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Research Article

Incidence of Pathogenic Strains of *Staphylococcus Aureus* Isolated from Diary Farms in Ferizaj, Kosovo

Healthcare

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Abstract

Pathogenic strains of Staphylococcus aureus often contaminate fresh milk produced in dairy farms. This study evaluates the occurrence of Staphylococcus aureus in milk produced in 10 farms located in the regions of Ferizaj, Kosovo. Early the milk samples were tested with "California test" for mastitis. All samples confirmed positive with this test are analyzed with ISO standard method for isolation of Staphylococcus aureus couagulase positive strains. From 2013 to 2014 were analyzed one hundred and eight milk samples from individual cows showing subclinical mastitis. S. aureus strains were detected in 26/108 or 24% of milk samples. 16 out of 26 or 61.5% of pathogenic strains of S. aureus showed atypical characteristics in incubated cultures. Results confirmed the potential transmission of staphylococcal food poisoning to consumers via milk affected by subclinical mastitis, mainly when raw milk is ingested. Subclinical mastitis remain a health probem of dairy cows in Ferizaj and consequently a real threat to the customer as a result of pathogenic strains of Staphylococus aureus present in fresh milk.

1. Introduction

Mammary glands infected by *S. aureus* are the main cause of milk contamination (Adesiyun et al., 1998). Mastitis in dairy cows is one of the most frequently infection caused by a large variety of microorganisms. Raw milk is an ideal growth medium for *Staphylococcus aureus* (Bendahou at al., 2008). Milk and its derivates are considered vehicles of *Staphylococcus aureus* for infection in humans. *S. aureus* is an important foodborne pathogen causes a wide variety of diseases in humans and animals (Zakary et al., 2012).

In dairy cattle, *S. aureus* is frequently associated with subclinical mastitis and may contaminate milk and other dairy products (Bianchi et al., 2014) Couagulase positive strains of this pathogen are mostly high virulence and ability to produce toxins. *S. aureus* produces several staphylococcal virulence factors, including enterotoxins (SEA to SEE and SEG to SEQ), as well as other toxins, such as exfoliative toxin A and B, and toxic shock syndrome toxin (Bianchi et al., 2014). Staphylococcal food poisoning is recognized as a cause of foodborne diseases (Jorgensen et al., 2005). The increase of milk production observed in Kosovo in recent years has resulted with improvements in several management techniques for dairy cattle, and has been followed by investments in the improvement of the microbiological quality of milk on dairy farms. However, little is known about the presence of mastitis caused by pathogenic bacteria in refrigerated raw milk from dairy cows. The aim of present study was to investigate the occurrence of *S. aureus* isolated in raw milk from individual dairy cows with subclinical mastitis in region of Ferizaj in Kosovo. Contaminated milking equipment and the hands of the milkers are also common vehicles of transmission (Katsuda et al., 2005). Although pasteurization kills *S. aureus* cells, thermostable entertoxins generally retain their biological activity. Cows with subclinical mastitis steadily excrete *Staphyloccus aureus* cells. When milk is consumed by man toxic-infections are frequent, but on the other hand the presence of this pathogen has impacts on milk processing. Implementation of veterinary sanitary conditions in cattle farms in Ferizaj leave much to be desired. Milk produced by cows in this area content high number of micoorganisms because of poor hygenic conditions applied in dairy farms. Various authors report in their studies that number of antibiotic resistant strains of *Staphylococcus aureus* is increased mainly for antibiotics as penicillin, methicillin and vancomycin. According to recent years is showed that the incidence of resistant strains in milk reaches 35% of tested isolates (Zecconi & Hahn, 2000; Paterson et al.,

2005). Resistance to penicillin, methicillin vancomycineis higher than other antibiotics and this comes as a result of mass using for the treatment of mastitis (Thaker et al., 2012).

In terms of sanitary conditions key factors influencing the environment and the contamination of animal body with strains *St. aureus* are related to milking equipments, farm conditions, health status, cooling and storage conditions of fresh milk as well as health status of personnel in dairy farms (White et al., 2005).

2. Materials and Methods

The study was conducted in dairy farms located in the region of Ferizaj in Kosovo. Accomplishment of sanitary, veterinary remains an engagment of veterinary inspection continuing to determine the production conditions of of fresh milk but also the health status of the herds of dairy cows. For this purpose, the inspections are carried out on a regular basis according to a model adopted in accordance with the veterinary and food legislation in 2014. In each farm, the milk samples are colected from milking cows. Milk samples were collected from each cow in aseptic manner in different farms after the milking process. At the end of the milking procedure, 300 ml milk samples is collected and sampled with sterile glass bottle. 108 milk samples taken from cows were transported to the laboratory in cooling temperature (4-8ºC) in the laboratory of Veterinary Institute in Pristina. Analytical control is realized applying firstly "California mastitis test" for detection of mastitis and then is carried out isolation and identification procedures of *St. aureus* according to ISO 6888-1, 1994. The serial dilutions of samples were prepared in accordance with IS0 6887-1, 1994. Samples were inoculted on Baird Parker agar (OXOID) prepared with egg yolk and teluriti emulsion (1%) (OXOID). From each serial dilution (10^{-2}, 10-^{3}.) were transferred with sterile pipette 0.1 ml of sample (milk) in each of two Baird Parker agar plates. This process is repeated for the 10^{-2} dilution and further decimal dilutions. Care was taken to spread as quickly as the volume of the sample evenly over the surface of Baird Parker agar plates. The incubuted plates were prepared at 37^{O} C for 24-48h. All plates were checked for the presence of couagulase positive *Staphyloccocus aureus* strains showed with typical and atypical characteristics. Suspect colonies of *St. aureus* were tested using API Staph strips (bio Merieux), hemolysis tests as well as couagulase test. Microscopic and biochemical tests were performed according to the method applied. In this case the value found was multiplied by a factor 10 for liquid samples. Besides analytical control, it was carried out the sanitary and veterinary inspection based on designed protocols to assess the compliance with the conditions mentions in specific legislation.

3. Results and Disscusion

From the analytical control of 108 fresh milk samples collected from the cows in different dairy farms in Ferizaj in 2014 was achived isolation and identifiction of couagulase positive strains of *St. aureus*. Summaries of the results are presented below in Table 1.

Table 1. *Staphylococus aureus* positive cases confirmed by "California tests" in dairy farms and analytical control of fresh milk samples in the region of Ferizaj, Kosovo.

The number of samples of fresh milk in dairy farms in Ferizaj	The number of samples identified positive by California mastitis test	The number of positive samples contaminated with *Staphyloccocus aureus*	Atypical strains of *Staphylococcus aureus*	Typical strains of *Staphylococcus aureus*
108	41/108 (37.9%)	26/ 108(24%)	16/26(61,5%)	8/26 (33,4%)

The analytical control with "California mastitis test" of milk samples collected in dairy farms in the region of Ferizaj confirmed the presence of *Staphylococcus aureus* in 24% (24/108) of total samples. According

to this suty results the incidence of *Staphylococcus aureus* confirmed by "California mastitis test" reached the value 37.9% of the analyzed milk samples. Found values are influenced by the health status of cows and sanitary conditions of dairy farms (Gücükoğlu et al., 2014).

This study identified milking cows with frequent subclinical mastitis and postpartum periode remains with high incidence becaouse untreated cows are the source of contamination (Jones et al., 2006; Dewanand et al., 2007).

The study results clearly indicated that isolates of *Staphylococcus aureus* occupy 16.3% of positive cases confirmed by California mstitis test. Also by other authors who describe this pathogen, in fresh milk samples is reported that *Staphylococcus aureus* is a major cause of subclinical mastitis (Thaker et al., 2012).

The isolated strains appeared that 61,5% of atypical strains formed colonies demonstrating a clear atypical charachteristics. But there are many studies in world reporting the incidence of *Staphylococus aureus* as a cause of subclinical mastitis but comparison with the results in the present study remains difficult, because the presence of *S. aureus* as a subclinical mastitis causative agent is compromised by area, treatment and animal husbandry practices as well as hygienic conditions during milking and other factors relating to the environment.

4. Conclusions

This study conducted from 2013 to 2014 in dairy farms in Ferizaj, Kosovo confirmed the presence of coagulase positive strains of *Staphylococcus aureus* in 108 fresh milk samples. Milking cows with subclinical mastitis are source of contamination with *Staphylococcus aureus*. The study concluded that fresh milk produced by dairy farms in Ferizaj continues to be a source of contamination threatening the health of consumers.

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