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Article

Bacteriological quality of dry powder milk available in local markets of Bangladesh

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Abstract: Milk and milk powders are very rich in several nutrients and relished by everybody throughout the world. The present study was undertaken with the aim of investigating the bacteriological quality of locally available dry powder milk in Bangladesh. A total number of eight powder milk samples were collected from Gazipur city and its surrounding areas during the period from January 2017 to February 2017. The analysis comprised of enumeration of total viable bacterial count (TVBC), isolation of bacterial isolates and identification of pathogenic bacteria. Almost all the powder milk samples showed the total aerobic heterotrophic bacterial (TAHB) level above the standard acceptable range (>10^4 CFU/g). Both gram positive and gram negative pathogenic bacteria viz. *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus, pseudomonas* sp. and *Enterobacter* sp. were isolated from studied samples through morphological and biochemical characteristics. *Pseudomonas* sp. were confirmed by API 20E. These results highlighted the necessity to maintain appropriate sanitary and hygienic measures at each critical point in order to safeguard consumers from food borne pathogens.

Keywords: pathogenic bacteria; powder milk; commercial local market; identification

1. Introduction

Dairy powders are a popular commodity due to their long shelf life, ease of storage and versatile nature. As milk and dairy products are very rich in several nutrients, they are relished by everybody throughout the world. Variety of dairy powders can be produced such as whole milk powder (WMP), skimmed milk powder (SMP), whey protein concentrate (WPC), whey protein isolate (WPI), milk protein concentrate (MPC), milk protein isolate (MPI), casein and caseinates (Lagrange *et al*., 2015). Dairy powders can be used in fortification of other dairy products (Karam *et al*., 2013), as well as an ingredient in a wide array of foods including soups and sauces, confectionary (Sharma *et al*., 2012), infant formula, sports dietary supplements and in foods for health recovery (Gill *et al*., 2001; Lagrange *et al*., 2015). Moreover, the advantages of dry milk over liquid milk are better keeping quality, less storage space, and low shipping costs (Robert *et al*., 2015). However, when controlling microbial loads of dairy powders, the increased production may create safety and economic risks to the dairy sector.

Milk powder is made by removing water from liquid milk. Removal of water is necessary to reduce water activity for the prevention of microorganism growth. Powder milk has a long shelf life than raw milk. Skim milk powder has a maximum shelf life of about 3 years where as whole milk powder has a maximum shelf life of about 6 months (Flegam and Oluwaniyi, 2015). Now a days, a great emphasis is given on adding value to powder milk. The important quality parameters for milk powder are microbiological quality, and sensory characteristics, beside physical and chemical properties, which are mainly concerned with the content of moisture, fat, total protein, and non-protein nitrogen, lactose, titratable acidity, ash, and other nutrients such as calcium (Laszlo, 2007). The thermophilic organisms have ability to produce extremely heat resistant spores and

can have significant economic consequences when they exceed specification limits. Thus, they may result in down grading of the products (Anup and Rupesh, 2012).

Microbial pathogens which are of major concern in dried milk include *Bacillus cereus, Staphylococcus aureus* and *Salmonella*. These organisms may remain viable in milk powder for long period of time, and resume growth when the powder is reconstituted and stored at favorable temperature (Hafsa *et al*., 2013). Study performed in New Zealand for sample of milk powder revealed that it is contaminated by *Bacillus licheniformis and Bacillus subtilis* (Ronimus *et al*., 2006). Another study of sample from different countries were examined, the dominated isolate was *Anoxybacillus flevithermus* followed by *Bacillus licheniformis* (Rucket *et al*., 2004).

In our country in Bangladesh, the microbiological quality of dry milk powder is not known. There is no quality control system for the powder milk though they are popularly consumed by all age groups both in urban and rural areas. So, the present study was undertaken to assess the microbiological quality of powder milk, enumeration of the bacterial load, isolation and identification of the pathogenic bacteria from powder milk samples.

2. Materials and Methods

2.1. Sample collection

Eight powder milk samples were collected from different local market of Gazipur city and its surrounding area. The packets were cleaned by washing with sterile water followed by rubbing with 95% alcohol and opened in the laminar air flow using sterile scissors. All packaged samples were maintained with their expiration dates. The samples were preserved at 4ºC for detailed study.

2.2. Isolation of bacteria

Bacterial enumeration and isolation was carried out by spread plate method in Nutrient Agar media (NA) (Eklund and Lankford, 1967) at adjusted pH 6. The microbiological condition of safety and hygiene were then assayed using the methods recommended by International Commission on Microbiological Specifications for Foods (ICMSF, 2005). The sample (0.1ml) of each dilution was taken onto each sterile petridish and evenly spread on the solid nutrient medium and incubated at 37°C for 24 hours. After incubation, plates having well discrete colonies were selected for counting. Discrete bacterial colonies were isolated immediately after counting. Based on distinct colony morphology, further selection was made and isolates were purified by repeated streaking as well as stored in NA slants at 4°C for further analysis.

2.3. Microbial load determination

Microbial load was determined from the total number of discrete colonies counted after incubation. Isolated colonies were counted in colony formation unit (CFU per gram) as follows-Number of CFU/ g = Number of CFU/ (Volume plated in ml × total dilution used)

2.4. Identification of the isolates

The selected bacterial colonies were observed to study various characters *viz.* color, form, elevation, margin, surface, optical characters etc. according to Eklund and Lankford (1967). Bacterial colonies were cultured on different selective and differential media such as: MSA, SSA, EMB, MacConkey, Bouillon agar, King's B, Simon citrate etc. Different biochemical tests (Casein test, Fermentation test, Indole test, Starch hydrolysis test, Catalase test etc.) were also performed. Results of the physiological and biochemical tests of selected isolates were analyzed following Bergey's Manual of Systematic Bacteriology (Sneath *et al*., 1986), Bergey's Manual of Determinative Bacteriology (Buchanan *et al*., 1974), Manual of Microbiological Methods (SAB, 1957), Microbiological Methods (Collins *et al*., 1984) and Understanding Microbes (Claus, 1995). Coagulase test, endospore staining and API 20 E (Holmes *et al*., 1978) were used as a confirmation test of bacterial genus or species of *Staphylococcus aureus, Bacillus* sp. and *Pseudomonas* sp.

3. Results

A total of 24 bacterial isolates was obtained from powder milk samples. Mean heterotrophic bacterial load of the milk samples were ranged from $9.9x10^3$ to $32.7x10^4$ CFU/g on Nutrient Agar media. In case of powder milk, maximum heterotrophic bacterial counts were observed in the Sample-3 (Table1).While minimum heterotrophic bacterial counts were observed in the Sample-8 (Table1). According to guidelines elaborated by the International Commission on Microbiological Specifications for Foods (ICMSF, 2005), the total bacterial count in powder milk below 10^4 CFU/g is indicative of their acceptable quality, the counts of 10^4 to10^5CFU/g indicates their permissible quality, whereas bacterial count exceeding 10^5CFU/g is unacceptable. In view of

these guidelines, CFU results showed marginally acceptable quality of the analyzed milk samples (Table1). All the powder milk samples exceeded the acceptable limits except one. But none of them exceeded the unacceptable limit.

In the present study, *Staphylococcus aureus* (Plate1B), *Staphylococcus epidermidis* (Plate1B), *Bacillus* sp., *Enterobacter* sp. and *Pseudomonas* sp. were identified through their respective color in specific culture media. In addition, results of biochemical tests (Table3) of different isolates revealed that out of 24 isolates 18 isolates were gram positive while the rest were gram negative (Plate1D) and rod shaped. Among the gram-positive isolates 12 were cocci (Plate1C) and 6 were rod shaped. All the isolates were catalase positive, casein negative, lactose fermentation negative and indole negative. Out of 24 isolates only 8 isolates were positive in starch hydrolysis test (Plate1E) and 6 were positive in endospore staining which showed the presence of *Bacillus* sp. Only 6 isolates showed positive result in citrate test (Plate1F). Among 12 cocci shaped bacteria 9 were positive for coagulase test which confirmed the presence of *Staphylococcus aureus*. API 20 E test confirmed the presence of *Pseudomonas* sp.

Table 1. Bacterial load of different samples.

Samples	CFU (average)	m*	M*
Sample-1	$25x10^4$		
Sample-2	$22x10^4$		
Sample-3	$32.7x10^4$		
Sample-4	$8.3x10^4$	10^4	10^5
Sample-5	$19.9x10^4$		
Sample-6	$15.3x10^4$		
Sample-7	$3.4x10^4$		
Sample-8	$9.9x10^3$		

*m, acceptable level and values above it are marginally acceptable or unacceptable in the terms of the sampling plan and *M, a microbiological criterion which separates marginally acceptable quality from defective quality according to ICMSF. Bacterial load represents as CFU/g

Table 2. Bacterial isolates on different selective and differential media.

Bacterial isolates	MSA	EMB	Mac-conkey	SSA	Bouillon	Simon citrate	Kings B	BGA
PP 1	NG	Colorless	Pink	Pink	White	Blue	White	NG
PP 2	NG	NG	Pink	NG	White	Blue	White	NG
MP 3	Yellow	Colorless	NG	NG	White	NG	White	NG
MP 4	NG	Colorless	NG	NG	White	NG	White	NG
FP 5	NG	Colorless	Colorless	NG	White	NG	Greenish Yellow	NG
FP 6	Pink	NG	NG	NG	White	NG	White	NG
MP 7	Yellow	NG	NG	NG	White	NG	White	NG
DD 1	NG	Colorless	Pink	Pink	White	Blue	White	NG
DD 2	NG	NG	Pink	NG	White	Blue	White	NG
DD 3	NG	NG	NG	NG	White	NG	White	NG
DD 4	Yellow	NG	NG	NG	White	NG	White	NG
DD 5	NG	NG	Pink	NG	White	Blue	White	NG
NP 1	Pink	NG	NG	NG	White	NG	White	NG
NP 2	Yellow	NG	NG	NG	White	NG	White	NG
NP 3	Yellow	NG	NG	NG	White	NG	White	NG
NP 4	NG	NG	NG	NG	White	NG	White	NG
NP5	NG	NG	NG	NG	Off white	NG	White	NG
NP 6	NG	NG	NG	NG	Off white	NG	White	NG
NP 7	NG	NG	NG	NG	Off white	NG	White	NG
NP 8	Yellow	Colorless	NG	NG	White	NG	White	NG
NP 9	Yellow	NG	NG	NG	White	NG	White	NG
NP10	Yellow	NG	NG	NG	White	NG	White	NG
NP11	Pink	NG	NG	NG	White	NG	White	NG
NP12	Yellow	NG	NG	NG	White	NG	White	NG

NG* No Growth

Table 3. Biochemical characteristics of the bacterial isolates.

Isolates	Catalase test	Casein test	Fermentation test	Indole test	Starch hydrolysis	Gram staining	Endospore staining	Coagulase test
PP 1	+	-	-	-	-	-	NA	NA
PP 2	+	-	-	-	+	-	NA	NA
MP 3	+	-	-	-	+	+	-	+
MP 4	+	-	-	-	+	+	+	-
FP 5	+	-	-	-	-	-	NA	NA
FP 6	+	-	-	-	-	+	-	-
MP 7	+	-	-	-	+	+	-	+
DD 1	+	-	-	-	+	-	NA	NA
DD 2	+	-	-	-	-	-	NA	NA
DD 3	+	-	-	-	+	+	+	-
DD 4	+	-	-	-	+	+	-	+
DD 5	+	-	-	-	-	-	NA	NA
NP 1	+	-	-	-	-	+	-	-
NP 2	+	-	-	-	-	+	-	+
NP 3	+	-	-	-	-	+	-	+
NP 4	+	-	-	-	-	+	+	-
NP5	+	-	-	-	-	+	+	-
NP 6	+	-	-	-	-	+	+	-
NP 7	+	-	-	-	+	+	+	-
NP 8	+	-	-	-	-	+	-	+
NP 9	+	-	-	-	-	+	-	+
NP10	+	-	-	-	-	+	-	+
NP11	+	-	-	-	-	+	-	-
NP12	+	-	-	-	-	+	-	+

*NA – Not Available

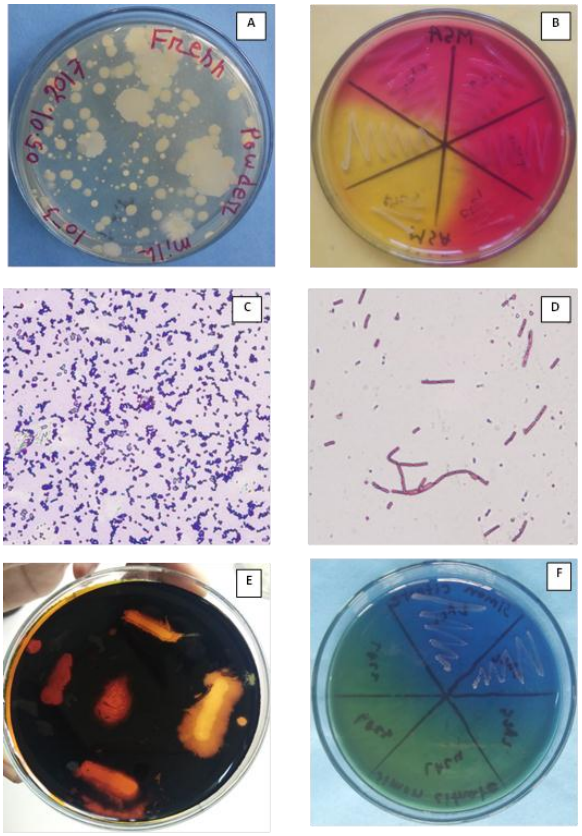

Plate 1. Bacterial colonies on nutrient agar (A), *Staphylococcus aureus* (yellow colonies) and *S. epidermidis* (pink colonies) on MSA media (B), gram staining represents gram positive (cocci shaped) *S. aureus* (C) and rod shaped gram negative *Enterobacter* (D), starch hydrolysis test (E), Simon citrate test (F).

4. Discussion

The knowledge of microbes and their evolution is highly imperative to ensure safety and quality of dairy products (Ahmed *et al*., 2014). Numerous studies have documented that powder milk could be contaminated by bacteria. The microbial count of dried milk powder has been reported by Salahudin and Naural (2006). Common contaminants identified in dairy powders include species of Bacilli, many of which are capable of forming endospores (Checinska *et al*., 2015). Taxa other than Bacilli have also been found to contaminate powdered dairy products includes *Clostridium halophilum, Klebsiella oxytoca* (Buehner *et al*., 2015), *C. perfringens, C. septicum, C. novyi/haemolyticum, C. sporogenes* (Barash *et al*., 2010), *Staphylococcus aureus* (Zhang *et al*., 2015), and *Cronobacter sakazakii* (Minami *et al*., 2012). These findings are in agreement to our study where we identified *Staphylococcus aureus, S. epidermidis, Enterobacter* sp., *Pseudomonas* sp. and *Bacillus* sp. from different samples. Similar finding was also reported by Hafsa *et al*. (2013). Pal (2011) identified *Staphylococcus aureus* which can be present in the cow's udder and teats, and consequently contaminate the milk.

The sources of microbial contamination of dry milk powder are many. Microorganisms that contaminate powder milk may come from air, dust, soil, water, insects, humans, storage containers and handling and processing equipment. Bacteria can originate from the soil (Heyndrickx, 2011), feces, bedding, feed, or milking equipment (Gleeson *et al*., 2013), or can enter the raw milk via contaminated teats, milking cups and bulk tanks. Additionally, contamination can occur during transport from the farm to the processing plant (Pantoja *et al*., 2011), and also within the processing facility itself from poor handling and contaminated equipment (Burgess *et al*., 2010; Faille *et al*., 2014). High numbers of microorganisms in the raw milk may result high numbers in the milk powder, and the decline in numbers as a result of exposure to heat, is offset by the removal of water in the powder (Ron *et al.*, 2006). Thus, improper cleaning and sanitation of dairy equipments will lead to food poisoning due to contamination by microbes (Pal and Mahendra, 2015). In addition, the formation of homogeneous or heterogeneous multicellular bacterial communities on the surface of processing equipment in the form of biofilms is a particular concern for the dairy processing sector and, when present, can lead to recurring problems of microbial contamination (Branda *et al*., 2001; Faille *et al*., 2014).

On the other hand, it has been shown that the spore-forming bacterial composition of raw milk differs considerably from their associated dairy powders (Miller *et al*., 2015), highlighting that the processing of milk into powder changes the composition of the specific spore-formers present. Post-production, powders can be stored for extended periods and in the absence of water, bacterial metabolic activity and growth is limited (Deng *et al*., 2012), thus preventing spoilage and product defects. However, under these conditions, bacterial spores can remain dormant until more favorable conditions are encountered, when germination and outgrowth can proceed (Setlow, 2003, 2014). In spray-drying the amount of heating to which contaminating bacteria may be exposed are insufficient for the drying process to decontaminate the milk even in respect of relatively heat-sensitive pathogens such as *Salmonella*. Besides, post-processing contamination of the powder milk from food production environments occur. Therefore, milk for spry-drying should be pasteurized first and care should be taken that there is no possibility of recontamination between pasteurizer and drier (Harrigan, 1998).

5. Conclusions

From the present study it can be concluded that dry powder milk can be frequently contaminated by pathogenic and spore forming bacteria. Both pre pasteurization and post pasteurization contamination occur. Therefore, low cost, simple and easy methods should be developed to detect the spoilage producing bacteria in milk and milk products. Such methods would be very beneficial to poor resource countries like Bangladesh. All milk and liquid product should be pasteurized prior to concentration before drying and post processing contamination should be checked. Finally, it is imperative to monitor every step of food production, from handling of raw products to preparation of finished foods in order to protect consumers from food borne illness.

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Conflict of interest

None to declare.

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