

Microbiological Quality Assessment of a Local Milk Product, Kwacha Golla, of Bangladesh

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Received April 28, 2008; Accepted September 11, 2008

Different types of milk products, such as kwacha golla, mawa, cheese, curd, and chocolate are popular in Bangladesh. However, the microbiological safety of these products is poorly understood. This study was performed to assess the microbiological quality of kwacha golla, a local milk product. Kwacha golla samples were collected from ten different areas of Rajshahi and Kushtia regions, and the quality of the each sample was assessed using various parameters including standard plate count, total coliform, fecal coliform, total fungi, and spore-forming bacteria, as well as food-borne microorganisms. Out of 300 samples, total coliform was detected at 56.66% (n=300), exceeding the minimum allowable limit of 36.66%. Similarly, experiments were carried out with fungi and food-borne pathogens including *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp., and *Staphylococcus aureus*. Results revealed 85.33, 53, and 49.33% of the samples were contaminated by fungi, *E. coli*, and *L. monocytogenes*, respectively. However, all samples showed no contaminations of *Salmonella* sp. and *Staphylococcus* sp. Therefore, this study could be helpful to the people of Bangladesh by providing information on the possibility of a major health problem caused by the consumption of kwacha golla.

Key words: food-borne pathogens, microbiological quality assessment, milk product

Illness caused by the consumption of contaminated foods has a detrimental impact on the economy and the public health worldwide [Mead *et al.*, 1999]. So far, many pathogenic microorganisms, such as *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* sp., and *Candida* sp., have been reported as the causal agents of food-borne diseases and/or food spoilage [McCabe-Sellers and Samuel, 2004; Anonymous, 2005]. Contamination of raw and/or processed foods usually occurs during the production, sale, and distribution of the foods [Deak and Beuchat, 1996]. Therefore, it is

important to determine the risks associated with the consumption of various milk products. To produce food with a low number of microorganisms, it is necessary to assay not only the final product, but also the processing equipments, packaging, and raw materials.

Public health problems associated with the consumption of unpasteurized cow milk and raw-milk products have been well documented [Cody *et al.*, 1999; Baird-Parker and Bruce Tompkin, 2000; De Valk *et al.*, 2000; De Buyser *et al.*, 2001; Harrington *et al.*, 2002]. Moreover, there is no evidence that the risk from unpasteurized goat's or ewe's milk is any lower [Hutchinson *et al.*, 1985; Kulshrestha, 1990; Allerberger *et al.*, 2001; McIntyre *et al.*, 2002]. Pathogenic microorganisms can gain access to milk either by fecal contamination or through direct excretion from the udder into the milk.

Kwacha golla, a popular sweetmeat in Bangladesh and its neighboring countries, is made with cow milk, buffalo milk or mixed milk through thermal evaporation of the

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Abbreviations: cfu, colony-forming units; LB, lactose broth; LTB, lauryl tryptose broth; MPN, most probable number

milk into 65-70% solids in an open pan. Generally, five times more milk is required for the production of a set amount of kwacha golla. Although the origin of kwacha golla is not known, it has been prepared for centuries in the Indian subcontinent as the base material for sweets. It is sold in city markets and other public places for immediate preparation of food or for later utilization without further processing or preparation [Mosupye and Von Holy, 1999]. Contamination of milk and milk products is largely due to unhygienic conditions. Preparation of these products is mostly based on traditional method without consideration of nutritional and microbiological standards of the raw materials. Such conditions allow many microorganisms easy access to the milk products. The quality of milk product is determined based on the composition and hygiene of the product. Due to its complex biochemical composition and high water activity, milk and milk products serve as an excellent medium for the growth and proliferation of many kinds of microorganisms. Therefore, in the processing of milk product, some of the microorganisms may cause undesirable effects on food or can contaminate the food. Although kwacha golla is a readily available, inexpensive, and nutritious food, several issues were raised over its safety and quality [Bryan *et al.*, 1988; Dawson and Canet, 1991; Moy *et al.*, 1997; Mosupye and Von Holy, 1999]. There is a growing demand for unpasteurized raw milk products by the consumers in Bangladesh. However, no microbiological work on kwacha golla has so far been done. The aim of this study was to assess the microbiological status and the abundance of representative food-borne pathogens such as *E. coli*, *L. monocytogenes*, *S. aureus*, and *Salmonella* sp. in kwacha golla. This study will aid in the evaluation of the general sanitary practices prevailing during processing and handling of foods and determination of the potential source(s) of contamination.

Materials and Methods

Sample collection. Thirty kwacha golla samples were collected from ten different shops located in Kushtia and Rajshahi regions including Mirpur, Kushtia, Varamara, Natore, Irshardi, Poradha, Pabbna, Lalpur, Putia, and Rajshahi towns. Two hundred grams of each sample was collected in separate sterile polythene bag and stored at 4°C in a refrigerator for further use.

Culture method. Each kwacha golla sample (20 g) was weighed aseptically into sterile conical flask and added with 180 mL of ringer solution. The mixture was then homogenized in a blender at 600 rpm for 5-10 min and diluted up to 10⁻⁶ times using sterilized distilled water. All diluted samples were inoculated in three kinds of

culture media (LB, plate count agar, and dextrose tryptone bromo cresol purple agar) and incubated at 37°C for 24 h. After successful growth of microorganisms, the colonies were counted using a colony counter (Yc-2A, Prma Optical Works Ltd., Tokushima, Japan). To isolate the endospore-forming bacteria, LB culture was heated at 80°C for 10 min before being used to inoculate the culture media. Vegetative cells were killed by this treatment, whereas the endospore-forming bacteria survived.

Techniques for the isolation of pure microorganism cultures. Two effective procedures, pour-plate and streak-plate techniques, were used for the isolation and subsequent identification of the microorganisms.

Measurement of growth. Microbial growth was determined by observing the increase in mass or numbers. Analytical procedures were used to determine the cell mass including dry weight, chemical analysis for protein or nitrogen concentration, turbidity, and cloudiness. Viable count, expressed as cfu, is an estimate of the number of living microorganisms in a given material. Only plates with 30 to 300 colonies can be used. cfu was calculated according to the following equation:

$$\text{cfu} = \frac{\text{no of colony/mL} \times \text{Dilution Factor} \times \text{TV}}{\text{Amount of solute dissolve/TV}}$$

where, TV is the total viable count.

Techniques and equipments used to obtain total counts of microorganisms in the materials include the direct microscopic count, proportional counting, counting chamber, and electronic counting devices. For determination of the total mesophilic bacteria count, total coliform, fecal coliform, and the standard procedure of International Commission on Microbiological Specifications for Foods [1986; 2002] methods were used.

Detection of coliform. A three-tube MPN method was performed to identify the coliform bacteria. For presumptive test using LTB, 20 g of the sample was weighed aseptically and diluted up to 10⁻⁶. Each 1 mL of the food homogenate was inoculated into the tubes containing 10, 100, and 1000 mL LTB broth. A Durham tube was placed inversely inside the tube. The whole apparatus was incubated at 37°C for 24-48 h. Tubes that produced gas were put aside, and those showing no gas production were re-inoculated for additional 24 h. Gas-producing samples grown in a brilliant-green lactose bile broth were collected for further confirmation test as follows. One loop full of the presumptive positive broth was added to the fermentation tube containing brilliant green bile broth medium and incubated for 48 h at 35°C. Gas production during incubation indicates positive result of the confirmation test.

Detection of *Salmonella* sp. Twenty-five grams of

each Kwacha Golla sample was diluted with sterile distilled water and inoculated on corn dextrose agar medium and incubated at 37°C for 24-48 h. Pale-colored colony and H₂S production were observed, indicating the presence of *Salmonella* sp.

Detection of *Staphylococcus* sp. Twenty-five grams of each kwacha golla sample was diluted with sterile distilled water and inoculated onto the MacConkey agar (Difco Laboratories, Detroit, Michigan) and incubated at 37°C for 24-48 h. Change of the medium color to yellow indicates lactose fermentation.

Detection of fungi. To select a particular fungus, 10⁻⁶ times diluted samples were inoculated in the potato dextrose agar medium and incubated at room temperature for 48-72 h. The plates were screened for the presence of discrete colonies, and these colonies were counted using a colony counter.

Detection of *E. coli*. The samples were inoculated onto the MacConkey agar (Difco laboratories) and incubated aerobically at 37°C for 24 h. The plates were observed for the growth of *E. coli*. A single, isolated colony was picked and subcultured again on the MacConkey agar for purification of the isolate. Simultaneously, another single colony with similar characteristics was picked, smeared, and Gram-stained for the examination of staining and morphological characteristics of the isolate using a bright field microscope. The cultural characteristics of the isolates were confirmed by inoculating the pure colonies on blood agar, nutrient agar, nutrient broth, and violet red bile agar. Biochemical tests were performed to confirm the presence of *E. coli* using catalase test, Simmon's citrate agar, sugar fermentation on triple sugar iron agar, gelatin liquefaction, indole production, nitrate reduction, urease production, Voges proskaur, methyl red, and presumptive test.

Detection of *Listeria* sp. For the identification of *Listeria* sp. in the food samples, the techniques

recommended by the International Standards Organization were used. Colonies suspected to be *Listeria* were transferred onto the pre-dried plates of trypticase soy-yeast extract agar (Difco, Becton Dickinson and Company, Sparks, MD) and incubated at 37°C for 18-24 h. The putative *Listeria* colonies were characterized using Gram staining, motility, catalase test, characteristics of haemolysis, carbohydrate utilization, and Christie Atkins Munch Peterson test following the standard methods.

Results and Discussion

A series of studies were carried out on the various microbiological parameters of a local milk product kwacha golla collected from Kushtia and Rajshahi regions of Bangladesh. The bacteriological analyses of the milk product samples were performed at Biotechnology and Genetic Engineering Department of Islamic University, Bangladesh, and the experimental results are summarized in Tables 1-3.

The term 'total count' refers to the sum of living and dead organisms. Because viable counting requires incubation time, it may be necessary to determine the total count to estimate the potential viable count. The total count also gives an estimate of the total number of microorganisms to which a substance has been exposed. The highest bacterial count was found in the sample from Putia area (4385.66×10^7 cfu/g), while the lowest was from the Lalpur area (37.0×10^7 cfu/g) sample (Fig. 1).

Total coliform count indicates the hygienic standard and the storage quality of the milk product, rather than the presence of human pathogens. Special care is required during the production and handling of the milk-based products in order to lower the coliform count. Among the ten different study areas, the highest the coliform count was found at Varamara (9.86 MPN/g), whereas the lowest was at Rajshahi town (0.78 MPN/g) (Table 1). On the

Table 1. Microbiological quality assessment of kwacha golla collected from cities of Bangladesh

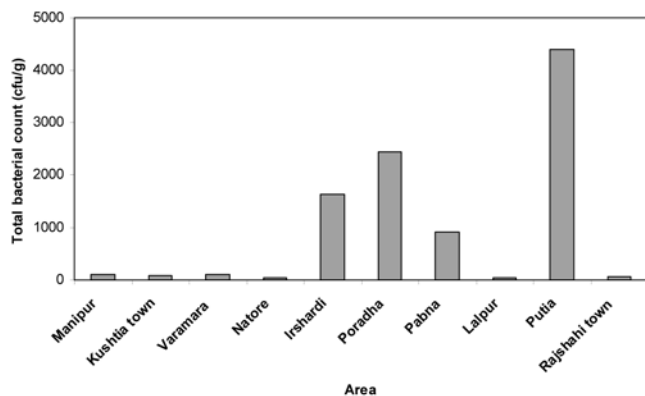
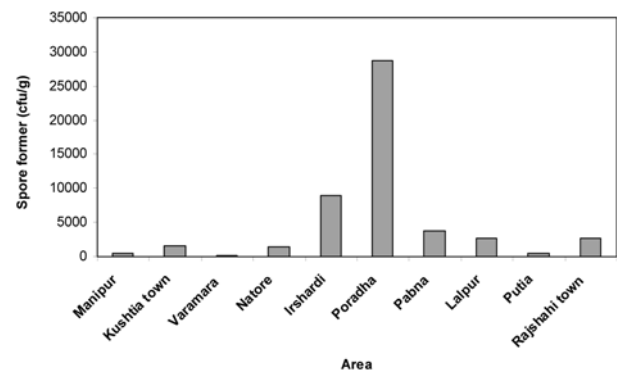
Area	Total bacterial count (cfu/g)	Total coliform count (MPN/gm)	Spore former (cfu/g)	<i>Staphylococcus</i> sp.	<i>Salmonella</i> sp.	Total mold (cfu/g)
Mirpur	97.89×10^7	9.06	487.65×10^3	Nil	Nil	51532.66
Kushtia town	89.33×10^7	2.51	1582.66×10^3	Nil	Nil	15500.52
Varamara	100.63×10^7	9.86	137.56×10^3	Nil	Nil	8186.61
Natore	51.66×10^7	0.00	1462.66×10^3	Nil	Nil	28569.61
Irshardi	1624.33×10^7	0.00	8945.3×10^3	Nil	Nil	21973.97
Poradha	2439.33×10^7	7.63	28754.0×10^3	Nil	Nil	29463.38
Pabna	915.33×10^7	3.12	3746.56×10^3	Nil	Nil	6355.39
Lalpur	37.0×10^7	5.85	2668.42×10^3	Nil	Nil	16564.57
Putia	4385.66×10^7	6.46	435.76×10^3	Nil	Nil	17216.56
Rajshahi town	67.50×10^7	0.78	2684.62×10^3	Nil	Nil	1520.42

Table 2. Overall percentages of *E. coli* and *L. monocytogenes* contamination in kwacha golla samples

Area	No. of collected samples	<i>E. coli</i> contaminated samples	<i>L. monocytogenes</i> contaminated samples	<i>E. coli</i> contamination (%)	<i>L. monocytogenes</i> contamination (%)
Mirpur	30	23	22	76.67	73.33
Kushtia town	30	21	12	70.00	40.00
Varamara	30	09	15	30.00	50.00
Natore	30	17	17	56.67	56.67
Irshardi	30	18	13	60.00	43.33
Poradha	30	16	10	53.33	33.33
Pabna	30	13	09	43.33	30.00
Lalpur	30	15	15	50.00	50.00
Putia	30	19	20	63.33	66.67
Rajshahi town	30	08	15	26.67	50.00

Table 3. Percentage of samples contaminated with different types of microorganisms

No. of total samples	Types of Bacteria/ Fungi	No. of positive samples	Contaminated samples (%)
300	Total coliform	170	56.66
300	Spore-forming bacteria	300	100
300	Fungi	250	85.33
300	<i>Escherichia coli</i>	159	53.00
300	<i>Listeria monocytogenes</i>	148	49.33
300	<i>Staphylococcus</i> sp.	00	00

**Fig. 1. Distribution of total bacteria in different areas.****Fig. 2. Distribution of spore-forming bacteria in different areas.**

other hand, milk product samples from Natore and Irshardi areas were free from coliform contamination (Fig. 4). None of the samples contained fecal coliform. Coliform organisms are present in the non-pasteurized market milk. Coliform count up to 10 MPN/g or /mL is acceptable in the milk products [Wehr and Frank, 2004]. These suggest that kwacha golla sold in various areas can be considered as a hygienic food in terms of coliform count.

The highest count of spore-forming bacteria was obtained from the samples of the Poradha area (28754.0×10^3 cfu/g) and the lowest was from the area of Varamara (137.56×10^3 cfu/g) (Fig. 2). The acceptable standard

count of spore-forming bacteria was $<10,000$ cfu/mL (Nørrung, 2000). The results of the present study showed that the kwacha golla samples from nine areas contained spore-forming bacteria below the standard acceptable level except for the samples from the Varamara area. Therefore, it could be said that kwacha golla milk product sold in most cities were hygienic.

Representative food-borne pathogens including *Salmonella* sp., *Staphylococcus* sp., *E. coli*, *Listeria* sp., and fungi (mold), which cause food-borne illnesses [Kulshrestha, 1990], were assayed. *Salmonella* is a type of a pathogenic organism. Salmonellosis may result following the ingestion of viable cells of the member of the genus

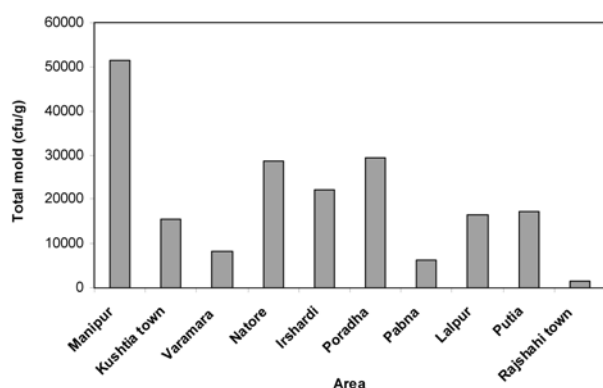


Fig. 3. Distribution of total molds in different areas.

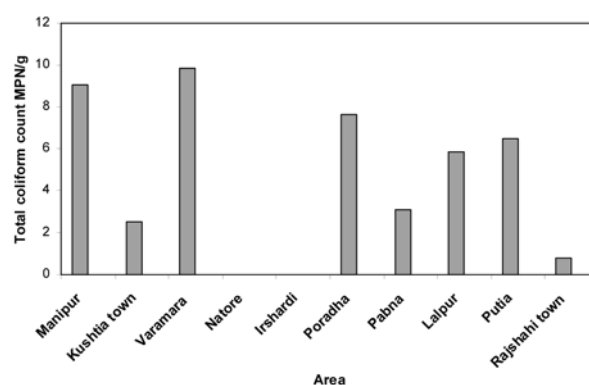


Fig. 4. Distribution of total coliform in different areas.

Salmonella; it is the most frequently occurring bacterial food infection [Barro *et al.*, 2002]. In addition to the typical food-poisoning salmonellosis, typhoid fever and paratyphoid fever can also occur following the consumption of *Salmonella*-contaminated food [Bhan *et al.*, 2005]. One of the most commonly occurring food poisoning incidences is caused by the ingestion of the enterotoxin formed in food during the growth of certain strains of *S. aureus*. The toxin was termed as enterotoxin, because it causes gastroenteritis or inflammation of the lining of the intestinal tract. All samples tested negative for the presence of *Salmonella* and *Staphylococcus*.

Mold growing on foods, with its fussy or cottony appearance, and sometimes colored, is familiar to everyone. Generally, moldy or “mildewed” food is considered unfit to eat. Although molds are involved in the spoilage of many kinds of foods, some molds are useful in the manufacture of certain foods or as ingredients of food [Carroll, 2003]. The highest mold count was found in the kwacha golla samples from the Mirpur area (51532.66 cfu/g), and the lowest count was in the Rajshahi town (1520.42 cfu/g) (Fig. 3). The mold standard plate count was 100 cfu/g [Tarakchi *et al.*, 2003]. Therefore, all milk product samples tested exceeded the maximum allowable level of fungi.

Results of the present study revealed that the kwacha golla samples were contaminated by the two leading food-borne microorganisms, *E. coli* and *L. monocytogenes* (Fig. 4); the highest *E. coli* contamination, 23 out of 30 samples (76.67%), was recorded in the samples from the Mirpur city area, whereas the lowest was found in the samples obtained from Rajshahi town, 8 out of 30 samples (26.67%) (Table 2), indications that kwacha golla products sold in various city markets are highly contaminated with *E. coli*.

Indeed, indigenous sweet products are commonly manufactured and consumed in Bangladesh; the method of production, handling, transportation, and marketing of

the products are entirely depended upon the local traditional system used. Such system could pose a favorable environment for bacterial contamination. The unclean hands of workers, poor quality of milk, unhygienic conditions of the manufacturing unit, and inferior quality of the materials used and water supplied for washing the utensils could be the sources accelerating the bacterial contamination of milk products and the post-manufacturing contamination as well [Kumar and Sinha, 1989; Masud *et al.*, 1989; Kulshrestha, 1990]. However, although *E. coli* is a commonly occurring organism in milk and milk-based products, the presence of *E. coli* in milk and milk products as a possible cause of food-borne disease is insignificant, because *E. coli* normally is a ubiquitous organism [Hahn, 1996].

The presence of *L. monocytogenes*, a food-borne pathogen, can be found in a wide variety of raw and processed foods including milk and dairy products [Rocourt and Cossart, 1997]. The highest *L. monocytogenes* contamination was recorded from the samples of Mirpur city area, which were 22 out of 30 (73.33%) samples, and the lowest contamination, 9 out of 30 (30.0%) samples, was recorded in samples from Pabna city area (Table 2).

L. monocytogenes is responsible for the severe food-borne illness, listeriosis. Listeriosis has recently been recognized to be one of the emerging zoonotic diseases and is contracted mainly through the consumption of *Listeria*-contaminated foods [Kulshrestha, 1990; Farber, 2000; Cordano and Rocourt, 2001]. Increasing evidence suggests that substantial numbers of the human listeriosis cases are attributable to the food-borne transmission of *L. monocytogenes* [Kulshrestha, 1990; Nørrung, 2000]. In the present study, 49.33% of the food samples examined were found to be positive for *L. monocytogenes*, and 85.33 and 53.0% of the samples were contaminated with fungi and *E. coli*, respectively (Table 3). However, all samples were free from the contamination of *Salmonella* sp. and *Staphylococcus*, comparable with the results of

the surveys undertaken in other countries [Uyttendaele *et al.*, 1999; Farber, 2000; Inoue *et al.*, 2000; Cordano and Rocourt, 2001; Dhanashree *et al.*, 2003]. The present study showed the possibility of a significant public health hazard linked to the consumption of foods contaminated with *L. monocytogenes*.

In conclusion the milk product kwacha golla sold in various areas of Bangladesh is safe in terms of spore-forming and coliform bacteria, but not from fungi, *E. coli*, and *L. monocytogenes*, which may cause pathogenicity in the kwacha golla products. As kwacha golla is produced through thermal evaporation of raw milk, contamination could occur during storage and use of unhygienic utensils. Therefore, monitoring the hygienic conditions of the concerned shops is of utmost importance to prevent the microbial contamination.

Steps that should be taken to prevent the microbial contamination of foods include regular monitoring or inspection of the overall hygienic condition following the recommendations of the Bangladesh Standards and Testing Institution and the International Commission on Microbiological Specifications for Foods, as well as an appropriate heat treatment of the food during preparation for eradication the food pathogens in the milk samples.

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