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Short Communication

Occurrence of *Staphylococcus aureus* in Milk Based Sweet Products Consumed in Karachi, Pakistan

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Abstract. A total of 290 samples of milk-based products of Khoya (75), Qalaqand (75), Rabri (65) and Rusmalai (75) were tested for occurrence of *Staphylococcus aureus*. The total staphylococcal counts ranged from 1.0×10^3 to 5.7×10^4 cfu/g, 1.0×10^2 to 6.0×10^3 cfu/g, 1.0×10^3 to 3.1×10^4 cfu/g and 1.3×10^3 to 4.0×10^3 cfu/g in Khoya, Qalaqand, Rabri and Rusmalai samples, respectively. All the samples tested were found contaminated with staphylococci, however, the percentage of *S. aureus* was low as compared to coagulase -ve staphylococci. Only 6.7% of Rusmalai samples (only 1.72% of all the samples tested) were found free of coagulase +ve *S. aureus*. About 8% of Khoya (6 out of 75), 28% of Qalaqand (21out of 75), and 40% of Rabri (26 out of 65) samples were found to be hazardous for human health as they contained coagulase +ve *S. aureus* whereas, none of the Rusmalai samples was found contaminated with coagulase +ve *S. aureus*.

Keywords: *Staphylococcus aureus,* Staphylococci, khoya, qalaqand, rabri, rusmalai

Foods at highest risk of contamination with *S. aureus* and subsequent toxin production are those that are made by hand and require no cooking. Milk and dairy products owing to their nutritional properties, provide good medium for the growth of microorganisms hence are highly liable to contamination (O'Mahony, 1979). Rabri, Khoya, Qalaqand and Rusmalai are traditional dairy products manufactured from raw milk that are produced and widely consumed in many Asian countries, and wherever people from the subcontinent are residing. These products are produced in bakeries and other sweet meat shops where most of the time hygiene and safety of the products are not given due consideration.

The present study was carried out to determine the occurrence of staphylococci especially *S. aureus* in samples of Khoya, Qalaqand, Rabri, and Rusmalai, the type of sweets often implicated for staphylococcal food poisoning (Wilson and Salyers, 2002) as a lot of handling is involved in their preparation. Staphylococcal toxins are resistant to heat and cannot be destroyed by cooking (Cimolai, 2008). The pathogenicity of *Staphylococcus* is also associated with coagulase positivity (Matthews *et al.,* 1997). Food contamination with Staphylococci has been reported by de Luca *et al*. (1997); while permissible limits of coliform and *S. aureus* in milk and milk products have been reported by Jayarao *et al.* (2004) and de Louvois and Rampling (1998).

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Samples of Khoya (n=75), Qalaqand (n=75), Rabri (n=65) and Rusmalai, (n=75), were randomly purchased from the markets of different localities of Karachi, Pakistan and were transported to the laboratory in an ice box with ice packs within 2 h of purchase.

Sample preparation. The sample preparation was carried out according to the method described by Bacteriological Analytical Manual Online US FDA (2010). 50 g of sample was weighed and homogenized in 450 mL peptone water. This was labeled as 1:10 dilution which is also the stock or homogenate. This was further serially diluted to $1:10^5$. A volume of 0.3, 0.3 and 0.4 mL sample was surface plated on Baird-Parker agar containing egg-yolk tellurite emulsion (Oxoid) and then incubated at 37 °C for 24 h to enumerate Staphylococci and *S. aureus.* Plates containing 30-300 black colonies were selected and counted. The average was taken and the number obtained was multiplied by four and then by the inverse of the dilution factor. This gave the number of colony forming units per gram of a sample (cfu/g).

Identification of coagulase positive and coagulase negative staphylococci. Colonies with typical *S. aureus* morphology were examined microscopically, gram stained, tested for catalase reaction and inoculated on Mannitol salt agar and incubated at 35 °C for 24 h. Following incubation, Mannitol fermenting organisms which showed a yellow zone surrounding their growth were isolated onto agar slants for biochemical tests and confirmed with an agglutination Staphytect latex agglutination (Oxoid).

Coagulase test. Slide coagulase tests of all isolates were performed by emulsifying few pure colonies of staphylococci from agar on undiluted plasma. Tube coagulase test was performed by diluting the plasma in freshly prepared normal saline (1:6). Three to four pure colonies were emulsified in 1 mL of diluted plasma and tubes were incubated at 37 °C. Readings were taken at the intervals of 1 h, 2h, 3h, 4h ands 24 h at room temperature and clot formation was observed. *S. aureus* ATCC# 29213 was used as control strain.

Figure 1 presents the results on the microbiological quality of milk based sweet products in terms of counts of Staphylococci (both coagulase -ve and +ve) in them. The results show that only 5 out of 75 (about 6.7%) of Rusmalai samples were found free of coagulase +ve *S. aureus*. All the samples of Khoya, Qalaqand, and Rabri were found contaminated with staphylococci including coagulase +ve *S. aureus;* however, the percentage of coagulase +ve *S. aureus* was low as compared to coagulase -ve staphylococci.

According to the Public Health Laboratory Standards (Gilbert *et al.,* 2000) foods can be divided into four categories i.e. satisfactory, acceptable, unsatisfactory, and hazardous on the basis of their staphylococcal counts (Table 1). All the sweet samples tested in the present study were found contaminated with staphylococci except for five Rusmalai samples. The staphylococcal counts ranged from 1.0×10^3 to 5.7×10^4 cfu/g in Khoya samples, from 1.0×10^2 to 6.0×10^3 cfu/g in Qalaqand samples, from 1.0×10^3 to 3.1×10^4 cfu/g in Rabri samples, and from 1.3×10^3 to 4.0×10^3 cfu/g in Rusmalai samples, though the percentage of *S. aureus* was low as compared to total staphylococci (Fig. 2). The counts in Khoya (75 samples) ranged from 1.0×10^3 to 5.7×10^4 cfu/g. Figure 2 reveals the results on the occurrence of coagulase +ve and/or coagulase –ve *S. aureus* in milk based sweet products. About 8% of Khoya (6 out of 75), 28% of Qalaqand (21out of 75), and 40% of Rabri (26 out of 65) samples were found to be hazardous for human health as they contained coagulase + ve *S. aureus* in them.

The study showed that *S. aureus* was present in 24.5% samples with counts ranging from 3×10^2 to 4×10^4cfu/g. This is a low percentage as compared to total staphylococci, also lower than the data obtained in other studies carried

Table 1. Guidelines for quality of dairy products for *Staphylococcus aureus* according to Public Health Laboratory Standards (Gilbert *et al.,* 2000)

Satisfactory	Acceptable	Unsatisfactory	Unacceptable/ potentially hazardous
< 20 cfu/g	20 - <100 cfu/g	100 - $<10^4$ cfu/g	$>10^4$ cfu/g

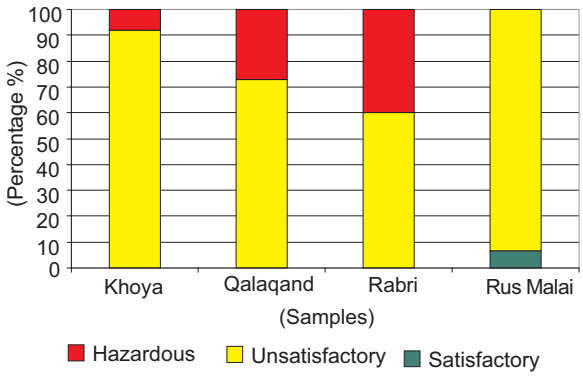

Fig. 1. Cumulative bar diagram showing the microbiological quality of sweet product samples according to PLHS, gILBERT ET AL., 2000 guidelines.

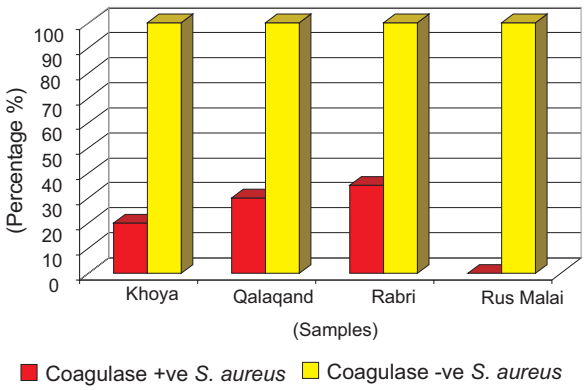

Fig. 2. Cumulative diagram showing the comparison of *S. aureus* and staphylococci occurrence in sweet product samples.

out in countries other than Pakistan (Bendahou *et al.,* 2009; Bennett and Lancett, 1998; de Luca, 1997; Gilmour and Harvey, 1990).

The results of the present study suggest that these sweets may not be completely safe for the health of consumers and at times may pose threats for community health. The alarming levels of coagulase +ve *S. aureus* in milk based sweet products warrant for some strict food safety regulations to be imposed on manufacturers of such products.

Strict sanitary measures must be imposed on the manufacturers of such products in order to prevent the public from some serious health hazard.

References

Bendahou, A., Abid, M., Bouteldoun, N., Catelejine, D., Lebbadi, M. 2009. Enterotoxigenic coagulase positive *Staphylococcus* in milk and milk products, lben and jben, in northern Morocco. *Journal of Infection in Developing Countries,* **3:** 169-176.

Bennett, R.W., Lancette, G.A. 1998. *Staphylococcus aureus*. In: *Bacteriological Analytical Manual.* 8th edition, (chapter 12),543 pp.

Cimolai, N. 2008. MRSA and the environment: implications for comprehensive control measures. *European Journal of Clinical Microbiology & Infectious Diseases,* **27:** 481-493.

de Luca, G., Zanetti, F., Stampi, S. 1997. *Staphylococcus aureus* in dairy products in the Bologna area. *International Journal of Food Microbiology,* **35:** 267-270.

de Louvois, J., Rampling, A. 1998. One fifth of samples of unpasteurised milk are contaminated with bacteria. *British Medical Journal,* **316:** 625-627.

Gilbert, R.J., de Louvois, J., Donoven, T., Little, C., Nye, K., Ribeiro, C.D., Richards, J. 2000. Guidelines for the microbiological quality of some ready to eat foods sampled at point of sale. *Communicable Diseases and Public Health,* **3:** 163-167.

Gilmour, A., Harvey, J. 1990. Staphylococci in milk and milk products. *Journal of Applied Bacteriology, (*Symposium Supplement), **19:** 147-168.

Jayarao, B.M., Pillai, S.R., Sawant, A.A., Wolfgang, D.R., Hegde, N.V. 2004. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. *Journal of Dairy Science,* **87:** 3561-3573.

Makovec, J.A., Ruegg, P.L. 2003. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. *Journal of Dairy Science,* **86:** 3466-3472.

Matthews, K.R., Roberson, J., Gillespie, B.E., Luther, D.A., Oliver, S.P. 1997. Identification and differentiation of coagulase-negative *staphylococcus aureus* by polymerase chain reaction. *Journal of Food Protection,* **60:** 686-688.

O'Mahony, M. 1979. Psychophysical aspects of sensory analysis of dairy products: A critique. *Journal of Dairy Science,* **62:** 1954- 1962.

US-FDA. 2010. In: *FDA Bacteriological Analytical Manual,* (online). Chapter 1, 202 pp., US-Food and Drug Administration, USA.

Wilson, B.A., Salyers, A.A. 2002. *Bacterial Pathogenesis: A Molecular Approach,* 2nd edition,