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INCIDENCE OF STAPHYLOCOCCUS AUREUS AND COAGULASE POSITIVE STAPH. AUREUS IN RAW MILK IN KOSOVO

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Abstract
The presence study was conducted to determine the presence of *Staphylococcus aureus* and coagulase positive *S.aureus* in raw milk collected from different localities in Kosovo. We have examined 55 samples of raw milk cow taken from cattle farms. The *Staphylococcus aureus* was isolated in Baird Parker agar where typical and atypical colonies were selected and submitted to coagulase and complementary tests. Out of 55 samples of raw milk studied, 17 showed contamination by *Staphylococcus aureus* corresponding to 31% of the samples being contaminated. Out of 17 samples showed contamination by *S. aureus*, 5 of them were coagulase positive *S. aureus* or 29 % (or 9 % of total samples analyzed). Results clearly suggested a possibility of potential public health threat of *Staphylococcus aureus* resulting from contamination of milk with pathogenic bacteria is mainly due to unhygienic processing, handling and unhygienic environment.
***Key-words**: Staph.aureus, coagulase positive, incidence, raw milk.*

Introduction

Raw milk produced by cows at the temperature of cow's body is considered as one of the best mediums for growth of Staphylococcus aureus (Zecconi et al, 2000). Mammary glands infected by Staphylococcus aureus are the main cause of milk contamination (Jayarao et al, 2004). The presence of Staphylococcus aureus in raw milk generally comes from cows with mastitis, from handlers or from deficient hygiene. The capacity to coagulate plasma, the principal characteristic of *S. aureus*, is highly correlated to the capacity to produce enterotoxins harmful to the tissues of the contaminated host (Murray PR et al, 2006). Not all coagulase positive *S. aureus* produce toxin. In Norway, 48% of isolates from bovine raw milk and raw milk products were identified as SE producers (Loncarevic et al, 2005).

Milk and its products are common vehicles of *Staphylococcus aureus* transmission to humans (Lowy FD, 1998). The presence of *Staphylococcus aureus* shows up unsanitary conditions in the cattle herd and counts above 10^3cfu in milk increase the risk of staphylococcal toxin production more resistant to the heat processes of pasteurization. It's presence in foods can be a risk to human health, causing a public health problem, as these bacteria produce toxins that can cause toxic food infections (1-4). In France, a study of foodborne disease outbreaks from 1992-1997 found that *S. aureus* was the most frequent pathogen associated with milk related outbreaks (De Buyser et al., 2001). Food intoxications caused by using raw milk are well known and the majority holds *S. aureus* or its enterotoxins as responsible agents (J.H.Lee, 2003).

The aim of this study was to verify the presence of *Staphylococcus aureus* and coagulase positive *S.aureus* in raw milk.

Material and Methods

Sample collection

A total of 55 raw milk samples were collected from different regions in Kosovo. The samples collected aseptically were transferred to sterile recipients and transported to the laboratory under cold conditions, and analyzed within 24 hours.

Staphylococcus aureus research

To carry out the laboratory test were used ISO method 6888. Ten mililitres of each cheese sample were placed aseptically into a sterile plastic bags and 90ml peptone water were added to make 10^0dilutions. The bags were homoginezed in stomacher for 1 min. Then, 0.1ml from each dilutions (10^0, 10^{-2}, 10^{-4}…) were inoculated on the surface of Baird Parker agar and spread with a Drigalski spatula. The incubation was done at a temperature of 37 ºC

for 24-48 hrs. Appearance of shiny black colonies with an opaque ring, surrounded by a clear halo were considered to be presumptive *S. aureus*. The suspected *S. aureus* colonies were submitted for Gram staining, oxidase test, catalase test and also were further identified biochemical profile, using api Staph.

Coagulase test

Five typical colonies were selected for seeding in tubes containing BHI and the incubation was done at a temperature of 35 ºC for 24 hours. From each tube cultivation in BHI, 0.3ml was transferred to sterile tubes containing 0.5ml of rabbit plasma. The incubation was done at a temperature of 35 ºC for 6 hours.

Results and discussion

The microbiological analysis of raw milk samples for presence of *S. aureus* and coagulase positive *S. aureus* are presented in Tables 1 and 2.

Out of 55 samples of raw milk analyzed, 17 of them were contaminated by *Staphylococcus aureus*, corresponding to 31% of samples contaminated, at a range of $2.5x10^2$cfu/ml to $4.6x10^5$cfu/ml. Out of the 17 contaminated samples of raw milk, 4 had levels of *Staphylococcus aureus* corresponding to 10^2 cfu/ml; 3 had levels of 10^3cfu/ml; 7 counts of 10^4cfu/ml and 3 samples of 10^5cfu/ml (Tables 1, 2).

Out of 17 samples showed contamination by *S. aureus*, 5 of them were coagulase positive *S. aureus* or 29 % (or 9% of total samples analyzed).

Table 1. Staph.aureus and coagulase positive Staphylococcus aureus in the examined raw milk samples

Sample type	No. of samples	*Staph. aureus*	Coagulase positive *Staph. aureus*
Rawmilk (cow)	55	17 (31%)	5 (29%)

Table 2. Counts of S. aureus and coagulase positive S. aureus of positive analyzed raw milk samples

Sample No	*S.aureus(cfu/ml)*	*Coag.positive S. aureus*	*Sample No.*	*S.aureus (cfu/ml)*	*Coag.positive S.aureus*
1.	$3.2x10^2$	+	10.	$2.5x10^2$	
2.	$2.5x10^3$	+	11.	$2.4x10^4$	
3.	$1.3x10^4$		12.	$5.1x10^4$	+
4.	$4.5x10^5$		13.	$4.6x10^5$	
5.	$1.2x10^3$	+	14.	$1.5x10^3$	
6.	$1.6x10^4$		15.	$5.1x10^2$	
7.	$5.1x10^4$		16.	$1.2x10^4$	+
8.	$3.5x10^2$		17.	$1.5x10^5$	
9.	$4.7x10^4$				

Of the 5 contaminated samples of raw milk, one had coagulase positive *Staph. aureus* counts corresponding to $3.2x10^2$cfu/ml; 2 with 10^3cfu/ml ($2.5x10^3$cfu/ml and$1.2x10^3$cfu/ml) and 2 with 10^4cfu/ml ($1.2x10^4$cfu/ml and $5.1x10^4$cfu/ml, Table. 2).

The incidence of *S. aureus* in raw milk depending on the health status of the milking cows and other factors (Adesiyun et al, 1998). The high incidence of *Staphylococcus aureus* is indicative of poor hygienic measures during production, handling and distribution.

Considering that three of samples had counts of *S. aureus* 10^5cfu/ml, andin other hand five sample were coagulase positive *S. aureus*, the raw milksamples analyzed may be a serious risk to the health of the population (Fig 1.). It is generally considered that the numbers of *S. aureus* need to be $>10^5$ cfu/g or ml product for the production of sufficient toxin to cause illness (Le Loir et al. 2003, Pinchuk et al. 2010).

S. aureus contamination can occur from raw milk produced from cows suffering from mastitis, food handlers who are carriers of *S. aureus*, or poor hygiene practices (O'Brien et al., 2008).

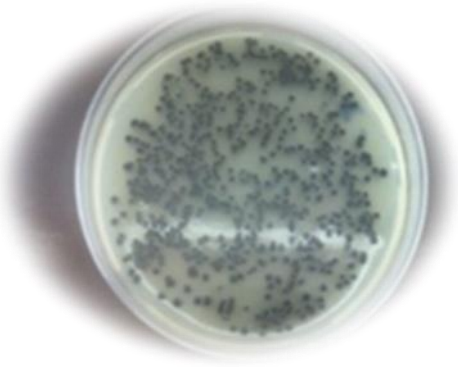

Fig.1 Staphylococcus aureus colonies on BPA

Fig.2 Coagulase positive test

Negligence of hygienic condition such as improper cleaning of bulk tank, dirty udders, milking equipments, cooking temperature, milk handling technique and improper storage will increase the proportion Gram positive and Gram negative bacteria in the bulk tank milk (Vasavada, 1988).

Conclusion

Coagulase positive *Staphylococcus aureus* was identified in 29% of raw cow milk samples collected and analyzed from different regions of Kosovo. Contamination of raw milk produced by cattle farms in Kosovo remains a possible source of food intoxication because of the high number of *S. aureus* in milk.

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