MICROBIAL QUALITY ANALYSIS OF RAW MILK SOLD IN BIKANER

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ABSTRACT

Present study was conducted to analyze the microbial quality of raw milk sold in Bikaner. One hundred milk samples were collected from various sources like dairy farms, milk pooling booths, and local milk vendors from the different areas of Bikaner, Rajasthan. For determining the microbial quality of raw milk samples standard plate count, psychrophilic count and total coliform count were performed. The mean Standard plate counts for dairy farms, pooled milk sources and local vendors was 7.02×10^5, $8.21 \times 10^6$and 1.6×10^7, respectively. On the basis of ISI standard for standard plate count 13% of the milk samples were found to be of good quality, 39% were of fair quality and 48% were of poor bacteriological quality. Standard plate counts showed non significant difference among the various sources of raw milk samples, dairy farms, pooled milk sources and local vendors (P value = 0.116). Mean psychrophilic counts were found 3.15×10^5, 4.21×10^5 and 1.38×10^6 for dairy farms, pooled milk sources and local vendors, respectively. Coliform count showed 10 samples were of satisfactory grade while remaining 90 samples belonging to all these groups as dairy farms, pooled milk sources and local vendors were of unsatisfactory grade when compared with ISI 1962 grades.

Key words: Bacteriological quality, milk, SPC, psychrophili c count, total coliform count, Chi square

Introduction

Milk is the normal mammary secretion derived from complete milking of healthy milch animal without either addition there to or extraction there from. It shall be free from colostrum (Food Safety and Standards Authority of India, 2009). Milk is a perfect food, readily digested and absorbed. It is a sole natural food for infants and children. It is chiefly a valuable source of good quality protein, fat, carbohydrates, vitamins and minerals. Protein in diet supply the amino acids required for growth of infants and children (Srinivasan *et al*., 2017).

Microbial contamination in milk may occur from various sources like within the udder, exterior of the udder, storage equipment, from animal's body, litter, floor, flies, insects, rodents, water supply, milker, milk utensils, atmosphere etc.(Solomon *et al*., 2013; Khan *et al*., 2008; Edward and Inya, 2013).

Total bacterial count is a good indicator for monitoring the hygienic conditions practiced during production, handling and collection of milk. Coliform count on the other hand especially associated with the level of sanitary conditions during production and subsequent handling, since they are mainly of faecal origin (Lal, 2019).

The current trend in the dairy industries is to reduce the frequency of milk collection; thus, the refrigerator storage of milk had been lengthened from two to five days to prior to heat treatment (O'Brien and Guinee, 2011). The presence of certain types of psychrophilic bacteria in milk causes various off flavours and odors; this lower, quality and value (Rao, 1966).

Materials and Methods

The present research work was conducted in the Department of Veterinary Public Health College of Veterinary and Animal Science, Bikaner, Rajasthan, University of Veterinary and Animal Sciences, Bikaner, India.

Sample collection

One hundred milk samples were collected from various sources like dairy farms (30), pooled milk sources (30) and local milk vendors (40). For the present study, milk samples collected from above sources were immediately brought to laboratory under cold conditions. Samples were subjected to different types of microbiological examinations.

Materials and Methods

Collected one hundred milk samples were investigated for their bacteriological quality on the basis of Standard plate count (SPC), APHA (1966), Psychrophilic count (APHA, 1962), Coliform count (NCFA, 1962). Counting in a colony counter and the counts were expressed as colony forming unit CFU/ml of sample.

Results and Discussion

Out of 30 milk samples collected from dairy farms 6 (20%), 16 (53.33%) and 8 (26.67%) milk samples were categorized as good, fair and poor, respectively (Table 1). Whereas out of 30 milk samples collected from pooled milk sources, 2 (6.67%), 9 (30%), 19 (63.33%) samples and out of 40 samples collected from local vendors 5 (12.5%), 14 (35%) and 21 (52.5%) samples were categorized as good, fair and poor, respectively as per ISI standards 1962. It is evident that higher percentage of samples from Dairy farms were categorized as good and fair quality as compared to samples collected from pooled milk sources and local vendors. The higher percentage of poor grade milk from pooled milk sources and local vendors as evident in present study may be due to the poor hygienic conditions in comparison to dairy farms that usually follows hygienic measures for maintaining quality of milk.

Chi square test (7.39) declared no significant (P>.05) association between sources of milk samples and standard

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plate count.

It is concluded that out of hundred milk samples screened in the present study none of the sample was in category of very good grade as per the standards prescribed by ISI (1962) and IDF (1967). Joshi (1996) and Mehra (2007) conducted the similar study on raw milk samples collected from various sources in Bikaner and also found none of the sample felled in category of "very good" grade

The results of present investigation indicated that milk of poor grade was observed in majority of milk samples and this falls in line with the findings as observed earlier by Mehra (2007) while Megha and Annadurai (2014) found only four samples of poor grade and other 21 samples were in fair grade and does not fall in line with present findings. Mistry *et al.* (2015) also found 53.33% raw milk samples of the good category and only 20% samples were of poor grade which was also not meet our current findings.

Out of 30 samples collected from dairy farms, 6 (20%), 6 (20%), 8 (26.67%), 5 (16.66%), 5(16.66%) samples had psychrophilic count in the range of 10-10^2, 10^2-10^3,10^3-10^4,10^4-10^5 and greater than 10^5 per ml of milk, respectively while none of the sample was having zero psychrophilic count

Out of 30 samples from pooled milk sources 8 (26.67%), 6 (20%), 6 (20%) and 10 (33.33%) were having psychrophilic count in the range of 10^2-10^3,10^3-10^4,10^4-10^5 and greater than 10^5 per ml of milk, respectively (Table 2). Out of 40 samples collected from local vendors 5 (12.5%), 5 (12.5%), 12 (30%) and 18 (45%) samples were having psychrophilic count in the range of10^2-10^3,10^3-10^4,10^4-10^5 and greater than 10^5 per ml of milk, respectively. Out of 30 milk samples from pooled milk sources and 40 samples from local vendors not a single sample showed psychrophilic count in the range of zero and 10-10^2 per ml of milk. The value of chi square test (8.69) revealed no significant (P>.05) association between sources of milk and psychrophilic count.

Psychrophilic count

The results of psychrophilic count for 23% samples which showed psychrophiles in the range of 10^4-10^5 are higher in comparison with the results of Joshi (1996) and Mehra (2007) who observed 17, 20 and 18 per cent samples with the psychrophilic count as observed in the range as mentioned above.

Lingathurai and Vellathurai (2013) examined 60 raw milk samples, collected from Madurai, South India and revealed mean counts per ml for psychrotophs were 5×10^3 cfu/m land it was much lower than our findings as present study showed the mean count 12.9×10^5 cfu/ml. The value of chi square test (8.69) revealed no significant (P>.05) association between sources of milk samples and psychrophilic count.

Total coliform count

Out of hundred milk samples analysed, 10 samples had zero to 10 coliform count, while 26, 17, 21,14 and 12 samples had coliform count in the range of 10-10^2, 10^2-10^3,10^3-10^4,10^4-10^5 and greater than 10^5 per ml of milk, respectively (Table 3). Out of 30 milk samples examined from dairy farms 5 (16.67%) samples had zero to 10 coliform count, whereas 10 (33.33%), 6 (20%), 5 (16.67%), 3 (10%) and 1 (3.33%) samples had coliform count in the range of 10-10^2, 10^2-10^3, 10^3-10^4, 10^4-10^5 and greater than 10^5 per ml of milk, respectively. Out of 30 milk samples examined from pooled milk sources 2 (6.67%) samples had zero to 10 coliform count, whereas 8 (26.67%), 3 (10%), 6 (20%), 7 (23.33%) and 4 (13.33%) samples had coliform count in the range of 10-10^2, 10^2-10^3, 10^3-10^4, 10^4-10^5 and greater than 10^5 per ml of milk, respectively while out of 40 milk samples examined from local vendors (7.5%) samples had zero to 10 coliform count, whereas 8 (20%), 8(20%), 10

Table 1: Standard plate count (SPC) of raw milk samples collected from various places in Bikaner

Range of SPC per ml of milk (ISI, 1962)	Grades	Milk samples			Total
		Dairy farms	Pooled milk	Local vendors	
< $2x10^5$	Very good	-	-	-	-
$2x10^5$-$1x10^6$	Good	6 (20%)	2 (6.67%)	5 (12.5%)	13 (13%)
$1x10^5$-$5x10^6$	Fair	16 (53.33%)	9 (30%)	14 (35%)	39 (39%)
>$5x10^6$	Poor	8 (26.67%)	19 (63.33%)	21 (52.5%)	48 (48%)
Total milk samples		30	30	40	100
Chi square value = 7.39		P value = 0.116		Non significant (NS)	

Table 2: Psychrophilic count of raw milk samples collected from various places in Bikaner

Range of Psychrophilic count per ml of milk (ISI, 1962)	Milk samples collected from various sources			Total
	Dairy farms	Pooled milk	Local vendors	
0	-	-	-	-
10 -$1x10^2$	6 (20%)	-	-	6(6%)
$1x10^2$-$1x10^3$	6 (20%)	8 (26.67%)	5 (12.5%)	19 (19%)
$1x10^3$- $1x10^4$	8 (26.67%)	6 (20%)	5 (12.5%)	19 (19%)
$1x10^4$- $1x10^5$	5 (16.66%)	6 (20%)	12 (30%)	23 (23%)
>10^5	5 (16.66%)	10 (33.33%)	18 (45%)	33 (33%)
Total milk samples	30	30	40	100
Chi square value = 8.69	P value = 0.191			Non significant (NS)

Table 3: Total coliform count of raw milk samples collected from various sources in Bikaner

S. No.	Range of coliform count per ml of milk	Milk samples collected from various sources			Total
		Dairy farms No. (%)	Pooled milk No. (%)	Local vendors No. (%)	
1.	0-10	5 (16.67%)	2 (6.67%)	3 (7.5%)	10
2.	10-10^2	10 (33.33%)	8 (26.67%)	8 (20%)	26
3	10^2-10^3	6 (20%)	3 (10%)	8 (20%)	17
4.	10^3-10^4	5 (16.67%)	6 (20%)	10 (25%)	21
5.	10^4-10^5	3 (10%)	7 (23.33%)	4 (10%)	14
6.	More than 10^5	1(3.33%)	4 (13.33%)	7 (17.5%)	12
Total		30	30	40	100
Chi square value = 10.51			P value = 0.396		Non significant (NS)

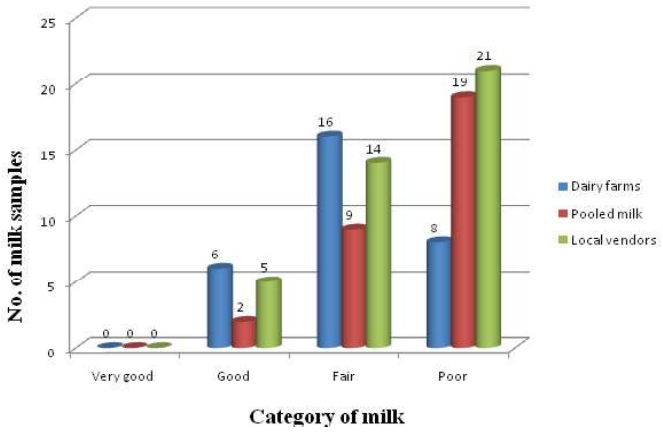

Fig. 1: Standard plate count of milk samples from various sources

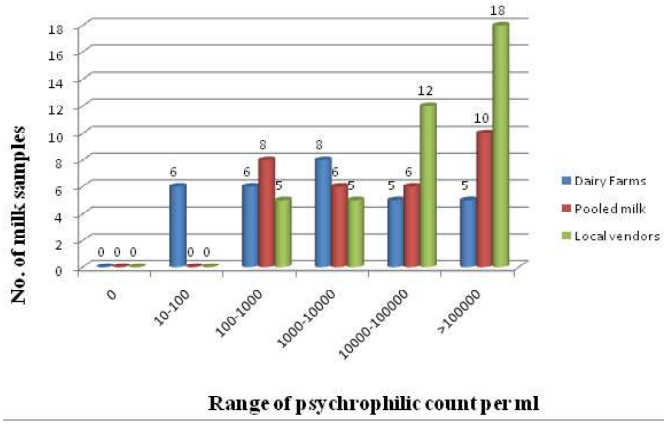

Fig. 2: Psychrophilic count of milk samples from various sources

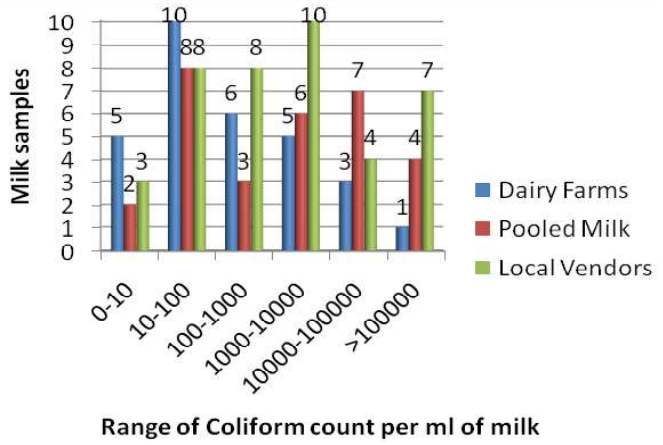

Fig. 3: Total coliform count of milk samples from various sources

(25%), 4 (10%) and 7 (17.5%) samples had coliform count in the range of 10-10^2, 10^2-10^3, 10^3-10^4, 10^4-10^5 and greater than 10^5 per ml of milk, respectively. The value of chi square test (10.51) revealed no significant (P>.05) association between sources of milk and total coliform count, as P value was found 0.396.

Kumar (1990), Opsomer (2001) and Mehra (2007) screened the raw milk samples and found that the majority of milk samples were of unsatisfactory grade. Mehra (2007) screened the raw milk samples from Bikaner city and found none of the sample in the range of 0 to 10 and 3 samples in range of 0 to 10 while in present study 10 samples were found within the range of 0 to10 coliforms count per ml of milk and 26 samples out of hundred samples were in the range of 10 to 100 count per ml of milk.

Results of present study indicate that the Coliform bacteria are present in higher per cent of the milk samples and these were of unsatisfactory grade, it determines the hygienic quality and poor status of milk which is consumed by public of Bikaner city.

Recommendations

It is inferred that the persons engaged in production and milking procedures must be suggested to follow the strict hygienic methods so the microbial contamination of raw milk can be reduced and to prevent economic losses to producers and consumers. As milk is also used for sweets and other products on large scale in this city in comparison to any of the state in India, the results of the present study will definitely be beneficial for improving the milk quality. Emphasis should be given on clean milk production (CMP). Raw milk consumption should be avoided. Boiling of milk before consuming should be done.

References

American Public Health Association (1962) Standard Methods for the examination of dairy products.11th ed. Inc. New York.

American Public Health Association (1966) Standard methods for the examination of dairy products.12th ed. Inc. New York.

Edward KC and Inya IM (2013) The Microbial Quality of Raw Milk from four locations in Abia State, Nigeria. *IOSR J. Pharma. Bio. Sci*. **5** (3): 30-33.

Food Safety and Standards Authority of India (2009) Food safety and standards rules 2009. Retrieved from http://old.fssai.gov.in/Portals/0/FSSAI_Rules_December 2009.pdf

International Dairy Federation (1967) Bacteriological quality of milk store at low temperature. *Inter. stand*. FIL-IDF **39**: 1966-1967.

Indian Standard Institution (1962) Bacteriological analysis of milk in Indian standards: methods of test for dairy industry. **8-**25.

Joshi R (1996) Bacteriological examination of raw milk and cream with special Sreference to isolation of pathogens of public health significance (Thesis submitted to Rajasthan University of Veterinary and Animal Sciences, Bikaner).

Khan MTG, Zinnah MA, Siddique MP, Rashid MHA, Islam MA and Choudhury KA (2008) Physical and microbial qualities of raw milk collected from Bangladesh Agricultural University dairy farm and the surrounding villages. *Bang. J. Vet. Medi*. **6** (2): 217-221.

Kumar S (1990) Bacteriological examination of bovine raw milk with special reference to isolation of some pathogens of public health significance. M. V. Sc Thesis, Haryana Agriculture University, Hisar.

Lal D (2019) Detection of adulteration and quality evaluation of market milk and raw milk collected from market in Varanasi city (Doctoral dissertation, Department of animal husbandry and dairying institute of agriculture sciences Banaras Hindu University Varanasi).

Lingathurai S and Vellathurai P (2013) Bacteriological quality and safety of raw cow milk in Madurai (South India). *Bangladesh J. Sci. Ind. Res*. **48** (2): 109-114.

Megha SV and Annadurai B (2014) Isolation and identification of

proteolytic bacteria from raw milk samples. *Global J. Bio-Sci. Biotechol*. **3**(4): 391-397.

Mehra S (2007) Bacteriological examination of buffalo raw milk and cream to isolate the organisms of public health significance. (Doctoral dissertation, Rajasthan University of Veterinary and Animal Sciences, Bikaner).

Mistry TB, Patel ND and Shaikh NM (2015) Bacteriological Quality of Raw Buffalo milk from different villages in Bardoli, Gujarat, India. *Int. J. Curr. Micrbiol. Appl. Sci.* **4**(4): 874-884.

Nordic Committee on Food Analysis NCFA (1962) Publication of Royal Veterinary and Agricultural University, Copenhaegan, Denmark, No. 44.

O'brien B and Guinee TP (2011) Milk Seasonal effects on processing properties of cows' milk. *In*: Fuquay, J. W. (Ed) Encyclope Encyclopedia of Dairy Sciences. 2nd ed. Academic Press, San Diego. 598-606.

Opsomer G, Vliegher SDE, Laurens J, Hoflack G, Beeckaman D and Kruif ADE (2001) Elevated number of coliform bacteria in the bulk milk due to chronic *klebsiella oxytocu* mastitis. *Vet. Bullet.* **71**(7): 4768.

Rao KM (1966) Sources of psychrophilie bacteria in a dairy plant with an emphasis on air. M.S. thesis. University of Tennessee, Knoxville.

Srinivasan C, Sathya P, Vijayaragavendiran S, Muralikrishnan, Kamalahasan, Bharathi, SV and Prakash (2017) Milk Adulteration and It's Detection Methods. *Int. J. Sci. Environ. Technol.* **6**(1): 483 - 486.

Solomon M, Mulisa M, Yibeltal M, Desalegn G and Simenew K (2013) Bacteriological quality of bovine raw milk at selected dairy farms in Debre Zeit town, Ethiopia. *J. Food Sci. Technol. Res.* **1**(1): 1-8.