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A review on the presence of *Staphylococcus aureus* in cheese

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Abstract

Up to now, 24 different antigenic serotypes of Staphylococcal enterotoxins have been described, but classical enterotoxins (A, B, C, D and E) are responsible for 95% of staphylococcal food poisonings. *Staphylococcus aureus* (*S. aureus*) is an ubiquitous microorganism that cause symptoms of poisoning such as abdominal cramps, nausea, vomiting and diarrhea after 2-4 hours following ingestion of toxins present in food. Cheese is a suitable substrate for the development of *S. aureus* due to its high nutritional content. The reason for the emergence of *S. aureus* in cheese are the inability to pasteurize the milk of animals exposed to mastitis infection with high amounts of *S. aureus*, the inadequate starter culture activity, the post-pasteurisation contamination and the unfavorable conditions for product processing and storage. During the production and maturation of many cheese species, the development of *S. aureus* and the production of enterotoxin in some cases have been investigated by some researchers for its importance of sanitation. In this study, the general characteristics of *S. aureus*, the presence in cheese, epidemiology and protection-fighting methods have been compiled.

Keywords: Cheese, *Staphylococcus aureus*, enterotoxin, toxication

Peynirde *Staphylococcus aureus* varlığının incelenmesi üzerine bir derleme

Özet

Stafilokokal Enterotoksin'lere ait bugüne kadar 24 farklı antijenik serotip tanımlanmıştır fakat stafilokokal gıda zehirlenmelerinin % 95'inden klasik enterotoksinler (A, B, C, D ve E) sorumlu tutulmaktadır. *Staphylococcus aureus* (*S. aureus*), gıdada bulunan toksinlerinin alınımını takiben 2-4 saat sonra abdominal kramplar, bulantı, kusma ve diyare gibi zehirlenme semptomlarına neden olan ubiquiter özelliğe sahip bir mikroorganizmadır. Peynir, yüksek seviyedeki besinsel içeriğinden ötürü *S. aureus*'un gelişimi için uygun bir substrattır. *S. aureus*'un peynirde ortaya çıkışının sebebi olarak yüksek miktarda *S. aureus* içeren mastitisli sütlerin pastörize edilmeden peynire işlenmesi, kullanılan starter kültür aktivitesinin yetersiz olması, sütün pastörizasyon sonrası kontaminasyonu ile ürünün işlenmesi ve depolanması sırasındaki uygun olmayan şartlar düşünülmektedir. Birçok peynir türünün üretimi ve olgunlaşması sırasında *S. aureus*'un gelişimi ve bazı vakalarda enterotoksin üretimi, sanitasyon öneminden dolayı bazı araştırmacılar tarafından incelenmiştir. Bu çalışmada *S. aureus*'un genel karakteristikleri, peynirdeki varlığı, epidemiyolojisi ve koruma-mücadele yöntemleri derlenmiştir.

Anahtar kelimeler: Peynir, *Staphylococcus aureus*, enterotoksin, zehirlenme

1. Introduction

Production of milk and dairy products, which is an industry chain based on animal husbandry, is of great importance because these products have not an alternative to sustain the human life in a healthy manner. Especially milk is only food ingredients necessary for the first life-span of mammals and humans, and almost all of the nutrients needed by the organism can be fully and adequately supplied due to its composition. Milk contains

all the basic components of food: carbohydrates, proteins, fats, minerals and vitamins [1]. The milk and dairy sector has a large producer and consumer base. Milk has an important place in the food sector, to not spoil in a short time by applying various technologies currently being processed, stored and transported [2]. Cheese is an important milk product with its nutritional value and unique flavor and aroma, which has a very important place both in terms of food sector and consumer in the processing of milk.

Cheeses made from raw milk are more preferred than those made from pasteurized milk because they have a stronger and richer flavor intensity. Traditionally, cheese made from raw milk is considered to have lost or reduced the levels of harmful microorganisms during the ripening process [3, 4]. The ripening process plays a natural selective role because of some components such as organic acids, hydrogen peroxide and bacteriocin produced by lactic acid

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bacteria [5]. On the other hand, there is an outbreak of *Listeria monocytogenes*, *Staphylococcus aureus* (*S. aureus*) producing enterotoxin, *Salmonella spp.* and *Escherichia coli* O157: H7 originating from the contamination of the cheese directly or indirectly. *S. aureus*, one of the pathogenic microorganisms that can be found in cheese, constitutes a significant part of the poisonings originating from cheese.

S. aureus, a gram-positive opportunistic pathogen, can lead to a number of diseases ranging from skin lesions to septicemia or meningitis. Some species of *S. aureus* can produce staphylococcal enterotoxin (SE) in food and cause staphylococcal food poisoning (SFP). The SEs are formed during the growth of *S. aureus* in foods. The symptoms of SFP such as abdominal cramps, nausea, vomiting and diarrhea, occurs 2-4 hours after consumption of contaminated foods and vary in severity depending on individual health status [6, 7]. To date, many antigenic serotypes have been described in SE's (A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R and U), but classical enterotoxins (A, B, C, D and E) are responsible for 95% of food poisonings [8, 9]. Enterotoxin A (SEA) is the most common serotypes among classical enterotoxins. The ability of *S. aureus* and toxins to form illness occurs under the influence of a number of extrinsic (eg, atmospheric, temperature) and intrinsic (e.g, pH, water activity) factors [10].

During the production and ripening of many cheese species, the development of *S. aureus* and, in some cases, the production of enterotoxin have been investigated by some researchers because of the importance of sanitation. The results obtained differed under the influence of a number of factors such as the type and nature of the cheese and the activity of the starters [11]. Therefore, it is not possible to make generalizations about the activity of *S. aureus* in cheese. In general, when the number of *S. aureus* is over 10^5 per gram during the production of cheese, SE production occurs and it is considered that there is a risk of intoxication [12]. Factors such as milk, starter culture, coagulation and whey formation, salting and brine conditions, maturation conditions, which play an important role in the cheese making process, have different effects on the development of *S. aureus* and enterotoxin formation [11]. The microbiological values of cheese in Turkey are stated in the Turkish Food Codex Microbiological Criteria Regulation and according to this regulation, M value was determined as 1×10^5 cfu/mL for coagulase positive staphylococci in cheeses made from raw milk and 1×10^2 cfu/g in cheeses made from pasteurized milk [13].

In this study, the presence of *S. aureus* in cheese, cheese-induced staphylococcal epidemics and protection-fighting methods were compiled.

1.1. General characteristics of *S. aureus*

Staphylococci are bacteria characterized by cocci that form multiple grape-like clusters on a plane with a diameter of 0.5-1.5 μm . Staphylococci are non-motile, non-spore-forming, aerobic or facultative anaerobic microorganisms. Many staphylococcal strains need complex nutrition, whereas they generally require organic nitrogen sources containing essential amino acids such as arginine, valine, thiamine and nicotamide, which contain 5-12 amino acids [14, 15]. Members of this class can be distinguished from streptococci having a different cell wall structure by being catalase positive and oxidase negative. Staphylococci have tolerance to high salt concentrations and resistance to heat. Pathogenic staphylococci can be commonly identified by coagulase producers and thus by the ability to coagulate blood. This feature allows the distinction of bacteria such as *S. aureus*, *S. intermedius* and *S. hyicus* from other

staphylococci such as coagulase negative *S. epidermidis* [16-18].

S. aureus forms smooth, convex, bright, rounded colonies in gray, grayish-yellow to yellowish orange with β -hemolysis property in blood agar. In the identification of *S. aureus*, thermonuclease (TNase) production helps to identify as well as the production of coagulase [19]. All coagulase positive bacterial species except *S. delphini* produce TNase, only *S. aureus* ferment mannitol aerobically and anaerobically producing protein A and acetone at the same time. SE's are heat-resistant proteins produced by *S. aureus* and some other coagulase-positive bacteria such as *S. intermedius* and *S. hyicus* [20].

1.2. The survival of *S. aureus* in cheese

Milk containing *S. aureus* in high amounts is processed without pasteurization, inadequate starter culture activity, post-pasteurisation contamination, and inappropriate conditions for processing and storage of the product may lead to poisoning from cheeses [21]. The development of *S. aureus* and the production of enterotoxin are known to occur in several hours after it has been in cheese vat, whereas the development and toxin production *S. aureus* is known to be associated with many factors in cheese. The high count of *S. aureus* in milk that made cheese facilitates the resistance of this bacterium to the inhibitor-affecting factors during production. On the other hand, as the number of competitive microorganisms in the milk increases, the inhibition of *S. aureus* becomes so easy [22]. In a study examining the viability of *S. aureus* in white cheese, it was reported that the number of *S. aureus* declined to an average of 10^2 cfu/g in the cheese made without starter culture at the end of the 24-hour production period and at the same time the count did not change remarkably during the production process [23]. According to the same study, it was reported that brine with a concentration of 14 and 18% did not affect the viability of *S. aureus* whereas the number of viable cells in cheeses with starter culture was lower. Selçuk [24] reported that she placed the white cheese made by adding about 1.2×10^4 cfu/mL *S. aureus* strain and 0.5% starter culture (*Streptococcus lactis* and *Lactobacillus casei*) into pasteurized brine containing 14, 15, 16 and 17% in 30, 60 and 90 days to mature. It has been reported that the addition of *S. aureus* and starter cultures did not significantly alter the total number of bacteria, but the number of total bacteria increased significantly from the 15th day to the 60th day of ripening and significantly decreased on the 90th day of ripening. It was stated that adding of starter culture and 90 days ripening of *S. aureus* contaminated cheese had the lowest number of *S. aureus* and that there was no significant effect of saline salt concentrations on the number of lactic acid bacteria. However, with the maturation of the cheese, it was noted that the number of lactic acid bacteria was significantly increased and that the highest number was in the maturation period of 90 days. In a similar study [25], the effects of *Lactobacillus plantarum* BG33 on the development of *Staphylococcus aureus* ATCC 6538 were investigated on days 6, 18, 23, 39, 59, 80, and 90th days of ripening time and it has been reported that the effect of *L. plantarum* BG33 on *S. aureus* is insignificant ($p > 0.01$). Yücebay [26] studied the effects of probiotic lactic acid bacteria of *Lactobacillus brevis* (*L. brevis*) BG18 and *Pediococcus pentosaceus* (*P. pentosaceus*) BH105 strains on the development of *S. aureus* ATCC 6538 during white cheese production. According to the research, it was found that the antibacterial effect of *L. brevis* was started after 648 hours and it had a significant effect on the development of *S. aureus* ($p < 0.01$) until the end of storage and that *P.*

pentosaceus BH105 bacterium had a statistically insignificant ($p > 0.01$).

In another study [27], 9 groups (90 samples) of white cheese were tested for survival time of *S. aureus* during the 63 days ripening period. It was reported that the number of *S. aureus* isolated from fresh cheese samples during 7-14 days, which was the first slice of ripening, was high and there was a significant decrease in the number of *S. aureus* in the 42-63 day period. It was stated that *S. aureus*, which was suppressed by pasteurization in fresh cheese samples, could gain activity again and stayed alive until the 42th day of ripening. When *S. aureus* reaches high counts ($> 10^6$ cfu/g) in foodstuffs (cheese or whey), toxins are released. Cheese vats can carry a long term risk due to low acidity, because *S. aureus* can reach high enough levels to produce enterotoxin. The use of lactic acid bacteria (LAB) in cheese making can reduce the likelihood of toxin-infections that may arise from *S. aureus*. However, even with the use of contaminated starter cultures, it is known that *S. aureus* causes a small number of epidemics. The rapid pH drop caused by the using of active starter culture is a preventive factor for *S. aureus* reaching the number required for enterotoxin production [28]. Baran et al. [29] reported that the counts of *S. aureus* decreased ($p < 0.05$) in all of the cheese samples which were stored at two temperature (4 and 12 °C) during 90 day ripening period. The reduction in the *S. aureus* count was found 2.5 times lower in cheeses ripened at the higher temperature, but the temperature was determined that had no significant effect on *S. aureus* survival ($p > 0.05$). They also stated that SE could not be detected in the cheeses during ripening.

There are few studies on *S. aureus* viability in different cheeses other than white cheese produced in Turkey. In one of these studies, a study [30] on *S. aureus* viability was carried out during a 90 day ripening period in which Van Otlu cheese that 10^5 cfu/mL *S. aureus* was inoculated to raw milk. The number of *S. aureus* began to decrease after the 15th day and then decreased to 10^2 cfu/g on the 90th day of ripening. It was noted that the number of *S. aureus* that initially rose to 10^7 cfu/g and then decreased to lower levels. The effect of flavoring substances, storage conditions and storage time on the viability of *S. aureus* has been investigated in Sürk cheese, another regional cheese. Aroma enhancers have been reported to have no effect on the development of *S. aureus* whereas storage conditions and storage time have reduced the number of *S. aureus* [31]. In a study Ozer et al. [32] claimed that the effect of different salt concentrations from 12.5% to 17.5% on Urfa cheese had no effect on the viability of *Yersinia enterocolitica*, *Escherichia coli* O157: H7, *Shigella flexneri* and *Salmonella enteritidis* bacteria but *Bacillus cereus* and *S. aureus*, were affected by salt concentrations.

There are a number of studies on the presence and growth activities of *S. aureus* in various types of cheese produced in different countries of the world and they present the threats to public health of the probable presence of *S. aureus* in cheeses. Vernozy-rozand et al. [33] reported that the number of *S. aureus* significantly decreased in a study examining the presence of enterotoxigenic *S. aureus* inoculated at different concentrations (4, 5 and 6 log (cfu/mL)) to lactic cheese produced from raw goat milk and at the end of the 42-day maturation, *S. aureus* was completely absent in samples of cheeses, contrary to this result, they reported that the number of aerobic mesophilic microorganisms increased. In another study [34] the growth of *S. aureus* and enterotoxin production were observed in semi-hard cheese produced from raw cow milk. Coagulase positive staphylococci inoculated according to the study showed rapid growth during the first 6 hours. An increase of less than 0.5 log cfu/mL was reported between 6 and 24

hours. The maximum level reached on the 1st day in cheese was 2.82-6.84 log cfu/g. Researchers reported that pH in Saint-Nectair cheese should be 5.8 or less in the first 6 hours and 6.3 or less in Salers cheese to limit the growth of *S. aureus*.

In a study [35] of the effects of lactic starter inoculation, heating of clot and ripening temperature on the behavior of *S. aureus* in Manchego cheese, a reduction in *S. aureus* of 5.8 fold at the end of 60 days maturation in 1% *Streptococcus lactis* cheeses was noted. According to the same study, it was reported that *S. aureus* found in the cheese produced by applying 30 °C temperature to the coagulant was 4-5 times lower than the ones prepared by applying from 36 °C to 40. It was emphasized that cheese ripened at 10 and 20 °C had 10 and 100 times less count of *S. aureus* than cheese ripened at 5 °C, respectively. Tuckey et al. [34] reported that microorganisms were concentrated in the coagulant and the amount of microorganisms increased until salted, in a study initially investigating the viability and growth of 1×10^5 *S. aureus* in Cheddar, Colby, Swedish type, Limburger, and Cottage type cheeses. It has also been reported that there has been another increase during the first 21 days of ripening after the clot was released. It was emphasized that the amount of *S. aureus* was continuously decreasing during ripening but never zero.

Meyrand et al. [36] observed for 41 days in a study conducted on the possible presence of *S. aureus* and staphylococcal enterotoxin A in camembert type cheese produced from raw milk. It has been reported that in cheese with a starting amount of *S. aureus* of more than 10^3 cfu/g, the amount at the 22nd hour shows an approximate 1 log reduction relative to the amount at the end of the 42 day ripening period. Staphylococcal enterotoxin A levels ranged from 1 to 3.2 ng g⁻¹ in the initial amounts of inoculum between 10^3 - 10^6 cfu/g, whereas it was not detected in the level of 10^2 cfu/g on cheese.

Hasalliu et al. [37] found that *S. aureus* in 72 of the samples in a study of *S. aureus* prevalence in 176 cheese samples kept in different conditions in Albania. Of these samples, 48 were reported that microbiological loading ranging from 10^2 - 10^4 log cfu/g was maintained at a temperature above 10 °C and 24 samples were stored below 10 °C, ranging from 10^2 - 10^3 log cfu/g. They found that the levels they found were less than the critical limit of 10^5 cfu/mL for toxin production, but the risk of enterotoxin formation in cheeses kept at temperatures above 10 °C. Ahmed et al. [38] reported that the viability of *S. aureus* during the production of Domiati cheese decreased with the decrease in pH.

Hamama et al. [39] have examined the viability of *S. aureus* in Iben, a traditional Moroccan cheese producing by adding *Lactococcus lactis* (*Lc. Lactis*), which produces nisin. *S. aureus* strains producing enterotoxin C at levels of 10^3 and 10^5 cfu/mL were inoculated and it was reported that despite the rapid decrease in the amount of *S. aureus* in cheeses producing with *Lc. lactis*, they have maintained their vitality for a long period in the cheeses where nisin-producing bacteria were not found. Enterotoxin C was detected in cheese with high initial *S. aureus* concentration (10^5 cfu/mL) for 3 days. Santos and Genigeorgis [40] reported that *S. aureus* at 10^5 cfu/mL in raw or pasteurized supernatant resulted in enterotoxin production in Minas cheese produced using starter or without starter. Delbes et al. [41] studied the viability and enterotoxin production of *S. aureus* in three types of cheese (Saint-Nectaire, Registered Saint-Nectaire and Registered Salers cheese). It was reported that *S. aureus* showed a rapid improvement in all types of cheeses during the first 6 hours, an increase of 0.5 log cfu/mL between 6 and 24 hours. Enterotoxins were detected only on the first day in two Salers cheeses when the

number of *S. aureus* was 5.55 log cfu/g and 5.06 log cfu/g, respectively. For this reason it was reported that the pH value is due to the high value of the first 6 hours.

1.3. Epidemiology of *S. aureus* in cheese

Foodborne staphylococcal intoxications are one of the most common causes of foodborne bacterial diseases in many countries. Dairy products carry a suitable substrate quality for the development of *S. aureus* due to high content of nutritional component. The main reasons for such products to cause foodborne illness are:

- the presence of coagulase positive staphylococci in the raw milk;
- direct contamination in the production process;
- cross-contamination after production.

The first epidemic report of foodborne outbreaks from staphylococci was made in 1884 by Vaughan and Sternberg in Michigan (USA) [42-44]. It has been reported that this food poisoning case was accompanied by staphylococci contaminating cheese. The authors commented on this case that "It seems not improbable that the poisonous principle is a ptomaine developed in the cheese as a result of the vital activity of the above-mentioned *Micrococcus* or some other microorganisms which had preceded it, and had perhaps been killed by its own poisonous products". With this phenomenon, the first 37 foodborne staphylococcal poisoning cases were identified and reported to be an important reference source in subsequent years [45]. Cheese-borne staphylococcal food poisonings have been reported in these cases and various cases have been reported in different countries around the world. In Brazil, 50 people were reported ill with the consumption of Minas cheese in the first of two cases of food poisoning involving 378 individuals. Symptoms of food poisoning occurred within two hours with the consumption of cheese. In the second poisoning case, in which 328 individuals were affected, clinical manifestations followed diarrhea and vomiting following consumption of raw milk. Analysis of unpasteurized milk and cheese samples revealed that *S. aureus* was detected in amounts ranging from 2.4×10^3 to 2.0×10^8 cfu/g and that it produced SEA, SEB, and SEC toxins. It was suggested that the source of specific enterotoxins in the two cases showed that the source was food workers in the first case and the second case was bovine mastitis [46]. In 2009, Ostyn et al. [47] reported six outbreaks of *S. aureus* in France, consisting of 23 cases with gastrointestinal symptoms between October 29 and November 14. They had observed nausea, vomiting, abdominal cramps and diarrhea following cheese consumption in 23 person of 26. Enterotoxin type E was detected in microbiological and molecular studies on consumed cheeses and this was the first outbreak of staphylococcal enterotoxin type E in France. It has been reported that 359 staphylococcal food poisoning cases have been detected in the UK Food Hygiene Laboratories between 1969-1990 [48]. And also, the number of *S. aureus* in the food samples were 3.0×10^7 log cfu/g on average. The presence of staphylococcal enterotoxin of 38 were reported in two cases of cheese poisoning, although the live *S. aureus* bacteria could not be detected in the samples.

Yücel and Anıl [49] have determined the frequency of coagulase-positive staphylococci (CPS), coagulase negative staphylococci (CNS) and the antimicrobial resistance of these strains in raw milk and cheese samples obtained from various companies and dairy in Ankara. Of the raw milk and cheese samples that were examined, 236 were CPS, 94 were CNS and 330 staphylococci were isolated in total. Among

the CPS strains, *S. aureus* was the second most common strains in raw milk and cheese samples with 35.0% rate. In, another study Gökmen et al. [50] reported that 40 (26.66%) samples of about 150 different cheese types sold in Istanbul were positive for *S. aureus*. Enterotoxin was found in 25 of the collected samples, also. They identified the staphylococcus species in a total of 640 samples taking from raw milk, pasteurized milk, feta cheese, personnel and other equipment in four companies in Konya, Turkey. They reported that *S. aureus* and *S. intermedius* were the predominant species of staphylococcus isolates in which coagulase positive isolates 144 were coagulase positive and 181 were coagulase negative. They also stated that they found staphylococcal enterotoxin levels below detectable levels in all milk and cheese samples. It has been reported that the results obtained indicate a lack of sanitation in the production of white cheese and a risk in public health. In another study, Sancak et al. [51] investigated the presence of enterotoxigenic *S. aureus* strains and the presence of enterotoxin in Van herby cheeses. A total of 50 samples of herby cheese were analyzed and *S. aureus* was reported to be detected at 8.4×10^1 to 5.2×10^4 cfu/g in only 7 (14%) of the samples. Of the isolated 7 (14%) *S. aureus* isolate, 3 (42.8%) were found to have enterotoxin C but no toxin was found in any sample. According to the results obtained, they found that although the enterotoxin could not be detected in the examined herby cheese samples, 14% of the samples had *S. aureus* and 42.8% of these isolates were enterotoxigenic, suggesting that these cheeses could constitute a potential risk for food poisoning.

1.4. Protection and Prevention against *S. aureus*.

Although food workers are the main source of food contamination in food poisoning cases, environmental and environmental surfaces may be a source of contamination with *S. aureus*. It is known that consumption of enterotoxins by some species of *S. aureus* has been known to cause food poisoning in humans due to insufficient heat treatment (60 °C, 140 °F, or above) or insufficient cooling (7.2 °C, 45 °F or below) [52, 53]. The staphylococci are ubiquitous and therefore can not be eliminated in environment. Total destruction or a significant decrease in the number of bacteria during the development, harvesting, processing, packaging and storage of food is the overall goal. Some of the same methods used to control organisms on the foods are used separately or in combination to protect foods. Staphylococci; when exposed to lethal doses of heating, cooling, drying, radiation or chemical treatments, they can be completely destroyed or damaged at sublethal doses [54]. Although these organisms are ideal to be completely destroyed, sublethal damage can allow organisms to regenerate and multiply under favorable conditions. The basic measures to protect cheese from contamination by *S. aureus* can be summarized as follows:

- Storing raw foodstuffs in freezing,
- Preventing cross-contamination between raw and baked goods,
- Refrigerated and stored baked products should be kept below 7 °C,
- Careful washing of hands and contact surfaces before food preparation,
- Washing and cleaning kitchen utensils with a solution of hot detergent after contact with raw foodstuffs,
- Realistic control for the dissemination and effectiveness of HACCP implementations,
- Sampling specialist from environmental sources at regular intervals,

- Compliance with the duration and dosage of disinfectants,
- Performing porter examination on food workers routinely,

2. Conclusion

It can be seen that a standard generalization about the viability of *S. aureus* in cheese species cannot be made in the obtained findings. Factors such as pH, temperature and starter culture activity have been identified as important factors affecting the viability of *S. aureus* in cheese and toxin production. Despite being a reliable nutrient, the enterotoxin production potential of *S. aureus* to survive in low-water activities has led to the cheese-risky class of products. In countries where the national surveillance system is not sufficiently developed, the degree of danger associated with this situation is not clearly known. Therefore, it is thought that further studies on the presence, viability and conditions affecting the production of enterotoxin of *S. aureus* in different types of cheese are required.

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