

The prevalence of *Staphylococcus aureus* in milk-based food products

Dr Krishan Bihari Verma,

ABSTRACT: For the presence of staphylococci, specifically *Staphylococcus aureus*, we have tested 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 strawberry-flavored yoghurt milk, and 50 samples of fresh butter (Rajo). We collected all of the samples for the microbiological investigation straight from the stores in the market. With levels of 100, 65, 5, 75, and 60 CFU/ml of *Staphylococcus aureus* bacteria, none of the yogurt milk samples tested met the requirements set forth by the Codex Alimentarius. All of the other food items that were tested for staphylococci met the existing requirements.

Keywords: bovines, ovines, their milk, staphylococci, and *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;

Bohačenko *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. aureus* 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 staphy-

lococci 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 – Stafylo-test (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 Vernozy *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” foods

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

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

Product	Examined 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

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

REFERENCES

Genotyping of *Staphylococcus aureus* isolated from bovine mastitis by Annemüller C., Lämmle Ch., and Zschock M. (1999). Published in *Vet. Microbiol.*, 69, 217–224. "Sheep milk as a potential source of staphylocotoxigenesis in humans" (in Slovak) was written by Beličková E. in 1999. [Completed Report.] Institution of Veterinary Medicine, Košice. 19 pages of research. In 1993, Beličková *et al.* suggested methods to decrease the microbial contamination of food (in Slovak). [Completed Report.] University of Veterinary Medicine Research Institute, Košice. 55 pages. In 1999, Beličková *et al.* conducted research on the ecology of major pathogens in imported raw materials and food (in Slovak). [Completed Report.] Veterinary Medicine Research Institute in Košice. Bhatti A.R. and Micusan V.V. (1999): Staphylococcal enterotoxins A and B specific anti-peptide monoclonal antibodies produced and characterized. The article is published in the *Journal of Microbiological Methods*, volume 35, pages 143 to 149. In 1999, Boháčenko, Erban, and Schwartz conducted an experiment at SETUZA, Ltd. that included integrating the Critical Points

System (HACCP) with ISO 9002 in the manufacturing technology of plant edible oils. The article may be found in the *Czech Journal of Food Science*, volume 7, pages 113. Pathogenic microorganisms in milk and milk products: situation in France and Europe (Brisabois A., Lafarge V., Brouillaud A., deBuyser M.L., Collette C., Garin-Bastuji B., Thorel M.F., 1999). (*Rev. Sci. Tech.*, Off., International Epiz., 16, 452–471). Official publication of the Slovak Republic's Ministry of Agriculture (1998): Vol. XXX, Section 21, Supplement No. 3. The logical nutrition perspective on milk and milk products (in Slovak) by Burdová, O. (1999). University of Veterinary Medicine, Košice, *Proc. Hygiene Alimentorum XX*, 45–47. Codex Alimentarius (1998): Slovak Republic's Ministry of Agriculture Bulletin, Vol. XXX, Section 21, Supplement No. 3–4 of the Second Part of the Food Codex (FC). Hazard Analysis and Critical Control Points in Eggs and Egg Products (Curtis A., 1998). In *Egg. Ind.*, 103, 8–12. The authors of the 1999 Slovak article "Staphylococci as contaminants of milk and milk products" are Udríková, Burdová, and

Pilipčinec. The proceedings of the XXth conference on food and water hygiene were held at the University of Veterinary Medicine in Košice, and the pages 69–70 were used. Research on the spread of enterotoxigenic strains of *Staphylococcus aureus* in animal products (in Slo-vak) conducted by Elečko J., Beličková E., and Vasič M. in 1998. [Report 05, Final] Veterinary Medicine Research Institute, Košice. 29 pages.

Characteristics of dairy cows associated with the isolation of *Staphylococcus aureus* from quarter samples, published in 1995 by Enevoldsen, Grohn, and Thynsen. Research in Dairy, 62, 69-81.

Concerns about the presence of *Staphylococcus aureus* in food pertaining to sheep's milk and products made from it were discussed in a 1979 publication by Grieger, Pakánová, and Bednarčíková. Annotated Abstracts of the XXI World Veterinary Congress, Moscow, Food Hygiene and Veterinary Public Health, 19.

The Slovak version of the article "Hygiene of Milk and Milk Products" was published in 1990 by Grieger et al. and authored by Holec, Burdová, Krčál, Lukášová, Matyáš, and Pleva. Bratislava, Príroda. 307-317.

A novel member of the SMR family encoding multidrug resistance: the *Staphylococcus* *gac* H gene product (1998) by Heir, Sundheim, and Holck. The cited work is FEMS Microbiol. Lett., 163, 49–56.

Studies on the colonization of *Staphylococcus aureus* in rabbits from flocks with and without chronic staphylococcosis were conducted by Hermans K., DeHerdt P., Devriese L.A., Hendrick X.W., Go- dard C., and Haesebrouck F. in 1999. Journal of Veterinary Microbiology, 67, 37–46.

In 1999, Jensen et al. published an article titled "Risk Factors for Hospital-Acquired *Staphylococcus aureus* Bacteremia" in the Arch. Int. Med. journal, which included the following pages: 1437–1444.

Šutiak V. and Kanjuka A.I. (1990) discussed many issues related to the use of antibiotics in veterinary medicine. (Veterinary Science, 40, 206–207).

The clonal distribution of methicillin-resistant

Staphylococcus in Poland was studied by Leski et al. (1998). With contributions from Olivera, Trzcinski, Santos Sanches, Aires de Sousa, Hryniewicz, and de Lencastre. The article may be found in the Journal of Clinical Microbiology, volume 25, pages 2532–3539. The presence of mastitis pathogens (yeasts and bacteria) is correlated with higher cell counts in bovine milk, according to a study by Moretti, Pasquali, Mencaroni, Boncio, and Piergili Fioretti (1998). Published in the Journal of Veterinary Medical Biology, volume 45, pages 129 to 132.

A study conducted by Nishijima, Namura, and Nakagawa (1997) examined the susceptibility of *Staphylococcus aureus* bacteria to antibacterials when isolated from various skin infections. Journal of International Medical Research, 25, 1–7.

Factors influencing the efficacy of selected dry cow treatment in curing severe mastitis in dairy cows, by Osteras, Edge, and Martin (1999). This article is published in the Journal of Dairy Science, volume 82, pages 1221–1231.

The RIDASCREEN commercial enzyme immunoassay kit was evaluated in 1994 by Park, Akhtar, and Rayman for the detection of staphylococcal enterotoxins A, B, C, D, and E in food. Applied Microbiology, 677-681. In 1995, Paulsen et al. characterized the staphylococcal multidrug export protein *QacC* molecularly. Brown, S.J., Skurray, and Paulsen, I.T. The citation is from the Journal of Bacteriology, volume 77, pages 2827 to 2833.

Protein A gene polymorphism investigation in *Staphylococcus aureus* strains derived from bovine subclinical mastitis(1999) by Pozza M.CH.D., Ricci A., and Vicenzoni G. The cited entry is "J. Dairy Res." with the pages 449–453.

The use of Western immunoblotting for the detection and analysis of Staphylococcal enterotoxin A in food was described by Rasooly (1998). Article published in the International Journal of Food Microbiology, volume 4, pages 205–212.

Journal Article: Factors linked to

bacteriological cure after dry cow treatment for subclinical Staphylococcal mastitis (Sol et al., 1994). Published in the Journal of Dairy Science, volume 77, pages 75 to 79.

This is Slovak Technical Norm 56 0089 (ISO 6888) from 1999. The study of microbes.

Protocol for the elimination of Staphylococcus aureus germs. Colony counting method (in Slovak).

In 1994, Vernozy et al. conducted an investigation on the presence of staphylococcal coagulase negative (S.C.N.) shells in cheese samples from the Rhone-Alpes region. The study aimed to identify the shells, determine their antibiotype, and determine their endotoxinogenicity. Med. Rev., 145, 107–113.

The acriflavine disk test was evaluated in 1998 by Wallace, Queen, Hoblet, and Hogan to distinguish Staphylococcus aureus from other staphylococci found in bovine milk. The sentence references JAVMA, 213, pages 394 to 398.

Fast and accurate identification of Staphylococcus species in milk using enzyme-linked immunosorbent assays based on monodisperse magnetic beads; Yazdankhah, Hellemann, Ronningen, and Olsen (1998). This sentence is paraphrased from Vet. Microbiol., 62, 17–26.

In 1999, Yazdankhah, Solverod, Simonsen, and Olsen developed and tested an immunomagnetic separation-ELISA to identify Staphylococcus aureus thermostable nuclease in composite milk. As a result, the study was published. The article may be found in Vet. Microbiol., volume 67, pages 113 to 125.

Arrival: 00–09–08. Approved following revisions: 00→12→11.

Author to Contact:

The contact information for MVDr. Eva Beličková, PhD. is as follows: Slovak Republic, 040 01 Košice, Military Veterinary Research Institute, Kukučínova 2, Tel., fax + 421 95 678 57 62, and hp@nexta.sk.