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Analysis of Quality of milk in samples Collected from Different Regions of Dehradun

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Abstract

The present study is aimed to analyze the milk quality and microbial infection in milk sold at different regions of Dehradun. Thirty random raw milk samples were collected from dairy owners from 30 different regions of Dehradun. A total number of 30 samples were analyzed for physical appearance, quality and microbial infection. 90% of milk samples were white in appearance and 10% of the samples were yellow in appearance. The pH range varies from 6.7-6.9. Microbial Count was determined by Std.Plate Count (SPC) as per BIS Std and compared with the standard chart of microbiological quality of raw milk .Out of 30 samples 79% of raw milk were found in the Category of Good quality, 11% of milk were of average quality and 10% were of the Poor quality .Microbial Characterization by Gram's staining technique, motility test and biochemical test revealed that all the 30 sample were Containing E.coli, 26 samples were Containing Klebsiella Species and Lactobacillus Species respectively, 21 were Containing S.aureus,15 were Containing Salmonella Species and 25 were containing other bacterial strains .The present study concludes that microbial contamination in raw milk is increasing very fast in Dehradun region and it is responsible for poor health of citizens. The measures should be adopted to reduce contamination which will cause positive impact on the health of individuals.

Keywords: Raw milk samples, Milk quality, Microbial contamination.

1. Introduction

It is not exactly known when man started utilizing milk of other animals for his benefit, but the importance of milk in our diet has been recognized since Vedic times.

The annual report of 2010-11 published by National Dairy Development Board (NDDB) identified India as biggest producer of milk products. The estimated milk production for 2010-11 is 121 million tones. Country has almost reached 17%of world milk production. (Chakravorty, S. and Chakravarty, A., 2011). Milk has been recognized as an almost complete food for man as it is a source of essential nutrients like carbohydrate, protein, fat, vitamins and minerals. Milk is required for promoting growth and maintenance of health. It can however also serve as a vehicle for the transmission of chemicals and other impurities. (Chandra H., *et al.,* 2008) A study conducted nationwide has documented that almost two third of the milk sold and consumed in India is adulterated by contaminants such as skim milk powder and detergent, but impure water is also the highest contaminant. (Wadekar Sanjeevani B., 2011) According to National Survey on Milk Adulteration conducted by FSSAI (India) in 2011, water is the most common adulterant followed by detergent in milk. A FSSAI in study conducted in 2012 revealed that almost two third milk samples were found to be adulterated in which one third were from villages. Of these 16.7% were packet or branded milk and rest were loose milk samples from dairies. In the urban areas, around 68.9% of the milk was found to be adulterated with detergent, water, urea and skim milk powder. (Kandpal S., 2012). In Uttarakhand, 88% of milk samples were found to be adulterated. Despite the laws governing the quality and sale of milk existing in India for decades, the adulteration of milk has not been checked completely.

2. Materials and Methods

Collection of the samples:

Thirty raw milk samples were collected from dairy owners from 30 different regions of Dehradun. The samples were collected from nearby areas in the morning to be transported easily without any delay. The samples were collected in 50 ml screw capped sterilized bottles (Borosil). Every care has

been taken to avoid contamination during processing and transport (Judkins, H. F. and Mack, M. J., 1955)

Analysis of milk samples:

The raw milk samples were analyzed for physical appearance, quality, presence of adulterants and microbial infection. Color and pH of all samples were checked .The microbial contamination was identified using standard plate count method. The bacteria were isolated using selective media and identified by help of various biochemical tests. The two bacteria were reconfirmed using PCR kits from Hi-media.

3. Results and discussion:

90% of milk samples were white in appearance and 10% of the samples were yellow in appearance. The pH range varies from 6.7-6.9 (Table No-1). Microbial Count was determined by Std.Plate Count (SPC) as per BIS Std and compared with the standard chart of microbiological quality of raw milk (Table No- 02).Out of 30 samples 79% of raw milk were found in the Category of Good quality, 11% of milk were of average quality and 10% were of the Poor quality (Table No-3).Microbial Characterization by Gram's staining technique, motility test and biochemical test revealed that all the 30 sample were Containing *E.coli*, 26 samples were Containing *Klebsiella* Species and *Lactobacillus* Species respectively, 21 were Containing S.*aureus*,15 were Containing *Salmonella* Species and 25 were containing other bacterial strains (Table No-4).

Among the isolated bacterial species which shows the order as: E.coli>Micrococcus >lactobacillus species / Klebsiella species >S.aureus> Salmonella.

Out of 174 bulks tank milk samples from U.S Diaries, 75 milk samples were detected as *salmonella* positive through Real-time PCR method. Additionally, 23.2% of the raw milk samples contained potentially pathogenic forms of *E.coli* and up to 4.2% contained a combination of Virulence factors in their *E.coli* populations that was indicatives of presence of 0157:H7(Van Kessel *et.al*., 2011) .

Recently poor quality of milk and antibiotics resistance is also observed in Hardwar city in Uttarakhand by *Singh and Chaudhary 2012* Out of 60 raw milk samples 31.67% were detected as *E.coli*and 6.67% were detected as *salmonella* and remaining were the other bacterial strains, *E.coli* forms *E.aerogenes.* (Singh, P. and Chaudhary, P., 2012)

In a study done by Mohammad Nassir Abbar *et.al* in 2013 in Peshawar district of Pakistan; the fresh cow milk collected from different farms were heavily Contaminated by bacteria with a mean total plate count (TPC) of $12.5x10^6$ CFU/ml. The highest average value of TPC was found in milk from the eastern region with $13.9x10^6$ CFU/ml. While the lowest average value of $11.7x10^6$ $CFUml^{-1}$ was detected from milk obtained from western region. (Mohammad Nassir Abbar *et. .al*., 2013)

The incidence of Salmonella spp. in local raw milk was still low, as only 8 of 60 samples were found positive for this organism, Samples from southern region of the district seem to have a higher rate of the isolation (31%).While the lowest (1%) was milk samples from eastern region. All Salmonella are of public health concern having the ability to produce infection ranging from a mild Self-Limiting form of Gastroenteritis to septicemia and life threatening typhoid fever. (Oliver SP *et al.,* 2005)

In countries like England and Wales there are reports of frequent out breaks of Salmonellosis associated with the consumption of raw milk and products. (De Buyser ML *et al*., 2001)

Salmonella spp. and *E.coli* were detected in 28/75 (37.33%) and 68/75(90.67%) raw milk samples respectively, indicating a significant prevalence ($P<0.005$) in the 2 districts. The highest *Salmonella*spp., prevalence was observed in bangata (42.11%) followed by Kikwe (31.58%). *E.coli* prevalence was high in all wards showing 94.73% followed by Kikwe (88.88%) and Akeri (84.21%) wards respectively. Among the surveyed area highest prevalence of *E.coli* was observed in Bangata with colony count of $8.0x10^3$ CFU/ml followed by SokoniII with count of $7.2x10^3$ CFU/ml. (Addis Ababa farms) he has done study on prevalence of *Salmonella* on raw milk samples, out of 195 raw milk samples 21 were detected as Salmonella spp. This study also reveals the prevalence of *salmonella* in apparently healthy lactating dairy cows is larger (10.76%) as compared to other studies.

Hence, Lactating cows could have potential source of salmonella infection for individual working in dairy farms and for the community at large. Reported a prevalence of 7.1% from apparently health slaughter cattle, which is less than the present report. (Alemayehu D *et al*., 2003)

According to the study conducted to study incidence of *E.coli* in cow's milk in Khartoum state (North India) out of 100 samples, 63 were detected as *E.coli* positive (Asmahan A Ali and Warda S Abdelgadir, 2011). Out of 30 total raw milk samples, 12 *Salmonella* samples were found PCR positive and 28*E.coli* was found PCR positive.

Table 1:-Physical appearance of milk

S.No	Name of the place	Color	Ph
01.	Bindal	White	6. 6
02.	Bhauwala	Light Yellow	6. 7
03.	Clock Tower	Light Yellow	6. 7
04.	G. M. S. Road	Yellowish White	6. 8
05.	Kumar Mandi	Yellowish White	6. 9
06.	Kishan Nagar Chowk	White	6. 8
07.	Laxmipur	White	6. 9
08.	Motibazar	Yellowish White	7. 0
09.	Mazra	Light Yellow	6. 6
10.	Panditwari	Light Yellow	7. 0
11.	Patel Nagar	White	6. 9
12.	Premnagar	White	6. 6
13.	RaghavVihar	White	6. 7
14.	Rajpur	Yellowish White	6. 8
15.	Shivpuri	White	6. 7
16.	Shuklapur	White	6. 9
17.	Smith Nagar	White	6. 8
18.	Sudhowala	White	7. 0
19.	Thakurpur	Yellowish White	7. 0
20.	Yamuna Colony	White	6. 9
21.	Khurbura	White	6. 7
22.	Tilak Road	White	6. 8
23.	Fish Market	White	6. 6
24.	GadiCantt	Yellowish White	6. 6
25.	KaulaGarh	Light Yellowish White	6. 9
26.	Rajendra Nagar	White	6. 7
27.	VasantVihar	White	6. 9
28.	Neranjanpur	White	6. 8
29.	Saharanpur Chowk	Yellowish White	7. 0
30.	Mazra	White	6.8

Table 2 BIS Standard for raw milk plate count (SPC) (IS: 1479-1977, PART 111)

Count/ml	Quality/Grade
<200, 000	Better
2,000,001-1,000,000	Good
1,000,000-50,00,000	Average
>5,000,000	Poor

Table 3 Analysis of raw milk quality

Total milk samples	Mean bacterial count(CFU/ml)	No. of samples	Quality grade of milk
30	$15x10^{-4}$	9	Good
	$20x10^{-5}$	11	Average
	$41x10^{-6}$	10	Poor

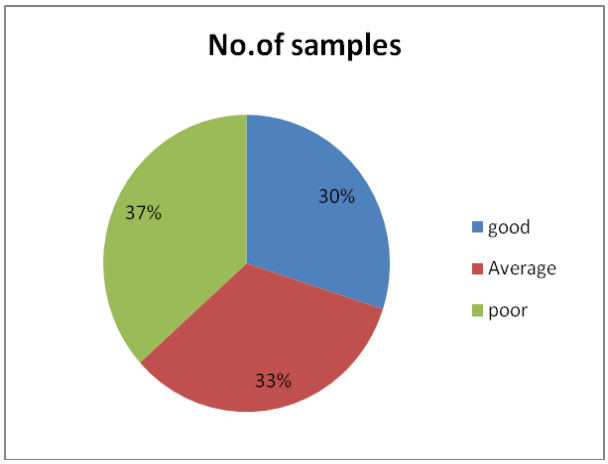

Fig: 1- Analysis of Raw milk Quality

Table 4: Microbial Examination of raw milk samples

Bacterial Isolate	Total Positive Samples
Lactobacillus sp.	22
Klebsiella sp.	22
Escherichia coli	30
Micrococcus	26
Salmonella sp.	15
Staphylococcus aureus	21
Other bacterial strains	25

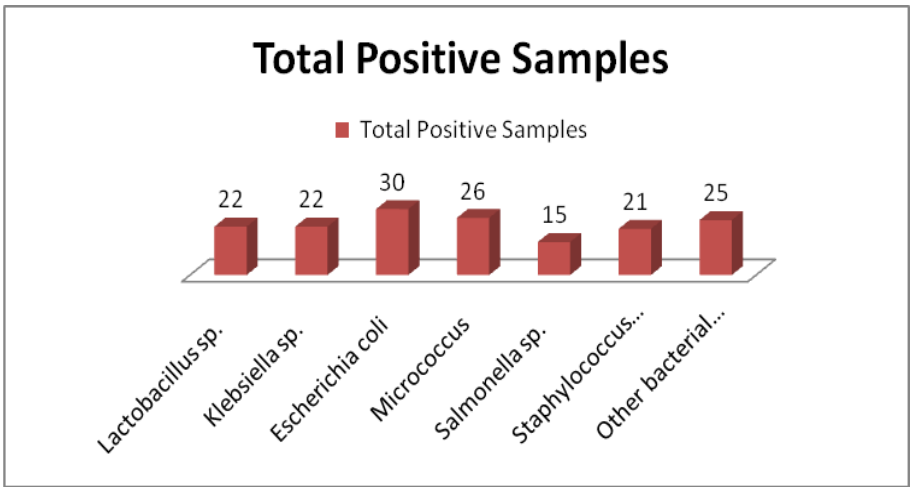

Fig: 2 Microbial Examination of raw milk samples

Table 5 Biochemical test of isolated bacteria

Bacterial species	Indole Test	Methyl Red Test	Voges-Proskaur Test	Citrate utilization Test	TSI Test	Catalase Test	Urease Test
E.coli	+ve	+ve	-ve	-ve	A/A with gas.	+ve	-ve
Klebsiella sp.	-ve	-ve	+ve	+ve	A/A with gas.	+ve	+ve
Micro coccus	-ve	-ve	-ve	+ve	-ve	-ve	+ve
Lactobaci-llus sp.	-ve	-ve	+ve	-ve	A/A with no gas.	-ve	-ve
Salmonell-a sp.	+ve	-ve	+ve	+ve	Al/A with H2S	+ve	-ve
S.aureus	-ve	+ve	-ve	-ve	A/A with no gas.	+ve	-ve

A= Acid, **Al =** Alkaline

Table 6: PCR result of isolated bacteria

Bacteria	No. of Samples	PCR result
E.coli	28	Positive
Salmonella	12	Positive

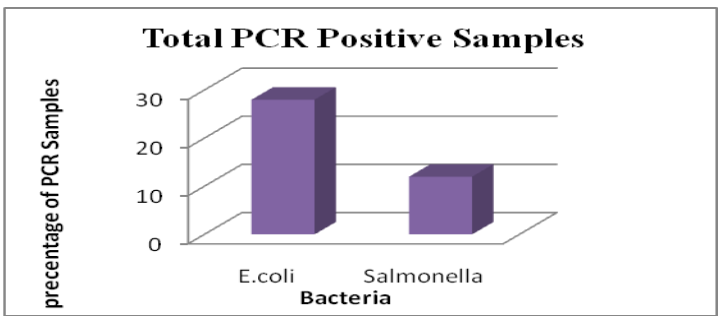

Fig: 3- PCR result of isolated bacteria

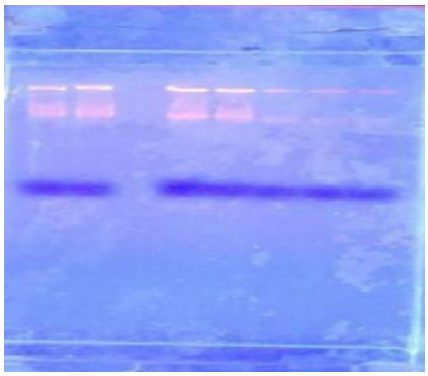

Fig.4: Gel image of *salmonella* and *E.coli*

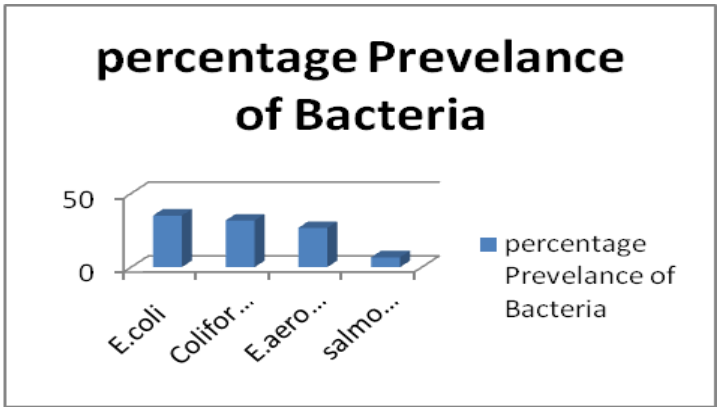

Fig.5: Bacterial prevalence in cows' raw milk

4. Conclusion

On the basis of data obtained in the present study, conclusion may be drawn that Microbial load in milk distributed in Dehradun is increasing very fast. The principle cause of microbial filth in milk is due to milking from diseased udder of the cattle, contaminated milking instruments, improper washing and improper storage conditions. Lack of awareness and negligence are still observed in this area which might be the reason of microbial contamination in milk and milk products, improving animal health reducing antimicrobial use in animal husbandry, implementation to restrict the use of antibiotics in animals, application of modern technologies may improve the current situation which will establish India as largest and best quality milk producer in the world.

References

1. Chakravorty, S., Chakravarty, A. (2011), An Investigation of adulteration in milk obtained from different localities of Varanasi city, The Indian Journal of Research Anvikshiki. 5, 120-123.
2. Chandra H., Srivastava, J., Tripathi, M.K., Rai, N., Chauhan, S, Singh, A. (2008), Contaminated milk production in the villages of district Dehradun (UK), India. ICFAI Journal of Life Sciences. 2(1), 51-59.
3. Judkins, H. F. and Mack, M. J. (1955), The Principle of dairying. 3rd Rev. John Wiley & Sons, Inc. NY. pp. 31.
4. Khan, M. T. G., Zinnah, M. A., Siddique, M. P., Rashid, M. H. A., Islam, M. A. and Choudhury, K. A. (2008), Physical and microbial qualities of raw milk collected from bangladesh agricultural university dairy farm and the surrounding villages. Bangl J Vet Med, 6 (2), 217–221.

5. Wadekar S. B., Chavan, B. R., Menkudale, G. V. (2011), Survey on adulteration of the milk received from government milk scheme in Nanded town. Interlink Research Analysis. 1,32-35.

6. Kandpal, S. D., Srivastava, S. K., Negi, K. S. (2012), Estimation of quality of raw milk (open & branded) by milk adulteration testing kit. Indian Journal of Community Health, 24 (3),188-192.

7. Van Kessel J. S, Karns J. S, Gorski L, McCluskey B. J., Perdue M. L. (2004), Prevalence of salmonella L. monocytogenes and fecal Coliforms in bulk tank milk on U.S. diaries. Journal Diary science 87,2822-30.

8. Singh, P. and Chaudhary, P. (2012), Comparative assessment of milk borne pathogens and their antibacterial sensitivity.Int J pharmaceutical Sci Res, 3, 3778-3782.

9. Muhammad N. A., Baharullah K., Sajid, A., Taiseer Ul Islam, Qaiser J., Shahzad M. (2013), Biochemical and Bacteriological Analysis of Cows' Milk Samples Collected from District Peshawar Pakistan. In International Journal of Pharmacy science. Rev. 21(2) ,221-226..

10. Oliver, S. P., Jayarao, B. M. Al-Media, R. A. (2005), Food-borne pathogens in milk and the diary environment food safety and public borne pathogens and Diseases, 2115-1129.

11. De Buyser, M. L., Dufour, B., Maire, M., Lafarge, V. (2001)**,** Implification of milk and milk products in food borne diseases in France and in different Industrialized countries. Int. J. of food Microbiology, 67, 1-17.

12. Alemayehu, D., Molla, B., Muckle, A. (2003), Prevalence of salmonella species isolated apparently from healthy slaughter cattle in Ethiopia Trop. Animal Health production 35,309-319.

13. Asmahan A Ali and Warda S Abdelgadir (2011), Incidence of *E.coli* in cow's raw milk in Khartoum State. British J. of Dairy sciences 2(1), 23-26.