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Microbial Study of Some Milk with Special Reference to Coli form Bacteria*

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Abstract: Cheese, Borhanii and Whey samples of Dhaka Metropolitan City studied for the justification of the quality and hygiene. The range of Coliform bacteria were ranged from $5.24x10^5$ to $0.5x10^4$ cfu in Borhanii, $6x10^3$ to $5.0x10^3$ CFU in Cheese and $2.98x10^5$ to $1.8x10^4$ CFU in whey respectively in our samples. Antibacterial sensitivity tests were carried out with five different antibiotics against bacterial isolates. All isolates were resistant to Streptomycin and sensitive to Gentamycin. The majority of the isolates were sensitive to Chloramphenicol and Tetracycline. Four isolates were resistant to Ampicillin.

Key words: Borhanii, cheese, whey, antibiotics, coliform, LF, NLF, load, bacteria, milk products

Introduction

Milk is an excellent growth medium for microorganisms, which when permitted to grow, will produce changes that render the milk unfit for human use. Milk and milk products are used as an ideal food for human, particularly for children through out the world (Varro *et al*., 1981). The US public health service code stipulates that pasteurized milk and milk products contain no more than 20,000 bacteria mL^{-1} and no more than 10 Coliform bacteria mL^{-1} (Anonymous, 1977).

Members of *Salmonella* produce a variety of diseases from taking milk and milk products (Bell *et al*., 1976). Milk and milk products such as raw milk creams may contain different type of pathogenic bacteria such as *Bacillus cereus, Streptococcus* sp., *E. coli, Klebsiella pnumoniae, Enterobacter aerogenes, Pseudomonas flurosence, Pseudomonas aurosa, Salmonella and Shigella* etc., (Marth, 1969). Even though, sanitation and handling are under regulatory controlled but some milk products poisoning has been found (Credit *et al*., 1972). Milk selected into the udder of healthy cows is sterile (Bell *et al*., 1976). Freshly drawn milk may have pathogens and it may be further infected during handling (Marth, 1969). There are various forms of milk products in different parts of the world likely cheese, whey, cream, butter, icecream, yogurt, borhani etc. Acid production in cheese retards growth of bacteria that cause undesirable fermentation in cheese. The food values of different cheeses are different. From our estimation we get highest protein and energy content in hard cheddar type and lowest in cottage type in average. High concentration of sugar is inhibitory to the growth of some bacteria and might retard or prevent the growth of some survivors of the heat treatments. Condensed whey, called whey semisolids is another concentrated dairy products, as is condensed buttermilk, called semisolid butter milk. Numerous out breaks of food poisoning attributed to *Salmonella* or *Staphylococci* from milk powder are evidenced that do on occasion survive in the final product (Frazier and Westoff, 1978). *Pseudomonas* and *Alcaligens* can cause surface spoilage. Some strains of

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Bacillus cereus have been reported to cause food poisoning (Nygren, 1962). Members of genus *Salmonella* produce a variety of infections in cow and also transmitted to man from taking milk and milk products (Marth, 1969).

Many bacterial species associated with milk and milk products are responsible for vomiting, bacterimia, pneumonia, meningitis and food poisoning (Schalm *et al*., 1917). Whey was tested for inhibition of in vitro adhesion of radio labeled *S. fimbrio* bearing *Escherichia coli*. To human ileostomy glycoproteins, which provide a model for human intestinal mucus (Ouwehand *et al*., 1995).

Multi drug resistant *Salmonella typhimurium* DT 104 emerged as a cause of Samonellosis in Yakima and Mexican style soft cheese made with unpasteurized milk is an important vehicle for *Salmonella typhimurium* DT 104 transmission (Rodrigo *et al*., 1999).

Few reports are available in the literature about the survival of *E. coli* and *P. paucimobilis* in Yogurt and fermented milk (Hekmat and Macmohoan, 1997). However, due to their metabolic versatility and common recovery from milk-based products (Wessels *et al*., 1989; Shelly *et al*., 1986; Postupa and Aldova, 1984) they are likely to survive in low pH dairy foods (Cangella *et al*., 1999).

The incidence of *Salmonella typhimurium* infections was associated with consumption of Quesco Fresco cheese (Hersh and Yakima, 1999.). The urban people of Bangladesh frequently consume the milk products like cheese, whey and borhani. Borhani is a milk product of Bangladesh contains the spicy ingredients (Curd, Master oil, *Capsicum* powder, *Mentha* paste, *Coriandrum sativum* powder, *Piper nigrum* paste and taste salt) (Kabir, 1978). Therefore the present study was undertaken to assess the sensitivity pattern of the organisms under antibiotics treated conditions.

Materials and Methods

Samples were collected from different community centers, hotel, restaurants and footpath vendors of the Dhaka Metropolitan City (Table 1). The microbial colonies were taken by dilution factor (1:100) plate procedure (Greenberg *et al*., 1980). Membrane filter technique was done by the machine (Memmert Gmbh Co) and Mac.Conkey Agar medium was used for counting Coliform bacteria colonies. The dilution sample was prepared sterile conical flask under pH controlling and shaken vortex stirrer (VF1 Jurk and Kunkel Gmbh and Co Germany). Serial diluted samples were poured in duplicated and sterilized plates and then were incubated at 37°C in an incubator (Memmert Gmbh Co) maintaining a high level of humidity inside for 24 h. Lactose fermentation test was done at 44°C for 24 h to identify fecal coliforms.

Nine isolates of bacteria were selected on the basis of their morphological (Table 2) and biochemical characteristics (Table 3) and nature of arrangement through microscopic studies after Gram staining with Nikon Microphot microscope (Japan). The method of Eklund and Lankford (1967) was followed regarding this staining. Among the isolates, three isolates were selected from

Table 1: Quantitative determination of total bacterial count of Borhani, Whey and Cheese

	Site	CFU/100 mL Sample (CFU g^{-1} in case of cheese)
Borhanii	Gulistan Raj Hotel, Gulistan	$5.24x10^4$
	Mugal Birani House, Gulistan	$5.2x10^4$
	Dhaka Birani House, Dhaka	$2.0x10^4$
	Sagar community center, Dhaka	$0.5x10^4$
Whey	Lal bag morh, Lal bag	$2.98x10^5$
	Chak super market, Dhaka.	$1.8x10^4$
	Gulistan Morh, Dhaka	$4.1x10^4$
Cheese	Chankharpul, Dhaka	$6.0x10^3$
	New market, Dhaka	$5.0x10^3$

Table 2: Morphological characteristics of the colonies of the selected isolates on Mac Conkey Agar medium

		Characteristics of the colonies and staining					
Sample	Isolates	Color	Form	Surface	Elevation	Margin	Gram staining
Cheese	*Serratia* sp.	Slight brick red	Circular	Smooth	Effuse	Entire	Negative, Rod
	Un identified	Brick red	Circular	Concentric	Convex	Entire	Negative, Rod
	Klebsiella sp.	Brick red	Irregular	Smooth	Viscous	Undulate	Negative, Rod
Whey	*Alcaligens*	White	Circular	Smooth	Raised	Entire	Negative, Rod
	Serratia sp.	Slight red	Circular	Smooth	Effuse	Entire	Negative, Rod
	Alcaligens sp.	White	Circular	Smooth	Flat	Entire	Negative, Rod
	Un identified	Brick red	Circular	Smooth	Flat	Undulate	Negative, Rod
	Alcaligens sp.	White	Circular	Smooth	Convex	Entire	Negative, Rod
Borhani	*Hafnia* sp.	Slight red	Circular	Smooth	Raised	Entire	Negative, Rod

Table 3: Biochemical characteristics of the isolates

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13
Serratia sp.	+	+	-	-	+	+	+	+	+	-	++	-	+
Un identified	+	-	-	-	+	+	+	-	+	-	-	-	+
Klebsiella sp.	+	+	-	+	-	+	-	+	+	-	Minute bubble	-	+
Alcaligens sp.	+	-	-	-	+	+	-	-	+	-	++	+	-
Serratia sp.	+	+	-	-	+	+	+	-	+	+	++	-	+
Alcaligens sp.	+	-	-	-	+	+	-	-	+	-	-	-	+
Un identified	+	-	-	-	+	+	-	-	+	-	++	+	+
Alcaligens sp.	+	-	-	-	+	+	-	-	+	-	++	+	-
Hafnia sp.	+	+	-	-	+	+	-	-	-	-	++	-	+

1 = Catalase 2 = VP Test 3 = Methyl red 4 = Starch hydrolysis
5 = Motility test 6 = Simmon's citrate test 7 = Gelatin hydrolys 8 = Casein Hydrolysis
9 = Citrate utilizati 10 = Indole formatio 11 = Deep glucose 12 = Oxidase
13 = Nitrate reduction

cheese, one from borhani and five from whey. Nutrient agar, blood agar and macconkey agar were used as media. The organisms were tested for antibiotic sensitivity. Nutrient agar plates were used for this purpose. Using cotton swab, test organisms were inoculated. Different types of antibiotic disks such as tetracycline (TE-30) ampicilin (AMP-25), chloramphenicol (C-30) and streptomycin (S-25) were placed on the inoculated plates. After 24 h of incubation, inhibition zones were measured. To determine the sensitivity pattern, measured diameter of the inhibition zones were compared to that of standard (Doye, 1994; Cheesbroug, 1984).

Results and Discussion

Counting of coliforms in different samples is shown in Table 4. A total of 24 isolates from 14 samples nine were finally selected for study. From Table 5 we found the record of Fecal, NLF and LF coliforms in different samples. Whether or not non-hygienic water is responsible for excess count; water samples were examined and found that count was not high although this result is dissimilar with Khan and Alim (1983). So, it might be non-hygienic environment or any special ingredients.

About 60.00% isolates were sensitive, 33.33% were resistant and 6.66% showed the intermediate susceptibility against applied antibiotics. Among five antibiotics streptomycin showed highest resistance (100%). gentamycin showed highest sensitivity (100%). In case of cholramphenicol 88.88% isolates were sensitive, 11.11% were resistant and there was no intermediate sensitive (Table 6). More than 50% gram-negative bacilli of clinical sources in Ethiopia were found as resistant to Chloramphenicol (Gedebou, 1983).

Table 4: Colony count in different samples (cfu/100 mL)

Borhanii		Borhanii water		Cheese		Whey	
Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest
$5.24x10^5$ to	$0.5x10^4$	$2x10^3$ to	$1x10^3$	$6x10^3$ to	$5x10^3$	$2.98x10^5$ to	$1.8x10^4$

Cfu: Colony forming unit

Table 5: Grouping of the isolates

Isolates	NLF	LF	Fecal
Serratia sp. (B4)	-	+	+
	-	+	+
Un identified (C14)			
Klebsiella sp. (C 17)	-	+	+
	-	+	-
Alcaligens (C18)			
	+	-	+
Serratia sp. (G1)			
	-	+	+
Alcaligens sp. (G12)			
	+	-	+
Un identified (G 13)			
	-	+	+
Alcaligens sp. (G 14)			
	+	-	+
Hafnia sp. (G 19)			
Serratia sp. (C 21)	Not done		
	Not done		
Un identified (C 22)			
Klebsiella sp. (C 23)	Not done		

NLF: Non Lactose Fermenter, LF: Lactose Fermenter

Table 6: C/S test for the selected isolates by using different antibiotics

	Inhibition zone (mm) and Sensitivity				
Isolates	Tetracycline (TE-30)	Ampicillin (AMP-25)	Gentamycin (CN-10)	Chloramphenicol (C-30)	Streptomycin (S-25)
Serratia sp.	12R	15R	19S	22S	16R
Un identified	19S	0 R	17S	20S	15R
Klebsiella sp.	19S	12R	20S	30S	18R
Alcaligens sp.	23S	14I	21S	27S	19R
Serratia sp.	22S	24R	20S	25S	18R
Alcaligens sp.	8R	23S	20S	25S	17R
Unidentified	30S	27S	25S	20R	16R
Alcaligens sp.	23S	13I	18S	30S	16R
Hafnia *sp.*	17I	9R	20S	23S	18R

***R-resistant, S-susceptible, I-intermediate

But in this study, it was dissimilar to that reported by Cody *et al*. (1999) who performed such kind of sensitivity test. For *Salmonella serotype typhimurium* DT 104. Milk products might have different groups of microorganisms including spoilage and pathogenic ones. An attempt had been made also to isolate spoilage and pathogenic bacteria from some milk products and to characterize them and test for sensitivity pattern. Borhanii is served in many localities. Borhanii was served with high bacterial count which posses a severe threat to the health of the consumers.

Among three isolates from cheese 75% are sensitive to tetracycline, 25% sensitive to ampicillin, 100% sensitive to gentamycin, 100% sensitive to chloramphenicol, 100% resistant to streptomycin. Among five whey isolates 60% sensitive to tetracycline, 60% sensitive to ampicillin cent percent

sensitive to gentamycin, 80% sensitive to chloramphenicol and cent percent resistant to streptomycin. Among one isolate from cheese was sensitive to tetracycline, gentamycin and chloramphenicol but in case of streptomycin it was resistant.

Above measurement was performed measuring the inhibition zone of these antibiotic disks in (mm) (Table 6).

Conclusions

The experimental results presented here emphasize the need for the establishment of an efficient quality control for the safe guard of human health as well as careful selection of antibiotics for drug resistant strains.

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