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Original Research Article

# Characterization of Pathogenic Bacteria from Raw and Pasteurized Milk of Different location of Dhaka city in Bangladesh

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**Abstract:** The aim of study was to characterize the bacteriological quality of locally available raw milk and pasteurized milk. A total number of thirty two raw milk and pasteurized samples were collected from Dhaka city and its surrounding areas. For the determination of sanitary, total viable bacterial count (TVBC), total coliform count (TCC) and total staphylococcal count (TSC) analysis was performed. The highest TVBC, TCC and TSC were 6.8 x 105 cfu/ml, 85cfu/ml & 4.5x105 cfu/ml, respectively. In order to observe the morphological characteristics, biochemical characteristics and milk quality, gram staning, MR, VP, Indole, Citrate, Urease, Catalase, Oxidase and Starch hydrolysis was carried out. All the sample from raw and pasteurized milk gave starch hydrolysis, oxidase and indole negative result but catalase positive. Indicating the presence of S*higella dysenteriae, Klebsiella pneumonia, Salmonella spp. Proteus spp. Klebsiella spp. Salmonella spp, E.coli Pseudomonas spp*. This survey indicates that most of the raw milk samples were not satisfactory in course of public health standard as some pathogenic bacteria were detected from these samples. Proper processing regarding pasteurization recommended to milk samples. Attention must be paid to save the raw milk as well as the pasteurized milk from the thread of milk quality related problems and public health concern.
**Keywords:** Pasteurization, raw milk, *Staphylococcus*, *E. coli* and *Salmonella species*.

## INTRODUCTION

Milk is one of the widely consumed products. Milk contains carbohydrates, proteins, lipids and other organic compounds and inorganic salts in water. Milk is a suitable medium for contamination by microorganisms and support the rapid growth and multiplication of bacteria at favorable temperatures. There are different types of bacteria which deteriorate the quality of milk. The genera *Achromobacter*, *Aerobacter*, *Alcaligenes*, *Escherichia*, *Flavobacterium*, *Pseudomonas*, and *Vibrio* are the most commonly found as members in milk product [1-3]. It is necessary to use very great care in the collection and handling of milk samples to prevent any extraneous contamination and to control the growth of organisms during transportation and storage of the milk. Temperature has a key role in the spoilage of milk [4]. If milk is produced in poor hygiene conditions then it contains increased numbers of psychrotrophic bacteria in the total microbial population [5, 6] and under low temperature contain proteinases and lipases of psychrotropic bacteria undergoes spoilage of milk product [7]. The psychrotrophs are readily killed by HTST pasteurization which is the most effective method of reducing the risk of contamination and spreading of disease. Depending on the efficiency of pasteurization process the shelf life of pasteurized milk is determined [8]. The activation of the thermoresistant enzymes or spores is responsible for repeat contamination takes place after pasteurization from same bacteria as raw milk after heat treatment [9-12]. For the growing children and expectant mothers daily diet milk is an essential part and is nutritious food for human beings. So the presence of pathogenic bacteria can harm the whole community. For this reason harmful bacteria characterization is a burning question in recent days throughout the world. Bacteriological safety of milk continues to be a topic of concern in the dairy industry and public health communities. In order to provide safe and healthy milk products, the Hazard Analysis and Critical Control Points (HACCP) system should be implemented starting from milk collection, through processing and storage. Microbial exposure assessments are critical components of the risk analysis [13]. The main aim of the study was to isolate and identify the bacteria in milk, and to enumerate the bacteria in milk, and eventually to check the quality of milk.

## MATERIALS AND METHODS

### Sample collection

The samples were collected [14] into sterile bottle and preserved in the deep freeze at the temperature of -4$^{o}$C. Milk samples were transported in polythyne paper to keep cool in transportation. The

samples were diluted with sterile normal saline. One successive dilution was inoculated in plates. The plates for the determination of TBC were incubated at 37°C for 24 h.

**Isolation of pathogenic organisms**

Raw milk and pasteurized milk samples were collected in sterile sample bottles. Samples were streaked on the surface of the Eosin methylene blue and Nutrient agar plates and Mannitol salt agar plates described by [15]. The plates were incubated at 37°C for 24-48 hrs, pure bacterial culture were isolated from each milk samples**.**

**Gram stating**

One loop full isolated colony was taken and staining and reagent were applied as described by [16].

**Methylene Blue Reductase test**

Methylene Blue Dye Reduction Test, commonly known as MBRT test is used as a quick method to assess the microbiological quality of raw and pasteurized milk [16].

**Identification of isolates by biochemical test**

The pathogenic bacteria isolates were confirmed by MR, VP, indole, urease, Citrate Utilization test, Oxidase test, Catalase test and Starch hydrolysis test according to Bergey's manual of determinative bacteriology [17].

**RESULTS**

**Isolation of bacteria from culture plate**

Presence of different types of bacteria found in culture plates. Highest number of coliform count observed followed by *Staphylococcus, E. coli* and *Salmonella species*. Microbial studies were carried out for the detection of total viable count (TVC), coliforms and staphylococcus. The twenty five raw milk samples were examined in present study. In raw milk sample one highest number of viable bacterial count, coiliform count and staphylococcus count was found. In addition, pasteurized milk sample TVBC is same for the entire sample, but total Coliform count was higher for milk vita sample and Total staphylococcus count was higher for farmfresh milk sample.

**Table 1: Percentage of bacterial count**

| Sl no. | Types of microorganisms (cfu/g) | Percentage (%) |
|---|---|---|
| 1 | Coliform Count | 100 |
| 2 | Staphylococcus species | 40 |
| 3 | Salmonella species | 33 |
| 4 | E. coli | 40 |

**Table 2: Total bacterial count in different culture media**

| Sample | Total viable bacterial count (TVBC) | Total coliform count (TCC) | Total staphylococcus count (TSC) |
|---|---|---|---|
| Raw milk 1 | $6.8 \times 10^5$ | 85cfu/ml | $4.5 \times 10^5$ |
| Raw milk 2 | $4.8 \times 10^5$ | 65 cfu/ml | $2.3 \times 10^5$ |
| Raw milk 3 | $5.2 \times 10^5$ | 72cfu/ml | $3.2 \times 10^5$ |
| Raw milk 4 | $2.4 \times 10^5$ | 34cfu/ml | $0.3 \times 10^5$ |
| Arong | <10cfu/ml | 1 | 3 |
| Milk Vita | <10 cfu/ml | 6 | 2 |
| Farm fresh | <10cfu/ml | 3 | 4 |
| Pran | <10cfu/ml | 5 | 2 |

**Methylene Blue Reductase test**

Methylene Blue Dye Reduction Test is based on the fact that the blue colour of the dye solution added to the milk get decolourized when the oxygen present in the milk get exhausted due to microbial activity. The sooner the decolourization, more inferior is the bacteriological quality of milk assumed to be. It was found that pasteurized milk sample from arong and farm fresh was fair quality; on the other hand milk from pran and milk vita showed poor and very poor quality respectively given in table 3.

**Table 3: Result of Methylene blue reductase test**

| Sample name | Reduction time (h) | Grade |
|---|---|---|
| Arong | 4 | Fair |
| Farm fresh | 3 | Fair |
| Pran | 1 | Poor |
| Milk vita | ½ | Very poor |

**Biochemical test result**

All the sample from raw and pasteurized milk gave starch hydrolysis, oxidase and indole negative result but catalase positive. The result of all biochemical tests are showed in table 4.

**Table 4: Biochemical test result**

| Sample | MR test | VP test | Indole test | Citrate test | Urease test | Catalase test | Oxidase test | Starch hydrolysis test | Organisms name |
|---|---|---|---|---|---|---|---|---|---|
| Raw-1 | + | - | - | - | - | + | - | - | ***Shigella dysenteriae*** |
| Raw-2 | - | + | - | + | + | + | - | - | ***Klebsiella pneumoniae*** |
| Raw-3 | + | - | - | + | - | + | - | - | ***Salmonella spp.*** |
| Raw-4 | + | - | - | + | + | + | - | - | ***Proteus spp.*** |
| Milk vita | - | + | - | + | + | + | - | - | ***Klebsiella spp.*** |
| Arong | + | - | - | + | - | + | - | - | ***Salmonella spp*** |
| Pran | + | + | - | - | - | + | - | - | ***E.coli*** |
| Farm fresh | - | - | - | + | - | + | - | - | ***Pseudomonas spp.*** |

**Bacterial growth on different Media plates**

**Fig-1: Growth of isolates on Eosin Methylene blue agar (EMB) and Plate count agar (PCA)**

**Fig-2: Urease test, Citrate test, Oxidase test and Catalase test**

## DISCUSSION

Milk and milk products are ideal foods for all age groups in both rural and urban people all around the world. Milk might also be contaminated with pathogenic bacteria or bacterial toxins which may serve as vehicle for the transmission of diseases to humans such as salmonellosis, diarrhea, food poisoning, tuberculosis etc. Psychrotrophic bacteria remain a significant economic problem for the dairy industry [18-20]. Still some microorganisms are used in the preparation and preservation of milk products such as yogurt; cheese etc. [21]. In this experiment we have examined two eighty milk samples. One hundred and forty are raw milk from different dairy farm of Dhaka city and another one hundred and forty samples collected from milk processing company of Bangladesh, as Milk Vita, Farm fresh, Arong and Pran . The result of the study indicated that most of the sampled dairy cows were reared under unclean environmental conditions and poor udder preparation [22]. Raw milk might be contaminated from manure, soiled bedding and soil [23].

According to the study it can be said that presence of microorganisms in the pasteurized milk was less then raw milk, in methylene blue reductase test was performed for detection of the quality of milk sample, pasteurized milk sample (Arong) was found to be of fair quality, milk vita sample was found very poor Quality and its reduction time was 30min, adoption of good sanitation practices and application of HACCP will definitely improve the quality of milk. From the result it can be conclude that after increasing temperature and timing in heating process, the growth of microorganisms were decreasing. Pasteurization value was increased with increasing heating time. Finally, it can be said, milk is contamination by different types of microorganisms might be reducing by adequate milking and transport measurement [24]. Robinson [25] suggested that prior to using detergents, it is essential that the equipment be washed with cold water hot water repeatedly to remove contaminants. The high presence of Escherichia coli in the milk samples imply that fecal contamination could have occurred and subclinical ill cows might have served as the causes of the microbial contamination. Higher incidences of different pathogenic microorganisms were observed in raw as well as pasteurized milk.

Proper processing regarding pasteurization recommended to milk samples. Attention must be paid to save the raw milk as well as the pasteurized milk from the thread of milk quality related problems and public health concern. Further research is needed to make the strategies of eliminating the problems related to raw milk as well as pasteurized milk.

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