# Microbial Quality of Raw and Pasteurized Milk Samples Collected From Different Regions of Madurai District, (T.N.) India

## R.Sudhasaravanan[1]*and S. Binukumari[2]

*[1,2]P. G. and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore-641 029, Tamilnadu, India*

***Abstract:*** *Milk is the fluid normally secreted by female mammals for the nourishment of their young ones. It is a compulsory part of daily diet for the expectant mothers as well as growing children and also serves as good medium for microbial growth and contamination 240 raw milk samples and 72 pasteurized milk samples from different places of Madurai District for a period of six months were analysed for microbial quality. Among the raw milk samples only 19.1% of samples were good quality and 28.3% are very poor quality. In the pasteurized milk samples 81.9% of samples were good for human consumption. The bacteria isolated from milk samples includes Lactobacilli, Staphylococcus aureus, Escheritia coli, Bacillus subtilis , Salmonella typhi, and feacal coliforms.*

***Keywords:*** *Raw milk, pasteurized milk, MBRT, coli forms, public health, Madurai*

## I.    Introduction

Milk is one of the complete foods which there seems to be no adequate substitute. Milk has good quality protein and is a unique substance in that it is consumed as fluid milk with minimal processing and also it is the raw material used to manufacture a wide variety of products . Milk may be modified by condensing, drying, flavouring, fortifying, demineralization and other treatments, lactation. It starts its journey in the udder of a mammal as a sterile substance, but as it passes out of the teat, it is inoculated by the animal's normal flora. Being a nutritionally balanced food stuff with a low microbial load (less than 10000 ml) when drawn from the udder of a healthy cow milk gets contaminated at various stages including the cow itself, the milker (manual as well as automated) i.e. the milker's hand or milking equipment, storage vessels and water supply particularly when used for adulteration. The microbial load may increase up to 100 fold or more once the milk is stored for some time at ambient temperature. The growth of microorganisms in milk causes disintegration of fat, protein and lactose and also makes the product unsuitable for drinking.

Milk is nutritious food for human beings, also serves as a good medium for the growth of many microorganisms, especially Lactobacillus, Streptococcus, Staphylococcus and Micrococcus sp. Bacterial contamination of raw milk can originate from different sources from animals such as air, milking equipment, feed, soil, feces and grass ([1]). Milk micorflora includes spoilage and pathogenic microorganisms. Many milk borne diseases such as tuberculosis, brucellosis and typhoid fever are known ([2]).

Milk is spoiled by a wide range of microorganisms some of which are pathogenic and are responsible for milk borne diseases. The milk is very easily contaminated if collected unhygeinically and handled carelessly leading to quick spoilage ([3]),[(4)] and is often contaminated by Escherichia coli bacteria under poor sanitary conditions which can affect public health. The coliform group of bacteria is defined as the indicator (faecal coliform) of suitability of milk for consumption (Standard method committee, 1981).

## II.    Materials And Methods

240 raw milk and 72 pasteurized milk samples were collected form diverse locations of Madurai district and surrounding villages in sterile screw cap tubes. After collection, the samples were transported to the laboratory on ice in sterile condition and processed for MBRT and coli form test with in three hours. In the methylence blue reduction (MBRT) test 1 ml of methylene blue (1:25,000) is added to 10ml of milk. The tube is sealed with rubber stopper and slowly inverted three times to mix. It is placed in a water bath at 35°C and examined at intervals up to 6hrs. The time taken for the methylene blue to become colorless is the methylene blue reduction time (MBRT). The methylene blue reduction test depends upon the ability of bacteria in milk to grow and to consume the dissolved oxygen, which reduces the oxidation reduction potentials in the medium([8]).

**Isolation of Microorganisms from milk samples**: Serial dilutions of samples were made up to 10-6 in nutrient broth and MacConkey broth. Samples were plated in duplicate using pour plate technique. 0.5ml of the diluted

sample was delivered by pipette in to 19.5 ml of enriched agar. Plates were inverted in an incubator at 37°C for 24 to 48hrs. Total viable counts were carried out on nutrient agar. Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media.

**Characterization of isolates from milk samples:** At intervals, colonies on the incubated plates were picked and purified by repeated sub-culturing by streaking on the desired media with a sterile wire loop. The strategy consisted of picking one colony to represent every visible different morphology on each plate. A maximum of 5 colonies were obtained per sample, which were examined microscopically for Gram's reaction and colony morphology (shape, size, colour, texture) using 24h old cultures. Motility and biochemical tests were performed. Appropriate positive and negative controls were used to make distinction positive and false-positive reactions.

 **Identification of isolates from milk samples:** Identification was based on growth on selective agar and broth, colony morphology, Gram's reaction,  Biochemical test results and criteria for disregarding negative cultures. Results were analyzed using Bergy's manual, and other methods for the identification ([5)], ([6)], ([7])

# III.     Results and Discussion

The results are presented in tables 1 and 2. A critical perusal of the table 1 reveals that out of 240 raw milk samples tested, 52 (21.6%) samples were found to be good, 67 (27.9%) samples were fair. The highest numbers of samples were found to be poor 71 (29.5.%) and very poor 72 (30.0\%). Out of 72 pasteurized milk samples, highest number of samples were found to be good 59 (81.9%) only 3 (4.1%) samples were found to be very poor, 4 (5.5%) samples were poor. Chatterjee et.al., (2006) reported that the raw milk contained higher number of micro flora probably due to contamination from the animal.

Bacteria found in manure, soil and water may enter milk due to dairy utensils and milk contact surfaces. Present study showed that 53% and 49% of raw milk samples were of very poor & poor category but in case of pasteurized milk samples, 86% of the samples were of good quality due to pasteurization .

# IV.     Tables

### Table I. Microbiological quality of can/pot milk supplied to coimbatore city (T.N)

| Month | Total No. of samples | Quality of milk | | | |
|---|---|---|---|---|---|
| | | VeryPoor | poor | fair | good |
| January | 40 | 6(15.0%) | 8(20.0%) | 15(37.5%) | 15(37.5%) |
| February | 40 | 7(17.5%) | 10(25.0%) | 14(35.0%) | 12(30.0%) |
| March | 40 | 9 (22.5%) | 11(27.5%) | 13(32.5%) | 10(25.0%) |
| April | 40 | 14(35.0%) | 14(35.0%) | 11(27.5%) | 5 (12.5%) |
| May | 40 | 16(40.0%) | 16(40.0%) | 6 (15.0%) | 6 (15.0%) |
| June | 40 | 20(50.0%) | 12(30.0%) | 8 (20.0%) | 4 (10.0%) |
| Total | 240 | 72 | 71 | 67 | 52 |
| | | (30.0%) | (29.5%) | (27.9%) | (21.6%) |

### Table II. Microbiological quality of pasteurized milk supplied to coimbatore city (T.N)

| Month | Total No of samples | Quality of milk | | | |
|---|---|---|---|---|---|
| | | Very poor | poor | fair | good |
| January | 12 | 0(0%) | 0(0%) | 2(16.6%) | 10(83.3%) |
| February | 12 | 0(0%) | 0(0%) | 1(8.3%) | 11(91.6%) |
| March | 12 | 0 (0%) | 0(0%) | 2(16.6%) | 10(83.3%) |
| April | 12 | 0(0%) | 0(0%) | 0(0%) | 12 (100%) |
| May | 12 | 1(8.3%) | 2(16.6%) | 0 (0%) | 9 (22.5%) |
| June | 12 | 2(16.6%) | 2(16.6%) | 1 (8.3%) | 7 (58.3%) |
| Total | 72 | 3 | 4 | 6 | 59 |
| | | (4.1%) | (5.5%) | (8.3%) | (81.9%) |

### Table III. Bacteria present in raw milk samples supplied to coimbatore city (TN)

| Month | Total No of samples | Number of colonies appeared | | | | | |
|---|---|---|---|---|---|---|---|
| | | Lactobacilli | Staphylococcus aureus | Escheritiacoli | Bacillus subtilis | Salomonella typhi | Fecal coliforms |
| January | 40 | 63 | 43 | 71 | 13 | - | 3 |
| February | 40 | 57 | 41 | 21 | 12 | 3 | - |
| March | 40 | 49 | 37 | 22 | 15 | 4 | 3 |
| April | 40 | 51 | 49 | 27 | 14 | | - |
| May | 40 | 73 | 59 | 36 | 21 | 6 | 5 |
| June | 40 | 87 | 63 | 40 | 26 | 7 | 5 |
| Total | 240 | 380 | 292 | 164 | 101 | 20 | 12 |

## V.    Conclusion

The study indicated that the dominant microbial flora associated with poor milk samples in and around Coimbatore District, (T.N.) were in the order of Lactobacilli, Staphylococcus aures, Escherichia coli, Bacillus subtilis, Salmonella typhi, Faecal coliforms. In pasteurized milk samples also very poor quality were recorded only in May and June months.

## Acknowledgements

## References

[1].   Torkar KG and Teger SG., The Microbiological quality of raw milk after introducing the two day's milk collecting system. Acta Agri. Slovenica. 92(1),(2008), 61- 74.

[2].   Goff J.P., Horst R.L., Effects of the addition of potassium or sodium, but not calcium, to preparations on milk fever in dairy cows. Journal of Dairy Sciences 80, (1997),176-186.

[3].   Prajapati J.B., Fundamentals of Dairy Microbiology. Akta Prakashal Nadiad, Gujarat, India, (1995), 4-45

[4].   Chatterjee S.N., Bhattacharjee I., Chatterjee S.K. and Chandra G., Microbiological examination of milk in Tarakeswar, India with special reference to coliforms. African Journal of Biotechnology 5, (2006),1383 – 1385

[5].   Barrow GL and Feltham KA., Cowan and Steel Manual for the identification of Medical Bacteria, 3rd Edn, Cambridge (1993).

[6].   De Silva ZN., Cunha AS., Lins MC., Carneior L., Almeida AC and Queuro ML., Isolation and serological identification of enteropathogenic Escherichia coli in pasteurized milk in Brazil. Rev. Saude publica. 35 (4): 375-379.

[7].   Ellis Dl and Goodacre R detection, identification, and enumeration methods for spoilage yeasts. In; Food Spoilage organisms, Blackburn C de W (Ed)., CRC Press LLC. (2006), Pp:28-54.

[8].   Benson.JH., Microbiological Application. Laboratory manual in general Microbial, 8th Edn, , (2002) ,1-478.

[9].   S.B. Javaid, J.A. Gadahi, M. Khaskeli, M.B. Bhutto, S. Kumbher and A.H. Panhwar, Physical and chemical quality of market milk sold at Tandojam, Pakistan. Pakistan Veterinary Journal. 29(1) ,2001,27- 31.

[10].   K.P.Talaro, Foundations in Microbiology 5th ed. (New York: McGraw-hill Higher education, 2005, 815-817.

[11].   M.L. Srivastava, Handbook on milk microbiology, (Delhi: Daya Publishing house, 2002) 216pp.

[12].   N.N. Potter and J.H. Hotchkiss, Food science 5th ed. (New York Chapman and Hall ITP), ,1995,279.

[13].   R. Lawley, Liquid milk products, LFRA Microbiology Handbook :Dairy products, 2nd ed. (Surrey,UK: Leather head food Research Association), 2001, 1-15.

[14].   S.C Murphy and K.J. Boor, Trouble shooting sources and causes of high bacteria counts in milk, Dairy food environmental sanitation, 20,2000, 606-611.