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Isolation of staphylococci from milk and cream sold at the Kars market and detection of their enterotoxigenicity

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Summary

The aim of the study was isolation of staphylococci from milk and cream sold at the Kars market and detection of their enterotoxigenicity and possible risk for public health. A total of 160 staphylococci from 40 milk samples and 52 staphylococci from 30 cream samples were isolated. 22 (13.75%) isolates from 160 milk isolates and 9 (17.3%) isolates from 52 cream isolates were determined using human plasma as coagulase-positive staphylococci. From all the milk isolates, 4 (2.5%) of them were resistant to methycilline and one of them was resistant to vancomycin. Similarly, from all the cream samples 6 (11.54%) of them were resistance to methycilline and one (1.92%) of them was resistant to vancomycin. A total of 60 staphylococci isolates were investigated for their enterotoxigenicity. From 38 milk isolates and 22 cream isolates, 6 isolates and only one isolate respectively were found to produce staphylococcal enterotoxin (SE). One staphylococci isolated from cream samples produced only SEA, one staphylococci produced SEA, SEB, SEC, and one staphylococci produced SEA, SEB, SEC and SEE isolated from milk samples. Two isolates produced SEE and one isolate produced SEA from milk samples.

In conclusion, investigated milk and cream samples are potentially the cause of staphylococcal food poisoning and can be dangerous for public health. For this reason, milk and cream should be supplied from healthy animals and milk and cream production, transporting this product to the market and keeping it their has to be done in a cold condition accordance to hygiene rules. At the same time milk producers and workers should be informed and educated of possible health risks.

Keywords: enterotoxin, staphylococci, CNS, CPS, milk, cream

Food-borne disease is of major concern worldwide. It has been reported that among the predominant bacteria involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. The foods that are often involved in staphylococcal food poisoning differ widely from one country to another. Among those foods 8% of the staphylococcal food poisonings reported between 1969 and 1990 were due to milk products in United Kingdom (19), milk products and especially cheeses were responsible for 32% of the cases in France in a two year period (1999-2000) (13). Coagulase-negative staphylococci (CNS) have been identified as significant pathogens in a variety of clinical situation and lately they have been started to identify as an important source of food borne disease along with coagulase-positive staphylococci (CPS) (4, 5, 7). Previously only CPS, namely *Staphylococcus aureus* have been associated with food poisoning because of their production of staphylococcal enterotoxins. However researches show that CNS can also produce enterotoxins (5, 6, 16). The aim of the study was isolation of CNS along with CPS from milk and cream samples and investigation of their enterotoxigenicity and possible risk for public health.

Material and methods

Sample collection. A total of 70 raw milk (40) and cream (30) samples was obtained from Kars market. One tenth of samples and 1 : 10 dilution in saline were surface-plated on Baird Parker (BP) agar (Oxoid, CM 0275), supplemented with egg yolk telluride emulsion (Oxoid, SR0054), incubated for 48 h at 37°C ± 1. After incubation five suspected colonies of staphylococci were sub cultured again BP agar and incubated for 48 h at 37°C ± 1 for identification and then they were sub cultured in Brain Heart Infusion Broth and incubated for 24 h at 37°C ± 1.

Identification of staphylococcal strains. The isolates were identified on the basis of cultural characteristics. Gram's stain reaction and the results of catalase, tube

coagulase test, using human and bovine plasma, were performed.

Tube coagulase test (human plasma) negative, catalase positive, gram positive coccal isolates were further analyzed to differentiate between coagulase negative staphylococci (CNS) and micrococcus isolates. For this purpose, glucose fermentation (GF), acid production from glycerol (GA) and response to furazolidone and bacitracine antibiotics were used. Glucose fermentation and GA was determined by the method described by Baker (3). Susceptibility to a 100 µg furazolidone disk (Oxoid, CT 0122B) and 10 units basitracine (Oxoid, CT 0005B) were determined using the standartised Bauer-Kirby disk diffusion method. Method was performed using Mueller Hinton Agar (Oxoid, CM0337) with 5% defibrinated sheep blood. Zone sizes at growth inhibition were measured in millimeters after 24 h of incubation at 36°C ± 1. Each organism was inoculated onto L of a Mueller Hinton plate so that total of four different organisms could be tested on one plate.

Susceptibility to antibiotics. Susceptibility to antibiotics was tested the disk diffusion method as described above with Mueller Hinton Agar. Methicilline (5 µg, Oxoid, CT 0029 B) and vancomycin (30 µg, Oxoid, CT 0058 B) antibiotic disks were used. The plates were incubated at 36°C ± 1 for 24 h.

Detection of Staphylococcal toxins. Staphylococcal enterotoxins were detected by the sandwich enzyme immunoassay test kit RIDA SCREEN SET A, B, C, D, E (R-Biophorm AG, D-64293, Germany). Test was performed by following the manufacturer's instructions.

Results and discussion

A total of 350 isolates, 200 isolates from milk and 150 isolates from cream samples, were examined. A 173 isolates from milk and 53 isolates from cream samples were identified as catalase positive. A 13 isolates from the milk samples and one isolate from the cream samples were suspected being micrococcus. The tube coagulase test was performed using freshly prepared human and bovine plasmas shown different results (tab. 1).

Antibacterial resistance. Resistance to two antimicrobial agents was detected in all CPS and CNS isolates. From all the milk isolates, four (2.5%) were resistance to methicillin, only one isolate (0.62%) to vancomycin. From all the cream isolates, 6 (11.54%) isolates were resistance to methicillin, one (1.92%) to vancomycin.

Toxin production. A total of 60 isolates, from 211 isolates, were investigated for their enterotoxigenicity. Results shown that 6 isolates from 38 milk isolates and only one isolate from 22 cream isolates produce staphylococcal enterotoxin (SE). Some characteristics of SE positive isolates and the type of toxin they produce were given in tab. 2.

Staphylococci were isolated from 39 milk samples out of 40 and from 11 cream samples out of 30. Alisarli et al. (2) found that only 88% raw milk contained staphylococci. Umoh et al. (17) also found that 58 of the 93 samples from nomadic herds and all the samples (43 samples) from settled herds contain staphylococci. In this study it was observed that more number of isolates, from bovine milk and cream, coagulated bovine plasma than human plasma. Adesiyun and Shebu (1) reported that bovine plasma was superior to human and rabbit plasma in detecting coagulase production by *S. aureus* strains of animal origin while human and rabbit plasma were better for strains from foods. In another study, from 84 staphylococci 70 of them found to be coagulate bovine plasma while only 50 of them coagulate human plasma (17). Enterotoxigenic staphylococci have been frequently isolated from milk, cream and cheese samples (12, 14, 18). In this study found that 6 isolates of the 38 milk isolates and only one isolate of the 22 cream isolates have an ability to produce staphylococcal enterotoxin. Only one isolate of the 6 enterotoxigenic staphylococci was given coagulase positive reaction with human plasma while 2 of them coagulated bovine plasma. Umoh et al. (17) were only detected enterotoxin production among *S. aureus* strains. Wieneke (19) found that 3 isolate of the 50 *S. aureus* isolates from milk samples produce SE. In another study done in Turkey, shown that two pasteurized milk samples were contain SEA from 250 pasteurized milk samples (12). Kayihura et al. (10) similarly isolated 3 SEA producing *S. aureus* from 99 pasteurized milk samples. Several staphylococcal species other than *S. aureus* reportedly produce SEs. For example, among the coagulase negative species, *S. cohnii*, *S. epidermis*, *S. xylosus* and *S. haemolyticus* have been isolated from

Tab. 1. Coagulase test results of staphylococcal isolates

Samples (source of plasma)	Number of Isolate	Number of CPS (%)	Number of CNS (%)
Milk (human plasma)	160	22 (13.75)	138 (86.25)
Milk (bovine plasma)	160	38 (23.75)	122 (76.25)
Cream (human plasma)	52	9 (17.30)	43 (82.70)
Cream (bovine plasma)	52	11 (21.16)	41 (78.84)

Tab. 2. Some characteristics of SE positive isolates and the type of toxin they produce

Isolate code	Coagulase human plasma	Coagulase bovine plasma	Hemolise	Enterotoxin produced				
				A	B	C	D	E
Milk 3c	+	+	β-hemolise	–	–	–	–	+
Milk 6e	–	–	Hemolise	+	–	–	–	–
Milk 7d	–	+	β-hemolise	+	+	+	–	–
Milk 10a	–	–	Hemolise	+	+	+	–	+
Milk 12a	–	–	Hemolise	–	–	–	–	+
Milk 32a	+	–	β-hemolise	–	–	+	–	–
Cream 6b	–	–	Hemolise	+	–	–	–	–

ewe's milk and found to produce one or several SEs (4). Enterotoxin E was similarly produced by 10 of 187 CNS isolated from goats milk, whey and cheese (18). Carmo et al. (6) also found that the presence of coagulase-negative staphylococci in counts exceeding 2.0×10^8 cfu g^{-1} and the production of enterotoxins SEC and SED in analysis of the raw milk. In our study 5 of the 7 isolate were CNS and they produce one or more SEs. Staphylococcal enterotoxin producing staphylococci were also isolated frequently from milk products (9, 11, 15). Research show that the most frequently detected enterotoxin are SEA from food poisoning cases (8) and in our study also 4 of the 7 enterotoxigenic staphylococci produce SEA.

In conclusion, the key factors in the manufacture of good quality dairy food are starting with top quality raw milk and cream materials. For this; cows and teat of cows must be healthy, milking has to be done hygienically, milk has to be carried and stored in cold places and risk assessment program must be performed. Staphylococci can indeed be easily eliminated from foodstuffs by heat treatment or by competition with other flora whereas SE resists most of the treatments used during food processing. Nevertheless, SE production rather than *S. aureus* itself should be taken into account in risk assessment. Staphylococcal species other than *S. aureus* are also investigated in routine tests.

References

1. *Adesiyun A. A., Shebu L. M.*: Detection of staphylocoagulase using plasmas from various animals. Vet. Microbiol. 1985, 10, 387-392.
2. *Alisarli M., Sancak Y. C., Akkaya L., Elibol C.*: Investigation of Staphylococcus aureus isolation and thermonuclease activity and enterotoxin formation in some dairy desserts. Turk. J. Vet. Anim. Sci. 2003, 27, 1457-1462.
3. *Baker J. S.*: Comparison of various methods for differentiation of staphylococci and micrococci. J. Clin. Microbiol. 1984, 19, 875-879.
4. *Bautista L., Gaya P., Medina M., Nune M.*: A quantitative study of enterotoxin production by sheep milk staphylococci. Appl. Environ. Microbiol. 1988, 54, 566-569.
5. *Breckinridge J. C., Bergdoll M. S.*: Outbreak of foodborne gastroenteritis due to a coagulasnegative enterotoxin-producing staphylococcus. New Engl. J. Med. 1971, 284, 541-543.
6. *Carmo L. S., Dias R. S., Linardi V. R., Sena M. J., Santos D. A., Faria M. E., Pena E. C., Jett M., Heneine L. G.*: Food poisoning due to enterotoxigenic strains of staphylococcus present in Minas cheese and rawmilk in Brazil. Food Microbiol. 2002, 19, 9-14.
7. *Crass B. A., Bergdoll M. S.*: Involvement of coagulase-negative Staphylococci in toxic shock syndrome. J. Clin. Microbiol. 1986, 23, 43-45.
8. *Evenson M. L., Hinds M. W., Bernstein R. S., Bergdoll M. S.*: Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. Int. J. Food Microbiol. 1988, 7, 311-316.
9. *Hayes P. R.*: Food Microbiolgy and Hygiene. Chapman-Hall, London 1995.
10. *Kayihura M., Kaburia H. F. A., Arimi S. M., Lindguist K. J.*: Staphylococcal enterotoxin „A" in raw and pasteurized milk. East. Afr. Med. J. 1987, 64, 177--182.
11. *Kısa Ö., Albay A., Erol İ., Sırıken B., Esin N., Gün H., Yurtyeri A.*: Kremalı pastalardan izole edilen koagulaz pozitif stafilokokların enterotoksin oluşturma özelliklerinin VIDAS yöntemiyle belirlenmesi. Ankara Üniv. Vet. Fak. Derg. 1996, 43 405-411.
12. *Küplülü Ö., Sarımehmetoğlu B., Kaymaz, Ş.*: Pastörize sütlerde ELISA tekniği ile stafilokokkal enterotoksin varlığının belirlenmesi. Turk J. Vet. Anim. Sci. 2002, 26, 631-637.
13. *Le Loir Y., Baron F., Gatier M.*: Staphylococcus aureus and food poisoning. Genet. Molecular Res. 2003, 2, 63-76.
14. *Müller M.*: Untersuchungen zum Vorkommen von Staphylococcus aureus in Rohmilch und Weichkäse and zur Bildung von Staphylokokken-Enterotoxinen mit immuchemisschen und molekular biologischen Methoden. Diss. Vet. Med., Freie Univ. Berlin 1996.
15. *Sumner S., Albrecht A. J., Peters D. L.*: Occurence of enteroxigenic strains of Staphylococcus aureus and enterotoxin production in bakery products. J. Food Prot. 1993, 56, 722-724.
16. *Udo E. E., Al-Bustan M. A., Jacob L. E., Chugh T. D.*: Enterotoxin production by coagulase-negative staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisining. J. Med. Microbiol. 1999, 48, 819-823.
17. *Umoh V. J. V., Adesiyun A. A., Comwolk N. E.*: Enterotoxigenicity of staphylococci isolated from raw milk obtained from settled and nomadic herds around Zaria, Nigeria. Revue Méd. Vét. 1984, 43, 43-47.
18. *Vernozy-Rozand C., Mazuy C., Prevost G.*: Enterotoxin production by coagulase-negative Staphylococci Isolated from goats milk and cheese. Int. J. Food Microbiol. 1996, 30, 271-280.
19. *Wieneke A. A., Roberts D., Gilbert R. J.*: Staphylococcal food poisoning in the United Kingdom 1969-1990. Epidemiol. Infect. 1993, 110, 519-531.

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