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Microbiological quality assessment of raw milk and water samples from Noakhali district, Bangladesh

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Abstract: This study was carried out to evaluate the microbial quality of raw milk and water samples taken at four different local shops in Maijdee area (Noakhali District), Bangladesh. Milk is a valuable food-stuff consumed on daily basis and is highly prone to bacterial contamination. Contamination with pathogenic microorganisms is most alarming for potable water and hence it is needed to detect the bacterial contamination in local drinking water. Microbiological quality of milk samples was analyzed using Total Viable Bacterial Count (TVBC), Total Coliform Count (TCC) and Yeast-mold Count techniques. Compare to four stores, store 3 shows the high contamination value in milk samples (TVC 12.48×10⁵ cfu/ml, TCC 6.4×10⁵ cfu/ml, yeast- mold count 3.48×10² cfu/ml and 4.85×10² cfu/ml) whereas store 1 is liable to water samples (TVC 12.09×10⁵ cfu/ml, TCC 4.81×10⁵ cfu/ml, yeast- mold count 2.7×10² cfu/ml and 2.24×10² cfu/ml). These outcomes accentuate applying and sustaining proper hygiene practice throughout the manufacturing and distribution to prevent health risks of the rural people.

Keywords: water quality; milk contamination; total viable bacterial count (TVBC); total coliform count (TCC); yeast-mold count

1. Introduction

A major constituent of diet is milk which quality reliance is essential to a community’s health. Milk may get contaminated at various stages such as from cow, extraneous dirt or unclean processing and thus it can contain few organisms (Hayes et al., 2001). Though milk emits nutritional quality and health benefits, it can also serve as an ideal medium for the growth of a wide variety of bacteria (Parekh and Subhash, 2008). Again, milk can be contaminated by improper pasteurization or recontamination. The detection of Coliform bacteria and other pathogens in milk indicates a plausible contamination of bacteria from milk during production, pasteurization and marketing (Bonfoh et al., 2003). Water is life and 60% of the drinking water is based on surface water resources (Chauret et al., 1995). Surface water bodies are presumed to be more vulnerable to fecal contamination than groundwater reservoirs as natural soil protection and filtration is absent in surface water bodies and possible short distances between the occurrence of contamination and water extraction (Kistemann et al., 2001). Total Viable Bacterial count (TVBC), also known as total viable count (TVC), is designed to provide an estimation of total number of aerobic organisms in a particular food or drink sample. A series of dilutions of the food/ drink in saline solution is mixed with an agar medium and incubated at 35°C for 48 hr. Each visible colony is the result of multiplications of a single cell on agar (FAO, 2009). Depending on the product, a high standard plate count may indicate that the product may have been prepared unhygienically or stored inappropriately (NSW Food Authority, 2013). The microbiological assessment of food lead to the classification of microbiological quality into one of the three classes- Satisfactory, Borderline and Unsatisfactory (Centre for...
Food Safety, 2014). Coliform bacteria (Enterobacteriaceae) are aerobic, gram-negative and facultative anaerobic, rod-shaped lactose fermentative bacteria that can produce acid (Clesceri, 1998) whereas fecal Coliform is useful indicator of fecal contamination that usually live in the intestines of warm-blooded animals. These coliform bacteria can survive for extended time periods outside the intestines of warm-blooded animals. E. coli and other fecal coliform bacteria can remain viable for several months in water and stream sediments (Davis et al., 2005). Yeasts and molds are another cause to spoil of fermented milk in which the low pH provides a selective environment for their growth (Fleet, 1990; Rohm et al., 1992). Good manufacturing practices should contain but 10 yeast cells and should have a shelf life of 3–4 weeks at 5 °C (Giudici et al., 1996). The aim of the study is to say the overall microbiological quality and to determine the microbial load in milk and water sample collected from local stores.

2. Materials and Methods

2.1. Sample collection

Four different types of milk as well as water samples were collected from local shops situated in various points of Maijdee city, Noakhali. Approximately 500 ml of each sample were taken aseptically in separate screw-capped wide mouthed plastic containers and then kept at 4 °C in a sample collector box and were immediately transported to the laboratory for the analysis of several microbiological parameters and after that samples were kept at 0–4 °C (refrigerator) for further study but not longer than 48 hours.

2.2. Culture method of total plate count

25 ml of each sample were weighted and aseptically added in 225 ml of sterile Buffer Peptone Water then homogenized the mixture in a blender at 600 rpm for 10-15 min and diluted up to $10^{-5}$ 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 was inoculated into Plate Count Agar 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).

2.3. Culture method of total coliform

1 ml and 0.1 ml of sample to 3/5 tubes of Lauryl Tryptose Broth (LST) media (each tube contains 10 ml of media). LST tubes were incubated at 35 °C for 24 ± 2 hours for gas production (i.e., displacement of medium in fermentation vial or effervescence when tubes are gently agitated). Gas-negative tubes were re-incubated for an additional 24 hours and confirmed test on all presumptive positive (gas) tubes (ISO 4831:2006. and ISO 7251:2009. Geneva). From each gas forming LST tube, a loopful of suspension was transferred to a tube of Brilliant Green Bile Broth (BGLB) media (each tube contains 10 ml of media). BGLB tubes were incubated at 35°C and examined for gas production at 48 ± 2 hours. If gas-positive BGLB tube showed a pellicle, Gram staining was performed to ensure that gas production was not due to Gram-positive, lactose-fermenting bacilli. Calculation was done by Most Probable Number (MPN) of coliforms based on proportion of confirmed gas producing LST tubes for three consecutive dilutions. 10 ml, 1 ml and 0.1 ml of sample were added to 3 tubes of LST-MUG (4-methylumbelliferyl-beta-D-glucuronide incorporated into lauryl tryptose broth) media (each tube contains 10 ml of media). Tubes were then incubated at 35°C for 24 ± 2 hours for gas production. Gas-negative tubes were re-incubated for an additional 24 hours and confirm test was performed on all presumptive positive (gas) tubes. Both positive and negative tubes were incubated for 24 to 48 ± 2 hours at 35 °C. Positive colonies were counted by culturing them in Plate Count Agar (PCA) media.

2.4. 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. Using a sterile pipette, 0.1 ml of the test sample or 0.1 ml of the initial suspension was transferred to one Rose Bengal Chloramphenicol Agar (RBC) agar plate. Using a fresh sterile pipette, 0.1 ml of the first decimal dilution was transferred to a second RBC agar plate. These operations with subsequent dilutions were repeated, using a new sterile pipette for each decimal dilution. The liquid over the surface of the agar plate was spread with a sterile spreader until the liquid is completely absorbed into the medium. Then the prepared plates were incubated aerobically, lids uppermost, in an upright position in the incubator at 25 °C ± 1 °C for 5 days.
3. Results and Discussion

3.1. Total viable bacterial count (TVBC) in milk and water samples

Total microbial load of any sample is an important hygienic indicator for food and food staffs. Figure 1 represents the Total Viable Bacterial Count of different samples.

All milk samples had high microbial load ranged in between $7.0 \times 10^5$ to $1.24 \times 10^6$ cfu/ml which is higher than the acceptable level suggested by BSTI (Bangladesh Standards and Testing Institution). This bacterial load is normally due to poor cleaning system of milking. In other study, the bacterial count in milk samples was from $7.5 \times 10^4$ to $1.24 \times 10^6$ cfu/ml (Hossain et al., 2010; Hasan et al., 2015) revealed that total viable count (TVC) of bacteria for raw milks was from log 6.29 to log 5.87. Raw milk contained an average TVC of $1.338 \times 10^7$ cfu/ml and Pasteurized milk contained an average TVC of $2.95 \times 10^4$ cfu/ml was depicted by Hussaini et al. (2014). In present study, values ranged in between $7.88 \times 10^5$ to $1.21 \times 10^6$ cfu/ml for water samples higher than the acceptable level suggested by BSTI (Bangladesh Standards and Testing Institution). Among the samples, the highest count was seen in the store-1 and the lowest from Store-4. These results indicate that these examined water samples are not suitable for drinking without purifying. Sarker et al. (2016) found that the bacterial count in water samples was from $17.80 \times 10^6$ to $11.46 \times 10^6$ cfu/ml while bacterial colonies in tap water ranges from the $1.05 \times 10^4$ to $2.55 \times 10^8$ cfu/ml was observed by Hameed et al. (2015). A study was conducted to evaluate the quality of drinking water samples from the four different drinking water sources namely open wells, bore wells, can water and tap water by Sailaja et al. (2015) and showed that the total viable count of the Open well water is highest ($95 \times 10^4$ bacteria/ ml) and lowest in are also high in open well water ($10.75 \times 10^5$ spores/ cm).

3.2. Total coliform count (TCC) in milk and water samples

Coliforms were found in all the milk and water samples of the four stores. The range of Coliform count for milk samples is from $6.39 \times 10^5$ to $5.85 \times 10^6$ cfu/ml and for water samples is from $4.81 \times 10^5$ to $2.62 \times 10^6$ cfu/ml (Figure 2).

Figure 1. Total viable bacterial count of milk and water samples.

Figure 2. Total Coliform count of milk and water samples.
Coliform count of these milk samples can give the consideration of it as an unhygienic food and pose a serious threat to the public health. Ogot et al. (2015) studied that among all the examined samples, Coliform count in 60% of the raw milk samples was >50000 cfu/ml while the number is reduced after boiling and only 40% of the boiled samples had >50000 cfu/ml Coliforms. Another study showed that the total Coliform and E. coli count was ranged from 3.74 log cfu/ml to 4.07 log cfu/ml and from 2.06 log cfu/ml to 2.98 log cfu/ml respectively for milk samples (Mohamed et al., 2017). Banik et al. (2011) published that the range of total Coliform count in raw milk samples collected from different areas of Dhaka city was from 8×10⁶ cfu/ml to 1.0×10⁴ cfu/ml. Another study was conducted by Ohanu et al. (2012) and he expressed an evidence of Coliform contamination in all the samples of sachet and tap water.

3.3. Average count of yeast and mold in milk and water samples

Average count of yeast and mold is another hygiene indicator that reveals the cleanliness of a sample. The results of average Yeast count and Mold count of this study are set out at figure 3 and 4. These results show the hygiene quality of these samples were low as higher number can deteriorate the sensory properties of milk and influence the taste defect and foreign off-flavor. Mohamed et al. (2017) enumerated another study of yeast and molds and count of yeast-mold ranged significantly from 5.28 to 5.96 log cfu/ml and with mean value 5.54 log cfu/ml.

![Figure 3. Average count of yeast in milk and water samples.](image1.png)

![Figure 4. Average count of mold in milk and water samples.](image2.png)

Another study showed that yeasts and molds count of retailer shops milk samples (2.17×10⁴ ± 5.22×10⁵ cfu/ml) was significantly high followed by dairies (1.94×10⁴ ± 3.86×10³ cfu/ml) and dairy farm milk samples (1.80×10⁴ ± 4.42×10³ cfu/ml) (Shah et al., 2016). A study conducted by Mohamed et al. (2017) showed some filamentous fungal species such as Aspergillus spp., Penicillium spp., Fusarium spp. and Mucor spp., and additionally showed presence of some yeasts such as Saccharomyces cerevisiae and Candida spp. This study showed that milk samples were comparatively more microbiologically contaminated than the water samples. Microbial count may be high due to milk in dirty udders, unclean conditions of milking, dirty housing environment and failing to
cool milk rapidly at less than 40 °F. Most water sources in this study were also microbiologically polluted. The effects were attributed to poor source of water protection, poor sanitation and low level of hygiene practices and lack of monitoring and healthcare awareness. Drinking water must be microbes free otherwise it can pose health hazard.

4. Conclusions
Availability and comparative lower price of milk and water in local stores make people to drink them. However, the findings of present study reveal that the water and milk samples are contaminated with microbial load so that these are not good for health. In further study, identification of the organisms makes it more significant to aware peoples about standard regulations as well as legislations to ameliorate their health.

Conflict of interest
None to declare.

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