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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Microbiological quality of some common dairy beverages available in Dhaka University campus of Bangladesh

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Abstract: Dairy beverages are nutritious and also highly susceptible to bacterial contamination posing a threat to human health. In recent years, production and consumption of dairy beverages are rapidly increasing in Bangladesh; however, the presence of bacteria including human pathogens in commonly consumed dairy beverages has not been evaluated before. Here, we focused on microbiological quality of some dairy beverages available in Dhaka University campus. We examined 25 samples from five different items locally named as Lassi, Labang, Borhani, Pistachio nut sherbet, and Strawberry milkshake. Non-selective medium PCA and different selective agar media such as MacConkey, SS, EMB, and TCBS, and Cooked Meat were used for isolation. Antibiotic resistance was assessed by disk diffusion method against Ampicillin, Colistin, Ciprofloxacin, Levofloxacin, Ceftriaxon, and Gentamycin. In PCA medium, Labang and Strawberry milkshake, respectively, showed the highest (3.6×10^7 CFU/mL) and the lowest (1.76×10^3 CFU/mL) bacterial count. In MacConkey and EMB media, the highest bacterial growths were, respectively, shown by Pistachio nut sherbet (1.22×10^4 CFU/mL) and Lassi (2.96×10^3 CFU/mL) whereas no growth was observed for Borhani. Strawberry milkshake was found with the highest bacterial prevalence such as *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas* *Bacillus*, *Micrococcus*, and *Streptococcus*



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Nushrat Alam Biva was a research assistant at food microbiology lab led by the pioneer food scientist Dr. Sharmin Rumi Alim, Professor, Institute of Nutrition and Food Science, University of Dhaka. As a dedicated researcher, she kept up her effort, from planning to documentation, while assessing microbiological quality of common dairy beverages available in Dhaka University campus. As an independent researcher, Biva is also engaged with other research teams working with community nutrition. Apart from food analysis, her research interest covers infant and young child feeding (IYCF), nutritional status assessment and nutritional data analysis. She provided effort to evaluate IYCF practices among underprivileged and refugee population in Bangladesh. In a team, her current endeavor consists of reviewing nutrition policy and programs through supporting to literature review and field level investigation. She dreams of conducting large scale nutrition and food related researches at national and international level.

PUBLIC INTEREST STATEMENT

Unlike other areas of the Dhaka city, dairy beverages are widely available and consumed in Dhaka University campus although their microbiological quality was not assessed. In this study, we analyzed five common dairy beverages locally named as Lassi, Labang, Borhani, Pistachio nut sherbet, and Strawberry milkshake and investigated if the samples contained harmful microorganisms and also how these microorganisms react against common antibiotics. We found all beverage samples contained health risk factors although the number and variety of bacteria differed by the types of beverages. It was alarming that bacterial isolates such as *Shigella*, *Aeromonas*, *Micrococcus*, *Bacillus*, *Streptococcus*, and *Proteus* were multi-drug resistant to antibiotics such as Ampicillin and Colistin. However, further research could be carried out to investigate the underlying causes of microbial contamination and also to identify the effective ways for ensuring wholesomeness. Findings suggest relevant stakeholders should be aware of food safety issues to avoid foodborne illness.

whereas Borhani and Labang contained the lowest variety of bacteria. Isolates such as *Shigella*, *Aeromonas*, *Micrococcus*, *Bacillus*, *Streptococcus*, and *Proteus* were multi-drug resistant to Ampicillin and Colistin. Findings indicated that examined samples contained health risk factor. Findings may help relevant stakeholders to consider food safety issues of dairy beverages during processing, marketing, and consumption.

Subjects: Microbiology; Beverages; Dairy Science; Food Microbiology;

Keywords: dairy beverage; contamination; antibiotic resistance; bacteria; Dhaka University

1. Introduction

Dairy beverages are made from milk or a part of milk constituents such as fat globules. Common dairy beverages in Bangladesh include Lassi, Labang, Milkshake, Faluda, Custard, Matha, Borhani, Pistachio-nut sherbet, etc. Dairy beverages in Dhaka University campus are widely available in fast food shops, restaurants, canteens, cafeterias, and street vendors and consumed by the people of all categories in the campus.

Dairy beverages are ideal for adding values in terms of nutrition and functional additives and generally perceived by consumers as nutritious products (Pereira, 2014). However, milk and milk products are highly susceptible to microbial contamination due to high content of nutrients such as minerals, good-quality proteins and fats (Robinson, 2005). Pathogens can be present even in freshly drawn milk and can spread further due to improper treatment (Marth, 1969; Sanaa, Poutrel, Menard, & Serieys, 1993). Further contaminations occur due to poor hygiene and sanitary practices during preparation, marketing, and consumption. Evidence suggests that milk and dairy products in Dhaka city are contaminated with pathogens many of which were found to be resistant to commonly used antibiotics (Banik, Das, & Uddin, 2014; Marjan, Kanta Das, Kishore Munshi, & Noor, 2014). Antibiotic resistance of the pathogens has become an alarming issue in recent years. A growing list of infections such as pneumonia, tuberculosis, blood poisoning, gonorrhoea, and foodborne diseases—are becoming harder, and sometimes impossible, to treat due to antibiotic ineffectiveness. Apart from dairy products, antibiotic-resistant pathogens were identified in a wide variety of food items such as vegetables, fruits, bakery products, juices, meats, fishes, and street foods in Dhaka city (Ali, Khan, & Saha 2011; Mrityunjoy et al., 2013).

Preparation of dairy beverages requires a number of raw materials primarily milk and water. In addition to milk, water quality in Dhaka city also represented high content of pathogens (Acharjee et al., 2011; Islam, Begum, & Nili, 2010; Mahub, Nahar, Ahmed, & Chakraborty, 2011; Talukdar et al., 2013). Use of contaminated raw materials may also pose serious health hazards to the final products (Nicolas et al., 2007). Dairy beverages are popular food items in Dhaka University area and the demand is increasing due to taste and nutritional values. But the consumers are often unaware of the health risks and, moreover, loyal to have their food in their favorite restaurants and places. This study will focus on the presence of pathogens and their drug resistant properties in some common dairy beverages in Dhaka University campus which may be useful for the consumers to choose healthy items and for the policy makers to consider food safety issues.

2. Materials and methods

2.1. Sample collection

A total of 25 samples, five samples from each of five dairy beverage items locally named as Labang, Lassi, Borhani, Strawberry milkshake, and Pistachio nut sherbet were collected randomly from five different nooks and corners of Dhaka University campus and adjacent areas. Samples were collected from canteen, restaurant, fast food shops, and street vendor. The producers used simple technology such as measuring the required ingredients proportionately into a mixing container and mixing accordingly to prepare the final products. The ingredients were mostly measured by eye estimation.

In this study, we collected five samples, prepared in different batches, of a particular beverage items from a particular selling point/shop which was randomly selected from a total of 21 identified selling points/shops in the study area. Samples were taken in sterile containers and were kept in ice-boxes and transported to the laboratory for further examination. Labang, Lassi, and Borhani contain fermented dairy products whereas Strawberry milkshake and Pistachio nut sherbet were non-fermented beverages. It was a general observation that dairy beverages were prepared in unhygienic environment with raw materials such as milk, yogurt, water, ice, syrup, sugar, mint, salts, etc. Samples were collected and analyzed during June to August 2018.

2.2. Sample processing

Samples were taken out from the sterilized container and placed in a sterilized petri-dish by pipetting out 10 mL. One milliliter of liquid sample was taken and transferred into sterilized cotton plugged test tubes containing 9 mL of 0.1% peptone water. Then, they were mixed thoroughly by shaking 20 times. This time the solution was allowed to stand for 5–10 min. Thus, the initial dilution of homogenate was prepared and from this homogenization, further serial dilutions were prepared. The samples were diluted at 10 fold dilution up to 10^{-6} according to American Public Health Association (APHA) sample dilution guidelines (American Public Health Association [APHA], 1992).

2.3. Bacteriological study

For bacterial isolation, the spread plate method was followed in this experiment. To identify the isolated bacteria; cultural, morphological and biochemical characteristics were studied using Bergey's Manual of Determinative Bacteriology, 9th Edition (Holt, Krieg, Sneath, Staley, & Williams, 1994). Different types of non-selective and selective agar such as Plate Count Agar (PCA) for viable count, MacConkey (MC) agar for gram-negative enteric bacteria, *Salmonella-Shigella* (SS) agar for different species of *Salmonella* and *Shigella*, Eosine-Methylene Blue (EMB) agar for coliform bacteria, and Thiosulphate Citrate Bile Sucrose (TCBS) agar selective for *Vibrio* were used for isolation. Cooked Meat Media was used for *Clostridium* and *Listeria*, and Potato Dextrose Agar (PDA) was used for observing fungal growth. From each tube of serially diluted sample suspension, 50 μ L was transferred to the petri-dishes and tubes which were prepared with PCA, MacConkey, EMB, SS, TCBS, and PDA and were incubated for 24–48 h at 37°C. The bacterial colonies grew on different types of medium were collected and maintained in nutrient slant agar for further analysis. Morphological, cultural, gram staining and some biochemical tests such as Kligler's Iron Agar (KIA) test, Motility test, Indole test, Urea (MIU) test, Catalase and Oxidase tests were performed for the identification of bacterial isolates.

2.4. Antibigram

Total 34 isolates were found among which 27 were gram-negative bacteria and seven were gram-positive. Gram-negative isolates of bacteria from five samples were selected for antibiotic susceptibility test. Six common antibiotics such as Ampicillin (AMP10), Colistin (CT10), Ciprofloxacin (CIP5), Levofloxacin (LE5), Ceftriaxon (CRO30), Gentamycin (GEN10) were used for each type of bacteria to observe the sensitivity and resistance toward antibiotics. Finally, Multiple Antibiotic Resistance (MAR) index was calculated as the ratio of number of antibiotics to which the isolate showed resistance to total number of antibiotics to which the isolate was exposed (Krumperman, 1983). The MAR index is a good tool for health risk assessment which identifies if isolates are from a region of high or low antibiotic use. A MAR index with 0.2 indicates a "high-risk" source of contamination (Rochell & Paul, 2016).

3. Results

3.1. Colony morphology, phenotypic, and biochemical traits

After 24 h of incubation, total viable count determined the estimation of total coliforms in the samples. Green metallic sheen on naked eye indicated the presence of *E. coli* on EMB plate. Red opaque colonies on SS agar plate primarily indicated *Shigella*. Biochemical tests gave the confirmation for the identification of bacterial isolates.

We found a wide range of viable count (from zero to 3.6×10^7 CFU/mL) in the samples using differential media (Table 1). All samples but Pistachio nut sherbet, which represented maximum growth in MacConkey, represented maximum bacterial growth in PCA medium. The highest bacterial load was calculated for Labang (3.6×10^7 CFU/mL) in PCA whereas no growth was shown by Borhani in MacConkey and EMB media.

All samples exhibited bacterial growth in Cooked Meat Agar but not in TCBS media (Table 2). Lassi, Labang and Borhani are fermented products thus the total colony count in PCA might be partially contributed by starter culture. Other media such as MacConkey, EMB, SS, and Cooked Meat Agar showed contamination. However, the highest fungal growth was observed in Pistachio nut sherbet in PDA media whereas no fungal growth was observed in Labang.

Different biochemical tests were conducted to identify bacterial species in the samples. We found the highest variety of bacterial species was present in Strawberry milkshake whereas Borhani and Labang contained the lowest variety of bacteria (Figure 1).

All the samples but Pistachio nut sherbet contained both Gram-positive and Gram-negative type of bacteria whereas only Gram-negative bacteria was identified in Pistachio nut sherbet. However, *E. coli* and *Pseudomonas* was the most common in all the samples. *Lactobacillus* was found in fermented products as it was present in the starter culture.

3.2. Antibiotic susceptibility and Multiple Antibiotic Resistance (MAR) index

Disk diffusion method was used to observe antibiotic resistance properties of the isolates. Bacteria which showed no clear zone or very small diameter (<7 mm) of clear zone indicated resistance against the antibiotic used. Clear zone with a diameter of 7 mm and more than 7 mm, respectively,

Table 1. Bacterial count (CFU/mL) in the samples using PCA, MacConkey and EMB media

Sample	PCA	MacConkey	EMB
Lassi	4.96×10^5	5.2×10^3	2.96×10^3
Strawberry milkshake	1.76×10^3	1.18×10^3	1.32×10^3
Borhani	7.4×10^4	No count	No count
Pistachio nut sherbet	5.36×10^3	1.22×10^4	2.56×10^3
Labang	3.6×10^7	5.0×10^2	6.6×10^2

*Note: Average value was considered while calculating the bacterial count.

PCA: Plate Count Agar, EMB: Eosine-Methylene Blue.

Table 2. Detection of the presence of bacteria in the samples by SS, TCBS, cooked meat media and fungus by PDA medium

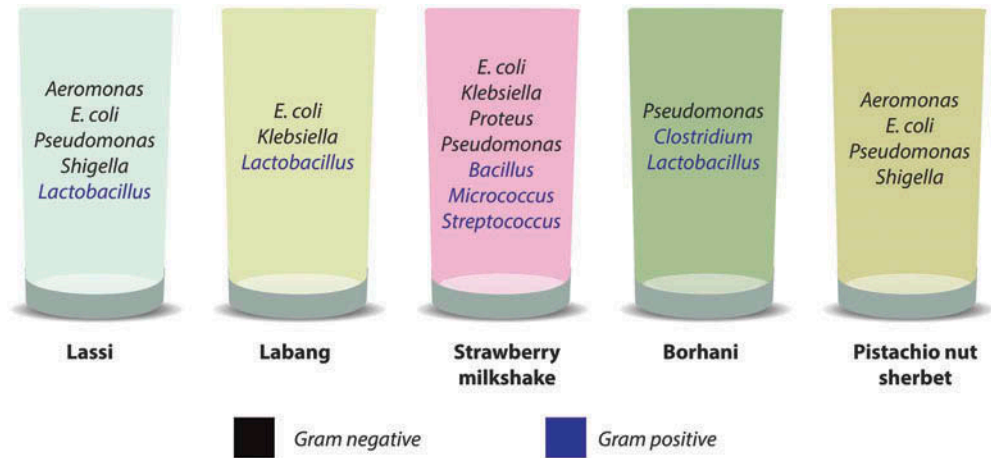
Samples	SS	TCBS	Cooked Meat	PDA
Lassi	++*	-	++	+
Strawberry milkshake	+	-	+++	++
Borhani	-	-	++	++
Pistachio nut sherbet	+++	-	+++	+++
Labang	+	-	+++	-

*Note: + for low growth, ++ for moderate growth, +++ for high growth, and— for no growth.

Mode value was considered while calculating the degree of microbial growth.

SS: *Salmonella-Shigella*, TCBS: Thiosulphate Citrate Bile Sucrose, PDA: Potato Dextrose Agar.

Figure 1. Identified bacterial isolates in the studied dairy beverages.



indicated the intermediate sensitivity and full sensitivity to the antibiotics. We found most of the isolates showed resistance against both Ampicillin and Colistin (Table 3). Antibiotics other than Ampicillin and Colistin were found to be effective against all the bacterial isolates.

The Multiple Antibiotic Resistance (MAR) index of the 27 gram-negative isolates was measured to see the ability of the isolate to resist the antibiotics used in our study. In our study, MAR values indicated that every isolate showed resistance to at least one antibiotic. We found *E. coli* and *Pseudomonas* showed the highest resistance against Colistin, a third generation antibiotic. The lowest MAR value (16.67%) was calculated for *Shigella* whereas all other bacterial isolates such as *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Shigella*, and *Aeromonas* showed the same MAR value (33.33%).

4. Discussions

In our study, total viable count in PCA medium was ranged from 1.76×10^3 CFU/mL to 3.6×10^7 CFU/mL. In MacConkey agar medium, the growth of bacteria was ranged from zero (no growth) to 1.22×10^4 CFU/mL and in EMB medium it was from zero (no growth) to 2.96×10^3 CFU/mL. All samples showed turbidity and gas formation in Cooked Meat medium. On the other hand, no growth was observed in TCBS. Except Borhani, all other samples showed positive growth in SS which means *Salmonella* or *Shigella* was present. Except Labang, other samples showed fungal growth in PDA medium. As all five samples of a particular dairy beverage item were collected from a particular selling point/shop, there was no big difference in total microbial count despite the samples were produced in different batches. There are other researches which employed similar sampling process that we have followed in this study (Khan, Islam, Chowdhury, & Alim, 2015; Uddin, Motazzim-ul-Haque, & Noor, 2011). Research showed some juices and sherbets sold by the street vendors in Dhaka University campus area contained potential microbial hazards whereas the use of unhygienic water and raw materials, insanitary surroundings and equipment, fruit flies and airborne dust were reported as the risk factors of contamination (Khan et al., 2015).

Evidence showed microbiological examination of different dairy products such as lassi, mattha, and yogurt in Dhaka city represented a total viable bacterial load ranging from 10^2 – 10^4 CFU/mL which is very similar to our findings that ranged 10^3 – 10^5 CFU/mL in Lassi, and 10^3 – 10^4 CFU/mL in Pistachio nut sherbet (Malek, Akter, Ahmed, & Uddin, 2015). Our findings showed the high contamination in most of the samples by *E. coli*, *Pseudomonas*, *Bacillus* spp, *Streptococcus* spp, *Klebsiella* spp, and *Aeromonas* spp. Other studies also reported similar findings that milk and dairy product samples in Dhaka city were contaminated with yeast and bacteria including *Bacillus subtilis*, *Bacillus cereus*, *Streptococcus pyogenes*, *Streptococcus* spp and *E. coli* indicating that the poor hygiene practices have not been improved over the time (Uddin et al., 2011).

Table 3. Resistance of the bacterial isolates against the examined antibiotics

Isolates	Gram characteristics	Antibiotics							
		AMP	CIP	CT	LE	GEN	CRO		
<i>E. coli</i> (9)*	-	3	0	5	0	0	0	0	
<i>Klebsiella</i> (5)	-	3	0	2	0	0	0	0	
<i>Proteus</i> (1)	-	1	0	0	0	0	0	0	
<i>Pseudomonas</i> (4)	-	2	0	3	0	0	0	0	
<i>Shigella</i> (2)	-	0	0	1	0	0	0	0	
<i>Aeromonas</i> (5)	-	3	0	3	0	0	0	0	
<i>Micrococcus</i> (1)	+	1	0	1	0	0	0	0	
<i>Bacillus</i> (1)	+	1	0	1	0	0	0	0	
<i>Streptococcus</i> (1)	+	1	0	1	0	0	0	0	
<i>Clostridium</i> (1)	+	0	0	1	0	0	0	0	

*Prevalence of bacteria in the samples.

AMP: Ampicillin, CIP: Ciprofloxacin, CT: Colistin, LE: Levofloxacin, GEN: Gentamycin, CRO: Ceftriaxon.

We found the presence of multi-drug resistant microorganisms in different samples. Although all the strains showed sensitivity to Ciprofloxacin, Gentamycin, Levofloxacin and Ceftriaxon, resistance was observed against either Ampicillin or Colistin. Resistance against Colistin, a third generation antibiotic, is alarming because it insinuates inappropriate use of drug which might cause the development of resistance in other types of bacteria through horizontal gene transfer by which an antibiotic resistance gene can be the agent of an outbreak by transferring resistance to multiple unrelated pathogens (Huddleston, 2014; Klümper et al., 2015). Other researchers identified that raw milk samples and dairy products in Dhaka city contain pathogens which were resistant to multiple drugs (Bhowmic, Saha, & Khan, 2006; Malek et al., 2015; Marjan et al., 2014). Another study revealed that *E. coli* isolated from raw milk exhibited 100% resistance against Rifampin and Tetracycline (Uddin et al., 2011). In our study, we identified resistance against both Ampicillin and Colistin by *E. coli*, *Pseudomonas*, *Klebsiella*, *Proteus*, *Shigella*, *Aeromonas*, *Micrococcus*, *Bacillus*, and *Clostridium*.

It is mentionable that no microbial growth was observed in Borhani using MacConkey and EMB media. Besides, Borhani and Labang also represented the lowest variety of bacterial content. The reasons may be the products were fermented and the antimicrobial properties of raw materials such as spices, herbs, and mint used in their preparation (Ceylan & Fung, 2004; Johnson, Wesely, Kavitha, & Uma, 2011; Shylaja & Peter, 2004). However, further investigations could be carried out to observe the antimicrobial properties of other beverage products prepared by using these types of raw materials.

Not only in Bangladesh but food safety is also becoming a serious global concern, for the global burden of food born diseases is following an increasing trend (Devleesschauwer, Haagsma, Mangen, Lake, & Havelaar, 2018; Rahman, Sultan, Rahman, & Rashid, 2015). For ensuring food safety, an approach such as Food Safety Act-2013 which has already been approved deserves appreciation. However, evidence showed that there were repeated failures to translate the food safety regulations into practices (Ali, 2013a; Ali, 2013b). To prevent such bacterial and fungal contamination, importance should be given on Good Hygiene Practices (GHP) as defined in the CODEX document on “General Principles on Food Hygiene” in combination with HACCP, the basis for safe food production (Codex Alimentarius, 1997). Necessary steps should be taken by Government health and food safety agencies. Dairy beverages producers and vendors should be educated on proper hygienic production and handling. Available data from different countries could be reviewed to identify the best solution for controlling foodborne illness as well as antibiotic-resistant pathogens in dairy beverages.

5. Conclusion

Apart from good nutritional value, a variety of tastes and availability make the dairy beverages a popular choice in Dhaka University campus. It is highly deserved that these products should be free from harmful microorganisms. Findings demonstrate that examined dairy beverage samples contained pathogens which are capable of producing foodborne illness. Some pathogens were multi-drug resistant that could be considered as an alarming public health issue. Drinking of such contaminated dairy beverages might cause illness to consumers. There are scopes to further researches for investigating the underlying causes of contamination and also the possible ways to ensure the hygienic production and marketing. It is recommended that relevant stakeholders should be aware of food safety issues of dairy beverages to avoid any possible foodborne illness in the future.

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Competing interests

Authors declare no competing interest.

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Supplemental Material

Supplemental data for this article can be accessed [here](#).

Correction

This article has been republished with minor changes. These changes do not impact the academic content of the article.

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