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

Mastafa Heilo Jabber Al-musawi*, Mushtaq Talib Shawi Al-Rubaye, Maryam Hosseini****

Department Clinical Laboratory Science, College of Pharmacy, University of Al-Mustansiriyah.

****Department of Genetic, Faculty of Biology, Shahid Beheshti University, Tehran, Iran***

Email: maryamhosseini83@gmail.com

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.*

الكشف الجزيئي عن بكتريا المكورات العنقودية (*Staphylococcus aureus*) في الحليب ومنتجاتة في مدينة الاهواز

***مصطفى حلو جابر، **مشتاق طالب و **مريم حسيني**

*** فرع العلوم المختبرية السريرية، كلية الصيدلة، الجامعة المستنصرية**

**** فرع الوراثة، كلية علوم الحياة، جامعة شهيد بهشتي، طهران، إيران**

الخلاصة:

تعد بكتيريا المكورات العنقودية (*Staphylococcus aureus*) من اكثر المسببات لحالات التسمم الغذائي والامراض لدى الانسان والمواشي على حد سواء. ويعد الحليب ومنتجاته من أهم وسائل العدوى بالبكتريا اعلاه كون الحليب ومنتجاته من اكثر الاغذيه استهلاكاً من قبل الانسان. وقداجريت هذه الدراسة في مدينة الاهواز لغرض اثبات وجود هذا البكتريا في الحليب ومشتقاته في تلك المدينة عن طريق قياس (Coagulase) وهو من أفرازات هذه البكتريا المذكورة.

تمّ جمع 460 عينه من مشتقات الحليب المختلفة بصورة عشوائية من مدينة الأهواز.وقد فحصت تلك العينات عن طريق أجراء الفحوصات المايكروبايولوجية والمتضمنة أستخلاص الحمض النووي المنقوص الأوكسيجين (DNA) لبكتريا ال(*Staphylococcus aureus*) من جميع العينات بأستخدام تقنية ال(PCR) ل (Primer) خاص.

أثبتت النتائج النهائية للبحث أن 127 (27.61%) من عينات مشتقات الحليب التي جمعت مسبقاً هي ملّوثة ببكتريا ال (*Staphylococcus aureus*) كما يلي: اللبن الخاثر24.5%، الدهن الحر 19%، الزبد والقشطة 14.5%، اللبن 12.5%، الجبن 10% وأخيراً الحليب 5%.

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التلوث العالي للحليب ومشتقاته بالبكتريا أعلاه في مدينة الاهواز الذي يحتاج الى اتباع برامج سيطرة أكثر فعالية خلال عملية انتاج الحليب ومشتقاته، والنتائج المتوخات من هذا البحث هي ذات فائدة من أجل أعداد خطط تهدف للسيطرة أو الوقاية من انتشار هذه البكتريا في منظومة صناعة الحليب ومشتقاته.

كلمات المفتاح : *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].

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Molecular analysis:

DNA of each strain of *S. aureus* was extracted according to the standard protocol [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 tri-phosphates, 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- 1: Primers used for amplification of *S. aureus* 16srRNA gene

Gene	Primer Sequence	Size of product (bp)
16s-rDNA	F : GTAGGTGGCAAGCGTTACC R : CGCACATCAGCGTCAG	228

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) [11].

Results:

The present research findings pertain to the isolation of *S. aureus* from dairy products of Ahvaz province in Iran. Out of 460, 127 (27.61%) samples were confirmed as *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).

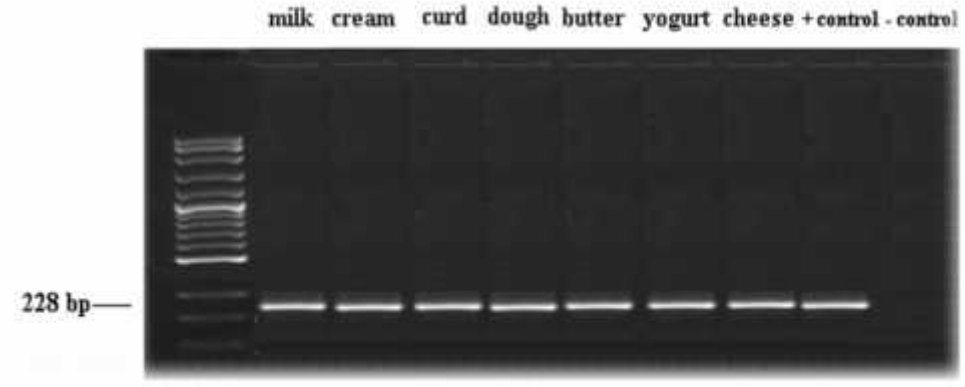

Figure- 1:Agarose gel electrophoresis of polymerase chain reaction (PCR) of *S. aureus* 6srRNA gene.

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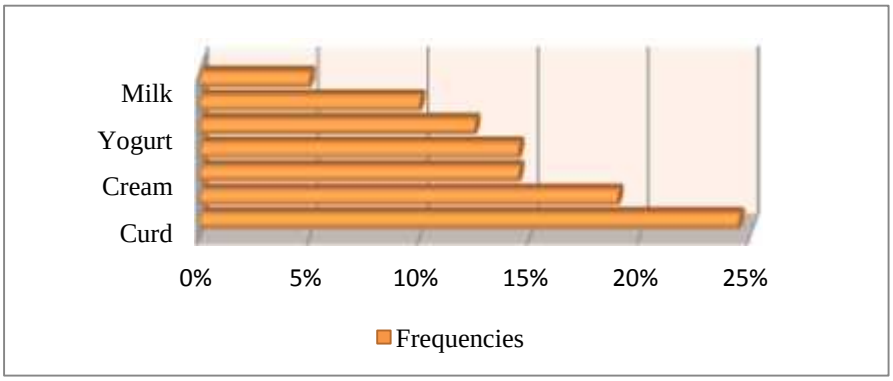

Figure-2:Frequencies of *S. aureus* in dairy samples evaluated by the genotypic and phenotypic method.

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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