Detection of Enterotoxin Genes and Methicillin-Resistance in *Staphylococcus aureus* Isolated from Water Buffalo Milk and Dairy Products

Erdem Saka and Goknur Terzi Gulel

Abstract: The aim of this study was to analyze the presence of genes encoding staphylococcal enterotoxins and methicillin resistance in *Staphylococcus aureus* isolates obtained from water buffalo milk and dairy products. A total of 200 samples (100 raw milk, 50 clotted cream, and 50 cheese samples) was collected from different dairy farms and smallholders in Samsun, Turkey. All samples were analyzed using the standard procedure EN ISO 6888-1 and isolates were confirmed for the presence of the target 16S rRNA specific for *Staphylococcus* genus specific and *nuc* gene specific for *S. aureus* species by PCR. *S. aureus* was identified in 30 of 100 milk (30%), 9 of 50 clotted cream (18%), and 17 of 50 cheese (34%) samples. A total of 99 isolates was confirmed as *S. aureus*. Genotypic methicillin resistance was evaluated using PCR for the *mecA* gene. Out of 99 isolates, nine (9%) were found to be methicillin resistant (*mecA* gene positive). Twelve out of 99 (12%) *S. aureus* isolates were found positive for one or more genes encoding the enterotoxins. The gene coding for enterotoxin, *sea*, was the most frequent (five isolates, 41.6%), followed by *sec* (two isolates, 16.6%), *sed* (1 isolates, 8.3%) and *see* (1 isolate, 8.3%). While three isolates (25%) contained both *sec* and *sed*, none of the samples was positive for *seb*. In conclusion, the presence of *se* gene-positive and methicillin-resistant *S. aureus* in buffalo milk and products revealed that consumption of these products is a potential risk of foodborne infection in this region.

Keywords: enterotoxins, methicillin resistance, milk, *Staphylococcus aureus*, water buffalo

Practical Application: Enterotoxigenic and methicillin-resistant *S. aureus* (MRSA) in milk and dairy products is an important public health problem. Especially in traditional dairy products, Staphylococcal enterotoxins may cause food poisoning due to consumption of raw or unpasteurized milk products.

Introduction

Staphylococcus aureus is a Gram-positive, facultative anaerobic coccal bacterium that is frequently located on the skin, and in the nose and respiratory tract of human and animals (Bergdoll, 1989; Otto, 2010). Staphylococcal food poisoning occurs as a result of consuming foods containing sufficient amounts of enterotoxins (Argudin, Mendoza, & Rodicio, 2010). *S. aureus* can lead to a wide variety of diseases ranging from minor skin infections, nausea, vomiting, and diarrhea to life-threatening diseases such as pneumonia and septicemia (Liu et al., 2011). Several foodborne outbreaks of *S. aureus* intoxications have been reported to be associated with the consumption of contaminated milk and dairy products in many countries (Asoa et al., 2003; Ostyn et al., 2010).

S. aureus is a ubiquitous organism that can be found in the air, water, milk, sewage, and milking equipment (Bergdoll, 1989). The main sources of contamination of raw milk are dairy animals with mastitis, animal skin, and mammary glands (Vautor, Abadie, Guibert, Huard, & Pepin, 2003). Milking equipment, cheese-making machinery, and improper food handling are considered other causes of contamination (Fagundes, Barchesi, Filh, Ferreira, & Oliveira, 2010; Jorgensen, Mork, & Rorvik, 2005). *S. aureus* has been isolated in raw milk, cheese, ice cream, clotted cream, and butter all over the world (Fagundes et al., 2010; Gucukoglu, Cadirci, Terzi, Kevenk, & Alisarli, 2013; Jakobsen, Heggebo, Sunde, & Skjervheim, 2011; Jorgensen et al., 2005; Rahimi & Alian, 2013). *S. aureus* is often isolated from raw milk products because the milk is heated only slightly without proper pasteurization during traditional homemade cheese production. The presence of *S. aureus* has been reported in dairy products, especially at the stage from milk to curd (Arques et al., 2005; Jakobsen et al., 2011).

Buffalo milk is an important source of protein. It is accepted to be superior in comparison to cow milk because it contains less water, a higher total solids content, higher fat content, and higher calorie content (Soysal, 2006). The Turkish Statistical Institute (TUIK) (2016) has reported that the total number of buffaloes in Turkey reached about 142,000 in 2016, of which 63,300 were dairy buffaloes. In addition, it was reported that the average buffalo milk production reached 63,000 tons during the same period. Buffalo milk is generally consumed as yoghurt, clotted cream, butter, and cheese in Turkey. *S. aureus* has been reported in buffalo milk by several researchers (D'Apice, Fenizia, Capparelli, Scala, & Iannelli, 1996; Rahimi & Alian, 2013).

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Table 1–Coagulase positive *Staphylococcus* counts in buffalo milk and dairy products samples.

Coagulase-positive *Staphylococcus* count (CFU/g)	Number of samples			Total samples
	Milk (n = 100)	Cheese (n = 50)	Clotted cream (n = 50)	
$<10^2$	35	21	40	96
10^2–10^3	46	8	10	64
10^4–10^5	19	17	–	36
$10^5>$	–	4	–	4

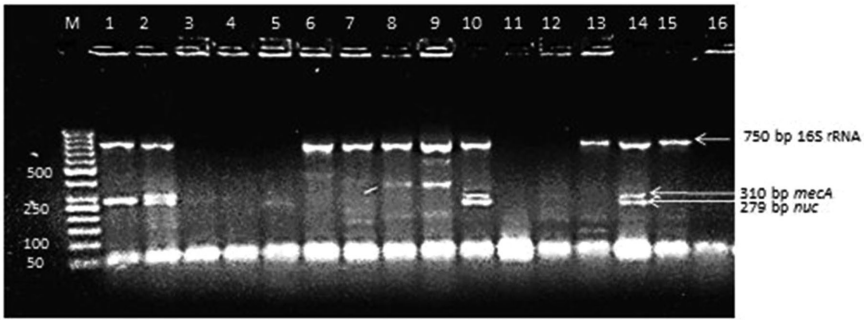

Figure 1–PCR amplification for the detection of 16S rRNA, *nuc* and *mecA* gene of *S. aureus*. Line M: 50 bp DNA marker; Line 1: Positive control 16S rRNA (750bp) and *nuc* (279bp) gene (*S. aureus* ATCC 43300); Line 2: Positive control *mecA* (310 bp) gene (*S. aureus* ATCC 46300); Line 3: Negative control (sterile deionized water); Line 4, 5, 11, 12, 16: *S. aureus* negative milk isolates; Line 6, 7, 8, 9, 13, 15: 16S rRNA gene positive milk isolates; Line 10, 14: 16S rRNA, *nuc* and *mecA* gene positive milk isolates.

S. aureus produces several virulence factors including enterotoxins (SEs), exfoliative toxin (ET), toxic shock syndrome toxin (TSST-1), thermonuclease, hemolysins, hyaluronidase, lipases, and coagulase (Iandolo, 1989; Sandel, & McKillip, 2004). The staphylococcal enterotoxins (SEs) have been classified into five classical serological types (SEA, SEB, SEC, SED, and SEE). These classical SEs have been reported to be responsible for 95% of staphylococcal food poisoning outbreaks (Bergdoll, 1983). In the last 2 decades, new types of SEs and SE-like (SEl) toxins (SEG to SEI, SElJ, SEK to SET, SElU, SElV, SElX, and SElY) have been reported (Bania et al., 2006; Chian, Chang, Lin, Yang, & Tsen, 2006; Ono et al., 2017; Wilson et al., 2011). Among the enterotoxins, SEA is the most common enterotoxin-related cause of staphylococcal food poisoning worldwide, followed by SED and SEB (Argudin et al., 2010).

Methicillin-resistant *S. aureus* (MRSA) has been a primary epidemiological and clinical problem globally in the last 10 years (Mimica et al., 2007). The *mecA* gene encodes for penicillin binding protein 2a (PBP2a), which determines resistance to methicillin (Beck, Berger-Bachi, & Kayser, 1986). The *mecA* gene is highly conserved in staphylococcal strains. Detection of *mecA* is considered to be the gold standard in determining resistance to β-lactam antibiotics in general. The *mecA* gene is responsible for the resistance to methicillin and is used as an indicator for resistance to the other β-lactams (Maes, Magdalena, Rottiers, Gheldre, & Struelens, 2002).

MRSA has become endemic worldwide in hospitals and has also spread into local communities (Goetghebeur, Landry, Han, & Vicente, 2007). MRSA, including strains of animal origin, has been frequently observed in dairy products such as raw milk or traditional cheeses (Peton & Le Loir, 2014; Wendlandt, Schwarz, & Silley, 2013). Studies to date have revealed that MRSA, either livestock-associated (LA-MRSA), community-associated (CA-MRSA), or hospital-associated (HA-MRSA), can be present in/on food that is intended for human consumption (Peton & Le Loir, 2014; Rhee & Woo, 2010; Wendlandt et al., 2013).

Methicillin resistance in *S. aureus* can be also sourced from the newly described *mecA* homologue *mecC* gene and other factors ensuring resistance. Several studies revealed the presence of *mecA* negative, but phenotype-positive MRSA strains in milk and food samples. Li, Zhou, Wang, Xue, and Zhao (2015) reported that seven *S. aureus* isolates derived from milk samples displayed a MIC value higher than >4 μg/mL to oxacillin though they did not carry *mecA* or *mecC* genes.

The objectives of this study were (1) to investigate the presence of *S. aureus* in buffalo milk and dairy products in Samsun, Turkey, (2) to confirm the presence of 16S rRNA and *nuc* genes using PCR, (3) to evaluate the classical enterotoxin genes (*sea*, *seb*, *sec*, *sed*, and *see*) of *S. aureus* isolates, and (4) to assess the methicillin resistance of these isolates based on the presence of the *mecA* gene.

Materials and Methods

Sample collection

In this study, 100 samples of buffalo milk and 100 dairy products (50 buffalo clotted cream and 50 buffalo cheese samples) obtained from 30 randomly selected farms and smallholders in four different districts in Samsun were used as material. Totally, 200 samples were analyzed from November 2012 to May 2013 by examining 30 to 35 samples each month. All samples were transported to the laboratory in containers with ice bags within 1 to 2 hr.

The selected farms in our study were classified as small (five to 10 heads animals), medium (10 to 50 heads animals), and large (50 or more animals) scales according to the buffalo numbers they contained. All samples were obtained from farms that use hand-milking technique and make traditional production. The daily average production of buffalo milk in the farms that the samples were collected was about 3 to 5 kg per animal.

Traditional buffalo cheese is produced from raw milk. The milk is filtered and drained. The raw milk (10 kg) is heated to 38 °C to 40 °C, to which 1 to 3 mL of cheese yeast is added and mixed. It is left for fermentation for 2 hr. The curd is cut into small pieces and filtered. Coagulants formed after fermentation are placed in

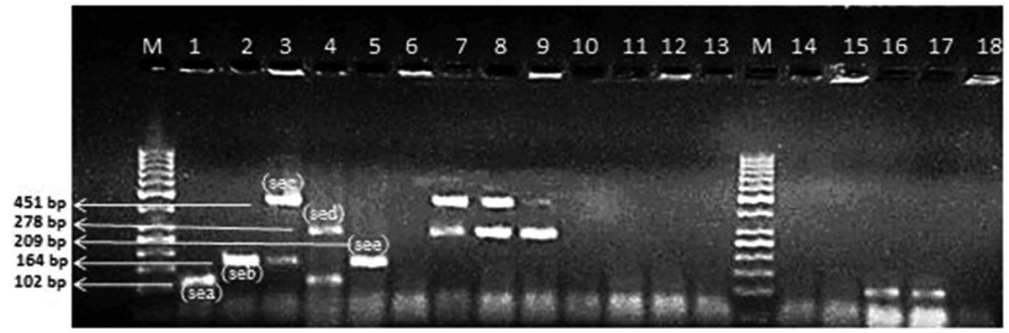

Figure 2–PCR amplification for the detection of *se* genes. Line M: 50 bp DNA marker; Line 1: Positive control *sea* (102 bp) (*S. aureus* NCTC 10652); Line 2: Positive control *seb* (164 bp) (*S. aureus* NCTC 10654); Line 3: Positive control *sec* (451 bp) (*S. aureus* NCTC 10655); Line 4: Positive control *sed* (278 bp) and *sea* (102 bp) (*S. aureus* NCTC 10656); Line 5: Positive control *see* (209 bp) (*S. aureus* ATCC 27664); Line 6: Negative control (sterile deionized water); Line 7, 8, 9: *sec* (451 bp) and *sed* (278 bp) positive milk isolates; Line 10, 11, 12, 13, 14, 15, 18: *se* gene negative milk isolates; Line 16, 17: *sea* (102 bp) positive milk isolates.

special sealed bags. Three kilograms of cheese are produced from 10 kg buffalo milk.

In the production of traditional buffalo clotted cream, the milk is filtered first and boiled at 90 °C to 95 °C for 3 to 4 h in such a way that it does not stick to bottoms and edges by mixing. After this process, the milk is poured to pans that have a depth of 10 cm in such a way that provides foamy and porous cream formation from a certain height. The pans are cooled to 40 °C and again heated to 70 °C with a brief heating. Afterwards, they are kept in cool conditions for 24 hr. Small pieces of ices are sprinkled to harden the cream layer.

Detection of coagulase-positive staphylococci and *S. aureus*

All samples were analyzed for the presence of coagulase-positive staphylococci (CPS) and *S. aureus* according to EN ISO 6888-1 (ISO, 1999). The samples were cultured on Baird-Parker (BP) agar and incubated at 37 °C for 24 and 48 hr. At the end of fermentation, 3 to 5 typical and atypical suspected colonies were collected from each BP agar. All of the isolates were analyzed by PCR for 16S rRNA, *nuc* and *mecA* genes.

Detection of 16S rRNA, *nuc* and *mecA*

Genomic DNA extraction was performed using proteinase K (P2308, Sigma-Aldrich, St. Louis, MO, U.S.A.) and lysostaphin (L7386, Sigma-Aldrich) according to Unal et al. (1992). *Staphylococcus* genus specific 16S rRNA gene amplifying primers (F:5′-AACTCTGTTATTAGGGAAGAACA-3′ and R:5′-CCACCTTCCTCCGGTTTGTCACC- 3′) (Maes et al., 2002; Zhang et al., 2004) were used along with species specific thermonuclease (*nuc*) gene amplifying primers (F:5′-AGCCAAGCCTTGACGAACTAAA GC-3′ and R:5′-GCG ATTGATGGTGATACGGTT-3′) (Brakstad, Aasbakk, & Maeland, 1992). The *mecA* gene was amplified using the primers (F:5′-GTAGAAATGACTGAACGTCCGATAA-3′ and (R:5′-CCAATTCCACATTGTTTCGGT CTAA-3′), as described by Geha, Uhl, Gustaferro, and Persing (1994). All primers were synthesized by Iontek Inc. (Turkey). *S. aureus* ATCC 43300 (16S rRNA and *nuc*) and *S. aureus* ATCC 46300 (*mecA*) were used as positive controls.

A multiplex PCR was performed in a final volume of 50 μL containing 1× PCR buffer (Sigma-Aldrich), 2 mM $MgCl_2$ (Sigma-Aldrich), 0.2 mM dNTP (Sigma-Aldrich), 0.6 μM 16S rRNA primers, 0.4 μM *nuc* primers, 0.4 μM *mecA* primers, 2 U Taq-Polymerase (Sigma-Aldrich), and 5 μL of target DNA. PCR amplification conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 2 min, annealing at 52 °C for 2 min, extension at 72 °C for 2 min, and final extension at 72 °C for 7 min in a thermal cycler (Bio-Rad MJ Mini-PTC-1148). DNA fragments were separated on 1.5% agarose in Tris-borate-EDTA (TBE) buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) and stained with ethidium bromide at 0.5 μg/mL. Electrophoresis was carried out at 90 V for 1 hr (BioRad Power Pac-Basic, Singapore; BioRad electrophoresis tank, Wide Mini, Singapore). PCR amplicons were visualized under UV illumination (Wise-UV Wuv-L50, DAIHAN Scientific, Seoul, Korea). The 16S rRNA, *nuc* and *mecA* genes were visualized at 750, 279, and 310 bp, respectively (Figure 1).

Detection of staphylococcal enterotoxin genes

Detection of enterotoxin genes was performed by multiplex PCR according to previously published primer sequences (Mehrotra, Wang, & Johnson, 2000). *S. aureus* National Collection of Type Cultures (NCTC) 10652 (*sea* positive), *S. aureus* NCTC 10654 (*seb* positive), *S. aureus* NCTC 10655 (*sec* positive), *S. aureus* NCTC 10656 (*sed* and *sea* positive), and ATCC 27664 (*see* positive) were used as positive controls. The PCR reaction mixtures and amplification conditions were done as described above for the 16S rRNA, *nuc* and *mecA* genes. For the detection of enterotoxin genes, 0.4 μM *sea*, *seb*, *sec*, and *see* primers, 0.8 μM *sed* primers, and 2.5 U Taq-Polymerase were used. After electrophoresis, *sea*, *seb*, *sec*, *sed*, and *see* genes were visualized at 102, 164, 451, 278, and 209 bp, respectively.

Statistical analysis

Statistical analyses were carried out using SPSS 11 software for Windows (SPSS Inc., Chicago, IL, U.S.A.) to evaluate *Staphylococcus* spp. counts in milk and dairy products. Significant differences were assessed by one-way ANOVA with *post hoc* Tukey's test. *P*-values less than 0.05 were regarded statistically significant.

Results and Discussion

Coagulase-positive *Staphylococcus* counts

The results of enumeration of coagulase-positive staphylococci in milk and dairy products are shown in Table 1. The population of coagulase-positive staphylococci was found to be higher than

1×10^2 CFU/g, the acceptable limit according to Turkish Food Codex, in 65% of milk samples, 58% of clotted cream samples, and 20% of cheese samples. Fifty-two percent of the 200 examined samples were found to exceed the specified limits (Anonymous, 2011). In a previous study, Costa et al. (2012) reported a higher proportion (40%) of cheese samples exceeding the acceptable limits for CPS compared to our results.

On the other hand, Can and Celik (2012) found lower results in the cheese samples they examined and found that 9.5% exceeded the acceptable limits. The reason for differences of CPS incidence may be due to using raw or unpasteurized milk in the dairy production, poor personnel hygiene, or cross contamination.

Prevalence of *S. aureus*

S. aureus was detected in 56 of 200 (28%) samples. As shown in Table 2, among the *S. aureus* positive samples, 30 (30%) were obtained from milk, nine (18%) were from clotted cream, and 17 (34%) were from cheese in this study. A total of 555 *Staphylococcus* spp. presumptive colonies was obtained from BP agar. These isolates were confirmed for the presence of 16S rRNA and *nuc* gene. A total of 283 of isolates from 126 samples showed amplification of the 750 bp region of the 16S rRNA gene for *Staphylococcus* spp. and 99 isolates from 56 samples showed amplification of the 279 bp region of the *nuc* gene (Figure 1). Among these *nuc* gene positive *S. aureus* isolates, 57 were obtained from milk, 9 from clotted cream, and 33 from cheese (Table 2).

The highest prevalence of *S. aureus* was found in cheese (34%), followed by milk (30%); the least frequently contaminated product was clotted cream (18%). Statistical evaluation showed that there were significant differences ($P < 0.05$) of the presence of *S. aureus* in the milk, clotted cream, and cheese samples.

The results of our study are similar to those of other researchers who found a *S. aureus* prevalence of 28% to 33% in buffalo milk (Abo-Shama, 2014; Pamuk, Yildirim, Seker, Gurler, & Kara, 2012; Sharma, Sharma, & Malik, 2011; Singh & Prakash, 2010). In another study conducted by Alisarli and Solmaz (2010), *S. aureus* was detected in 38% of teat skin samples. However, Rahimi and Alian (2013) found *S aureus* in 17.5% of buffalo milk, which is a lower rate than that found in the present study. On the other hand, other studies have demonstrated higher isolation (75% to 96.2%) rates in cow and goat milk samples than the counts presented here (Jorgensen et al., 2005; Rall et al., 2008). The reason for the different rates might be that milk was obtained from dairy animals with mastitis, or due to differences in milking hygiene, milking equipment, storage conditions, and transportation of the milk.

In the present study, *S. aureus* was identified in 17 of 50 (34%) cheese samples. This result is in agreement with those reported by other investigators (Manfreda, Mioni, & De Cesare, 2005). However, in contrast to our study, Andre et al. (2008) detected *S. aureus* in 70.8% of cheese samples in Brazil and Basanisi et al. (2016) found *S. aureus* in 41.1% from goat and sheep cheese samples in Italy. The reason for this might be due to the use of raw milk from unhealthy animals, inadequate pasteurization during home-made traditional cheese production, contaminated cheese-making equipment, and improper food handling.

In our study, the lowest prevalence of *S. aureus* contamination was found in clotted cream (18%). This may have occurred since, during the preparation of clotted cream, high heat (90 °C to 95 °C) treatment is applied. This treatment might be effective at inactivating bacteria in milk. These results are in agreement with the data reported in the other study by Mashouf, Hosseini, Mousavi, and Arabestani (2015).

Table 2–Prevalence of 16S rRNA, *nuc*, *mecA*, and enterotoxin genes of *S. aureus* isolated from water buffalo milk and dairy products.

Sample	No. of *Staphylococcus* spp. presumptive colonies in Baird parker agar		PCR						No. of *Staphylococcal* enterotoxins		Enterotoxin genes					
			Staphylococcus spp. (16S rRNA)		*S. aureus* (*nuc*)		Methicillin resistance (*mecA*)									
	Samples	Isolates	Samples	Isolates	Samples	Isolates	Samples	Isolates	Samples	Isolates	*sea*	*seb*	*sec*	*sed*	*see*	*sec* + *sed*
Milk ($n = 100$)	98	349	76	167	30	57	4	8	6	8	2	–	2	–	1	3
Clotted cream ($n = 50$)	26	45	15	27	9	9	–	–	1	1	–	–	–	1	–	–
Cheese ($n = 50$)	48	161	35	89	17	33	1	1	2	3	3	–	–	–	–	–
Total ($n = 200$)	172	555	126	283	56	99	5	9	9	12	5	–	2	1	1	3

Prevalence of the *mecA* gene

Genotypic detection of methicillin resistance of *S. aureus* isolates was determined by the presence of *mecA* gene. The presence of MRSA in food is not routinely investigated. Therefore, limited data about the prevalence and genetic spread of MRSA in dairy products are present. In our study, the *mecA* gene was detected in 9 (9%) of 99 of the *S. aureus* isolates from milk (8 isolates) and cheese samples (one isolate), but it was not detected in clotted cream samples.

Similar to the findings of our study, a study conducted in Afyon, Turkey, by Pamuk et al. (2012) reported that out of 360 buffalo milk and milk products samples, 9 (9.2%) of the 97 *S. aureus* isolates were genetically carrying *mecA* gene. In contrast to our study, Nam et al. (2011) reported that, out of 402 *S. aureus* isolates obtained from mastitic milk cows in Korea, 17 (4.2%) were genotypically MRSA. In another study conducted by Normanno et al. (2007a), the *mecA* gene was detected in six (0.4%) of 160 *S. aureus* isolates (from milk, dairy products, meat, and meat products) in Italy.

Prevalence of staphylococcal enterotoxin genes

Enterotoxin genes were detected in 12 out of 99 (12%) isolates. Among these, five (5/12; 41.6%) of them (two from milk and three from cheese) were positive for *sea*, 2 (2/12; 16.6%, from milk) were positive for *sec*, 1 (1/12; 8.3%, from clotted cream) was positive for *sed*, one (1/12; 8.3%, from milk) was positive for *see*, and three (3/12; 25%, from milk) were positive for *sec* + *sed*. None of the samples were positive for *seb* (Table 2, Figure 2).

In this study, the results show that 12% (12/99) of the *S. aureus* isolates obtained from buffalo milk and dairy products were found SEs positive (possessed one or two genes). These results are similar to previous studies, such as the work of Neder, Canavesio, and Calvinho (2011) who found that 11.76% of *S. aureus* isolates obtained from milk samples in Argentina were enterotoxigenic. Kuplulu, Sarimehmetoglu, and Celik (2004) reported that 35 (16.4%) of 214 cheese samples were contaminated with enterotoxigenic staphylococci species in Turkey. Similar values were detected by Korpysa-Dzirba and Osek (2014). It was found that 20 (11.9%) out of 168 *S. aureus* isolates were positive for one or more classical *se* genes. However, contrary to our study, Ertas, Gonulalan, Yildirim, and Kum (2010) found enterotoxin genes (*sea*, *seb*, *sec*, *sed*) in 13 (3.02%) out of 80 isolates from sheep cheese and dairy-based desserts. The presence of SEs, the expression of enterotoxin genes, was identified in 12 (2.8%) out of 80 isolates by using the ELISA technique. In the study of Carfora et al. (2015), 77 colonies of 93 were found positive for the "classical SEs" coding genes (*sea*, *seb*, *sec*, or *sed*) by PCR. The SEs/SE*l*s genes most frequently detected were *sed*, present in 40% of the isolates, followed by *sec* (34.3%).

Our results indicate that the most frequently isolated enterotoxin gene was *sea* (41.6%), similar to the other studies (Dores, Dias, Arcuri, Nobrega, & Ferreira, 2013; Mashouf et al., 2015; Morandi, Brasca, Lodi, Cremonesi, & Castiglioni, 2007). In agreement to our study, Rall et al. (2008) reported that in the raw milk isolates the gene *sea* was the most frequent (16 isolates, 41%), followed by *sec* (eight isolates, 20.5%), *sed* (five isolates, 12.8%), *seb* (three isolates, 7.7%), and *see* (two isolates, 5.1%). SEA, either alone or together with other SEs/SEls, is the most common cause of staphylococcal food poisoning worldwide (Do Carmo et al., 2004). Many studies have reported that SEA, SEC, and SED type toxins are the most commonly produced toxins in milk and dairy products (Jorgensen et al., 2005; Morandi et al., 2007; Neder et al., 2011; Normanno et al., 2007b).

In our study, interestingly, *see* gene was detected in 8.3% of tested isolates. Several other studies reported that none of tested *S. aureus* isolates from milk and dairy products harbored the *see* gene (Arcuri et al., 2010; Carfora et al., 2015; Jorgensen et al., 2005; Poli et al., 2007; Zouharova & Rysanek, 2008). SEE has been rarely reported in foods, and its existence in staphylococcal food poisoning outbreaks has only been determined in rare occasions. However, this enterotoxin has also been associated with outbreaks in the United States and other countries (Arcuri et al., 2010; Carfora et al., 2015; Ostyn et al. 2010; Wieneke, Roberts, & Gilbert, 1993).

Our data show that the *seb* gene was not detected in any of the isolates. Different from our study, Gucukoglu et al. (2013) reported that in the ice cream isolates, the most frequently detected enterotoxin gene was *seb* ($n = 9$, 69.2%) followed by *sea* ($n = 1$, 7.6%), *sed* ($n = 1$, 7.6%), *seb* + *sed* ($n = 1$, 7.6%), and *sea* + *seb* + *sed* ($n = 1$, 7.6%), respectively. The *sec* gene was not detected in any of these isolates.

Moreover, contrary to our study, Dores et al. (2013) found the presence of the *seb* gene in one isolate only and they reported that all other genes (*sea*, *sec*, *sed*, and *see*) were not detected in any isolate. Several studies described that none of the investigated isolates from bovine and goat milk and dairy products harbored the gene *seb* (Akineden et al., 2001; Lyra et al., 2013; Pexara, Solomakos, Sergelidis, Angelidis, & Govaris, 2016). In other studies, however, the *seb* gene was detected in *S. aureus* isolates from milk and dairy products at various frequencies (Arcuri et al., 2010; Jorgensen et al., 2005; Peles et al., 2007; Rall et al., 2008; Zouharova & Rysanek, 2008). The prevalence of enterotoxin-producing *S. aureus* isolates in milk and dairy products samples differs among studies. This might be due to differences in the source of sampling, and geographical differences.

Conclusion

The results of this study showed that buffalo milk and its products were contaminated with enterotoxigenic and MRSA. Furthermore, the most frequently enterotoxin gene was *sea*. The presence of *S. aureus* in raw milk and products may be considered a potential health risk for food poisoning. Therefore, it is recommended that consuming raw milk and low heat-treated milk products should be avoided.

Acknowledgments

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