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Quality Assurance and Public Health Safety of Raw Milk at the Production Point

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Abstract: In order to get an insight on the microbial quality assurance of milk at the point of production (farmer's level), a total of 240 raw milk samples were collected from farmers belonging to three farmer dairy societies of Kerala, viz; FS₁, FS₂ and FS₃ and were examined to estimate the microbial load per ml of milk and also to detect the presence of bacterial pathogens of public health significance. The quality of each samples were evaluated by estimating the Total Viable Count (TVC), Coliform Count (CC), Psychrotrophic Count (PC), Faecal Streptococcal Count (FSC) and Yeast and Mould Count (YMC). The samples collected from the farmers belonging to society 2 (FS₂) had the highest microbial load with respect to the TVC, CC, PC and YMC with highly significant ($p \leq 0.01$) difference between mean TVC, CC and PC of the samples obtained from the FS₁ and significant ($p \leq 0.05$) difference between mean YMC of FS. The samples belonging to FS₃ has the highest mean FSC. Among the pathogenic isolates, *E. coli* was detected in 76 (31.6%) samples and the isolates consisted of the serotypes 05, 024, 025, 068, 084, 087, 0103, 0116, 0125, 0145, 0157 and 0172. *Stap. aureus* was isolated from 84 samples (35%), *Yersinia* from 118 and *L. monocytogenes* from only one sample. Among spoilage causing organisms viz., *Aeromonas*, *Pseudomonas aeruginosa*, *Lactobacillus* and *Proteus*, the isolates obtained were 88, 10.8, 83.7 and 5%, respectively. In order to identify the Critical Control Points (CCP) of microbial contamination of milk the samples of air, water, hand wash, udder wash and utensil rinsing obtained from the farmers were evaluated and observed high counts from samples of udder and hand wash indicative of milk contamination.

Key words: Coliform count, CCP, contamination, *E. coli*, milk, quality assurance

INTRODUCTION

Milk has a high nutritive value, not only for the new-born mammal and for the human consumer, but also for microbes. The quality and safety of market milk begins with the milk producer (farmers). Even with higher levels of quality, some consumer groups challenge and question the microbial safety of the milk supply which affects the milk producer's profits and staying power. To overcome it, the farmer must recognize the potential issues and know the interrelationship of the issues to milk production. Milk gets contaminated at various stages be it from the cow, milker (manual as well as automated), extraneous dirt, environment or unclean process water (Hayes *et al.*, 2001). Such significant contribution to milk contamination from sources (such as milking equipment) was reported by McKinnon *et al.* (1990). The microorganism, which may gain entry to milk, can multiply and bring about either spoilage or render them unsafe due to potential health hazards (Chye *et al.*, 2004). The threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable (Foster, 1990). Thus it is very much essential to know how

milk gets contaminated and milk being a major part of human diet, its microbial quality is all the most important. The present study thus aimed to determine the hygienic conditions and possible sources of contamination associated with cow-milking from randomly selected group of farmers (belonging to members of milk societies in Palakkad district, Kerala, India) by the enumeration, detection and isolation of a variety of indicator and spoilage organisms from milk and enumeration of microbes from contaminating sources.

MATERIALS AND METHODS

In order to get an insight on the microbial quality and the presence of bacterial pathogens of public health significance in milk at the point of production (farmer's level), the samples were collected from farmers and subjected for evaluation of the microbial load per ml of milk and also for isolation of the pathogenic and spoilage causing bacterial pathogens. The study was undertaken in Palakkad district, Kerala, India. This is a district in Kerala with highest number of cattle population and where cattle rearing serve as an important occupation. A total of 240 milk samples were collected from 24 farmers belonging to three farmer societies of the district viz; FS₁, FS₂ and FS₃. The three societies were selected based on the high contribution of milk supply to the general public and where a minimum of 200 registered producers were involved as member. From each society 8 farmers were selected on the basis that cattle farming serve as an important source of their livelihood and the collection of the sample from each farmer was repeated ten times. Each sample consisted of about 500 mL milk, collected in clean sterile conical flask and brought to the laboratory in an insulated container.

At the time of collection of milk from the farmer, water samples used by farmers to clean utensil or animal were also collected according to the procedure described by BIS (1978) and the utensil rinses were collected according to the procedure described by Evancho *et al.* (2001). Hand washing of the milker and the udder washing of the each animal were also taken. Air sample was also collected before and after the process of milking by sedimentation method as described by Evancho *et al.* (2001). All the samples were brought to the laboratory in an insulated container and were tested immediately for microbial quality.

In order to estimate the microbial load per milliliter of milk, each sample was thoroughly mixed and 25 mL of the sample was transferred to 225 mL of 0.1% peptone water (diluent) so as to form one in 10 dilution of the sample. Further 10 fold serial dilutions were prepared. Dilutions were made up to 10⁻⁶ and selected dilutions of each sample were used for the estimation of various microbial loads per ml of sample. All aseptic precautions were taken during collection and processing of milk samples. Each sample was tested to estimate the Total viable count or TVC (Mortan, 2001), coliform count or CC (Kornacki and Johnson, 2001), faecal Streptococcal count or FSC (as per the method described by the Nordic Committee on Food Analysis 1968), yeast and mould count or YMC (Beuchat and Cousin 2001) and psychrotrophic count or PC (Consin *et al.*, 2001), respectively. The count per ml was expressed as log₁₀ cfu mL⁻¹. Air sample was tested for total count and YMC whereas samples from other sources were evaluated for TVC, CC, ECC (BIS, 1980) and FSC. All milk samples were also subjected for the isolation and identification of *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Aeromonas*, *Yersinia*, *Pseudomonas*, *Lactobacillus* and *Proteus*. Isolation was done according to the standard methodologies recommended by the American Public Health Association (2001) and BIS (1980). All the suspected colonies were selected and transformed to suitable growth medium and stored under refrigeration and the isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions as described by Barrow and Feltham (1993). *Escherichia coli* Isolates were serotyped at National Salmonella and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh. The data obtained from the above studies were subjected to statistical analysis following procedure described by Rangaswamy (1995).

RESULTS AND DISCUSSION

Microbial Counts of Milk

The microbial counts of milk samples collected from the farmer's level belonging to the three societies are given in Table 1. Total Viable Count (TVC) serves as an important criteria for evaluating the microbial quality of various foods and also degree of freshness of food. Analysis of variance test of the data revealed highly significant ($p < 0.01$) difference between the mean TVC count of samples belonging to three societies. The highest mean count was recorded from FS₂ and the lowest count in milk samples belonging to FS₁. The critical difference test revealed highly significant ($p < 0.01$) difference between the mean counts of milk samples FS₁ and FS₂ and the samples from FS₁ and FS₃. The count of the milk samples was similar to those obtained in the studies conducted by Lues *et al.* (2003) and Chye *et al.* (2004).

Statistical analysis of mean coliform count revealed significant ($p < 0.05$) difference between mean count of milk samples from farmers of the three societies. The highest count was seen in FS₂ ($3.20 \pm 0.06 \log_{10} \text{ cfu mL}^{-1}$) and lowest mean count was seen in milk samples belonging to farmers of society 1 (FS₁). The critical difference test revealed significant ($p < 0.05$) difference between the mean counts of milk samples FS₁ and FS₂. Khalilur *et al.* (2002) also obtained the mean coliform count of $3.38 \log_{10} \text{ cfu mL}^{-1}$ from raw milk collected from Aligarh city.

Psychrotrophs are widely distributed in raw milk. The presence of psychrotrophic bacteria in milk could be of great significance as these organisms may grow and proliferate during storage at even low temperature such as 5°C and bring about spoilage conditions. Statistical analyses of the data revealed highly significant ($p < 0.01$) difference between mean count of samples. Highest count was seen in the samples of FS₂ ($5.30 \pm 0.09 \log_{10} \text{ cfu mL}^{-1}$) and the lowest mean count was seen in milk samples FS₃ ($4.86 \pm 0.08 \log_{10} \text{ cfu mL}^{-1}$). The psychrotrophic count at $5 \log_{10} \text{ cfu mL}^{-1}$ as seen in FS₂ was also observed in the findings of Misra and Kuila (1989). The critical difference test revealed highly significant ($p < 0.01$) difference between the mean counts of milk samples belonging to farmers of society FS₁ and FS₂ and the samples from farmers of society 2 and FS₃. The source of psychrotrophic bacteria in raw milk as non-sterile farm utensils and equipments, coats of cows and water supplies. Thus hygienic practice in milking barn minimizes psychrotrophic counts.

Enterococci (Faecal Streptococcal count, FSC) provide a better index of food sanitary quality than coliform since these organisms are more resistant to adverse processing conditions like low and high temperature. Statistical analysis of the data revealed highly significant ($p < 0.01$) difference between the mean count of samples of the farmers of the three societies. The lowest mean count was seen in milk samples belonging to farmers of society 1 ($1.76 \pm 0.13 \log_{10} \text{ cfu mL}^{-1}$) and the highest count in the samples of farmers of society 3 ($2.80 \pm 0.08 \log_{10} \text{ cfu mL}^{-1}$). The critical difference test revealed highly significant ($p < 0.01$) difference between the mean counts of milk samples FS₁ and FS₂ and the samples from FS₁ and FS₃.

Yeast and mould are widely distributed in the environment and may be found as a part of the normal flora of foods or in inadequately sanitized utensils or as an air-borne contaminants. These can

Table 1: Mean microbial counts of milk from farmers of the three societies

Source of milk samples	Mean±SE (Log ₁₀ cfu mL ⁻¹)				
	TVC	CC	PC	FSC	YMC
FS ₁	6.10±0.17 ^b	2.97±0.05 ^b	4.90±0.07 ^b	1.76±0.13 ^b	3.11±0.05 ^a
FS ₂	6.57±0.18 ^a	3.20±0.06 ^a	5.30±0.09 ^a	2.66±0.27 ^a	3.12±0.06 ^a
FS ₃	6.40±0.16 ^a	3.13±0.06 ^a	4.86±0.08 ^a	2.80±0.28 ^a	2.77±0.05 ^b

N = 80 sample from each farmers society, Figures bearing the same superscript do not differ significantly, TVC: Total Viable Count, CC: Coliform Count, PC: Psychrotrophic Count, FSC: Faecal Streptococcal Count, YMC: Yeast and Mould Count

cause public health problems by the production of toxic metabolites. Analysis of variance test of the data revealed significant ($p < 0.05$) difference between mean count of samples of the farmers of the three societies. The lowest mean count was seen in milk samples belonging to FS₃ ($2.77 \pm 0.05 \log_{10}$ cfu mL⁻¹) and the highest count in the samples FS₂. However Lues *et al.* (2003) obtained higher average yeast count (2.3×10^6 cfu mL⁻¹) from 60 selected households from Botshebelo Township. The critical difference test revealed significant ($p < 0.05$) difference between the mean counts of milk samples FS₁ and FS₃ and of the samples FS₂ and FS₃.

Interpretation on the Quality of Raw Milk

In India raw milk is graded by Indian Standards (BIS, 1977). According to the criteria for total counts prescribed by BIS: 1479 (1977) 27% samples were graded as good and 64% as fair quality and remaining (9%) as poor quality. The study conducted by Singh *et al.* (1994) observed 37.73% as poor quality. With regard to coliform standards only 15% of raw milk confirmed with the Indian standards, 1977 and were considered satisfactory.

Isolation and Identification of Pathogenic and Spoilage Organisms from Milk

In the present investigation, 240 milk samples were collected from 24 farmers belonging to three societies and evaluated for the presence of pathogenic and spoilage organisms. The respective number and percentages are indicated below (Table 2).

All the samples of milk samples collected from farmers revealed the presence of Staphylococci. However, coagulase positive *Staphylococcus aureus* was isolated from 84 samples (35%). *S. aureus* has been associated with food poisoning outbreaks with the consumption of raw milk (Carmo *et al.*, 2002). The presence of the organism in milk indicates the poor hygienic practices and health conditions of animal. *E. coli*, a member of the family Enterobacteriaceae, is a part of normal flora of the intestinal tract of humans and animals. The isolates obtained (76 number) were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli. Of the 76 isolates, 8 isolates belonged to serotype 0172, nine of serotype 024, three of 0157, two each isolate of serotype 025, 0125 and 0145. Apart from this there were three isolates of serotype 0103, one each of serotype 05 and 068. Remaining isolates were 084 (7), 087(3), 0116 (9). However some isolates were Rough (16) and Untypable (10). From the isolates, FS₃ revealed maximum contamination of the organism (43% of the total isolates). The serotype 05 and 0172 belong to Enterohaemorrhagic group. Similarly Sharma *et al.* (1995) also reported the isolation of serotype 05 from raw milk. Infection with Enterohaemorrhagic group of *E. coli* in man causes diarrhea, haemorrhagic colitis and haemolytic ureamic syndrome (HUS). The serotype 0157 belongs to EHEC. In severe infection this serotype causes disease which is characterised by acute renal failure, haemolytic anaemia and thrombocytopenia. *L. monocytogenes* could be isolated from only one samples obtained from the farmer belonging to member of society 2 (FS₂). Such low incidence of *L. monocytogenes* in milk was also reported by Bhilegaonkar *et al.* (1997) and Chye *et al.* (2004). *Yersinia enterocolitica* is an emerging pathogen of importance of milk-borne disease. It is one of the few human pathogens that can grow at refrigerated temperature and its presence in milk is of great public health significance. On the basis of colony morphology on Yersinia Selective agar, a total of 118 isolates were found to contain typical yersinia colonies, however none of the sample on biochemical characterization revealed the presence of the *Yersinia enterocolitica*. Rohrbach *et al.* (1992) had obtained *Y. enterocolitica* from 15.1% of raw milk.

Isolation of Spoilage Organisms

Aeromonas was isolated from 88% samples. However, only three samples revealed the presence of *A. hydrophila* and the remaining samples were *A. caviae*. *Aeromonas*, a psychotropic organism, is associated with food borne gastroenteritis in immunocompromised individuals. The virulent strains of

Table 2: The number and percentage of isolates of pathogenic and spoilage organisms

Samples	No. of isolates	Percentage of isolates
<i>Staphylococcus aureus</i>	84	35.00
<i>Escherichia coli</i>	76	31.60
<i>Listeria monocytogenes</i>	1	0.42
<i>Aeromonas</i> spp.	211	88.00
<i>Yersinia</i> spp.	118	49.20
<i>Pseudomonas aeruginosa</i>	26	10.80
<i>Lactobacillus</i> spp.	201	83.70
<i>Proteus</i> spp.	12	5.00

Table 3: Microbial load from various contamination sources of milk

Sources	Bacterial count (Log ₁₀ cfu mL ⁻¹)			
	TVC	CC	ECC	FSC
Milk pail	2.81±0.27	0.70±0.25	ND	1.42±0.16
Udder and teat wash	5.21±0.33	2.20±0.24	1.41±0.26	1.36±0.16
Hand wash	3.86±0.30	2.56±0.35	1.20±0.20	2.27±0.31
Water	3.10±0.21	1.90±0.21	0.57±0.30	0.77±0.11

TVC: Total Viable Count; CC: Coliform Count; ECC: Escherichia Coli Count; FSC: Faecal Streptococcal Count

Table 4: Mean microbial counts of air samples

Samples Process	Collection	Mean±SE (cfu/ft ³ /min)	
		TVC	YMC
Milking of animals	Before	66.10±3.58	22.36±0.84
	After	119.83±3.90	29.17±1.49

TVC: Total Viable Count; YMC: Yeast and Mould Count

A. hydrophila posses enterotoxic, cytotoxic and haemolytic properties whereas, *A. caviae* has been identified with enterotoxin production. *Pseudomonas* is an important gram negative psychrotrophs which can cause flavour defects in milk. Out of 26 isolates the presence of pigmented colonies were identified and characterized as *P. aeruginosa*. The samples collected from farmers of the three societies revealed the presence of *Lactobacilli* spp in 84% samples. The organisms have the ability to ferment sugars with the production of considerable amount of lactic acid. *Proteus* (5%) produces amoeboid colonies that show swarming phenomenon on solid medium and has a spoilage causing potential as it leads to the formation of cadaverine which produces foul odour in the milk samples. Garg *et al.* (1977) obtained 4 isolates of *proteus* from 57 raw milk examined from different sources of Hissar city.

Assessment of Critical Control Points of Microbial Contamination of Milk

The external microbial contamination of milk is one of the most important causes for milk spoilage and human health hazards. The presence and extent of microbes in environmental samples, utensils used in the milking parlour, hand wash of milker and the udder wash from the animal and their role in the contamination of milk was determined. The load of microorganism in milk from various contamination sources viz. water, air, utensil, milker and animal is given Table 3 and 4.

Water used in by farmers to wash utensils, hand etc. had mean total viable count at the level of 3 log cfu mL⁻¹. The coliform count of water samples was 1.90±0.21 (Table 3). Palaniswami *et al.* (1988) obtained the mean coliform count of tap water in the dairy farm, as 72 MPN/mL. *E. coli* was not detected in most of the water samples however mean count obtained was 0.57±0.30 log cfu mL⁻¹. Lopes and Stamford (1997) found 60% samples of water used to clean the milk equipment revealed the presence of coliform. The mean faecal streptococci count was 0.77±0.11 log₁₀ cfu mL⁻¹, thus indicating faecal contamination of water.

The farmers of various house holds use Aluminium or stainless steel utensils for collecting milk. Dirty or unhygienic pails carry pockets of bacteria in the soil deposits and thus are unsafe for collecting milk. In the present study coliform and faecal streptococcal contaminants was evident in

some utensils, however none of them revealed the presence of *E. coli*. Milk pail samples revealed the mean total viable count at the level of 2 log cfu mL⁻¹ (Table 3). McKimmon *et al.* (1990) reported that the mean bacterial count of the milking equipment rinse was at the level of 4.4×10⁷ m⁻². In the study, coliforms were present at the level of one log cfu mL⁻¹ level in samples of milk pail.

Hand washings of milker had high CC and FSC when compared with other CCP. The detection of *E. coli* and faecal streptococci in the hand wash of the workers indicated the poor hygienic practices of the milker. However some milkers practice hand washing with soap and water before milking. The unhygienic procedure observed from milker are improper trimming of nails, practice of coughing, spitting or sneezing during milking and unclean clothes. Palaniswami *et al.* (1988) found the coliforms counts of milkers hand wash as 113 per 100 cm².

Samples of washings taken from animal udder and teat also revealed contamination from *E. coli*, faecal streptococcus and coliform. The TVC obtained was high (5.21±0.33 log₁₀ cfu mL⁻¹). None of the udder from which milk samples were collected revealed the presence of ulcer or other mastitic conditions.

Mean total viable count of air samples collected from the milking barn was higher after the process of milking (Table 4). Mean fungal counts were also found to be increased considerably after the above process. Aggarwal and Srinivasan (1980) found that fall of mould conidia per plate per min from hand milking byre ranged between zero to 24.5.

Critical Control Points of Raw Milk Produced at Farmer's Level

Critical control points in production of raw milk and control measures are shown in Fig. 1. Although milk secreted from udder is free from microorganism, the contamination occurs as it passes through ducts and from reservoirs of udder and from external contamination from milker, utensils or from environment. The critical control points of bacterial contamination during production raw milk in the households of farmers of are given in Fig. 1. Assessment of CCP revealed that maximum contamination of raw milk collected from farmers was due to improperly cleaned udder and also through unhygienic milkers. Contamination from the environment (air, water) was also evident.

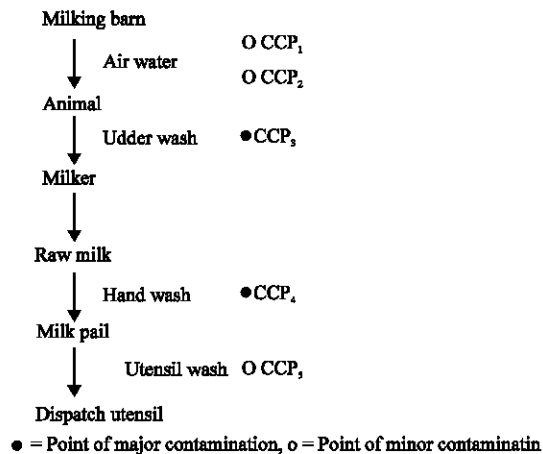


Fig. 1: Critical control points in production of milk at farmers level. CCP₁ = Environment sanitation, CCP₂ = Chlorination, Environment sanitation, CCP₃ = Healthy animal, disinfection of udder, CCP₄ = Milkers personal hygiene, Hygienic milking process and CCP₅ = Periodic cleaning of utensils

CONCLUSIONS

The hygienic practices followed during the production of milk at the point of production (farmers level) needs an improvement with regard to reduction in microbial count and overcoming the impact of the harmful pathogens like *E. coli* and *S. aureus*. The presence of coliform, faecal streptococci and *E. coli* in milk indicated contamination of milk from the environmental sources and also from human and animal sources. Apart from this the evidence of spoilage microorganism like pseudomonas, proteus etc was also seen. Hence strict hygiene and health education needs to be implemented to minimize contamination occurring in the milking barn and the milk. Thus the concept of quality and food safety (HACCP) management system needs to be strictly adopted. Thus the study provides the basis for hygienic milk production and how and where control measures needs to be adopted so as to minimize microbial contamination of milk. Further the study will also serve as the indicator for estimating the average shelf life of milk by knowing the initial quality of milk and changes with regards to microbial and sensory quality.

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