Incidence Of Staphylococcus Aureus And Its Enterotoxins In Various Cheeses Sold At Retail Markets Of Izmir City

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Abstract

In this study, the incidence of S.aureus and staphylococcal enterotoxins in various cheeses sold at retail markets of Izmir city was examined. Some physicochemical properties of samples were also evaluated. Seventy five cheese samples composed of kashar cheese, Erzincan and Izmir tulum cheeses, white cheese (feta cheese), örgü and Van otlu cheeses were analyzed. S.aureus was detected in the range of 1.0 x10^1cfu g^{-1} to 3.0 x10^5 cfu g^{-1} at 18.7 % of samples. Enterotoxin analyses were performed with Reversed Passive Latex Agglutination (RPLA) kits. Number of enterotoxin detected samples were 17 and the distribution of toxins were in the order of SEA, (10 samples; 58.82%), SEB, (7 samples; 41.17%), SEC, (2 samples; 11.76 %) and SED (1 sample; 5.88%). Results showed that cheeses especially that made from raw milk would pose a staphylococcal intoxication risk to public health. In conclusion, a great attention should be given to bacteriological standards of milk that used in cheese production and contamination must be avoided through strict adherence to good manufacturing practices in dairy plants.

Keywords: Cheeses, S. aureus, Enterotoxin,

Özet

İzmir İlinde Satılan Bazı Peynirlerde S.aureus **ve Enterotoksinlerinin Bulunma Sıklığı**

İzmir ilinde satılan bazı peynirlerde S.aureus ve enterotoksinlerinin varlığının ve bulunma sıklığının araştırıldığı bu çalışmada çeşitli firma ve mandıraların ürettiği beyaz, kaşar, tulum, örgü ve Van otlu peynirlerinden oluşan toplam 75 örnek incelenmiştir. Örneklerin % 18.7'sinde 1.0 x10^1cfu g^{-1} ile 3.0 x10^5 cfu g^{-1} arasında değişen düzeylerde S.aureus tespit edilmiştir. Örneklerdeki enterotoksin analizi RPLA (Reverse Passive Latex Agglutination) ticari test kiti ile yapılmıştır. 17 örnekte toksin tespit edilmiştir. Örneklerde en çok tespit edilen enterotoksin tipi SEA (10 örnek, 58.82 %) olmuştur. Onu sırasıyla SEB, (7 örnek; 41.17%), SEC, (2 örnek; 11.76 %) ve SED (1 örnek; 5.88 %) izlemiştir. Elde edilen bu sonuçlar peynirlerin özellikle çiğ sütten üretilen peynirlerin Staphylococcus intoksikasyonları oluşumunda halk sağlığı açısından risk oluşturabileceğini göstermektedir Bu nedenle peynir üretiminde kullanılacak olan sütün bakteriyolojik standartlara uygunluğuna ve peynir işletmelerinde oluşabilecek bulaşmaların önlenmesi için iyi üretim tekniklerine ve düzenli temizliğe önem verilmelidir.

Anahtar kelimeler : Peynir**,** S. aureus, Enterotoksin

INTRODUCTION

Staphylococcus aureus is a common food poisoning bacterium which has been implicated as aetiological agent in several food poisoning outbreaks associated with milk and cream, cheeses, cream filled pastries, butter, ham, sausages, canned meat, salads, cooked meal and sandwich fillings (1). Various examples of staphylococcal food poisonings are described by some researchers. (2,3,4,5,6,7). Although process conditions such as low pH and a_w values could minimize the growth of pathogens in cheeses, S. aureus growth and enterotoxin production may take place if uncontrolled fermentation and/ or personnel misbehaviour occurred during process. Also post pasteurization contamination and temperature abuse during transport and storage might result in high levels of pathogenic microorganisms in cheeses (8). Risks vary with the type of cheeses, whether raw or pasteurized milk is used for processing and the length of ripening period (9). The foods that are most often involved in staphylococcal food poisonings differ widely from one country to another (1). Data about the outbreaks of S. aureus intoxications in Turkey is limited since in many cases, the type of food involved in food borne diseases and pathogens associated with are not notified to the public health services.

There are some reports related to microbiological and physicochemical properties of various type of cheeses including white cheese, kashar cheese, tulum cheese, örgü and Van otlu (with herbs) cheeses sold at different regions of Turkey (10,11,12,13,14,15,16,17).

The purpose of this work was to study the incidence of S.aureus and staphylococcal enterotoxins in different cheese samples sold in Izmir city. Some physicochemical parameters such as acidity, moisture and salt contents of the products have been also evaluated.

MATERIALS AND METHODS

Sample collection

A total of 60 cheese samples composed of white cheese (15), kashar cheese (15), tulum cheese (5 Izmir pickled type, 10 Erzincan type), orgu cheese (10) and Van otlu (herby) cheese, (5) were purchased within their original packages consisted of 4 different brands. Also 15 samples consist of white (5), kashar (5) and tulum (3 Izmir pickled type, 2 Erzincan type) cheeses were collected from local bazaar produced by different small manufacturers. **Enumeration of** S. aureus:

Samples (10g) were weighed into sterile stomacher bags, diluted with prewarmed 90 ml of 2 % sodium citrate (Riedel de Haën 25116) solution and homogenized in a Stomacher Lab-Blender 400 (Seward Medical,) for 2 min. They were further diluted with 0.1 % (w/v) peptone water (Oxoid L37). 1ml of initial suspensions were spread on the surface of three Baird Parker Agar (Oxoid CM 275) plates, supplemented with tellurite and egg yolk emulsion, also 0.1 ml of further decimal dilutions were spread on two parallel plates of BPA. Plates were incubated at 37°C for 30-48 h. Suspected colonies were identified through the coagulase test (18).

Physicochemical analysis

The pH value was measured according to Mc Sweeney and Fox (19) by using a glass electrode pH meter (N.E.L., Mod 821). Acidity was determined according to Turkish standard TS 591 (20).

Dry matter content was determined gravimetrically according to TS 5311 (21) and salt content was determined according to TS 591 (20).

Detection of Enterotoxins

Reversed Passive Latex Agglutination commercial test kit (Oxoid SET.RPLA, TD900) were applied for the detection of enterotoxins in cheese samples. This method is given as a standard method for detection of staphylococcal enterotoxins in Canadian Health Protection Branch. Samples were prepared according to procedures given in the instructions of kit.

2.5 Statistical analysis

Analysis of variances, correlations and nonparametric tests were performed using SPSS/PC version 10 (SPSS Inc.)

RESULTS

A total of 75 cheese samples were analysed for the presence of S. aureus and its enterotoxins. Fourteen cheese samples from the out of 75 contained S.aureus ranging between 1 x 10^1 to 3 x 10^5 cfu g^{-1}. Enterotoxins have been detected in 17 samples in which staphylococcal enterotoxin A (SEA) was the most frequently detected toxin type (10 samples; 58.82 %), followed by SEB (7 samples; 41.17 %), SEC, (2 samples; 11.76 %), SED (1 sample; 5.88 %). No correlation has been observed between the presence of enterotoxins and S.aureus in different cheese samples. However level of S.aureus was found to be statistically significant depending on the brand of cheese samples ($P<0.05$).

The results of physicochemical analysis of the samples are given in Table 3 and 4. The pH values of cheeses collected from markets varied from 5.27 to 6.91 with a mean value of 6.16. Dry matter content showed a great variability in samples depending on the cheese type ranging between of 32.22 % to 81.01 % (w/w). Acidity and salt contents of samples were changed from 0.15 % to 2.56% and 1.75 % to 6.52 % (w/w) respectively. The acidity, salt and dry matter contents of the bazaar samples were found higher than samples purchased from markets. Also changes in the all physicochemical characteristics of different cheese samples were found to be statistically significant depending on cheese type and brand of samples ($P<0.05$).

DISCUSSION

The presence of S.aureus is considered as the tool to asses the hygienic conditions in dairy products and its presence at a level of 10^5 cfu g^{-1} is perceived as an evidence of enterotoxin production and food poisoning risk in foods. Number of S.aureus in analysed cheese samples was below this level (10^5 cfu g^{-1}) except two samples (one kashar and one white cheese samples) collected from bazaar (data not given). However staphylococcal enterotoxins have been detected in 17 cheese samples including those containing high numbers of S.aureus. No viable S.aureus cells were recovered from 9 of 17 toxin detected cheese samples (Table1 and Table 2). In these circumstances it could be thought that raw milk initially contaminated with the toxin was used in the process or once the toxins produced, S.aureus cells were died off by any reasons. This study as well as others showed that most type of cheeses sold at the retail markets of different regions of Turkey do not sustained the S.aureus index set-up in Turkish standards for cheeses (11, 12, 13, 15, 16, 17). Enterotoxin presence in pasteurised milk, milk powder and cheese has been demonstrated by several studies carried out in Turkey and other countries (5, 22, 23, 24, 25, 26).

Level of enterotoxins in 17 toxin detected samples was assumed to be higher than 1 ng g^{-1} since the sensivity of RPLA kit reported to be 1 ng g^{-1} of food (27). It was reported that the amount of enterotoxin causing intoxications depends on weight and individual sensivity but it is generally agreed that 0.1-1 µg kg^{-1} will cause illness in a human (28) However an unusual outbreak was reported in which wild mushrooms in vinegar containing 10 ng SEA and 1 ng SED per gram was condemned as the source of food poisoning outbreak (29). So that cheese samples containing more than 1ng g^{-1} toxin posses a risk from the standpoint of public health.

There was no significant difference between the physicochemical characteristics of toxin detected and non-detected samples of same cheese types ($P<0.05$). As Todd et al. (25) reported that, pH value of enterotoxin detected Swiss-type cheese did not differ significantly from normal value of this cheese. The physicochemical properties of analysed cheese samples greatly varied depending on cheese brand (Table 3). When market and bazaar cheese samples were compared, the differences in the mean values of pH, acidity and salt contents of white cheese have been found to be statistically significant where as statistical difference observed in the dry matter content of kashar cheeses and acidity and/or salt content of tulum cheeses (Table 4). This results as well as others (11, 14, 30) show that physicochemical properties of different cheese types sold in Turkish retail markets greatly vary probably due to the non-standardized manufacturing conditions in small dairy plants.

In conclusion, the results of this study which are consistent with the finding of others indicate that contamination of cheese by S.aureus and its enterotoxins represents a significant potential health hazard. The manufacture of cheese and other dairy products from raw milk provides an opportunity for incidence of staphylococcal food poisonings. Therefore cheese must be produced from pasteurised milk and post process contamination must be avoided through strict adherence to good manufacturing practices. In addition bacteriological standards established for the milk and cheese must be strictly observed by effective quality control programs exercised by authorised body and manufacturer itself.

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Table 1 Distribution of S.aureus and Its Enterotoxins in Cheeses Collected from Markets

	White Cheeses			Kashar Cheeses		
Brands[a]	S.aureus[b] cfu g^{-1}	Toxin Detected Samples[c]	Toxin type	S.aureus cfu g^{-1}	Toxin Detected Samples	Toxin type
A(n:5)[f]	<10	1	A,D	<10	0	-
B(n:5)	<10	1	B,C	<10	0	-
C(n:5)	<10	1	A	2.6 x10^2	0	-
	Örgü Cheeses			**Van Otlu Cheeses**		
B(n:5)	<10	0	-	<10	1	A
D(n:5)	<10	0	-			
	Tulum Cheeses[e]					
A(n:5)[f]	6.0 x10^2	3	A(2)[d] C(1)			
B(n:5)	<10	2	B(1) A(1)			
C(n:5)	1.7 x10^2	0	-			

[A]The differences for S.aureus loads in cheeses from same brand were significant (P<0.05). [b] Mean value of S.aureus counts of 5 samples [c] No significant differences was detected in the enterotoxin occurrence of different cheeses with Kruskal Wallis test (P<0.05). [d] The number of this type toxin detected samples given in parenthesis [e] Cheeses from A brand are Izmir tulum type.[f] Number of samples.

Table 2: Distribution of S.aureus and Its Enterotoxins in Cheeses Collected from Bazaar (n=5)

White Cheeses			Kashar Cheeses		
S.aureus[b,c] cfu g^{-1}	Toxin Detected Samples[d]	Toxin type	S.aureus cfu g^{-1}	Toxin Detected Samples	Toxin type
3.3 x 10^3	5	A(3)[e] B(3)	6.0 x10^4	1	B
Tulum Cheeses[a]					
4.2 x 10^3	2	A(2)[e] C(1)			

[a] 2 samples were Erzincan tulum cheese type other samples were Izmir tulum cheese type. Toxins were detected in Izmir tulum cheeses. [b] Mean value of n samples [c] S.aureus loads differs significantly from the stand point of cheese type (P<0.05) [d] No significant differences was detected in the enterotoxin occurrence of different cheeses with Kruskal Wallis test (P<0.05. [e] Number of this type toxin detected samples given in parenthesis.

Table 3 Physicochemical Characteristics of Different Brands of Cheese Samples Collected from Markets [a]

	White Cheeses			Kashar Cheeses			Tulum Cheeses[b]			Orgu Cheeses		Vanotlu Cheese
Brand	A (n=5)	B(n=5)	C(n=5)	A(n=5)	B(n=5)	C(n=5)	A(n=5)	B(n=5)	D(n=5)	B(n=5)	D(n=5)	B(n=5)
pH	5.7±0.3[a]	5.9±0.2[a]	6.2±0.1[b]	6.3±0.0[a]	6.3±0.1[a]	6.8±0.1[b]	6.1±0.3	5.8±0.1[a]	5.8±0.18[a]	6.4±0.2[a]	6.4±0.1[a]	5.9±0.1
Acidity[c]	1.3±0.2[a]	1.1±0.1[a]	1.1±0.1[a]	1.7±0.2[a]	0.79±0.1[b]	0.2±0.0[c]	0.7±0.0	2.0±0.3[a]	1.9±0.2[a]	0.9±0.1[a]	0.8±0.1[a]	1.9±0.5
Salt[d]	3.1±0.5[ab]	2.6±0.6[b]	3.5±0.5[a]	2.6±0.7[a]	2.1±0.3[ab]	1.9±0.1[b]	2.7±0.5	4.3±0.3[a]	3.7±0.6[a]	5.1±0.7[a]	5.6±0.6[a]	4.7±0.5
Drymatter[e]	45.0±1.8[a]	67.1±2.4[b]	51.1±5.7[c]	34.4±1.3[a]	40.7±7.4[ab]	44.0±4.6[b]	53.6±4.4	47.0±2.4[a]	58.0±3.2[b]	78.1±2.7[a]	75.7±1.8[a]	63.3±3.7

[a] Mean values from n samples of cheeses ± standard deviation. Means of same cheese type in the same row with different letters show statistically significant differences depending on brand with Waller and Duncan test (P<0.05)

[b] Cheeses from A brand are Izmir tulum type and others are Erzincan Tulum type.[c] % lactic acid [d] on wet basis (g 100g^{-1}) [e] g 100g^{-1}

Table 4 Physicochemical Characteristics of Market and Bazaar Cheese Samples [a]

	White Cheeses		Kashar Cheeses		Izmir Tulum Cheeses		Erzincan Tulum Cheeses	
	Market (n=15)	Bazaar (n=5)	Market (n=15)	Bazaar (n=5)	Market (n=5)	Bazaar (n=3)	Market (n=10)	Bazaar (n=2)
pH	6.1±0.3[a]	6.4±0.4[a]	6.5±0.2[a]	6.3±0.2[a]	6.1±0.2[a]	6.1±0.3[a]	5.8±0.1[a]	6.2±0.3[a]
Acidity[b]	1.2±0.2[a]	3.4±0.5[b]	0.9±0.6[a]	0.37±0.2[a]	0.68±0.1[a]	1.9±0.3[b]	1.9±0.3[a]	2.19±0.0[a]
Salt[c]	3.1±0.6[a]	6.4±0.6[b]	2.2±0.5[a]	2.7±0.6[a]	2.7±0.5[a]	8.9±1.1[b]	4.0±0.5[a]	8.00±0.3[b]
Drymatter[d]	54.4±10.2[a]	48.2±6.6[a]	39.7±6.2[a]	53.4±5.2[b]	53.6±4.4[a]	55.7±7.1[a]	52.5±6.3[a]	55.7±3.6[a]

[a] Mean values from n samples of cheeses ± standard deviation. Means of same cheese type in the same row with different letters show statistically significant differences with Mann-Whitney test (P<0.05)

[b] % lactic acid [c] on wet basis (g 100 g^{-1}). [d] g 100 g^{-1}.