Culture and Molecular Detection of *Staphylococcus aureus* in Dairy Products of Ahwaz

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Abstract:

*Staphylococcus aureus* is one of the most important bacteria that cause food poisoning and disease in humans and herds. Milk and milk products are important part of the human diet also support the growth of pathogenic organisms. The present study was aimed to determine the prevalence of coagulase positive *Staphylococcus aureus* in different kinds of dairy products of Ahvaz Province.

460 dairy product samples randomly were collected from Ahvaz Province. The samples were analyzed for the presence of *S. aureus*. Positive samples screened by microbiological tests. DNA extracted from all isolates and the PCR carried out using specific primers for *S. aureus*.

The results indicated that 127 (27.61%) of dairy samples were contaminated by *Staphylococcus aureus* (curd 24.5%, dough 19%, butter and cream 14.5%, yogurt 12.5%, cheese 10%, milk 5%).

High prevalence of *Staphylococcus aureus* contamination in milk and milk products of Ahvaz suggests that more control measures should be applied during dairy production. The obtained results are useful for designing strategic plans of prevention and control program against *Staph. aureus* in dairy ecosystem.

Keyword: *Staphylococcus aureus*, dairy products, Culture, PCR.
Introduction:
Milk has been reported as a common food that may cause staphylococcal poisoning [1]. Milk and milk products like cheese and curd are widely consumed and market for them has existed in many parts of the world for many generations. Contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions [2]. Staphylococcus aureus from the Staphylococcus genus of Micrococcaceae family is a leading cause of community-acquired infections in humans and a cause of mastitis and skin diseases in milk producing animals [3]. S. aureus is commonly resident on skin and mucous membranes of cattle [4]. Despite its generally benign nature, in changing circumstances, as response to damage and exposure of structures below the epithelial or mucosal surface, S. aureus can behave as a pathogen (3-4). Illness through S. aureus range from minor skin infection such as pimples, boils, cellulites, toxic shock syndrome, impetigo, and abscesses to life threatening disease such as pneumonia, meningitis, endocarditis and septicemia [2]. Long-term surveys suggest that the significance of S. aureus in the dairy industry has remained unchanged [5]. Under certain environmental conditions, many S. aureus strains are able to produce a variety of enterotoxins, which can cause food poisonings [3].

These toxins are not inactivated by pasteurisation or other heat treatment of milk. S. aureus can also be a zoonotic pathogen, even though it is generally regarded as rather host-specific. However, direct transmission of S. aureus between cows and humans was reported [6].

Presence of S. aureus in milk products which constitute a public health hazard can be detected by simple molecular analysis. Based on the culture methods and PCR technique the prevalence of local milk products Staphylococcus aureus contamination was detected in several provinces of Iran [7-9]. The aim of present study was to investigate the occurrence of coagulase positive Staph. aureus in bovine dairy at Ahvaz province, Iran.

Materials and Methods
Collection of samples:
A total of 460 samples randomly collected from Ahvaz province (70 of milk, 65 of curd, top milk, dough, butter, cream, yogurt and cheese). Each dairy sample was collected in a sterile screw cap bottle (20 ml).

Media and growth conditions:
The samples were immediately taken to the laboratory for bacteriological analysis. S. aureus was isolated by using the technique given by Baird Parker [10]. Enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37°C for 24–48 hours. Appearances of jet black colonies surrounded by white halo were considered to be presumptive S. aureus.

Microbiological analysis:
Black colonies with transparent zone were produced in Baird-Parker, confirmation tests including gram staining, coagulase, catalase, DNase and manitol salt agar were carried out for identification of S. aureus [10].
Molecular analysis:

DNA of each strain of \textit{S. aureus} was extracted according to the standard protocol \cite{12}. The collected DNA was precipitated, described by electrophoresis on agarose gel and then stored at -20°C. PCR reactions were performed in reaction buffer (10x), MgCl2 (2 mM) in a total volume of 50 μl, containing 1 μl of template DNA, 0.2 mM of mixed deoxynucleotide triphosphates, 1 unit of Taq DNA polymerase (Fermentase) and 20-30 pM each of the primers of 228 bp of 16srRNA (CinnaGen Co.) (table-1).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Size of product (bp)</th>
</tr>
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<tbody>
<tr>
<td>16srDNA</td>
<td>F : GTAGGTTGGCAAGCGTTACC</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>R : CGCACATCAGCGTCAG</td>
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PCR was performed under the following conditions: initial denaturation at 94°C for 5 min, subsequently followed by 30 cycles of 94°C for 60 s, 56°C for 30 s, and 72°C for 90 s with a final extension of 10 min at 72°C. Amplification products were separated by electrophoresis on agarose gel 1.5% stained with ethidium bromide (0.5 μg/ml) in Tris - borate - EDTA TBE. Photographs of gels were taken under ultraviolet (UV) device (Gel Doc) \cite{11}.

Results:

The present research findings pertain to the isolation of \textit{S. aureus} from dairy products of Ahvaz province in Iran. Out of 460, 127 (27.61%) samples were confirmed as \textit{S. aureus} contaminated on the basis of morphological, biochemical and molecular characterization (Fig-1). The most frequently contaminated sample was curd (24.5%) and the least frequently contaminated was milk (5%) (Fig-2).

![Figure- 1:Agarose gel electrophoresis of polymerase chain reaction (PCR) of \textit{S. aureus} 16srRNA gene.](image)
Discussion:

The existence of *S. aureus* in foods and dairy products was confirmed in the 19th century \(^{(13)}\). In 1941, Barber described the symptoms of food poisoning, resulting from *S. aureus* contamination \(^{(14)}\). On the other hand, milk is a complex biological fluid containing a wide variety of constituents and possessing unique physical and chemical properties that can be a nutritive medium for the multiplication of various contaminating microorganisms \(^{(14)}\).

In the current study *S. aureus* isolates are differentiated on the basis of microbial and biochemical characteristics and confirmed by PCR technique. In agreement with another study, we found that more than 27% of all dairy products were contaminated by *S. aureus* \(^{[7,8]}\).

Although the frequency of raw milk sample was more than milk products, the frequency of contamination to *S. aureus* of raw milk was significantly less than other samples (5%). As a result, the shedding of bacteria from the infected mammary glands of dairy animals is most likely the primary source of *S. aureus* contamination of milk and dairy products. Contamination may also occur during the cooling, storage, and serving procedures. Thus, hygienic control measures should be considered between prepare of milk to dairy products. Curd and dough had the most frequencies of contamination to *S. aureus* indicated to inappropriate method for producing of them (24.5% and 19%, respectively).

The results indicate to ensure contamination free milk products, consciousness and care is required from the point of generation to the point of consumption of these widely consumed milk products. Effective programs for contamination control that promote dairy food safety are based on identifying the pathogens present, developing effective tools to control contamination of pathogens and observing practices that reduce the risk of contamination of dairy products. Also, PCR is a rapid, sensitive, specific and inexpensive method; we suggest that it can be replaced to traditionally assays for detecting *S. aureus*.

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