Molecular Typing of *Staphylococcus aureus* Strains from Iranian Raw Milk and Dairy Products by Coagulase Gene Polymorphisms

Azar Asadollahi Dehkordi¹, Elahe Tajbakhsh²*, Forough Tajbakhsh³, Faham Khamesipour³, Manouchehr Momeni Shahraki³ and Hossein Momeni⁴

¹Graduated of Microbiology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

²Departments of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

³Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

⁴Student of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Copyright © 2015 Azar Asadollahi Dehkordi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Abstract**

*Staphylococcus aureus* (*S. aureus*) is one of the most important pathogen in food poisoning and causes gastroenteritis in humans. This study was carried out to determine coagulase gene (coa gene) polymorphisms among *S. aureus* strains in raw milk and dairy products. A total of 320 samples of raw milk from cow, sheep and goat and 350 samples of traditional dairy products were investigated for *S. aureus* contamination and their genotypes of coa gene. Among the 320 raw milk samples, *S. aureus* were detected in 88 (27.5%) samples, and in the 350 dairy products, 87 samples (24.8%) were positive for *S. aureus*. *S. aureus* strains isolated from dairy products belong to genotype I and VIII, 45.9% and 18.3% respectively. Among *S. aureus* strains from raw milk samples, coa gene polymorphisms were observed only in cow milk samples and genotype I and VIII.
were observed in 37.5% and 14.5% of strains, respectively. In this study only two genotypes (I and VIII) were observed among \textit{S. aureus} strains in cow raw milk and dairy products in Chahar Mahal VA Bakhtiyari province, Iran. The genotype I is the most prevalent genotype among \textit{S. aureus} strains in Iran.

**Keywords**: Coagulase gene polymorphisms, Dairy products, Raw milk, Restriction Fragment Length Polymorphism

1 Introduction

\textit{Staphylococcus aureus} (\textit{S. aureus}) is a common cause of food poisoning and staphylococcal gastroenteritis occurs worldwide [1]. Some \textit{S. aureus} strains can produce heat stable enterotoxins and these enterotoxins can cause staphylococcal food poisoning (SFP) [2]. Milk is an excellent growth medium for a large number of microorganisms, including \textit{S. aureus}. \textit{S. aureus} can be shed into the milk from udders with mastitis. Also, \textit{S. aureus} contamination of milk and dairy products can occur during the cooling, storage, and serving procedures [3, 4]. Coagulase test is the principal criterion used by the clinical microbiology laboratory for the identification of \textit{S. aureus} isolates from human infections [5]. All strains of \textit{S. aureus} can produce coagulase [6]. \textit{S. aureus} species like to other bacteria consist of several subtypes, which differ from each other in virulence gene factors [7, 8]. Because predominant strains in a herd usually have a distinct genetic profile, determining these subtypes could be helpful in control of \textit{S. aureus} infections [9]. Several molecular techniques have been developed and used for identification and comparison of \textit{S. aureus} isolates in epidemiological studies. Among these methods, coagulase gene (coa gene) typing is considered a simple and effective method for typing \textit{S. aureus} isolates from human patients and bovine mastitis milk [5, 10, 11]. Epidemiological studies based on analysis of the coagulase (\textit{coa}) gene have shown that \textit{S. aureus} isolates could be divided into a number of subtypes [12]. The sizes and DNA restriction endonuclease site polymorphisms at the 3’ coding region of the coa gene have been utilized in PCR-based restriction fragment length polymorphism (RFLP) analysis of \textit{S. aureus} [5, 13, 14]. The aim of our study was to determine the prevalence of coagulase positive \textit{S. aureus} strains and their genotyping of coa gene in raw milk and dairy products in Chahar Mahal VA Bakhtiyari province, Iran.

2 Materials and methods

2.1 Sampling
A total of 320 samples of raw milk (cow milk, n=200; sheep milk, n=60; goat milk n=60) and 350 samples of traditional dairy products (50 samples of sarshir (traditional cream), dough, n=50; butter, n=50; yoghurt, n=50; cheese, n=50; whey,
Molecular typing of Staphylococcus aureus strains

n=50 and cream, n=50) were collected randomly from different point of ChaharMahal VA Bakhtiyari province in Iran. All the samples were collected under aseptic condition and transferred in a container containing ice cubes to the laboratory of microbiology at Islamic Azad University, Shahrekord Branch.

2.2 Isolation and identification of S. aureus
A dilution of 1:10 from whey, yogurt, butter and sarshir samples in sterile physiological saline was used for microbial culture. Also the cheese samples were diluted 1:10 in 0.02% sodium citrate solutions. The raw milks and dough samples were used directly for culture. For the selective enrichment of S. aureus, 1 ml of each sample was added in Giolitti–Cantoni broth (Merck) with 1% potassium tellurite and cultures were incubated in 37°C for 24 h. Then the samples were subcultured on Baird–Parker agar and incubated in 37°C for 24-48 h. The presumptive colonies of S. aureus were identified by gram stain, catalase, coagulase and DNase tests [15].

2.3 DNA extraction and coa Polymerase Chain Reaction (PCR)
DNA was prepared using a genomic DNA purification kit (Fermentas, Germany) according the manufacturer’s recommendations. Amplification of the 3’ end of the coa gene was performed using primers reported by Aslantas et al. [12] and primer sequences showed in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Size of Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COA</td>
<td>COAG2: CGA GAC CAA GAT TCA ACA AG COAG3: AAA GAA AAC CAC TCA CAT CA</td>
<td>730-1050</td>
</tr>
</tbody>
</table>

PCR for detection of coa gene, was performed in a 50-μl reaction mixture containing 1-2 μl of template DNA approximately 500 ng/μl), 5 μl of 10× PCR buffer (750 mM Tris HCl (pH 8.8) 200 mM (NH4) 2SO4, and 0.1% Tween 20), 200 μM of each primer. The PCR reaction was performed in a thermocycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) using the following cyclic conditions: initial denaturation at 95 °C for 2 min, 30 cycles of 30 s each with denaturation at 95 °C, 2-min annealing at 58 °C, 4-min extension at 72 °C, and a final 7-min extension at 72 °C [12].

2.4 Coagulase gene typing by RFLP method
The PCR products were digested with AluI (Fermentas) for restriction analysis. For this aim, 12.5 μl of PCR products was mixed with 10 U of enzyme and 10×1.5 μl restriction buffer and then incubated at 37°C overnight [12].

2.5 Statistical analysis
Statistical analysis using SPSS software version 16 by chi-square test. A p value less than 0.05 were regarded as significant.
3 Results

Out of the 350 dairy products, 87 samples (24.85%) were positive for *S. aureus*. Eleven samples (22%) of traditional sarsheir, 11 samples (22%) of dough, 16 samples (32%) of butter, 11 samples (22%) of yogurt, 9 samples (18%) of cheese, 18 samples (36%) of whey and 11 samples (22%) of cream were contaminated with *S. aureus*. The whey samples had the highest (36%) and the cheese samples had the lowest (18%) contamination rate (*p* < 0.01). Also, there was a significant difference in contamination rate between whey and traditional sarsheir, whey and dough, whey and yogurt, whey and cream, and butter and cheese (*p* < 0.05). Among the 320 raw milk samples, *S. aureus* was detected in 88 (27.5%) samples. Contamination of *S. aureus* was observed in 48 samples (24%) of cow milk, 14 samples (23.3%) of sheep milk, and 26 samples (43.3%) of goat milk.

The presence of coa gene was confirmed by using COA2 and COA3 primers mentioned in the materials and methods. Amplification of COA2 and COA3 gene were shown 730 bp and 970 bp fragments in all the 175 *S. aureus* strains. The result is shown in Figure 3. Coa gene polymorphism were observed in 25 *S. aureus* strains of cow milk samples, which 18 strains (37.5%) had three bands (160, 320 and 490 bp) and were located in genotype I, and 7 strains (14.5%) had two bands (240 and 490 bp) and were located in genotype VIII (*p* < 0.05). Coa gene polymorphisms were not observed among the *S. aureus* strains isolated from goat and sheep milk.

**Figure 3.** Agarose gel electrophoresis of *S. aureus* coa gene PCR products. Lane M: 1 kb DNA ladder, line 1 negative control. Lanes 2–4: different sizes of *S. aureus* coagulase PCR products. (2–4 positive samples with 970-bp fragment; 5–7 positive samples with 730-bp fragment)

Out of the 87 *S. aureus* strains of dairy products, coa gene polymorphisms were observed in 56 strains. Genotype I and VII were detected in 40 (45.9%) and 16
Molecular typing of *Staphylococcus aureus* strains

(18.3%) of strains respectively. There was a significant difference between the frequency of genotype I and VIII among studied dairy products (p<0.05). The results are shown in Table 2 and Figure 4.

**Table 2.** Coagulase genotypes of *S.aureus* strains isolated from dairy products and raw milk.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PCR product (bp)</th>
<th>RFLP (bp)</th>
<th>N (%) in cow milk</th>
<th>N (%) in dairy products</th>
<th>N (%) in goat milk</th>
<th>N (%) in sheep milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>970</td>
<td>490-320-160</td>
<td>18 (37.50%)</td>
<td>40 (45.97%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>810</td>
<td>410-240-160</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>810</td>
<td>490-240-80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>810</td>
<td>490-240-160</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>890</td>
<td>410-240-160-80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>810-1050</td>
<td>490-410-320-160</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>890</td>
<td>490-410</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VIII</td>
<td>730</td>
<td>490-240</td>
<td>7 (14.50%)</td>
<td>16 (18.39%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IX</td>
<td>730</td>
<td>410-320</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 4.** Agarose gel electrophoresis, RFLP of the *coa* gene *S.aureus* strains isolated from dairy product and raw milk samples. Line M 100 bp DNA ladder, line 1 negative control, lines 2–5 genotype I *coa* gene, lines 6–7 genotype VIII *coa* gene

**4 Discussion**

The importance of milk and other dairy products in the nutrition and health of human is a consensus subject all over the world. The *S. aureus* is an important opportunistic foodborne pathogen in human and animals, which can contaminate
dairy product and cause considerable economical and individual damages. In the recent years, coa gene polymorphism has been used by several studies for genotyping of \(S.\) aureus strains and this method regarded as simple and accurate technique for genotyping of \(S.\) aureus \([5, 10, 16-18]\).

In this study only two genotypes (I and VIII) were observed among ChaharMahal VA Bakhtiyari province. The genotype I was the predominant genotype. These findings are in accordance with a previous study by Momtaz et al. in this region \([19]\). In another report by Saei et al. \([20]\) in Tabriz and Urmia regions of Iran, similar results have been observed. These findings show that the genotype I is the predominant genotype among \(S.\) aureus strains in Iran, especially in ChaharMahal VA Bakhtiyari province. This genotype appears to play a major role in pathogenicity of \(S.\) aureus in bovine mastitis and food poisoning.

Aslantas et al. \([12]\) in Turkey and Raimundo et al. in Australia \([21]\) reported that the genotype I have been predominant among \(S.\) aureus strains in dairy products and bovine mastitis. The presence of a few predominant genotypes among \(S.\) aureus strains may be caused by their higher resistance to the host immune factors like neutrophils activities, while rare genotypes have lower resistance to host immune system \([22, 23]\).

The coa gene in \(S.\) aureus strains of goat and sheep did not show any polymorphism. This result showed that \(S.\) aureus strains of goat and sheep did not have any restriction site for AluI enzyme in the variable region of coa gene. Point mutations can alter this region and may cause this phenomenon \([24]\). The high percentage of \(S.\) aureus contamination in dairy products can cause by cross-contamination of dairy products after production, and also bovine mastitis may be the source of contamination. For reducing bacterial contamination, dairy producers have to observe hygiene and sanitation specially HACCP in their production centers.

References


Molecular typing of Staphylococcus aureus strains


Received: January 11, 2015; Published: February 17, 2015