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Bacteriological Study of Khoa Sold in Gwalior and Morena City (Madhya Pradesh) in Relation to Public Health

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Abstract : A study was conducted to determine bacterial contaminants/pathogens in Khoa samples sold in Gwalior and Morena city in Madhya Pradesh. Total Fifty samples of Khoa brought at random were cultured on several media. Bacterial colony counts were also made. Predominant organisms isolated were *Staphylococcus* and *Streptococcus* species. The viable counts obtained ranged from 1.3×10^4 to 2.1×10^6 CFU/g. Contamination of Khoa by pathogenic bacteria could be an important factor of gastrointestinal illness in the consumers. Adequate consumer protection can be achieved by measuring the microbiological data of product.

Key words : Khoa, Bacteriological study, Immunocompromised, *Staphylococci*

Introduction :

Khoa or partially desiccated milk is a traditional Indian milk product. It is used in preparation of variety of sweets, vegetable curry etc. In India, Khoa is prepared by condensing milk by regular heating to remove water. During preparation of khoa temperature of milk is raised enough to destruct most of the vegetative cells of bacteria. The keeping quality of the product is adversely affected by thermoduric organisms and organisms acquired during storage. The product is manufactured by traditional method without any regard to quality of raw material used and hygienic storage. Under these conditions, microorganisms find access to product and contaminate it. Khoa is perishable food product and has short shelf-life. Its high nutritional value and high water activity (0.96) is conducive for the growth of bacteria (Sawhney *et al*., 1994). Microbial content of heat dried dairy product depends on the temperature and time of preheating evaporation process, contamination and growth during storage. Psychrotrophic bacteria also affect keeping quality and flavor of heat-treated product, some psychrotrophs produce heat tolerant enzymes and cause spoilage, both before and after heating. Microbes can produce undesirable effects like change in odor, color, taste, and texture of food. Beside this, contamination of products with pathogenic bacteria can result into outbreaks of gastrointestinal infection this pose great threat to consumers. Several studies carried out in different part of India evidenced that pathogenic organism as *Staphylococcus aureus*, *Bacillus cereus,* often contaminate Khoa (Gilmour and Marvey, 1990; Gill *et al.*, 1994; Mandokhot and Garg, 1986). The most

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important source of contamination of Khoa is probably the human. The contaminants can reach the products either during cooling or handling after cooking. Keeping in view the above facts, the present study is designed to examine the bacterial load of Khoa sold at Gwalior and Morena city.

Materials and Methods :

In the present study total 50 samples of khoa were collected in pre-sterilized containers from different shops of local market of Gwalior and Morena city and then these samples were transported in ice bucket to Department of Microbiology at College of Life Sciences, Cancer Hospital and Research Institute, Gwalior. Each sample of khoa was processed as follows under sterile condition: 1 g of khoa is mixed with 10 ml of physiological saline and homogenize with the aid of mortar pestle. Characterization of isolated strains was carried out using standard method (Cheesbrough, 1984; Halt *et al*., 1994). Inoculation of processed sample was carried out immediately on nutrient agar, MacConkey agar (HiMedia), Mannitol salt agar (HiMedia), DNase agar (HiMedia), Blood agar (HiMedia), Esculin azide broth (HiMedia). After incubation at 37^0 C ± 1^o C for 24 hrs the identification of the colonies grown was made by putting up various biochemical and pathogenecity test.

Samples were also subjected to methylene blue reduction test and SPC. To assess the bacterial colony count, dilutions of homogenized samples were made in normal saline and then inoculation was made on plate count agar. Plates were incubated at 37^0C ± 1^0C for 24 hrs. The colony forming units per gram (CFU/g) of the original samples were obtained by multiplying the counts obtained with dilution factor.

Results :

In the present study total 50 samples of khoa were examined to determine bacterial load. All the 50 samples studied have bacteriological count ranging from 1.3×10^4 to 2.1×10^6 CFU/g (Table 1). Results of Methylene blue reduction test indicates 60% samples are of poor quality and 40% samples are of fair quality.

After 24 hours of incubation, different types of colonies develop over nutrient agar. These samples were identified using standard techniques according to standard manual (Cheesbrough, 1984 and Halt *et al*., 1994). Out of 50 samples (Fig.3), 47 were showing presence of *Staphylococcu*s species; 34 were found to be *Streptococci*; 04 were found to be *Proteus*; 04 are showing presence of *Lactobacilli*; 03 showed presence of *Serretia*; *Pseudomonas* sp. was present in 03 while *Enterobacter* and *Klebsiella* were present in one sample only (Table 2).

Discussion :

Food products serve not only as source of nutrition but also as substrates for the growth of microorganisms. The growth of microorganisms causes food spoilage. It may result in food-borne illness. In tropical countries raw milk and its various products are responsible for many outbreaks of gastrointestinal tract. It is also reported that immuno-compromised individuals are prone to

Table 1 : Bacteriological study of Khoa

Data mean of three separate determinations

S. No.	Code No. of Sample*	City	Viable count/g	Gram staining reaction				Bacterial Isolates
				Gram positive		Gram negative		
				Rods	Cocci	Rods	Cocci	
1	G1	Gwalior	03.5×10^4	-	+	-	-	A, B
2	S1	Gwalior	07.8×10^4	-	+	-	-	A
3	G2	Gwalior	10.5×10^4	-	+	-	-	A, B
4	S2	Gwalior	8.3×10^4	-	+	+	-	A,B, D, G,H
5	R1	Gwalior	6.8×10^4	-	+	+	-	A,B, D, E,G
6	C1	Gwalior	5.9×10^4	-	+	-	-	A, B
7	R2	Gwalior	4.7×10^4	-	+	-	-	A, B
8	R3	Gwalior	1.1×10^5	-	+	-	-	A, B
9	C2	Gwalior	4.5×10^4	-	+	-	-	A, B
10	G3	Gwalior	7.2×10^4	-	+	+	-	A,B, D,G, H
11	G4	Gwalior	9.1×10^4	-	+	+	-	D,F , H
12	K1	Gwalior	6.0×10^4	-	+	-	-	A, B
13	K2	Gwalior	7.1×10^4	-	+	-	-	A, B
14	M1	Gwalior	5.2×10^4	-	+	-	-	A, B
15	M2	Gwalior	5.8×10^4	-	+	-	-	A, B
16	N1	Gwalior	8.0×10^4	+	+	-	-	A, B ,C
17	N2	Gwalior	7.0×10^4	+	+	-	-	A, B, C
18	C3	Gwalior	8.8×10^4	+	+	-	-	A, B, C
19	C4	Gwalior	9.0×10^4	-	+	-	-	A, B
20	C5	Gwalior	6.5×10^4	+	+	-	-	A, B, C
21	K3	Morena	8.0×10^4	-	+	-	-	A
22	M3	Morena	1.9×10^4	-	+	-	-	A
23	J1	Morena	0	-	-	-	-	-
24	J2	Morena	8.3×10^4	-	+	-	-	A, B
25	A1	Morena	7×10^4	-	+	-	-	A, B
26	L1	Morena	1.35×10^5	-	+	-	-	A, B
27	L2	Morena	3.0×10^4	-	+	-	-	A, B
28	J3	Morena	2.0×10^4	-	+	-	-	A, B
29	R4	Morena	4.0×10^4	-	+	-	-	A, B
30	K3	Morena	0	-	-	-	-	-
31	R5	Morena	8.0×10^4	-	+	-	-	A
32	S3	Morena	6.5×10^4	-	+	-	-	A
33	J3	Morena	2.1×10^5	-	+	-	-	A
34	G5	Morena	3.8×10^4	-	+	-	-	A, B
35	L3	Morena	3.1×10^4	-	+	-	-	A, B
36	A2	Morena	2.3×10^4	-	+	-	-	A, B
37	C6	Morena	1.3×10^4	-	+	-	-	A, B
38	S4	Morena	9.0×10^4	-	+	-	-	A, B
39	L3	Morena	1.4×10^4	-	+	-	-	A, B
40	B1	Morena	3.0×10^4	-	+	-	-	A, B

41	A3	Morena	12.2×10^4	-	+	-	-	A
42	B2	Morena	6.0×10^4	-	+	-	-	A, B
43	K1	Morena	7.2×10^4	-	+	-	-	A, B
44	B3	Morena	4.0×10^4	-	+	-	-	A
45	G6	Morena	1.8×10^4	-	+	-	-	A
46	S5	Morena	4.4×10^4	-	+	-	-	A
47	G7	Morena	4.1×10^4	-	+	-	-	A, B
48	G8	Morena	7.1×10^4	-	+	-	-	A
49	B4	Morena	6.2×10^4	-	+	-	-	A
50	G9	Morena	6.5×10^4	-	+	-	-	A

Data mean of three separate determinations
Sample* = Code no of Khoya sample, + = Present, - = Absent,
A = *S*taphylococcus sp., B = *Streptococcus* sp ,
C = *Lactobacillus* sp, D = *Proteus* sp., F = *Klebsiella* sp.,
E = *Enterobacte*r sp., G = *Serretia* sp. H = *Pseudomonas* sp.

Table 2 : Bacterial isolates in Khoa samples

S. No.	Name of Bacteria	No. of samples with the Bacteria
1	*Staphylococcus* sp.	47
2	*Streptococcus* sp.	34
3	*Lactobacillus* sp.	04
4	*Proteus* sp.	04
5	*Pseudomonas* sp.	03
6	*Serretia* sp.	03
7	*Enterobacte*r sp.	01
8	*Klebsiella sp.*	01

food-borne infection (Altekruse *et al.*, 1994). It is reported that food borne infection in individuals with HIV + can be life threatening. In this study high degree of bacterial contamination is found. Bacterial colony count assesses the number of viable bacteria in khoa; positive MBRT also confirms the degree of contamination. Misra and Kulia (1988) reported coliforms count from 10 CFU/g to 1.0×10^2 CFU/g in sandesh sweet prepared from khoa. Reddy *et al.*, (1983) reported SPC of 5×10^3 CFU/g to 2.1×10^5 CFU/g in khoa from Hissar market. Sen and Rajorhia (1989) reported that product sandesh showed count between 3×10^5 CFU/g to 7.5×10^7 CFU/g.

Staphylococcus species was the prominent organism isolated from majority of samples. The literature studied in present study evidenced that *Staphylococcus* species is frequently occurring organism in sweet-based milk products such as Khoa, rabri, gulabjamun, etc (Grewal and Tiwari, 1990; Hamama and Tatini, 1991). Cows excrete *Staphylococcus* from their udder but *Staphylococcus* is heat sensitive and its presence indicates that it is acquired from hands of khoa-sellers. It is reported in case of cheese, contamination comes from hands of sellers. Strains of *Staphylococcus* can cause gastroenteritis via production of heat stable enterotoxin

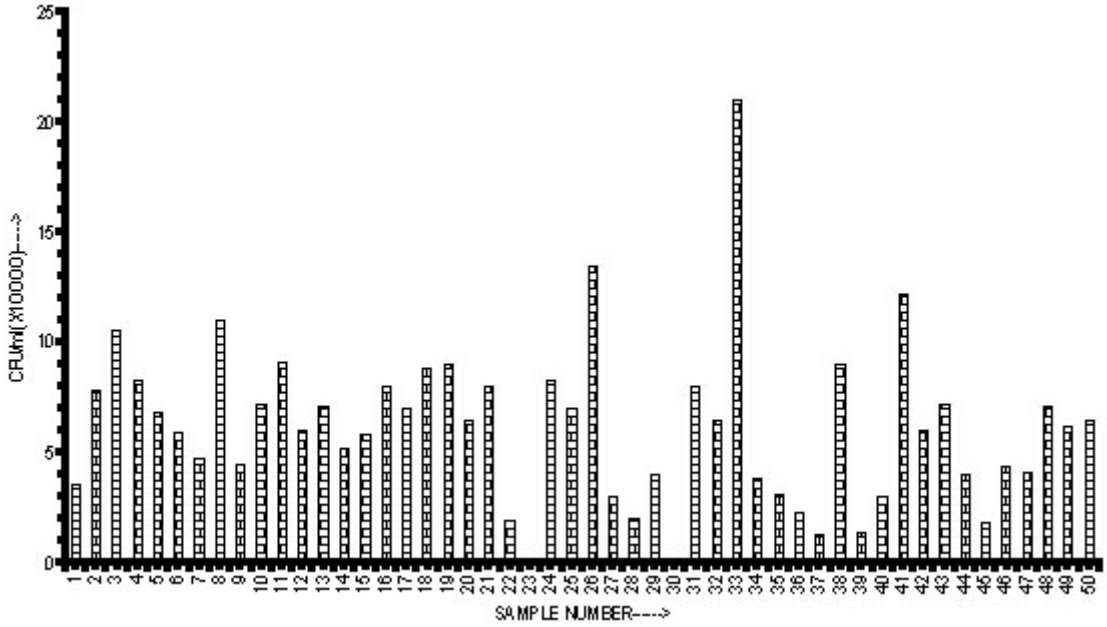

Fig. 1 : Bacterial colony count of k*hoa s*ample

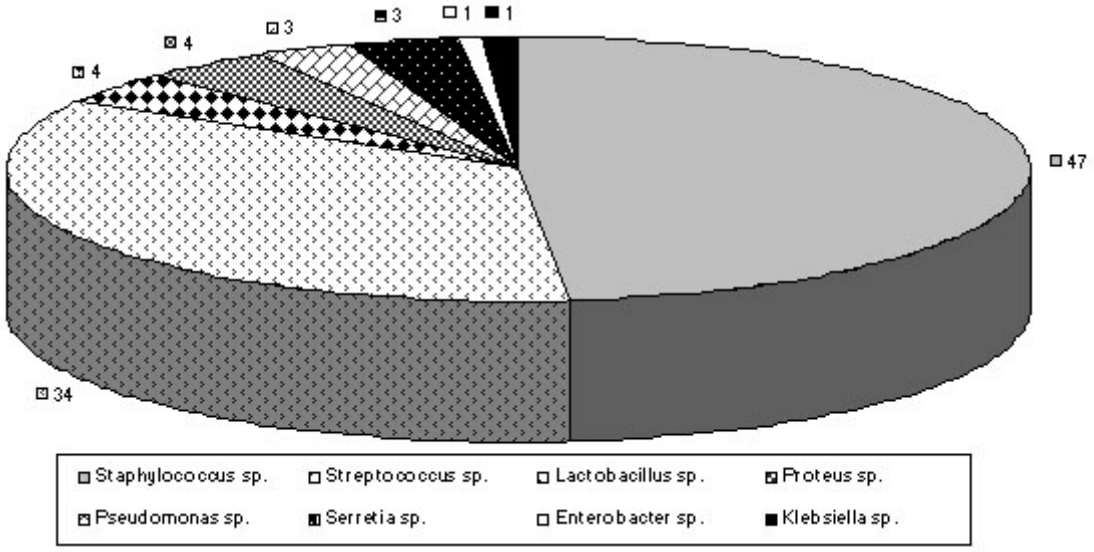

Fig. 2 : Total number of bacteria found in studied samples.

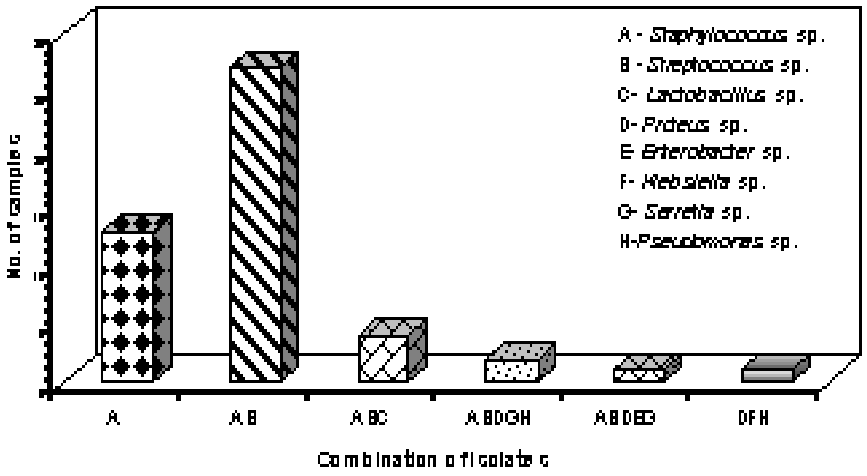

Fig. 3 : Simultaneous isolation of different bacteria in analyzed samples

(Payne and Wood, 1974). Millions of peoples are victims of food-borne illness resulting from ingestion of toxin produced by food associated *Staphylococcus.*

Streptococcus species was also present in 68% of samples. It is a thermoduric organism; its presence indicates that it may survive after heating process. In the present study *Pseudomonas, Proteus, Serretia*, *Enterobacter* sp., *Klebsiella* sp. were found in khoa samples. All these organisms are potential pathogens and fewer than hundred microorganisms can cause disease (Kumar and Sinha, 1989). *Streptococci* and *Lactobacilli* have fermentative metabolism; they can cause souring of khoa. *Pseudomonas* is proteolytic and lipolytic, it can also change the colour of khoa.

Haq *et al* reported the presence of fecal and non-fecal contaminants in raw milk sample of Khoa. *E. coli* is frequently occurring organism in milk products like Mawa/Khoa, dahi, cheese etc (Haq *et al.*, 1995). Soomro *et al* reported high percentage of Khoa contamination by *E. coli (*Soomoro *et al.*, 2002*)*. It is ubiquitous organism; its pathogenic strains could be hazardous to consumers (Hahn, 1996). However, *E. coli* species was not isolated in studied samples.

Bacteria are not homogeneously distributed in Khoa; number of different factors makes it difficult to enumerate population present in a given lot. In addition, physical attributes of food matrix may make difficult detection of bacteria and processing steps like heating; dehydration also affect the degree of bacterial growth and development. Even with statistially significant sampling technique, it is difficult to analyze whether a whole lot is pathogen free or contaminated.

Results of present study (Fig.1 and 2) revealed that the problem of Khoa contaminations exists in Gwalior city as heavy bacterial contamination is found in all samples. The unhygienic condition of preparation of these foodstuffs and water used for washing of utensils has enhanced the bacterial contamination of milk and milk products (Johnson, 1961). The sample contamination can be attributed to the practice of preparing large bulk of product in advance prior to the requirement and storage of the product at room temperature for long duration (Vaishnavi *et al.*, 2001). Keeping in view the public health importance of consumer, more hygienic preventive measures are needed to reduce bacterial load, so as to increase quality of product.

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References :

Altekruse S., Hyman F., Klontz K., Timbo B. and Tollefson L. (1994) : Food borne bacterial infections in individuals with the human immunodeficiency virus. *South Med J.* **87,** 69-73.

Cheesbrough M. (1984) : Medical Laboratory Manual for Tropical Countries, Vol II : Microbiology, Butterworth and Co. (Publishers) Ltd.

Gill J.P.S., Joshi D.V. and Kwatra M.S. (1994) : Qualitative bacteriological survey of milk and milk products with special reference to *Staphylococcus aureus*. *Indian J. Dairy Sci.* **47,** 680.

Gilmour A. and Marvey J. (1990) : Staphylococci in milk and milk products. *Symp. Series, Soc. for Applied Bacteriology* 1475-1665.

Grewal J.S. and Tiwari I.R. (1990) : Microbiological quality of rasmalai. *J Food Sci. Technol* **27,** 178-179.

Hahn G. (1996) : Pathogenic bacteria in raw milk-situation and significance In: Bacteriological quality of raw milk. *Intl. Dairy Federation, Brussels (Belgium)* 67-83

Halt J.G., Crieg N.R., Smith P.H.A., Staley J.T. and Williams S.T. (1994) : Bergey's Manual of Determinative Bacteriology (IXth Ed), Williams and Wilkins Publishers.

Hamama A. and Tatini S.R. (1991) : Enterotoxigenicity of *Staphylococcus aureus* isolated from Moroccan raw milk and traditional dairy products. *Microbiology Aliments and Nutrition* **9,** 263-267.

Haq I., Mahmod F., Qadeer M.A., Mukhtar H. and Iqbal (1995) : A microbial examination of khoa. *Biologia* **41,** 127-132.

Johnson J.E. (1961) : Studies on the pathogenesis of *Staphylococcal* infection. *J. Exp Med* **113,** 249-259.

Kumar V. and Sinha R.N. (1989) : Incidence of coliforms in indigenous milk products. *Ind J Dairy Sci.* **42,** 579-580.

Mandokhot V.V. and Garg S.R. (1986): Market quality of Khoa Burfi and Pera: A critical review. *J Food Sci. Technol.* **22,** 299.

Misra K.A. and Kulia K.R. (1988) : Microbiological quality of Burfi and Sandesh. *Asian Journal of Dairy Research* **7(1),** 51-55.

Payne D.N. and Wood J.M. (1974) : The incidence of enterotoxin production in strains of *Staphylococcus aureus* isolated from food. *J Applied Bacteriol.* 319-325.

Reddy G.R., Reddy K.S., Mandokhot U.V. and Chandiramani N.K. (1983) : Survey and growth of microflora in khoa at different storage conditions In: *Proceedings of the*

Symposia on Biology of Virus Plasmids and Biotechnology; Microorganisms in Agriculture and Ecology, 24th Annual conference of Association of Microbiologist of India, Hyderabad Nov 13.

Sawhney I.K., Patil G.R., Kumar B. and Rao K.J. (1994) : Evaluation of glycerol as a water activity modifier in Khoa. *J Food Sci Technol* **31,** 252.

Sen C.D. and Rajorhia S.G. (1989) : Quality characterstics of sandesh marketed in Calcutta. *Ind. J. of Dairy Sci*. **42(2),** 241-24.

Soomoro A.H., Arain M.A., Khaskheli M. and Bhutto B. (2002) : Isolation of E. coli from raw milk and milk products in relation to public health sold under market conditions at Tandojam. *Pak J of Nutrition* **1,** 151-152.

Vaishnavi C., Singh S., Grover R. and Singh K. (2001) : Bacteriological study of Indian cheese (paneer) sold in Chandigarh. *Ind. J. of Med. Microbi*. **19(4),** 224-226.