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Isolation and quantification of microorganisms from some common milk products within Dhaka city, Bangladesh

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While milk is well known to be a balanced diet with its high nutritional values, conversely milk and milk products may serve as potential substrate for the growth and proliferation of a range of microorganisms which in turn fatally influences mass public health. Current study attempted to examine the likelihood of microbial contamination within some common milk products consumed by the locality of the city of Dhaka, Bangladesh. All samples exhibited the presence of bacterial and fungal contamination within a range of 10^2-10^4 cfu/mL and 10^2-10^3 cfu/mL, respectively. Among specific pathogens, *Staphylococcus* spp. was noticed to be the predominant ones and was recovered from 9 samples out of 20 samples in a range of 10^2-10^3 cfu/mL. *Klebsiella* spp. and *Vibrio* spp. were found within 6 and 9 samples, respectively. Products were also found to be contaminated with *Vibrio* spp. Study of antibiotic susceptibility test revealed that all the pathogenic bacteria were resistant against most of the commonly used antibiotics of which several isolates showed multi-drug resistant (MDR) trait. Therefore, the presence of pathogenic bacteria with the drug-resistance property in tested milk and milk products overall imparted the necessity of maintaining standardized hygienic handling and processing means for better management of public health.

Key words: Milk; Bacteria; Fungi; Quality; Drug resistance

Milk has long been known to be the most nutritious as well as balanced food being rich in proteins, fats, carbohydrates, vitamins, minerals, essential amino acids, etc. (1). However, milk may serve as an ideal substrate for the growth and survival of an array of bacteria and fungi, thereby leading to the public health threat (2-9). Microorganisms present in milk and milk products may influence the flavour, taste and texture of the finished forms of foods (10). Usually, the lactic acid bacteria (LAB; i.e., *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Enterococcus*) are the dominant microbial population in bovine, goat, sheep and buffalo milk, which are usually available prior to pasteurization (10). Psychrotrophic microbial populations, which particularly establish themselves during cold storage, are also a major component and frequently include *Pseudomonas* and *Acinetobacter* spp. (11-13). Undesirable microorganisms including Gram negative psychrotrophs such as *Pseudomonas* spp., *Achromobacter* spp., *Aeromonas* spp., *Serratia* spp., *Alcaligenes* spp., *Chromobacterium* spp., *Salmonella* spp., *Brucella* spp., *Mycobacterium bovis*, *Listeria monocytogenes* and *Campylobacter jejuni*, *Flavobacterium* spp. and yeasts such as *Candida* spp., can cause spoilage and hence render the raw or even the processed milk unsuitable for human consumption (14, 15).

Indeed, milk borne pathogenic microorganisms have been globally well reported to trigger various disease outbreaks resulting in fatality (1, 16-18). Microorganisms may gain access into milk and milk products from a variety of sources, including the milking apparatus, surrounding air condition, soils, water or moisture content, etc. (1, 19, 20). The bovine teat surface is known to harbor varieties of bacteria (19, 21, 22). Moreover, the unhygienic handling from the operators may largely account for the microbial contamination of milk.

Contamination of milk and milk products by pathogenic microorganisms is a global health concern; however, its fatal impact on human and animal health in the developing countries including in Bangladesh has not yet been extensively resolved except a few research works (1, 14). Since the constituents of milk and milk based products are adequate enough to support the microbial growth and replication, the dairy foods intensely demand a careful microbiological examination for the quality assurance for the sake of consumer safety. Some of the previous local researches within Dhaka city, Bangladesh revealed that raw or un-pasteurized milk and milk products could be very efficient vehicle for bringing a large number of people into contact with potential microbial hazards with an ultimate effect in the onset of various diseases (1, 14, 15). Therefore, the safety of milk products in context to the onset of food borne diseases stands as a major global health issue, especially in the developing countries where production of milk and milk product usually takes place

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under poor hygienic practices (14, 15). Moreover, food contamination with antibiotic-resistant bacteria is a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance (23-25). Certain antibiotics, however, are critical to human medicine because there is no other drugs available to treat human infections caused by multi drug resistant pathogens (26-28). Current study further endeavored to chalk out the microbial content with demonstration of their drug resistant traits within some popular consumable milk products within the community inhabiting the city of Dhaka.

MATERIALS AND METHODS

Sample collection and processing. A total of 10 milk and milk products were collected aseptically from different shops within the city of Dhaka maintaining the standard procedure of sampling (1). Samples were transported immediately (approximately within 1 hour) to the laboratory for microbiological analysis. Prior to the estimation of bacterial and fungal load, samples were subjected serial dilutions up to 10^{-2}.

Microbiological analyses. For the enumeration of total viable bacteria and fungi, an aliquot of 0.1 mL of each suspension was introduced onto the nutrient agar (NA) plates and Sabouraud dextrose agar (SDA) plates by means of spreading in order to isolate and quantify the total viable bacterial count (TVBC) and fungi, respectively (1). The NA plates were incubated at 37 °C for 18 to 24 hours and the SDA plates were incubated at 25 °C for 48 to 72 hours, respectively. For the estimation of specific pathogenic bacteria, from the dilution of 10^{-2} of each sample, 0.1 mL of suspension was spread onto MacConkey agar, mannitol salt agar (MSA) and cetrimide agar media for the enumeration of *Escherichia coli*, *Staphylococcus* spp. and *Pseudomonas* spp., consecutively. All the plates were incubated at 37 ^{o}C for 24 hours. Appearance of the typical colonies such as pink colonies on MacConkey agar, yellow colonies on MSA and colonies with greenish pigmentation on cetrimide agar was analytical for the growth of *E. coli* or *Klebsiella* spp., *Staphylococcus* spp. and *Pseudomonas* spp., consecutively (29).

Enrichment procedure. For the detection of *Salmonella* spp. and *Vibrio* spp., samples were subjected enrichment for isolation and identification of these bacteria (30). One ml sample was added to selenite cystein broth (SCB) and alkaline peptone water (APW), respectively, and incubated at 37 ^{o}C for 6 hours, and then 0.1 mL from each of the enriched broth was spread over Salmonella-Shigella agar (SSA) and thioglycollate citrate bile salt (TCBS). Appearance of small blackish colonies after incubation for 24 hours at 37 ^{o}C was indicative of the presence of *Salmonella* spp., while the large (2-4 mm) and slightly flattened, yellow colonies on the TCBS agar denoted the presence of *Vibrio* spp. Finally the confirmative biochemical tests were conducted to ensure the identity of the isolates (29, 30).

Study of antibiogram. Isolated *E. coli*, *Vibrio* spp., *Pseudomonas* spp. and *Staphylococcus aureus* were subjected to antibiotic susceptibility assay against different groups of antibiotics in vitro by the Kirby-Bauer method (31). Drug resistance was observed against Penicillin (10 µg), amoxicillin (10 µg), Impenem (10 µg), cefixime (5 µg), Chloramphenicol (30 µg), gentamicin (10 µg); ciprofloxacin (5 µg), sulphamethoxazole-trimethoprim (25 µg), Vancomycin (30 µg), azithromycin (15 µg), Erythromycin (15 µg). From overnight culture plate, a small portion of a fresh colony was transferred to Muller-Hinton broth and incubated at 37 °C for 4 to 5 hrs until the growth reached to the equivalent turbidity standard of McFarland (0.5 standards). Muller- Hinton agar plates were seeded properly by spreading the inocula using sterile cotton swab. Discs (OXOID, UK) were placed gently at a proportionate distance from each other using a sterile needle. The plates were then incubated overnight at 37 °C and zones of inhibition (if any) were measured and interpreted as susceptible, intermediate and resistant (32).

RESULTS AND DISCUSSION

In the field of food microbiology sector in Bangladesh, while lots of works regarding food safety and security issues have been addressed; the specific identification strategy of microbial contaminants in the milk and milk products is still in its infancy (1, 8, 14, 26, 33-36). Nevertheless, a few earlier researches reported that microbial contamination in milk and milk products could take place from three principal sources: inside the udder; the exterior of the udder and the surface of milk handling; and storage equipment (15, 37).

Current investigation also showed a scenario of huge microbial contamination in most of the samples. All samples were found to harbor the total viable bacteria and, were biochemically identified (Tables 1 & 2). Although *E. coli*, *Salmonella* spp. and *Shigella* spp. were not found in any sample, 6 samples were found to be contaminated with *Klebsiella* spp. (10^2 cfu/mL), belonging to the same family. *Staphylococcus* spp. were found in approximately about half (9) of the samples in a range of 10^2-10^3 cfu/mL. On the other hand, *Vibrio* spp. and *Pseudomonas* spp. were present in 9 and 7 samples, respectively in an average of 10^2 cfu/mL (Table 1).

In this study, all the pathogenic isolates showed multi-drug resistance (MDR) phenotype (Table 3). Isolates of *Vibrio* spp. and *Pseudomonas* spp. were found to be highly resistant (88.88%) against all the used antibiotics except imipenem. *Staphylococcus aureus* isolates were found to be resistant against 8 different antibiotics and sensitive to ciprofloxacin, gentamicin and imipenem. *Klebsiella* spp. was found to be susceptible towards imipenem, gentamicin, erythromycin and chloramphenicol, respectively (Table 3). Results of this study showed a close link with previous study conducted by Marjan et al. (1). The research group showed that all the pathogenic isolates exhibited the MDR phenotype. Overall, according to the current study results, the presence of microorganisms in the studied samples is sufficiently indicative of severe health risk upon consumption of the dairy products tested unless appropriate microbiological measures are not taken. Sufficient legislative actions are thus of major clinical significance.

CONCLUSION

In cohort with the previous study findings on the microbiological analysis of milk and milk products carried out in the same laboratory, present study revealed the presence of a range of pathogenic bacteria which were of public health significance. Maintenance of proper hygiene during handling and processing of milk products as well as proper application of sterilization procedure such as pasteurization and UHT could ensure food quality and most importantly consumers' safety.

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TABLE 1. Prevalence of pathogenic microorganisms in Milk and milk products

Sample name	TVB (cfu/mL)	Total fungal count	*E. coli* (cfu/mL)	*Klebsiella* spp. (cfu/mL)	Fecal Coliform count (cfu/mL)	*Pseudomonas* spp. (cfu/mL)	*Staphylococcus* spp. (cfu/mL)	*Vibrio spp.* (cfu/mL)
Sweetened yogurt	1.20×10^2	2.0×10^2	0	2.0×10^2	0	Nil	Nil	3.0×10^2
Sweetened yogurt	3.4×10^2	1.65×10^2	0	9.0×10^2	0	Nil	Nil	Nil
Matta	4.0×10^2	8.5×10^2	0	0	0	8.2×10^2	Nil	Nil
UHT Strawberry Yogurt Milk	8.0×10^2	9.0×10^2	0	0	0	7.0×10^2	9.0×10^2	2.8×10^2
Cream	1.35×10^2	3.0×10^2	0	0	0	2.4×10^2	Nil	1.1×10^2
Cream	2.20×10^2	2.20×10^2	0	0	0	1.6×10^2	2.4×10^2	2.6×10^2
Sour card	2.60×10^2	8.0×10^2	0	0	0	Nil	Nil	2.7×10^2
Sour Card	8.5×10^1	2.20×10^2	0	6.0×10^2	0	Nil	Nil	2.3×10^2
Butter	2.10×10^2	1.60×10^2	0	0	0	1.7×10^2	Nil	3.0×10^2
Butter	4.0×10^2	3.4×10^2	0	0	0	3.0×10^2	Nil	Nil
Pasteurized milk	1.2×10^3	1.0×10^2	0	2.0×10^2	0	0	1.0×10^2	0
Pasteurized milk	2.9×10^3	4.5×10^2	0	9.0×10^2	0	0	0	8.0×10^2
UHT milk	2.6×10^4	3.0×10^3	0	0	0	0	0	0
UHT milk	2.4×10^3	8.0×10^2	0	0	0	0	0	0
Mango milk	6.0×10^2	5.0×10^2	0	0	0	0	3.0×10^2	0
Mango milk	2.8×10^4	1.2×10^3	0	0	0	5.0×10^2	1.0×10^3	4.0×10^2
Chocolate milk	2.0×10^4	7.0×10^3	0	0	0	0	4.0×10^2	0
Chocolate milk	1.0×10^4	1.0×10^2	0	6.0×10^2	0	0	8.0×10^3	0
Normal Lassie	1.8×10^3	2.0×10^2	0	0	0	0	1.0×10^2	0
Mango lassie	2.6×10^2	2.0×10^2	0	0	0	0	4.0×10^2	0

***Microbial limit** (38, 39, 40)
Pasteurized Milk: SPC 5×10^5 cfu/g, Coliform-5 cfu/mL
UHT Milk: Commercially sterile
Milk product: SPC 5×10^5 cfu/g, Coliform-100 cfu/g

TABLE 2. Confirmative biochemical tests for the isolates

Assumed Organism	TSI			H_2Sreaction	Indole test	MR test	VP test	Citrate Test	Motility	Oxidase test
	slant	Butt	gas							
Klebsiella spp.	Y	Y	-	-	-	-	-	-	-	+
Pseudomonas spp.	A	A	-	-	-	-	-	+	-	-
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+

TSI	Triple Sugar Iron Test
Y	Yellow (Acid)
R	Red (Alkaline)
MR	Methyl red
VP	Voges-Proskauer
A	Acidic reaction
K	Alkaline reaction
ND	Not done
+	Positive
-	Negative

TABLE 3. Study of antibiogram

Antibiotics	Pathogenic Isolates							
	Klebsiella spp. N=6		*Vibrio* spp. N=9		*Staphylococcus aureus* N=9		*Pseudomonas* spp. N=7	
	R	S	R	S	R	S	R	S
CIP	83.33%	16.66%	88.88%	11.11%	55.55%	44.44%	85.71%	14.28%
CEF	83.33%	16.66%	88.88%	11.11%	11.11%	88.88%	71.42%	28.57%
AML	83.33%	16.66%	55.55%	44.44%	88.88%	11.11%	85.71%	14.28%
IPM	16.66%	83.33%	88.88%	11.11%	11.11%	88.88%	14.28%	85.71%
PEN	83.33%	16.66%	66.66%	33.33%	88.11%	11.11%	85.71%	14.28%
TMP-SUL	50%	50%	66.66%	33.33%	66.66%	33.33%	71.42%	28.57%
GEN	16.66%	83.33%	88.88%	11.11%	88.88%	11.11%	71.42%	28.57%
AZI	83.33%	16.66%	11.11%	88.88%	88.88%	101.11%	85.71%	14.28%
ERY	33.33%	66.66%	11.11%	88.88%	88.88%	11.11%	85.71%	14.28%
CHL	33.33%	66.66%	88.88%	11.11%	55.55%	44.44%	71.42%	28.57%
VAN	83.33%	16.66%	11.11%	88.88%	66.66%	33.33%	85.71%	14.28%

CIP = ciprofloxacin (5 µg); CEF = Cefixime (5 µg); AML = Amoxicillin (10 µg); IPM = Imipenm (10 µg); Penicillin = (10 µg) TMP-SUL = Sulfamethoxazole, (25 µg); GEN = Gentamicin (10 µg); AZI = Azithromycin (15 µg); ERY = Erythromycin (15 µg); CHL = Chloramphenicol (30 µg); VAN = Vancomycin (30 µg); ND = Not Done; R = Resistant; S = Sensitive

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