OCCURRENCE OF STAPHYLOCOCCUS AUREUS AND COAGULASE POSITIVE STAPH. AUREUS IN ARTISANAL CHEESE IN KOSOVO

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Abstract

The aim of this study is to verify the occurrence of Staphylococcus aureus in artisanal cheese, for which 45 samples of artisanal cheese were analyzed from 3 regions of Kosovo. The Staphylococcus aureus was isolated in Baird Parker agar where typical and atypical colonies were selected and submitted to coagulase test and identified biochemical profile, using the api Staph. Out of 45 samples of artisanal cheese studied, 18 showed contamination by Staphylococcus aureus corresponding to 40% of the samples being contaminated. Out of 18 samples showed contamination by S. aureus, 10 of them were coagualase positive S. aureus or 55.5 % (or 22% of total samples analyzed). The presence of this pathogenic microorganism indicates a potential health hazard to those who consume this artisanal cheese from these regions.

Key words: *staph.aureus, coagulase positive, occurrence, artisanal cheese*

1. INTRODUCTION

Milk and dairy products contain all the nutritional components that make milk and milk products an important part of the human diet, but, on the other side, the nutrients also support the growth of pathogenic organisms. Cheeses are ready-to-eat (RTE) food products that do not undergo any further treatment to ensure their safety before consumption. Approximately 10% of cheese in Europe is made from raw milk (Beuvier et al., 2004), presenting a considerable potential risk to public health.

Recently there have been more than a few reports of cheese food infections, and food poisoning cases reported. Many such outbreaks have been associated with *Staphylococcus aureus* (Jorgensen et al. 2005, Williamson et al. 2005). Staphylococcus aureus was the third most common cause of confirmed food poisoning in the world till the year 2003 (Acco et al.,2003).

In 2006, the consumption of contaminated cheese accounted for the 0.4% of the total foodborne outbreaks in Europe (EFSA, 2008).

Staphylococcal food poisoning is one of the most common food-borne diseases worldwide (Balaban N et al, 2000) resulting from the ingestion of staphylococcal enterotoxins preformed in food by enterotoxigenic strains of coagulase – positive staphylococci, mainly *S. aureus*.

The organism is a common cause of mastitis in dairy cattle, and it can also enter the milk supply from sores on the teats of dairy cattle or from the hands and nasal discharges of dairy farmers and workers.

The present study attempts to assess the occurrence of *S. aureus* in traditional cheese produced using unpasteurized cow milk and collected from unorganized sectors in Kosovo. In Kosovo, there are many unorganized sectors (private enterprises whose activities are not regulated under legal provision) where milk and milk products are produced by traditional methods and these products are usually consumed locally.

2. MATERIAL AND METHODS

2.1. *Sample collection*

A total of 45 artisan cheese samples were collected from different regions in Kosovo, from September to December 2014. The samples were collected aseptically, transferred to sterile plastic bags and transported to the laboratory under cold conditions, and analyzed within 24 hours.

2.2. *Staphylococcus aureus research*

To carry out the laboratory test were used ISO method 6888. Ten grams of each cheese sample were placed aseptically into a sterile plastic bags and 90ml peptone water were added to make 10^{-1} dilutions. The bags were homoginezed in stomacher for 1 min. Then, 0.1ml from each dilutions (10^{-1}, 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.

2.3. *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.

3. RESULTS AND DISCUSSION

The microbiological analysis of artisan cheese samples for presence of *S. aureus* and coagulase positive *S. aureus* are shown in Tables 1, 2 and Fig.1.

Out of 45 samples of artisan cheese analyzed, 18 of them were contaminated by *Staphylococcus aureus*, corresponding to 40% of samples contaminated, at a range of $1.5x10^2$ to $1.8x10^6$ cfu/g. Out of the 18 contaminated samples of cheese, 4 had levels of *Staphylococcus aureus* corresponding to 10^2 cfu/g; 2 had levels of 10^3cfu/g; 8 counts of 10^4cfu/g; 2 samples of 10^5 cfu/g and 2 sample 10^6 (Tables 1, 2).

Out of 18 samples showed contamination by *S. aureus*, 10 of them were coagulase positive *S. aureus* or 55.5 % (or 22% of total samples analyzed).

Table 1. *Staph. aureus* and coagulase positive *Staphylococcus aureus* in the examined cheese samples.

Sample type	**No. of samples**	***Staph. aureus***	**Coagulase positive *Staph. aureus***
Artisanal cheese	45	18 (40%)	10 (22%)

Table 2. Counts of *S. aureus* and coagulase positive *S. aureus* of positive analyzed cheese samples

Sample No	**S.aureus(cfu/g)**	**Coag.positive S. aureus**	**Sample No.**	**S.aureus (cfu/g)**	**Coag.positive S.aureus**
1.	$2.0x10^2$	+	10.	$2.5x10^2$	
2.	$8.5x10^3$	+	11.	$2.4x10^4$	+
3.	$1.3x10^4$		12.	$5.1x10^4$	+
4.	$6.5x10^5$	+	13.	$3.6x10^5$	
5.	$3.2x10^3$	+	14.	$1.5x10^6$	
6.	$1.6x10^4$		15.	$5.1x10^2$	+
7.	$1.1x10^4$		16.	$2.2x10^4$	+
8.	$1.5x10^2$		17.	$1.8x10^6$	
9.	$4.7x10^4$	+	18.	$4.2x10^4$	+

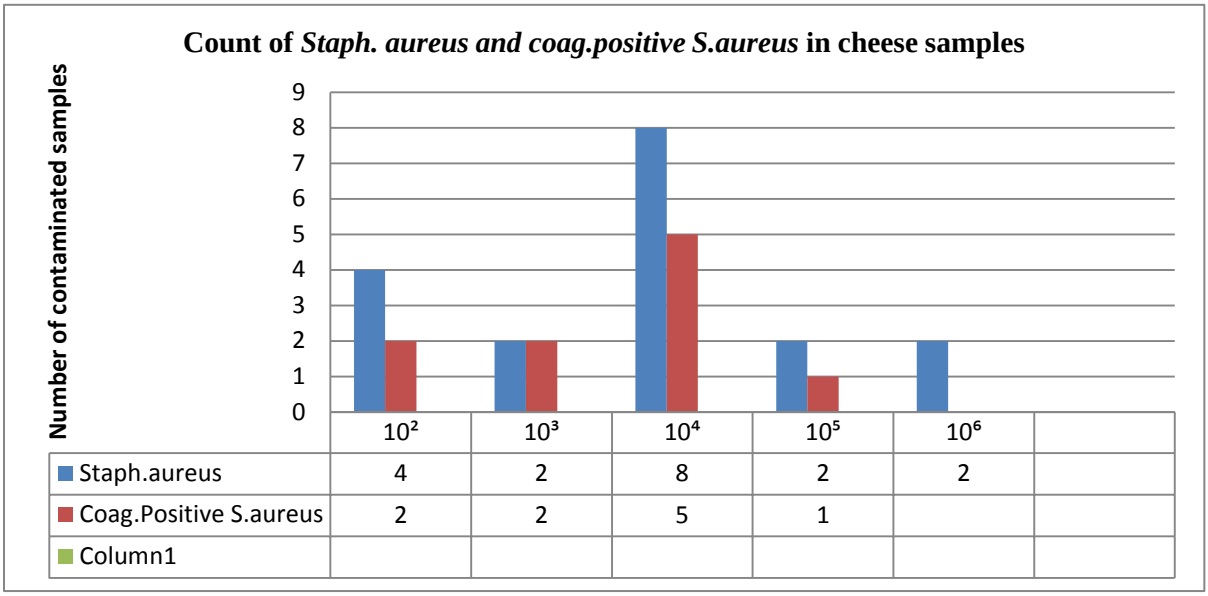


Figure1. Count of *Staphylococcus aureus* and coagulase positive S. aureus in samples of contaminated artisanal cheese cfu/g.

Of the 10 contaminated samples of cheese, 2 had coagulase positive *Staph. aureus* counts corresponding to 10^2cfu/g; 2 with 10^3 cfu/g; 5 with 10^4cfu/g and 1 sample 10^5cfu/g (Figure 1).

Considering that two of samples had counts of *S. aureus* 10^5 cfu/g, one 10^6 cfu/g and in other hand one sample coagulase positive *S. aureus* with 10^5 cfu/g, the cheese consumed in these regions 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 of cheese 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). Staphylococcal food poisoning is caused by ingestion of a heat-stable toxin produced by coagulase-positive *Staphylococcus aureus*.

The presence of coagulase – positive *Staphylococcus aureus* in a food give an indication about its contamination from skin, mouth or handling the food, but inadequately cleaned utensils or equipment may also a source of contamination. Staphylococcal food poisoning is a major form of food borne illness and appears to be so as time goes on when the environmental conditions are favorable for growth and multiplication of Staphylococcal.

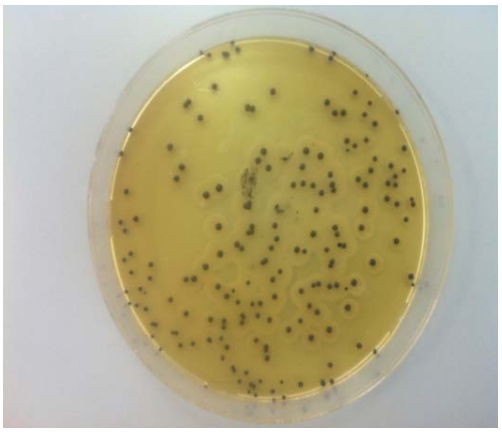


Fig.1 Staphylococcus aureus colonies on BPA



Fig.2 Coagulase positive test

In villages and unorganized sectors in Kosovo, cheese is prepared by traditional methods that increases the probability of contamination during the processing and may cause the transfer of undesirable microorganisms in the end of products. If milk products are contaminated with *S. aureus*, they mean a serious health problem. Artisanal cheese taken for study was produced without pasteurization milk which may play an important role on that levels of contamination with *S. aureus*.

4. CONCLUSION

The presence of *S. aureus* and coagulase positive *S.aureus* in the analyzed cheese samples seemed to be related to the use of raw milk and unhygienic production processes and storage conditions. For controlling of such contamination, it is essential to ensure high safety standards such as raw milk quality, effective pasteurization processes, hygienic production and storage conditions, proper cleaning, and sanitation processes in production facilities.

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