



## ISOLATION OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* FROM FULL CREAM POWDER MILK SOLD UNDER MARKET CONDITIONS AT DHAKA, BANGLADESH AND THEIR ANTIBIOTIC SUSCEPTIBILITY

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### ABSTRACT

Milk and milk products are ideal foods for all age groups in both rural and urban people all around the world. This study reports microbiological status of powder milk samples and antibiotic susceptibility pattern of *E. coli* and *Staphylococcus aureus* isolated from powder milk samples collected from different area of Dhaka, Bangladesh. Twelve samples were collected and seven of them were found acceptable according to codex Alimentarius and ICMSF in terms of total viable count and total coliform. *E. coli* was isolated from 11 samples and *Staphylococcus aureus* was isolated from 6 samples. *E. coli* isolated were resistant to 5 antibiotics and *Staphylococcus aureus* isolates were resistant to 6 antibiotics. Hygienic conditions during production and post-processing should be improved according to HACCP (Hazard Analysis and Critical Control Points) guidelines to improve the microbiological quality and safety of powder milk products.

**Keywords:** Powder milk, Quality, antibiotic susceptibility, *Escherichia*, *Staphylococcus*

### 1. INTRODUCTION

Milk is a compulsory part of daily diet for the expectant mothers as well as growing children. Milk is valued because it is an important source of many of the nutrients essential for the proper development and maintenance of the human body [1]. Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatment contamination [2]. Undesirable microbes that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms, lactic acid bacteria, yeasts, and molds. In addition, various bacteria of public health concern such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphylococcus aureus* may also be found in milk and dairy products [3]. For this reason, increased emphasis should be placed on the microbiological examination of milk and dairy foods. Microbiological analyses are critical for the assessment of quality and safety, conformation with standards and specifications, and regulatory compliance [4].

Pathogenic bacteria in milk have been a major factor for public health concern since the early days of the dairy industry. Many diseases are transmissible via milk products. Traditionally raw or unpasteurized milk has been a major vehicle for transmission of pathogens [5]. The health of dairy

herd and milking conditions basically determine the milk quality. Another source of contamination by microorganisms is unclean teats. The use of unclean milking and transport equipments also contributed to the poor hygienic quality [6]. Although the microorganisms in full cream milk powder owing to their low moisture content cannot grow and thus do not play any direct role in their spoilage, their occurrence in these products is of great significance they serve as an index of hygienic standards maintained during Production, Processing, packaging and handling [7].

This study investigates the microbiological quality of powder milk sold in local market of Dhaka City, Bangladesh and to isolate *E. coli* and *Staphylococcus aureus* from powder milk and to determine their antibiotic susceptibility pattern.

### 2. MATERIAL AND METHODS

#### 2.1. Study area and sample collection

Twelve full cream powder milk sample of different brand were collected for microbial analysis from Dhaka city. The sampling period was from March 2012 to May 2012. Milk samples were collected in sterile container (bottles). Samples were transported to the laboratory without delay and preserved in a cool and dry place till analysis.

#### 2.2. Preparation of samples

Serial dilutions of samples were made up to  $10^{-7}$  in sterile normal saline.

### 2.3. Enumeration of microorganisms

Each milk sample was tested for total bacterial count, total coliforms count (TC), *E. coli* count (FC), total staphylococcal count (TSC) and yeast and mold count (YMC). The total viable bacterial count was carried out by the pour plate technique according to ISO/DIS 4833-1:2009. Total coliform count (TCC) was performed in most probable number technique according to ISO 4831:2006. *E. coli* count (EC) was done by most probable number technique according to ISO 7251:2005. Total staphylococcal count was done according to ISO 6888-1:1999 and Yeast and Mold count was done according to ISO 6611:2004.

#### 2.3.1. Isolation of *E. coli* and *Staphylococcus aureus*

25 gm milk powder sample was added to 225 ml buffered peptone water and incubated at 37°C overnight. For the isolation of *E. coli*, the enriched sample was cultured on selective medium Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hours. Morphologically typical colonies (at least 4/plate) producing metallic sheen were taken into nutrient agar for further identification. For isolation of *S. aureus*, enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37°C for 24-48 hours. Appearances of jet black colonies surrounded by white halo were considered to be presumptive *S. aureus* [8].

#### 2.3.2. Identification of *E. coli* and *Staphylococcus aureus*

The shape and type of Gram reaction are microscopically studied using 18 hour culture from agar plate. The biochemical tests involved Klingler's Iron (KIA) Agar, Simmon's Citrate Slant, Motility Indole Urease (MIU), Lysine Iron agar (LIA), Urea broth, Peptone water, Methyl Red (MR), Voges Proskauer (VP), Nutrient Nitrate Broth (NB), carbohydrate fermentation test was done for lactose, sucrose, glucose and starch, Oxidase, and Catalase tests. Identification of isolates obtained in pure culture was based on Gram staining, biochemical characteristics and growth pattern on selective and differential media and; according to the procedures recommended in the Bergey's Manual of Determinative Bacteriology [9, 10].

#### 2.3.3. Antibiotic susceptibility testing

Antibiotic susceptibility was tested by the standard agar disc diffusion technique [11] on Mueller Hinton agar using commercial discs (Oxoid, UK). The following antibiotics with the disc strength in parentheses were used: Ciprofloxacin (Cip, 5µg), Gentamycin (Gen, 10µg), Nalidixic acid (Nal, 30µg), Nitrofurantoin (Nit, 300 µg), Cefuroxime (Cem, 30µg), Azithromycin (Azt, 15 µg), Imipenem (Imp, 10µg), Cefixime (Cep, 30µg), Ceftriaxone (Cef, 30µg), Cotrimoxazol (Cot, 25µg), Ampicillin (Amp, 10µg), Erythromycin (Ery, 15µg). A control strain of *E. coli* ATCC 25922 was included in each

plate. Antimicrobial breakpoints and interpretation were taken from the CLSI standards [12].

## 3. RESULTS

### 3.1. Total Viable Bacterial Count (TVBC)

The highest total viable bacterial count ( $2.36 \times 10^7$  cfu/gm) was found in sample no-1, collected from Uttara and lowest total bacterial count was  $7.3 \times 10^2$  cfu/gm, which had been collected from Mohammadpur (sample 9) (Table 1). The variation in TVBC of the milk may be due to the hygienic maintenance during production.

### 3.2. Total Coliform Count (TCC)

The presence of coliform bacteria in milk is a common indicator of fecal contamination. The highest coliform bacterial count was found in sample no. 1, collected from Uttara (460 MPN/gm) and lowest total coliform count was 39 MPN/gm which was collected from Asulia (sample 12) (Table 1).

#### 3.2.1. *E. coli* count

*E. coli* was isolated from 11 milk samples out of 12 powder milk samples. The highest *E. coli* count was found in sample no. 10, collected from Mirpur (53 MPN/gm) and lowest total coliform count was <3 MPN/gm which was collected from Jatrabari (sample 2) (Table 1).

### 3.3. Total Staphylococcal Count (TSC)

Coagulase positive staphylococci may cause human disease through the production of toxins. The formation of effective levels of toxin requires a high number of microorganisms (approximately  $10^5$ - $10^6$  microorganisms per ml of food). In this experiment, staphylococci were found in 6 powder milk samples out of 12 tested samples. The highest staphylococcal count was found in sample no. 1, collected from Uttara ( $3.6 \times 10^3$  cfu/ml).

### 3.4. Yeast & Mold count

All the milk samples were contaminated with yeast and molds indicating lack of hygiene in production and post-processing. The highest yeast and mold count was found in sample no. 11, collected from Savar ( $4.31 \times 10^3$  cfu/gm) and lowest yeast and mold count was  $2.5 \times 10^2$  cfu/gm which was collected from Jatrabari (sample 2) (Table 1).

### 3.5. Isolation and Identification of *E. coli* and *Staphylococcus aureus*

*E. coli* was isolated from 11 samples and *Staphylococcus aureus* was isolated from 6 powder milk samples. All *E. coli* isolates showed positive reaction in citrate utilization, Voges-Proskauer and catalase test and negative reaction in indole, methyl red, H<sub>2</sub>S production, motility, urease and oxidase test,

those characteristics are typical of *E. coli*. All the *Staphylococcus aureus* isolates showed positive reaction in methyl red, voges-proskauer and catalase test and negative reaction in indole, H<sub>2</sub>S

production, citrate utilization, motility, urease and oxidase test which are typical biochemical characteristics of *Staphylococcus aureus*.

**Table 1: Microbiological quality of full cream milk powder of 12 different brands**

Sample	Total Viable Bacterial Count (cfu/g)	Total Coliform Count (MPN/g)	Total <i>Staphylococcus</i> Count (cfu/g)	<i>E. coli</i> count (MPN/g)	Yeast and Mold count (cfu/g)
1	2.36x10 <sup>7</sup>	460	3.6x10 <sup>3</sup>	24	1.1x10 <sup>3</sup>
2	1.9x10 <sup>5</sup>	150	<10	<3	2.5x10 <sup>2</sup>
3	4.4x10 <sup>4</sup>	95	<10	11	6.23x10 <sup>2</sup>
4	3.5x10 <sup>3</sup>	93	1.5x10 <sup>2</sup>	35	2.36x10 <sup>2</sup>
5	4.7x10 <sup>3</sup>	64	<10	44	7.2x10 <sup>2</sup>
6	6.1x10 <sup>6</sup>	120	4.3x10 <sup>3</sup>	15	3.62x10 <sup>3</sup>
7	3.4x10 <sup>3</sup>	75	<10	9.4	8.3x10 <sup>2</sup>
8	1.7 x 10 <sup>3</sup>	95	2.2 x 10 <sup>2</sup>	6.1	6.9x10 <sup>2</sup>
9	1.5 x 10 <sup>5</sup>	160	2.8 x 10 <sup>3</sup>	75	2.36x10 <sup>3</sup>
10	5.8x10 <sup>4</sup>	64	<10	53	3.5x10 <sup>3</sup>
11	8.1x10 <sup>5</sup>	160	1.8x10 <sup>2</sup>	39	4.31x10 <sup>3</sup>
12	7.3x10 <sup>2</sup>	39	<10	9.3	3.6x10 <sup>2</sup>

**Table 2: Antibiotic susceptibility of isolated *E. coli* and *Staphylococcus aureus***

Name of Antibiotic	<i>E. coli</i> (% resistant)	<i>S. aureus</i> (% resistant)
Ciprofloxacin (Cip, 5μg)	25	33
Gentamycin (Gen, 10μg)	30	28
Nalidixic acid (Nal, 30μg)	85	67
Nitrofurantoin (Nit, 300 μg)	23	29
Cefuroxime (Cem, 30μg)	79	58
Azithromycin (Azt, 15 μg)	18	22
Imipenem (Imp, 10μg)	22	25
Cefixime (Cep, 30μg)	42	48
Ceftriaxone (Cef, 30μg)	55	47
Cotrimoxazol (Cot, 25μg)	36	32
Ampicillin (Amp, 10μg)	83	72
Erythromycin (Ery, 15μg)	88	69

### 3.6. Antibiogram of the Isolates

Antibiotic resistance has increased worldwide that leading to failures in treatment of human infectious diseases. Resistance against antibiotics by pathogenic bacteria is a major concern in the anti-infective therapy of both humans and animals. The Kirby-Bauer disk diffusion test was used in this experiment to determine whether the isolated organisms were susceptible or resistant to a selection of antimicrobial agents. Antibiotic resistance pattern of *E. coli* and *Staphylococcus aureus* isolates are shown in table 2.

*E. coli* isolates were resistant to nalidixic acid (85%), cefuroxime (79%), ceftriaxone (55%), ampicillin (83%) and erythromycin (88%). *Staphylococcus aureus* isolates were resistant to nalidixic acid (67%), cefuroxime (58%), cefixime

(48%), ceftriaxone (47%), ampicillin (72%) and erythromycin (69%).

## 4. DISCUSSION

Milk powder is used for many purposes including making ice cream, curd, custard, pudding and other milk-based food preparation irrespective of its use in normal liquid milk preparation and even for the preparation of infant food in some instance [13]. Recent and reliable data about the microbiological quality of dried milk powder in Bangladesh is lacking though many previous studies reports contamination of raw milk, liquid milk and other milk products with pathogenic microorganisms [1, 2]. In view of the above, a microbiological study had been conducted in order to investigate the microbiological quality of dry milk powder that is available in local market of Dhaka city of Bangladesh.

Total viable count ranged from  $7.3 \times 10^2$ - $2.36 \times 10^7$  cfu/gm. According to Codex Alimentarius [14] guideline, powder milk should contain less than  $5 \times 10^4$  cfu/gm & according to ICMSF [15] (International Commission on Microbiological Specifications for Foods) it should contain less than  $3 \times 10^4$  cfu/gm. In this study, seven out of twelve samples were acceptable in terms of total viable count.

Total coliform count of the samples ranged 39-460 MPN/gm. ICMSF recommends coliform count less than  $10^2$  MPN/gm. The seven samples acceptable in terms of total viable count were found to contain acceptable range of coliform.

Eleven out of the 12 samples were found contaminated with *E. coli* & 6 out of 12 samples contained *Staphylococcus aureus*. Total *Staphylococcus* count ranged  $1.5 \times 10^2$ - $3.6 \times 10^3$  cfu/gm & *E. coli* count ranged 9.3-53 MPN/gm. Yeast & mold count ranged  $2.36 \times 10^2$ - $4.31 \times 10^3$  cfu/gm. There is no specification yet been set by the Codex or the ICMSF in this regard, however, the presence of high number of staphylococci in dry powder milk indicates poor post-processing sanitation since their presence is often used as a post-processing hygienic indicator.

Amongst food poisoning organisms, *S. aureus* and *E. coli* were isolated. This could be due to water used in manufacture, unhygienic hawking habits, storage environment and not necessarily failure of GMP. *S. aureus* has been linked to gastroenteritis by producing enterotoxins, boils, skin infections, (pneumonia, deep abscesses and meningitis in debilitated persons). Eleven *E. coli* and 6 *Staphylococcus aureus* were isolated and identified by biochemical tests. All the isolated *Staphylococcus aureus* were coagulase-positive.

*E. coli* isolates were resistant to nalidixic acid (85%), cefuroxime (79%), ceftriaxone (55%), ampicillin (83%) and erythromycin (88%). *Staphylococcus aureus* isolates were resistant to nalidixic acid (67%), cefuroxime (58%), cefixime (48%), ceftriaxone (47%), ampicillin (72%) and erythromycin (69%).

From the present study, it can be concluded that the microbiological quality of most of the powder milk samples collected from different areas of Dhaka city were satisfactory in terms of total viable count and total coliform count but some pathogenic bacteria such as *E. coli* and *Staphylococcus* spp were detected from the samples. The presence of *S. aureus* and *E. coli* will render milk unfit for human consumption, since sufficient number of these organisms will cause infection and intoxication [16]. Multiplication and production of *S. aureus* would however, depend upon environmental factor like time, temperature, relative humidity and duration of storage and food factors, potential water activity (aw), moisture contents, nutrients present, additives used and associated microflora like

*S. aureus* and coliforms and fecal coliforms. Frequent use of antibiotics should be stopped as antibiotic resistant strains are continuously increasing. Because *S. aureus* is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents, the presence of this bacterium or its enterotoxins in full cream powdered milk is an indication of poor sanitation, post processing contamination. *E. coli* has been linked to diarrheal diseases, urethrocystitis, prostatitis, pyelonephritis [16].

It was apparent from the present study that microbiological quality of some of the dry milk products was acceptable although most of the samples tests were contaminated with *Staphylococcus*. The international agencies like the Codex and the ICMSF did not fix any limit for *Staphylococcus* in dry milk. Since, powder milk is consumed mainly by children in Bangladesh, therefore, a Standard Sanitation Operating Procedure (SSOP) should be maintained, which is a prerequisite program of Hazard Analysis and Critical Control Point (HACCP), in order to minimize the risk of contamination for safety purpose.

## 5. CONCLUSION

There is no microbiological limit in Bangladesh standard for milk powder (BDS CAC 207: 2008). In terms of International guidelines such as Codex Alimentarius and ICMSF, powder milk products of studied in this study are moderately acceptable though presence of *Staphylococcus* indicates lack of hygiene in production. Hygienic conditions during production and post-processing should be improved according to HACCP (Hazard Analysis and Critical Control Points) guidelines to improve the microbiological quality and safety of powder milk products.

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