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Prevalence of *Escherichia coli* in Different Milk Products from Lahore, Pakistan

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Abstract:

Dairy foods are an important part of the diet as a source of good-quality protein and several vitamins and minerals. Due to its complex biochemical composition and high water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. A total 120 samples were collected, 60 from area of high sanitation and 60 from area of poor sanitation. After processing the samples with different detecting methods and techniques, we came to know that all the samples of dairy products collected from both sites in both turns were positive for Escherichia

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coli. Escherichia coli in samples of dairy products collected from site A were 1.25±4.65×10³, 1.62±4.91×10³, 5.97±5.45×10³, 7.84±6.18×10³ and 11.65±5.71×10³ respectively, Whereas in those of site B were 3.24±6.71×10³, 3.12±5.53×10³, 7.41±5.22×10³, 9.02±3.91×10³ and 13.41±8.46×10³ respectively. Conclusively, Escherichia coli in samples of dairy products collected from site B were 19.9%, 15%, 14.4%, 11.8% and 17.6% respectively higher than those of site A.

Key words: Dairy Products, Escherichia coli, Prevalence

Introduction

Dairy foods are an important part of the diet as a source of good-quality protein and several vitamins and minerals. In addition, milk products may have both positive and negative health effects (Givens 2008). The consumption of dairy foods and calcium in dairy foods has been suggested to be beneficial in the regulation of body weight (Major *et al.* 2008), although results from intervention studies are inconsistent (Barba and Russo, 2006). It has also been indicated that milk consumption and intake of dairy proteins are inversely related to the risk of hypertension (Ruidavets *et al.* 2006), and intervention studies have shown a blood pressure–lowering effect of milk products and milk peptides (Jauhiainen and Korpela 2007). Some epidemiologic studies have suggested an inverse relation between intake of dairy products and components and prevalence of the metabolic syndrome (Pfeuffer and Schrezenmeir 2006), which has not been apparent in other studies (Snijder *et al.* 2008).

The wide array of available dairy foods challenges the microbiologist, engineer, and technologist to find the best ways to prevent the entry of microorganisms, destroy those that do get in along with their enzymes, and prevent the growth and activities of those that escape processing treatments. Troublesome spoilage microorganisms include aerobic

psychrotrophic Gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria. The type of spoilage microorganisms differs widely among dairy foods because of the selective effects of practices followed in production, formulation, processing, packaging, storage, distribution, and handling (Ledenbach and Marshall 2009).

The quality of milk is determined by aspects of composition and hygiene. Due to its complex biochemical composition and high water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. Therefore in the processing of milk, some of them may produce undesirable effects and some micro-organisms produce food infections which can either carry the pathogens that will increase the likelihood of infection of the consumer's food (Oliver *et al.* 2005). Milk is a major part of human food and plays a prominent role in the Pakistani diet. Approximately 50 percent of the milk produced is consumed as fresh or boiled, one sixth as yogurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as Ice cream, Khoa, Burfi and Gulabjaman (Anjum *et al.* 1989). Indigenous sweet based products like Khoa, Gulabjamun, Rasgulla are highly susceptible to variety of microorganisms because of high nutritive value and complex chemical composition (Soomro 2002).

Escherichia coli is often used as marker organism. Recovery and counting of *Escherichia coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and toxigenic microorganisms which constitute a public health hazard. *Escherichia coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *Escherichia coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al.* 2004).

The output value of dairy and dairy products is

increasing day by day. Considering its economic potential, extensive and intensive exploitation of milk and milk products can both contribute to the nutrient requirements of the people and increase the income of farmers. In view of the growing public awareness about food safety and quality, knowledge of the microbial and chemical composition of milk is of great significance for further development of its hygienic processing into high quality consumer products. Until now, data on such aspects is scant and scattered. Considering the above facts the present study was designed to isolate the opportunistic pathogen *Escherichia coli* from different milk products sold under market conditions at Lahore.

Materials and Methods

Sample Collection

A total of 120 samples of different milk products (Rasgulla, Gulabjamun, Burfi, Khoa and Dahi) 24 each, were collected from different localities of Lahore, Pakistan. Half of the samples were collected from those shops dealing in sweets which had extreme hygienic facilities and these were denoted as A1 and A2 sites. Whereas half of the samples were collected from those ones which had substandard sanitation and these were denoted as B1 and B2 sites. Three samples of each product were collected in each turn from each site in sterilized containers and were brought in ice box to the laboratory for isolation and enumeration of *Escherichia coli*.

Isolation of *Escherichia Coli*

Processing of Samples

Under aseptic condition three dilutions of each sample were made by thoroughly mixing 0.1 ml of slurry of a given sample in 9.9 ml of the autoclaved water in a sterilized tube that was labeled as “A”, thus producing a dilution of 1: 10^2. The second dilution made by similarly suspending 0.1 ml of water from “A”

in 9.9 ml of sterile water in tube "B". This produced a dilution of 1: 10^4. Like wise a third dilution (1: 10^6) was made employing the serial dilution.

Media Preparation

Nutrient agar, and MacConkey agar medium was used for detection, isolation and enumeration of *Escherichia coli* per manufacturer's protocol.

Spread Plate Method

Six solidified agar petriplates of MacConkey agar were taken and 0.1 ml of each dilution from samples was spread evenly with the help of sterile, bent glass rod on a separate plate. All the plates were incubated at 37°C up to 24 hours.

Observation and Enumeration of Bacterial Colonies

After 24 hours of incubation, pink colored colonies on the plates were counted with the help of a colony counter and plates having 30 to 300 colonies were selected for estimating colony forming units (CFU).

Gram Staining and Biochemical Tests

The samples were processed for Gram staining. Bacteria were then grown on MacConkey agar medium. The MacConkey agar was prepared according to Merck (1996). The test tubes were filled 1/3 with the MacConkey agar medium, cotton plugged and autoclaved. When the broth cooled, the culture was picked up with the help of a sterilized loop and inoculated in the broth. The tube's necks were sterilized on flam. They were then cotton plugged and incubated for 24 hours at 37 ° C. the bacterial growth thus obtained was then used for various identification tests.

Catalase Test

Catalase reagent was prepared by mixing 3 ml of hydrogen peroxide with 7 ml of distilled water. A loopful of the bacterial

culture from the plate was picked up on the slides and few drops of the reagent were added to it. Effervescence indicated the presence of catalase enzyme (Benson 1994).

Indole Production Test

Bacterial culture was inoculated in tryptone water and incubated for 2 days at 37°C. Kovacs reagent was prepared by dissolving 5 gm of p-dimethylaminobanzaldehyde in a mixture of 75 ml of n-amyle alcohol and 25 ml of concentrated HCl. A few of this reagent were added to the broth culture. Appearance of rose pink colored ring indicated indole production (Collins and Lyne 1995).

Lactose and Mannitol Fermentation Test

An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red lactose/Mannitol broth. The inoculated tube was incubated at 35-37°C for 24 hours and the results were determined. A positive test consisted of a color change from red to yellow, indicating a pH change to acidic.

Urease Test

An inoculum from a pure culture was transferred aseptically to a sterile tube containing 3 ml sterile Christensen's modified urea broth and incubated at 35-37°C for 3-12 hours. Appearance of pink color indicated a positive result.

Results

Enumeration of *Escherichia Coli*

The number of C.F.U/gm of *Escherichia coli* in samples of Rasgulla, Gulabjamun, Barfi, Khoa and Dahi (Curd) collected from site A1 in the first turn were $1.34\pm3.47\times10^3$, $1.41\pm5.32\times10^3$, $5.73\pm5.49\times10^3$, $7.43\pm6.72\times10^3$ and $11.35\pm3.42\times10^3$ respectively, Where as in the samples collected from site A2 were $1.55\pm7.16\times10^3$, $1.56\pm3.63\times10^3$, $6.03\pm8.39\times10^3$, $6.94\pm4.52\times10^3$ and $12.16\pm7.54\times10^3$ respectively as shown in

table 1.

Table 1: No. of C.F.U/gm of *E. coli* isolated from different dairy products collected from sites A1 and A2 in the first turn.

Collection Sites	Sample Type	Total Samples	No of contaminated samples	Percentage of contamination	C.F.U/gm
A1	**Rasgulla**	6	6	100%	$1.34\pm3.47\times10^3$
	Gulabgamun	6	6	100%	$1.41\pm5.32\times10^3$
	Barfi	6	6	100%	$5.73\pm5.49\times10^3$
	Khoa	6	6	100%	$7.43\pm6.72\times10^3$
	Dahi (Curd)	6	6	100%	$11.35\pm3.42\times10^3$
A2	**Rasgulla**	6	6	100%	$1.55\pm7.16\times10^3$
	Gulabgamun	6	6	100%	$1.56\pm3.63\times10^3$
	Barfi	6	6	100%	$6.03\pm8.39\times10^3$
	Khoa	6	6	100%	$6.94\pm4.52\times10^3$
	Dahi (Curd)	6	6	100%	$12.16\pm7.54\times10^3$

Values of C.F.U/gm are means of three replicates ±S.E.M.
A1= Gourmet sweets Chung.
A2= Gourmet sweets Thokar Niaz Baig.

The number of C.F.U/gm of *Escherichia coli* in samples of Rasgulla, Gulabjamun, Barfi, Khoa and Dahi (Curd) collected from site A1 in the second turn were $0.98\pm2.63\times10^3$, $1.66\pm3.33\times10^3$, $6.17\pm3.77\times10^3$, $8.81\pm5.61\times10^3$ and $10.97\pm5.73\times10^3$ respectively, Where as in the samples collected from site A2 were $1.12\pm5.33\times10^3$, $1.87\pm7.34\times10^3$, $5.95\pm4.13\times10^3$, $8.18\pm7.87\times10^3$ and $12.11\pm6.12\times10^3$ respectively as shown in table 2.

Table 2: No. of C.F.U/gm of *E. coli* isolated from different dairy products collected from sites A1 and A2 in the second turn.

Collection Sites	Sample Type	Total Samples	No of contaminated samples	Percentage of contamination	C.F.U/gm
A1	**Rasgulla**	6	6	100%	$0.98\pm2.63\times10^3$
	Gulabgamun	6	6	100%	$1.66\pm3.33\times10^3$

	Barfi	6	6	100%	$6.17\pm3.77\times10^3$
	Khoa	6	6	100%	$8.81\pm5.61\times10^3$
	Dahi (Curd)	6	6	100%	$10.97\pm5.73\times10^3$
A2	**Rasgulla**	6	6	100%	$1.12\pm5.33\times10^3$
	Gulabgamun	6	6	100%	$1.87\pm7.34\times10^3$
	Barfi	6	6	100%	$5.95\pm4.13\times10^3$
	Khoa	6	6	100%	$8.18\pm7.87\times10^3$
	Dahi (Curd)	6	6	100%	$12.11\pm6.12\times10^3$

Values of C.F.U/gm are means of three replicates ±S.E.M.
A1= Gourmet sweets Chung.
A2= Gourmet sweets Thokar Niaz Baig.

The number of C.F.U/gm of *Escherichia coli* in samples of Rasgulla, Gulabjamun, Barfi, Khoa and Dahi (Curd) collected from site B1 in the first turn were $3.73\pm8.91\times10^3$, $2.87\pm4.47\times10^3$, $7.34\pm6.53\times10^3$, $9.11\pm3.24\times10^3$ and $13.6\pm9.21\times10^3$ respectively, Where as in the samples collected from site B2 were $2.98\pm4.55\times10^3$, $3.03\pm9.12\times10^3$, $8.05\pm5.55\times10^3$, $8.98\pm4.98\times10^3$ and $12.37\pm8.17\times10^3$ respectively as shown in table 3.

Table 3: No. of C.F.U/gm of *E. coli* isolated from different dairy products collected from sites B1 and B2 in the first turn.

Collection Sites	Sample Type	Total Samples	No of contaminated samples	Percentage of contamination	C.F.U/gm
B1	**Rasgulla**	6	6	100%	$3.73\pm8.91\times10^3$
	Gulabgamun	6	6	100%	$2.87\pm4.47\times10^3$
	Barfi	6	6	100%	$7.34\pm6.53\times10^3$
	Khoa	6	6	100%	$9.11\pm3.24\times10^3$
	Dahi (Curd)	6	6	100%	$13.6\pm9.21\times10^3$
B2	**Rasgulla**	6	6	100%	$2.98\pm4.55\times10^3$
	Gulabgamun	6	6	100%	$3.03\pm9.12\times10^3$
	Barfi	6	6	100%	$8.05\pm5.55\times10^3$
	Khoa	6	6	100%	$8.98\pm4.98\times10^3$
	Dahi (Curd)	6	6	100%	$12.37\pm8.17\times10^3$

Values of C.F.U/gm are means of three replicates ±S.E.M.
B1= Rahman sweets Chung.
B2= Rahman sweets Thokar Niaz Baig.

The number of C.F.U/gm of *Escherichia coli* in samples of

Rasgulla, Gulabjamun, Barfi, Khoa and Dahi (Curd) collected from site B1 in the second turn were $3.14\pm9.21\times10^3$, $3.45\pm5.16\times10^3$, $6.95\pm2.13\times10^3$, $8.89\pm3.96\times10^3$ and $14.81\pm6.82\times10^3$ respectively, Where as in the samples collected from site B2 were $3.11\pm4.16\times10^3$, $3.12\pm3.35\times10^3$, $7.28\pm6.68\times10^3$, $9.09\pm3.44\times10^3$ and $12.89\pm9.64\times10^3$ respectively as shown in table 4.

Table 4. No. of C.F.U/gm of *E. coli* isolated from different dairy products collected from sites B1 and B2 in the second turn.

Collection Sites	Sample Type	Total Samples	No of contaminated samples	Percentage of contamination	C.F.U/gm
B1	**Rasgulla**	6	6	100%	$3.14\pm9.21\times10^3$
	Gulabgamun	6	6	100%	$3.45\pm5.16\times10^3$
	Barfi	6	6	100%	$6.95\pm2.13\times10^3$
	Khoa	6	6	100%	$8.89\pm3.96\times10^3$
	Dahi (Curd)	6	6	100%	$14.81\pm6.82\times10^3$
B2	**Rasgulla**	6	6	100%	$3.11\pm4.16\times10^3$
	Gulabgamun	6	6	100%	$3.12\pm3.35\times10^3$
	Barfi	6	6	100%	$7.28\pm6.68\times10^3$
	Khoa	6	6	100%	$9.09\pm3.44\times10^3$
	Dahi (Curd)	6	6	100%	$12.89\pm9.64\times10^3$

Values of C.F.U/gm are means of three replicates ±S.E.M.
B1= Rahman sweets Chung.
B2= Rahman sweets Thokar Niaz Baig.

Table 5: Overall comparison of C.F.U/gm of different dairy products of sites A and B.

Sample Type	Sample Site	C.F.U/gm	Sample Type	Sample Site	C.F.U/gm
Rasgulla	**A**	$1.25\pm4.65\times10^3$	**Barfi**	**A**	$5.97\pm5.45\times10^3$
	B	$3.24\pm6.71\times10^3$		**B**	$7.41\pm5.22\times10^3$
Gulabgamun	**A**	$1.62\pm4.91\times10^3$	**Khoa**	**A**	$7.84\pm6.18\times10^3$
	B	$3.12\pm5.53\times10^3$		**B**	$9.02\pm3.91\times10^3$
Dahi (Curd)	**A**	$11.65\pm5.71\times10^3$			
	B	$13.41\pm8.46\times10^3$			

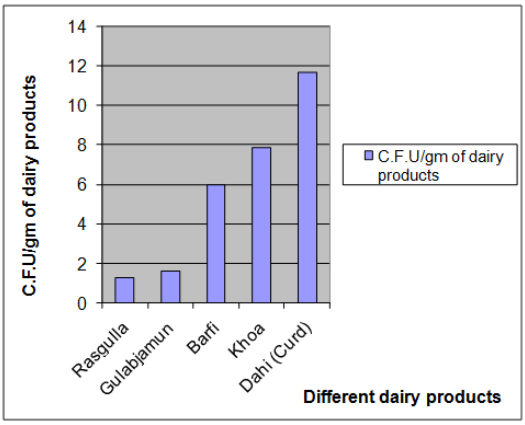

Fig 1: C.F.U/gm of different dairy products of site A.

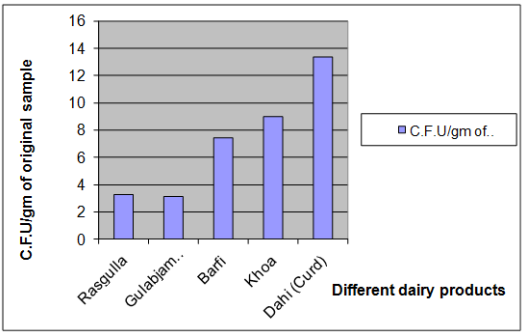

Fig 2. C.F.U/gm of different dairy products of site B.

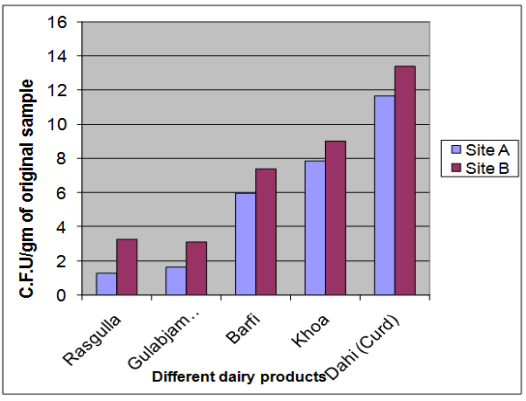

Fig 2: Overall comparison of C.F.U/gm of different dairy products of sites A and B.

Biochemical Analysis

All the samples of Rasgulla, Gulabjamun, Barfi, Khoa and Dahi (Curd) collected from sites A1 and A2 in first and second turns were positive for *Escherichia coli* (Table 6).

Table 6. Biochemical analysis of *E. coli* isolated from different dairy products collected from sites A1 and A2 in the first turn.

Collection Sites	Sample Type	Round	Biochemical Analysis				
			Catalase	Indole	Lactose	Mannitol	Urease
A1	Rasgulla	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Gulabgamun	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Barfi	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Khoa	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Dahi (Curd)	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
A2	Rasgulla	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Gulabgamun	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Barfi	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Khoa	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Dahi (Curd)	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve

A1= Gourmet sweets Chung. **A2**= Gourmet sweets Thokar Niaz Baig.

All the samples of Rasgulla, Gulabjamun, Barfi, Khoa and Dahi (Curd) collected from sites B1 and B2 in first and second turns were positive for *E. coli* (Table 7).

Table 7. Biochemical analysis of *E. coli* isolated from different dairy products collected from sites B1 and B2 in the first and second turn.

Collection Sites	Sample Type	Turn	Biochemical Analysis				
			Catalase	Indole	Lactose	Mannitol	Urease
B1	Rasgulla	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Gulabgamun	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Barfi	1st	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve

	Khoa	**1st**	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Dahi (Curd)	**1st**	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
B2	**Rasgulla**	**1st**	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Gulabgamun	**1st**	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Barfi	**1st**	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Khoa	**1st**	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve
	Dahi (Curd)	**1st**	+ve	+ve	+ve	+ve	-ve
		2nd	+ve	+ve	+ve	+ve	-ve

B1= Rahman sweets Chung. **B2**= Rahman sweets Thokar Niaz Baig.

Discussion

Many sweets samples indicated higher *Escherichia coli* contents up to $13.41\pm8.46\times10^3$ per gram of orignal sample. Bacterial load of such foods depend not only on the initial contamination levels but cross-contamination during handling and processing of the produce, nature of the substrate in turn of supporting viability and growth of microorganisms concerned and the incubation period and temperature exert great influences in this regard (Beuchat 2002). Usually different components of the food are prepared several hours before serving. Thus it may be speculated that keeping the products produce at ambient temperature, vigorous traffic activities and sanitation conditions might have caused highest *Escherichia coli* contents in these samples.

Recent food-borne disease outbreaks emphasize the importance of screening various food produce especially those that are vulnerable to contamination and those which are ready to eat, or used for products not undergoing any processing steps. Scientific data show that there has been an increase in the number of outbreaks of food-brone diseases in connection with consumption of fresh produce (Beuchat 2002, De-Roever 1998).

The present results indicated a striking difference of

C.F.U/gm of *E. coli* between covered and uncovered foods. Yogurt (Dahi) is mostly sold in the open environment where the chances of contaminations are much greater and as a result indicated much higher contents of *Escherichia coli* then those ones that are mostly sold under hygienic conditions. Moreover the results of advanced and backward areas were much variable depicting higher *Escherichia coli* contents in backward areas. Here it might be speculated that in backward areas contamination become increased due to lack of hygienic facilities.

Another interesting point noted that the number of C.F.U/gm in Rasgulla and Gulabjamun were quite less than those of Barfi and Khoa of both collection sites. Here the "logic" might be that former foods are continuously dipped in a sugar broth and this sugar broth might served as a bacteriostatic agent and thus the contamination could not increased in these samples.

The unclean hands of worker, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk products and the post manufacturing contamination (Bhat *et al.* 1948; Marrier 1973; Tariq Masud *et al.* 1988; Kumar and Sinha 1989; Grewal and Tiwari 1990; Kulshrestha 1990).

Measures to minimize the risk of microbial contamination at all point from the field to the table thorough good manufacturing practices would be the most effective strategy to assure that fresh produce is safe for human consumption (Blumenthal *et al.* 2000).

It is quite obvious that *Escherichia coli* contents of the samples worked out in this study were much higher than the permissible limits. Chances of the produce to carry enteric pathogens pose higher health risks for the consumers. Strict measure to prepare and store the produce are recommended to

be taken seriously by local public health authorities for controlling the enteric infections spread by such contaminated food.

Almost all recent data reported results for samples being positive or negative for *Escherichia coli*. Regarding the enumeration of *Escherichia coli* there is no available data. Thus the present data report these informations for the first time. So this data might be very helpful in future studies.

Conclusion

The results obtained from this study were that milk products available to the consumer have high *Escherichia coli* contamination. More strict preventive measures should be taken to prevent milk products from various contaminations. For this, regular sterilization of dairy equipments, washing of utensils, milker's hands, udders, eradication of diseased animals, pasteurization/boiling of milk is required before collection and distribution for consumption and product making.

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