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

D. Sylejmani*, A. Hamidi

Faculty of Agriculture and Veterinary, University of Prishtina, Boul. ”Bill Clinton”, 10000, Kosovo

*corresponding author: afrim.hamidi@uni-pr.edu

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 10^3 cfu 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 10^0 dilutions. The bags were homogenezed 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 °C.
for 24-48 hrs. Appearance of shiny black colonies with an opaque ring, surrounded by a clear halo were considered to be presumptive \textit{S. aureus}. The suspected \textit{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 °C 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 °C for 6 hours.

**Results and discussion**

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

Out of 55 samples of raw milk analyzed, 17 of them were contaminated by \textit{Staphylococcus aureus}, corresponding to 31% of samples contaminated, at a range of $2.5 \times 10^2$ cfu/ml to $4.6 \times 10^5$ cfu/ml. Out of the 17 contaminated samples of raw milk, 4 had levels of \textit{Staphylococcus aureus} corresponding to $10^3$ cfu/ml; 3 had levels of $10^4$ cfu/ml; 7 counts of $10^5$ cfu/ml and 3 samples of $10^6$ cfu/ml (Tables 1, 2).

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

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of samples</th>
<th>Staph. aureus</th>
<th>Coagulase positive Staph. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk (cow)</td>
<td>55</td>
<td>17 (31%)</td>
<td>5 (29%)</td>
</tr>
</tbody>
</table>

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


Considering that three of samples had counts of \textit{S. aureus} $10^6$ cfu/ml, and in other hand five sample were coagulase positive \textit{S. aureus}, the raw milk samples analyzed may be a serious risk to the health of the population (Fig 1.). It is generally considered that the numbers of \textit{S. aureus} need to be $>10^6$ cfu/g or ml product for the production of sufficient toxin to cause illness (Le Loir et al. 2003, Pinchuk et al. 2010). \textit{S. aureus} contamination can occur from raw milk produced from cows suffering from mastitis, food handlers who are carriers of \textit{S. aureus}, or poor hygiene practices (O’Brien et al., 2008).
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.

**References**


