Prevalence of enterotoxigenic Staphylococcus aureus in organic milk and cheese in Tabriz, Iran

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Received: June 2014, Accepted: September 2014

ABSTRACT

Background and Objectives: Staphylococcal food poisoning is a gastrointestinal disease, which is caused by consumption of contaminated food with enterotoxins produced by Staphylococcus aureus (SEs). Milk and its products are known sources of food borne diseases. This study was carried out to evaluate the prevalence of enterotoxigenic S. aureus strains in organic milk and cheese in Tabriz - Iran.

Materials and Methods: A total of 200 samples (100 milk samples and 100 cheese samples) were collected from farms and milk collection points in Tabriz - Iran. The samples were cultured and identified by standard bacteriological methods, then PCR was performed to detect sea gene.

Results and Conclusion: Staphylococcus aureus was found in 27% of all samples (milk and cheese). Results of PCR showed that 12.96% of S. aureus isolates possessed sea gene. It suggested the potential public health threat of S. aureus resulting from contamination of dairy products. So, efforts are required to improve safety standards for preventing staphylococcal food poisoning.

Keywords: Staphylococcus aureus, Enterotoxin, Organic Milk, Cheese.

INTRODUCTION

Staphylococcus aureus is an important food-borne pathogen involved in a variety of invasive diseases (1). It produces many toxins as well as non-toxic enzymes and can facilitate bacterial attack and proliferation in the body of host (2). Some strains produce staphylococcal enterotoxins (SEs) that can cause food poisoning if food containing preformed SE is ingested. Symptoms of staphylococcal food poisoning (SFP) have a rapid onset (2 to 6 h) and may include vomiting, stomach pain and diarrhea (3). Enterotoxins from S. aureus strains can be classified into 18 serological types: A-U (except S, F and T)(2,4-9). The SEs are a group of heat stable and pepsin resistant exotoxins encoded by genes in the chromosome, pathogenicity island, phages or plasmids (2). For production of sufficient amount of toxin to cause intoxication symptoms, 10⁵ CFU/ml or CFU/g enterotoxigenic strains of bacteria are needed (10). SEs are low molecular weight proteins (MW 26,900 – 29600 KD) (11). SEA is a leading cause of food intoxication (12) and is an extremely potent gastrointestinal toxin, as little as 100 ng is sufficient to cause symptoms of intoxication (13).

Milk and milk products have frequently been implicated in staphylococcal food poisoning and contaminated raw milk is often involved (2, 3). S. aureus mastitis is a serious problem in dairy
production and infected animals may contaminate bulk milk. Human handlers, milking equipment, the environment and the udder and teat skin of dairy animals are other possible sources of bulk milk contamination (3, 14). There are several methods for detection of enterotoxigenic bacteria. PCR is recommended for detection of *S. aureus* enterotoxin genes (15).

Surveys to detect classical enterotoxins and to identify enterotoxin genes in *S. aureus* from milk and milk products have been conducted in many counties including Italy, Norway, Turkey, Brazil and Iran (Tehran) (16-20). However, there are no published reports about presence of SEA gene in milk and its products in Tabriz-Iran. Therefore, this study was conducted to investigate the presence of SEA gene of *S. aureus* in organic milk and cheese using PCR method in Tabriz-Iran.

**MATERIALS AND METHODS**

**Specimen collection.** A total of 200 samples (100 milk samples, 100 cheese samples) were collected from milk collection points and food stores in Tabriz, Iran. The samples were collected in sterile containers, immediately kept in an ice box and transported to the laboratory.

**Microbiological analysis.** The samples were investigated microbiologically for the presence of *S. aureus*, according to the standard method No. 6806-1 of the Institute of Standards and Industrial Research of Iran (ISIRI). The milk and cheese samples were diluted in ringer and pepton water, respectively. From each diluted solutions produced, 1 ml was transferred to Brain- Heart Infusion broth and incubated at 37°C for 24 h. Baird- Parker agar supplemented with potassium tellurite and egg yolk emulsion was used for isolation of *S. aureus*. After 24-48 h incubation at 37°C, the BPA plates were analyzed for the presence of *S. aureus*, which appeared as black colonies with transparent zone. Biochemical confirmation tests (Gram staining, coagulase, catalase, oxidase, VP and growth on mannitol salt agar) were carried out for final identification of the suspected *S. aureus* isolates.

**DNA extraction and PCR experiments.** For DNA isolation, biochemically confirmed single colonies of *S. aureus* isolates on BPA were cultured on LB broth. DNA extraction procedure was done by a commercial kit (Cinna pure kit), according to the supplier’s instructions on the bacterial cells pellet from an overnight culture in LB broth. The primers used for the detection of nuc and sea genes are listed in Table 1.

Final confirmation of the strains was carried out using PCR with nuc gene. PCR reactions were performed in reaction buffer (10x), MgCl₂ (50mM) in a total volume of 25 μl, containing 5 μl of template DNA, 0.75 μl of each primers (10 pm), 0.25 μl of dNTP (10 mM), 0.2 μl of Taq DNA polymerase (5 unit/μl ) and 14.05 μl of ddH₂O. PCR was performed under the following conditions: initial denaturation at 94°C for 5 min, subsequently followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min with a final extention of 5 min at 72°C. The amplified products were shown by electrophoresis on 1% gel agarose containing ethidium bromide. Gels were viewed by UV Transillumination and photographed (Fig. 1).

After final confirmation of the isolates, PCR reaction was performed for sea gene. PCR reaction was conducted like the previous test but with some changes in annealing temperature (50°C for 1 min). The presence of 552 bp amplicon indicates the positive samples for sea gene in these isolates.

**RESULTS AND CONCLUSION**

In the present study, *S. aureus* was observed in 54 (27 %) out of 200 samples: 9 from milk and 45 from

| Primer name | Nucleotide sequence | Product size | Reference |
|-------------|---------------------|--------------|-----------|
| Nuc F       | 5’-GCGATTTGATGGTGATACGGTT-3’ | 279 bp       | 22        |
| Nuc R       | 5’-AGCCAAGCCTTGACGAACTAAAGC-3’ | 279 bp       |           |
| Sea F       | 5’-TTGCGTAAAAAGTCTGAATT-3’ | 552 bp       | 23        |
| Sea R       | 5’-ATTTACCGAAGGTTCTGTAGA-3’ | 552 bp       |           |
cheese samples. Total *S. aureus* counts determined between $10^2$ and $10^6$ CFU/ml in samples. The number of bacteria in contaminated samples by CFU/ml is shown in Table 2. For further molecular confirmation of *S. aureus* colonies, PCR reaction for *nuc* gene was performed. All 54 isolates carried *nuc* gene. DNA from those isolates was examined for the presence of *sea* gene. Results showed that 7 out of 54 *S. aureus* isolates harbored *sea* gene (Table 2).

Several researchers have reported different results of the number of bacteria in contaminated dairy products by CFU/ml (10, 20, 21). Variations in *S. aureus* count in dairy products may depend on sanitary precautions during milk processing chain. The existence of *S. aureus* in food and dairy products was previously confirmed by Imani Fooladi in Iran (20). SEA is the most common enterotoxin recovered from food poisoning outbreaks in the USA (77.8% of all outbreaks)(24).

In the current study, we used Baird- Parker agar for isolation of *S. aureus* from dairy products. This method was also employed by Boerema *et al.* (25), Rall *et al.* (26), and Ertas *et al.* (27), in same products. Considering the findings of Brakstad *et al.* (22), and comparing them to our study, it can be stated that the PCR for amplification of the *nuc* gene has potential for rapid diagnosis and confirmation of *S. aureus* isolates. The present study aimed to evaluate the prevalence rate of *sea* gene in organic milk and cheese in Tabriz, Iran. Outside of Iran, various results have been reported on the presence of *S. aureus* and its enterotoxins in milk and its products (28-31).

In the current study, we found that 27% of the organic milk and cheese samples were contaminated by *S. aureus*. PCR results for *sea* gene, showed that 12.96% of the isolates possessed *sea* gene. Similar level of incidence was reported by Holeckova *et al.* (32), Imani Fooladi *et al.* (20) and El-Jakee *et al.* (33). In contrast to the above results, Ertas *et al.* (27) reported lower incidence of *sea* gene (1.6%). This discrepancy could be attributed to the improvements in the handling and sanitary procedures during milking and its processing. It was concluded that even though the level of microorganisms in dairy products was not sufficient to cause disease, the presence of toxins could be considered a potential risk for public health.

Several conditions such as delay in processing, inadequate refrigeration, poor personal hygiene and post process contamination are associated with staphylococcal growth and enterotoxin production (34). Therefore it is essential to ensure high safety standards for preventing staphylococcal food poisoning. In conclusion, the results of this study provides some important preliminary data about the prevalence of enterotoxigenic *S. aureus* in organic milk and cheese in Tabriz, Iran. Consumption of organic milk and specially cheese is still widespread and could cause a potential risk to public health, so an effective reduction of contamination levels could be achieved by improving sanitation and hygiene procedures. Further investigations are needed to evaluate the production of other toxins in milk and cheese.

### Table 2: Prevalence of *sea* gene in *S. aureus* isolated from organic milk and cheese.

| Types of samples | Sample size | No. of *S. aureus* isolates | Distribution CFU/ml | No. of enterotoxigenic *S. aureus* (sea gene) |
|------------------|-------------|-----------------------------|---------------------|---------------------------------------------|
| Milk             | 100         | 9                           | $6(66.6%)$          | 2                                           |
| Cheese           | 100         | 45                          | $26(57.7%)$         | 5                                           |
| Total            | 200         | 54(27%)                     | -                   | 7 (12.96%)                                  |

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**IRAN. J. MICROBIOL.** Vol. 6, No 5 (October 2014), 345-349
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