* genes were detected in 9.7% of the CNS isolates. Vernozy-Rozand et al. (1996) also detected *see* genes in 5% of CNS isolates from goat milk and cheese. In the present study, *sed* gene was detected in 1.6% of the CNS isolates, and *tst* gene was detected in 11.3% of the CNS isolates. Zell et al. (2008) identified *sed* gene in 14.3% of CNS isolates from various food samples, which is higher than the figure found in the present study. The studied virulence genes were detected with remarkable prevalence in *S. chromogenes* and *S. caprae* among the CNS isolates from goat milk in this study (Table 3).

Table 4 Distribution of the studied gene profiles in staphylococci isolates

Isolate	Number	*icaA*	*icaD*	*icaA + icaD*	*sec + sed + tst*	*sec + tst*	*tst*	*see*	*sed*
S. aureus	12	-	5	7	4	2	1	-	
Total CNS	62	15	8	13	-	-	7	6	1

In this study the presence of *icaA* and *icaD* genes in the *S. aureus* isolates was clearly higher than in the CNS isolates (Table 4). Carriage of the high prevalence of *ica* genes may cause resistance potential against host immune defence. The *S. aureus* slime gene data of this study were approximately the same as those found in other studies (Namwar et al., 2013; Zmantar et al., 2008). Basanisi et al. (2016) reported that *icaA* was present in 94.6% of the *S. aureus* isolated from sheep and goat cheeses. On the other hand, the prevalence of *icaA* gene (45.2%) was higher than that of *icaD* gene (33.9%) among CNS isolates, which is contrary to the results of some studies of CNS isolates in cases of clinical mastitis (Darwish and Asfour, 2013). The prevalence of *S. caprae icaA* (60.0%) and *icaD* (53.3%) genes in CNS isolates in cases of clinical mastitis in goats was lower than that reported by d'Ersu et al. (2016) (93% and 100%, respectively). The discrepancy in the findings of the two studies may be due to the origin of the isolates.

In the present study, no exfoliative toxin (*eta* and *etb*) genes were detected in the *S. aureus* and CNS isolates from goat bulk milk samples and goat cheese. Similarly, Salasia et al. (2004) and Karahan et al. (2009) did not detect *eta* and *etb* genes in *S. aureus* isolated from bovine milk and bovine mastitis milk samples. On the other hand, a study by Zell et al. (2008) found *eta* production in 2 (5.7%) of 35 CNS isolates from food and starter culture. The authors suggested that the prevalence of *eta* and *etb* genes might differ depending on the geographic region. The inability to detect some toxin genes in the isolates in this study may also be due to the nominal number of examined isolates to moderate.

For practical clinical evaluations in foodborne poisoning cases, toxin gene-positive staphylococci should be considered as toxin producers because *in vivo* toxin production cannot be ignored (Schmitz, 1998). Differences in the presence and prevalence of the studied virulence genes may have originated from the kinds and numbers of examined samples, the reservoirs or origins of the strains in the various geographical regions and the differences in detection methods and in their sensitivities.

Conclusion

In this study, toxin genes and slime genes were detected in *S. aureus* isolates from goat milk and goat cheese. In some CNS isolates, both toxin genes and slime genes were identified, whereas only slime genes were detected in other CNS isolates. These results are the preliminary result of pathogenic gene comparison of *S. aureus* and CNS. Fewer CNS isolates with toxin genes were isolated from cheese samples than from milk samples. Training of workers in dairy plants and hygiene measures during milking and manufacture of dairy products are of paramount importance to prevent staphylococcal contamination and ensure the safety of dairy products. The potential risk of enterotoxigenic CNS should not be ignored with regards to food safety and public health. In future studies of staphylococcal toxin gene detection, the presence of major and minor toxin genes and other virulence genes such as adhesins should be investigated to evaluate from a broader perspective.

Acknowledgements

This study was funded by Mustafa Kemal University Scientific Research Fund (Project Number: 14161). We also thank Rachid Achek (High National Veterinary School, Issad Abbes Avenue, Qued Smar, Algiers, ALGERIA) for the positive control strains.

References

Akineden Ö, Hassan AA, Schneider E and Usleber E 2008. Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. Int J Food Microbiol. 124 (2): 211-216.

Anonymous, 1999. "Microbiology of Food and Animal Feeding Stuffs -- Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (Staphylococcus aureus and Other Species) Part 1: Technique Using Baird-Parker Agar Medium," 6888-1:1999, International Standart Office, Geneva, Switzerland.

Argudin MÁ, Mendoza MC and Rodicio MR 2010. Food Poisoning and *Staphylococcus aureus* Enterotoxins. Toxins. 2(7): 1751-1773.

Balaban N and Rasooly A 2000. Staphylococcal enterotoxins. Int J Food Microbiol. 61(1), 1-10.

Basanisi MG, Nobili G, La Bella G, Russo R, Spano G, Normanno G and La Salandra G 2016. Molecular characterization of Staphylococcus aureus isolated from sheep and goat cheeses in southern Italy. Small Ruminant Research. *135*, 17-19.

Bergdoll MS 1983. Enterotoxins. In Staphylococci and staphylococcal infections. C. S. F. Easmon and C. Adlam (ed.), Academic Press, Inc., New York, N.Y. p. 559-598.

Cantekin Z, Ozmen GO, Demir M, Yılmaz Er Z, Solmaz H and Ergun Y 2016. Detection of Causative Agents in Goat Mastitis and their Antibiotic Resistance in Hatay Region. Van Vet J. 27 (2): 79-83.

Chu C, Yu C, Lee Y and Su Y 2012. Genetically divergent methicillin-resistant *Staphylococcus aureus* and sec-dependent mastitis of dairy goats in Taiwan. BMC Vet Res. 8: 39.

Chu CS, Huang HH, Chiang SH, Chou CC, Lai JM, Shih WL, Changchien CH, Lin HC, Chuang ST, Su, Y. C. 2017. Investigation into antimicrobial resistance, enterotoxin and cassette chromosome gene of Staphylococcus aureus isolates from humans, cows and goats in Taiwan. Thai J Vet Med. 47(4): 481-492.

Coton E, Desmonts MH, Leroy S, Coton M, Jamet E, Christieans S, Donnio PY, Lebert I and Talon R 2010. Biodiversity of coagulase-negative Staphylococci in French cheeses, dry fermented sausages, processing environments and clinical samples. Int J Food Microbiol. 137(2-3): 221-229.

Darwish SF and Asfour HAE 2013. Investigation of biofilm forming ability in staphylococci causing bovine mastitis using phenotypic and genotypic assays. Sci World J.

d'Ersu J, Aubin GG, Mercier P, Nicollet P, Bémer P and Corvec S 2016. Characterization of *Staphylococcus caprae* clinical isolates involved in human bone and joint infections, compared with goat mastitis isolates. J Clin Microbiol. 54 (1): 106-113.

Doğruer G, Sarıbay MK, Aslantaş Ö, Kireççi E, Ergün Y, Ülkü A and Demir C 2016. The Prevalance, Etiology and Antimicrobial Susceptibility of the Microorganisms in Subclinical Mastitis in Goats. Atatürk Üniversitesi Veteriner Bilimleri Dergisi.11(2): 20-24.

Even S, Lero S, Charlier C, Zakour NB, Chacornac JP, Lebert I, Jamet E, Desmonts MH, Coton E, Pochet S, Donnio PY, Gautier M, Talon R and Le Loir Y 2010. "Low Occurrence of Safety Hazards in Coagulase Negative Staphylococci Isolated from Fermented Foodstuffs. Int J Food Microbiol. 139: 87- 95.

Irlinger F 2008. Safety Assessment of Dairy Microorganisms: Coagulase-Negative Staphylococci. Int J Food Microbiol. 126(3): 302-310.

Kamber U 2007. The traditional cheeses of Turkey: cheeses common to all regions. Food reviews international. 24(1): 1-38.

Karahan M, Açık MN and Çetinkaya B 2009. Investigation of toxin genes by polymerase chain reaction in *Staphylococcus aureus* strains isolated from bovine mastitis in Turkey. Foodborne Pathog Dis. 6 (8): 1029-1035.

Kloos WE and Bannerman TL 1994. Update on Clinical Significance of Coagulase-Negative Staphylococci. Clin Microbiol Rev. 7(1): 117-140.

Ladhani S, Joannou CL, Lochrie DP, Evans RW and Poston SM 1999. Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev. 12 (2): 224-242.

Le Loir Y, Baron F and Gautier M 2003.Staphylococcus aureus and Food Poisoning. Genet Mol Res. 2(1): 63-76.

Letertre C, Perelle S, Dilasser F and Fach P 2003. Identification of a new putative enterotoxin SEU encoded by the egc cluster of Staphylococcus aureus. J Applied Microbiol. 95(1): 38-43.

Lyra DG, Sousa FGC, Borges MF, Givisiez PEN, Queiroga RCRE, Souza EL, Gebreyes WA and Oliveira CJB 2013. Enterotoxin-encoding genes in *Staphylococcus* spp. from bulk goat milk. Foodborne Pathog Dis. 10 (2): 126-130.

Marrack P and Kappler J 1990. The staphylococcal enterotoxins and their relatives. Science. 248(4956): 705-711.

McCormick JK, Yarwood JM and Schlievert PM 2001. Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol. 55: 77-104.

McLauchlin J, Narayanan GL, Mithani V and O'neill, G 2000. The detection of enterotoxins and toxic shock syndrome toxin genes in Staphylococcus aureus by polymerase chain reaction. J Food Protect. 63(4): 479-488.

Mehrotra M, Wang G, and Johnson WM 2000. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol. 38 (3):1032-1035.

Namvar AE, Asghari B, Ezzatifar F, Azizi G and Lari AR 2013. Detection of the intercellular adhesion gene cluster (ica) in clinical *Staphylococcus aureus* isolates. GMS Hyg Infect Control. 8 (1): 1-4.

Pehlivanlar Onen S and Aygun O 2017. Enterotoxin producing ability and antimicrobial susceptibility of coagulase-negative staphylococci isolated from goat milk, cheese and salted yoghurt in Turkey. IJSTR. 6 (10): 200-206.

Podkowik M, Park JY, Seo KS, Bystroń J and Bania J 2013. Enterotoxigenic potential of coagulase-negative staphylococci. Int J Food Microbiol. 163(1): 34-40.

Rosengren A, Fabricius A, Guss B, Sylven S and Lindqvist R 2010. Occurrence of foodborne pathogens and characterization of *Staphylococcus aureus* in cheese produced on farm-dairies. Int J Food Microbiol. 144(2): 263-269.

Salasia SIO, Khusnan Z, Laemmler C and Zschöck M 2004. Comparative studies on pheno- and genotypic properties of *Staphylococcus aureus* isolated from bovine subclinical mastitis in central Java in Indonesia and Hesse in Germany. J Vet Sci. 5 (2): 103-109.

Sambrook J and Russel DW 2001. Molecular Cloning: A Laboratory Manual. 3rd ed., CSHL Press, New York.

Schmitz FJ, Steiert M, Hofmann B, Verhoef J, Hadding U, Heinz HP and Köhrer K 1998. Development of a multiplex-PCR for direct detection of the genes for enterotoxin B and C, and toxic shock syndrome toxin-1 in Staphylococcus aureus isolates. J Med Microbiol. 47: 335-40.

Taponen S and Pyorala S 2009. Coagulase-negative staphylococci as cause of bovine mastitis-not so

different from Staphylococcus aureus? Vet Microbiol. 134(1-2): 29-36.

TUIK 2017: tuik.gov.tr: 22.12.2017.

Valle J, Gomez-Lucia E, Piriz S, Goyache J, Orden JA and Vadillo S 1990. Enterotoxin production by staphylococci isolated from healthy goats. Appl Environ Microbiol. 56(5): 1323-1326.

Vasudevan P, Nair MKM, Annamalai T and Venkitanarayanan KS 2003. Phenotypic and genotyping characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet Microbiol. 92(1-2):179-185.

Veras, JF, do Carmo LS, Tong LC, Shupp JW, Cummings C, dos Santos DA, Cerqueira MMOP, Cantini A, Nicoli JR, Jett M 2008. A study of the enterotoxigenicity of coagulase-negative and coagulase-positive staphylococcal isolates from food poisoning outbreaks in Minas Gerais, Brazil. Int J Infect Dis.12(4): 410-415.

Vernozy-Rozand C, Mazuy C, Prevost G, Lapeyre C, Bes M, Brun Y and Fleurette J 1996. Enterotoxin production by coagulase-negative staphylococci isolated from goats' milk and cheese. Int J Food Microbiol. 30(3): 271-280.

Xing X, Zhang Y, Wu Q, Wang X, Ge W and Wu C 2016. Prevalence and characterization of *Staphylococcus aureus* isolated from goat milk powder processing plants. Food Control. 59: 644-650.

Yasan Ataseven Z and Gülaç N 2014. Süt ve Süt Ürünleri 2014. Ankara: Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü, TEPGE No: 233, 2014.

Zell C, Resch M, Rosenstein R, Albrecht T, Hertel C and Götz F 2008. Characterization of toxin production of coagulase-negative staphylococci isolated from food and starter cultures. Int J Food Microbiol. 127(3): 246-251.

Zmantar T, Chaieb K, Makni H, Miladi H, Abdallah FB, Mahdouani K and Bakhrouf A 2008. Detection by PCR of adhesins genes and slime production in clinical *Staphylococcus aureus*. J Basic Microbiol. 48(4): 308-314.

บทคัดย่อ

การศึกษายีนส์ท็อกซินและสไลม์ในเชื้อสตาฟิโลค็อกคัสที่แยกได้จากน้ำนมแพะ และชีสที่ทำจากน้ำนมแพะในภาคใต้ของประเทศตุรกี

เซฟดา เปห์ลิแวนลาร์ โอเนน[1] ซาเฟอร์ แคนตีคิน[2] ไฟซา เมบ์เขาท์[3] ออสมาน อาร์กัน[4] ยาซา เออกัน[5]*

วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อศึกษาการปรากฏของเชื้อแบคทีเรียสตาฟิโลค็อกคัส ออเรียส และเชื้อสตาฟิโลค็อกคัสกลุ่มที่แสดงผลเป็นลบต่อการทดสอบด้วยเอนไซม์โคแอ็กกูเลซ (CNS) ที่แยกได้จากน้ำนมแพะและชีสที่ผลิตจากน้ำนมแพะ และศึกษายีนส์ท็อกซินและสไลม์ของเชื้อชนิดนี้ร่วมด้วย เชื้อแบคทีเรียสตาฟิโลค็อกคัส ออเรียส และ CNS ที่แยกได้ ถูกนำมาตรวจวิเคราะห์ด้วยวิธีโพลีเมอเรสเชนรีแอคชั่น (PCR) เพื่อวิเคราะห์ความชุกของเอ็นเทอโรท็อกซิน (sea seb sec sed และ see) ท็อกซิกชอล์กซินโดรม (TSS) ท็อกซิน (tst) เอ็กโฟลิเอทีฟท็อกซิน (*eta* และ *etb*) และ ยีนส์สไลม์ (*icaA* และ *icaD*) *sec sed และ tst* พบร่วมกันใน 4 (33.3%) ตัวอย่าง *sec* + ยีนส์ *tst* พบร่วมกันใน 2 (16.7%) ตัวอย่าง และ ยีนส์ *tst* พบใน 1 (8.3%) ตัวอย่าง ของเชื้อแบคทีเรียสตาฟิโลค็อกคัสออเรียสจำนวน 12 ตัวอย่าง อย่างไรก็ดียีนส์ see ถูกพบใน 6 (9.7%) ตัวอย่าง ยีนส์ *sed* และ ยีนส์ *tst* ถูกตรวจพบใน 1 (1.6%) ตัวอย่าง และ 7 (11.3%) ตัวอย่าง จากเชื้อ CNS จำนวน 62 ตัวอย่าง ตามลำดับ ไม่พบยีนส์ *sea seb see eta* หรือ *etb* ในเชื้อสตาฟิโลค็อกคัสออเรียสที่แยกได้ ในขณะที่ไม่พบยีนส์ *sea seb sec eta* หรือ *etb* ในเชื้อแบคทีเรียในกลุ่ม CNS ที่แยกได้ อย่างน้อย ยีนส์ ica จำนวน 1 ตัวอย่าง ปรากฏในเชื้อสตาฟิโลค็อกคัส ออเรียส ทั้งหมด และใน 36 จาก 62 เชื้อแบคทีเรียในกลุ่ม CNS การปรากฏของยีนส์ icaA และ icaD เชื้อสตาฟิโลค็อกคัส ออเรียส สูงกว่าเชื้อแบคทีเรียในกลุ่ม CNS อย่างชัดเจน โดยสรุป ยีนส์ท็อกซินและสไลม์ถูกตรวจพบในเชื้อสตาฟิโลค็อกคัส ออเรียส และเชื้อแบคทีเรียในกลุ่ม CNS ที่แยกได้จากน้ำนมแพะและชีสที่ผลิตจากน้ำนมแพะ ความเสี่ยงของเชื้อแบคทีเรียในกลุ่ม CNS ที่มีสารพิษเอ็นเทอโรท็อกซินไม่ควรถูกละเลย เช่นเดียวกับเชื้อแบคทีเรียชนิดสตาฟิโลค็อกคัส ออเรียส ในเรื่องเกี่ยวกับอาหารปลอดภัยและงานด้านสาธารณสุข

คำสำคัญ: สตาฟิโลค็อกคัสออเรียส สตาฟิโลค็อกไค ยีนส์ท็อกซิน น้ำนมแพะ ชีสที่ผลิตจากน้ำนมแพะ

[1]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Hatay, Turkey

[2]Department of Microbiology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Hatay, Turkey

[3]École Nationale Supérieure Vétérinaire d'Alger, Algiers, Algeria, scamilia2000@yahoo.fr

[4]Program of Food Technology, Keban Vocational School, Firat University, Elazig, Turkey

[5]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Mustafa Kemal, Hatay, Turkey

***ผู้รับผิดชอบบทความ** E-mail: zcantekin@hotmail.com*