Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2115

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **3 (6):** 87-92 (2015)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Microbiological Assessment of Different Milk Samples from Mannargudi, Thiruvarur (DT)

N. Uma Maheswari* and K. Sabitha

PG and Research Department of Microbiology, Sengamala Thaayar Educational Trust, Women's College, Mannargudi, Tamil Nadu, India

*Corresponding Author E-mail: umasamyamf@ gmail.com

ABSTRACT

The objective of the study was to analyze the relationship of Total bacterial Count, Total cell Count and Total Psychrotrophic Count as an indicator of each microbiological quality of milk sample. A total of 50 raw milk samples (Set1, Set2, Set3) were collected from Mannargudi, Thiruvarur (DT),Total Coliforms Counts are 420 cfu/ml in Set I (Raw Milk),100cfu/ml in Set II (Pasteurized Milk), 320cfu/ml in Set III (Packet Milk). Followed by Total bacterial Counts are 520cfu/ml (Set 1 milk), 120cfu/ml in (Set 2 Milk) and 326cfu/ml (Set3 milk). Psychrotrophic bacteria Counts are 400cfu/ml (Set 1 milk), 80cfu/ml (Set 2 milk) and 200cfu/ml (Set 3 milk), are also done by the samples. Identified Microorganisms are (Staphylococcus, Streptococcus, Lactobacillus, and E. coli). Finally all the Counts are high in Set1 that is raw Milk samples.

Key words: *Raw Milk, Packet Milk, Psychrotrophic bacteria, Total bacterial count, Total Coliforms.*

INTRODUCTION

Milk is a complex fluid consisting of fats, proteins, lactose and minerals. Among these, proteins play very important role in the texture of dairy products[1]. Most contaminants of milk are coliforms and psychrotrophic group of microorganisms . Psychrotrophic bacteria present in raw milk include the gram negative genera (*E.coli*, *serratia*) and the gram positive genera (*Bacillus*, *Clostridium*), psychrotrophic bacteria are becoming increasingly dangerous to the dairy industries because they produce extracellular heat resistant lipases and proteases[2-3].

Milk is widely consume as nutrient food and it is excellent medium for the growth of microorganisms such balanced diet. Milk becomes contaminated with several types of microorganisms which originate from the soil, water, or skin and hair of the animals or utensils or from the milk maid. Bacterial contamination is brought about by bacteria, virus and parasites[4]. All food carry contaminating microorganisms from natural sources in most instances contamination begins from the start of handling by humans and this continues till the product is consumed[5].

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with microorganisms therefore the microbial content of milk is a major feature in determining its quality[6].

Cite this article: Maheswari, N.U. and Sabitha, K., Microbiological Assessment of Different Milk Samples from Mannargudi, Thiruvarur (DT), *Int. J. Pure App. Biosci.* **3 (6):** 87-92 (2015). doi: http://dx.doi.org/10.18782/2320-7051.2115

Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil and grass[7]. The number and types of microorganisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health[8]. Pasteurization cannot guarantee the absence of microorganisms, when they are present in large numbers in raw milk or due to post-pasteurization contamination[9].

Examination for the presence and number of specific microorganisms is, therefore, and integral part of any quality control and quality assurance plan and it may be applied to a number of areas: raw materials, intermediate samples, finished products, or environmental /equipment sites. Milk –borne and milk–product borne outbreaks represent 2-6% of bacterial food- borne outbreaks reported by surveillance systems from several countries. Therefore the present study was aimed to investigate the Microbiological assessment on Milk samples[10].

MATERIALS AND METHODS

Sample Collection

Milk samples were collected from mannargudi, Thiruvarur District. In sample are grouped into three sets based on the place of collection. In the case of raw milk, about 50 ml of raw milk were collected in sterile glass bottle either directly from the udder in cases of individual cows or from the milk tank or milk containers. Samples were then kept in an ice box and transported directly to the laboratory. Tryptone glucose yeast extract agar and violet ret bile agar were prepared one ml of samples was separately plated into plate count agar. The plates were incubated at 37˚C for 24-72 hrs. Packet milk samples are purchased from Lock shops nearby Mannargudi, Thiruvarur (DT).

Each milk samples were serially diluted individually with sterile from 10^{-1} to 10^{-2} one ml of each sample was separately plated into plate count agar, Violet Red Bile Agar (VRBA) and Tryptone Glucose Yeast Extract Agar (TGYEA) in three replicates with control respectively for Total Bacterial Count (TBC), total coli forms count (TCC) and Psychrotrophic bacterial count (PBC).

Total Bacterial, Psychrotrophic Cell Count

After 7 days the total bacterial cell count method was done and the number of bacteria was identified in milk sample.

Total coliform count (TCC), Total bacterial count (TBC) and Psychrotrophic bacterial count (PBC) Quality of milk samples were done as per the standard biochemical methods. Identify organisms *Staphylococcus, Streptococcus, E. coli* and *Lactobacillus.*

Total Coliforms Count

Total Coliforms count (TCC) is a non regulated test that has been used historically to assess milk production practices such as milk refrigeration. Milking machine sanitation, and per milking udder hygiene[11-14]. Coli form count is a practical indicator of milking hygiene because it is easy and inexpensive to perform (the test can be performed on the form), and it is often correlated with the population of other bacteria in BTM[15-16]. However, because coli form bacteria populations can increase rapidly under some conditions, it is important to distinguish between the level of initial contamination and increased CC that may be the result of incubation in the milk handling system after milk harvest.

PresumptiveTest

According to ISO/CD[(17)], the Laureltryptose broth was used as the media for the presumptive test for total coliforms count. Peptone water used as a diluents; this result in a dilution of 10^1, 10^2, 10^3, 10^4 and 10^5. A Durham tube was inserted into each Laureltryptose tube. 1ml of each dilution was pipette into 3 Laureltryptose tube. All tubes were incubated at 35 to 37˚C for 48 hr and then examined for gas formation in the Durham tubes.

Confirmed Test ForColiforms

Each positive (gassing) Laurel Tryptose tube was gently agitated and a loopful of suspension was transferred to tube of brilliant green bile broth. All the tubes with were incubated at 35 to 37˚C any gas formation in Durham's tubes with slight turbidity in the media was regarded as positive confirmed test. Results were interpreted using The MPN tables based on combination of confirmed gassing of Laurel Tryptose broth tubes for three consecutive dilutions.

Confirmed Test for *E. coli*

Eosin methylene blue (L-EMB) agar was used the plates were streaked with a loopful of suspension from confirmed positive brilliant green bile broth culture. Plates were incubated at 35˚C for 18 to 24hrs. discrete dark centered nucleated colonies with or without metallic sheen were regarded as a positive test. Two colonies or more were picked from each (L-EMB) agar plate and transferred to nutrient agar slants for morphological examination of all gram negative short rods or cocci were identified.

RESULTS AND DISCUSSION

In our study was highlighted that Microbiological Assessment of different Milk Samples from Mannargudi, Thiruvarur(DT), Milk samples are Set 1 Raw milk, Set 2 Pasteurized Milk, and Set 3 Pocket Milk samples. Assessment of milk quality was done by means of Methylene blue reductase test (MBRT). The time taken for color change from blue to white, which indicates reduction.Color changes was noticed within stipulated time (Table 5).

Total Bacterial Count

Total bacterial count method was used to Count the number of bacteria present in Milk sample. The highest total bacterial count 520 cfu/ ml was found in Set 1 raw milk sample, 120 cfu/ml in Set 2 Pasteurized Milk and 326 cfu/ml in Set 3 Packet Milk samples, (Table 5).

Total Coliform count

The presence of Coli form bacteria, such as *E. coli*, in milk is a indicator of fecal contamination *E.coli* was isolated from samples. Total Coli forms Counts are 420 cfu/ml in Set I(Raw Milk), 100 cfu/ml in Set II (Pasteurized Milk), and 320 cfu/ml in Set III (Packet Milk) (Table 2,5).

Isolation and Identification of Microorganisms

Bacteria

Different selective media were used for the isolation of bacteria. They are five types of bacteria were isolate from Raw milk, Pasteurized milk and Packet milk. The bacteria can be identified based on the Morphological and biochemical test. The identified bacteria and organisms for *E. coli*, *Staphylococcus*, *Streptococcus* and *Lactobacillus* (Table 2).

From our study clearly reported that, Total bacterial count, coli form count and Psychrotrophic bacterial count was highest in raw Milk followed by Set 3 Packet Milk samples. Our results are in agreement with the findings of, Coli form Count was higher in hot season, this showed that the majority of the raw Milk in the state was within the accepted limits[18].

Our findings were similar to microbial populations in milk varieties at the time of Processing has a significant influence on shelf life, spoilage and Packaging well as on the other dairy products. Also reported Psychrotrophic bacteria usually account for more than 90% of the total Microbial Population in cooled raw milk[19].

Table - 1. Morphological and Cultural Characteristics of Bacterial Isolated from Raw and Pasteurized Milk Samples and Packet Milk

Isolated Organism	Morphological Characteristics	Cultural Characteristics
E. coli	Gram negative, Rod, Motile	Non- spore forming Aerobic and Facultative anaerobic
Staphylococcus	Gram positive, Cocci, pairs chain, Non motile	Aerobic and Facultative anaerobic temperature range 37 ˚ C, p^H 7.4 to 7.9
Streptococcus	Gram positive, Short chains and pairs, Non motile	Non - sporing, anaerobic
Lactobacillus	Gram positive, Rod chain, and pairs, Non motile	Ping color, lactic acid bacteria group p^H – 5.0

Table - 2. Biochemical Characteristics of Bacterial Isolates from Raw, Pasteurized and Packet Milk Samples

Isolated Organisms	Indole	MR	VP	Citrate	Catalase	Urease
E. coli	+	+	-	-	+	+
Staphylococcus	-	+	+	-	+	+
Streptococcus	-	-	+	-	+	-
Lactobacillus	-	-	+	-	-	+

+ indicates Positive; - indicates Negative

Table - 3. Total Psychrotrophic Bacterial Count

Sample	Dilution	Psychrotrophic Bacterial Count (cfu/ml)	
T_1	10^{-4}	400	250
T_2	10^{-5}	200	80
T_3	10^{-6}	250	200

T_1, T_2, T_3—Set1,2 and 3 Milk samples

Table-4. Methylene Blue Reductase Test

Sample No.	Methylene Blue Reductase Test			
	Decolourization time	Grade	Decolourization Time	Grade
T_1	1.30 hours	Poor	2.30 hours	Good
T_2	2 hours	Poor	2.40 hours	Good
T_3	4.30 hours	Good	6 hours	Excellent

T_1, T_2, T_3—Set1,2 and 3 Milk samples

Table 5. Total bacterial count

S. No.	Raw Milk	Total Bacterial Count Cfu/ml
T_1	520	160
T_2	120	100
T_3	326	150

T_1, T_2, T_3—Set1,2 and 3 Milk samples

CONCLUSION

The lack of knowledge about clean milk production, use of unclean milking equipment and lack of Potable water for cleaning purpose were some of the factors which contributed to the Poor hygienic quality of raw cows- milk at farms and at collection centers, in the three regions of the state. Due to the facts that Psychrotrophic bacteria are the main microbial causative agents of Spoilage of milk and dairy products, and that some of them are considered the opportunistic pathogenic bacteria, effective control of processing conditions have to be required. The higher counts of PBC/TCC and TBC in milk samples in this study indicate the poor sanitation practices that may be at farm level or the poor maintenance of containers used for transporting the milk. Further investigation to find out the stage of contamination of milk samples is under progress.

In sense of quality, psychrotrophic bacteria have become major problem for today's dairy industry as leading causes of spoilage and significant economic losses. This review focuses on the impact of psychrotrophs on quality problems associated with Raw milk, Pasteurized milk, Packet milk as well as on the final dairy products. Our study was extended to identification of moulds from milk samples and detection of after toxin production.

Acknowledgements

Authors highly acknowledge the support provided by the The Managing Trustee; STET Women's College, Sundarakkottai, Mannargudi for the completion of this work

REFERENCES

1. Adams, D.M. Breach, Effect of Psychrotrophic Bacteria From Raw milk on Milk Proteins and Stability of milk proteins to Ultrahigh Temperature. *Journal of Dairy Science*, **59:** 823 – 827 (1997).
2. Causin, M.A., Presence and activity of Psychrotrophic Microorganisms in Milk and dairy Products. *Journal of Food Protection*, **45:** 172-207 (1982).
3. Cempirikova, R., Psychrotrophic Vs. total bacterial counts in bulk milk samples. Veternary Medicine Czech, **47:** 227 – 233 (2002).
4. Champagne, C. P. and Laing R., Psychrotrophs in dairy products, their effect and their control. Crit. Rev. *Food Sci. Nutrition*, **34:** 1-30 (1993).
5. IDF Bulletin Psychrotrophsin milk and milk products. International Dairy Federation, Brussels, E-Doc **68:** (1976).
6. Stepanick, L., Psychrotrophs and their enzymes in milk and dairy Products. Quality Aspects Trends Food SicTe, **8:** 35-41 (1997).
7. Janstova, B. and Drackova., Effect of *Bacillus Cereus* Enzymes on the milk Quality following Ultra High Temperature Processing. *Alta Veterinarian Brno*,**73:** 400 (2006).
8. Randolph, H., Identifying Spoilage bacteria and potential Shelf-life problems. Dairy Food/Find Article, 1-3. (2006).
9. Santana, E.H.W., Beloti, V., Gusmao, V.V., Milk contamination in different points of dairy process. Seminar, Cynic's Vagaries, Londrina **25:** 349-358 (2004).
10. Boor, K.J. and Murphy, S.C., Microbiology of market milks. U Knjizi: Dairy microbiology Handbook, The Microbiology of Milk Products, edited by Robinson, R.K, third edition, Wiley Interscience, New York, 91 -122 (2002).
11. Kurwijiila, R.L, Hansen K.K., Mocha H.J.S., The bacteriological quality of milk From hand and machine Milked dairy Herds in Meguro, Tanzania. *Afar Lives Res.* **2:** 59-67 (1992).
12. Marino, M., Mainframe, M. and Rondinini, G., Presence of Coliform bacteria and *Escherichiacoli* in raw milk and soft cheeses produced in Friuli Venetia Giulia: Industrial Latte **33:** 15-24(1997).
13. Vyletelovaaaa, M., Benda, P., Hanus, O. and Kopuneczp, Determination of count of bacteria in Cool milk Samples and their relation to total counts of microorganisms. Czech. J, *Food, Sic*, 216-222 (1990).
14. Tavaria, F.K. Reis, P.J.M., Effect of dairy farm and milk refrigeration on microbiological and micro structural characteristics of matured serratia Estrela cheese. *International Dairy Journal* **16:** 895-902 (2006).
15. Pesek., Psychrotrophic microflora of cooled raw milk stored on farms supplying two dairies in South Bohemia. Sbornik Univerzita Zemed. Budejovice **9:** 67-75 (1992).
16. Randolph, H., Identifying Spoilage bacteria and potential Shelf-life problems. Dairy Food/Find Article, 1-3 (2006).

17. Walker, S.J., Major Spoilage Microorganisms in milk and Dairy product. *Journal of the Society of Dairy technology*, **41:** 91-92 (1988).
18. Law, B.A., Review of the progress of dairy Science: Enzymes of Psychrotrophic bacteria and their effects on milk and milk products. *Journal of Dairy Research,* **46:** 173-176 (1979).
19. Washam, C.J., Olson, HC., Heat- resistant Psychrotrophic bacteria isolated from Pasteurized milk. *Journal of Food Production,* **40:** 101-108 (1997).