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Bacteriological quality and safety of raw cow milk in Madurai (South India)

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Abstract

The microbiological quality and safety of raw milk from 60 dairy farms in Madurai were determined. Milk samples were collected at 60 centers from four regions, namely northern, eastern, western and southern (NEWS) according to stratified random sampling design. Samples were analyzed for Total plate count (TPC), psychrotrophs, thermophiles, *Staphylococcus aureus*, coliform, *Escherichia coli* 0157: H7 and *Salmonella.* The mean counts per ml for TPC, psychrotrophs and thermophiles were $12.5x10^6$, $5x10^3$ and $6.85x10^3$ respectively. From the 60 milk samples tested, coliform bacteria contaminated approximately 90% and 70% were *E. coli* positive, with mean counts ranged from $10^3$to 10^4 cfu ml^{-1}. *S. aureus* was isolated from more than 61.7% of the samples and the mean count per ml was $6.2x10^3$. Meanwhile, *E. coli* 0157: H7 was also detected in 39 (65%) samples. However, *Salmonella* was only detected in 8 (13.3%) of the samples with the southern region having the highest frequency of isolation.

Keywords: Indian cow milk; Microbial quality; Safety; TPC; *E. coli; S. aureus*

Introduction

Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections / intoxications in consumers (Murinda *et. al*., 2004 and Oliver *et. al.,* 2005). Microbes may gain entry into raw milk directly from dairy cows experiencing sub clinical or clinical mastitis (Rodojcic and Necev, 1991), from the farm environment particularly the water source (Eberhart, 1977) and utensils used for the storage of milk on farm or during transportation (Freedman, 1977).

A number of bacteria including *S. aureus, Escherichia coli* and *Salmonella* have been recovered from raw milk (De Buyser *et. al.,* 2001) and some of these have been determined to be pathogenic and toxicogenic, and implicated in milk- borne gastroenteritis (Bergdoll, 1979: De Buyser *et al.* 2001 and Maguire *et. al.,* 1992). In recent year's *E. coli* 0157: H7 strain has become very important milk-borne pathogen and cattle are considered its main reservoir (Betts, 2000 and Karmali, 1989).

In India raw milk is traditionally consumed at the small farms where it is produced or fermented into different products. During scaling up, the hygienic aspects are not always sufficiently considered. The risk of contaminated and pathogen containing products could therefore be even greater than when the milk is processed at household level (FAO / WHO, 1997). The delayed time of milking process performance and low hygienic conditions were possible to grow the microorganisms. The contamination leads to pathogenic microbes grows well the milking media. No matter how fast the microorganisms multiply, the contamination would not be detected until the incubation time is over and the contamination sample is taken for analysis.

The importance of various etiological agents in milk borne disease has changed dramatically over time. However, more than 90% of all reported cases of dairy related illness continued to be of bacterial origin, with at least 21 milk borne or potentially milk borne diseases currently being recognized (Bean *et. al*., 1996). Pathogens that have been involved in food borne outbreaks include *Salmonella, Staphylococcus aureus* and *E. coli.* The presence of these pathogenic bacteria in milk emerged as major public health concerns, especially for those individuals who still drink raw milk (Riser, 1998). Most recently *E. coli* 0157: H7 has become serious threat to the dairy industries ranging from mild diarrhoea to potentially fatal hemolytic uremic syndrome (HUS), hemorrhagic colitis and thrombotic thrombocytopenic purpura (Wells *et. al.,* 1991: Bleem, 1994 and Coia *et. al.,* 2001). Keeping fresh milk at an elevated temperature together with unhygienic practices in the milking process may also result in microbiologically inferior quality. Apparently, these are

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common practices for small-scale Asian produce fresh milk and sell it to consumers (Chye *et. al*., 1994).

The output of dairy and dairy products from India is increasing day by day in their international market. Considering its economic potential, extensive and intensive exploitation of cow milk can both contribute to the nutrient requirements of the Indian public 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, information on such aspects is scant and scattered. Thus this study was carried out to investigate the microbiological quality and safety of local cow milk.

Materials and methods

A total 60 raw cow milk samples were collected from 60 dairy farmers who send their milk-to-milk centers (MC) in Madurai. Farmers involved in the study were chosen according to stratified experimental design, where by Madurai was divided in to four regions. Samples were collected in the early morning.

Approximately 100-200 mL milk was aseptically sampled from containers (Pails, buckets or Churns) of bulk milk from each individual farmer into a sterile bottle. It was collected immediately after milking using hand or machine in to bulk milk containers at ambient temperature (28-30^{o}C). Samples were delivered to the laboratory in a cool box at less than 4^{o}C within 1-2 h of collection and tested immediately upon arrival.

Microbiological analysis

Initially, 25 mL of sample were dispensed into a sterile bag containing 225 mL of sterile water and homogenized with stomachar (Bagmixer 400. Interscience). Subsequent serial decimal dilutions of milk were prepared in saline water. Enumeration of total plate count, psychrotrophs, thermophiles, coliform, *E. coli* and *S. aureus* were carried out as described by standard methods of the American Public Health Association (Vanderzant and Splittstoesser, 1992). To enumerate the numbers of coliforms bacteria and *E. coli* in milk, a three tube most probable number (MPN) technique was employed. Positive tube from MPN was streaked onto Eosine Methylene Blue (EMB) agar and then incubated overnight at 35^{o}C. Typical isolates were confirmed based on their iMViC pattern. Baird Parker Agar (Hi Media, India) was used for quantitative detection of *S. aureus*. Representative colonies with typical black appearance and surrounded by clear zone were picked and subjected to catalase and coagulase tests (Staphylex, Oxoid).

Detection of *Salmonella* was carried out according to the International Standard Organization protocol (ISO, 1990), and typical *Salmonella* colonies were confirmed using API 20E test kit (This kit used as identification for Enterobacteriaceae and other non-fastidious Gram-negative rods, which uses 21 standardized and miniaturized biochemical tests and a database). Milk samples (25 mL) were inoculated into 225 mL modified tryptic soy broth with Novobiocin (Hi Media, India) and incubated overnight at 35^{o}C. Approximately 0.1mL of the broth then was streaked on to the surface of sorbitol MacConkey Agar (Hi Media, India) colorless colonies from SMAC Agar were streaked onto a modified EMB agar before confirmed with *E. coli* 0157: H7 latex test (Hi Media, India).

Statistical analysis

Bacterial load and mean counts of coliform, *E. coli* and *S. aureus* were statistically analyzed by one way Analysis of Variance. Significant differences between treatments were determined using Tukey's multiple range test at P =0.05 with the help of SPSS 11.5 software.

Results and discussion

Fresh cow milk collected from different farms were heavily contaminated by bacteria with a mean total plate count (TPC) of 12.5x10^{6} cfu mL^{-1} (Table I). The highest mean value of TPC was found in milk from the eastern region with 13.9 x10^{6} cfu mL^{-1}, while the lowest mean value of 11.7x 10^{6} cfu mL^{-1} was detected in milk obtained from the western region. Results from the analysis of variance (ANOVA) suggested that there was a significant difference ($p \leq 0.05$) in bacterial loads between the two regions. The presence of bacteria in milk samples may not be due to infection of the udder itself, but arise from the teat duct (Ledford, 1998). The bacteria can be carried into milk duct of the cow during milking by suction of the milking machine and then flushed out during subsequent milking without causing clinical symptoms of infection. A TPC less than 10^{6} cfu mL^{-1} is used as a basic standard by milk centers in the price incentive program.

The milking process, especially the equipment associated with it introduces the greatest proportion of microorganism in cow milk (Olson and Mocquot, 1980). According to

Table I. Bacterial load and mean counts of coliform, *E. coli* and *S. aureus* in raw cow milk sample collected from local dairy farmers in Madurai district

Region	Bacterial count (cfu mL^{-1})					
	Total plate count ($x10^6$)	Psychrotrophs ($x10^3$)	Thermophiles ($x10^3$)	Coliform ($x10^4$)	*E. coli* ($x10^3$)	*S. aureus* ($x10^3$)
Southern (n=15)	12.0^{ab}	1.4^{f}	4.1^{de}	14.0^{a}	7.5^{c}	4.2^{de}
Western (n=15)	11.7^{b}	7.1^{c}	6.2^{d}	11.5^{b}	2.7^{e}	9.0^{c}
Eastern (n=15)	13.9^{a}	8.4^{c}	11.0^{b}	5.5^{d}	2.4^{e}	8.5^{c}
Northern (n=15)	12.7^{a}	3.2^{e}	6.1^{d}	3.7^{e}	1.0^{f}	3.3^{e}
Mean count	12.5	5.0	6.85	8.67	3.4	6.25

Within columns, mean ± SD followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$.

Aumaitre (1999), the health of the dairy herd, milking and pre storage conditions are also basic determinants of milk quality. Bacteria may enter milk through the udder and most of the organisms in raw milk are contaminants from the external surface of udder, milking utensils and handlers (Ayres *et. al.,* 1980). Various types of equipment and utensils, such as milking machines, pails, cans and milk churns are used in handling milk on the farm. In order to reduce contamination of milk, utensils used for milking should be rinsed, cleaned using detergent and disinfected immediately after use (Dodd and Phipps, 1994: FAO and WHO, 1997). The results for psychrotrophs and thermophile contamination in raw milk are shown in Table I. Counts for psychrotrophs and thermophiles ranged between 10^3 and 10^4 cfu mL^{-1} with an average count of $5.0x10^3$ and $6.85x10^3$ cfu mL^{-1}, respectively. Samples taken from the eastern region had a significantly higher ($p<0.05$) psychrotrophic count as well as thermophilic load, as compared with other regions. Nevertheless, the high TPC was not significantly correlated with the number of psychrotrophs ($r=0.42$) and thermophiles ($r = 0.48$).

The psychrotrophs count was considered lower than the count for milk produced in temperate countries, which could reach as high as 10^6 cfu mL^{-1} (Reinheimer *et. al.*, 1990). Generally, psychrotrophic organisms were represented by both Gram-negative and Gram-positive bacteria such as, *Pseudomonas, Flavobacterium, Bacillus, Clostridium* and *Mycobacterium* (Cousin, 1982: Sorhaug and Stepaniak, 1997). Champagne *et. al*. (1994) indicated that the quality of dairy products may be affected by heat resistant enzymes or metabolites secreted by psychrotrophs in raw milk during the cold storage.

Table II displays mean counts of coliform, *E. coli* and *S. aureus* of locally produced raw milk. Nearly 90% of the samples collected were contaminated by Coliform bacteria (Table III), with a mean number of colonies 88.3 percent. The existence of coliform bacteria may not necessarily indicate a direct fecal contamination of milk, but more precisely as an indicator of poor hygienic and sanitary practices during milking and further handling. *E. coli* was isolated from 42 (70%) of the milk samples tested, with none of the regions supplying milk free from the organism (Table III). Samples with the highest prevalence (80%) of *E. coli* originated from the Western zone, while the lowest prevalence (60%) was detected in milk from northern region. Although global importance of *E. coli* as a causative agent for diarrhoeal illness has decreased markedly over the past 50 years following the implementation of improved sanitary practices, it is still the major cause of illness in under- developed nations (Riser, 1998). Detecteion of *E. coli* in milk often reflects fecal contamination although environmental coliforms have also been detected in milk (Shehu and Adesiyun, 1990).

Table II. Contamination of raw cow milk samples by Coliform, *E. coli* and *S. aureus*

Region	No. of sample tested	No. of positive sample (%)		
		Coliform	*E. coli*	*S. aureus*
Southern	15	13 (86.7)	10 (66.7)	9 (60)
Western	15	14 (93.3)	12 (80)	10 (66.7)
Eastern	15	13 (86.7)	11 (73.3)	8 (53.3)
Northern	15	13 (86.7)	9 (60)	10 (66.7)
Total	60	53 (88.3)	42 (70)	37 (61.7)

Nearly 61% of the milk samples analyzed were positive *S. aureus* with a frequency of detection ranging from 53% in Eastern region to 67% in western and northern regions which showed a significantly higher *S. aureus* count than other

regions (Table III). These, may most probably due to some of the samples from the regions were highly contaminated with *S. aureus* and also due to the differences in milking technique. However, the rate of isolation of the organism was very much lower than (40%) reported from other tropical countries (Umoh *et. al.,* 1990 and Adesiyun *et. al.,* 1995). Leonard and Markey, 2008 stated that *S. aureus* is widely recognized as a major causative agent of clinical and subclinical mastitis in dairy cattle. Overall 39 of 60 (65%) milk samples tested were positive for *E. coli* 0157: H7; in raw milk samples collected from the northern region was the highest 73.3% followed by samples from eastern and western regions with prevalence of 66.7% respectively (Table IV). The prevalence of *E. coli* 0157: H7 in local milk seems to be higher than (76%) the published data reported by Adesiyun *et. al.* (1995) and Padhye and Doyle (1991). The difference in the frequency may be partially due to the fact that in the present study, selective enrichment medium was used before streaking onto Sorbitol MacConkey agar.

Table III. Contamination of raw cow milk samples by Coliform, *E. coli* and *S. aureus*

Region	No. of sample tested	No. of positive sample (%)		
		Coliform	*E. coli*	*S. aureus*
Southern (n=15)	15	13 (86.7)	10 (66.7)	9 (60)
Western (n=15)	15	14 (93.3)	12 (80)	10 (66.7)
Eastern (n=15)	15	13 (86.7)	11 (73.3)	8 (53.3)
Northern (n=15)	15	13 (86.7)	9 (60)	10 (66.7)
Total	60	53 (88.3)	42 (70)	37 (61.7)

Although the consumption of undercooked group beef is still the traditional mode for *E. coli* 0157: H7 infection, illness resulting from ingestion of contaminated raw milk is increasing. The environmental niches for *E. coli* 0157: H7 have not yet been clearly established. However, dairy cattle appear to be a major reservoir for this pathogen, even though with a very low prevalence (Wells *et. al.,* 1991 and Garber *et. al.* 1999). *E. coli* 0157: H7 is apparently confined to the intestinal tract of dairy cattle and perhaps other animals as well. Given the higher possibility for contamination of milk at dairy farms, consumption of such raw milk should be avoided. Flushing animal houses with water to remove manure are fairly common practice in most dairy farms. Although it is effective and quickly removes manure, this practice may distribute fecal flora throughout the farm environment, thus exposing large number of animals to the organism. All aspects of hygienic handling, strict maintenance of refrigeration at lower than 4^{o}C and effective control measures are all primary concerns for quality assurance in the dairy industry (Sorhaug and Stepaniak, 1997).

The incidence of *Salmonella* spp in local raw milk was still low, as only 8 of 60 milk samples were found positive for this organism (Table IV). Samples from southern region of the district seem to have a higher rate of isolation (3%), while the lowest (1%) was milk samples from eastern region. All salmonellae are of public health concern having the ability to produce infection ranging from a mild self-limiting form of gastroenteritis to septicemia and life threatening typhoid fever (ACDP, 2001). Thus, although their occurrence in local milk is low, they still pose a health risk to consumer if milk is consumed without any heat treatment. This problem is particularly evident in developed countries like England and Wales, where the most frequently reported outbreaks were salmonellosis associated with the consumption of raw milk and products (De Buyser *et. al.,* 2001).

Table IV. Prevalence of pathogens in raw cow milk in Madurai

Region	No. of sample tested	No. of positive sample (%)	
		E. coli 0157: H7	*Salmonella* spp
Southern	15	8 (53.3)	3 (20)
Western	15	10 (66.7)	2 (13.3)
Eastern	15	10 (66.7)	1 (6.7)
Northern	15	11 (73.3)	2 (13.3)
Total	60	39 (65)	8 (13.3)

Since the microbiological limits of raw milk are not established in this country: it is very likely that milk should often be tested, if found positive for pathogens then withheld from human consumption. The production of high-quality milk and safe milk should be of great importance to the economy of the farmer and the sustainability of the dairy industry in this country.

Conclusion

Therefore, poor milk quality has often been considered as one of the major reasons for losses and results in deduced income for the stallholder dairies in Madurai.

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