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Microbial Quality Assurance of Milk During Production, Processing and Marketing

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Abstract: The microbial quality assurance of milk was done by evaluating 200 samples of milk from production point (dairy farm = 60), processing plant (60) and from market (80). Out of 60 samples of raw milk analyzed, 20 each from individual animal after complete milking (IAM), Pooled milk (PM) and chilled milk (CM), the PM revealed maximum contamination with highly significant ($p\leq 0.01$) difference between mean Total viable count (TVC) and Coliform count (CC) and significant ($p\leq 0.05$) difference between mean Psychrotrophic count (PC) and Faecal Streptoccal count (FSC) of milk from IAM. From the sample of milk (20 each) collected from pasteurization plant viz. immediately after heating (AHM), after pasteurization (Pasteurized milk or PAM) and after packaging (Packaged milk or PGM) the mean TVC was high in PGM. Only 40% of PGM was graded satisfactory based on coliform standards prescribed by BIS. On comparison of PGM obtained from dairy plant with the retail brands revealed that PGM had highly significant ($p\leq 0.01$) difference and lesser TVC, CC and PC in comparison with the brands B, C and D. The samples of the brand B had highest mean TVC. The highest mean CC, ECC, PC and FSC were seen in the samples of brand C. Evaluation of various critical points of bacterial contamination of milk from production and processing sites revealed high microbial count from milk pail, milkers hand washings and package machine wash indicating an important sources of contamination.

Key words: Contamination, milk, microbial quality, pasteurization

INTRODUCTION

Milk, being a major constituent of the diet, its quality assurance is considered essential to the health and welfare of a community. Milk may contain few organisms when it leaves the udder, also milk gets contaminated at various stages be it from the cow, milker (manual as well as automated), extraneous dirt or unclean process water (Hayes *et al*., 2001). The threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable (Foster, 1990). With the aim of minimising milk-associated health hazards, restrictions and legislation on the marketing of unpasteurised milk have been introduced in most countries (European Commission, 2000). However, this does not necessarily guarantee the safety of milk products. Outbreaks of milk-borne diseases have occurred despite pasteurisation, caused either by improper pasteurization or by recontamination (DaSilva *et al*., 1998). Several food borne disease outbreaks have been linked to pasteurized milk and traced to inadequate pasteurization or post-pasteurization contamination (ICMSF, 1998). The detection of coliform bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used

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(Bonfoh *et al*., 2003). Thus the objective of the study deals with ensuring the safety of pasteurized milk by evaluating the milk quality, hygienic conditions and possible sources of contamination associated with milk during production, pasteurization and marketing.

MATERIALS AND METHODS

In order to get an insight on the microbial quality of milk produced at the point of production (farm level), processing line (pasteurization plant) and at the time of marketing (retail brands) the milk samples were collected and examined for the microbial counts. The study was conducted in a dairy farm and pasteurization plant located in Thrissur, Kerala. The processing plant is located at a distance of 500 m from the farm and receives milk from the dairy farm morning and evening. Raw milk of evening and next day morning collection are pasteurized and packed at one time in this plant. Market samples were collected by selecting 4 different brands commonly available in Thrissur market. A total of 200 milk samples consisting of raw milk collected from individual cows after complete milking or IAM (20), pooled samples or PM (20), chilled milk before pasteurization or CM (20) and milk at various stages of pasteurization viz., immediately after heating section or AHM (20), pasteurization or PM (20), packaging or PGM (20) and 20 samples from 4 different brands (Brands' A, B, C, D) available in market were assessed for the microbial quality. The samples were tested for total viable count (TVC), coliform count (CC), *Escherichia coli* count (ECC), psychrotrophic count (PC), faecal streptococcal count (FSC) and yeast and mould count (YMC).

Approximately 500 mL milk samples were collected from all sources at a time. The samples were brought to the laboratory in an insulated container. In order to estimate the microbial load per mL 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. The procedure used for estimation of TVC, CC, ECC, FSC, YMC and PC was as per the method described by Mortan (2001), Kornacki and Johnson (2001), BIS (1980), the Nordic Committee on Food Analysis (1968), Beuchat and Cousin (2001) and Consin *et al*. (2001), respectively. The count was expressed as $\log_{10}$ cfu/mL.

In order to assess the critical control points of microbial contamination of milk during milking, samples of tap water used in milking barn, washings of milk pail, milkers hand, milking machine, milk strainer and milk cans were collected and subjected to estimation of TVC, CC, ECC and FSC. The samples of air before and after milking, before and after pasteurization and packaging were evaluated for total count and YMC. Also, water and washings of workers hands and equipment of the pasteurization area, washings of packaging materials, packaging machine and storage crate were also collected and analysed for the bacterial load. Air sample was collected by sedimentation method as described by Evancho *et al*. (2001). Samples of water were collected as per the procedures described by BIS (1978). Rinse samples were collected following procedure described by Evancho *et al*. (2001).

The data obtained from the above studies were subjected to statistical analysis following procedure described by Rangaswamy (1995). Comparison of milk from farm and processing line was done by students t test and between brands was done by ANOVA.

RESULTS AND DISCUSSION

Total Viable Count (TVC)

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 the data revealed highly significant ($p<0.01$) difference between mean TVC of the samples from IAM and PM and also between the samples of IAM and CM (Table 1). The mean TVC of the IAM and PM samples in the present study was about one log lower than that reported by Jolly *et al*. (2000). The author also reported high mean count in PM when compared to individual animal milk samples from the same source. The process of chilling of PM in the dairy plant and storage at 7-10°C for 17 h. did not significantly increase the total viable count due to the presence of antimicrobial substance in milk and the effect of low temperature. The difference between the mean TVC of milk obtained from various stages of pasteurization was highly significant ($p<0.01$). PGM samples had highest mean count. There was highly significant ($p<0.01$) difference in count of PAM and PGM which may be attributed to post pasteurization contamination which includes improperly cleaned pasteurizer equipment, storage tank and packaging units, package materials and working personnel. Among retail samples the highest mean count was seen in the samples belonging to brand B and the lowest from samples belonging to brand A. The critical difference test revealed highly significant ($p<0.01$) difference between the mean counts of brand A and B, A and C, D and A. Comparison of this brands with PGM revealed highly significant ($p<0.01$) difference between the counts of brands B, C and D.

Coliform Count (CC)

The analysis of the data of mean CC of raw milk revealed highly significant ($p<0.01$) difference between mean counts of IAM and PM and between IAM and CM (Table 1). Similar variation in the count of individual and pooled milk samples was made by Palanniswami *et al*. (1988). The samples of PM had a highest mean coliform count. Coliform organism in milk can gain entry through environmental and faecal contamination and also coliform can rapidly build up in moist, milky residues on the milking equipment and become a major source for contamination of milk (Robinson, 2002). The coliform organism was absent in cent per cent of AHM. However 20% of PAM and 60% of PGM revealed the presence of the organism whose mean counts are depicted in Table 1. Highly significant ($p<0.01$) difference was observed between the mean counts of PAM and PGM. Among the brands, coliform count was highest in brand C and lowest was seen in the samples of the brand A. The critical difference test revealed high significant ($p<0.01$) difference between the mean counts of the samples from A and B, A and C, A and D, C and D and also from the mean counts of PGM with the counts of brands B, C and D.

***Escherichia coli* Count (ECC)**

The highest *Escherichia coli* count was seen in chilled milk samples. The presence of this organism in milk is a clear indication of the poor hygienic practices followed during the production and handling of milk, since this organism is of intestinal origin in man and animal. The *Escherichia coli* count was detected only in 10% packaged milk. The presence of the organism in pasteurized milk indicates insufficient pasteurization or post pasteurization contamination of milk. Highly significant ($p<0.01$) difference was observed between the mean count of brands. The lowest mean *E. coli* count was seen in samples belonging to brand A and the highest count in brand C. The critical difference test revealed highly significant ($p<0.01$) difference between the mean counts of the brand A and B, A and C and B and D, B and PGM and C and PGM. The presence of *E. coli* in pasteurized milk may cause a variety of illness from mild diarrhoea to severe hemorrhagic colitis (Table 1).

Psychrotrophic Count (PC)

In connection with the holding of milk at relatively low temperatures, psychrotrophic bacteria are of special importance. Analysis of the mean psychrotrophic count of revealed that significant ($p<0.05$) difference between mean counts of IAM and PM and also between that of CM and IAM. The samples of chilled milk had a highest mean count as the psychrotrophs thrive and grow under refrigerated temperatures. The psychrotrophic count at 5 $\log_{10}$ cfu mL^{-1} was also observed in the

Table 1: Microbial counts of milk at the point of production, processing line and market milk

Source of milk	Mean±SE (log_{10} cfu mL^{-1}) TVC	CC	ECC	PC	FSC	YMC
Microbial counts of raw milk samples						
IAM	5.14 ± 0.13^{a}	1.83 ± 0.22^{a}	1.01 ± 0.21	4.69 ± 0.24^{a}	1.89 ± 0.08^{a}	1.58 ± 0.27
PM	5.58 ± 0.14^{b}	3.24 ± 0.19^{b}	0.63 ± 0.31	5.42 ± 0.07^{b}	2.59 ± 0.11^{b}	1.86 ± 0.19
CM	5.70 ± 0.13^{b}	3.10 ± 0.17^{b}	1.05 ± 0.29	5.56 ± 0.14^{b}	2.57 ± 0.12^{b}	1.84 ± 0.24
Microbial counts of milk at various stages of pasteurization						
AHM	3.00 ± 0.15^{a}	ND	ND	1.20 ± 0.22^{a}	0.23 ± 0.10^{a}	0.11 ± 0.07^{a}
PAM	3.64 ± 0.15^{b}	0.25 ± 0.19^{a}	ND	2.15 ± 0.14^{b}	0.70 ± 0.16^{b}	0.23 ± 0.11^{a}
PGM	4.76 ± 0.15^{c}	0.98 ± 0.36^{b}	0.31 ± 0.21	4.16 ± 0.09^{c}	1.06 ± 0.18^{b}	0.62 ± 0.13^{b}
Microbial counts of retail samples						
PGM	4.76 ± 0.15^{b}	0.98 ± 0.36^{c}	0.31 ± 0.21^{b}	4.16 ± 0.09^{c}	1.06 ± 0.18	0.62 ± 0.13
Brand A	4.72 ± 0.26^{b}	1.22 ± 0.28^{c}	0.75 ± 0.27^{b}	4.45 ± 0.32^{c}	1.10 ± 0.16	1.24 ± 0.23
Brand B	5.77 ± 0.10^{a}	2.42 ± 0.28^{ab}	1.83 ± 0.27^{a}	5.40 ± 0.42^{ab}	1.42 ± 0.24	0.75 ± 0.12
Brand C	5.42 ± 0.26^{a}	3.16 ± 0.19^{a}	2.25 ± 0.28^{a}	5.72 ± 0.15^{a}	1.61 ± 0.26	0.80 ± 0.21
Brand D	5.33 ± 0.17^{a}	2.20 ± 0.25^{b}	0.65 ± 0.15^{b}	4.92 ± 0.25^{b}	0.80 ± 0.20	0.65 ± 0.25

N = 20 per sample; Mean values with the diffirent superscripts are significantly diffirent at level $p<0.05$ or $p<0.01$ (as given in text), ND: Not Detected, IAM: Milk from Individual animal, TVC: Total Viable Count, PM: Pooled milk, CC: Coliform Count, CM: Chilled Milk, ECC: *Escherichia coli* Count, AHM: Milk after heating section, PC: Psychrotrophic Count, PAM: Pasteurized Milk FSC: Faecal Streptococcal Count, PGM: Packaged Milk, YMC: Yeast and Mould Count

findings of Misra and Kuila (1989). Psychrotrophic counts in AHM shows highly significant ($p<0.01$) variation from the count of PAM and PGM. Similar findings was reported by Eneroth *et al*. (1998) where maximum recontamination by gram-negative psychrotrophs occurred due to post pasteurization contamination by the filling procedure of the pasteurized milk. A highly significant ($p<0.01$) difference was observed in the mean count of the samples from all brands. The highest count was found in the samples belonging to brand C and the lowest count in the samples of brand A. The critical difference test revealed highly significant ($p<0.01$) difference between the mean counts of the samples from A and B, A and C, A and D, B and PGM, C and D, C and PGM and also significant ($p<0.05$) difference between D and PGM. Gopi *et al*. (2001) also found variation in psychrotrophic counts from retail brands available in Chennai and the count ranged between 12.50 to 99.33×10^{4} cfu mL^{-1}.

Faecal Streptococcal Count (FSC)

The mean faecal streptococcal count of raw milk samples revealed a significant ($p<0.05$) difference between mean counts of IAM and PM and also between the counts of IAM and CM: If better sanitary practices are followed in the farm with minimal faecal contamination lesser will be the count, as these organisms are the inhabitants of the intestines of man and animals. The faecal streptococcal count was minimum in heated milk and maximum in packaged milk and the difference between the mean count of the samples was highly significant ($p<0.01$) (Table 1). However the presence of the organism in heated milk indicates the survival of the organism after heating. The count of packaged milk sample was similar to that observed by Latha and Nanu (1997). The FSC count was highest in samples of brand B. The lowest mean count was observed in the samples of brand D.

Yeast and Mould Count

The mean yeast and mould count of PM samples had the highest mean count. The mean count was lowest for the milk of individual animals. The count obtained in the present study was lesser than that observed by Lues *et al*. (2003). Analysis of the mean count revealed a significant ($p<0.05$) difference in the YMC of PAM and PGM and also between PAM and AHM. The count of PGM was much lower to that obtained by Arora and Sudarsanam (1986). High YMC indicates unsanitary conditions of handling and contamination from environment. The highest YMC was seen in samples from brand A and lowest count in the samples of the brand D. The count was not detected from 57.14, 50.0, 35.72 and 21.44% of the samples of brand D, B, C and A, respectively. Although counts of yeasts and moulds were found, their growth in milkis rather uncommon, as the pH of milk is neutral, causing bacteria to predominate (Pitt and Hocking, 1997).

Grading of Milk

In India raw milk is, graded by Burean of Indian Standards (1977). According to the criteria prescribed by BIS (1977) for TVC 46.7% samples were graded as very good, 36.6% as good and 16.7% as fair quality. None of the samples were graded poor. Singh *et al*. (1994) observed that 28.75% of the milk samples were graded very good. Among pasteurized milk 55% of PGM were within 30,000 mL^{-1}, the standards prescribed by Indian Standards (1992). Among the retail pasteurized brands none of the sample from brand B met the standards. Only eight, three and two samples from brands A, C and D, respectively, met the standards prescribed.

According to Bureau of Indian Standards (1977) for coliform organisms revealed 75% of raw milk confirmed with the standard. However none of the pooled and chilled milk sample examined met the standards. For pasteurized milk, BIS (1992) coliform counts absent in 1:10 dilution are considered satisfactory. In the present investigation revealed 40% samples of packaged milk as satisfactory. Of the samples of retail brands' A, B, C and D the per cent which met standards were 64.28, 21.44, 14.29 and 7.14%, respectively.

Microbial Load from Various Sources of Contamination of Milk

The presence and extent of microbes in environmental samples, utensils used in the farm, hand wash of milker and workers in the dairy plant, the wash sample of equipments and other sources like strainer, storage crate and package material and their role in the contamination of milk was determined. The mean TVC, CC, ECC and FSC of various CCPs collected from dairy farm and dairy plant are given in Table 2a and b. Water used in dairy plant had the highest mean total viable count whereas water from dairy farm samples had the highest mean coliform count. Palaniswami *et al*. (1988) obtained the

Table 2a: The mean microbial counts of various CCPs collected from dairy farm and dairy plant

	Bacterial count (log_{10} cfu mL^{-1})			
Sources	TVC	CC	ECC	FSC
Dairy farm water	3.60±0.17	1.45±0.59	0.80±0.27	2.15±0.27
Dairy plant water	3.84±0.26	1.12±0.23	ND	2.10±0.21
Milk pail	3.81±0.27	1.10±0.25	0.73±0.35	1.42±0.16
Milking machine	3.37±0.33	0.55±0.24	0.41±0.26	1.36±0.16
Milk can	2.29±0.30	0.74±0.35	ND	0.47±0.31
Milkers hand wash	5.05±0.29	1.92±0.37	0.72±0.23	2.38±0.30
Working personnel hand wash	4.13±0.31	0.80±0.27	0.51±0.33	1.92±0.17
Pasteurization equipment	3.03±0.34	0.68±0.32	ND	0.79±0.37
Packaging equipment	5.12±0.24	1.70±0.44	ND	2.11±0.27
Package material	2.82±0.18	0.32±0.31	ND	0.43±0.28
Storage crate	3.70±0.17	0.73±0.34	ND	1.00±0.38
Strainer sample	2.26±0.18	ND	ND	0.58±0.37

Table 2b: The mean microbial counts of air samples from dairy farm and dairy plant

Samples		Mean±SE (cfu/ft^2/min)	
Process	Collection	TVC	YMC
Milking of animals	Before	98.0±3.58	16.67±0.84
	After	127.83±3.90	23.17±1.49
Pasteurization	Before	34.0±2.2	9.5±0.76
	After	56.3±3.21	12.16±1.37
Packaging	Before	41.66±2.74	13.17±1.07
	After	73.66±2.23	18.66±2.44

ND: Not Detected, TVC: Total Viable Count, CC: Coliform Count, ECC: *Escherichia coli* Count, FSC: Faecal Streptococcal Count, YMC: Yeast and Mould Count

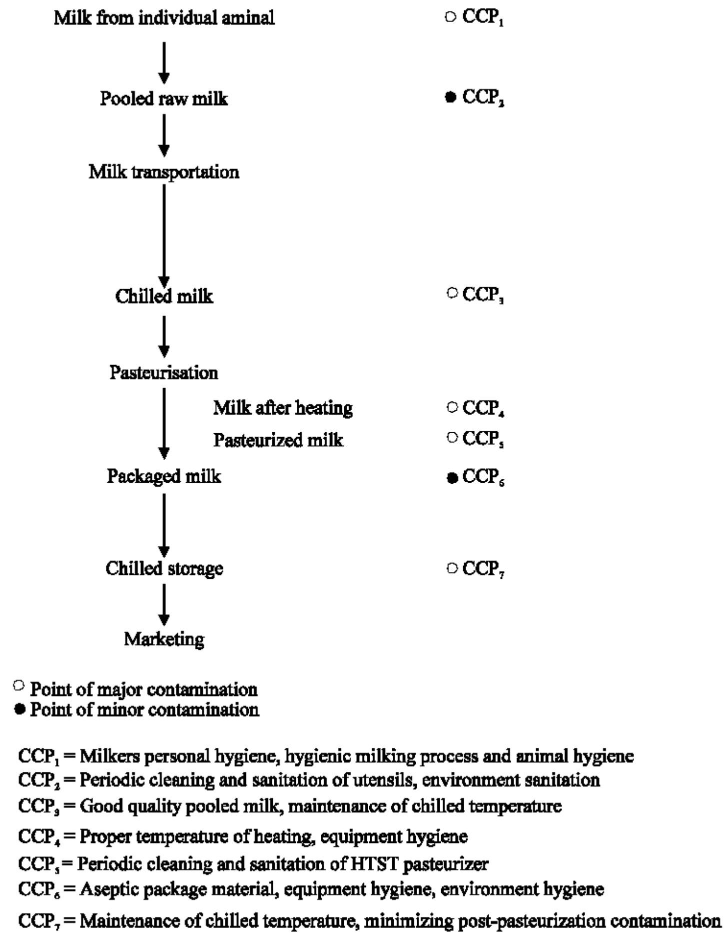

Fig. 1: Critical control points in production of pasteurized milk

mean coliform count of tap water in the dairy farm, as 72 MPN mL^{-1}. *E. coli* was not detected in the water samples of the dairy plant. In the study only 30% of water used in the plant revealed the presence of the coliforms however, Lopes and Stamford (1997) found 60% samples of water used to clean the milk equipment revealed the presence of coliform. Milk pail samples revealed the high mean total viable count, whereas milk can had a comparatively low count. Patel *et al*. (1993) obtained average total plate count of $1.7\pm0.44\times10^6$/can and that of coliform count $0.1\pm0.04\times10^6$ cfu/can. Coliforms were present at the level of one log cfu mL^{-1} level in samples of milk pail. The coliforms count was more in milk pail in comparison with the count of milk can and milking machine. This was in consonance with findings of Palaniswami *et al*. (1988). *E. coli* was not detected in samples of milk can.

Hand washings of personnel involved had CC, ECC and also FSC. Highest mean total count was obtained from milkers hand wash. The detection of *E. coli* and faecal streptococci in the hand wash of the workers indicated the poor hygienic practices of those personnels. Among the equipment wash sample examined, Packaging machine had the highest total viable count (4.91 ± 0.24 $\log_{10}$ cfu mL^{-1}) when compared to the count of pasteurization equipment. *E. coli* were not detected in any of the samples. The mean faecal streptococcal and coliform count was more (at the level of 1 $\log_{10}$ cfu mL^{-1}) in washings collected from packaging machine. Coliforms were also present in samples of package material and storage crate whereas the organism was not detected in strainer samples.

Mean total viable count of air samples collected from the milking barn, pasteurization room and packaging room was higher after the process of milking, pasteurization and packaging. Mean fungal counts were also found to be increased considerably after the above process. Aggarwal and Srinivasan (1978) found that fall of mould conidia per plate per minute from hand milking byre, machine milking byre and milk pasteurization unit ranged between zero to 24.5, zero to seven and zero to six per plate per minute. Highest count of mean TVC and YMC was seen from air samples collected from dairy farm. The counts obtained in the present study from various processing areas were well above the standards prescribed by APHA (Hickley *et al*., 1992).

Assessment of CCP of Pasteurized Milk

The critical control points of bacterial contamination during production of pasteurized milk are given in Fig. 1. The assessment of critical control points revealed that maximum contamination was seen when the milk samples were pooled and packaged. The contamination of pooled samples was due to the contribution of microbes by handling of milk by different milkers, where the personnel hygiene and cleanliness varied and also by transferring milk from different containers, air and water acting as source of contamination as detailed above. The process of packaging of pasteurized milk revealed next major sources of contamination which may be attributed to improper cleaning of equipment.

CONCLUSION

The evaluation of microbial quality of raw milk produced in the farm and pasteurized milk revealed that milk was contaminated on pooling and after pasteurization process. Hence it is necessary to minimize microbial contamination which can be achieved through healthy animal and milker and hygienic practices followed in dairy plant. High microbial counts and the occurrence of pathogens is likely to affect the keeping quality and safety of raw milk as well as products derived from it. The achievement of hygiene in dairy farm directly influences the production's economic result and health safety perspectives in humans. It is therefore critically important to ensure high quality raw milk produced from healthy animals under good hygienic conditions and that control measures are applied to protect human health. Therefore, it is recommended that training and guidance should be given to farms' owners and their workers responsible for milking and employees in pasteurization plant and emphasizes the need for hygienic practices at the farms and dairy plant. Meanwhile, information on health hazards associated with contaminated raw milk should be extended to the public, so that consumption of untreated/ improperly treated raw milk could be avoided.

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