Asian J. Med. Biol. Res. 2015, 1 (2), 292-296; doi: 10.3329/ajmbr.v1i2.25624

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Article

Microbial analysis of raw and pasteurized milk from selected areas of Dinajpur, Bangladesh

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Received: 26 July 2015/Accepted: 13 September 2015/ Published: 30 September 2015

Abstract: Milk is highly vulnerable to microbial contamination and consequently is easily perishable. The aim of the present study was microbial analysis of raw and pasteurized milk. For this, a total of 32 milk samples (12 from raw milk and 20 from pasteurized milk) were collected from 8 sources. Among them three were raw (R1 to R3) sources and rest five were pasteurized milk (P1 to P5) sources. Each of the collected samples was investigated during the period from May to December, 2012. All milk samples were subjected to total viable count (TVC), total coliform count (TCC), Total Staphylococcus count (TSC) and Gram's staining to determine the loads of microbes in raw and pasteurized milk. Total viable counts (TVC) range of 12 raw milk samples (R, R2 and R3) were 1.3 $x10^6$ to 7.4 $x10^5$ cfu/ml. The presence of *Escherichia coli* in the raw milk samples were from 2.3 $x10^2$ to 9.4 $x10^2$ cfu/ml, but the presence of *Staphylococcus* were from 5.9 $x10^2$ to 7.9 $x10^2$ cfu/ml. Whereas, the range of TVC for five brands of pasteurized milk (P1, P2, P3, P4, and P5) were from 1.8 $x10^4$ to 9.8 $x10^4$ cfu/ml, TSC were from 2.8 x10 to 8.6 $x10^2$ cfu/ml and TCC from 1.01 $x10^2$ to 9.1 x10 cfu/ml. Therefore, it can be concluded that high counts of bacteria were found in raw milk and pasteurized milk. The government therefore should conduct frequent inspection of the marketed milks to check whether they meet the minimum legal standards and should monitor the overall hygienic condition surrounding the production and handling of milk. Realistic standards for the raw milks need to be devised and appropriate training should be given to the raw milk producers in hygienic handling of milk.

Keywords: raw milk; pasteurized milk; microbial quality; TVC

1. Introduction

Milk is highly vulnerable to bacterial contamination and hence is easily perishable (Kim *et al.*, 1983; Steele *et al.*, 1997). Milk as well as dairy products are important sources of food borne pathogens and numerous epidemiological reports have implicated inadequate heat treated milk and raw-milk products are the major factors for illnesses caused by food-borne pathogens (De Buyser *et al.*, 2001; Harrington *et al.*, 2002). So it is mandatory this product to be obtained as cleanest possible and not to harm the consumers' health (Dan *et al.*, 2008). The presence of pathogenic bacteria in milk emerged major public health concerns (Ryser, 1998). Many milk borne epidemics of human diseases are spread through consumption of contaminated milk (Parekh and Subhash, 2008). Few examples of the known milk -borne diseases are bovine tuberculosis, brucellosis, anthrax, listeriosis, salmonellosis, leptospirosis, Q fever, campylobacteriosis and E. coli O157:H7as an emerged new milk -borne bacterial pathogen reported recently with a very serious health effects (Sivapalasingams *et al.*, 2004). To protect consumers and public health against these milk -borne infections it require proper hygienic milking and milk handling procedures.

Common bacteria reported to be isolated from milk include *Staphylococcus* spp., *Listeria* spp., *Salmonella* spp., *E. coli*, *Campylobacter* spp., *Mycobacterium* spp., *Brucella* spp., *Coxiella burnetii*, *Yersinia* spp., *Pseudomonas aeroginosa* and *Corynebacterium ulcerans*. Others are *Proteus* spp., *Leptospira* spp., Clostridium spp., *Streptococcus* spp, *Klebsiella* spp., *Enterobacter* spp. and *Bacillus* spp. (Shirima *et al*., 2003; Sivapalasingams *et al.*, 2004; Al-Tahiri, 2005;

Milk is a perishable product and an ideal medium for the growth of a wide variety of bacteria (Parekh and Subhash, 2008).When it is secreted from a healthy udder, raw milk contains only a very few bacteria of about 500 to 1,000 bacteria per milliliter (Omore *et al.*, 2005; Pandey and Voskuil, 2011). After milking enviromental contamination occurs ,which in turns increases the total bacteria count up to 50,000 per ml or may even reach several millions bacteria per milliliter (Pandey and Voskuil, 2011). That count level indicates a very poor hygienic standard of milk during milking a nd handling or milk of a diseased animal. The presence of coliform bacteria particulary *E. coli* in raw milk is an indicator of feacal contamination which implies poor hygienic conditions and unsanitized environment since these bacteria are of faecal origin.

In developing countries, most of the milk is produced by mallholder farmers dominated by local herds of cattle (Pandey and Voskuil, 2011). Their milking units are widely distributed throughout in rural areas with a poor infrastructure, while most of the markets and customers are in urban areas. Therefore, the need for good hygienic practices and a streamlined collection, handling and transport system is important but has been always a challenge (Pandey and Voskuil, 2011). However, milk contains a natural inhibitory system or temporary germicidal or bacteriostatic properties which prevents a significant rise in the bacteria count during the first 2 - 3 hours (Swai and Schoonman, 2011; Pandey and Voskuil, 2011). Thus the evaluation of microbial quality in milk is vital. The aim of the present study was conducted to investigate occurrence and microbial load in raw and pasteurized milk and also compare among different brands of pasteurized milk.

2. Materials and Methods

2.1 Study area

The study was conducted during the period from May to December, 2012. The milk samples were collected from different retail markets and farm house of Dinajpur area, Bangladesh.

2.2. Collection of sample

A total of 32 milk samples were collected from 8 sources. 12 raw milk samples from three sources (designated as R1 to R3) of local dairy farm, remaining 20 samples from five (P1 to P5) sources of different commercial packaged pasteurized milk were selected for this study. Aseptic techniques were strictly maintained during sample collection. Raw milk samples were collected in sterile glass bottle and packaged pasteurized milk were in sterial containers which were kept cool in ice boxes and immediately transported to the microbiology laboratory of Hajee Mohammad Danesh Science and Technology University, Bangladesh.

2.3. Sample processing and isolation of microbes

Prior to taking samples, milk containing bottle and packet were held firmly and shaken thoroughly for proper mixing of the milk samples. To get milk the packed was cut open with the help of sterile scissors. The cut is so made obliquely at one of the corners that there is sufficient opening for the sterile pipette to enter easily into the pasteurized milk inside. To obtain countable plate with individual colonies the samples were diluted using simple 1/10 dilutions. For a 1/10 dilution 1 part sample was added to 9 parts diluents (Distilled water) then mixed thoroughly. A series of dilutions was made as desired, and from one or more of these dilutions, known amounts were inoculated in an agar by pour plate techniques.

2.4. Identification of microbes

The forgoing prepared milk samples were bacteriologically examined in order to determine the total viable count (TVC), total coliform count (TCC), and total Staphyloccus count (TSC) on plate count agar (PCA), MacConkey agar (MCA) and Staphylococcus agar no-110 media, respectively. Plates exhibiting colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total count. The total count was expressed as the number of organism of colony forming units per ml (CFU/ml) of samples according to ISO (1995). All the data obtained during the study were analyzed statistically.

2.5. Statistical analysis

Data on the total viable count, coliform and staphylococcal count/ml of collecting sample from each point were subjected to Chi-Square Test using Microsoft Excel 2007 program. Significant differences of the data were established by least significant difference at the 5% level of significance.

3. Results

A total of 32 liquid milk samples, 12 raw milk samples from dairy farm, chilling center and local market denoted as R1, R2 and R3, respectively with 4 in each case and rest 20 pasteurized milk samples from different shops under specific brand name denoted as P1, P2, P3, P4 and P5 with 4 in each case too, were collected. The liquid milk samples were subjected to bacteriology laboratory of Hajee Mohammad Danesh Science and Technology University (HSTU) to determine the microbial load. The results of bacterial distribution in the collected samples are as follows.

3.1. Total viable count (TVC)

Total Viable Count (TVC) of bacteria was carried out on plate count agar media using pour plate techniques. The results, presented in Table 1, showed that the average TVC (cfu/ml) were 7.4 $x10^5$ (log5.87), 1.3 $x10^6$ (log 6.13) and 1.9 $x10^6$ (log 6.29) for raw milks collected from different sources, R1, R2 and R3, respectively. The counts for pasteurized milk samples, P1, P2, P3, P4 and P5 were 9.8 $x10^4$ (log 4.99), 6.8 $x10^4$ (log 4.83), 1.9 $x10^5$ (log 5.29), 1.8 $x10^4$ (log 4.27) and 5.3 $x10^4$ (log 4.72), respectively.

3.2. Total coliform count (TCC)

The average measures of TCC (cfu/ml) of milk samples were 2.3 $x10^2$ (log 2.37), 2.6 $x10^2$ (log 2.42) and 9.4 $x10^2$ (log 2.97) for raw milks collected from different sources, R1, R2 and R3, respectively (Table 1). The results for pasteurized brand milks, P1, P2, P3, P4 and P5 were 1.6 $x10^2$ (log2.21), 9.1 x10 (log 1.9), 3.4 $x10^2$ (log 2.54), 0 and 1.01 $x10^2$ (log 2.00).

3.3. Total staphylococcal count (TSC)

Staphylococcus agar no-110 medium was used for the enumeration of total staphylococcal count (TSC) in the milk samples. In the raw milks the TSC (cfu/ml) were 7.9 $x10^2$ (log 2.90), 5.9 $x10^2$ (log 2.77) and 6.1 $x10^2$ (log 2.78) for R1, R2 and R3, respectively. The values of TSC (cfu/ml) of pasteurized milks were 8.6 $x10^2$ (log 2.93), 6.4 $x10^2$ (log 2.81), 4.4 $x10^2$ (log 2.64), 8.5 $x10^2$ (log 2.93) and 2.8 $x10^1$ (log 1.44) for P1, P2, P3, P4 and P5, respectively.

3.4. Microscopic examination

In Gram's staining under microscope, smears from plate count agar, MacConkey agar and Staphylococcus agar no-110 Medium were examined. In case of PCA, there were presence of both Gram's positive and Gram's negative organisms with different arrangements. From MCA, Gram's negative, pink color, mostly rood shape organisms were revealed. For Staphylococcus agar no-110, Gram's positive, violet color, short cocco-bacilli or rod were found within bundles and singly arranged.

Table 1. The average counts of TVC, TCC and TSC in raw and different brands of pasteurized milk.

	Sources of Milks	Numbers of Tested Sample	Total Viable Count/ml		Total Coliform Count/ml		Total Staphylococcus count/ml	
			cfu/ml	Log10	cfu/ml	Log10	cfu/ml	Log10
	R1	4	7.4 $x10^5$	5.87215627	2.3 $x10^2$	2.37199091	7.9 $x10^2$	2.901730692
	R2	4	1.3 $x10^6$	6.13273984	2.6 $x10^2$	2.42813479	5.9 $x10^2$	2.770852012
	R3	4	1.9 $x10^6$	6.29501701	9.4 $x10^2$	2.97312785	6.1 $x10^2$	2.788875116
Level of	Significance			***		NS		NS
	P1	4	9.8 $x10^4$	4.99343623	1.6 $x10^2$	2.218798	8.6 $x10^2$	2.938894818
	P2	4	6.8 $x10^4$	4.83537345	9.1 x10	1.95904139	6.4 $x10^2$	2.810736437
	P3	4	1.9 $x10^5$	5.29003461	3.4 $x10^2$	2.54157924	4.4 $x10^2$	2.64615857
	P4	4	1.8 $x10^4$	4.27760921	0	0	8.5 $x10^2$	2.933234129
	P5	4	5.3 $x10^4$	4.72733789	1.01 $x10^2$	2.00432137	2.8 $x10^1$	1.447158031
Level of	Significance			***		NS		NS

4. Discussion

The investigation on microbial analysis of raw and different brands of pasteurized milk was conducted to evaluate milk samples obtained from two important sources viz., raw and pasteurized milk. PCA, MCA and Staphyloccocus agar no-110 were used for the enumeration of bacteria from milk in agreement with other workers (Mhone *et al.*, 2011; Hossain *et al*., 2010). The study revealed that the total viable counts (TVC) for raw milk were ranges from Total viable counts (TVC) range of 12 raw milk samples (R1, R2 and R3) were 1.3 $x10^6$ to 7.4 $x10^5$ cfu/ml. The presence of *Escherichia coli* in the raw milk samples were from 2.3 $x10^2$ to 9.4 $x10^2$ cfu/ml, but the presence of *Staphylococcus* were from 5.9 $x10^2$ to 7.9 $x10^2$ cfu/ml. Whereas, the range of TVC for five brands of pasteurized milk (P1, P2, P3, P4, and P5) were from 1.8 $x10^4$ to 9.8 $x10^4$ cfu/ml, TSC were from 2.8 x10 to 8.6 $x10^2$ cfu/ml and TCC from 1.01 $x10^2$ to 9.1 x10 cfu/ml., with an average of 1358333 cfu/ml which is corresponding to the findings of Dan (2008). The average count (cfu/ml) for pasteurized milk was observed in samples belong to P1-9.8 $x10^4$ (log 4.99), P2-6.8 $x10^4$ (log 4.83), P3-1.9 $x10^5$ (log 5.29), P4-1.8 $x10^4$ (log 4.27) and P5-5.3 $x10^4$ (log 4.72). It was evident from the result that the average TVC count was nearly matched to Lingathorni (2009). Average counts for *E. coli* in raw milk samples were 481 cfu/ml where as in pasteurized milk *E. coli* counts for P-1,P-2,P-3,P-4, P-5 were 1.6 $x10^2$ cfu/ml (log2.21), 9.1 x10 (log 1.9) cfu/ml, 3.4 $x10^2$ cfu/ml (log 2.54), 0 and 1.01 $x10^2$ cfu/ml (log 2.00), respectively. Total Staphylococcal count in the raw milks ranged from 5.9 $x10^2$ to 7.9 $x10^2$ cfu/ml. These counts were less than the findings of Ghose and Maharajan (2002) where the mean Staphylococcal counts were $4.7x10^6$ cfu/ml in raw milk but higher than that of Srairi *et al.* (2006) where TSC ranged from <30 cfu/ml. In contrast to different recommended standard for milk foods, sanitary condition of milks sample used for this study were not satisfactory. United State standards recommended, each ml of raw milk for pasteurization must have less than $3x10^5$ cfu/ml (Coast *et al.*, 2004; Heinemann *et al.*, 1999). Unfortunately, the results of TVC ranged from 1.3 $x10^6$ to 7.4 $x10^5$ cfu/ml is almost far greater than the United State standard. The bacteria count in the pasteurized milk samples ranged from 1.8 $x10^4$ to 9.8 $x10^4$ cfu/ml, much higher than that recommended by BSTI and USPHS, i.e., not exceeding 20,000 cfu/ml (BSTI, 2002; Jay, 2003). The reason for high bacteria count in the pasteurized milks may include defective pasteurization machinery, survival of pasteurization and post-pasteurized contamination such as poor processing and handling conditions and/or poor worker hygiene. Coliforms are considered as indicator organisms because their presence in food indicates some form of contamination. The coliforms standards for Grade 'A' pasteurized milk and milk products should not be over 10/ml (BSTI, 2000) and for certified pasteurized milk should not be over 1/ml. The present investigation however reported significantly high coliforms count than that recommended by BSTI (2002). *E. coli* causes severe diarrhea in newborns and adolescents and originates from mastitis (Kornalijnslijper *et al.*, 2004). However, the raw and pasteurized milk of this study are not only top grade but also some kinds of them are unusable. In consideration of comparative figure of microbial load in the best quality raw milk to different brands of Pasteurized milk, all pasteurized milk contained lower TVC count. For TCC the P-3 brands pasteurized milk contain higher count than the best quality raw milk. In case of *Staphylococcus* count surprisingly P-1, P-2, P-4 contain high bacteria count than raw milk sample.

5. Conclusions

It was concluded from the study that microbial loads of raw milk were not satisfactory. Therefore, it could be assume that the handler of raw milk do not maintain good personal hygiene. All most all brands of pasteurized milk tested in this study were of low quality based on BSTI standard. Presence of *E. coli* and *Staphylococcus* spp. were of public health concern. Government therefore should conduct frequent inspection of the marketed milks to check whether they meet the minimum legal standards and should monitor the overall hygienic condition surrounding the production and handling of milk. Realistic standards for the raw milks need to be revised and appropriate training should be given to the raw milk producers in hygienic handling of milk.

Conflict of interest

None to declare.

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