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MONITORING OF HYGIENIC STATUS OF RAW MILK MARKETED IN LAHORE CITY, PAKISTAN

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ABSTRACT

Milk is an important source of nutrition and its hygienic quality is important from public health point of view. The quality of the milk was assessed by monitoring aerobic viable bacterial count, coliform count and methylene blue reduction (MBR) test. Milk is procured from rural or suburban areas, transported at ambient temperature to different marketing points of Lahore city and sold as loose raw milk to consumers. The milk samples collected from these marketing points of Gulberg, Iqbal, Ravi and Gunj Bukish towns, contained 8.5±0.6, 7.5±0.83, 5.4±1.6 and 5.1±1.7 counts (Log_{10}) of aerobic viable bacteria, respectively. It indicated that milk samples from Gulberg (100%), Iqbal (100%), Ravi (60%) and Gunj Bukish (53%) Towns contained the bacterial counts beyond the acceptable limits (10^6/ ml), respectively. There was no linear correlation between aerobic bacterial count and coliform count in samples collected from either of the towns. However, coliform count was higher than log $_{10}$7.6±1.7 in the milk samples collected from either of the towns. Although, there was no correlation between results of total aerobic bacterial count and that of MBR test in the market samples but higher dilution was having lower MBR time of the same sample. This is why negative correlation between aerobic bacterial count and MBR is still reliable and valid test for monitoring hygienic status of market milk.

Keywords: Methylene blue reduction test, Coliform count, Aerobic bacterial count, Raw milk.

INTRODUCTION

Milk of cattle, buffalo, goat, sheep, camel, yak, llama, mare etc contains almost same but varying concentration of the chemical constituents. Milk differs widely in composition due to different factors including species of animal, breed, individuality, stage of lactation, frequency of milking, age, seasonal variations, feed, interval of milking, disease and abnormal conditions and administration of drugs and hormones (Ensminger, 1993). Milk is produced in rural or suburban areas of the country and is transported from point of production to cities mainly through middle men called dodhies. The milk obtained from healthy animal's udder is free from pathogenic bacteria but some of the animals in field condition may be suffering from sub-clinical mastitis and are excreting the causative agent in milk (FAO, 2008). Such milk contaminates the bulk milk. Moreover, fresh milk may get microbial contamination from utensils, animal skin, environment, or water used for adulteration etc (FAO, 2008). Milk is enrichment medium to support growth of contaminating microbes. During transportation of milk at ambient temperature, the contaminated microbes may multiply and deteriorate the quality of loose milk (Schmidt and Van-Vleck, 1982). In this study, hygienic quality of raw milk is monitored by determining microbial load in milk samples collected from different towns of Lahore city.

MATERIALS AND METHODS

Source of samples: Loose milk samples (60) were collected from four towns: Gulberg Town, Iqbal Town, Ravi Town and Gunj Bukish Town (15 samples from each) of city Lahore during month of September, 2008. Each sample (250 ml) was collected in labeled sterilized plastic (autoclave-able) bottles and transferred through cold chain system (crushed ice) to WTO-Quality Operations Laboratory, University of Veterinary and Animal Sciences, Lahore.

Total viable aerobic bacterial count: Total viable aerobic bacterial count and coliform count of the milk samples was determined as described by Colins *et al*. (1989). Each of the samples was diluted ten fold in properly labeled 20 ml glass tubes containing 9 ml sterile normal saline as diluent. Separate sterilized glass pipette (10 ml) was used for dilution of the sample. One ml sample from tube dilution number 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} was transferred to surface of nutrient agar and Eosin Methylene Blue (EMB) agar plates (triplicate for each). The sample on the plate was spread evenly using sterile glass spreader. After incubation of each plate at 37^0C for 48 hours, total colony forming units (cfu) of aerobic bacterial and coliform count, respectively were recorded from the plate having 30-300 colonies. The

result was interpreted as total cfu of aerobic and coliform bacterial count.

Methylene blue reduction test: Hygienic status of the raw market milk samples was also determined using methylene blue reduction (MBR) test (Benson, 2002). One ml of methylene blue solution (1:25000) was transferred to labeled and sterilized 20 ml screw caped test tube containing 10 ml of each of the samples. The tube was caped and gently inverted three times to mix up the dye with milk sample. Each of the tube was incubated at 37^0C and examined after every 2 hours up to 8 hours. The time taken by the methylene blue in milk to become colorless was recorded.

Correlation of total viable aerobic bacterial count and methylene blue reduction time: For total aerobic bacterial count in loose milk samples, inter-laboratory comparison of randomly selected 5 samples was conducted. Moreover, each of the five samples was also diluted using sterilized milk as diluent and total aerobic bacterial count was determined. Methylene blue reduction test (MBR) was also conducted on each dilution of each sample (Chatterjee *et al*., 2006).

Statistical analysis: Bacterial counts of each town were transformed in to log values then data were analyzed through one way analysis of variance (Steel *et al*., 1997). Correlation between total aerobic counts and coliform counts of each sample of different towns were determined using statistical analysis program SPSS 10.

RESULTS AND DISCUSSION

Milk secreted from healthy animals is usually free from bacteria but may get contamination from dairy farm environment, external surface of teat/udder or animal body, utensils, utensil cleaning water, contaminated added water, etc (Bramley, 1982). However, milk collected from animals suffering from mastitis is highly contaminated with pathogenic bacteria such as S*treptococcus uberis, E. coli, staphylococcus species*, etc (Bramley, 1982; Leigh, 1999). Milk is enrichment medium to support growth of contaminated bacteria leading to high total bacterial count (Prajapati, 1995). In the present study, milk samples collected from Gulberg, Iqbal, Ravi and Gunj Bukish Towns contained high number of total aerobic bacterial counts. There was no significant difference ($P>0.05$) in number of bacterial counts in samples collected from either of the towns (Table-1). Milk supplied to former towns (Gulberg and Iqbal Town) is transported at ambient temperature from far distance (up to 100 kilometer) and contaminated bacteria might have multiplied and attained a high number during transportation. The milk supplied to Ravi and Gunj Bukish Towns is transported from Sheikhupura road (maximum 40 kilometer distance). These results are in line with Hayes *et al*. (2001) who recorded high total bacterial counts in milk samples collected from bulk milk supply. These high counts are linked with un-hygienic milk handling, contamination from animal bedding, mixing of normal milk with the milk collected from the animal suffering from *Streptococcus uberis* induced mastitis, etc. Total bacterial count is a rough gauge to measure the quality of milk, herd health, efficacy of farm sanitation, milk handling and storage /transportation temperature. It indicated that the bacterial counts in milk samples from Gulberg (100%), Iqbal (100%), Ravi (60%) and Gunj Bukish (53%) towns were beyond the acceptable limits (10^6/ ml) respectively (Table-2). Milk marketed in metropolitan cities might have handled carelessly and is thus poor quality in terms of hygienic quality.

Coliform counts were abundantly high in milk samples collected from all the towns of the city (Table-1). Coliform counts in milk samples collected from Gulberg and Iqbal Towns were significantly higher than those of samples collected from Ravi and Gunj Bukish Towns ($p<0.01$). Its high count could be attributed due to unhygienic collection of milk from individual house holds and handled carelessly during transportation. Addition of coliform contaminated water in milk during transportation could be plausible reason of its high count. Coliform bacteria have minimum generation time and multiply at rapid rate to reach its number up to unhygienic level. There was low correlation ($r = 0.008$ and 0.057) between coliform and total aerobic bacterial counts. In milk samples collected from Ravi and Gunj Bukish town. However, the corresponding values ($r = 0.653$ and 0.544) were higher for milk samples collected from Gulberg and Iqbal town, respectively. Coliform, such as E. coli, and other gram negative bacteria (Pseudomonas species) are also common on the dairy sheds. Coliform causing mastitis is also found abundantly in bovine feces, animal bedding and herd environment. These organisms are frequently found in water used for cleaning the equipment, collection and storage of milk (Hogan *et al*., 1989). High coliform count in bulk milk could be due to the milk collected from the animals suffering from coliform induced mastitis. One infected animal may shed *E. coli* as high as 10^8 colony forming unit (cfu) per ml of milk. However, it is unlikely because E. coli induced mastitis is apparent or clinical so no one milk such animals and mix with the milk from healthy animals. Increased number coliform count in milk could be due to contamination with fecal and bedding material. Used animal bedding contains 10^8-10^{10} cfu/gram (Hogan, *et al*., 1989).

In raw milk samples, there was very low correlation ($r= 0.081$) between total viable count and methylene blue reduction test (Table 4A). These results are in agreement with Sale (2005), who quoted many reasons of the poor correlation. Total aerobic count

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represents aerobic as well as aero-tolerant bacteria/facultative anaerobes. The MBR test depends upon the ability of milk bacteria to grow and to consume dissolved oxygen which reduces the oxidation-reduction potential of the medium and decolorizes the methylene blue (Benson, 2002). Aero-tolerant bacteria that are homolactic acid producing or curd forming, grow on nutrient medium but do not utilize oxygen and hence are not expressed in MBR test (Lim, 1989). This could be

Table-1: Comparison of aerobic bacterial and coliform counts of towns of Lahore city (log_{10})

Name of the Town	Aerobic bacterial count Mean±SD	Coliform count Mean±SD
Gulberg	8.5±0.6[a]	8.4±0.36[a]
Iqbal	8.2±0.5[a]	7.5±0.83[a]
Ravi	7.4±1.4[a]	5.4±1.6[b]
Gunj Bukish	7.6±1.7[a]	5.1±1.7[b]

Figures in each column having similar superscript are not significantly different (p>0.01)

Table-2: Grading of milk samples of Lahore city (log_{10}) on the basis of aerobic bacterial counts

Grade of milk (n=15)	Gulberg Town	Iqbal Town	Ravi Town	Gunj Bukish Town
A	0	0	1(7)	0
B	0	0	0	0
C	0	0	5 (33)	7(47)
D	15 (100)	15 (100)	9(60)	8(53)

Figures in parenthesis indicate percent of the particular grade of milk
Grade A: Less than 10,000/ml, Grade B: 10,000 to 300,000/ml
Grade C: 300,000 to 1000,000/ml Grade D: More than 1000,000/ml

Table-3: Correlation between aerobic bacterial and coliform count in milk samples of Lahore city

Gulberg Town		Iqbal Townn		Ravi Town		Gunj Bukish Town	
Aerobic bacreial count ($xl0^7$)	Coliform count ($x10^5$)	Aerobic bacreial count ($xl0^7$)	Coliform count ($x10^5$)	Aerobic bacreial count ($xl0^7$)	Coliform count ($x10^5$)	Aerobic bacreial count ($xl0^7$)	Coliform count ($x10^5$)
10.0	12.0	20.0	0.9	4.400	0.0180	0.034	0.0010
15.0	5.7	51.0	0.9	3.300	0.0830	90.000	34.0000
20.0	17.0	10.0	2.7	0.280	0.0025	3.200	0.0310
7.0	0.1	8.0	2.0	2.600	0.0830	0.041	0.0013
11.0	8.8	20.0	10.0	0.003	0.0003	0.130	0.0370
28	12.0	20.0	21.0	340.000	0.0480	4.300	0.0390
92	61.0	8.9	4.1	16.000	0.1000	0.910	0.0038
72	18.0	0.9	0.0	0.500	0.0040	0.410	0.0066
61	51.0	40.0	13.0	15.000	0.9700	670.000	0.0210
71	31.0	80.0	35.0	0.500	0.0010	76.000	50.0000
79	47.0	15.0	11.0	32.000	19.0000	1.100	0.0270
61	33.0	18.0	31.0	0.800	0.0020	3.100	0.0001
15	56.0	30.0	15.0	5.300	0.1200	5.300	0.0120
68	51.0	55.0	11.0	0.520	0.7300	0.520	0.0073
57	67.0	0.0	0.0	0.120	0.0006	0.530	0.0037
Correlation =0.653		Correlation =0.544		Correlation =0.008		Correlation =0.057	

Table-4A: Aerobic bacterial and metylene blue reduction test in milk samples of lahore city

Aerobic bacterial count ($xl0^7$)	MBR Time (Hours)
15.0	0.50
28	7.35
7.0	1.50
11.0	2.15
61	5.50
79	0.5
68	4.5
79	0.30
61	1.5
68	2
Correlation = 0.081	

Table-4B: Aerobic bacterial and metylene blue reduction test in milk samples of lahore city

Aerobic bacterial count	MBR Time (minutes)
$1.5xl0^9$	<30
$1.5xl0^8$	50
$1.5xl0^7$	100
$1.5xl0^6$	150
$1.5xl0^5$	170
$1.5xl0^4$	>480
Correlation = - 0.856	

plausible reason that MBR does not represent the true bacterial counts of raw milk. However, there was strong correlation (r= 0.865) between total aerobic count and MBR timings with in the same sample at different dilutions (Table 4B). Based upon these facts, MBR test is still considered as valid and reliable test for screening of milk tanks.

It may be concluded that loose raw milk supplied to Lahore city is un-hygienic and of poor quality. Methylene blue reduction test is a reliable and rapid test (MBR) for monitoring hygienic status of market milk.

Note: In this case, one milk sample processed for total aerobic bacterial count. The sample was diluted 10 fold and processed for methylene blue reduction (MBR) test. It was noted that MBR time (in minutes) increases with increasing dilution of the milk sample.

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