ISSN : 2393-8188 (print)
2393-8196 (online)
www.milliyasrcollege.org.journal.php

BACTERIAL ANALYSIS OF RAW AND PACKED MILK OF BEED CITY

Syed Zubair Hussaini[1], M. Shaker[2],Gulve R.M[2], M.Asef Iqbal[3*]

1 Mrs. K.S.K. College of Food Technology Beed
2 Dept. of Microbiology,Mrs. K.S.K. College, Beed
3 Dept. of Microbiology, Milliya Arts, Science & Mgmt. Sci. College, Beed
Corresponding Author: e_bareed@yahoo.co.in

ABSTRACT: Milk is a nutritious food for human beings. It also serves as a good medium for the growth of many microorganisms such as Staphylococcus and Coliform.To determine the presence and levels of microbes in unexpired pasteurized milk and raw milk from randomly selected markets in beed city

The average total variable count of raw milk is $1.33x10^7$ cfu/ml. The average of total coliform count is $1.05X10^1$ cfu/ml. The average total variable counts of pasteurize milk is $2.94x10^4$ cfu/ml; the average of total coliform count is absent.

Key words: raw milk, pasteurized milk. Milk quality, TVC.TCC.MBRT

INTRODUCTION

Milk is an extremely nutritious food. It is an aqueous colloidal suspension of proteins, fats and carbohydrates that contains numerous vitamins and minerals. Many of the pathogenic bacteria encountered do not grow well in milk but remain viable for undesirable lengths of time (Sangoyomi, et al 2010). Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. (Sangoyomi, et al 2010)

Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits et al., 2008). The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health (Rogelj, 2003). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits et al., 2008). Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley, 1990).

Milk is the lacteal secretion of mammals, which are the complete foods on Earth. In India, the term ‘milk’, refers to cow or buffalo milk or a combination of the two (De, 2004), widely accepted as a national drink due to the benefit associated with it. Chemically, milk is a complex mixture of fat, protein, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet (Haug et al., 2007). Milk considered as an attractive source of energy, proteins, and calcium for infants, young children and elderly people who have few alternative sources available for nutrients; even it is a part of daily diets of most of the people.

The rural families produced the major parts of the unprocessed milk marketed to the urban consumers. The rural milk producers were not acting as retailers instead some intermediary act as retailers, these urban retailers were delivered milk door- to- door without cooling containers, by their own vehicles or sell it in milk booth. Door-to-door raw milk deliveries in the urban areas commonly practiced with virtually no quality control at all levels. Poor hygiene and mal practices of the intermediary of these products were responsible for poor quality of milk distribution in the community (Siva and Sannabhati1994).

Measurement of bacterial numbers in milk is of interest because they are indicator of poor milk hygiene production or ineffective pasteurization of milk. Some microbes such as gram negative Psychrotrophs, Coliforms and other pathogenic bacteria such as Escherichia Coli ,Staphylococcus aureus may also be found in milk (Tatini, S.R. and Kauppi, K.L. 2003).The hygienic quality of milk at the point of production is also of importance from both public health and consumer perception points of view. For milk to be produced with a low bacterial count the temperature must be kept low until the point of processing. (Silva, T.M. et al 2010). Contaminations of raw milk and the consequent high bacterial count in milk originates from milking wet dirty udders, the milking system used, the cooling and storage temperature and the holding time (Murphy, S.C. and. Boor, K.J. 2000)..8The bacterial count is a useful method to measure milk quality, a bacterial count ranging between 9×10 - 9×10 6 cfu/ml is acceptable (Salman, A. and Elnasri, H. 2011) , and the mean standard plate count of raw milk is 1.29×10 6 cfu/ml , (Ramanjaneyulu, G. and Vyas, S. H. 1985) but when milk was pasteurized it was reduced to 1.2×10 4 cfu/ml. Grade A milk has a count less than 1×10 5 cfu/ml and grade B milk has count with less than 3×10 5 cfu/ml (IDF) (1994) , but the majority of pasteurized milk sample has a count of 1×10 3 cfu /ml. (László,V. 2003).

The objective of this work was to evaluate the level of microbiological contamination of raw milk samples & pasteurized milk sample taken from beed city local market.

This study was carried out to investigate the quality of raw milk & pasteurized milk sold in beed city.

We wished to found out the differences in number of micro-organisms between raw milk & pasteurized milk samples.

MATERIAL AND METHODS

Sample collection:-

Ten raw milk samples and 10 pasteurized milk samples were collected form locations of BEED CITY 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 total coliform count & total viable count within one day hours.

Microbiological analysis

In the methylene 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 o 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 grading of milk sample's on the basis of methylene blue reduction test in different milk samples are presented Table. (Benson,2002). 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.

Enumeration of Microorganisms

Determination of Total Viable Count

Different dilution of milk sample ranging from 10^1 to 10^6 was prepared by using sterile peptone water. For the determination of Total Viable Count 0.1 ml of each dilution was inoculated on Nutrient Agar using a sterile pipette for each dilution.

The diluted sample was sprayed as quickly as possible on the surface of the plate with sterile glass spreaders.

1 sterile spreader was used for each plate the plates were then kept in an incubator at 35°C.for 24-48 hrs.

After incubation, plates showing 30 to 300 colonies were counted. The average number of Colonies in a particular dilution was multiplied by dilution factor to obtain the TVC

Enumeration of Total Coliform Count (TCC)

For the enumeration of Total Coliform, TVC method was employed for TCC method.

MacConkey Agar plates were used as above. The milk sample were inoculated on MacConkey Agar & incubated aerobically at 37°C for 24 hrs. The plates were observed for the growth of *Escherichia Coli*. Single isolated circular pink colony was picked and subcultures on MacConkey Agar for purification of the isolate simultaneously another single colony showing similar characters was picked for the Gram Staining, morphological characters of the isolates using bright field microscope.

All milk samples were subsequently sub cultured on .to Eosin Methylene Blue (EMB) Agar, for primary screening of *E. coli* and incubated aerobically at 37°C for 24 hrs. suspected colonies of E.Coli (greenish metallic sheen appearance with dark centers) were identify biochemically, (Cappuccino 2007).

The cultured characteristics of the isolate were confirmed by inoculating the pure colonies on Blood Agar, Nutrient Agar, Nutrient Broth and Violate Red Bile Agar (Table 3).

Biochemical test were performed to confirm the E. coli using catalase test, Simmons Citrate Agar, Sugar fermentation on Triple Sugar Iron Agar, Gelatin liquefaction, lodole production, Nitrate reduction, Urease Production Voges Proskaur, Methyl Red and Presumptive test.

RESULT AND DISCUSSION

STATISTICAL ANALYSIS

All microbial counts were converted to the base – 10 logarithm of the number of colony forming units per ml of milk samples (log cfu/ml), and from these means their standard deviations were calculated.

The micro flora of raw milk & pasteurized milk is presented in Tables 1 and 2.

Table 1 Micro flora of raw milk

Milk sample	**TVC/ml**	**TCC/ml**
R1	$1.08x10^7$	$0.43x10^1$
R2	$1.42 x10^7$	$1.65 x10^1$
R3	$1.48 x10^7$	$1.07 x10^1$
R4	$1.39 x10^7$	$0.45 x10^1$
R5	$1.46 x10^7$	$1.06 x10^1$
R6	$1.32 x10^7$	$0.26 x10^1$
R7	$1.19 x10^7$	$0.31 x10^1$
R8	$1.45 x10^7$	$0.19 x10^1$
R9	$1.31 x10^7$	$0.1 x10^1$
R10	$1.28 x10^7$	$0.95 x10^1$

Table 2 The micro flora of pasteurized milk

Milk sample	**TVC/ml**	**TCC/ml**
P1	$2.7x10^7$	Absent
P2	$2.9 x10^7$	Absent
P3	$3.2 x10^7$	Absent
P4	$2.6 x10^7$	Absent
P5	$3.7 x10^7$	Absent
P6	$2.5 x10^7$	Absent
P7	$3.8 x10^7$	Absent
P8	$2.1 x10^7$	Absent
P9	$2.9 x10^7$	Absent
P10	$3.1 x10^7$	Absent

Note: - P=, R= pasteurized milk

No significant differences were observed with respect to the average counts of *E. coli*. Raw milk

contained an average TVC of $1.338x10^{7}$ cfu/ml &TCC of 0.647 $x10^{1}$ cfu/ml. Pasteurized milk contained an average TVC of $2.95x10^{4}$ cfu/ml &TCC of absent.

The result of Methylene-Blue-Reduction time are noted in Table No.3 &4

Table 3 Microbiological quality of raw milk supplied to Beed City

Milk Sample	M.B.R. time hour	Remark about Quality of milk
R1	1.30	Fair
R2	1.15	Fair
R3	1.00	Fair
R4	1.20	Fair
R5	1.10	Fair
R6	1.25	Fair
R7	1.15	Fair
R8	1.20	Fair
R9	1.30	Fair
R10	1.00	Fair

Table 4 Microbiological quality of pasteurized milk Supplied To Beed City

Sample Milk	M.B.R. time hour	Remark about Quality of milk
P1	6.30	Good
P2	7.00	Good
P3	6.00	Good
P4	6.15	Good
P5	6.45	Good
P6	7.15	Good
P7	6.45	Good
P8	6.30	Good
P9	7.00	Good
P10	6.30	Good

Biochemical tests and cultural characterstic E.coli on different medium of were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation tests (Table 5& 6).

Table 5 cultural characterstic E.coli on different medium

Media used	Culture Character
MacConkey Agar	Smooth, circular pink colonies with spreading growth
Blood Agar	Non hemolytic, grey white moist, glistening opaque, circular, convex colonies with entire edge.
Nutrient Agar	Colorless and yellowish white, circular, smooth colonies with entire edge.
Nutrient Broth	Organism showed uniform turbidity.
Violet Red Bile Agar	Small, circular pink colonies.
Eosin Methylene Blue Agar	Green metallic sheen with dark center

Table 6 Biochemical tests of E.coli.

Biochemical Test	Reaction
Castalase	+ve
Simmon's citrate	-ve
TSI	A/A + gas
Gelatin liquefaction	-ve
Indole Production	+ve
Nitrate Reduction	+ve
Urease	-ve
Voges Porskaur	-ve
Methyl Red	+ve
Presumptive test	+ve

The literature reviewed in the present study shown evidence that *Escherichia coli* are frequently occurring organisms in milk. The methods of production, transportation, handling and sale of milk are entirely unhygienic the raw milk poses a great hazard to public health without adapting hygienic measures because of possibilities of contamination with *E. coli* the result of milk sample shows that all sample were contaminated with *E. coli.* The unclean hands of workers, poor quality of milk, unhygienic

conditions of manufacturing units, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk product and the post manufacturing contamination (Bhat et. al., 1948; Marrieer, 1973; Triq Masud et. al., 1988) although *E. coli* frequently occurring organisms in milk and its products, the incidence of these species of *E. coli* itself in milk and milk product as a possible for food born disease is insignificant because *E. coli* normally is ubiquitous organisms (Hahn, 1996).

The microbiological quality was only marginally acceptable with respect to the total bacteria count. The presence of pathogenic and indicator bacteria, such as *E. coli, indicate* that the growth of these organisms may lead to a hazard against public health. Therefore practice and regulations, such as on-site pasteurization and implementation of HACCP following established standards, should be introduced to facilitate the production of cow milk of high quality and safety.
The bacterial isolates observed in this study are suspected to contaminate the sample from various sources, which could be due to poor handling and storage after milk collection. The environment, utensils used the state of hygiene of the animal from which the milk was collected and the sanitary conditions of the milk collectors are all possible source of contamination.

It is recommended that the milk collection should be done with utmost hygienic measure and that milk should be pasteurized immediately after collection to reduce the load of bacteria especially the pathogenic ones. Government should endeavor to assist the poor fulani milk producer, in buying and getting these product into a collection centers were proper equipment for pasteurization are provided before the products get to the consumer, in view of the danger inherent in this product. Hygiene measures assume a decisive importance in food safety management (Untermann, 1998). Isolation of bacterial pathogen from dairy farms and from outbreaks of human disease substantiates the hypothesis that packed food samples are reservoir of food borne pathogens (Oliver *et al*., 2005). It is therefore; critically important to ensure high quality milk production from healthy animal, therefore it is recommended that training should be given to farm owner and worker responsible for milking process.

The results of the present study indicate that strict preventive measures should be adopted to ensure contamination free milk products for the good health of all consumers. For this, consciousness and care is required from the point of generation to the point of consumption of these widely consumed milk products.

ACKNOWLEDGEMENTS

The authors are thankful to the joint secretary of our college management,Principal of our college, other staff of Department of Food & industrial Microbiology for providing Laboratory facilities and encouragement.

REFERENCES

1. *Sangoyomi TE, Owoseni AA and Okerokun O. African Journal of Microbiology Research. 2010, 4(15), 1624-1630*
2. *Coorevits, A./ De Jonghe, V./ Vandroemme, J./ Reekmans, R./ Heyrman, J./ Messens, W./ De Vos, P./ Heyndrickx, M. Comparative analysis of the diversity of aerobic-spore-forming bacteria in raw milk from organic and conventional dairy farms. System. Appl. Microbiol. 2008, in press.*
3. *Rogelj, I. Mleko. In: Mikrobiologija živil živalskega izvora (Eds.: Bem, Z./ Adamič, J./ Žlender, B./ Smole Možina, S./ Gašperlin, L.). Ljubljana, Biotehniška fakulteta, Oddelek za živilstvo, 2003, 515–538.*
4. *Bramley, A.J./ McKinnon, C.H. The Microbiology of Raw Milk. In: Dairy Microbiology, I, (Ed.: Robinson, R.K.). London, New York, Elsevier Applied Science, 1990, 171.*
5. *De S (2005). Market milk. In: Outlines of Dairy Technology. 19th ed. Oxford University Press (Oxford).*
6. *Haug A, Høstmark AT and Harstad OM (2007). Bovine milk in human nutrition – a review. Lipids in Health and Disease 6 25. Available: http://www.lipidworld.com/content/6/1/25 [Accessed on 2 February 2012*
7. *Siva CY and Sannabhati SS (1994). Dung and milk cans as sources of aerobic and anaerobic Bacterial spore contamination of raw milk. Indian Journal of Dairy Science 47 401-405.*
8. *Tatini, S.R. and Kauppi, K.L. (2003), in: Encyclopedia of Dairy Sciences H. Roginski, J.W. Fuquay and P.F. Fox (eds.) Vol. 1. (Academic Press and Elsevier Science, Amsterdam, Boston, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo. pp. 74-79.*
9. *Silva, T.M.; Oliveria, R.L.; Barbosa, L.P. (2010). Body components of young crossbred Boer goats fed licury oil (Syagruscoronata) in the diet. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v.62, n.6, p.1448-1454, 2010.*
10. *Murphy, S.C. and. Boor, K.J. (2000). Trouble-shooting sources and causes of high bacteria counts in raw milk. Dairy Food Environ.Sanit. 20:606-611.*
11. *Salman, A. and Elnasri, H. (2011) . Somatic cell count, total bacterial count and acidity properties of milk in Khartoum State, Sudan.*

Journal of Cell and Animal Biology Vol. 5 (10), pp. 223-230.

12. *Ramanjaneyulu, G. and Vyas, S. H. (1985). Keeping quality of heat treated milk at room temperature .. Gujarat . India Asian.J. of dairy pp 20-22.*
13. *International Dairy Federation(IDF) (1994). Pasteurization and other heat treatment processes in: Recommendation for the hygienic manufacture of milk and milk based products. Bulletin of the International Dairy Federation N 292 . Belgium. In food and Microbilogy . Academic press .-I N C. or Lando,Floreda.*
14. *László,V. (2003). Microbiological quality of commercial dairy products Communicating Current Research and Educational Topics and Trends in Applied Microbiology . A. Mendez-Vilas (Ed)*
15. *Benson JH., Microbiological Application. Laboratory manual in general Microbial. 8th Edn. 1-478, (2002)*
16. *Hahn, G. Pathogenic bacteria in raw milk situation and significance. In: Bacteriological quality of raw milk. Brussels (Belgium), Int. Dairy Federation, 1996, 67–83.*
17. *Masud, T./ Ather, H.I./ Azhar Chushti, M./ Amim Shah, M. Microbiological studies on indigenous dahi with special reference to public health. Aust. J. Dairy Technolo., 1988, 8–13.*
18. *Utermann F. (1998). Microbial hazards of food.Food Controll.9:119-126.*
19. *Oliver S. P., Jayarao B. M. and Almeida R. A. (2005). Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathogenic Dis. 2 (2): 115-129*
20. *Bhat J.V., Sethna K. and Fernandes F. (1948). Ind. J. Dairy Sci. 49.*