Original Research

Bacteriological Quality Assessment of Market Milk Sold in Parbhani City, Maharashtra

Rajesh Kumar Sahu[1*] and A. M. Chappalwar[2]

College of Veterinary Science, Parbhani, MAFSU, Nagpur, Maharashtra, INDIA

[1]Department of VPH
[2]Department of LPT

***Corresponding author:** rajeshwarrior32@gmail.com

Rec. Date:	Feb 21, 2018 07:38
Accept Date:	Apr 09, 2018 16:29
DOI	10.5455/ijlr.20180221073846

Abstract

The research was carried out to evaluate the microbiological quality of local market milk sold by vendors in Parbhani city, Maharashtra. A total of hundred milk samples (from January to March 2015) were collected randomly from vendors of Parbhani city and analyzed for their microbial quality. Milk samples were processed for total plate count as well as for isolation of selected pathogens such as Staphylococcus spp, Streptococcus spp, Escherichia coli, Enterobacter spp, Clostridium spp and Salmonella spp. The mean value for total plate count was 6.61 ± 0.13x10^{5}cfu/ml, with minimum 2.73 ±0.15 x10^{5}cfu/ml and maximum 8.83 ±0.08 x10^{5}cfu/ml count. Differential organisms E. coli, Staphylococcus, Streptococcus and Enterobacter spp was identified from 38 percent, 4 percent, 58 percent and 66 percent of the market samples, while Clostridium spp and Salmonella spp were not found in any of the market milk samples. The microbiological quality of milk was judged marginal and indicates the need for improved hygienic standards.

Key words: *E. coli*, Market Milk, Microbiological Quality, *Staphylococcus*

How to cite: Sahu, R., & Chappalwar, A. (2019). Bacteriological Quality Assessment of Market Milk Sold in Parbhani City, Maharashtra. International Journal of Livestock Research, 9(4), 68-75. doi: 10.5455/ijlr.20180221073846

Introduction

Milk is balanced food having high biological value in nature due to which it gains significance in diet of immense population on earth. At the same time it is an excellent medium for growth of an organism. Raw milk (RM) often contains microorganisms which may cause food borne diseases (Headrick *et al.*, 1998). Fresh milk drawn from a healthy cow normally contain low number of microorganisms (less than 1000 ml^{-1}). But contamination of milk and milk products mostly occurs at milk collection places due to manure, water, soil, milkman hands, utensils and flies, which results in increase of 100 folds or more times microbial

load at normal temperature, due to which raw milk may contain organisms over 2,000,000 cfu/ml before processing and may reach to poor quality also (Kameni *et al*., 2002). Presence of food-borne pathogens in milk results due to the direct contact of milk with contaminated sources in the dairy farm as well as excretion from the udder of an infected animal (Zubeir *et al.,* 2006), which creates majority of hygienic quality problems. Major reason of presence of higher bacterial counts is due to poor production hygiene or slackened pasteurization (Harding, 1999). Improper storage of milk, fluctuation in the temperature during storage/ transportation of milk from village to city for selling to fulfill daily need may encourage a growth of microorganisms and there is a tremendous increase in the microbial load (Sharpe and Bramley, 1977). Adulteration especially addition of contaminated water is a potent source for the bacterial contamination of the milk. Occurrence of pathogenic bacteria results in changes in flavor / appearance of raw milk due to their activities may even cause serious health problem (Younis, 2003). The presence of certain pathogenic bacteria such as *Salmonella* spp, *Escherchia coli, Staphyllococcus aureus* (Ryser, 1998) and *Escherichia coli* and *Streptococcus* spp (Lingathurai *et al.,* 2009) in milk emerged major public health threats.

Standard plate count, coliform and enterobacteria represent microbiological quality of milk and milk products (Szita *et al.*, 2008). Almost all half of the total coliform is attributed to *faecal coliforms* including *E. coli* indicating great possibility of the occurrence of enteric pathogens in milk (Grimaud *et al.,* 2007). However organisms *E. coli* and *Streptococci* are most common contaminants of fecal origin and could be an important factor of gastrointestinal infection including food poisoning and food borne illness (Kumar and Prasad, 2010). *E. coli* is most frequently contaminating organism which is reliable indicator of fecal pollution. *Clostridium* organisms are typical colonizer of sediments i.e. fecal contamination of fresh water; hence it also has been suggested as an alternative indicator of fecal pollution. *Streptococcus* and *Staphylococcus* organisms mostly derived from udder of unhealthy animal i.e. mastitis. Moreover presence of food borne pathogens creates major threat to human health. However higher bacterial counts always indicates poor hygiene during production (Harding 1995), which may cause food borne diseases (Ammara *et al.,* 2009).

In view of growing public awareness about food safety and its quality at the same time to avoid the health and economic losses of consumers due to food borne pathogens present study was aimed to determine the microbial quality of milk sold in Parbhani city, Maharashtra with an objective to investigate the occurrence and load of microorganisms in milk with special reference to food borne pathogens.

Materials and Methods

Raw Material

A total of hundred milk samples were collected from vendors of local market in Parbhani city during Jan-Mar, 2015. Two hundred and fifty mililitre (250ml) milk of each sample was collected in sterilized glass

DOI 10.5455/ijlr.20180221073846

bottle and immediately brought to the laboratory of College of Veterinary and Animal Sciences, Parbhani by maintaining cold chain (4°C) using icebox and then they were analyzed for microbial assessment.

Determination of Total Viable Count (TVC)

As per the method described by Association of Official Analytical Chemists (AOAC, 1997) total viable count of milk samples was calculated. Pour plate method was used for determination of TVC in which 0.1 ml of 10^{-5} dilutions was added. Molten plate count agar was added to the petri plates and the plates were incubated for 24 hr at 37°C. Incubation was done at 37°C. After 24 hrs, the colonies were counted using digital colony counter. A separate plate was used for each dilution of milk samples. The TVC of each milk sample was calculated by using following formula.

Where,

$$\text{CFU/sq. cm} = \frac{\sum \text{C}}{[n_1 + (0.1 x n_2) \times \text{d}]}$$

Σ C = Total number of colonies counted from all plates
n_1 = No. of plates of lower dilution
n_2 = No. of plates of higher dilution
d = Dilution factor

Determination of Differential Count

Collected milk samples were processed for determination and isolation of different food borne pathogens. Selective media from Hi-media were used for isolation of differential organisms viz. *E. coli*, *Streptococcus* spp, *Staphylococcus* spp, *Enterobacter* spp, *Clostridium* spp *and Salmonella. E. coli*, *Streptococci*, *Staphylococci*, *Enterobacter* spp and isolation was carried out as per the method of American Public Health Association (APHA, 1984).

Preparation of Dilutions

Representative milk sample of 10 ml was pipette out using a sterile pipette from each collected milk sample and homogenized in sterile bag containing 90ml normal saline solution. These diluents were used for the preparation of subsequent decimal dilutions.

Isolation of *E. coli*

Organism *E. coli* was isolated by using 0.1 ml inoculum of 10^{-4} and 10^{-5} dilutions of milk samples on eosine methylene blue (EMB) agar plates. The inoculum was spread by means of L-shape spreader and plates were kept overnight at 37°C for incubation. Bluish purple coloured colonies with greenish metallic sheen were considered indicative of *E. coli.*

DOI 10.5455/ijlr.20180221073846

Isolation of *Streptococci*

A quantity of 0.1 ml inoculum of 10^{-4} and 10^{-5} dilutions was inoculated on Slantez and Bartley Medium (SBM) plates. The inoculum was spread by means of L-shape spreader. The plates were kept at 37°C for 24 hrs for incubation. Maroon red colored colonies were considered as *Streptococci* colonies.

Isolation of *Clostridium* spp

Sodium polymyxin sulpha-diazine (SPS) agar was used as selective medium for the *Clostridia spp* and was isolated as per the method described by Angelotti *et al.* (1962). The organisms *Clostridia* were grown anaerobically in the tubes of SPS agar. A quantity of 0.1 ml inoculum 10^{-4} and 10^{-5} dilutions was allowed to trap in between the two layers of SPS agar to maintain the anaerobic conditions in the test tube. The tubes were sealed by paraffin films and incubated in the incubator for 24 hrs at 37°C. Black cottony wool growth in between two layers indicates presence of *Clostridia* in the samples.

Isolation of *Staphylococcus* spp

A quantity of 0.1 ml inoculum of 10^{-4} and 10^{-5} dilutions out of six serial dilutions was inoculated on Baired Parker Agar (BPA) plates. The inoculum was spread by means of L-shape spreader. The plates were kept at 37°C for 24 hrs for incubation. After incubation grey black colonies on the plate were considered as positive.

Isolation of *Enterobacter* spp

A quantity of 0.1 ml inoculum of 10^{-4} and 10^{-5} dilutions was inoculated on Eosin & Methylene Blue (EMB) plates. The inoculum was spread by means of L-shape spreader. The plates were kept at 37°C for 24 hrs for incubation. Red purple colour colonies without greenish metallic shine were considered as colonies of *Enterobacter* spp.

Isolation of *Salmonella* spp

Isolation of *Salmonella* spp was done by using the method of Andrews (1992). The procedure was followed as pre-enrichment, enrichment and incubation period. Pre enrichment was done by using 1 percent Buffered peptone water (BPW) at 37^0C for 24 hours. Pre-enriched sample was inoculated in Tetrathionate Broth (TTB) for enrichment and incubation was done for 24 hours at 42^0C. Streaking of TTB grown bacteria was done on Xylose Lysine Deoxycholate (XLD) agar and incubated for 24 hour at 37^0C. Pink colonies with or without black centers were considered as positive for *Salmonella*.

Result and Discussion

The finding with respect to the microbiological quality was found to be poor. An observation with respect to the mean value of Total viable count of market milk is presented in Table 1 and prevalence rate of food borne pathogens were presented in Table 2.

DOI 10.5455/ijlr.20180221073846

Table 1: Mean value of total viable count of raw milk

Milk Samples Collection	No. of Samples Collected	Total viable count (TVC)Mean ± SE (cfu/ml) x 10^5	Minimum Total viable count (cfu/ml)x 10^5	Maximum Total viable count (cfu/ml) x 10^5
Vendors of Parbhani city	100	6.61 ± 0.13	2.73± 0.15	8.83±0.08

Table 2: Prevalence rate of differential microorganisms

Market Milk	*E. coli*	*Streptococcus* spp	*Staphylococcus* spp	*Enterobacter* spp	*Clostridium* spp	*Salmonella spp*
Sample tested	100	100	100	100	100	100
Samples showing presence	38	4	58	66	0	0
Prevalence	38%	4%	58%	66%	0	0

The microbial content of milk indicates the hygienic level during milking and wholesomeness of udder of individual animal (Speer, 1998). Tested milk samples showed 6.61 ± 0.13x10^5cfu/ml, mean ± standard deviation of total plate count with minimum and maximum count 2.73 ±0.15 x10^5cfu/ml and 8.83 ±0.08 x10^5cfu/ml respectively. The details of TVC counts was shown in Fig. 1.

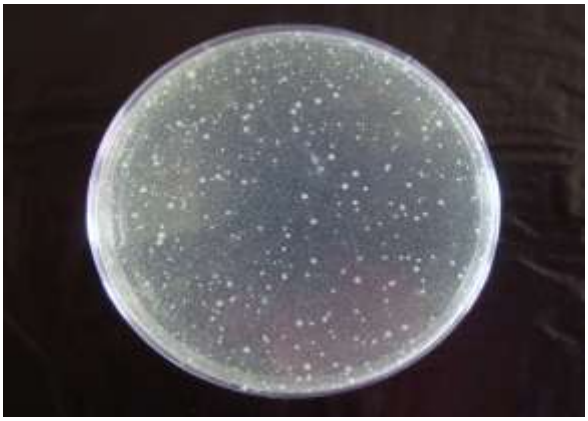

Fig 1: Plate showing total viable counts (TVC)

The TVC of tested market milk was higher than the permissible limit in the raw milk i.e. 2x10^5 cfu/ml, since samples were put in the very poor quality and marked as grade-D. The animal may be the main source of contamination that probable result in higher number of microbial flora in raw milk. Bacteria can also easily enter through the dairy utensils due to the contact of surfaces with water, soil, manure etc. and further transferred to milk. Therefore, possible reason for the higher counts could be infected udder of milking animals, unhygienic milking procedure and/ or equipment and unclean water having high microbial count used for cleaning of utensils & animal. Inappropriate storage of milk can also be a cause for increment in microbial load (Sahu *et al*., 2016). Similarly, Lingathurai *et al.* (2009) reported 5.84 log cfu/ml total counts

DOI 10.5455/ijlr.20180221073846

in cow milk of Madurai, Tamil Nadu. Monika and Poonam (2013) noticed lower total viable count in milk sample during the preservation period i.e. 4.19+0.69 to 6.35+0.11 log cfu/ml. Higher counts were observed by Manzoor *et al.* (2012) in milk samples collected from market milk sample collected during transportation to consumers from three different areas of Abbottabad city.

The findings obtained with respect to the prevalence study after isolation and identification of differential organisms revealed the presence of organisms *E. coli*, *Staphylococcus* spp, *Streptococcus* spp *and Enterobacter* spp respectively in 38, 4, 58, 66 percentages of milk samples. Kumar and Prasad (2010) reported presence of *E. coli* and *Staphylococcus* spp in 26 percent milk and milk products samples collected from Pantnagar, these findings are almost analogous with the present study for *E. coli* organism, but lower for *Staphylococcus* spp. Contrarily, Lingathuria *et al.* (2009) found *E. coli* and *Stahyloccous* spp in 80 percent cow milk sample collected from different locations of Madurai city, Tamil Nadu, which reflect higher percentage of positive samples than the present findings. Kivaria *et al.* (2006) observed lower results during quality evaluation of raw milk marketed by milk selling points (MSPs) in Dar es Salaam region and reported $8.2 \pm 1.9 \times 10^6$ cfu/ml mean value for TBC and major bacterial isolates i.e. *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus agalactiae* in 6.3 percent, whereas *Enterobacter aerogenes* and *Enterococcus faecalis* were 5.6 percent and 4.7 percent in milk samples respectively. Pathak *et al.* (2012) were found significantly lower values for presence of pathogenic organisms such as *Enterobacter spp* in 6.92, 15.3, 5.88 and 8.06 percent, *Escherichia coli in* 18.46, 18.37, 17.65, 20.97 percent and *Staphylococcus* spp in 3.08, 6.12, 7.84, 8.06 percent in boiled bovine milk collected from urban consumers and open market areas of Jabalpur during summer, monsoon, post monsoon and winter season respectively than the present findings. Presence of these organisms is a horizontal source of human health deterioration due to food poisoning. Source of these pathogenic organisms may be due to the fecal contamination, unhygienic conditions during milk production, poor sanitation and cleanliness of environment as well as utensils.

None of the sample from local market of Parbhani city was found positive for *Salmonella* spp and *clostridium* spp. which was in agreement with the Chandrashekhar *et al*. (2010) for *Salmonella* spp in milk sample of Kumargani, Faizabad, whereas Lingarthurai and Vellathurai (2010) for *clostridium* spp in milk samples from Madhurai.

Conclusion

The milk is considered to be as the richest medium for the growth of microorganisms. Improper rearing condition, processing practices and slackness in storage and distribution leads to rise in the growth of pathogenic microorganism. Collected samples were found positive towards presence of *E. coli*, *Streptococcus*, *Staphylococcus* and *Enterobacter*. Effective measures such as good hygienic practices at

DOI 10.5455/ijlr.20180221073846

production point, maintenance of cold chain during storage, transportation and proper pasteurization of milk before consumption should be done to avoid spoilage of milk and to prevent the consumers from risk.

References

1. Ammara H, Amjad I, and Shahid Mahmood. (2009). Microbiological and physicochemical analysis of different UHT milks available in market. *Afr. J. Food Sci.*, 4: 100-106.
2. Andrews. (1992). Manual of food quality control 4(1). Microbiological analysis: published by food and agriculture organization of the UN.
3. Angelotti R, Hall HE, Foter M and Lewis KH. (1962). Quantitation of Clostridium perfringens in foods. *Appli Micro*., 10:193-199.
4. AOAC. Association of Official Analytical Chemists. (1997). Official Methods of Milk Analysis. 16th Edition, 3rd Revision Washington, USA.
5. APHA. (1984). In compendium of methods for the microbiological examination of foods, 2nded. American Public Health Association, Washington, D.C.
6. Callon C, Gilbert FB, Cremoux RD, Montel MC. (2008). Application of variable number of tandem reat analysis to determine the origin of *S. Aureus* contamination from milk to cheese in goat cheese farms. *Food Control*, 19: 143-150.
7. Chandra Shekhar, Motina E and Sunil Kumar. (2010). Microbiological quality of raw milk and its public health significance. *J Dairy Foods* Home Sci., 29 (1):15-18
8. Grimaud P, Sserunjogi ML and Grillet N. (2007). An evaluation of milk quality in Uganda: value chain assessment and recommendations, *African Journal of Food Agriculture Nutrition and Development*, 7: 5.
9. Harding F. (1995). Milk quality. Blackie Academic and Professionals, an imprint of Chapman and Hall, Glasgow, UK, 157-158.
10. Hayes MC, Raley RD, Murphy SC, Carey NR, Scarlett JM and Boor KJ. (2001). Identification and characterization of elevated Microbial counts in bulk tank raw milk. *Journal of Dairy Science*, 84:292-298.
11. Headrick ML, Korangy S, Bean NH, Angulo FJ, Altekruse SF, Potter ME and Klontz KC .(1998). The epidemiology of raw milk associated food borne disease out breaks reported in the United States, 1973 through 1992. *American Journal Public Health*, 88(8): 1219-1221.
12. Jayarao BM and Henning DR. (2001). Prevalence of Foodborne Pathogens in Bulk Tank Milk. *Journal of Dairy Science*, 84(10): 2157-2162.
13. Kameni A, Imele H and Mbanya NJ. (2002). An alternative heat treatment for milk pasteurization in Cameroon. *International. Journal of Dairy Technology*, 55(1):40-43.
14. Kivaria FM, Noordhuizen TM and Kapaga AM. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy, producers in the Dares Salaam region, Tanzania. *Tropical Animal Health Production*, 38:185-194.
15. Kumar R and Prasad A (2010). Detection of E. coli and Staphylococcus in milk and Milk products in and around Pantnagar. *Veterinary world*, 3(11):495-496.
16. Lingathurai S and Vellathurai P. (2010). Bacteriological quality and safety of raw cow milk in Madurai, South India. *Webmed Central microbiology*, 1(10): WMC001029
17. Lingathurai S, Vellathurai P, Ezil Vendan S. and Alwin Prem Anand A. (2009). A comparative study on the microbiological and chemical composition of cow milk from different locations in Madurai, Tamil Nadu. *Indian Journal of Science and Technology*, 2(2): 68-46.
18. Manzoor B, Tarique Tunio M, Shuming Y, Mohsina Z, Qiu J, Chen A and Chen G. (2012). Physicochemical and bacteriological changes in market raw milk during its transportation to consumers. *Indian Journal of Agro Veterinary and Medical Sciences*, 6: 480-486.

DOI 10.5455/ijlr.20180221073846

19. Monika Saxena and Poonam Rai. (2013). Microbiological and chemical analysis of raw, pasteurized and UHT milk during preservation in India. *International Journal of Chemistry Techology Research*, 5(6): 2804-2809.
20. Pathak AK, Varmas K and Soni A. K. (2012). Physio-chemical and microbial quality of boiled milk, Jabalpur. *International Journal of Food, Agriculture and Veterinary Sciences*, 2 (2) May – August:10-15.
21. Ryser ET. (1998). Public health concerns. In Marth EH, Steele JL, (Eds.), Applied Dairy Microbiology. Marcel decker, inc., New York, 263-403.
22. Sahu Rajesh Kumar, Satyasamparna Raut, A.M. Chappalwar C.D. Bhong and V.V. Deshmukh. (2016). Physio-chemical and bacteriological analysis of osmanabadi goat milk. Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 18(3): 615-618.
23. Sharpe ME and Bramley AJ. (1977). Incidence of pathogenic bacteria and viruses in raw milk. *Dairy Industries International*, 42(9): 24-26.
24. Szita G, Gyetvai B, Szita J, Gyenes M, Solymos N. (2008). Synthetic culture media evaluated for detection of coliforms bacteria in milk, cheese and egg melange. *Acta Veterinary Brno*, (77): 143-147.
25. Younis. (2003). Household Income and Expenditure Survey conducted by the Federal Bureau of Statistics (1998) and recently substantiated by Younis (2003), Government of Pakistan, Economic Advisor's Wing, Finance Division, Islamabad.
26. Zubeir El, Ibtisam EM, Kutzer P, Owni EL. (2006). Frequencies and antibiotic susceptibility patterns of bacteria causing mastitis among cows and their environment in Khartoum State. *Research Journal of Microbiology*, 1(2): 101-109.

DOI 10.5455/ijlr.20180221073846