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Short Communication

Microbiological examination of milk in Tarakeswar, India with special reference to coliforms

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A study was carried out to assess the milk quality in Tarakeswar, India with special reference to coliforms. By standard plate count (SPC) method, out of ten raw milk samples, the microbial colonies were found to be high in six samples and the colony content was low in rest four samples. In pasteurized milk samples, the colonies were low in seven samples and high in three samples. The methylene blue test performed for raw milk samples showed that out of ten samples, the five samples were poor, two samples were fair, two samples were good and only one sample was found to be an excellent. Out of ten pasteurized samples, nine samples were of good quality and one was found to be excellent. Bacterial colony was found to be opaque and metallic sheen in colour prepared from five raw milk samples, and by biochemical characterization it was identified as *Escherichia coli*.

Key words: Raw milk, pasteurized milk, methylene blue reduction test, standard plate count, coliforms.

INTRODUCTION

Milk is an important food of diet of vast population on earth, due to its high nutritional value for human beings. Milk is an excellent growth medium of microorganism when suitable temperature exists. If it is produced unhygenically and handled carelessly, it gets contaminated very easily leading to its early spoilage (Prajapati, 1995, Schmidt, Van Vleck, 1982). The introduction of a few pathogens into milk becomes a much more serious problem because of the ability of these substances to support tremendous increases in bacterial numbers. Many milk-borne epidemics of human diseases have been spread by contamination of milk by spoiled hands of dairy workers, unsanitary utensils, flies and polluted water supplies. The same thing can be said for improper handling of foods in the home, restaurants, hospitals, and other institutions.

Coliforms particularly *Escherichia coli* are frequently used in the microbiological analysis of food as an indicator of poor hygienic condition. Microbiological examination of milk is essential to find the degree of contamination with the dictions and enumeration of indicator organisms. The coliform bacteria are able to grow well in a variety of substrates and to utilize a number of carbohydrates and some other organic compounds as food for energy and a number of fairly simple nitrogenous compounds as a source of nitrogen. The coliform group of bacteria is defined as the indicator (faecal coliform) of suitability of milk for drinking (Standard Method Committee, 1981).Tarakeswar is an important pilgrimage center in East India. The present study has been designed to assess the milk quality with special reference to coliforms.

METHODOLOGY

Ten raw milk and ten pasteurized milk samples were collected from various sources (milk vendors, milk societies, shops, prosing unit, etc) in sterile screw cap tubes and prossed within 3 h. The milk samples were mixed well before diluting. The samples were the diluted to 1:1000 and 1:10000 by using sterilized phosphate buffered water. The diluted samples were mixed again by using

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Table 1. Grading of milk samples on the basis of methylene-blue reduction test in different milk samples.

Quality of milk	Decolourization time
Excellent	More than 8 h
Good	Between 6 hours and 8 h
Fair	Between 2 to 6 h
Poor	Less than 2 h

Table 2. Enumeration of microorganisms in different milk samples by standard plate count methods.

Sample No.	Colony forming units, cfu			
	Raw milk		Pasteurized milk	
	(1/1000)	(1/10,000)	(1/1000)	(1/10,000)
T-1	627	121	123	31
T-2	594	173	161	52
T-3	512	133	187	66
T-4	551	131	210	82
T-5	113	96	68	14
T-6	463	126	133	49
T-7	128	93	94	24
T-8	451	111	112	42
T-9	101	91	98	34
T-10	205	95	77	21

sterile pipette each time. A measured quality (0.1 ml) of the sample was transferred into each sterilized Petri plates, thoroughly mixed, and allowed to solidify. Eosin Methylene Blue (EMB) agar was used as media because it contains methylene blue which inhibits gram-positive bacteria (Atlas and Bertha, 1997). Gram-negative lactose fermenters (coliforms) that grow on this medium will produce "nucleated colonies" (dark centers). Colonies of *Escherichia coli* and *Enterobacter aerogenes* can be differentiated on the basis of size and the presence of a greenish metallic sheen. (Atlas, Parks and Brown, 1995).

E. coli colonies in this medium are small and have this metallic sheen, whereas *E. aerogenes* colonies usually lack the sheen and larger. After solidification, the plates were incubated at 37°C for 24 h. After incubation, the colonies were counted by standard plate count method (Grag and Usha Mandokt, 1997; Benson, 2002) and the results were recorded.

In the methylene blue reduction (MRBT) test 1 ml of methylene blue (1: 25,000) is added to 10 ml of milk. The tube is sealed with rubber stopper and slowly inverted three times to mix. It is placed in a water bath at 35°C and examined at intervals up to 6 h. The time it takes for the methylene blue to become colourless is the methylene blue reduction time (MBRT). The shorter the MBRT, the lower the quality of milk. The grading of milk samples on the basis of methylene blue reduction test in different milk samples are presented in Table

1 (Benson, 2002). The methylene blue reduction test depends upon the ability of bacteria in milk to grow and to consume the dissolved oxygen, which reduces the oxidation reduction potentials in the medium.

The screenings of coliforms of all the seven milk samples were analyzed by the method of Bhattacharya (1993). The characterization of bacteria was by gram staining by the method of (Cappuccino and Sherman, 1996). Biochemical tests such as indole production test (Cowans and Steel, 1993), methyl red test, carbohydrate fermentation test (Kannan, 1996; Atlas et al., 1995; Garbutt, 1997) were qualitatively estimated.

Table 3. Decolorizing time and grading of milk samples collected from different parts of Tarakeswar.

Sample No.	Raw milk		Pasteurized milk	
	Decolourization Time	Grade	Decolourization Time	Grade
T-1	1.30 h	Poor	6.55 h	Good
T-2	1.25 h	Poor	6.25 h	Good
T-3	1.42 h	Poor	6.27 h	Good
T-4	7.30 h	Good	6.35 h	Good
T-5	9.41 h	Excellent	12.56 h	Excellent
T-6	7.15 h	Good	7.45 h	Good
T-7	1.21 h	Poor	7.42 h	Good
T-8	5.46 h	Fair	7.26 h	Good
T-9	1.25 h	Poor	7.15 h	Good
T-10	5.30 h	Fair	6.42 h	Good

RESULTS AND DISCUSSION

Out of ten raw milk samples, the microbial colonies were found to be high in six samples (T-1, T-2, T-3, T-4, T-6 and T-8) and the colony content was low in rest four samples (T-5, T-7, T-9 and T-10). In pasteurized milk samples, the colonies were low in seven samples (T-1, T-5, T-6, T-7, T-8, T-9 and T-10) (Table 2). The methylene blue test performed for raw milk samples revealed that out of ten samples, the five samples were poor, two samples were fair, two samples were good and one sample was found to be excellent. Out of ten pasteurized samples, nine samples were of good quality and one was found to be excellent (Table 3). The raw milk contained higher number of microflora probably due to contamination from the animal. Bacteria found in manure, soil and water may enter milk due to dairy utensils and milk contact surfaces. If the milk contact surfaces are inadequately cleaned, bacteria may develop in large numbers. Present study showed that 60% of the raw milk samples were of poor category. But in case of pasteurized milk samples, 100% of the samples were of good quality due to killing of contaminating of microorganisms by pasteurization.

Bacterial colony was found to be opaque and metallic sheen in colour in 5 raw milk samples. The bacteria were found to be gram negative in nature and were small straight rods. Phase contrast microscopic observation revealed the motile nature of these bacteria. Biochemical tests showed that the bacteria were indole positive, methyl red positive, Voges-Proskauer test negative, citrate utilization test negative, glucose fermentation positive and lactose fermentation positive.

During milking operation, however, milk may be expo-

sed to contamination from the animal, especially the exterior of the udder and adjacent areas. Bacteria found in manure, soil, and water may enter from this source. Such contamination can be reduced by clipping the cow, and washing the udder with water or a germicidal solution before milking. Contamination of cow with manure, soil, and water may also be reduced by paving and draining barnyards, keeping cows from stagnant pools, and cleaning manure from the barns or milking parlors. Pasteurization kills pathogens that may enter the milk and improve the keeping quality of milk.

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