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Prevalence of *Staphylococcus aureus*, *Klebsiella spp.*, *Escherichia coli* and *Pseudomonas spp.* in some common Bangladeshi milk and dairy products

Partha Paul¹, Md. Zahid Al Hasan¹, Arup Kumar²,
Mohammad Hasan Chowdhury^{3*}, Md. Jayed Chowdhury⁴,
Nafisa Habib Purba³, Moumita Chakma³, Shah Md. Aatur Rahman¹

¹Department of Microbiology, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh, ²Materials Science Division, Atomic Energy Centre, 4-Kazi Nazrul Islam Avenue, Shahbag, Dhaka-1000, Bangladesh, ³Department of Food Technology and Nutrition Science, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh, ⁴Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

ABSTRACT

The study was done to isolate *Klebsiella spp.*, *Staphylococcus aureus*, *Escherichia coli* & *Pseudomonas spp.* from raw milk, processed milk (powdered milk & ultra-high-temperature milk) and dairy products (mango milk, and ice cream). During 3 months' time span of study, a total of ten samples were gathered where eight of the samples powdered milk (2), ultra-high-temperature milk (2), ice cream (2) & mango milk (2) were gathered from different places of Bangladesh & other 2 samples (raw milk) were collected from a cattle farm & individual households in & around Noakhali city. The samples were gathered under aseptic safety measures and were followed by plating on non-selective media (Nutrient broth) & selective media (Mannitol Salt Agar & Macconkey agar). The presumptive *Klebsiella spp.*, *Staphylococcus aureus*, *Escherichia coli* & *Pseudomonas spp.* isolates were identified by biochemical tests. Analysis of the result discovered that out of a total of 10 samples; raw milk (2), processed milk i.e. powdered milk (2), ultra-high-temperature milk (2) and dairy products i.e. mango milk (2) and ice cream (2) resulted in the isolation of 8 isolates (2.5%) of *S. aureus*, (12.5%) of *Klebsiella spp.*, (2.5%) of *Pseudomonas spp.* & (2.5%) of *Escherichia coli*. Results recommended a probability of potential public health threat of *Staphylococcus aureus*, *Klebsiella spp.*, *Escherichia coli* & *Pseudomonas spp.* resulting from contamination of milk and dairy products with pathogenic microorganisms is mainly due to unhygienic practices and conditions in the handling, production, and processing.

KEYWORDS: Raw milk, *Escherichia coli*, bacteria, Bangladesh

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*Corresponding Author:
Mohammad Hasan
Chowdhury
Email: mdhsnchowdhury@gmail.com

INTRODUCTION

Food-borne diseases (FBD) are characterized by the World Health Organization (WHO) as diseases of infectious or toxic nature brought about by or thought to be caused by the utilization of water or food. The pathogenesis of bacteria causing food-borne poisoning relies upon their capacity to produce toxins after ingestion (in the digestive tract) or intoxication (ingestion of preformed toxins in foodstuff) [1]. Among the bacteria predominantly engaged with these diseases, *Staphylococcus aureus*, *Klebsiella spp.*, *Escherichia coli* & *Pseudomonas spp.* are the main source of gastrointestinal infections resulting from the consumption of contaminated food.

Milk and dairy products are the prime habitats to complex microbial ecosystems; these are in charge of the wide varieties of taste, aroma, and texture of milk and dairy products. Contamination of milk and dairy products with pathogenic microorganisms is mainly due to handling, processing, and unhygienic condition. The presence of these pathogenic bacteria in milk and dairy products may pose a major health threat since the complex chemical composition and high nutritional content are hugely susceptible to a variety of microorganisms [2]. Many contaminants find their way to raw milk, from which they obtain access to dairy products [3,4].

Staphylococcus aureus is extraordinarily adapted to cause infection in humans. Around 50 % strain of this organism can

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deliver enterotoxins related to food poisoning [5]. Disease through *S. aureus* run from minor skin infection such as boils, pimples, cellulitis, toxic shock syndrome, impetigo, and abscesses to life-endangering disease such as endocarditis, pneumonia, meningitis, and septicemia [2]. Gastroenteritis is self-limiting with the person getting better in eight to twenty-four hours. Side effects include vomiting, nausea, diarrhea, and abdominal pain [6]. Chapaval et al. seen the formation of staphylococcal enterotoxins in milk when milk was stored at 37°C to 42°C temperatures or when introduced to variations in temperature [7]. On heating at normal cooking temperature, the bacteria may be put to death but the toxins remain active [8]. Staphylococcal enterotoxins are exceedingly resistant and are believed to be more heat resistant in foodstuffs than in a laboratory culture medium [9]. Some virulent *E. coli* can even guide to urinary tract infections, diarrheal disease, neonatal meningitis, diarrheal disease, and gastroenteritis [10]. They harm the host in a few stages: colonizing the intestinal mucosal surface, evading the defenses by the host, and ultimately multiplying themselves in numbers [11]. *Klebsiella* is deft pathogens and can give rise to extreme illnesses, for example, pneumonia, septicemia, urinary tract infection (UTI), and soft tissue infection. The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract (GIT) and the hands of a dairyman. On account of their capacity to spread quickly in the cattle farm environment, these microscopic organisms tend to cause severe diseases [12]. Milkers' hands, a surface of cows' cooling tanks, teats, and teat cups are related to contamination of raw milk with *Pseudomonas spp.* on farms with manual and mechanical milking systems, showing that regardless of the kind of milking system and season, proper hygiene procedures of equipment, utensils, and workers' hands are essential to avoid contamination of milk and, accordingly, improve milk quality [13]. The most well-known pathogen in this genus is *Pseudomonas aeruginosa* which causes a wide range of diseases, from easy-to-cure serious infections of burn patients, ear infections, to severe lung infections which lead to significant difficulties in cystic fibrosis patients [14,15]. Besides *Pseudomonas aeruginosa*, other species e.g., *Pseudomonas putida* or *Pseudomonas fluorescens* is likewise a reason for infections in clinical settings [16,17,18,19].

The objective of this research was to declare the occurrence of *Staphylococcus aureus*, *Klebsiella spp.*, *Escherichia coli* & *Pseudomonas spp.* in milk (raw milk, powdered milk & ultra-high-temperature milk) and dairy products (mango milk, and ice cream).

MATERIALS AND METHODS

Collection of Samples

From October 2018 to December 2018, a total of 10 samples which includes raw milk (2 samples), powdered milk (2 samples), ultra-high-temperature milk (2 samples), mango milk (2 samples) and ice cream (2 samples) were collected from groceries, street-vendors, and supermarkets at different cities in Bangladesh. Only raw milk (2 samples) were collected from cattle farms & individual households in & around Noakhali city. The

ice cream & mango-milk samples were got as sold to the public in a cleaned and sanitized refrigerator or deep freezer while raw milk samples were got as sold in the containers available at cattle farms & individual households. The samples were shifted as soon as possible to a laboratory of the Department of Microbiology, Noakhali Science & Technology University, Sonapur, Noakhali with a minimum of delay to be examined.

Isolation and Identification

With slight modification isolation of *S. aureus* was attempted according to Ahaduzzaman et al. [20]. For the isolation of *Staphylococcus aureus*, 0.1ml of a sample was inoculated in plates containing Nutrient Agar and incubated overnight at 37°C. Then sample from Nutrient Agar (HiMedia Pvt. Ltd.) was streaked on Mannitol Salt Agar (MSA) (HiMedia Pvt. Ltd.) and incubated for 24 hours at 37°C. Bacterial growth was observed after overnight incubation and the selection of positive samples colony was carried out based on the morphology of the colony and confirmed by Gram's staining.

With slight modification isolation of *E.coli* was attempted according to Cheesbrough [21]. For the isolation of *E. coli*, 0.1ml of all the samples were cultured primarily in Nutrient Agar (HiMedia Pvt. Ltd.) at 37°C for 18-24 hours, then subcultured onto the MacConkey Agar (HiMedia Pvt. Ltd.) by streak-plating technique and incubated at 37°C overnight. Bacterial growth was observed after overnight incubation and the selection of positive samples colony was carried out based on the morphology of the colony and confirmed by Gram's staining.

With slight modification isolation of *Pseudomonas spp.* was attempted according to Priyadharsini et al. [22]. For the isolation of *Pseudomonas spp.*, 0.1 sample was streaked on to Nutrient Agar (HiMedia Pvt. Ltd.) and MacConkey Agar (HiMedia Pvt. Ltd.) plate and incubated at 37°C for 24 hours. Next day individual colonies were selected and identified based on morphological, cultural, and biochemical characteristics.

With slight modification isolation of *Klebsiella spp.* was attempted according to Bruce et al. [23]. For the isolation of *Klebsiella spp.*, 0.1 sample was streaked on to Nutrient Agar (HiMedia Pvt. Ltd.) and Mac Conkey Agar (HiMedia Pvt. Ltd.) plate and incubated at 37°C for 24hours. Next day individual colonies were selected and identified based on morphological, cultural, and biochemical characteristics.

Morphological Characteristics

The smear was formulated from the isolated culture on a clean grease-free microscopic glass slide and stained with Gram's Method of staining. The stained smear was then observed under a microscope. Smear stated both Gram-positive & Gram-Negative, spherical cells arranged in irregular clusters resembling a bunch of grapes for *Staphylococcus aureus*; spherical cells arranged singly, in pairs, or short chains and sometimes in clusters for *Klebsiella spp.* & spherical cells arranged singly or in pairs for *Pseudomonas spp.* & *Escherichia coli* (Table 1).

Biochemical Examination

Biochemical tests were performed to confirm *Staphylococcus aureus*, *Klebsiella spp.*, *Escherichia coli* & *Pseudomonas spp.* using Catalase test, Oxidase test, Motility Indole Urease (MIU) test, IMViC tests, Nitrate test, Triple Sugar Iron (TSI) test & Carbohydrates tested include glucose, lactose, maltose, mannitol, sucrose, and xylose.

RESULTS & DISCUSSION

Analysis of the result showed that out of a total of 10 samples; raw milk (2), processed milk i.e. powdered milk (2), Ultra High-Temperature milk (2) and dairy products i.e. mango milk (2), ice cream (2) resulted in the isolation of 8 isolates (2.5%) of *S. aureus*, (2.5%) of *Klebsiella spp.*, (12.5%) of *Pseudomonas spp.* & (2.5%) of *Escherichia coli*.

The biochemical identification of *S. aureus*, *Klebsiella spp.*, *Pseudomonas spp.* & *Escherichia coli* were conducted by performing the biochemical test. The biochemical test was conducted as stated on Barger's manual of Determination Bacteriology. In this project, 16 biochemical tests have been done. Based on the result both the major gram-negative & positive pathogenic bacteria have been identified (Table 2).

Staphylococcus Aureus

Staphylococcus aureus could be isolated with percentages of 2.5 from investigated mango milk samples (Table 3).

The presence of *S. aureus* in mango milk may derive from mouth, skin, or nose of workers while handling the food in the processing plant. *S. Aureus* is a good personal hygienic indicator of staff with respiratory suppuration and infection [24]. *Staphylococcus aureus* is one of the key etiological agents of bovine mastitis and a high percentage of food handlers performing in dairy distribution is a nasal carrier of *S. aureus* [25]. The growth of *S.aureus* in food products is a prospective public health hazard since many strains can generate thermostable enterotoxins which can originate food poisoning if ingested as well as enterotoxins that perform on vomiting center in the brain via the vagus nerve [26].

Escherichia Coli

Data recorded in (Table 3) revealed that the prevalence of *Escherichia coli* was 2.5%; from examined raw milk samples.

The public health hazard of *E.coli* organism has been focused by several researchers as they have been implicated in human cases of epidemic diarrhea in infants, sporadic diarrhea, gastroenteritis in children as well as food poisoning [27,28]. The high prevalence of different organisms in the inspected samples may be due to the lack of cleanliness in handling, manufacturing, and distribution and ignorance of sanitary measures during storage. On the other hand, it is predicted that the storage temperature would influence the contamination of the imported samples.

Pseudomonas spp.

Data recorded in (Table 3) revealed that the prevalence of *Pseudomonas spp.* was 2.5 % from examined ice cream.

Table 1: Morphological and culture characteristics of *Staphylococcus aureus*, *Klebsiella spp.*, *Escherichia coli* and *Pseudomonas spp.*

Isolated bacteria	Gram staining	Culture characteristics on selective media
<i>Staphylococcus aureus</i>	Gram positive cocci (in clusters)	MSA: Yellow colonies; may have yellow halo around colonies.
<i>Klebsiella spp.</i>	Gram negative (in short chains)	MAC: Appears pink, large, glistening and mucoid.
<i>Escherichia coli</i>	Gram negative (singly)	MAC: Bright pink or red, flat, convex, dry.
<i>Pseudomonas spp.</i>	Gram negative (singly)	MAC: Colorless, flat, smooth, non-lactose fermenting colonies with regular margin.

Table 2: Biochemical tests for the identification of microbial isolates

Biochemical test		<i>Escherichia coli</i>	<i>Pseudomonas spp.</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella spp.</i>
Catalase		+	+	+	+
Citrate		-	+	-	+
Motility		+	-	-	-
Indole		+	-	-	-
Urease		-	-	-	-
Oxidase		-	+	-	-
Triple sugar iron	Slant	A	K	-	A
	Butt	A	K	-	A
	Gas	+	-	-	+
	H ₂ S	-	-	-	-
MR		+	-	+	-
VP		-	-	+	+
Glucose		AG	A	A	AG
Lactose		AG	-	A	AG
Xylose		A	-	-	-
Mannitol		AG	-	A	AG
Maltose		-	-	A	-
Sucrose		-	-	A	AG
Nitrate		+	+	+	+

+ = positive; - = negative; A=Acid; G=Gas; K=Alkaline.

Table 3: Incidence of some food poisoning microorganisms in the examined samples

	No. of Samples	<i>Escherichia coli</i>		<i>Pseudomonas spp.</i>		<i>Staphylococcus aureus</i>		<i>Klebsiella spp.</i>	
		No. of isolate (s)	Prevalence	No. of isolate (s)	Prevalence	No. of isolate (s)	Prevalence	No. of isolate (s)	Prevalence
Raw milk	2	1	2.5%	-	0	-	0	1	2.5%
Powdered milk	2	-	0	-	0	-	0	2	5%
UHT milk	2	-	0	-	0	-	0	-	0
Ice cream	2	-	0	1	2.5%	-	0	2	5%
Mango milk	2	-	0	-	0	1	2.5%	-	0

As shown by da Silva et al. [29] when considering *Pseudomonas spp.*, milkers' hands are one of the primary sources of milk contamination in properties with manual milking.

Total counts of *Pseudomonas spp.* can be decreased by choosing proper hygiene practices during the milking operation and storage of refrigerated raw milk [29,30]. Since *Pseudomonas spp.* can even influence the processing of ultra-high temperature treated dairy products [31,32], good hygienic practices can contribute to the quality of dairy products and in industrial productivity. To decrease the count of *Pseudomonas spp.* in milk, utensils & milking equipment ought to be cleaned with water of good quality, and disinfected immediately after use [33]. Production of glycosidase, lipase, phospholipase C, and proteinase enzymes in milk strongly damaged the milk fat protein-membrane causing milk rancid, fruity, bitter, off-flavor, and putrid which particularly due to the growth of these organisms in raw milk [34]. Other spoilage defects include slime and pigment production [35]. These unwanted changes render milk of inferior quality and unfit for human consumption or it may possess public health hazards [36,37]. Careful sanitary procedures coupled with the application of hazard analysis of critical control point (HACCP) programs among dairy farms during the production of milk as well as good personal hygiene should be adopted for enhancing its microbial safety.

Klebsiella spp.

Klebsiella spp. could be isolated with percentages of 2.5, 5.0, and 5.0 from examined raw milk, powdered milk & ice cream samples, respectively (Table 3).

Klebsiella spp. are commonly present in the soil, water rumen content, troughs, bedding, feces, holding pens & alleyways on dairy farms [38].

Existence of *Klebsiella spp.* throughout the dairy farm environment could be expected based on its frequent existence in feces [39,40], but participating farmers did not know about this. Fecal contamination of the udder, which may be caused by cow traffic via dirty alleyways and holding pens, is connected with an additional risk of the presence of *Klebsiella spp.* on teat skin during and before milking [41]. Meanwhile, hygiene of holding pens & alleyways should be allowed as an important feature of preventing mastitis due to *Klebsiella spp.*

CONCLUSION

Food poisoning due to *Pseudomonas spp.*, *S. aureus*, *Klebsiella spp.*, & *Escherichia coli* is of main concern in public health

programs worldwide. *Pseudomonas spp.*, *S. aureus*, *Klebsiella spp.*, & *Escherichia coli* may be available in milk and milk products as a result of milk gathered from the animal suffering from disease condition and excreting in milk or due to unhygienic conditions during processing, production, handling & storage of milk products, which are the major causes of foodborne diseases. Results undoubtedly indicated that raw milk, processed milk (powdered milk & Ultra High-Temperature milk) and dairy products (mango milk, and ice cream) present in the market were contaminated with *Pseudomonas spp.*, *S. aureus*, *Klebsiella spp.*, *Escherichia coli* & *Klebsiella spp.* posing a high risk of food poisoning. Thus more hygienic preventive practices are required to decrease bacterial contamination, to increase the wholesomeness of these milk and dairy products.

AUTHORS' CONTRIBUTIONS

PP, M.Z.A.H., A.K., M.H.C., M.J.C., N.H.P., M.C., and S.M.A.R. conceived, designed, and performed the experiments. M.H.C., and M.J.C. drafted the manuscript. All authors read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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