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Research Article

Investigation of *Staphylococcus aureus* and Enterotoxin Presence in Raw Milk of Production in Aydin Region, Turkey

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ABSTRACT

The purpose of this study is the investigation of the presence of *Staphylococcus aureus* and enterotoxin in raw milk for production found in different enterprises of Aydın province. Twenty-eight (28) swab samples taken from different points and 86 raw milk samples were examined for *S. aureus* count and staphylococcal enterotoxins (SET A, B, C, D, E Total) presence. *S. aureus* analyses in raw milk samples were conducted within the framework of TSE 6582 ISO 6888 standard. According to Turkish Food Codex (Number: 2009/14), all the samples of 86 total raw milk examined were determined to be appropriate in terms of *S. aureus* count. *S. aureus* analyses in raw milk samples were conducted within the framework of TSE 6582 ISO 6888 standard. In this study, ELISA test used for the presence of staphylococcal enterotoxins. The result of test, neither of 96 raw milk samples was contaminated with staphylococcal enterotoxin. As a result, it has been shown that enterotoxin was not detected in the raw milk examined but the presence of *S. aureus* in samples taken from various points of milk processing plants showed that public health is at risk. Therefore, it is necessary to give importance to production hygiene, cleaning and disinfection protocols and informing the staff at milk processing units.

Key words: *Staphylococcus aureus*, Enterotoxin, Raw milk, ELISA

INTRODUCTION

Staphylococci are important microorganisms in terms of public health since they have the ability to cause epidemics both in hospital infections and in the food sector. The most important strain of the genus *S. aureus*, which carries potential pathogenic properties for humans that express high toxicity enterotoxin and is effective on the gastrointestinal tract (Normanno *et al*., 2007).

Staphylococcal food poisonings are food-borne intoxications caused by enterotoxins that are effective on the gastrointestinal tract, synthesized by enterotoxigenic staphylococci during the reproduction of food. However, not all species have enterotoxigenic properties in food. For example, only 6.25% of *S. aureus* strains, the most important strains of the species, have enterotoxigenic character (Bergdoll, 1991).

Especially the proportion of staphylococcal food poisonings originating from dairy and milk products varies according to the nutrition habits of the countries. This result of Le Loir (2003) showed that milk and dairy-based poisonings in the UK between 1969 and 1990 accounted for 8%, in the USA between 1975 and 1982 1.4% of milk and dairy-based food poisonings, in 1999 and 2000, 32% of food poisonings originating from dairy products, especially cheese, were supported by poisonings caused by Staphylococci. Although the frequency of occurrences in various countries varies according to geographical conditions and nutrition habits, staphylococcal food poisoning causes many damages that continue with economic losses particularly in the health sector (Küplülü *et al*., 2002).

S. aureus, is known as the second or third pathogen for being the causative agent of food poisoning in the health sector, (Atanassova, 2001). In the US, 6-80 million people have been found to be affected by this pathogen every year and 9000 of these cases have been found to be fatal (Buzby, 1997; Le Loir *et al*., 2003).

The studies in various countries have shown that about one-third of food poisoning events originate from contagious populations with *S. aureus*, one of the most important species of the genus (Mutluer, 1993). It has been reported that the rate of *S. aureus* poisoning among foodborne microbiological diseases is 40% in Hungary, 45% in USA and 25-30% in Japan (Nakazawa, 1992).

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It is very important for public health to determine the presence of *S. aureus* and enterotoxins in milk and dairy products, as well as the consumption of milk and dairy products by children who are in the age of growing at the same time. Enterotoxin in dairy and dairy products may lead to food poisoning even at very low concentrations (0.5-0.75 ng/ml). The scope of this study is to determine the presence of *S. aureus* and enterotoxins by ELISA method in raw milk samples to be processed and *S. aureus* isolation in various points in different production facilities in Aydın province.

MATERIALS AND METHODS

In this study, 28 samples of swabs from different milk production facilities (including A, B, C and D facilities) and 86 samples of raw milk taken from milk collection tanks into sterile cups were brought to the Laboratory of Microbiology Department of Adnan Menderes University Veterinary Faculty under a cold chain and examined for the presence of *S. aureus* and staphylococcal enterotoxins. The collected swab samples and milk samples are shown in Table 1.

S. aureus isolation

In the raw milk samples analysed, the *S. aureus* count was performed within the framework of Turkish Standards Institute 6582 ISO 6888 (2001) standard. Samples were taken on aseptic conditions to 10 ml of the stomacher bags and homogenized for 2 minutes on the shaker by adding 90 ml sterile physiological peptone water. Serial dilutions of homogenized samples were prepared up to 10^{-2}. The sample dilutions and swab samples were inoculated onto Baird Parker agar containing Egg yolk-Tellurite Emulsion by spreading plate method and incubated for 48 hours at 37°C. After the incubation, characteristic grey and black coloured colonies surrounded by a transparent hare were evaluated as suspected *S. aureus* colonies. Suspected colonies were evaluated as *S. aureus* after gram staining of suspected colonies, DNase test, mannitol fermentation, slide coagulase test and catalase test.

ELISA test

Raw milk samples taken in aseptic conditions and brought to the laboratory in the cold chain were prepared as described in the commercial kit procedure (r-BioPharm® AG, Darmstadt, Germany, Art No.: R4105). As a positive control in our study, *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC (25923) was used.

RESULTS

In this study, 28 samples of swabs from different milk production facilities (including A, B, C and D facilities) and 86 samples of raw milk taken from milk collection tanks in May 2017 sterile cups were brought to the Laboratory of Microbiology Department of Adnan Menderes University Veterinary Faculty under a cold chain and examined for the presence of *S. aureus* levels and staphylococcal enterotoxins. In terms of the findings of the collected swab samples, from the samples of swab collected from the enterprises A, B, C, D; despite the application of CIP and other washing procedures, *S. aureus* at $5.8x10^1$ - $6.2x10^4$ cfu/ml was detected in samples taken from external storage tanks, inside tank, boiling cauldrons, fermentation lines, inside and inside of vehicle tanks. Of the 86 milk samples taken from the provinces of Aydin, 14 of them were pasteurized milks under conditions stated in the Communiqué of Raw Milk and Heat Treated Drinking Milks numbered TSI 2000/6, and 4 (29%) of the samples were *S. aureus* isolation was determined. No populations were identified that could synthesize the toxin in any of the isolates identified. All of 86 milk samples (100%) were found to be appropriate in terms of *S. aureus* number according to the Communiqué on Amendments in the Communiqué of Turkish Food Codex, 2009/14 numbered Communiqué on Raw Milk and Heat Treated Drinking Milk. The presence of staphylococcal enterotoxin (SET A, B, C, D, E Total) was not detected in any of the 86 samples examined (Table 2).

Table 1: The collected swab samples and milk samples

Enterprise	Swab samples	Raw milk samples
A	11	43
B	3	6
C	9	20
D	5	17
Total	28	86

Table 2: The results of isolation and ELISA test

Enterprise	*S. aureus* isolation		Staphylococcal enterotoxin
	Swab samples	Raw milk samples	
A	4	2 ($3.0x10^2$- $1x10^3$ cfu/ml)	-
B	3	-	-
C	7	-	-
D	4	2 ($2.1x10^1$- $1.0x10^3$ cfu/ml)	-
Total	28	5	0

DISCUSSION

Milk and dairy products are often preferred in daily nutrition and diet applications for a sustainable healthy life. Milk and dairy products are aimed at providing the physical needs of babies and children in the age of development and creating a significant part of their daily diet. However, the presence of microbial contaminants besides other contaminants in the milk also brings certain risks with respect to health. It is not desirable that various important pathogenic agents are present in milk and dairy products. One of these pathogenic agents is *S. aureus*. The presence of *S. aureus* in raw milk is very important in terms of community health (Nassasra, 2011).

Raw milk is also a suitable medium for *S. aureus* as it is for most pathogens. Even if milk is prepared under normal conditions and in compliance with hygienic rules, a certain number of microorganisms can be found. *S. aureus* is infected with the best-milked animal. Especially milk from mastitic animals is an important source of enteropathogenic *S. aureus* strains. *S. aureus*, found in raw milk, can come from the mammary canals of the animal, from the body, from air, from water, from milk and milk, and from the pipes used during storage (Gülbandılar, 2006; Çakır, 2007).

Different isolation rates have been reported in studies conducted on *S. aureus* in raw milk. It was found that 75% of cows' milk samples were contaminated with *S. aureus* in a study in which Norway examined the presence of enterotoxigenic *S. aureus* in cow and goat milk and in raw milk products, including cheese samples (Jorgensen *et al*., 2005). It was reported that 168 (38.4%) of 437 raw milk samples contained coagulase-positive *S. aureus* in a study conducted in Italy between 2000 and 2002 (Normanno *et al*., 2005). Another previous study stated that *S. aureus* isolated from 55 (35%) of 190 raw milk samples (Nohutçu, 2005) and 60 (100%) 60 raw milk samples (Gündoğan and Ataol, 2012). *S. aureus* isolates were isolated from products made from 26 of the raw milk of 86 samples. It was also found 38 (70%) *S. aureus* isolates from 54 raw milk samples by conventional culture methods in Brazil (Rall *et al*., 2008). It was detected that 218 (40.22%) of the 542 milk samples examined were *S. aureus* (Virgin *et al*., 2009). In another study, it was found that *S. aureus* in all 75 milk samples (100%) in Spain (Garcia *et al*., 2009). It was reported that 30 of the total 60 raw milk samples in the study they conducted in Kayseri province were not suitable due to the number of Turkish Food Codex 2000/6 numbered, Raw Milk and Heat Treated Drinking Milk Communiqué numbered *S. aureus* (Yılmaz and Gönülalan, 2010). It was reported 60 of the 100 milk samples examined (60%) with *Staphylococcus* spp. catalase and coagulase test, which 300 isolates were examined, determined that 42 (14%) isolates were *S. aureus* (Gönülalan and Ertaş, 2010). When previous studies are examined, *S. aureus* can be isolated at high rates in raw milk in general. The isolation of *S. aureus* at an acceptable level of 29% from 14 pasteurized raw milk in this study can be attributed to the sufficiency of hygiene quality in the milk bulk tanks in the sample enterprises. Although there is no *S. aureus* in the milk samples, the high *S. aureus* counts from samples from vehicle tanks indicate that heat treatment from the production facilities is being performed correctly, but CIP procedures and other hygienic applications are inadequate.

The growth of *S. aureus* and the formation of toxins in vigour are dependent on many factors. These are pH, salt content, a_w value, competitive microflora and the chemical content of the foodstuff. However, it has been stated that there is also an inhibitory or supporting effect of competitive bacterial flora in food (Sancak *et al*., 2006).

Generally, enterotoxigenic *S. aureus* reaches 10^6 cfu/g or more in food, and toxin can be produced in sufficient amount. The minimum number of cells that can produce enterotoxin at a minimum level that will cause intoxication in humans varies depending on the type of strain, type of toxin and substrate (such as the composition of food, temperature and other physical and chemical parameters). Detectable SEA can be produced by a low number of cells up to about 10^4 cfu/g. SEA, SEB and SED positive strains reached 4 ng/ml when the cell number was $6x10^6$ cfu/ml and the number of SEA produced was 1 ng/ml and increased to $3x10^7$ cfu/ml (Erol, 2007; Ünlütürk and Turantaş, 1999).

Studies on raw milk have reported the presence of enterotoxin at different levels. It was reported that 13 of the 42 bovine sputum contained enterotoxigenic *S. aureus* and that 5 of these isolates were positive for SEA, 3 isolates were positive for SED (Umoh *et al*., 1990). Sixty three (7.3%) of 852 samples were positive for enterotoxigenic *S. aureus* isolated from raw milk in France, and that isolates produced the most SED (Lamprell *et al*., 2004), unlike in the study. It was reported that the majority of SEC toxins were detected in the study of raw milk from Norway (Jorgensen *et al*., 2005). In a previous study, 362 isolates of milk and milk products were identified as *S. aureus* (Normanno *et al*., 2005) and 362 isolates were found to be enterotoxigenic in 217 (59.9%). SEC may be an important cause of staphylococcal intoxications due to the consumption of contaminating milk and dairy products, however in this study, SEC was not detected.

As a result of a previous study (Joffe and Baranovics, 2006), it was found that the samples of milk and dairy products sold in markets in Latvia were contaminated with *S. aureus* at 44.1%, mastitis milk at 25% and raw milk samples at 22.5%. Enterotoxigenic *S. aureus* was isolated from mastitis milk at the highest level of 77.3% and isolates produced the most SEA. As a result, it is stated that mastitis milk and milk products may play an important role in the contamination with enterotoxigenic *S. aureus.*

The researchers studied a model for the production of *S. aureus* and enterotoxin in UHT milk. They found that there was a positive correlation between toxin production and the number of bacteria until the number of *S. aureus* reached to $10^{6.5}$ cfu/ml was found in the pods according to the developed models (Fujikawa and Morozumi, 2006).

In a study of bovine and ovine milk in Italy, coagulase positive *S. aureus* has been detected from all 111 cases. Enterotoxin was detected in 58 (79%) of 37 cattle milk and 37 (97%) in 38 small cattle milk (Cremonesi *et al*., 2006). In another study, it was reported that 27 of the 106 *S. aureus* strains obtained from 408 milk samples were enterotoxigenic. 25 strains were reported to be positive for SEA, 2 strains were positive for SEB (Boynukara *et al*., 2008). Thirty seven samples of 60 raw milk samples in Kayseri province did not comply with the 2008/26 regulation on the maximum limits of contaminants in foodstuffs due to the presence of staphylococcal enterotoxins (Yilmaz and Gönülalan, 2010). The presence of SE in 28 milk samples of 100 milk samples was detected in a previous study (Gönülalan and Ertaş, 2010). The ratio of SE A, B, C, D were 10 (35.7%), 2 (7.1%), 5 (17.8%), 9 (32.2%) respectively.

In our study, the presence of staphylococcal enterotoxin was not found in any of the 86 raw milk samples taken from 4 different businesses. We concluded that *S. aureus* levels in our study could not reach the number for toxin release and production.

Conclusion

In the study, raw milk collected from the enterprises in Aydın province was examined for the presence of *S. aureus* and enterotoxin. None of the raw milk samples analysed contained staphylococcal enterotoxin, and in terms of the number of *S. aureus*, it was found in a safe level in terms of public health.

The general precautions that should be applied to reduce the risks of *S. aureus* in raw milk according to the data obtained in our study are mentioned below.

- Compliance with personal hygiene regulations when transporting, storing and processing units,
- Prevention of cross-contamination,
- The staff in contact with milk must have adequate hygiene training,
- Milk should be stored at appropriate temperatures.

As a result, it has been proved that considering the raw milk supplied from the enterprises in Aydın and offered for retail sale and consumer purchase does not present a risk for healthy nutrition. Manufacturers, retailers, consumers; it is necessary to be recognized as an important task and responsibility to inform the public with increasing responsibility by the responsible public institutions and organizations, media and non-governmental organizations on animal health, animal welfare, hygiene and sanitation about food safety and risks.

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