



Study of Prevalence of Toxic Shock Syndrome Toxin (*TSST-1*) and Methicilin Resistance (*MecA*) Genes of *Staphylococcus aureus* Isolates from Local Cheese in Northwest of Iran

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Abstract

Background: Staphylococcal food poisoning is one of the most common food borne diseases. Widespread incidence of antibiotic resistances of *Staphylococcus aureus* have been reported in the world. The bacterium causes this poisoning by producing different toxins.

Objectives: The aim of this study was to determine the presence of toxic shock syndrome toxin (*TSST-1*) and methicillin resistance (*mecA*) producing genes in isolated *S. aureus* from local cheese in northwest of Iran.

Methods: A total of 22 *S. aureus* samples, already identified by biochemical tests for diagnosis of *S. aureus*, were isolated from local cheese, and identified as *S. aureus* with proliferation of thermonuclease species-specific gene (*nuc*) by PCR method. To determine the frequency of *TSST-1* and *mecA*, genes were tested by PCR method.

Results: Of 22 *S. aureus* isolates, one case (4.54%) contained *mecA* gene and two cases (9.09%) possessed *TSST-1* gene. None of the tested isolates harbored the intended genes simultaneously.

Conclusions: The presence of *S. aureus* isolates in local cheese, which harbor toxic shock syndrome toxin (*TSST-1*) and methicillin resistance (*mecA*) genes, show that these isolates have high potential in producing toxic shock syndrome toxin and methicillin resistance; therefore, the use of procedures to reduce the bacterial contamination during the processing of dairy product is required.

Keywords: *Staphylococcus aureus*, *mecA*, *TSST-1*, Local Cheese

1. Background

Staphylococcus spp. belongs to the Micrococcaceae family and is a gram positive, non-spore bacterium. This bacterium is one of the important germs in medicine and veterinary medicine and some members of this genus are among the most common factors causing mastitis in cows and sheep (1). Some members of this genus are on the skin, skin glands, and mucosal members of animals, and through this they can transfer to animal products and environmental resources. *Staphylococcus aureus* is a species that not only causes pathogenicity in humans and animals, it can also play an important role in food spoiling (2). This bacterium is the most common factor of bacterial food poisoning and usually contaminates foodstuffs during preparation (3). Foodstuffs including milk, cheese, butter, creamy sweets etc. are a suitable media for *S. aureus* (4). The types of food that cause food poisoning dif-

fer from one country to another. For example, cheese was the most common factor of staphylococcal food poisoning between 1999 - 2000 in France (4). According to different studies, 1% - 5% of food borne intoxications are associated with milk consumption and dairy products, 53% of which are due to consumption of contaminated cheese. Cheese production has been practiced since a long time ago in Iran and still, there is much tendency to use local or unpasteurized cheese. A total of 20% of the milk produced in milk industries turn into cheese in Iran, much of which is devoted to the production of local cheese (5). One of the important hygienic problems in the production of local cheese is related to the abundance of microbial load due to the use of raw milk. Raw milk, after milking and during cheese production stages, is contaminated by various microbes such as *S. aureus* (6). Furthermore, this bacterium can contaminate milk via breast suffering clinical or subclinical staphylococcal mastitis. This bacterium can grow and duplicate

in milk and its products during production and storage stages, which leads to the production of pathogenic enterotoxins (7). *S. aureus* has many virulence factors, the most important of which are enterotoxins and toxic shock syndrome toxin (*TSST-1*) and have a significant impact on the host (8). Expansion of antibiotic resistance is another problem with which physicians deal, and due to antibiotic resistance strains emergence in *S. aureus*, the number of antibiotics gradually decreases for the treatment of its infections. Most of *S. aureus* isolates are resistant to penicillin, some of them are resistant to methicillin and nafcillin, and a few of them are resistant to vancomycin (9).

2. Objectives

Due to the importance of this subject and also high production and consumption of local cheese in Iran, the current study aimed to determine the presence of toxic shock syndrome toxin (*TSST-1*) and methicillin resistance (*mecA*) producing genes of *S. aureus* strains isolated from local cheese in northwest of Iran.

3. Methods

3.1. Samples

The present study was performed in the Microbiology Laboratory at the Islamic Azad University, Maragheh branch, in May 2017. In this study, we isolated 22 *S. aureus* strains from 100 local cheese in northwest of Iran, which already identified with biochemical experiments (e.g. Baird parker and Mannitol salt agar, Coagulase and Voges proskauer tests and gram staining).

3.2. DNA Extraction

DNA extraction was performed on 22 cultured isolates of *S. aureus* in brain heart infusion (BHI) (Merck, Germany) medium. One mL of bacterial culture centrifuged in 5000 g for five minutes and supernatant was poured off. Subsequent to an addition of 1 mL lysis buffer (Tris 1 M (pH = 7.5), NaCl 5 M, EDTA 0.5 M and C-TAB 2%) on mixed pellet, it was put into 85°C for 30 minutes (in water bath). In the next step, the supernatant was separated and 0.5 µL RNase was added to it. Later, it was kept at 37°C for 30 minutes. Then, an equal volume of isopropanol was added to the content of the vial, and it was kept at -20°C for 15 minutes, and then centrifuged in 12000 g resulting in some DNA samples sediment. Then, DNA samples were dried in lab temperature. Finally, dried DNA samples were dissolved in 50 µL of double distilled water (10). Nanodrop and electrophoresis on

1% agarose gel were used to determine the quantity and quality of extracted DNA samples.

3.3. PCR Test to Detect *Thermonuclease Gene (nuc)*

The polymerase chain reaction (PCR) method was done in 25 µL, including Master mix 12.5 mL PCR, 0.4 µM specific primers (Table 1), and DNA extracted containing 1 µL (50 ng). The polymerase chain reaction was performed with primary denaturation cycles at 94°C for four minutes, 32 cycles with denaturation step at 94°C for one minute, the primer annealing step at a temperature of 55°C for one min, extension at 72°C for one minute, and finally, an extension cycle at 72°C for 10 minutes. The PCR production was electrophoresed on 1.5% agarose gel. The extracted DNA of *S. aureus* PTCC1112 used as positive control and double distilled water was considered as negative control. The isolates were considered as *S. aureus* after definite diagnosis of *nuc* existence with a length of 275 bp.

3.4. PCR Test to Identify *TSST-1* and *mecA* Genes

This reaction was performed in a 20 µL volume, which includes 8.5 µL double distilled water, 2 µL buffer 10 X, 2.5 µL dNTPs (10 mM), 1 µL of each one of primers associated with *TSST-1* and *mecA* genes (0.5 µL of forward primer and 0.5 µL of reverse primer, 10 Mm) (Table 1), 2 µL MgCl₂ (50 mM), 2 µL Taq DNA Polymerase, and 1 µL extracted DNA. Proliferation of *TSST-1* and *mecA* genes in thermocycler was performed as following: primary denaturation at 95°C for four min, 32 cycles with denaturation stage at 95°C for one min, annealing stage at 52°C for one min, extension stage at 72°C for one min, and finally, a terminal extension stage at 72°C for one min. PCR products related to the proliferation of each one of *TSST-1* and *mecA* genes were evaluated by electrophoresis on 1% agarose gel and 100bp DNA ladder was used to determine the size of fragments. The extracted DNA of *S. aureus* PTCC1112 used as positive control and double distilled water was considered as negative control.

4. Results

The obtained results showed that all 22 isolated *S. aureus* strains were positive for the *nuc* gene (Figure 1).

Of 22 *S. aureus* isolates, one case (4.54%) contained *mecA* gene and two cases (9.09%) possessed *TSST-1* gene. None of the tested isolates harbored intended genes simultaneously. *S. aureus* PTCC 1112 (as standard bacterium) didn't possess the *TSST-1* gene (Figures 2 and 3).

Table 1. Characteristics of Specific Primers Related to the Genes Under Investigation

Primer Sequence	PCR Product (bp)	Ref
nuc	275	(11)
5'-GCGATTGATGGTGATACGGTT-3'		
5'-AGCCAAGCCTTGACGAACAAAGC-3'		
TSST-1	326	(12)
5'-ACCCCTGTTCCTTATCATC-3'		
5'-TTTTCAGTATTGTAACGCC-3'		
mecA	163	(12)
5'-ACTGCTATCCACCCTCAAAC-3'		
5'-CTGGTGAAGTTGTAATCTGG-3'		

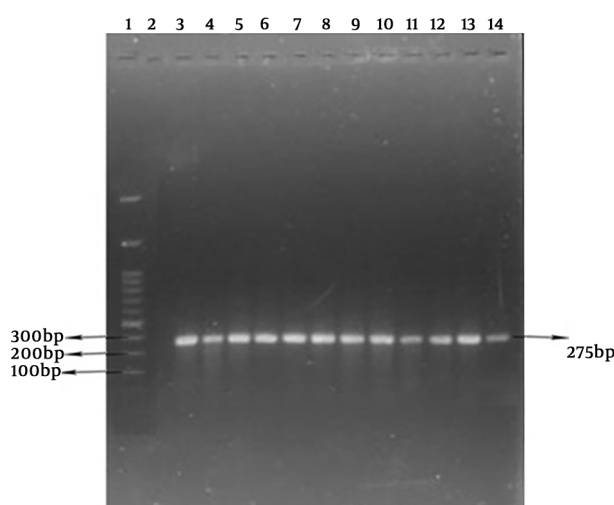


Figure 1. Lane 1 is marker (100 bp). Lane 2 is negative control (double distilled water). Lane 3 indicates *S. aureus* PTCC 1112 (positive control) that bands within 275 bp and is associated to *nuc* gene. Lanes 4 - 14 indicate positive samples.

5. Discussion

This research reported the frequencies of *mecA* (4.54%) and *TSST-1* (9.09%) genes. Eshraghi et al. (13), reported that 12% of *S. aureus* samples isolated from foodstuffs in central zones of Iran harbored the *mecA* gene. Morad Nia et al. (14), reported that 1.6% of *S. aureus* samples isolated from dairy products in northeast of Iran harbored the *mecA* gene, which agrees with the finding of the current study. Alizadeh and Amini (10) reported that 60% of *S. aureus* samples isolated from dairy products in Kerman province in Iran harbored the *mecA* gene. Pexara et al. (15), showed that the highest prevalence of methicillin-resistant *S. aureus* (MRSA) in milk and dairy products is found in Ethiopia (60.3%); whereas the MRSA prevalence in Asian countries is

variable (for instance, while it is 28.3% in Iran, it is very low in Korea and Japan). The MRSA prevalence in dairy products has been reported to be low in European countries. In addition, the strains prevalence in USA and Canada have been reported as zero to low (15). The MRSA prevalence varies in different countries in the world. This frequency is very low in some countries such as Norway and Netherlands (about 1%) and in some countries, such as India, it is reported between 0% - 10% (16). Study of prevalence of MRSA is one of the ways of assessing hygienic conditions in dairy cattle herds and public health hazards when there are antibiotic resistant strains (15). Arcuri et al. (17), reported that 11.4% of *S. aureus* samples isolated from Minas frescal cheese in Brazil possessed *TSST-1* gene. It has been also reported that the prevalence of *TSST-1* producing gene in *S. aureus* is between 0% - 37% in other parts of the world (18). The researches have shown that *TSST-1* is one of the agents involved in causing damage to breast tissue in clinical and subclinical mastitis in cows, sheep, and goats (19). The presence of *TSST-1* in domestic animals can be considered as one of the transmission routes to milk and foodstuffs. The researches have shown that 15.5% of *S. aureus* strains comes from the bovine mastitis possessed *TSST-1* gene in the northwest of Iran (20). Studies performed on *S. aureus* strains originated from dairy products, particularly in endemic strains of Iran, are limited in that in similar studies 66.25% (21) and 15.5% (20) of *S. aureus* strains originated from local dairy products harbored *TSST-1* gene, which is a relatively higher rate when compared to the current research.

Significant differences in the frequency of *TSST-1* and *mecA* genes in different studies may be due to different sources of isolation in different regions, their methods of investigation and sensitivity, and number and types of samples. The results of the current study indicate the difference in dispersion of *TSST-1* and *mecA* genes in *S. aureus*; this difference probably originates from geographical diversities and also differences in the ecological origin of the isolated strains (milk, human and different animals).

Footnotes

Authors' Contribution: All authors had an equal role in design, work, statistical analysis, and manuscript writing.

Conflict of Interests: The authors declared that they had no conflict of interests.

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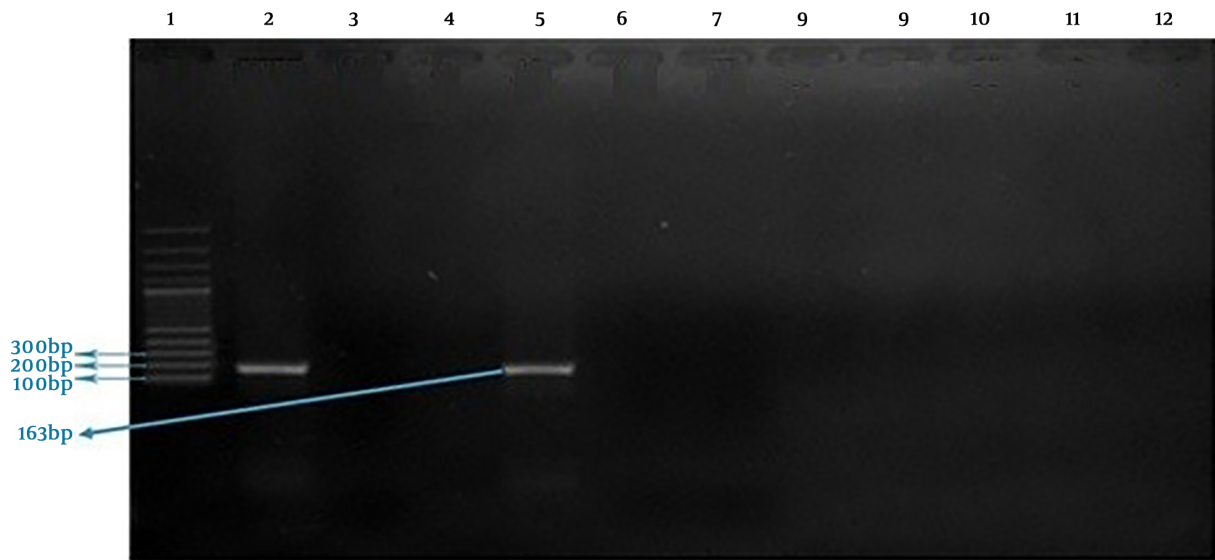


Figure 2. Lane 1 is marker (100 bp). Lane 2 indicates *S. aureus* PTCC 1112 (positive control) that bands within 163 bp and is associated to *mecA* gene. Lane 3 is negative control (double distilled water). Lane 5 indicates positive sample.

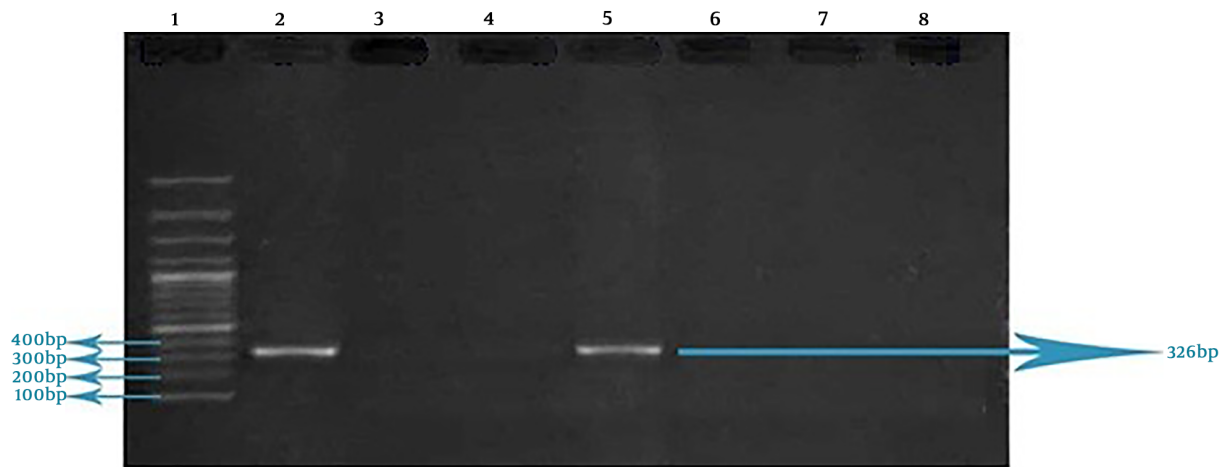


Figure 3. Lane 1 is marker (100 bp). Lanes 2 and 5 indicate positive samples that band within 326bp and are associated to *TSS7-I* gene. Lane 7 is negative control (double distilled water). Lane 8 is *S. aureus* PTCC 1112 (positive control) that didn't possess band within 326 bp.

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