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Enterotoxin A Gene Barrier *Staphylococcus aureus* Within Traditionally Dairy Products of Tehran

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Background: *Staphylococcus aureus*is a serious agent that often colonize dairy products all over the world. Staphylococcal enterotoxins are the essential causes of food poisoningin human societies. Enterotoxin type A is an importantstaphylococcal exotoxin.
Objectives: The aim of present study was to detect the enterotoxin producing *Staphylococcus.aureus* within different dairy products collected from Tehran, Iran.
Materials and Methods: Two hundreds twenty dairy products samples were collected from local dealers across the city. The samples were first screened for *S. aureus* contaminations. All isolated strains of *S. aureus* were then investigated for enterotoxin a gene, usind spesific primer sets.
Results: *Staphylococcus aureus* was isolated from 43% of dairy samples: 22% from milk and 18% from cheese samples. The *SEA* genes were detected in 10 isolates (22%) originated from raw milk and in two isolates (25%) from domestic cheese.
Conclusions: Since, the staphylococcal enterotoxins are heat stable, heat had no effect on the toxicity of the enterotoxins within positive samples. Our primer stets confirmed previous studies that introduced PCR as rapid, sensitive, and specific method for dairy products screening system. Our data showed that routine screening and surveillance is vital for different food materials including dairy products.

*Keywords:*Enterotoxin; Food Poisoning; *Staphylococcus aureus*; Dairy products

1. Background

Staphylococcus species are gram-positive and facultative anaerobe of micrococcaceae (1). *Staphylococcus aureus* is catalase positive and is able to grow in a wide range of temperature and pH as well as and sodium chloride concentrations of up to 15% (2). The bacterial cell wall is resistant to lysozyme and is sensitive to lysostaphin that specifically cleaves the pentaglycin bridges of *Staphylococcus* species (3). Most strains of *S. aureus* are capable of producing enterotoxin. Staphylococcal enterotoxins are single-chain, low-molecular weight (27-34 kDa) proteins. Although enterotoxins are mainly producedby some *S. aureus* strains, they might be produced by *Staphylococcusintermedius*, *Staphylococcus hyicus*, *Staphylococcus xylosus*, and *Staphylococcus epidermidis*. Almost 21 staphylococcal enterotoxins and enterotoxin-like peptides have been described (4). Enterotoxin genes are located in an open reading frame within the gene cluster (5). Staphylococcal enterotoxins belongs to pyrogenic toxin super antigens family. These superantigens bypass conventional antigen recognition by interaction with major histocompatibility complex (MHC) class II, located on the surface of antigen presenting cells (6, 7). *Staphylococcus aureus*might be found regularly in most anatomical sites as bacterial flora (8) and is usually present on external sites such as the nostrils or skin surface and transiently in the oropharynx and faces (9). About 30% to 50% of the human populations are healthy carriers (10). *Staphylococcus aureus* has been reported as one of the most common causes of foodborne diseases from different parts of the world (10). Staphylococcal food poisoning is an intoxication that occurs after consumption of food materials contaminated with enterotoxigenic strains. The symptoms including nausea, vomiting, and abdominal cramps with or without diarrhea have rapid onset (2-8 hours). The disease is usually self-limiting and typically resolves within 24 to 48 hours. Occasionally, it can be severe enough to warrant hospitalization, particularly when infants, elderly, or those with people are concerned. The most involved foods in foodborne intoxications are mainly meat and meat products, poultry and egg products, egg salads, fish, potatoes and pasta, pastry like cream and custard cake, and dairy products (11).

Healthy carriers of enterotoxin-producing *S. aureus* are regarded as the main source of food poisoning, via di-

rect contact or through respiratory secretions (9). Since *S.aureus* does not compete with indigenous microorganisms within raw milk and dairy products, staphylococcal contamination is usually associated with improper handling of food materials, followed by storage under poor environmental conditions (12). *Staphylococcus aureus* is also present in animals; dairy cattle (10), sheep, and goats, particularly if they are affected by subclinical mastitis, are likely the source of milk contamination (11). Air dust can also serve as vehicles for transferring *S. aureus* to dairy products (13). Milk is suitable substrate for enterotoxin producing *S. aureus* (14). In addition, enterotoxins can retain their biological activity even after pasteurization (15). Staphylococcal enterotoxins are highly resistant to heat; e.g. they retain some biological activities after 28 minutes at 121°C (16, 17). All genes of staphylococcal enterotoxins are located on accessory genetic elements including plasmids, prophages, *S. aureus* pathogenicity islands, or next to the staphylococcal cassette chromosome (18). Most of genes are mobile elements and their spread within *S. aureus* strains can considerably improve staphylococcal virulence (18). Staphylococcal enterotoxin A and B (*SeA* and *SeB*, respectively) are the most important agents in gastroenteritis. In some areas, more than 50% of food poisonings are caused by *SeA* (19). In the United States, *SeA* and *SeB* have been reported as the most common detected agents in food poisoning (20). Enterotoxin production needs long incubation period. Some factors such as pH, water activity, and culture substrates influence toxin production (21). Several procedures such as bioassay, immunologic, and molecular methods have been introduced for screening enterotoxin-producing strains (22). Some methods are based on direct detection of enterotoxin in the food. DNA amplification methods can detect the presence of enterotoxin genes, even before the expression (23). Previous research groups (24, 25) have reported the prevalence of staphylococcal enterotoxins A, B, C, D, and E within food materials by using molecular methods.

2. Objectives

We reported *SEA*-producing *S. aureus* isolated from dairy products collected during summer of 2013 from local markets of Tehran Province, Iran.

3. Materials and Methods

Dairy products samples were collected from different and random municipal parts of Tehran during summer of 2012. The research was performed on 220 cheese and milk samples. 120 milk samples (60 samples of sheep's milk, 30 samples of cow's milk, and 30 samples of goat's milk) and 100 samples were collected from Iranian traditional cheese. the samples were plated on Baird Parker agar (Merk) with 5% egg yolk tellurite emulsion (Merk) and incubated at 35°C for 48 hours (26). Appearance of jet-black colonies, which were surrounded by a white halo, were considered to be presumptive *S. aureus* and were subjected to further analysis by Gram's reaction and biochemical tests. Characteristic colonies were tested for catalase, MAST media, salt agar, and coagulase production. For final approval of all isolate, nuclease gene were analyzed by PCR (27).

BHI media were used for *Staphylococcus* isolation. The media were prepared according to manufacturer's instruction and were sterilized by autoclave for 15 minutes. All isolates were confirmed by standard bacteriologic methods (28). The confirmed isolates were then stored within 2 mL ependorf containing 15% glycerol BHI at -20°C until use (29). The genomic DNA were extracted from each *S. aureus* isolate by Small kits of genomic DNA for Gram-positive bacteria which prepared from Centre for Genetic Resources of Iran (30). All isolates of *S. aureus* were screened by specific primer sets. Forward and Reverse primers were selected from publications; forward 5-TGTATGTATGGAGGTGTAAC-3, Reverse 5-ATTAACCGAAGGTTCTGT-3. Primers were designed previously (31, 32), and its detection power were optimized routinely. PCR product size is 270 bp.

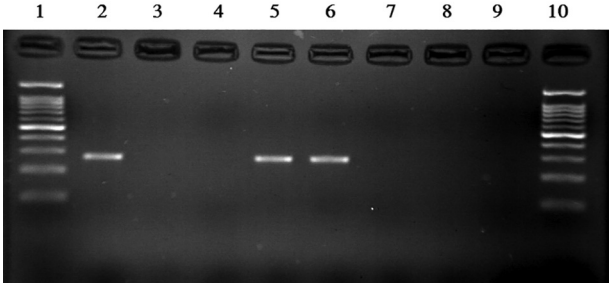
The PCR was performed with master mixture: 1 μL of template DNA, 10 μL of primer mixture (0.5 μM of each forward and reverse primer), and 25 μL of double distilled water with final volume of 50 μL (33). Thermo cycle program was adjusted asan initial denaturation for three minutes at 94°C. The number of cycles was 28 and each cycle included denaturation for 30 seconds at 94°C, annealing for 30 seconds at 50°C, and elongation for 30 seconds at 72°C. Additional ten-minute incubation at 72°C was applied for final elongation. Amplified PCR products were resolved within 2% agarose gel electrophoresis at 80 V for 45 minutes (34).

4. Results

The PCR product size and the profile of positive sample profile are shown in Figure 1.

The contamination with *S. aureus* was confirmed in approximately 28% of milk samples (Table 1).

Figure 1. Amplification of Staphylococcal Enterotoxin A *SEA*



wells 1 and 10; DNA markers, Well 2; Positive control, Well 3; Negative control, Well wells4, 7, 8 and 9) negative samples, Wells 5 and 6 positive samples.

Table 1. Prevalence of Staphylococcal Enterotoxin A Barrier *Staphylococcus aureus* within Dairy Product Samples [a,b]

Samples	Milk			Cheeses	Total
	Sheep	Cow	Goat		
***S. aureus* contaminated**	27 (45)	10 (33)	13 (43)	8 (8)	58 (26.36)
***SeA*-positive strains**	6 (22)	1 (10)	3 (23)	2 (2)	12 (5.45)
Total	60	30	30	100	220

[a] Abbreviation: *SeA*, Staphylococcal Enterotoxin A.
[b] Data are presented as mean ± SD.

The prevalence of coagulase-positive *Staphylococcus* was slightly more than 28%. The highest and the lowest contamination rates were observed in sheep and cow milk samples, respectively. The *SEA* gene was detected in 22% of sheep milk samples that was the highest rate between all milk samples (Table 1). Totally, 100 Cheese samples were analyzed and *S. aureus* was isolated from 8% of them. The staphylococcal isolates were also subjected to *SEA* replication by PCR, and the related gene was detected from 2% of samples.

5. Discussion

Coagulase-positive *Staphylococcus* within dairy products should be highlighted for health authorities. As our data showed, 58 out of 220 dairy samples were contaminated with *S. aureus*. Di Giannatale at el. (19) worked on 350 cases and showed that 49 cases of dairy production (14%) were contaminated with *S. aureus* and among them, 13.3% were in fresh cheese. The surveillance of staphylococcal contamination within foods has been applied globally as critical tactic in public health. Sharma et al. (35) reported that 41% of isolated *S. aureus* from milk bulk tank were capable of producing enterotoxin. In our investigation, only about 10% of staphylococcal isolates were positive for *SEA*. We assume the total assessment of enterotoxin instead of type specific assessment could be main reason of their higher rate of enterotoxin-positive strains. The variations of staphylococcal isolates and their ability to produce enterotoxins depend on the sources of food. Some researchers reported factors such as human interference, animal source of dairy products, and also the health standards of local procedures as major factors in milk and dairy contamination (8).

Our data showed that milk samples were more contaminated than cheese samples. It might be due to more handling of milk bulks in comparison to cheese. Totally, 45 milk samples were positive for *S. aureus* among which 10 isolates were *SeA* carrier. In contrast, only eight out of 100 cheeses samples were contaminated with coagulase-positive strains and *SeA* was detected in only two isolates. According to Rall et al.(33), 41% of isolated *S. aureus* from milk bulk tank produced *SeA*. However, the considerable differences between their results and the present study might be due to differences in geographic as well as study setting. Genotype and phenotype characterization of *S. aureus* isolates within dairy products (28) showed that nearly 10% of isolates were *SeA*, and 11% were *SeD* or *SeJ*. In our study, we focused on coagulase-positive staphylococcal contamination and *SeA* barrier isolates. When reusing the refrigerated foods, one should heat them properly especially if they are going to be kept for a long time because the enterotoxin-producing strains of *S. aureus* are not being inactivated through the low temperature heating procedures. On the other hands, simply warming at low temperature (20°C-45°C) activates the spores to vegetate and multiply rapidly. Last but not least, the quantity of contaminant bacteria is also a critical factor in risk assessment of microbial food contaminations. Although bacteria are limited to the foods, given the chance, they can easily spread around living areas. More researches are still required for understanding the true interactions between *S. aureus* and food matrix, and the mechanisms of enterotoxin production in foodstuffs. Qualified efforts are also needed for isolation and identification of more recent staphylococcal enterotoxins. Novel methods and more sensitive techniques are required for screening of enter toxigenic *Staphylococcus* within foodstuffs. Predictive models for *S. aureus* growth and enterotoxin production would be powerful tools for microbial risk assessment in food industries. Contamination of traditionally dairy products including raw and pasteurized milk obviously shows the potential risk to the public health. Many factors affect *S. aureus* growth and enterotoxin production in foodstuffs and further studies are still necessary in order to develop such predictive tools. Food materials must be stored at standard conditions, and be used within a recommended period.

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