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Original Research

Quality Control Tests Based Assessment of Raw Milk Samples Sold in Puducherry

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Abstract

A total of 100 milk samples were collected from different farms in Puducherry. Quality analysis was done based on the Methylene Blue dye reduction test and Standard plate count test. As per Methylene blue dye reduction test 30 %, 38 % 28 %, and 4 % of the samples were very good, good, fair, and poor in quality respectively. According to Standard Plate Count 28 %, 36 %, 26 %, and 10 % of the samples were very good, good, fair, and poor in quality respectively. This reflects the hygienic practices followed by the farmer at the farm level including animal health, environmental hygiene, and personnel hygiene. A strong correlation (r = 0.82) was observed between the Methylene blue dye reduction test and Standard Plate Count. Therefore, the Methylene blue dye reduction test can be used effectively, economically, and efficiently, to detect the quality of raw milk compared to costly and tedious microbiological methods.

Keywords: Milk, MBRT, SPC, Puducherry.

Introduction

Milk is one of the most nutritious foods because of its highly nutritive elements. The main constituents of milk are water, vitamins, lactose, indispensable amino acids, phospholipids, and trace elements (Pratelli *et al.,* 2024). India leads in milk production, surpassing all other nations with annual milk production of 230.58 million tons (2022-23) with a per capita availability of 459 grams/day and contributes 24% of global milk production (BAHS, 2023). The Puducherry state contributes around 50 thousand tons (2022-23) of milk with a per capita availability of 85 grams/day (BAHS, 2023). Milk quality is determined by its composition and hygienic practices followed during milking. The microbial quality of milk is determined by factors such as the hygienic environment in the farm, animal health, and equipment hygiene. Good milk hygiene practices such as maintaining clean and healthy cows, keeping a clean milking environment free of dust and mud, avoiding milking if the farmer is suffering from communicable diseases like diarrhoea or typhoid, not mixing colostrum and fore milk, washing hands with soap and clean water before milking, washing the udder with warm water and drying the udder with a clean dry cloth and use of clean containers for milking, will improve the quality of raw milk (De Silva *et al.,* 2016). Therefore, this study was carried out to determine the correlation between the methylene blue dye reduction test and with standard plate count method by assessing the microbial quality of raw milk sold in Puducherry and to suggest methods to improve the quality of milk.

Materials and Methods

Sample Collection

A total of 100 raw milk samples were collected aseptically from various locations in and around Puducherry UT which includes Ariyur, Pangur, Poraiyur, Thirukanchi, Kanuvapet, Villianur, Korkadu, Eambalam and Nathamedu. The samples were collected in sterile screw-capped bottles which were maintained at 4^0C with the help of ice pack. Further processing was done in the biosafety level II laboratory available at the Department of Veterinary Public Health and Epidemiology, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry.

Analysis of Microbial Quality of Raw Milk

The milk samples were analysed for their microbial quality by quality control tests namely the Methylene Blue dye Reduction Test (MBRT) and Standard Plate Count (SPC).

Methylene Blue Dye Reduction Test

About 1 ml of methylene blue solution was added to 10 ml of milk taken in a test tube. Contents were mixed gently by inverting the test tube 5 times and incubated in the water bath at 37^0C. The colour of milk was observed at every half an hour interval. It is based on the principle that, when methylene blue is added to milk, the redox potential gets reduced as bacteria grow and thereby methylene blue changes from blue to colourless state (leucomethylene). Time taken for discoloration is inversely proportional to the amount of bacterial contamination. Results were interpreted based on the IS:1479 (Part III), 1977 (Table 1).

Table 1: BIS Standards for MBRT

Time taken for decolouration	Quality of milk	Approximate no. of organisms
<1/2 hour	Poor	>20 million/ml
1-2 hours	Fair	5-40 lakhs/ml
3-4 hours	Good	1-5 lakh/ml
> 5 hours	Very good	<1 lakh/ml

Standard Plate Count (SPC)

SPC detects the number of viable bacteria present in milk and to thereby assess the microbial quality of milk. Three sterile test tubes containing 9 ml of normal saline were taken. One ml of milk sample was added to first test tube and mixed thoroughly which makes 1:10 dilution. Transfer 1 ml from first test tube to second tube which makes 1:100 dilution. Similarly, 1:1000, 1:10000 (or) more dilutions can be prepared as required. Using fresh pipette, 1 ml

was transferred from each dilution to 3 sterile petri plates. About 10-15ml of plate count agar which was cooled to 45^0C was poured into the petri plates and the contents were thoroughly mixed. After solidification, the plates were inverted and incubated at 37^0C for 24 hours. At the end of the incubation period, the plates were removed from the incubator and the colonies were counted. Results were interpreted based on the IS:1479 (Part III), 1977 (Table 2).

Table 2: BIS Standards for SPC (Raw milk)

Count in lakhs cfu/ml	Quality
< 2	Very good
2-10	Good
10-50	Fair
> 50	Poor

Statistical Analysis

The relationship between the MBRT and SPC was studied by measuring the correlation coefficient between the former and the latter using Microsoft Excel.

Results and Discussion

Several pathogenic bacteria including *S. aureus, Listeria* spp., *Escherichia coli, Bacillus cereus*, and *Salmonella* spp. have been recovered from raw milk. Many milk-borne infections in humans occur due to contamination of milk by unhygienic practices followed by the milker, adulteration of milk by non-potable water, and improper storage (Robi *et al.*, 2024). Thus, monitoring the quality of milk before consumption or processing is an essential prerequisite.

The results of the microbial quality of raw milk samples in this study by MBRT as per BIS guidelines were as follows: 30% of the total samples were of very good quality containing approximately <1 lakh organisms per ml of milk sample, 38% of good quality (1-5 lakh/ml), 28% were of fair quality (5-40 lakhs/ml) and 4% were of poor quality containing more than 20 million per ml of the milk sample (Table 3). These results were in concordance with Gupta *et al.* (2020) and Pawan *et al.* (2021) who reported that most of the raw milk samples in their study were of good and very good quality grade as per BIS guidelines. Further 32 % of the raw milk samples in this study were of fair and poor quality which may be due to unhygienic practices during milking, unhealthy animal and use of poor potable water.

Table 3: Grading of milk by MBRT

Commune	Number of samples	Very good	Good	Fair	Poor
Nathamedu	14	6 (42.85%)	4 (28.75%)	2 (14.29%)	2 (14.29%)
Embalam	12	4 (33.33%)	6 (50.00%)	2 (16.67%)	0 (0.00%)
Korkadu	8	4 (50.00%)	4 (50.00%)	0 (0.00%)	0 (0.00%)
Villianur	8	0 (0.00%)	4 (50.00%)	4 (50.00%)	0 (0.00%)
Kanuvapet	12	0 (0.00%)	2 (16.67%)	8 (66.66%)	2 (16.67%)
Thirukanchi	12	2 (16.67%)	4 (33.33%)	6 (50.00%)	0 (0.00%)
Pangur	12	8 (66.66%)	2 (16.67%)	2 (16.67%)	0 (0.00%)
Poraiyur	12	4 (33.33%)	8 (66.67%)	0 (0.00%)	0 (0.00%)
Ariyur	10	2 (20.00%)	4 (40.00%)	4 (40.00%)	0 (0.00%)
Total	100	30 (30.00%)	38 (38.00%)	28 (28.00%)	4 (4.00%)

The results of the microbial quality of raw milk samples by SPC as per BIS guidelines were as follows: 28% of the total samples were of very good quality containing approximately <2 lakh cfu per ml of milk sample, 36% were of good quality (2-10 lakh cfu / ml), 26% were of fair quality (10-50 lakhs cfu / ml) and 10% were of poor quality containing more than 50 lakhs cfu per ml of the milk sample (Table 4). In contrary to our findings, Rameshwar *et al.* (2022) reported 100 % fair quality raw milk in Madhya Pradesh, India whereas Singh *et al.* (2017) reported 100 % of very good quality raw milk in Lucknow, India. About 36 % of the milk samples in this study was of fair and poor quality which may be due to improper milking method and unhygienic managemental practices.

Table 4: Grading of milk by SPC

Commune	Number of samples	Very good	Good	Fair	Poor
Nathamedu	14	4 (28.57%)	6 (42.85%)	2 (14.29%)	2 (14.29%)
Embalam	12	4 (33.33%)	6 (50.00%)	2 (16.67%)	0 (0.00%)
Korkadu	8	4 (50.00%)	4 (50.00%)	0 (0.00%)	0 (0.00%)
Villianur	8	0 (0.00%)	2 (25.00%)	6 (75.00%)	0 (0.00%)
Kanuvapet	12	0 (0.00%)	2 (16.67%)	4 (33.33%)	6 (50.00%)
Thirukanchi	12	2 (16.67%)	2 (16.67%)	6 (50.00%)	2 (16.67%)
Pangur	12	6 (50.00%)	4 (33.33%)	2 (16.67%)	0 (0.00%)
Poraiyur	12	6 (50.00%)	6 (50.00%)	0 (0.00%)	0 (0.00%)
Ariyur	10	2 (20.00%)	4 (40.00%)	4 (40.00%)	0 (0.00%)
Total	100	28 (28.00%)	36 (36.00%)	26 (26.00%)	10 (10.00%)

The correlation between the MBRT and SPC was calculated using Microsoft Excel. A correlation coefficient of 0.82 was obtained which indicates a strong positive relationship between MBRT and SPC. As there is a strong correlation between MBRT and SPC, MBRT can be used in place of SPC at the farm level, as MBRT is efficient, effective, and economical compared to other costly and tedious methods like SPC.

Conclusion

The study yielded information about the quality of raw milk in Puducherry. Only 28 to 30% of very good quality milk was produced by the farmers which needs special attention. Creating awareness among the farmers about the need for clean milk production and the significance of hygienic quality of raw milk on public health. As MBRT test results showed a strong correlation with SPC results, MBRT can be improved and made available as a kit, so that farmers can make self-assessments of the milk they produce at the farm level.

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Contribution by Authors

All co-authors contributed equally.

Conflict of Interests

There is no conflict of interest.

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