

## Short Communication

# Evaluation of Bacteriological Contamination in Raw (Unprocessed) Milk Sold in Different Regions of Lahore (Pakistan)

MUHAMMAD FARHAN<sup>1</sup> AND SARAPHINE SALIK

Department of Biological Sciences, Forman Christian College, Lahore, Pakistan

<sup>1</sup>Corresponding author's e-mail: farhan\_fcc@hotmail.com; ssalikpk@yahoo.com

## ABSTRACT

This study evaluated 100 raw milk samples for bacterial contamination. Milk samples were collected from 10 main regions of District Lahore and tested for total plate count (TPC), total coliforms and faecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Lactobacilli* and *Salmonella*. The bacterial counts (cfu mL<sup>-1</sup>) were 2.1 x 10<sup>8</sup> to 6.1 x 10<sup>8</sup> for TPC, while 1.7 x 10<sup>5</sup> to 4.3 x 10<sup>5</sup> for *E. coli*, 7.1 x 10<sup>5</sup> to 12.6 x 10<sup>5</sup> for *S. aureus*, 2400 to 6800 for *Lactobacillus* and 3000 to 8200 for *Salmonella*. Coliforms and faecal coliforms were detected in all raw milk samples. Significant difference existed between regions for TPC. There was, no difference among regions for *E. coli*, *S. aureus*, *Lactobacilli* and *Salmonella*. Presence of coliforms and faecal coliform in raw milk showed the use of contaminated water, either for the washing of utensils or for mixing in raw milk. It is concluded that raw milk supplied in the study area was unfit for consumption unless processed using standard procedures.

**Key Words:** Milk; Hygiene; Coliforms

## INTRODUCTION

A large population of Pakistan uses the raw (unprocessed) milk, which is sold in local markets unpacked. Raw milk contains vast number of spoilage and pathogenic bacteria, which are serious health hazards. Even when drawn under aseptic condition, milk always contains microorganisms, which are derived from milk ducts in the udder. Different microorganisms present in raw milk include *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Flavobacterium*, *Micrococcus*, *Streptococcus*, *Corynebacterim*, *Lactobacillus* and coliforms (Cousins & Bramley, 1981). The lowest bacterial count registered for raw milk samples was 4 x 10<sup>7</sup> mL<sup>-1</sup>, while the highest was 1 x 10<sup>9</sup> mL<sup>-1</sup> (Mahari & Gashe, 1990).

It is generally assumed that raw milk stored below 100°C does not spoil; psychrotrophs mainly being responsible for spoilage. Psychrotrophic bacteria, originally derived from soil and water, are readily isolated from farm milking equipment, pipes and bulk milk tanks (Thomas *et al.*, 1971). The other bacteria that frequently encounter the milk causing disease are *Salmonella* (Greenwood & Hooper, 1983; Halpin-Dohnalck & Marth, 1989; D'Aoust, 1991), *Staphylococcus* and *Escherichia coli* (LeChevallier *et al.*, 1996; Shinoda *et al.*, 1997; Stark, 2000). The food laws governing the commerce of raw and processed milks in Pakistan are extremely deficient, being completely out of touch with the current scientific knowledge and general lack of awareness of food hygiene (Mohiuddin, 1996; Ahmad, 1999). Therefore, little attention has been paid to evaluate the milk for bacterial contamination. This study evaluates milk supplied in some regions of Lahore city (Pakistan) for bacterial contamination.

## MATERIALS AND METHODS

**Collection of samples.** Ten zones of District Lahore (Ghari Shau, Cantt, Gulberg, Defence, Model Town, Allama Iqbal Town, Ichra, Mughalpura, Shad Bagh & Wapda Town) were selected for the present study. Ten raw milk samples were then collected aseptically from each zone (in triplicate) randomly from 10 different shops directly from bulk tanks used for storage and selling were taken in sterilized 300 mL screw capped bottles and placed in ice bucket to keep temperature below 10°C during transportation.

**Samples analysis.** Samples were examined in Microbiology Lab., Forman Christian College, Lahore. Serial dilutions were prepared in 0.1% buffer peptone and 10<sup>-1</sup> dilution was prepared by mixing 9 mL buffer peptone water and 1 mL milk sample. Further dilutions (10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, etc.), were prepared by mixing 1 mL of previous dilution and 9 mL of 0.1% buffered peptone. Selective agars and 'pour plate' method was used for enumeration of 'total plate count (TPC)', total and faecal coliforms, *E. coli*, *Streptococcus*, *Salmonella* and *Lactobacillus*. For TPC Total Plate Count agar was used. A 12 to 15 mL of sterilized (121°C for 15 min.) rehydrated TPC-agar was mixed with 1 mL of appropriate milk dilution, in petriplates, which were incubated at 37°C for 48 h. Numbers of colonies were then counted and microorganisms were recorded as colony forming units per mL (cfu mL<sup>-1</sup>). Number of microorganisms = mL of dilution used x Number of colonies x Dilution factor used For *Escherichia coli*, *Streptococcus*, *Salmonella* and *Lactobacillus*, Eosine Methylene blue agar, Braid Parker agar, *Salmonella* Shigella agar were used, respectively with the same technique as used for TPC. For Total coliform detection, Lauryl Sulphate Tryptose (LST)

broth was used. In sterilized test tube, 9 mL of LST broth was added to Durham tubes (10 mm x 75 mm). Durham tubes were then inverted in test tubes. After that, 1 mL of appropriate dilution was added, mixed well and incubated at 37°C for 48 h to detect total Coliforms and at 44°C for detection of Faecal Coliforms. Tubes showing gas production were considered as the presumptive test for Total and Faecal coliforms. Total coliform bacteria were confirmed by using Brilliant Green Bile Broth and faecal coliforms were confirmed by using EC broth using same Durham tube technique of gas production.

**Statistical analysis.** Completely randomized design was used with 10 replicates. Means were compared by using Costat software.

## RESULTS AND DISCUSSION

**Total plate count.** The TPC ranged from  $2.1 \times 10^8$  to  $6.1 \times 10^8$  cfu mL<sup>-1</sup> with an average of  $4.2 \times 10^8$  cfu mL<sup>-1</sup> (Table I). Differences ( $P < 0.05$ ) were recorded in TPC among milk samples collected from different regions. According to the UK Standards (Anonymous, 1996), TPC more than  $10^4$  is considered un-satisfactory. Hence, the raw milk sold in Lahore does not meet the standards and is rather hazardous (Mahari & Gashe, 1990; Godefay & Molla, 2000; Simon & Hansen, 2001).

**Total coliforms and faecal coliform.** All raw milk samples were positive for the presence of coliforms and faecal coliforms. Presence of faecal coliforms confirmed the faecal contamination in raw milk. The possible source of faecal coliforms is sewage contaminated water. Farmers use the contaminated water for washing of their utensils and also for milk adulteration. These findings are similar with those of Reuben *et al.* (2003), Jayarao and Wang (1999) and Hoogkamp- Korstanje (2003).

**Escherichia coli.** *E. coli* count in raw milk ranged from  $1.7 \times 10^5$  to  $4.3 \times 10^5$  cfu mL<sup>-1</sup> (Table I). There was no difference ( $P > 0.05$ ) among the milk samples collected from different regions. Globally, it is recommended that the raw milk should not contain  $> 10^4$  cfu mL<sup>-1</sup> *E. coli* (Anonymous, 1996). The study showed that the raw milk in Lahore deviates greatly from the international standards.

Presence of such a high count of *E. coli* warrants the strict observation of hygiene rule at every step in the raw milk supply chain (Mechie *et al.*, 1997; Jayarao & Henning, 2001; Reuben *et al.*, 2003).

**Staphylococcus aureus.** *S. aureus* counts ranged from  $7.1 \times 10^5$  to  $12.6 \times 10^5$  cfu mL<sup>-1</sup> (Table I). There was no difference ( $P > 0.05$ ) among the milk samples collected from different regions. *S. aureus* count more than  $10^4$  has been reported as potentially hazardous (Anonymous, 1996). The presence of high *S. aureus* count in Gulberg region confirms that there is no relation between socio-economic status of regions and the *S. aureus* count. The findings are similar to those reported by Adesiyum *et al.* (1998).

**Lactobacillus.** The *Lactobacillus* count in raw milk ranged from 2400 to 6800 cfu mL<sup>-1</sup> with an average of 4533 cfu mL<sup>-1</sup> (Table I). There was no difference ( $P > 0.99$ ) among the milk samples. Samples collected from WAPDA town had the highest *Lactobacillus* count (4830 cfu mL<sup>-1</sup>) and those from Defence region had the lowest (4380 cfu mL<sup>-1</sup>). High *Lactobacillus* counts represent that there is lack of raw milk cooling immediately after milking. High *Lactobacillus* count also results in the early milk spoilage as compared to the low *Lactobacillus* count raw milk.

**Salmonella.** *Salmonella* count ranged from 3000 to 8200 cfu mL<sup>-1</sup> in raw milk (Table I). There was no difference ( $P > 0.87$ ) among the milk samples collected from different regions. This implied that milk sellers in all the regions lacked awareness of hygiene. The highest *Salmonella* count was observed in Cantt region (5560 cfu mL<sup>-1</sup>) and the lowest one from Ichra region (4600 cfu mL<sup>-1</sup>). As per UK standards, if *Salmonella* is detected in 25 g of food then it is potentially hazardous. Presence of high *Salmonella* count may lead to the occurrence of possible health hazards by consuming of raw milk (Vasavada, 1993; Warnick *et al.*, 2003).

## CONCLUSION

Raw milk in District Lahore is highly contaminated and do not meet the international raw milk standards. Presence of faecal coliform confirmed the mixing of contaminated water in raw milk. It is recommended that water free of faecal coliform should be used for cleaning

**Table I. Comparison of bacterial count between regions**

Region	Bacterial Count cfu mL <sup>-1</sup>				
	TPC	<i>E. coli</i>	<i>Streptococcus</i>	<i>Lactobacillus</i>	<i>Salmonella</i>
Ghari Shau	$3.38 \times 10^8$	$2.46 \times 10^5$	$9.81 \times 10^5$	$4.46 \times 10^3$	$5.23 \times 10^3$
Cantt	$3.99 \times 10^8$	$2.69 \times 10^5$	$1.19 \times 10^6$	$4.67 \times 10^3$	$5.56 \times 10^3$
Gulberg	$4.01 \times 10^8$	$3.11 \times 10^5$	$1.03 \times 10^6$	$4.41 \times 10^3$	$5.41 \times 10^3$
Defence	$3.92 \times 10^8$	$2.98 \times 10^5$	$1.02 \times 10^5$	$4.38 \times 10^3$	$5.39 \times 10^3$
Model Town	$3.95 \times 10^8$	$2.66 \times 10^5$	$9.72 \times 10^5$	$4.49 \times 10^3$	$5.37 \times 10^3$
Allama Iqbal Town	$4.11 \times 10^8$	$3.14 \times 10^5$	$9.74 \times 10^5$	$4.61 \times 10^3$	$5.19 \times 10^3$
Ichra	$4.73 \times 10^8$	$2.98 \times 10^5$	$9.52 \times 10^5$	$4.49 \times 10^3$	$4.60 \times 10^3$
Mughalpura	$3.64 \times 10^8$	$3.05 \times 10^5$	$9.89 \times 10^5$	$4.53 \times 10^3$	$5.18 \times 10^3$
Shadbagh	$3.29 \times 10^8$	$2.56 \times 10^5$	$1.01 \times 10^6$	$4.46 \times 10^3$	$5.40 \times 10^3$
WAPDA Town	$4.36 \times 10^8$	$2.93 \times 10^5$	$1.00 \times 10^6$	$4.83 \times 10^3$	$5.21 \times 10^3$

Each value is mean of 10

animal surfaces, utensils and equipment. No cleanliness, cooling, covering of bulk tank are some of the serious debacles. The presence of high bacterial count confirmed that there exists no significant relation between socio-economic statuses of region. Programs like 'good hygiene practices' and 'good farm practices' should be adopted at every step in milk handling and processing. Moreover, raw milk should not be used without processing (at least boiling).

## REFERENCES

- Adesiyun, A.A., I.A. Webb and H.T. Romain, 1998. Prevalence and characteristics of *Staphylococcus aureus* stains isolated from bulk and composite milk and cattle handlers. *J. Food Prot.*, 61: 629–32
- Ahmad, M., 1999. *Contaminated Milk a Risk for Consumers*. Daily The News, March 29, Lahore, Pakistan
- Anonymous, 1996. Microbial guidelines for some ready-to-eat foods sampled at point of sale: an expert opinion from the Public Health Laboratory Service (PHLS). *PHLS Microbiol. Digest*, 13: 41–3
- Cousins, C.M. and A.L. Bramley, 1981. The microbiology of raw milk, In: Robinson, R.K. (ed.), *Dairy Microbiology (The Microbiology of Milk)*, Vol. 1. Applied Science Publishers, London
- D'Aoust, Y.L., 1991. Pathogenicity of food borne *Salmonella*. *Int. J. Food Microbiol.*, 12: 17–40
- Godefay, B. and B. Molla, 2000. Bacteriological quality of raw cow milk from four dairy farms and a milk collection center in and around Addis Abada. *Berl. Munch. Tierarztl. Wochenschr.*, 113: 276–8
- Greenwood, M.H. and W.L. Hooper, 1983. Chocolate bars contaminated with *Salmonella napoli*: an infective study. *British Medical J.*, 286: 1394
- Halpin-Dohnalck, M.I. and E.H. Marth, 1989. *Staphylococcus aureus* production of extracellular compounds and behavior in foods. *J. Food Prot.*, 52: 267–82
- Hoogkamp-Korstanje, J.A., 2003. Nutrition and health infections caused by food. *Ned. Tijdschr. Geneesk.*, 147: 590–4
- Jayarao, B.M. and D.R. Henning, 2001. Prevalence of food borne pathogens in bulk-tank milk. *J. Dairy Sci.*, 84: 2157–62
- Jayarao, B.M. and L. Wang, 1999. A study of prevalence of gram-negative bacteria in bulk-tank milk. *J. Dairy Sci.*, 82: 2602–4
- LeChevallier, M.W., N.J. Werch and D.B. Smith, 1996. Full-scale studies of factors related to Coliform regrowth in drinking water. *Appl. Environ. Microbiol.*, 62: 689–93
- Mahari, T. and B.A. Gashe, 1990. A survey of the microflora of raw and pasteurized milk and the sources of contamination in a milk processing plant in Addis Ababa Ethiopian. *J. Dairy Res.*, 57: 233–8
- Mechie, S.C., P.A. Chapman and C.A. Siddons, 1997. A fifteen month study of *Escherichia coli* in dairy herds. *Epidemiol. Infect.*, 118: 17–25
- Mohiuddin, A., 1996. *Milk Packets to Go Up by Rupees 2 Per Liter from July*. The Dawn, 6<sup>th</sup> June, Lahore, Pakistan
- Reuben, A., H. Treminio, M.L. Arias and C. Chaves, 2003. Presence of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. in food from animal origin in Cost Rica. *Arch. Latinoam Nutr.*, 53: 389–92
- Shinoda, S., S. Yamamoto, K. Tomochika and S. Miyoshi, 1979. Enterohemorrhagic *Escherichia coli* infection. *Japanese J. Toxi. Environ. Health*, 43: 1–14
- Simon, M. and A.P. Hansen, 2001. Effect of various dairy-packaging materials on shelf life and flavor of pasteurized milk. *J. Dairy Sci.*, 84: 767–73
- Stark, K.D., 2000. Food safety achieved through herd management. *Schweiz. Arch. Tierheilkd.*, 142: 673–8
- Thomas, S.B., R.G. Druce and M. Jones, 1971. Influence of production conditions on the bacteriological quality of refrigerated farm bulk-tank milk. *J. Appl. Bacteriol.*, 34: 659–77
- Vasavada, P.C., 1993. Rapid methods and automation in dairy microbiology. *J. Dairy Sci.*, 76: 3101–13
- Warnick, I.D., J.B. Kaneene, P.L. Ruegg and C. Fossler, 2003. Evaluation of herd sampling for *Salmonella* isolation on Midwest and Northern US dairy farms. *Prev. Vet. Med.*, 60: 195–206

(Received 21 March 2007; Accepted 25 May 2007)