

Staphylococci plate counts in foods of milk origin

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ABSTRACT: We have examined 35 samples of fine cottage cheese, 14 samples of whole winter “bryndza”, 29 samples of Ondava cheese, 18 samples of skim kephir milk, 18 samples of whole acidophilous milk, 5 samples of yoghurt milk with strawberry flavour and 50 samples of fresh butter (Rajo) for the presence of staphylococci focusing on *Staphylococcus aureus*. All samples intended for microbiological analysis were taken directly from market establishments. None of the examined samples of yoghurt milk complied with the Codex Alimentarius because they contained *Staphylococcus aureus* bacteria on the level of 100, 65, 5, 75, and 60 CFU/ml. The other analysed food products satisfied the current standards with regard to the presence of staphylococci.

Keywords: cows; sheep; milk products; staphylococci; *Staphylococcus aureus*

INTRODUCTION

Contamination of dairy products with *Staphylococcus aureus* (*S. aureus*) bacteria may influence considerably their harmlessness, decrease their shelf-quality and endanger the health of consumers.

S. aureus causes diseases both in people and animals (Park *et al.*, 1994; Nishijima *et al.*, 1997; Moretti *et al.*, 1998; Leski *et al.*, 1998; Wallace *et al.*, 1998; Hermans *et al.*, 1999; Jensen *et al.*, 1999, etc.) and thus attracts considerable attention particularly from the point of view of food hygiene (Paulsen *et al.*, 1995; Yazdankhah *et al.*, 1998, etc.). The recent research in this field has focused on the direct detection of staphylococci enterotoxins in foods (Rasooly and Rasooly, 1998; Bhatti and Micusan, 1999; Yazdankhah *et al.*, 1999 and others).

Contamination of food products with *S. aureus* pathogens may result primarily from their presence in the basic raw material, milk. In such cases, the source of pathogen is the dairy cow or sheep (Sol *et al.*, 1994; Enevoldsen *et al.*, 1995; Moretti *et al.*, 1998; Elečko *et al.*, 1998; Annemüller *et al.*, 1999; Osteras *et al.*, 1999; Pozza *et al.*, 1999, etc.) or the milker (Beličková, 1999; Brisabois *et al.*, 1999).

Bacterial contamination of food products may also result from violation of technology and production hygiene rules (Grieger *et al.*, 1990; Dudriková *et al.*, 1999).

As the incidence of alimentary diseases in human population increases, the food inspection must follow a similar trend. Additional complications arise out of the constant expansion of international food trade, extension of the range of available foods, increase in tourism and the number of people taking advantage of canteens and other food serving establishments (Curtis, 1998; Heir *et al.*, 1998; Boháček *et al.*, 1999; Brisabois *et al.*, 1999, etc.).

As the liberalisation of trade on an international scale goes along with the liberalisation of food legislative, its global co-ordination is needed to reach a unified qualitative level (Kanjuka and Šutiak, 1990).

With regard to the diverse situation in import, production and distribution of foods in this country as well as decreased transparency and therefore also the efficacy of complex inspection we tried to determine the status of microbial contamination of some final milk products available on the market focusing on pathogens, particularly on the increasingly important *S. aureus*.

MATERIAL AND METHODS

The microbiological analysis of milk products performed in our study was based on the valid methods for detection and determination of staphylococci and *S. au-*

reus counts in raw materials and foods of animal origin (STN ISO 6888, 1999; Codex Alimentarius, 1998).

Samples of fine cottage cheese, whole winter “bryndza”, Ondava cheese, skim kephir milk, whole acidophilous milk, yoghurt milk flavoured with strawberry and fresh butter Rajo were purchased directly from market establishments.

Baird-Parker agar, produced by Imuna, Šarišské Michaľany (Slovak Republic), was used as a nutrient medium for microbiological detection.

Staphylococci and *S. aureus* counts were determined by spreading 0.1 ml of a suitably diluted sample onto the surface of Baird-Parker agar plates. The inoculated plates were incubated at 37°C for 48 hours. As staphylococci were regarded only black, glossy, convex colonies with a diameter of 1–1.5 mm were counted.

As *S. aureus* were regarded the colonies surrounded with a 1–2.5 mm lighter zone visible in the cloudy agar and with positive coagulase test PK – Stafylotest (Imuna, Šarišské Michaľany, Slovak Republic).

According to the Bulletin of the Ministry of Agriculture, Slovak Republic, Vol. XXX, section 21, suppl. No.3, 1998, no *S. aureus* are allowed in the final milk products.

RESULTS AND DISCUSSION

The results of microbiological detection of staphylococci in food samples of milk origin taken directly from the market are presented in Tables 1 to 4.

The microbiological analysis of fine cottage cheese samples for the presence of staphylococci, particularly *S. aureus*, is shown in Table 1. These bacteria were present in all 35 samples examined ranging from 9×10^2 to 1.07×10^4 CFU/g. Similar investigations were carried out by Elečko *et al.* (1998), who failed to detect *S. aureus* in any of 13 examined samples.

Another milk product tested was the whole winter “bryndza” (Table 2) produced as a mixture of stored (barrel) sheep cheese and fresh dairy cottage cheese. Staphylococci were observed in all samples, their counts ranging from 9.11×10^3 to 8.56×10^4 CFU/g. No *S. aureus* was found in any of the examined samples. Similar results were obtained by Grieger *et al.* (1979), Beličková *et al.* (1993) and others, who investigated “bryndza” for the presence of staphylococci.

Additional products tested for staphylococci were samples of Ondava cheese. None of the 29 samples examined showed the presence of staphylococci or *S. aureus* (Table 3), which is very important from the hygiene point of view. Similar results were presented by Vernozý *et al.* (1994), who investigated cheese for the presence of coagulase negative staphylococci.

Microbiological analysis of 18 samples of skim kephir milk and 18 samples of whole acidophilous milk showed (Table 3), that not a sample contained staphylococci, which indicated that the starting raw material, microbial culture and production hygiene were on a good level (Burdová, 1999).

Staphylococci including *S. aureus* have been determined also in 50 samples of fresh milk butter Rajo (Table 3).

Table 1. Counts of staphylococci and *S. aureus* in fine cottage cheese

Sample No.	Staphylococci (CFU/g)	<i>S. aureus</i> (CFU/g)	Sample No.	Staphylococci (CFU/g)	<i>S. aureus</i> (CFU/g)
1	9.45×10^2	0	19	9.40×10^3	0
2	1.02×10^3	0	20	7.80×10^3	0
3	9.00×10^2	0	21	7.35×10^3	0
4	9.85×10^2	0	22	1.00×10^4	0
5	7.46×10^3	0	23	1.07×10^4	0
6	8.50×10^3	0	24	4.70×10^3	0
7	7.90×10^3	0	25	5.55×10^3	0
8	1.02×10^4	0	26	5.05×10^3	0
9	6.76×10^3	0	27	5.25×10^3	0
10	6.76×10^3	0	28	4.35×10^3	0
11	6.90×10^3	0	29	5.50×10^3	0
12	5.75×10^3	0	30	5.05×10^3	0
13	6.45×10^3	0	31	4.25×10^3	0
14	4.35×10^3	0	32	5.45×10^3	0
15	9.45×10^3	0	33	4.85×10^3	0
16	8.50×10^3	0	34	4.90×10^3	0
17	6.25×10^3	0	35	6.10×10^3	0
18	1.05×10^4	0			

Table 2. Counts of staphylococci and *S. aureus* in "bryndza"

Sample No.	Staphylococci (CFU/g)	<i>S. aureus</i> (CFU/g)
1	9.50×10^3	0
2	1.01×10^4	0
3	9.11×10^3	0
4	1.02×10^4	0
5	6.76×10^4	0
6	8.56×10^4	0
7	6.70×10^4	0
8	6.40×10^4	0
9	4.30×10^4	0
10	5.98×10^4	0
11	6.22×10^4	0
12	5.70×10^4	0
13	6.40×10^4	0
14	5.78×10^4	0

Neither staphylococci nor *S. aureus* were found in the tested samples. This corresponds with the data of a number of authors that staphyloentero-toxicosis originating from butter occurs very rarely (Beličková *et al.*, 1999, *etc.*). Grieger *et al.* (1990) stressed that violation of production technology enhanced substantially the multiplication of micro-organisms in butter.

Yoghurt is one of the most widely consumed milk product (Grieger *et al.*, 1990, *etc.*) It is a nutritionally valuable food article with good organoleptic properties and longer shelf-life compared to milk. It is increasingly popular with children. All analysed samples of yoghurt milk with strawberry flavour (Table 4) contained staphylococci on the level of 2.89×10^2 CFU/ml, on average. All the tested samples were positive for *S. aureus* averaging 61 CFU/ml.

In conclusion, all analysed foods of milk origin complied with the current standards except for yoghurt milk with strawberry flavour in which *S. aureus* was found on the level of 100, 65, 5, 75, and 60 CFU in 1 ml of examined samples although the standard allows no presence of *S. aureus*.

The fact that of the wide range of analysed milk products the most popular and widely used product did not comply with the hygiene standard indicates clearly the need for systematic and on-the-spot inspection of this final milk product in order to identify the etiological focus of contamination and eliminate it efficiently in the interest of protection of consumers' health.

REFERENCES

Annemüller C., Lämmler Ch., Zschock M. (1999): Genotyping of *Staphylococcus aureus* isolated from bovine mastitis. Vet. Microbiol., 69, 217–224.

Table 3. Counts of staphylococci and *S. aureus* in investigated foods

Product	Examined samples	Positive samples	
		Staphylococci	<i>S. aureus</i>
Ondava cheese	29	0	0
Skim kephir milk	18	0	0
Whole acidophilous milk	18	0	0
Fresh milk butter Rajo	50	0	0

Table 4. Counts of staphylococci and *S. aureus* in yoghurt milk

Sample No.	Staphylococci CFU/g	<i>S. aureus</i> (CFU/g)
1	2.75×10^2	1.0×10^2
2	2.55×10^2	65
3	2.40×10^2	5
4	3.25×10^2	75
5	3.50×10^2	60

Beličková E. (1999): Sheep milk as potential source of staphyloenterotoxigenesis in humans (in Slovak). [Final Report.] Research Institute of Veterinary Medicine, Košice. 19 pp.

Beličková E., Siklenka P., Jacková A., Obšitníková D. (1993): Proposed measures for reduction of microbiological contamination of foods (in Slovak). [Final Report.] Research Institute of Veterinary Medicine, Košice. 55 pp.

Beličková E., Elečko J., Tóth L., Obšitníková D. (1999): Ecology of important pathogens in raw materials and food of animal origin (in Slovak). [Final Report.] Research Institute of Veterinary Medicine, Košice.

Bhatti A.R., Micusan V.V. (1999): Production and characterisation of anti-peptide monoclonal antibodies with specificity for Staphylococcal enterotoxins A and B. J. Microbiol. Meth., 35, 143–149.

Bohačenko I., Erban V., Schwartz W. (1999): Experiences with interconnection of the Critical Points System (HACCP) in production technology of plant edible oils and ISO 9002 in company SETUZA, Ltd. Czech J. Food Sci., 17, 113–120.

Brisabois A., Lafarge V., Brouillaud A., deBuyser M.L., Collette C., Garin-Bastuji B., Thorel M.F. (1999): Pathogenic germs in milk and milk products: situation in France and in Europe. Rev. Sci. Tech., Off., Int. Epiz., 16, 452–471.

Bulletin of the Ministry of Agriculture, Slovak Republic (1998): Vol. XXX, Section 21, Suppl. No.3.

Burdová. O. (1999): Milk and milk products from the point of view of rational nutrition (in Slovak). Proc. Hygiene Alimentorum XX, University of Veterinary Medicine, Košice. 45–47.

Codex Alimentarius (1998): Bulletin of Ministry of Agriculture, Slovak Republic, Vol. XXX, Section 21, Suppl. No. 3 to the 4th chapter of the 2nd part of FC.

Curtis A. (1998): HACCP in egg and egg products. Egg. Ind., 103, 8–12.

- Dudriková E., Burdová O., Pilipčinec E. (1999): Staphylococci as contaminants of milk and milk products (in Slovak). In: Proc. Hygiena Alimentorum XX, University of Veterinary Medicine, Košice. 69–70.
- Elečko J., Beličková E., Vasil' M. (1998): Epizootiological and epidemiological studies on circulation of enterotoxigenic strains of *S. aureus* in commodities of animal origin (in Slovak). [Final report 05.] Research Institute of Veterinary Medicine, Košice. 29 pp.
- Enevoldsen C., Grohn Y. T., Thynsen I. (1995): Dairy cow characteristic related to *Staphylococcus aureus* isolation from quarter samples. J. Dairy Res., 62, 69–81.
- Grieger C., Pakánová M., Bednarčíková E. (1979): Problems of the occurrence of *Staphylococcus aureus* in food with regard to sheep milk and sheep milk products. Summaries of XXI. In: World Veterinary Congress, Moscow, Food hygiene and Veterinary Public Health. 19.
- Grieger C., Holec J., Burdová O., Krčál Z., Lukášová J., Matyáš Z., Pleva J. (1990): Hygiene of Milk and Milk Products (in Slovak). Příroda, Bratislava. 307–317.
- Heir E., Sundheim G., Holck A. (1998): The Staphylococcus gac H gene product: a new member of the SMR family encoding multidrug resistance. FEMS Microbiol. Lett., 163, 49–56.
- Hermans K., DeHerdt P., Devriese L.A., Hendrick X.W., Godard C., Haesebrouck F. (1999): Colonization of rabbits with *Staphylococcus aureus* in flocks with and without chronic staphylococcosis. Vet. Microbiol., 67, 37–46.
- Jensen A., Wachmann C.H., Poulsen K.B., Espersen F., Scheibel J., Skinhoj P., Fridmot-Møller N. (1999): Risk factors for hospital-acquired *Staphylococcus aureus* bacteremia. Arch. Int. Med., 159, 1437–1444.
- Kanjuka A.I., Šutiak V. (1990): About some problems of antibiotics use in veterinary practice. Veterinářství, 40, 206–207.
- Leski A.T., Olivera D., Trzcinski K., Santos Sanches I., Aires de Sousa M., Hryniewicz W., de Lencastre H. (1998): Clonal distribution of Methicillin-resistant *Staphylococcus* in Poland. J. Clin. Microbiol., 2532–3539.
- Moretti A., Pasquali P., Mencaroni G., Boncio L., Piergili Fioretti D. (1998): Relationship between cell counts in bovine milk and the presence of mastitis pathogens (yeasts and bacteria). J. Vet. Med. B., 45, 129–132.
- Nishijima S., Namura S., Nakagawa M. (1997): Sensitivity to antibacterials of *Staphylococcus aureus* isolated from different types of skin infections. J. Int. Med. Res., 25, 1–7.
- Osteras O., Edge V.L., Martin S.W. (1999): Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J. Dairy Sci., 82, 1221–1231.
- Park C.E., Akhtar M., Rayman N.K. (1994): Evaluation of a commercial enzyme immunoassay kit (RIDASCREEN) for detection of staphylococcal enterotoxins A, B, C, D and E in foods. Appl. Environ. Microbiol., 677–681.
- Paulsen I.T., Brown M.H., Dunstan S.J., Skurray R.A. (1995): Molecular characterisation of the staphylococcal multidrug export protein QacC. J. Bacteriol., 177, 2827–2833.
- Pozza M.C.H.D., Ricci A., Vicenzoni G. (1999): Protein A gene polymorphism analysis in *Staphylococcus aureus* strains isolated from bovine subclinical mastitis. J. Dairy Res., 66, 449–453.
- Rasooly A., Rasooly R.S. (1998): Detection and analysis of *Staphylococcal enterotoxin A* in food by Western immunoblotting. Int. J. Food Microbiol., 41, 205–212.
- Sol J., Sampimon O.C., Snoep J.J., Schukken Y.H. (1994): Factors associated with bacteriological cure after dry cow treatment of subclinical *Staphylococcal* mastitis. J. Dairy Sci., 77, 75–79.
- STN 56 0089 (ISO 6888) (1999): Slovak Technical Norm 56 0089 (ISO 6888). Microbiology. General instructions for determination of *Staphylococcus aureus* bacteria count. Method of counting colonies (in Slovak).
- Vernozy C., Mazvy, Lapeyre C., Chantegrelet G., Richard Y. (1994): Etude des souches des staphylocoques à coagulase négative (S.C.N.) isolés de fromages de chèvre en région Rhône – Alpes: identification, antibiotypie et entérotoxinogénicité. Rev. Méd. Vét., 145, 107–113.
- Wallace R.L., Queen W.G., Hoblet K.H., Hogan J.S. (1998): Evaluation of an acriflavine disk assay for differentiating *Staphylococcus aureus* from other staphylococci isolated from bovine milk. JAVMA, 213, 394–398.
- Yazdankhah S.P., Hellemann A., Ronningen K., Olsen E. (1998): Rapid and sensitive detection of *Staphylococcus species* in milk by ELISA based on monodisperse magnetic beads. Vet. Microbiol., 62, 17–26.
- Yazdankhah S.P., Solverod L., Simonsen S., Olsen E. (1999): Development and evaluation of an immunomagnetic separation – ELISA for the detection of *Staphylococcus aureus* thermostable nuclease in composite milk. Vet. Microbiol., 67, 113–125.

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