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Study of Milk Extracted from Cows Related to *Staphylococcus aureus* by Culturing and PCR

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Abstract: *Staphylococcus aureus* is one of the most common causes of contagious bovine mastitis and it is the most economically important disease of dairy industries. In this study, 286 dairy cows from 21 traditional dairy cow farms of Tabriz region were selected. CMT was performed and 30.76% milk samples were positive. After identification of the coagulase positive bacteria, antibiotic sensitivity test using the disc diffusion method was done using Enrofloxacin, Sulfamethoxazole-Trimethoprim, Tetracyclin, Lincomycin, Ceftriaxone, Amoxicillin, Ampicillin and Penicillin. For coagulase positive bacteria, after extraction of DNA, PCR analysis, based on nuc gene, was used. The results of this research showed that in the mentioned herds, 21.59%of samples were coagulase positive *S. aureus* and 15.91 % were coagulase negative staphylococci. The antibiotic sensitivity test defined that 94.73% of *S. aureus* isolated from bovine mastitis were susceptible to Enrofloxacin, 89.47% to Sulfamethoxazole-Trimethoprim, 78.94% to Lincomycin and Tetracyclin and 42.10% to Ceftriaxone. On the other hand, all *S. aureus* isolates were resistant to Penicillin, Ampicillin and Amoxicillin. The results of PCR showed that 52.63 %(10 of 19 *S. aureus* by culture) were *S. aureus*. According to the results of this research, the culture method yielded high percentage of *S. aureus* compared to PCR, is time-consuming and the PCR reaction is sensitive and specific for the identification of *S. aureus.*

Key words: Milk · *Staphylococcus aureus* · Culturing · PCR

INTRODUCTION

Staphylococcus aureus is an important cause of mastitis in dairy cows. In fact *Staphylococcus aureus* causes one of the most common types of chronic mastitis. Though some cows may flare up with clinical mastitis (especially after calving) the infection is usually subclinical, causing elevated somatic cell counts (SCC) but no detectable changes in milk or the udder [1, 2]. The bacteria persist in mammary glands, teat canals and teat lesions of infected cows and are contagious. The infection spreads at milking time when *S. aureus*-contaminated milk from an infected gland comes in contact with an uninfected gland and the bacteria penetrate the teat canal [3, 4].

Infected cows' udders are the main reservoir from which S. aureus is transmitted to other cows in the herd and prevention of pathogen transmission from cow to cow reduces mastitis incidence [5]. Bovine mastitis is one of the most problematic diseases and continues to have major economic impact on the dairy industry throughout the world. Numerous agents can cause mastitis in dairy cows but *S. aureus* is the most common etiological agent of bovine mastitis. However, when mastitis control measures are implemented, new infections continue to occur and eradication of S. aureus intramammary infection is difficult to achieve. Infections that originate from sources outside of the mammary gland may contribute to the infection control problem [6]. Many sources of S. aureus exist, including housing materials and fodder, equipment and air, bovine skin, non bovine animals and humans [7]. Therefore the aim of present study was to determination the milk extracted from cows related to *Staphylococcus aureus* by culturing and PCR.

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MATERIALS AND METHODS

In this study, 286 dairy cows from 21 traditional dairy cow farms of Tabriz region were selected. After doing CMT (88/286, 30.76%), milk samples obtained from infected teats were transported to the microbiological laboratory in Faculty of Veterinary Medicine of Tabriz Azad University. The collected milk samples were cultured on selective media and identification of the suspected colonies were carried out according to [9-12]. Coagulase positive *S. aureus* isolates were tested for antibiotic sensitivity by disc diffusion method performed by Enrofloxacin, Sulfamethoxazole-Trimethoprim, Tetracycllin, Lincomycin, Ceftriaxone, Amoxicillin, Ampicillin and Penicillin [8]. For coagulase positive bacteria, after extraction of DNA, PCR analysis, based on nuc gene, was done using the following primers [9-12].

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 below, we ran the test in 35 cycles [9-12].

PCR Materials:

Template DNA	2 µl
dNTPs	1µl (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	1min (denaturation)
55°C	30s (annealing)
72°C	1.5min (extension)
Go to 2	37 cycles
72°C	5 min (final extension)

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 [9-12].

RESULTS

The results of this research displayed that 21.59% of the examined milk samples were coagulase positive *S. aureus* and 15.9 % were coagulase negative staphylococci (Table 1).

The antibiotic sensitivity test showed that 94.73% of *S. aureus* isolated from bovine mastitic milk samples were susceptible to Enrofloxacin 89.47%(revise with Table 2), Sulfamethoxazole-Trimethoprim 78.94% (revise with Table 2), Lincomycin, Tetracyclin 42.10% and Ceftriaxone. On the other hand, all the isolates were resistant to Penicillin, Ampicillin and Amoxicillin.

PCR results showed that 52.63% (10/19) of samples were *S. aureus* (Fig. 1).

Table 1: Results of the bacterial culture of the collected milk samples.

Samples	Number	%
Negative milk samples	12	13.64
Gram(+)cocci	43	48.86
Coagulase positive *S. aureus*	19	21.59
Coagulase negative staphylococci	14	15.91
Total	88	100.00

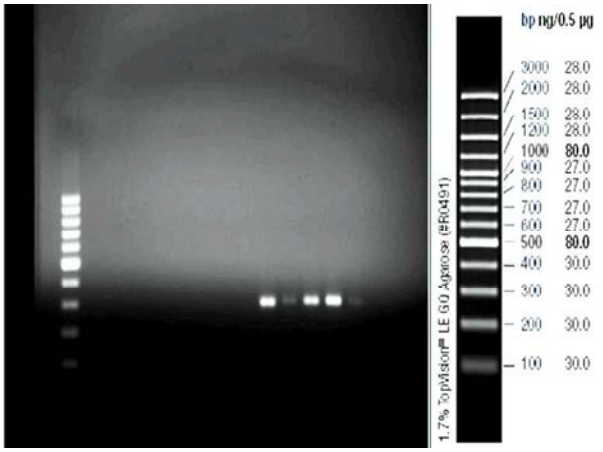

Fig. 1: 270bp PCR products

Table 2: Results of antibiogram test. Make the table horizontal and revise the results in the text with that in the table

Antibiotic	Enrofloxacin	Sulfamethoxazol-Trimethoprim	Lincomycin	Ceftriaxone	Penicillin	Amoxicillin
Sensitivity	94.73	89.47	78.94	42.1	0	0

DISCUSSION

Bacteriological analysis of CMT positive 88 milk samples showed that 12 samples (%13.64) were culture negative and 19 samples (%21.59) were coagulase positive staphylococci and 14 samples (%15.91) were Coagulase negative staphylococci. PCR results showed that 10 out of 19 (%52.63) coagulase positive staphylococci were recognized as *Staphylococcus aureus*.

Researches in the other countries sometimes lead to different results. For example, in a research that was administrated in 2003 by Krystina [9] in Poland, who worked on 40 milk samples obtained from cows suffering from mastitis using PCR method; in 29 cases (%72.5), *Staphylococcus aureus* was isolated. In another research in 2005, Sukru [13] in Turkey, working on 300 milk samples, isolated *Staphylococcus aureus* in %28.33 of the cases and coagulase negative Staphylococci in %20 of the cases. In 2008, Baranski and his colleagues in Poland, working on 41 cows suffering from mastitis, isolated 22 cases of coagulase negative staphylococci and 16 cases of *Staphylococcus aureus* [14].

In this research, Antibiogram test was performed on all coagulase positive staphylococci which showed the following results: 94.73, 89.47, 78.94 and 42.10% were sensitive to Enrofloxacilin, Trimethoprim/Sulfamethoxazol, Lincomicin & Tetracyclin and Ceftriaxone, respectively. Furthermore no susceptibility to three antibiotics; Penicillin, Amoxycillin and Ampicillin was observed. In a research carried by Sukru [13], 100% of isolated *Staphylococcus aureus* showed sensitivity of 85% Cefquinom, 84% to Amoxicillin and 100% to Tetracyclin and 95% of *Staphylococcus aureus* isolated from mastitis cases was resistant to Penicillin. In a research that was administrated on 288 cow milk collected from 8 farms in Poland in 2008 by Branski [14], the results obtained from antibiogram test, showed that 100% of the isolated coagulase negative Staphylococci, 87.6% of *Staphylococcus aureus*, 95.4% of *Streptococcus agalactiae* and 100% of *Streptococcus uberis* were sensitive to Cefquinom [14].

In practice, due to antibiotic resistance and also error probability in performing or interpreting the results of antibiogram test, it is considered a weak point in diagnosis of coagulase-positive Staphylococci using culturing method [14]. In these situations, using PCR method is very rewarding and compensates for the possible errors resulted from culturing and interpreting the antibiogram and nowadays it is commonly used in researches.

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