VETERINARSKI ARHIV 94 (1), 33-42, 2024 DOI: 10.24099/vet.arhiv.2038

Detection of enterotoxigenic *Staphylococcus aureus* in raw and pasteurized milk

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BASARAN KAHRAMAN, B., R. GECKINLI, S. AK: Detection of enterotoxigenic *Staphylococcus aureus* in raw and pasteurized milk. Vet. arhiv 94, 33,-42 2024.

ABSTRACT

The aim of this study was to investigate staphylococcal enterotoxins (SEs) by ELISA, and detect the five classical *sea, seb, sec, sed,* and *see* genes by real-time PCR in *Staphylococcus aureus* isolates from raw and pasteurized milk samples. *Staphylococcus* spp. were isolated from 98 out of 100 raw milk samples, and in 6 out of 100 pasteurized milk samples. On further biochemical tests, *S. aureus* was isolated in 48 samples (48%) of raw milk (n=100) and in one sample (1%) of pasteurized milk (n=100). Ten (10%) out of 100 raw milk samples were positive for at least one enterotoxin, and the most frequently observed SE was SEA (10%), followed by SEE (7%) and SEB (6%), but none of the isolates were positive for SEC and SED. At least one of the SEs gene types (*sea, seb, sec, sed, see)* was detected in 45 (93.8%; 45/48) *S. aureus* isolates from raw milk samples. *sec, sea, seb, sed*, and *see* genes were observed in 56.2%, 39.5%, 31.2%, 29.1% and 14.5% of strains respectively. The enterotoxin genes were the single type in 21 (46.7%) of the 45 isolates, there were two in 15 (33.3%), three in six (13.3%), four in two (4.4%), and one (2.2%) in all gene regions. The SE gene was not detected in the *S. aureus* (n=1) isolate from pasteurized milk. As a result of this study, the presence of enterotoxigenic *S. aureus* in raw milk was revealed, and it was pointed out that these SEs may contribute to cases of staphylococcal foodborne poisoning (SPF).

Key words: ELISA; staphylococcal enterotoxins (SEs); real-time PCR**;** staphylococcal foodborne poisoning (SPF)

Introduction

Human beings need high quality foods with high nutritional value in order to maintain their vital activities. Among these foods, one of the most preferred is milk. Milk is a special nutrient secreted from the mammary glands of female mammals and has a unique taste, smell, colour and consistency (SINGH and BENNETT, 2002). While many foods meet some of the nutrients needed by living things, milk contains many elements, including vitamins, antibodies, enzymes, etc. Since it is a product that contains these within itself, it can meet these needs on its own. Milk is a beverage and an essential nutrient for people of all ages. It is an important source of protein, carbohydrates, fats and minerals, particularly for individuals during growth. Milk and milk products meet approximately 30% of

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ISSN 0372-5480
Printed in Croatia

the fat and protein required for a healthy life. For this reason, milk and milk products are the most consumed nutrients in the world, as they are crucial at every stage of human life (CHAMBERS, 2005).

It has been pointed out that foods of animal origin, especially milk and milk products, are often associated with foodborne illness if appropriate sanitary and sanitary procedures are not followed during the production and marketing of these products. This is mainly because milk can serve as the perfect medium for the survival and growth of many different pathogens (AKINDOLIRE et al., 2015).

Milk has been considered a potential source for transmission of bacteria to humans, including staphylococci, a common source of contamination, particularly in the dairy supply chain, the environment, and final consumers (AKINDOLIRE et al., 2015). Staphylococcal enterotoxin (SE) was first described in 1959 (BERGDOLL, 1989). Staphylococcal food poisoning (SPF) is one of the most prevalent foodborne diseases in the world and is caused by the ingestion of enterotoxins produced in food by enterotoxigenic strains of the *Staphylococcus* species (EFSA, 2017). Moreover, enterotoxigenic *Staphylococcus aureus* in raw milk poses a potential health risk to consumers. The identification of such strains should be used as part of a risk analysis of milk and milk products (ZOUHAROVA and RYSANEK, 2008). Most of the *S. aureus* genome contains one or more enterotoxin genes (MERDA et al., 2020). Some of the genes seem to be more prevalent, and this seems to depend on the origin where the strains are isolated (BENKERROUM, 2018). Outbreaks involving classical enterotoxins (SEA-SEE) have been largely described in the literature.

The European Union One Health 2020 Zoonoses Report noticed that eleven EU Member States reported data on SEs. Eleven EU Member States (Bulgaria, Cyprus, Czechia, Estonia, Germany, Italy, Portugal, Romania, Slovakia, Slovenia and Spain) reported data on staphylococcal enterotoxins collected in contexts other than the framework of Regulation (EC) No 2073/2005. From an overall total of 267 batches tested, one was positive and was from 'ice cream and similar frozen desserts' collected at a 'processing plant' during an official sampling programme in Slovakia. Also, EFSA (2021) reported that there were no positive results from 1,269 samples collected by four EU Member States (Croatia, Estonia, Romania and Spain) at the distribution level (wholesale establishments and retail establishments). Out of 723 tested samples, only one sample (0.138%) of goat cheese made from raw or low-heat-treated milk collected at a processing plant in Spain was positive (EFSA, 2021).

In the light of all these data, the aim of this study was to present the immune-enzymatic detection of staphylococcal enterotoxins and the molecular detection of enterotoxigenic genes of *S. aureus* in raw and pasteurized milk in Istanbul, Turkey.

Materials and methods

Sampling. Between 2018 and 2019, a total of 200 milk samples were examined, 100 raw and 100 pasteurized, from 177 cows, 14 buffaloes, and 9 goats. The samples were collected from milk collection facilities, retail dairy stores, and the Food Control Laboratory Directorate, in 11 districts of Istanbul. The samples were transported to the laboratory in a cold chain with their sales packaging intact (taken from the stores), or in sterile containers (from the milk collection facilities) and analysed within 1-2 hours.

Staphylococcus aureus isolation. Samples were streaked directly onto Baird Parker Agar with 2% egg yolk tellurite emulsion (BPA, Oxoid, USA). After 48 h of incubation at 37°C, suspected colonies were subcultured on blood agar and incubated at 35°C for 24 hours to obtain pure isolates. Gram staining, catalase-oxidase tests, slide / tube coagulase, DNase test and a latex agglutination test (Plasmatec Laboratory, UK) were performed for identification of *Staphylococcus aureus* (ADWAN et al., 2006; GILANI et al., 2016).

Detection of staphylococcal enterotoxins (SEs - SEA, SEB, SEC, SED, and SEE). The production of SEs in milk samples was assessed with an ELISA detection kit (Ridascreen Set R-Biopharm AG, Germany).

To detect SEs, the isolates were cultured overnight aerobically in 10 mL nutrient broth

(Merck, Germany) at 37°C. Bacterial culture supernatants were collected by centrifugation at 4,000 x g for 10 min and used for detection of SEs. The assay was performed according to the manufacturer's recommendations (RAHIMI and GHASEMIAN SAFAI, 2010). The mean lower detection limit of the assay was 0.1 mg/mL. All experiments were performed in duplicate.

The microtiter strips were placed in the microwell holder and one hundred microliters of the prepared sample was added to the wells of the microtiter strip. After the positive control was added, the plate was gently mixed by shaking by hand and incubated at room temperature for 60 minutes. After incubation, the liquid was completely poured out of the wells. The washing procedure was performed twice. In the next step, one hundred microliters of diluted enzyme conjugate were added to the wells, the plate was mixed gently by shaking by hand, and incubated at room temperature for 60 minutes. The washing procedure was performed three times. After adding 50 microliters of substrate and chromogen to the wells, they were incubated at room temperature for 30 minutes in the dark. After incubation, 100 µL of stop solution reagent was added to each well. The wells were gently mixed by shaking the plate by hand, and absorbance was measured at 450 nm with the addition of a 30 min stop solution. The cut-off value was obtained by adding 0.15 to the mean value of the negative control.

Detection of enterotoxin genes (sea, seb, sec, sed, and see). S. aureus isolates were examined by Real-Time PCR to determine whether they harbour enterotoxin genes. DNA was subsequently extracted using a Roche MagNA Pure 96 System with MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Diagnostics, Germany), according to the manufacturer's instructions. DNA concentrations were measured spectrophotometrically. A LightCycler® 480 Probes Master kit (Roche Diagnostics, Germany) was used for amplifications. The following profile was used; a denaturation step at 95°C for 10 minutes, followed by 40 cycles of 10 seconds at 95°C, 30 seconds at 55°C, and 1 minute at 72°C (VARSHNEY et al., 2009; DEMIRCI et al., 2017). The sequences of the primers (IDT, USA) are shown in Table 1.

Table 1. Sequences, amplicon sizes of the primers, references and positive control used in real-time PCR

Target	Sequences	Amplicon size (bp)	References	Positive controls*
sea	5-AAAATACAGTACCTTTGGAAA-3 5-TTTCCTGTAAATAACGTCTTGC-3 AACGAATAAGAAAAATGTAACTGTTCAGGAGTTGGATC	92	DEMIRCI et al. (2017)	*S. aureus* ATCC 13565
seb	5-CGCATCAAACTGACAAACGA-3 5-CCGTTTCATAAGGCGAGTTG-3 TGTTCGGGTATTTGAAGATGG	243	DEMIRCI et al. (2017)	*S. aureus* ATCC 14458
sec	5-AATAAAACGGTTGATTCTAAAA-3 5-ATCAAAATCGGATTAACATTAT-3 TAGAAGTCCACCTTACAA	80	DEMIRCI et al. (2017)	*S. aureus* ATCC 19095
sed	5-TGATTCTTCTGATGGGTCTAA-3 5-GAAGGTGCTCTGTGGATAATG-3 TATGATTTATTTGATGTTAAGGGTGATTTTCCCGAA	115	DEMIRCI et al. (2017)	*S. aureus* ATCC 23235
see	5-ACCGATTGACCGAAGAAAAA-3 5-CTTACCGTGGACCCTTCAGA-3 TGCAAAGAGGCTTGATTGTG	264	VARSHNEY et al. (2009)	*S. aureus* ATCC 27664

*Positive controls were used also for ELISA and Real-time PCR assays.

Results

Isolation. Staphylococcus spp. were detected in 98 out of 100 raw milk samples and in six out of 100 pasteurized milk samples. On further biochemical tests, *S. aureus* was isolated in 48 (48%) of the raw milk and in one (1%) of the pasteurized milk samples.

Detection of staphylococcal enterotoxins (SEs). Staphylococcal enterotoxin was not found in any of the pasteurized milk samples. Ten out of 100 (10%) raw milk samples were positive for SEA, SEB, and SEE. Staphylococcal enterotoxin A (SEA) was produced by 10%, Staphylococcal enterotoxin B (SEB) was produced by 6%, and Staphylococcal enterotoxin E (SEE) was produced by 7%. None of the milk samples was positive SEC or SED (Table 2).

Detection of SE genes. At least one of the SE gene types (*sea, seb, sec, sed, see)* was detected in 45 (93.8%; 45/48) *S. aureus* isolates from raw milk samples. The genes detected were *sec* (n=27), *sea* (n=19), *seb* (n=15), *sed* (n=14), and *see* (n=7). *sec, sea, seb, sed,* and *see* genes were found in 56.2%, 39.5%, 31.2%, 29.1%, and 14.5% of the strains studied, respectively. The enterotoxin genes were the single-type in 21 (46.7%) of 45 isolates, there were two in 15 (33.3%), three in six (13.3%), four in two (4.4%), and one (2.2%) in all gene regions (Table 3). No SE genes were found in pasteurized milk samples.

Comparison of the enterotoxigenic gene regions detected by Real-Time PCR in *S. aureus* isolates and enterotoxin types detected in milk according to ELISA results is shown in Table 4.

Table 2. Number of Staphylococcal enterotoxin types detected by ELISA

Sample no	Staphylococcal enterotoxin				
	SEA	SEB	SEC	SED	SEE
29	+	-	-	-	-
47	+	-	-	-	-
50	+	-	-	-	-
108	+	+	-	-	+
126	+	+	-	-	+
130	+	+	-	-	+
133	+	-	-	-	+
174	+	+	-	-	+
192	+	+	-	-	+
198	+	+	-	-	+
Total (n=10)	10	6	-	-	7

Table 3. Enterotoxigenic gene regions by milk type

Milk type	*S. aureus* isolates number	Number of Isolates Carrying Enterotoxigenic Gene	Enterotoxigenic gene regions (n)
Raw milk (n=100)	48	45	*sea (6)* *seb (4)* *sec (9)* *sed (2)* *sea+sec (2)* *seb+sec (3)* *sea+sed (2)* *sec+sed (8)* *sea+seb+sec (1)* *sea+sec+sed (1)* *sea+seb+see (4)* *sea+seb+sec+see (2)* *sea+seb+sec+sed+see (1)*
Pasteurized milk (n=100)	1	-	-

Table 4. Number of enterotoxigenic gene regions and enterotoxin types detected by two methods

Isolate Number (n=45)	Real-Time PCR					ELISA				
	sea (n=19)	*seb* (n=15)	*sec* (n=27)	*sed* (n=14)	*see* (n=7)	SEA (n=10)	SEB (n=6)	SEC	SED	SEE (n=7)
n=3	+	-	-	-	-	-	-	-	-	-
n=4	-	+	-	-	-	-	-	-	-	-
n=9	-	-	+	-	-	-	-	-	-	-
n=2	-	-	-	+	-	-	-	-	-	-
n=2	+	-	-	+	-	-	-	-	-	-
n=1	+	-	+	+	-	-	-	-	-	-
n=8	-	-	+	+	-	-	-	-	-	-
n=2	+	-	+	-	-	-	-	-	-	-
n=1	+	+	+	-	-	-	-	-	-	-
n=3	-	+	+	-	-	-	-	-	-	-
n=3	+	-	-	-	-	+	-	-	-	-
n=4	+	+	-	-	+	+	+	-	-	+
n=2	+	+	+	-	+	+	+	-	-	+
n=1	+	+	+	+	+	+	-	-	-	+

Discussion

Staphylococcus aureus is a ubiquitous, opportunistic, and very versatile pathogen that produces a wide range of exoproteins with toxic outcomes for humans and animals (BENKERROUM, 2018). Among the exotoxins, SEs are the most commonly associated with food-borne intoxications (DE BUYSER et al., 2001). *S. aureus* enterotoxigenic strains are frequently isolated from milk and milk products, especially raw milk, and play an important role in public health in terms of SFP (KWON et al., 2004).

For this reason, research in various countries has determined the prevalence of *S. aureus* in raw milk samples. ADWAN et al. (2006) stated that 48% of 250 raw milk samples in Northern Palestine were contaminated with *S. aureus*. KORPYSA-DZIRBA et al. (2011) reported that 77 (32.5%) of 237 raw milk samples were contaminated with *S. aureus*. KOU et al. (2021) isolated 43.1% of *S. aureus* from 144 retail raw milk samples collected from four regions in China. Researchers emphasized that *S. aureus* can contaminate many raw milk sources, usually associated with mastitis or human carriers, and reported that it can lead to contamination of the finished product if proper food processing methods are not followed during milking or later. Many studies have reported varying rates from 42% to 75% for raw milk samples in Turkey (ERTAŞ and GÖNÜLALAN, 2010; YILMAZ and GÖNÜLALAN, 2010; GÜÇLÜKOĞLU et al., 2012).

In this study, *S. aureus* was isolated from 48% of commercially available raw milk (n=100) and 1% of pasteurized milk (n=100) samples. This data is compatible with the findings of other researchers. The fact that the percentage detected in the raw milk offered for sale is quite high, reveals the necessity of minimizing the *S. aureus* contamination of raw milk in order not to harm consumer health. It is thought that it contaminates the raw milk source through causative mastitis or human-induced contaminations, which have an opportunistic pathogenic character.

Various methods are employed for detection of SEs in raw milk. SEs are routinely assayed immunologically by ELISA, which is one of the most simple and sensitive methods (WU et al., 2019). RAHIMI et al. (2012) examined 72 raw milk samples collected from milk tanks in Iran by the ELISA method, and found that 20.8% of them were positive for SEs. They reported that the SE most often detected was SEA (16.7%), followed by SED (12.5%) and SEC (8.3%), and none of the isolates was positive for SEB and SEE. In addition, the researchers showed that 59.9% of *S. aureus* isolates were positive for SEs and the SE most often detected was SEC (28.1%), followed by SEA (26.7%) (NORMANNO et al., 2005). Many studies have also been carried out in Turkey. Researchers isolated 42% (42/100) *S. aureus* and found that 66.6% (28/42) of the milk tested were positive for enterotoxins (ERTAŞ and GÖNÜLALAN, 2010). YILMAZ and GÖNÜLALAN (2010) stated that they isolated 50% of *S. aureus* in raw milk sold on the market, and 61.7% of the milk was positive for SE. KEYVAN et al. (2020) reported that *S. aureus* was isolated at a rate of 57.5% from raw milk samples collected, but they detected enterotoxin in only two of these milk samples.

In the present study, it was determined that 10 (10%) out of 100 raw milk samples were positive for at least one enterotoxin, and the most frequently observed SE was SEA (10%), followed by SEE (7%) and SEB (6%), and also none of the isolates was positive for SEC and SED. These data are in agreement with NORMANO et al. (2005) and MORANDI et al. (2007), who reported frequent findings of SEA, which is the prevalent enterotoxin recovered from food-poisoning outbreaks. Some of our findings differ from those shown in studies in other countries; such studies suggest that SEC is the most commonly produced enterotoxin (JORGENSEN et al., 2005; MORANDI et al., 2007).

Considering that *S. aureus*, which has the ability to form enterotoxins in foods, occurs at the earliest in the early logarithmic phase of reproduction during the synthesis time of SE types, the presence of *S. aureus* detected in milk suggests that isolates may secrete enterotoxins depending on the time. The fact that SEs were not found in any of the pasteurized milk shows that the bacteria (except

in one milk sample) were completely eliminated during the pasteurization stage and there was no contamination in the later stages.

Another preferred and frequently used method is Real-time PCR to show the presence of the SE genes. In many studies, the genes responsible for *sea-see* production in *S. aureus* strains isolated from food samples have been investigated. JORGENSEN et al. (2005) determined that raw cow's and goat's milk was contaminated with *S. aureus* at a rate of 75% and 96.2%, respectively, in Norway. In addition, they found that 22.1% of the isolates of bovine origin and 57.3% of the isolates of goat origin contained enterotoxin genes and had the most *sec* genes. ZOUHAROVA and RYSANEK (2008) gave the rates of SEs genes from milk tanks as 27.1% *sea*, 10% *seb*, 4.3% *sed*, 2.9% sed, and 1.4% sec. MANSOUR et al. (2017) stated that 23 of 141 raw milk samples contaminated with *S. aureus* and six isolates had enterotoxigenic gene regions. Also, the researchers reported that they detected the *see* gene in all 6 isolates, *seb* in 2 isolates, *sec* in 2 isolates, and both *seb* and *sec* genes in 3 isolates.

Moreover, various studies have been carried out to present SE growth in raw milk in Turkey. BOYNUKARA et al (2008) reported that 25.5% (27/106) of *S. aureus* strains isolated from 480 cows with subclinical mastitis were the enterotoxigenic type, 25 isolates carried the *sea* and two isolates the *seb* gene, whilst none of them had the *sec* + *sed* gene. ERTAŞ and GÖNÜLALAN (2010) reported that 66.6% of contaminated milk contains enterotoxins. However, they reported that 73.8% of the isolates contained SE genes, 38.7% *sea*, 6.5% *seb*, 16.1% *sec*, 32.3% *sed* and 6.5% had *sea* + *sed* gene regions. They concluded that *S. aureus* might not secrete enterotoxins into milk despite having the relevant gene regions. GÜÇLÜKOĞLU et al. (2012) reported that 13.7% of the isolates had enterotoxigenic gene regions and stated that 71.4% of these genes were *sea*, 14.2% *seb* and 14.2% *sea* + *seb*. DEMIRCI et al. (2017) reported that the enterotoxigenic gene regions in three strains isolated from milk samples were one *sea*, one *sec* and one *sea* + *sec* + *sed*. As a result of this study, at least one of the *sea-see* enterotoxin gene types was determined in 93.8% of the isolates only obtained from raw milk samples and the most frequently detected gene was *sec*.

When Real-Time PCR (n=45) and ELISA results (n=10) were compared, the enterotoxin types detected as a result of ELISA were compatible with the enterotoxigenic gene regions (*sea-see*) detected in *S. aureus* strains isolated from the same milk. Although one or more enterotoxin gene type(s) were detected in 35/45 (77.8%) of the isolates, no enterotoxin was detected in the milk samples from which these isolates were obtained by ELISA. Ten strains were *sea*/SEA positive; six strains were *seb*/SEB positive by both methods. However, nine strains were *sea* and *seb* positive by PCR, but SEA and SEB negative by ELISA. *sec* and *sed* genes were detected by PCR but were nevertheless negative by ELISA. The results of both methods were identical concerning *see*/SEE.

RAHIMI et al. (2013) reported that 35% of *S. aureus* strains produced classical enterotoxins and emphasized that SEs and *se* genes are in close correlation with the *S. aureus* strain origin. Different explanations for the diverse rates of enterotoxigenicity found in several studies have been suggested. It was underlined that the discrepancy in the prevalence of enterotoxin encoding genes among studies may be due to numerous factors such as the origin of isolates, research sites, hygiene rules in different countries, and diagnostic methods. The contamination of *S. aureus*, especially in raw milk at any stage, is a potential source for a possible food poisoning, and the presence of enterotoxin gene types may become active in later periods even if no enterotoxin is found.

In the present study, only one *S. aureus* isolate was detected from pasteurized milk. Enterotoxin genes were not detected, and these data were considered a good result. Researchers emphasized that pasteurized milk is also important in food poisoning, pasteurization kills *S. aureus* cells, but thermostable SEs mostly maintain their biological activity. Therefore, it is thought that an effective screening method is needed to reveal the prevalence of these enterotoxic strains in foods in order to protect public health. (KÜPLÜLÜ, 2002; RAHIMI, 2012).

Conclusions

It was determined that a high number (93.8%; 45/48) of isolates carried the enterotoxin gene type(s), and some of these milks contaminated with *S. aureus* also contained enterotoxins released into the milk. Therefore, raw milk and milk products are important reservoirs of enterotoxin-producing *S. aureus*. This result also reveals the potential threat of raw milk, and shows that a monitoring program and preventive measures should be implemented for control purposes. Furthermore, the data obtained will be beneficial for risk assessment and public health management. In addition, education and provision of information for animal owners, producers, as well as consumers, should be undertaken in the community. For this reason, in order to decrease the risk of food poisoning caused by raw milk consumption, especially in terms of enterotoxin-producing *S. aureus*, the relevant institutions should carry out regular health inspections of raw milk, which should be standardized, keeping the collection, transportation, and sales management of these products under control.

Acknowledgements

Scientific Research Project Coordination Unit of Istanbul University-Cerrahpasa supported this project (ID: 27343).

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Received: 5 July 2022
Accepted: 13 October 2022

BASARAN KAHRAMAN, B., R. GECKINLI, S. AK: Detekcija enterotoksigene bakterije *Staphylococcus aureus* u sirovom i pasteriziranom mlijeku. Vet. arhiv 94, 33-42, 2024.

SAŽETAK

Cilj je bio u izolatima bakterije *Staphilococcus aureus* iz sirovog i pasteriziranog mlijeka pomoću ELISA testa istražiti enterotoksine stafilokoka (SEs) te uporabom PCR-a u stvarnom vremenu provjeriti prisustvo pet gena, *sea, seb, sec, sedi i see*. Bakterija *Staphylococcus* spp. izolirana je iz 98 od 100 uzoraka sirovog mlijeka i iz 6 od 100 uzoraka pasteriziranog mlijeka. Daljnjim je biokemijskim testiranjem *S. aureus* izolirana iz 48 uzoraka (48%) sirovog mlijeka (n=100) i iz jednog uzorka (1%) pasteriziranog mlijeka (n=100). Ukupno je 10 uzoraka (10%) od 100 uzoraka sirovog mlijeka bilo pozitivno na barem jedan enterotoksin, a najčešći je enterotoksin bio SEA (10%), zatim SEE (7%) i SEB (6%). Ni jedan od izolata nije bio pozitivan na SEC i SED. Najmanje je jedan tip gena za enterotoksine (*sea, seb, sec, sed, see*) otkriven u 45 izolata (93,8%, 45/48) *S. aureus* iz uzoraka sirovog mlijeka. Gen *sec* uočen je u 56, 2% sojeva, gen *sea* u 39,5% sojeva, gen *seb* u 31,2% sojeva, gen *sed* u 29,1% sojeva i gen *see* u 14,5% sojeva. Geni za enterotoksine pronađeni su kao pojedinačan tip u 21 od 45 izolata (46,7%), dva od 15 izolata (33,3%), tri od šest izolata (13,3%), četiri od dva izolata (4,4%) i jedan (2,2%) u svim regijama gena. Gen za enterotoksin nije pronađen u izolatu *S. aureus* (n=1) iz pasteriziranog mlijeka. Ovo je istraživanje pokazalo da se enterotoksigena bakterija *S. aureus* nalazi u sirovom mlijeku, pri čemu je naglašeno da enterotoksini mogu pridonijeti trovanju hranom uzrokovanom stafilokokima (SPF).

Ključne riječi: ELISA; enterotoksini stafilokoka (SEs); PCR u stvarnom vremenu; trovanje hranom uzrokovano stafilokokima (SPF)