Article

Microbiological quality assessment and acceptance of dairy products in Dhaka city

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Abstract: This investigation was carried out to evaluate the microbiological quality of the processed dairy products including borhani, matha/labang, sweet & sour yoghurt. Dairy products are consumed as desert and popular enough among the people. 50 of the different dairy samples were collected from street vendor and also from some branded shop in Dhaka city. The microbiological quality of the samples were analyzed and monitored according to criteria in European Commission Recommendations 2004/24/EC and 2005/175/EC, BSTI and USPHS. All the dairy products had high microbial load ranged 5.90×10^5 cfu/ml to 8.97×10^9 cfu/ml. Coliforms were found up to 10^9 dilutions in milk based drink products Borhani and Labang considered a serious threat to the public health. The mold contamination was much lower in Borhani and Labang compared to yoghurt 7.86×10^8 cfu/ml. Approximately 17% dairy products were contaminated by *Salmonella* spp. Majority of the dairy products (25.537%,) such as Yogurt, Borhani and Labang were contaminated by *Staphylococcus* spp. The presence of *Listeria monocytogenes* found in Yogurt, 7 out of 21 (21.515%) samples were contaminated. A minor number of milk based products were corrupted by *Shigella* spp. These results emphasize applying and maintaining good hygiene practices throughout the processing chain to prevent contamination and bacterial growth. It was concluded that the presence of some pathogens in milk based products with toxigenicity of some strains pose a health hazard to consumers. Thus, good hygienic practices, good manufacturing practices, HACCP with all other hygienic practices should be applied during processing and distribution for public health safety.

Keywords: dairy products; microbial quality; assessment; acceptance

1. Introduction

Now a days, one of the biggest economic problem is food spoilage, approximately one fourth of food around the world is spoiled by microbiological activity which is thought to be most significant medium of foodborne disease mainly caused by the lack of knowledge about food safety and sanitation, transmitting around the world (Huisin't Veld, 1996; WHO, 1999) and also have been the reason of high mortality and morbidity in all over Bangladesh for years (Haq and Rahman,1991; Henry *et al.*, 1990; Islam *et al.*, 1993; Luby *et al.*, 2006; Ram *et al.*, 2007; Saha *et al.*, 2009; Sheikh *et al.*, 2002). Milk is thinks of perfect, complete and one of the most nutritious foods for all aged people from child to old contains minerals along with calcium these are required bone growth and maintenance (Aneja *et al.*, 2002), despite that milk can be used as potential growth media for organisms which is full of nutrients that enhance the rate of duplication and production of toxin by microorganisms (Ruegg, 2003; Rajagopal *et al.*, 2005). A variety of organisms that can cause spoilage of milk products include some lactic acid bacteria, Gram negative bacteria, coliforms, yeast and fungus in addition some pathogenic bacteria like *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* and

toxic strains of *Staphylococcus aureus* also found in milk products (Tatini and Kauppi, 2003). Consummation of dairy products or milk contaminated by pathogens or toxic metabolites can lead to food borne infection and poisoning interestingly, few endotoxin pathogenic bacteria undergo lysis and release toxic metabolites in intestinal tract can lead to septic shock (Aneja *et al.*, 2002). These pathogens may directly or indirectly enter in milk and milk products which serves as complex biochemical composition and high water activity, milk and milk products serve as an excellent medium for the growth and replication of organisms during handling, processing, pre and post heat treatment, packaging etc. (Richter *et al.*, 1992). That is why microbiological analyses are crucial food safety, security, quality and conformation with standards and specifications (Vasavada, 1993). Most of the dairy products are produce in traditional manner by unpasteurized raw milk without consideration of nutritional and microbiological standards of the raw materials, which allows many bacteria to grow which can change the products flavor, color, consistency and odor. These incidences are decreased in half by Quality and safety management systems such as ISO and Hazard Analysis Critical Control Point (HACCP). In addition, Bangladesh Standard Testing Institution also working to improve the physical, chemical and microbiological quality of milk and dairy products to reach the acceptable quality ofwhich would be safe for consumption. The indicator bacteria and some other organisms in dairy products are now used to determining the safety and quality of milkand milk products (Uddin *et al.*, 2011). To reduce economic losses by the early detection of insufficient processing, packaging or refrigeration, microbiological assessments play an important role to in the dairy industry. The purpose of this study is to determine the microbiological quality and cause food-borne pathogens such as coliform, *L. monocytogenes*, *Staphylococcus* spp., *Salmonella* spp. and *Shigella* spp. of dairy product samples collected from various points of Dhaka city and identify the sources of contamination along with possible solution.

2. Materials and Methods
2.1. Study location and length of study
For this study all the experiments were conducted in Industrial Microbiology Lab, IFST, Bangladesh Council of Scientific and Industrial Research (BCSIR Laboratories, Dhaka) from February 2017 to July 2017.

2.2. Sample collection
Fifty different types dairy products such as Labang, Sweet Yogurt, Sour Yogurt and Borhani were collected from fifty street food vendors and local shops situated in various points of Dhaka city. Approximately 500ml/200g samples were taken aseptically in separate screw-capped wide mouthed plastic containers/polythene bag then kept at 4°C in a sample collector box and were immediately transported to the laboratory for the analysis of several microbiological parameters, if laboratory analysis was delayed due to the delayed arrival of samples, those samples were refrigerated at 0–4 °C until examination but not longer than 48 hours.

2.2. Microbiological culture method
2.2.1. Culture method of total plate count
25ml/g of each milk product samples were weighted and aseptically added in 225 ml of sterile Peptone Water (Difco^{TM}) then homogenized the mixture in a blender at 600 rpm for 10-15 min and diluted up to 10^{-10} times. The total viable bacterial count was carried out by the spread plate technique (ISO 4833:2003). The diluted sample (1 ml) of each dilution were inoculated into Plate Count Agar (Difco^{TM}) for Total Viable Bacterial Count (TVBC) using pour plate technique (Marjan *et al.*, 2014). The plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit in per ml (cfu/ml). Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media.

2.2.2. Culture method of coliform and *E. coli*
10 ml, 1 ml and 0.1 ml of sample to 3/5 tubes of LST for each amount. LST tubes were incubated at 35°C. Tubes were examined and record reactions at 24 ± 2 h for gas, i.e., displacement of medium in fermentation vial or effervescence when tubes are gently agitated. Re-incubated gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 2 h. and confirmed test on all presumptive positive (gas) tubes (ISO 4831:2006. and ISO 7251:2009. Geneva). From each gassing LST tube, transfer a loopful of suspension to a tube of BGLB broth, voiding pellicle if present. Incubated BGLB tubes at 35°C and examine for gas production at 48 ± 2 h. If gas-positive BGLB tube showed a pellicle, perform Gram stain to ensure that gas production was not due to Gram-positive, lactose-fermenting bacilli. Calculation was done by most probable number (MPN) of

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coliforms based on proportion of confirmed gassing LST tubes for 3 consecutive dilutions. 10 ml, 1 ml and 0.1 ml of sample were added to 3 tubes of LST-MUG for each amount. LST tubes were incubated at 35°C. Examine tubes and record reactions at 24 ± 2 h for gas. Gas-negative tubes were re-incubate for an additional 24 h and examine and record reactions again at 48 ± 2 h. Confirm test was performed on all presumptive positive (gas) tubes. Both positive and negative tubes were incubated for 24 to 48 ± 2 h at 35°C. Examine each tube for growth (turbidity, gas) then examine tubes in the dark under UV long-wave lamp (365 nm). A bluish fluorescence is a positive presumptive test for *E. coli.* From each gassing LST tube of the Presumptive test, transfer a loop-full of the suspension to a tube containing EC broth. EC tubes 24 ± 2 h at 44.5 °C were incubated and examine for gas production.The Completed test for *E. coli* was performed, gently agitate each gassing EC tube and streak for isolation, a loop-full to an EMB agar plate and incubate 18-24 h at 35°C. Plates for suspicious *E. coli* colonies were examined, i.e., dark centered and flat, with or without metallic sheen. Up to 5 suspicious colonies were transferred from each EMB plate to PCA slants and incubate for 18-24 h at 35°C and use for further testing. Identification of any 1 of the 5 colonies as *E. coli* is sufficient to regard that EC tube as positive; hence, not all 5 isolates may need to be tested.

2.2.3. Culture method of *Salmonella* spp. and *Shigella* spp.
25 gm of sample was added in 225 ml of Buffered peptone water and incubate at 37^0C for 18±2 h (ISO 6579:2002-1:2007 and ISO 21567:2004, Geneva). Then the pre-enriched culture was incubated in Modified semi-solid Rappaport-Vassiliadis agar plate at 41.5^0C for 24±2 h. the enriched culture then inoculated in xylose lysine deoxycholate agar or any other solid selective medium complementary to XLD agar. The presumptive salmonella colonies were confirmed by means of appropriate biochemical and serological tests.

2.2.4. Culture method of *Staphylococcus* spp.
25 g Sample stomatched in 225 ml Phosphate Buffered Saline/Alkaline Peptone Water Prepare serial dilutions by transferring 1 ml of previous dilution to 9 ml of diluents (ISO 6888-1:1999. ISO, Geneva). Aseptically transfer 1 ml sample suspension to plates of Baird Parker Agar and Spread inoculums over the agar surface using sterile bent glass rod. After 10 to 15 minutes, invert the plates and incubate at 35^oC for 48 hours. Select characteristic colonies (Black, gray, smooth, circular, convex, moist colony with opaque.

2.2.5. Culture method of *Listeria monocytogenes*
25 gm of sample was added in 225 ml of fraser broth with reduced concentration of selective agents at 30^0C for 24±2 h. then 0.1 ml of the primary enriched sample is transferred in fraser broth with full concentration of selective agent Fraser broth and incubate at 35^0C for 48±2 h (ISO 11290-1. ISO, Geneva). then from the primary selective medium one lop full of culture is streaked in Oxford agar and from the secondary selective medium one lop full of culture is streaked in PALCUM agar and incubate at 35^0C for 48±2 h. typical colonies of *Listeria* spp. grown in Oxford agar are grayish colonies surrounded by black halos and in PALCUM agar colonies are pink to purple color sometimes black centered an always black halos. After that *Listeria* spp. was confirmed by performing several appropriate biochemical tests.

2.2.6. Culture method of yeast and mold
The total yeast and mold count of the collected dairy products are enumerated according to ISO 21527-2:2008: Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and molds. Part-2 colony count technique in products with high water activity.

3. Results and Discussion
3.1. Total viable bacterial count (TVBC) and total coliform count (TCC)
The most important hygienic indicator for food and food stuffs is total microbial load of the samples. Total Viable Bacterial Count of the number of bacteria which were present in samples were presented in Figures 1, 2 and 3. All the dairy products had high Microbial load ranged in between 5.90×10^5cfu/ml to 8.97×10^9cfu/ml which is much higher than the acceptable level suggested by BSTI and USPHS (Bangladesh Standards and Testing Institution. 2002. Bangladesh standard: Specification for pasteurized milk and Jay 2003). In other study the bacterial count in milk samples were from 7.5×10^7 to 1.24×10^8cfu/ml (Hossain *et al.,* 2010). One of the members of coliform bacteria *Escherichia coli* in dairy products is a common indicator of fecal contamination, this point out the hygienic standard and the storage quality of the milk product, rather than the presence of human pathogens. During the production, handling and storage of the dairy products extra precaution is necessary in order to lower the coliform count. Figure-1, 2 and 3 indicate Total coliform count in milk based

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product, the highest the coliform count was found in Borhani and Labang whereas the lowest was in Yogurt especially in Sour Yogurt this is because Yogurt have lower water activity then Borhani and Labang as well as cultured dairy products have low pH which is pH of 4.5 or less, this prevents the growth of most pathogenic and spoilage organisms which require pH 7 for growth (Richter *et al.*, 1992). Coliforms were found up to 10^9 dilutions in milk based drink products Borhani and Labang sold in various areas can be considered as an unhygienic food in terms of coliform count and can a serious threat to the public health.

3.2. Average count of yeast and mold in dairy products

The total yeast & molds count is another indicator for food hygiene and the results found in the collected samples devastatingly serious as molds are responsible for aflatoxinic contamination. The mold contamination was much lower in Borhani and Labang in between 7.46×10^4 cfu/ml to 7.54×10^5 cfu/ml on the other hand 7.86×10^8 cfu/ml number of mold was found in Yogurt which crosses the normal level, the reason of much higher mold contamination in Yogurt may be because of low p^H which is around p^H 5 and perfect for molds. Interestingly, no yeast was found in any of the dairy products (Figure 4).

Table 1. Summery of biochemical test of *E. coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp. and *Listeria* spp.

Organism	TSI S	TSI B	TSI G	H₂S	Indole test	MR test	VP test	Citrate test	Motility test	Oxidase test	Catalase test	Urea	Gelatin	Starch
E. coli	Y	Y	+	-	+	+	-	-	+	-	+	-	-	-
Salmonella	R	Y	-	+	-	+	-	-	+	-	+	-	-	-
Shigella	R	Y	+	-	+/-	+	-	-	-	+	+	-	-	-
Staphylococcus	Y	R	+	-	-	+	-	+	-	-	+	-	-	-
Listeria spp.	Y	Y	-	-	-	+	+	-	+	-	+	-	-	-

TSI = Triple Sugar Iron Test, Y = Yellow (Acidic), R = Red (Alkaline), MR = Methyl Red, VP = Voges−Proskauer
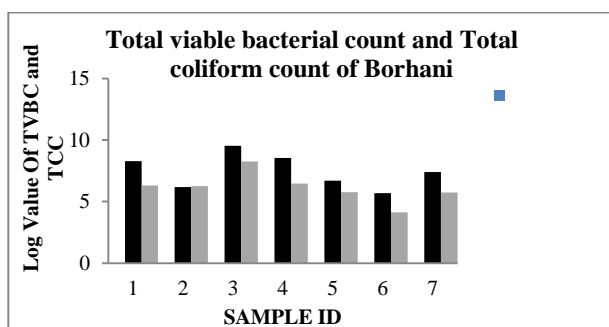

Figure 1. Total viable bacterial count and Total coliform count of Borhani.
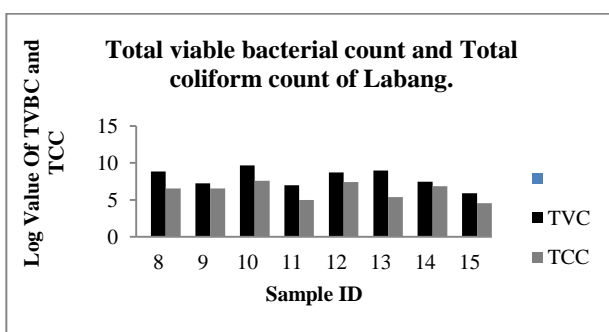

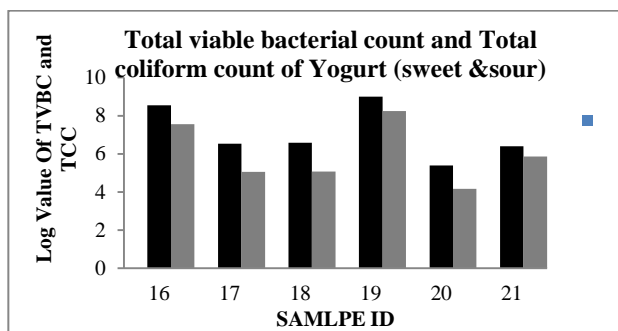**Figure 2. Total viable bacterial count and Total coliform count of Labang.**

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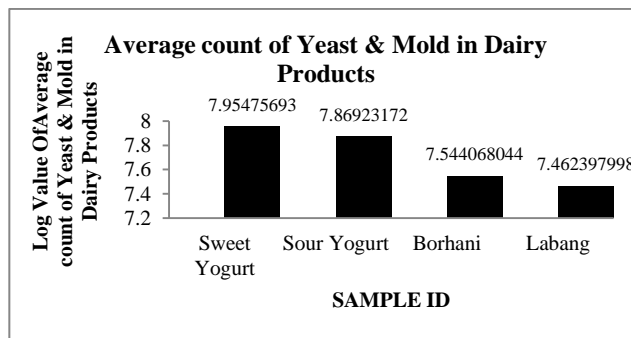**Figure 3. Total viable bacterial count and Total coliform count of Yogurt.**

Figure 4. Average count of Yeast & Mold in Dairy Products.
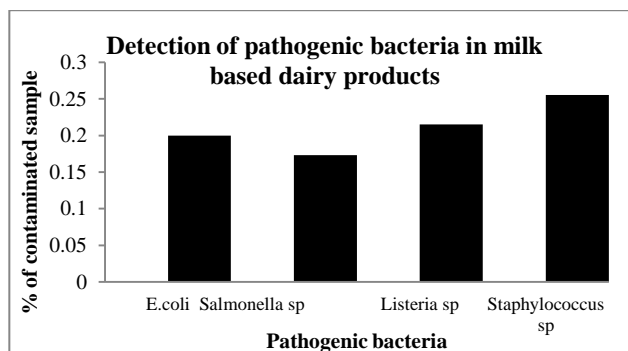

Figure 5. Detection of pathogenic bacteria in milk based dairy products.

3.3. Presence of other pathogenic bacteria

Some specific bacteria which are major food-borne pathogens including *Salmonella* spp., *Staphylococcus* spp., *Listeria* spp. and *Shigella* spp. (Kulshrestha, 1990) were cultured. The most common bacterial food infection caused by *Salmonella* spp. (Barro *et al.*, 2002). Moreover, the ingestion of *Salmonella* contaminated food can cause typhoid fever and paratyphoid fever (Bhan and Bhatnagar, 2005) alongside the typical food-poisoning salmonellosis. Approximately 17% dairy products were contaminated by *Salmonella* spp. Another most frequently occurring food poisoning incidence is caused by the consumption of some specific strains of *Staphylococcus aureus* responsible for the formation of enterotoxin formed in food, if there is a possibility of public health issues because of *Staphylococcus* spp., it is better to test for enterotoxins (Richter *et al.*, 1992). Majority of the dairy products (25.537%, Figure 5) such as Yogurt, Borhani and Labang were contaminated by *Staphylococcus* spp., the enumeration of coagulase positive Baird-Parker agar (BPA, Oxoid) (ISO 6888-1:1999) was use for cultivation, coagulase and enterotoxins test it is worth to be mention that small number of dairy product samples were coagulase and enterotoxin positive. The presence of *Listeria monocytogenes* can be found in a wide range of raw and processed foods including milk and dairy products (Rocourtand Cossart, 1997) and the highest *Listeria monocytogenes* contamination was found in Yogurt, 7out of 21 (21.515%) samples were contaminated. A minor number of milk based products were corrupted by *Shigella* spp.

3.4. Biochemical results for confirmation of dairy pathogens

For confirmation, biochemical tests were done for every pathogenic bacteria species. All pathogenic bacteria were unable to hydrolyzed Urea, Gelatin, Starch as well as MR test. *E. coli* and *Salmonella* spp. were capable of degrading Glucose, Sucrose, Lactose and Maltose, no pathogen except *Staphylococcus* spp. was able to degrade Manitol. Kliger's Iron Test was also done only for *E. coli* and *Salmonella* spp. where slant and butt was acidic, no H2S gas formed for *E. coli* whereas slant was alkali and butt was acidic, H2S gas formed for *Salmonella* spp. (Table 1). Some additional Biochemical Tests were also performed for identification of enterotixin producing *Staphylococcus* spp. those are Coagulase production, Thermonuclease production, Lysostaphin sensitivity, Anaerobic utilization of glucose, mannitol and give positive reactions for all those Biochemical Tests (Biochemical results for *Staphylococcus* spp. are not shown in this study).

4. Conclusions

The findings of this present study shown, heavy microbial load and coliform contamination in the dairy product samples collected from different point of Dhaka city which exceeded the microbiological acceptation level of dairy product. Other pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., and *Listeria monocytogenes* were also detected in several dairy products, indicate the hygiene quality of the samples were really poor. This will aware the public health department to improve and monitor the standard regulation and the quality of dairy products. Also Dhaka City Corporation (DCC) should arrange training and educational program for the producer of dairy product so that they can improve their food handling practices, minimum requirements of good hygienic and sanitary conditions and good storage conditions to reduce the risk microbiological contamination. The regulatory bodies should enact specific laws, legislations, rules and guidelines for production of dairy products to control microbiological quality, adulteration, sanitation and quality assurance of these products.

Conflict of interest

None to declare.

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