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# Toxigenic Genes of Coagulase-negative Staphylococci and *Staphylococcus aureus* from Milk and Dairy

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ABSTRACT

The study investigates the prevalence of *Staphylococcus aureus* and coagulase-negative staphylococci in raw milk and dairy products and assesses their toxin-related pathogenic potential and methicillin resistance. A total of 1015, raw milk (260) and dairy samples (325 cheeses, 180 yogurts, 140 ice creams, 110 butter samples) were collected and analyzed. The prevalence of *Staphylococcus aureus* and coagulase-negative staphylococci were 3.2% and 5.3% with mean counts of 3.46 and 3.16 log CFU/mL-g, respectively. Three (*sea, seb, see*) of five (*sea, seb, sec, sed, see*) staphylococcal enterotoxin (SE) genes, two (*tss, etb*) of four (*tss, pvl, eta, etb*) virulence-associated genes, and the absence of methicillin resistance (*mec*A) gene were defined by polymerase chain reaction. SE *sea* (6.9%), *seb* (2.3%) and *see* (1.1%) genes were detected in *Staphylococcus aureus* from one milk and seven different cheese samples. The presence of multiple enterotoxin genes (*sea* and *see*) was detected in a *Staphylococcus aureus* isolate from one cheese. However, the milk-sourced one coagulase-negative staphylococci possessed both the *tss* and *etb* virulence genes. The finding in this study indicates that the frequency of coagulase-negative staphylococci was higher than *Staphylococcus aureus* and moreover, toxin genes associated with human infections were assigned in coagulase-negative staphylococci while enterotoxin genes were determined among *Staphylococcus aureus*. In terms of food safety perspective, coagulase-negative staphylococci are ignored and they are not considered in standard food surveillance analysis. But the presence of virulent coagulase-negative staphylococci in foods is a public health concern. The results obtained from this study are significant as it demonstrates that pathogenic coagulase-negative staphylococci are found in foods, and provides data from Turkey. Additional research is required concerning coagulase-negative staphylococci in the food matrix and clinical isolates.

Keywords: Coagulase-negative staphylococci, Cheese, Milk, Enterotoxin, Virulence

## 1. Introduction

*Staphylococcus aureus* is one of the major human pathogens of worldwide bacteriological diseases. Amongst the different types of diseases, staphylococcal enterotoxins (SEs) primarily produced by certain *S. aureus* strains cause a foodborne intoxication referred to as staphylococcal food poisoning (SFP) through consumption of contaminated food (Chieffi et al. 2020). Enterotoxin-producing *S. aureus* strains are associated with food poisoning by producing heat-stable, protease- (such as pepsin and trypsin) and environmental-resistant toxins in foodstuffs (Ahmed et al. 2019; Mahanti et al. 2020). Although there are ten SEs identified, they are primarily classified into five classical types including SEA, SEB, SEC, SED and SEE. These five enterotoxins are responsible for 95% of staphylococcal intoxication cases. The remaining newly described SEG-SEI, SEIJ, SEIQ, SER-SET, and SEIU-SEIV enterotoxins are known to cause only 5% of food poisoning cases (Ahmed et al. 2019; Kou et al. 2021).

The pathogenicity of *S. aureus* strains is not only enterotoxin dependent. This pathogen also secretes strain-specific toxic shock syndrome toxin (TSST-1) which generates an immune hyper-response in the host, a leucocytolytic toxin Panton-Valentine leukocidin

(PVL), exfoliative toxins (*eta* and *etb*) implicated in human skin damage and also the toxins synthesized by most of the strains as α-toxin, γ-toxin, some leukotoxins, and phenol-soluble modulins (PSMs) (Elal Mus et al. 2019).

Antimicrobial resistance is another concern for *S. aureus* strains. In particular, methicillin resistance encoded by the *mec*A gene is widely considered to be a global health problem. The World Health Organization listed methicillin-resistant *S. aureus* as one of the three most difficult infectious agents due to its role in nosocomial infections (Kou et al. 2021).

The pathogen *S. aureus* exists in the mammary glands of dairy animals. Several risk factors associated with *S. aureus* include unhygienic milking procedures, inappropriate preventive techniques, and the lack of antiseptic use before and after milking (Aqib et al. 2018). The pathogenic *S. aureus* strains are found in raw milk and unpasteurized dairy products as a result of primer/seconder contamination (Mahanti et al. 2020). Moreover, several foods are contaminated with enterotoxigenic *S. aureus* via the hands of food handlers (Dorotíkova et al. 2022). European regulations (European Council Regulations 2005) and the Turkish Food Codex (TFC 2011) require the enumeration of coagulase-positive staphylococci and/or detection of SEs in dairy products and some specific foods. However, *S. aureus* toxins still remain one of the most important agents responsible for food poisoning. The European Union One Health Zoonoses Report revealed 43 *S. aureus* toxins outbreaks with 402 human cases in 2020. Four of these outbreaks were strong-evidenced and the number of people hospitalized totaled 32 in European Union Countries (EFSA & ECDC 2021). On the other hand, coagulase-negative staphylococci (CoNS) emerge in foods, especially ready-to-eat products. The presence of toxin genes and production ability among CoNS strains isolated from foods has been observed in recent years (Pyzik et al. 2019; Chajecka-Wierzchowska et al. 2020; França et al. 2021; Banszkiewicz et al. 2022). CoNS are a notorious opportunistic pathogen and can cause infections in immunosuppressed individuals and foreign material implantated patients (Becker et al. 2020). The presence of CoNS in food has become a growing concern in recent years.

The purpose of this study was to determine the distribution and pathogenic potential of CoNS and *S. aureus* in milk and dairy products. To this aim, some staphylococcal toxin genes associated with human infections/food intoxications and methicillin resistance gene were investigated.

## 2. Material and Methods

### *2.1. Bacterial isolation*

A total of 1015 milk and dairy samples were collected from May 2017 to July 2021 in the Bursa province of Turkey. Samples included raw milk (25 goats, 80 sheep, 155 cows), cheese (325), butter (110), yogurt (180), and ice cream (140). All samples were collected from local bazaars, supermarkets, and local delicatessens. The collected samples were transferred to the laboratory in a cooler with ice packs and prepared for analyses within the same day. The prevalence of *S. aureus* and CoNS was determined using the qualitative detection method according to ISO 6888-1. Briefly, 10 g was obtained randomly from each sample and placed into a sterile stomacher bag containing 90 mL of peptone water. Following homogenization, 0.1 mL of each serial dilution solution was spread in duplicate on Baird-Parker Agar (1.05406, Merck, Germany) supplemented with egg yolk tellurite emulsion and incubated at 37 °C for 48 h. After incubation, five black colonies surrounded by a clear zone from petri dishes were separated for identification. All isolates were subjected to the Gram staining and catalase test. Gram-positive, cocci-shaped, grape-like clusters and catalase-positive isolates were evaluated as *S. aureus*. For the test of coagulase tube test, colonies were taken by inoculation loop and transferred in Brain-Heart Infusion Broth (110493 Merck, Germany) and incubated at 37 °C for 20-24 h. 0.1 mL Brain-Heart Infusion Broth culture mixed with EDTA rabbit plasma (1.13306, Merck, Germany). The *S. aureus* reference strain ATCC 25923 was used for positive control of the coagulase-positive reaction. Tube contents check for coagulation at 37 °C for 4-6 h. The isolates were preserved in Brain-Heart Broth (1.10493, Merck, Germany) supplemented with 20% glycerol at -20 °C for further experiments. The isolates were further confirmed by using polymerase chain reaction (PCR).

### *2.2. PCR identification and detection of pathogenicity genes*

Total DNA from the strains was extracted by using Chelex 100 resin (Sigma Aldrich, USA). PCR was performed in a ThermoCycler (T100, Bio-Rad, USA). Each 25 µL reaction mixture consisted of 1 µL of template DNA, 5 µL of 1.25 U Hot Start Taq DNA polymerase (Bioron, Germany), 1 µL of 1.8 mM $MgCl_2$ (Fermentas, USA), 3µL of 10 mM Tris-HCl (pH 8.9), 4 µL of 200 µ*M* dNTPs (Biolabs, UK), 22 mM KCl, 1 µL of 0.5 mM of each primer (Oligomer, Turkey) and 7 µL of PCR grade water (EURx, Poland). The PCR procedure was carried out with firstly SA gene for confirmation of *S. aureus* isolates, and then the *sea, seb, sec, sed*, *see* genes for detection of enterotoxins and *pvl, tss, eta, etb, mec*A genes for determination of virulence traits. Multiplex-PCR was used to amplify *sea, seb, sec,*

*sed*, *see* genes and *tss*, *eta*, *etb* genes. The *S. aureus* reference strains ATCC 25923 for *sea*, ATCC 14458 for *seb*, ATCC 19095 for *sec*, ATCC 23235 for *sed*, ATCC 27664 for *see*, ATCC BAA-1747 for *pvl*, MN8 for *tss*, *eta, etb*, ATCC 43300 for *mec*A gene were used as a positive control. The PCR procedures for each primer pair were performed according to the related reference given in Table 1. PCR products were electrophoresed in 2% agarose gel and visualized by ethidium bromide staining. 100 bp DNA ladder (Genesta, Germany) was used as a marker.

**Table 1- Primers used in the confirmation of *S. aureus* and the determination of enterotoxin and virulence-associated genes**

| *Primers* | *Product size (bp)* | *Oligonucleotide sequence (5´-3´)* | *Thermal-cycle protocol* | *Reference* |
|---|---|---|---|---|
| *SA* | 108 | AATCTTTGTCGGTACACGATATTCTTCACG<br>CGTAATGAGATTTCAGTAGATAATACAACA | 94 *ºC 4 min - (94 ºC 45 s, 50 ºC 45 s, 72 ºC 1 min) x 30 cycles* - 72 *ºC 5 min* | Martineau et al. 1998 |
| *sea* | 102 | GGTTATCAATGTGCGGGTGG<br>CGGCACTTTTTTCTCTTCGG | 98 *ºC 30 s - (98 ºC 20 s, 60 ºC 20 s, 72 ºC 10 s) x 30 cycles –*<br>*72 ºC 5 min* | Mehrotra et al. 2000 |
| *seb* | 164 | GTATGGTGGTGTAACTGAGC<br>CCAAATAGTGACGAGTTAGG | | |
| *sec* | 451 | AGATGAAGTAGTTGATGTGTATGG<br>CACACTTTTAGAATCAACCG | | |
| *sed* | 278 | CCAATAATAGGAGAAAATAAAAG<br>ATTGGTATTTTTTTTCGTTC | | |
| *see* | 209 | AGGTTTTTTCACAGGTCATCC<br>CTTTTTTTTCTTCGGTCAATC | | |
| *tss* | 326 | ACCCCTGTTCCCTTATCATC<br>TTTTCAGTATTTGTAACGCC | 98 *ºC 30 s - (98 ºC 5 s, 57 ºC 10 s, 72 ºC 10 s) x 30 cycles –*<br>*72 ºC 5 min* | Mehrotra et al. 2000 |
| *eta* | 93 | GCAGGTGTTGATTTAGCATT<br>AGATGTCCCTATTTTTGCTG | | |
| *etb* | 226 | ACAAGCAAAAGAATACAGCG<br>GTTTTTGGCTGCTTCTCTTG | | |
| *pvl* | 433 | ATCATTAGGTAAAATGTCTGGACATGATCCA<br>GCATCAAGTGTATTGGATAGCAAAAGC | 98 *ºC 30 s - (98 ºC 5 s, 58 ºC 5 s, 72 ºC 10 s) x 30 cycles –*<br>*72 ºC 5 min* | Lina et al. 1999 |
| *mecA* | 519 | TGTCCGTAACCTGAATCAGC<br>GACAAACTCCCACCTATCGC | 98 *ºC 30 s - (98 ºC 5 s, 57 ºC 10 s, 72 ºC 10 s) x 30 cycles –*<br>*72 ºC 5 min* | Tsuchizaki et al. 2000 |

## 3. Results and Discussion

*S. aureus* is a worldwide zoonotic pathogen, which can be responsible for approximately 40% of bovine mastitis cases. However, *S. aureus* may exist in the milk of dairy animals after infection and so it contaminates other dairy products (Kou et al. 2021). In the present work, 33 *S. aureus* and 54 CoNS were recovered from 260 raw milk and 755 dairy samples (8.6%) by using the standard culture method, and all *S. aureus* isolates were confirmed by PCR. The mean count of *S. aureus* was 3.30 log CFU/mL-g for all isolated samples (data not shown). A summary of the mean counts by sample kind and coagulase activity results is presented in Table 2. A similar observation has been made in a study by Mercanoglu Taban et al. (2021) who isolated *S. aureus* in 9.4% of milk and dairy collected from Central Anatolia and the Mediterranean Regions of Turkey. Some studies, however, revealed high isolation rates from different countries. Tohoyesseu et al. (2020) found that 36.4% of fermented artisanal dairy products contained *S. aureus* in samples collected from Benin. Research conducted in Egypt showed that the isolation rate of *S. aureus* was 40.5% in milk and dairy products with a 4.12 log CFU/mL-g mean count (Ahmed et al. 2019). Another fairly high result with a 70% rate of *S. aureus* contamination in milk and dairy was published in Saudi Arabia (Alghizzi & Shami 2021).

**Table 2- Distribution and counts of staphylococci in the samples**

| *Sample* | *Staphylococci counts (log CFU/mL-g)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | | | | *Coagulase (-) staphylococci* | | | |
| | *No of samples* | *Min.* | *Mean ± SEM* | *Max.* | *No of samples* | *Min.* | *Mean ± SEM* | *Max.* |
| Milk (n=260) | 8 | 2 | 3.35±3.13 | 4.05 | 21 | 2 | 2.57±2.11 | 3.43 |
| Cheese (n=325) | 16 | 2 | 3.54±3.27 | 4.38 | 17 | 2 | 3.54±3.47 | 4.70 |
| Butter (n=110) | 1 | - | 2.30± | - | 2 | 3.16 | 3.25±2.60 | 3.34 |
| Ice cream (n=140) | 8 | 2 | 3.46±3.25 | 3 | 14 | 2 | 2.45±1.82 | 3 |
| Yoghurt (n=180) | - | - | - | - | - | - | - | - |
| All (n=1015) | 33 | 2 | 3.46±3.01 | 4.38 | 54 | 2 | 3.16±2.97 | 4.70 |

Min: Minimum, Max: Maximum, SEM: Standard error of the mean

The prevalence of staphylococci was the highest with an incidence of 15.7% in ice cream, followed by milk (11.2%), cheese (10.1%), and butter (2.7%). None of the yogurt samples contained staphylococci (data not shown). Table 2 presents the distribution of CoNS and *S. aureus* by sample kind. *S. aureus* was observed in 22 out of 140 (15.7%) ice cream samples in this study. Lower incidence rates were reported in China with an incidence of 4.2% (Zhang et al. 2022) and a higher result (74%) was obtained in Egypt (Ahmed et al. 2019). In the present study, 19 of 22 ice cream samples contaminated with *S. aureus* were fruit, chocolate pieces, or cocoa ice cream. The use of improperly washed fruits in ice cream production may be responsible high contamination rate. In our research, 29 (8 were *S. aureus*, 21 were CoNS) out of 260 raw milk samples were found to be contaminated. In Turkey Pehlivanlar Onen et al. (2017) found that 22 out of 40 raw milk samples contained CoNS. Studies on raw milk performed in Turkey showed that 64% of samples contained *S. aureus* (Yildirim et al. 2019), and 95 out of 725 raw milk samples contained *S. aureus* (Tavsanli & Cibik 2022). However, some other researchers reported the presence of *S. aureus* in heat-treated milk. *S. aureus* was determined in pasteurized milk samples by Dai et al. (2019), in 21 marked milk by Ahmed et al. (2019), and also isolated in composite milk by Mahanti et al. (2020). In the current research 17 CoNS and 16 *S. aureus* were identified from 325 cheese samples. The presence of *S. aureus* in various types of cheese was previously reported in Turkey (Pehlivanlar Onen and Aygun 2017; Elal Mus et al. 2019), Egypt (Ahmed et al. 2019; Zayda et al. 2020), Romania (Morar et al. 2021), and China (Cai et al. 2021). In this study, *S. aureus* was detected in 3 of 110 butter samples. Our results were lower than Ranjana et al. (2019), who isolated and identified 11 strains from plain and table butter. A previous study in China revealed that 12.4% of retailed yak butter samples collected from retail stores were contaminated with *S. aureus* (Zhang et al. 2021). We mostly analyzed fresh and homemade yogurt samples to determine the distribution of CoNS and *S. aureus* in this study. Research performed by Pehlivanlar Onen and Aygun (2017) in salted yogurt samples from Turkey showed the absence of staphylococci. This result is in agreement with our findings. Contrary to our yogurt analysis results, Ahmed et al. (2019), Tohoyessou et al. (2020) and Abdulrahman & Sanmi (2021) detected *S. aureus* among yogurt samples. The occurrence of *S. aureus* in dairy products may be caused by various factors, but it is commonly associated with the use of contaminated raw milk and endogenous starter cultures in the production process of dairy products, apart from asymptomatic worker carriers of *S. aureus* (Castro et al. 2020). In addition, the difference in *S. aureus* and CoNS prevalence in milk and dairy may be caused by geographical differences, hygienic conditions of preparation, and storage.

Staphylococci secret the coagulase enzyme and this enzyme is regarded as an indicator of the pathogenicity of *S. aureus* (Ahmed et al. 2019). In the current work, 33 (38%) of 87 *S. aureus* isolates tested positive for coagulase activity. In terms of coagulase activity, the other 54 isolates consisting of 10% of ice cream, 8.1% of milk, 5.2% of cheese, and 1.8% of butter isolates were classified as coagulase-negative. The prevalence of *S. aureus* and CoNS in samples is summarized in Table 2. Besides *S. aureus*, CoNS has been associated with rare cases of food poisoning. However, CoNS is regarded as a major reservoir for toxin production and antimicrobial resistance genes of *S. aureus* as a pathogen (Nasaj et al. 2020; França et al. 2021). Some CoNS isolates from different foods encode toxin-producing genes and these genes can be transmitted to other bacteria (França et al. 2021) Moreover, CoNS causes significant morbidity and socioeconomic losses worldwide (Becker et al. 2020; França et al. 2021).

**Table 3 - Distribution of toxin-related genes**

| Genes | | No of samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *S. aureus* | | | | | *Coagulase (-) staphylococci* | | | | |
| | | *Milk* | *Cheese* | *Butter* | *Ice cream* | *Yoghurt* | *Milk* | *Cheese* | *Butter* | *Ice cream* | *Yoghurt* |
| Enterotoxin genes | sea | 1[a] | 5[b] | - | - | - | - | - | - | - | - |
| | seb | - | 2[c] | - | - | - | - | - | - | - | - |
| | sec | - | - | - | - | - | - | - | - | - | - |
| | sed | - | - | - | - | - | - | - | - | - | - |
| | see | - | 1[d] | - | - | - | - | - | - | - | - |
| Other toxin genes | tss | - | - | - | - | - | 1[e] | - | - | - | - |
| | eta | - | - | - | - | - | - | - | - | - | - |
| | etb | - | - | - | - | - | 1[e] | - | - | - | - |
| | pvl | - | - | - | - | - | - | - | - | - | - |

[a]S37 isolate number, [b]S7, S14, S25, S44, S56 isolate numbers, [c]S38, S41 isolate numbers, [d]S25 isolate numbers, [e]S3 isolate number

The presence of enterotoxin genes in the *S. aureus* and CoNS isolates is shown in Table 3. SE *sea* gene was detected in one milk (S37) and five (S7, S14, S25, S44, S56) (three soft, one hard, and one semi-hard kind) cheese isolates of *S. aureus*. The SE *seb* gene was assigned in two soft kinds of cheese (S38, S41) and the SE *see* gene in one kind of hard cheese (S25) isolates of *S. aureus*. The *S. aureus* isolated from the hard cheese (S25) possessed both SE *sea* and *see* genes (Figure 1). The coagulase test was positive for all strains carrying the enterotoxin gene. The SE *sec* and *sed* genes were not detected in any isolate from milk and dairy in our research. Two different findings from Turkey showed that *S. aureus* isolates from traditional Turkish dairy-based milk desserts harbored *sea* (5 isolates), *seb* (5 isolates), *see* (1 isolate) and *sea*+*see* (2 isolates) genes (Gucukoglu et al. 2020) and CoNS isolate from goat's milk and cheese had *sed* (1 isolate), *see* (6 isolates); *S. aureus* had *sec* (6 isolates), *sed* (4 isolates) genes (Pehlivanlar Onen et al. 2018). An earlier report from China revealed that 12.9 % of *S. aureus* strains isolated from raw cow (*sea*, *sea*+*sec*, *sec* and *see* genes), horse (*see* gene) and camel (*sea*+*sec* gene) milk had several SE's genes (Kou et al. 2021). The results of research conducted in India demonstrated that *sei* and *seg* genes were found but *sea*, *seb*, *sec*, *sed,* and *see* genes were absent in raw bovine milk samples (Mahanti et al. 2020). A survey from Italy showed that *S. aureus* from raw milk samples harbored enterotoxin genes and these strains' ability to produce sufficient amounts of enterotoxin (Chieffi et al. 2020). Similar to our finding, Ahmed et al. (2019) described SE *sea* and *sed* genes in 13 cheese isolates, also both *sea* and *sed* genes in two different artisanal cheese isolates Zayda et al. (2020) suggested that *S. aureus* in Egyptian raw milk cheeses has *sea, seb, sec, sed, see* and *seg, seh, sei, sej, sep, ser* enterotoxin genes and the presence of multiple enterotoxin genes in isolates was observed in 40%. Again, the detection of various enterotoxin genes in artisan cheese was reported in Brazil (*sea* gene) (Castro et al. 2020) and China (*sea, seb, sec, sed, see* genes) (Cai et al. 2021). Kayili & Sanlibaba (2020) isolated 85 *S. aureus*

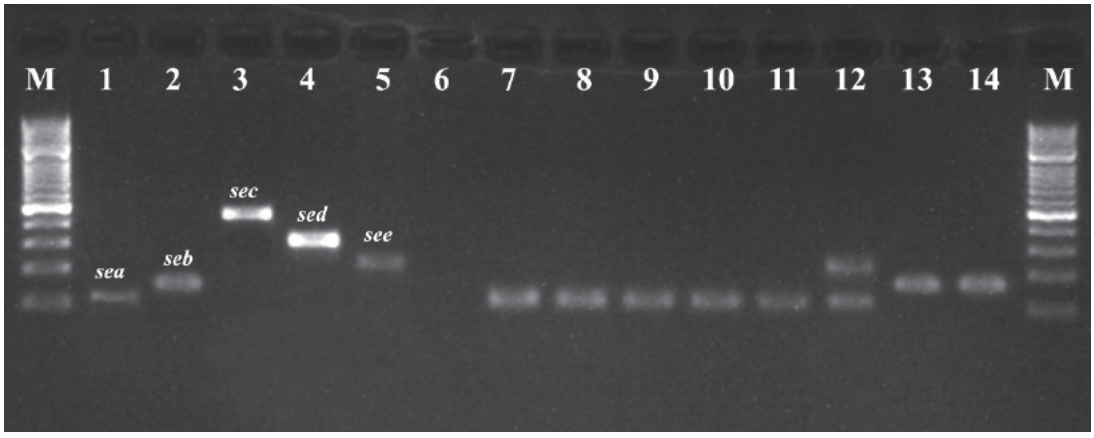


**Figure 1- Enterototoxin genes of *S aureus*. M: 100 bp DNA ladder; lane 1: *S aureus* ATCC 25923 for *sea*; lane 2: *S aureus* ATCC 14458 for *seb*; lane 3: *S aureus* ATCC 19095 for *sec*; lane 4: *S aureus* ATCC 23235 for *sed*; lane 5: *S aureus* ATCC 27664 for *see*; lane 6: negative control; lane 7-11: S7, S14, S37, S44, S56 - *sea* positive isolates; lane 12: S25 - *sea*+ *see* positive isolate; lane 13-14: S38, S41 - *seb* positive isolates**

from 387 cheese samples and none of the isolates had enterotoxin genes. In the same way, the absence of enterotoxin genes in *S. aureus* from milk and cheese was observed by Yildirim et al. (2019). On the other hand, Muneeb et al. (2021) described the presence of sea, seb, seg, and sei among CoNS isolate from retail market fish. SEs are a tremendously important issue for food safety because they can be active after 30 min boiling and may remain present at 121 ºC for 28 min. This means that SEs were not eliminated through normal pasteurization and sterilization methods. Thus, foods can be contaminated with SEs at production steps before packaging (Necidova et al. 2019). The presence of enterotoxigenic *S. aureus* in cheese which is a ready-to-eat food is a remarkable finding of our research.

The PCR results showed that the frequency of virulence-associated genes was relatively low among isolates. Only one CoNS strain (S3) from raw milk had both *tss* and *etb* virulence genes (Figure 2). The remaining isolates did not represent any investigated virulence genes. The distribution of toxigenic genes in the isolates is shown in Table 3. A study performed in Turkey demonstrated the presence of the *tst* gene (6 goat milk, 1 goat cheese sample) in CoNS and 7 *S. aureus* isolates. The absence of *eta*, *etb* genes (goat milk and cheese) in CoNS and *S. aureus* was also reported (Pehlivanlar Onen et al. 2018). A recent study indicated that one *S. aureus* in cow milk carried the *tst* gene and *pvl*, *eta*, *etb* toxin genes (Gharsa et al. 2019). In Brazil, Castro et al. (2020) declared that 14 (18.4%) artisanal cheese isolates of *S. aureus* harbored the *tsst*-1 gene. In Benin, Tohoyesseu et al. (2020) recorded *eta* and *etb* toxin production in coagulase-positive/negative staphylococci and, also *pvl* toxin secretion in 8.3% of *S. aureus* strains. Similar to our results, the *pvl* gene positive *S. aureus* was not detected in milk by Alghizzi and Shami (2021) but Sadat et al. (2022) reported *pvl* positive *S. aureus* in raw cow milk. The mass food surveillance work including milk and dairy products from China revealed that *S. aureus* isolates carried *tss* (14.3%), *eta* (21.9%), *etb* (12.3%), and *pvl* (16.7%) genes (Liao et al. 2018). Staphylococcal superantigens (SAgs) involve pyrogenic toxins such as *tsst*-1, and SE's, and toxic shock syndrome is caused by *S. aureus* strains that produce *tss*-1 toxin. This syndrome is characterized by rash, fever, hypertension, multiple organ malfunction, and may cause death (Abril et al. 2020). The occurrence of CoNS carrying *tss* and *etb* genes in milk is another considerable finding obtained in our study.

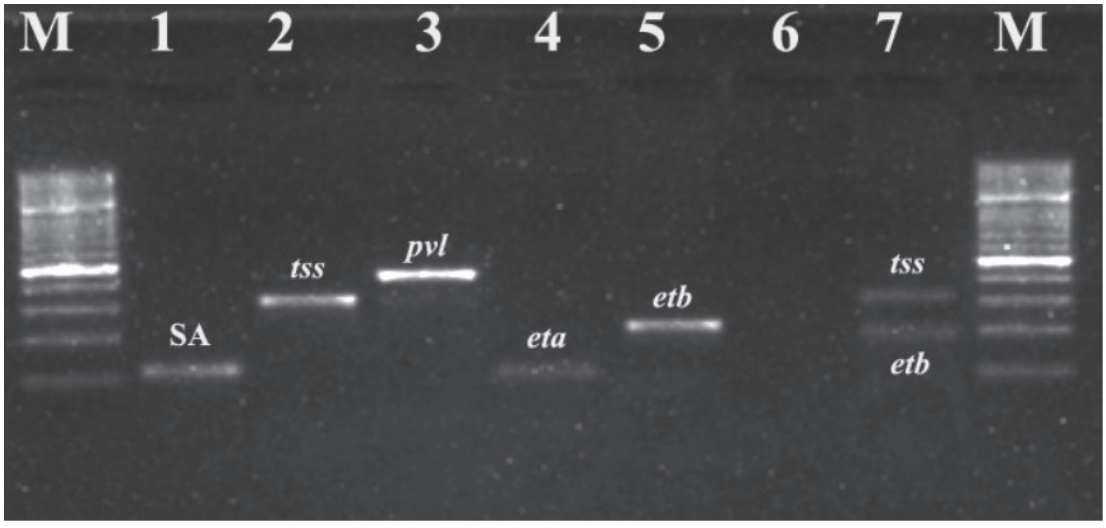


**Figure 2- PCR-based screening of virulence genes. Lane M: 100 bp ladder, Lane 1-5: positive controls of SA, *tss, pvl, eta*, and *etb* genes (*S. aureus* ATCC 19095 for SA gene, *S. aureus* ATCC BAA-1747 for *pvl* gene and *S. aureus* MN8 for *tss, eta*, and *etb* genes); lane 6: negative control; lane 7: S3 - *tss* and *etb* positive CoNS isolate**

The use of antibiotics still remains to treat or control mastitis, but the impact of antibiotic therapy on *S. aureus* decreases with the accelerating antimicrobial agent resistance across the world (Kou et al. 2021). In particular, methicillin-resistant *S. aureus* is another concern for food safety and public health (Elal Mus et al. 2019). In our research, all *S. aureus* and CoNS isolates were negative for the *mec*A gene responsible for methicillin resistance. Our findings were similar to those obtained by Guncuoglu et al. (2020) reporting the absence of the *mec*A gene carrying *S. aureus* in traditional Turkish dairy-based desserts. As in the current work, Castro et al. (2020) did not observe the *mec*A gene in artisanal cheese isolates of *S. aureus*. In contrast, some current reports indicate the presence of *mec*A gene carrying *S. aureus* isolated from milk and/or dairy products in Turkey (Elal Mus et al. 2019; Tavsanli & Cibik 2022), in Romania (Morar et al. 2021), in Egypt (Ahmed et al. 2019; Zayda et al. 2020; Sadat et al. 2022), in China (Cai et al. 2021; Kou et al. 2021; Zhang et al. 2021), in India (Mahanti et al. 2020), in Benin (Tohoyesseu et al. 2020) and in Algeria (Chenouf et al. 2021). Additionally, Chenouf et al. (2021) reported three *mec*A-positive CoNS isolates from milk in Algeria, and Pyzik et al. (2019) demonstrated that 27.5% of poultry-derived CoNS in Poland carried the *mec*A gene.

## 4. Conclusions

In conclusion, the present research has revealed the presence of several staphylococcal toxin genes in *S. aureus* and CoNS. Our main findings are the presence of *S. aureus* containing enterotoxin genes from ready-to-eat food (cheese) (S7, S14, S25, S38, S41, S44, S56) and CoNS isolate (S3) carrying TSST-1 and exfoliative toxin genes from milk. From a food safety perspective, the observation of virulent CoNS in foods is noteworthy. Through this work, the surveillance of toxigenic genes of CoNS and *S. aureus* contributed from Turkey. CoNS isolates are also an emerging concern for the transmission of toxins and other pathogenicity-related genes. In terms of one health approach commonly observed but ignored CoNS in animal-derived foods can pose risk to contaminated food consumers, hospitalized patients, juvenile and elderly people, etc. In short, this work acknowledges the pathogenicity of CoNS. The accurate assessment of this suspected bacteria would need further comparative research involving hospital/animal origin, and more data from foods.

*Data availability:* Data are available on request due to privacy or other restrictions.

*Authorship Contributions:* Concept: T.E.M., F.C., Design: T.E.M., F.C., Data Collection or Processing: T.E.M., G.E.S., B.E., Analysis or Interpretation: T.E.M., F.C., G.E.S., B.E., Literature Search: T.E.M., G.E.S., B.E., Writing: T.E.M., F.C., G.E.S., B.E.

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