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Contamination Rate of Marketed Raw Milk in Tabriz City to Coagulase Positive *Staphylococcus aureus* by Culture and PCR Methods

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Abstract: Milk has an outstanding nutritional quality but is also an efficient vehicle for transmission of diseases to humans and is an excellent medium for bacterial growth and an important source of bacterial infection when consumed without pasteurization. *S. aureus* is an important pathogen due to a combination of toxin-mediated virulence, invasiveness and antibiotic resistance. Milk is a good substrate for *S. aureus* growth and enterotoxin production. The aim of this study was to determination of contamination ate of raw milk to *Staphylococcus aureus* by Culture and PCR Method in Tabriz city. A total of 100 raw milk samples were collected from the milking bucket into a plastic or aluminum containers which were not well washed, no cooling system was applied at any level of the milk chain which may last for 5 h till milk reaches the consumer. The collected milk samples were cultured on selective media and identification of the isolated colonies identified by PCR Method. In this study, 30% raw milk contaminated to coagulase positive *Staphylococcus aureus* were detected. It can be concluded that raw milk is contaminated by this pathogen in this area as well as in other countries and might constitute a risk for *S. aureus* enterotoxin food poisoning.

Key words: Raw milk, coagulase positive, *Staphylococcus aureus*, culture, PCR, humans, growth

INTRODUCTION

Food poisonings are due to many factors. Bacteria are major causative agents of food borne diseases. *Staphylococcus aureus* is one of the main pathogen involved in food poisonings related to dairy products (Soomro *et al*., 2003). Staphylococcal food poisonings are occurred by Staphylococcal enterotoxins produced by *S. aureus* strains contaminating foodstuffs. *S. aureus* 0.5-1.59 m in diameter are spherical gram positive, non motile, non-spore forming facultative anaerobes which ferment most of the sugars except raffinose and salicin producing lactic acid during fermentation.

They are catalase and coagulase positive and flourish between a pH of 7.4-7.6. They exist in air, dust water, sewage, meat and meat products, poultry and egg products, salad such as tuna, chicken, potato and macaroni, bakery products such as cream filled pastries, cream pies, chocolates eclairs and sandwich fillings and in milk and milk products (Baird-Parker, 1962). Pathogenesis of *S. aureus* is due to repertoire toxins, exoenzyme adhesions and immune modulating protein that it produces while 20-30% of healthy people may carry this bacterium on their skin surface and nasal passage. It causes a variety of superlative infections by producing leukocidin, a toxin that destroys the white blood cells and leads to the formation of pus and toxinosis in humans. The presence of *S. aureus* in food causes food poisoning by releasing enterotoxins into the food and it can also cause toxic shock syndrome by release of super antigens into the blood stream (Soomro *et al*., 2003; Baird-Parker, 1962; Presscott *et al*., 2002; Ercolini *et al*., 2004).

The major observations is the wet poor hygienic practices in the farm and during marketing which contributes a lot to the quality of raw milk before it reaches the consumers. Accordingly, it was expected that milk would have a moderate to poor hygienic quality (Balaban and Rasooly, 2000). In Tabriz, milk is produced mostly in non-organized way and usually it is being supplied to the consumers from the urban and rural areas by milk vendors or from the groceries. The distribution of milk to the consumers is completely in poor hygienic conditions. On the other hand, milk is an excellent media for growth of a wide variety of bacteria (Holtfreter *et al*., 2004).

One of the requirements of production of the high quality milk is maintaining the bacteria count level of microorganisms in a product and to study the hygienic and sanitary conditions, under which milk was produced, handled, transported and processed (Salman and Elnasri,

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2011). Polymerase Chain Reaction (PCR) based analytical methods for ascertaining the occurrence of pathogenic or toxigenic microorganisms in food are widely recognized as capable of decreasing detection time and increasing the specificity and sensitivity. Currently, these advantages are gained after pre-enrichment steps when important pathogens are to be found because of unsatisfactory detection levels. *Staphylococcus aureus* is a food borne pathogen responsible for an intoxication resulting from the ingestion of food containing preformed heat-stable enterotoxins, usually produced by this microorganism and representing a sanitary risk when levels of specific bacterial counts at least as high as (105 CFU g) mL of sample are detected.

Staphylococcus aureus is a ubiquitous bacterium, both human and animal commensal (Ercolini *et al*., 2004). Consequently, many foods can be contaminated by this species, thus representing hazard for human health. PCR has been often experimented in milk and cheeses for the direct detection of *Staphylococcus aureus* (Ramesh *et al*., 2002; Tamarapu *et al*., 2001; Ercolini *et al*., 2004). The PCR-based detection of pathogens is made more difficult when raw material with high level of background microflora or complex food matrices are considered. Often when species-specific sequences from *rRNA* genes are chosen as target, falsepositive results may occur because of parallel amplification of target genes from closely related species. It is well known that different substances such as calcium ions, plasmin and proteins can inhibit amplification.

The efficiency of the approach can be also dependent on the specific nucleic acid targeted. Moreover, with particular regard to *Staphylococcus aureus*, not only the presence of the pathogen but also of the genes encoding for SEs production is important to evaluate as enterotoxins nonproducing strains may also occur (Ercolini *et al*., 2004; Ramesh *et al*., 2002; Tamarapu *et al*., 2001). The aim of this study was to determination of contamination rate of raw milk to *Staphylococcus aureus* by Culture and PCR Method in Tabriz city (Center of East Azerbaijan province).

MATERIALS AND METHODS

A total of 100 raw milk samples were collected from the milking bucket into a plastic or aluminum containers which were not well washed, no cooling system was applied at any level of the milk chain which may last for 5 h till milk reaches the consumer. The samples were collected aseptically from market agents and transported on ice to the Laboratory of Microbiology in Veterinary Faculty of Tabriz Branch, Islamic Azad University for analysis. The collected milk samples were cultured on selective media and identification of the suspected colonies were carried out according to (Krystyna *et al*., 2003; Lovseth *et al*., 2004; Stepan *et al*., 2004; Brakstad *et al*., 1992). For coagulase positive bacteria, after extraction of DNA, PCR analysis based on nuc gene was done using the following primers (Balaban and Rasooly, 2000; Holtfreter *et al*., 2004; Lovseth *et al*., 2004). Primer 1: 5-GCG ATT GAT GGT GAT ACG GTT-3, Primer 2: 5-AGC CAA GCC TTG ACG AAC TAA AGC-3. After extraction of DNA, we added the PCR materials to micro tubes and by using the PCR program, we ran the test in 35 cycles (Krystyna *et al*., 2003; Lovseth *et al*., 2004; Stepan *et al*., 2004; Brakstad *et al*., 1992). At last PCR products were separated based on their sizes, using Gel Electrophoresis Method. In this method agarose 1.5% with voltage of 85-100 was used (Krystyna *et al*., 2003; Lovseth *et al*., 2004; Stepan *et al*., 2004; Brakstad *et al*., 1992) (Table 1).

Table 1: PCR program

Parameters	Results
PCR materials	
Template DNA	2 μL
dNTPs	1 μL (10 mM)
Enzyme (Taq DNA polymerase)	1 μL (5U μL)
Buffer (10X)	6 μL
$MgCl_2$	2.5 μL (50 mM)
Primer	1 μL
D.W.	36.5 μL
PCR program	
95°C	10 min (initial denaturation)
94°C	1 min (denaturation)
55°C	30 sec (annealing)
72°C	1.5 min (extension)
Go to 2	37 cycles
72°C	5 min (final extension)

RESULTS AND DISCUSSION

The results of Culture Method in this research displayed that 30% of the examined milk samples were contaminated to coagulase positive *S. aureus* also, results belong to PCR Method demonstrated that all of these contaminated raw milk samples were infected to coagulase positive *S. aureus*. PCR results showed that 30% of samples were contaminated to *S. aureus* (Fig. 1).

Staphylococcus aureus is one of the most common agents in bacterial food poisoning outbreaks. It is also a major causative pathogen of clinical or subclinical mastitis of dairy domestic ruminants. Poultry, meat and egg products as well as milk and milk products have been reported as common foods that may cause staphylococcal food poisoning (Li Loir *et al*., 2003). Foods of animal origin especially, milk and dairy products are associated with food borne disease (Asao *et al*., 2003).

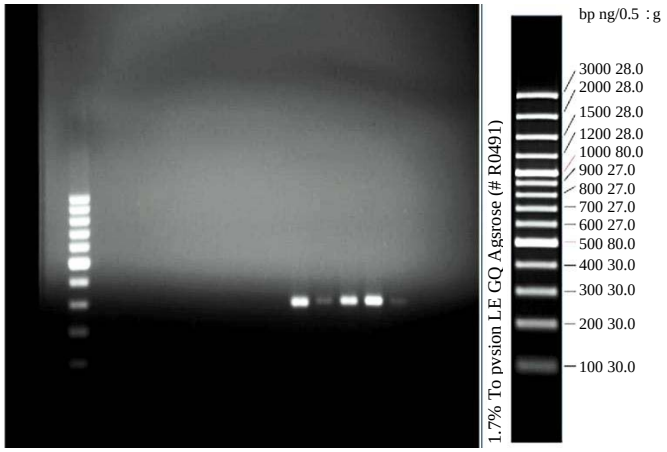

Fig. 1: 270 bp PCR products

Staphylococcus aureus is one of the commonest a etiological agents of bacterial diseases worldwide due to its ability to produce a broad range of exotoxins and other virulence factors. Among them, the Staphylococcal enterotoxins produced by some *S. aureus* strains are the main causal agents of one of the most widespread food borne intoxications, the Staphylococcal food poisoning and together with toxic shock syndrome toxin-1 are responsible for toxic shock syndrome and Staphylococcal scarlet fever (Balaban and Rasooly, 2000; Holtfreter *et al*., 2004). Contamination of dairy products with *S. aureus* may be due to the presence of this pathogen in the basic raw material milk. This is very important, especially in countries producing large amounts of milk products such as cheese. In Palestine, cheese is mostly prepared from unpasteurized cows and sheeps milk and therefore can contribute to the sources of staphylococcal food poisoning. To the knowledge, this is the 1st survey to estimate the prevalence of enterotoxigenic *S. aureus* from raw milk used for human consumption in Palestine. It can be concluded that raw milk is contaminated by this pathogen in this area as well as in other countries and might constitute a risk for *S. aureus* enterotoxin food poisoning (Adwan *et al*., 2005). This result was consistent with previous reports from Japan, Poland and Slovakia where 64-85% of the enterotoxigenic *S. aureus* isolates recovered from raw poultry meat or different food samples and manufacturers harbored the toxin gene (Kitai *et al*., 2005; Bystron *et al*., 2005; Holeckova *et al*., 2002).

CONCLUSION

Dairy animals with subclinical *S. aureus* mastitis may shed large numbers of *S. aureus* organisms into the milk. However, contamination of raw milk and milk products from human handling or from the environment during manufacture also is possible. These contaminations may cause important public health risks. Therefore, greater attention should be given to bacteriological standards for the milk that is used in cheese and ice cream production (Tasci *et al*., 2011).

When scaling up food production from household level to industrial level, general hygienic practices need to be integrated into the process. Teaching and training programs for those working at the dairies can possibly improve the situation.

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