Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/April-2014/10.pdf

RESEARCH ARTICLE
Open Access

Bacteriological quality of raw cow milk in Shahrekord, Iran

Abdolmajid Fadaei

Department of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran. Tel: +98-381-3330299, Fax: +98-381-3334678, Email: ali2fadae@yahoo.com

Received: 02-02-2014, Revised: 12-03-2014, Accepted: 19-03-2014, Published online: 15-04-2014

doi: 10.14202/vetworld.2014.240-243
How to cite this article: Fadaei A (2014) Bacteriological quality of raw cow milk in Shahrekord, Iran, *Veterinary World* 7(4): 240-243.

Abstract

Aim: The aim of this study was to evaluate the rate of contamination with *Escherichia coli*, coliforms, and *Staphylococcus aureus* in raw milk from Shahrekord city, Iran.

Materials and Methods: In this study, 300 raw milk samples were collected randomly from five regions, namely northeast, east, southeast, south, and southwest regions of Shahrekord city according to stratified random sampling design. Samples were analyzed for Total plate count (TPC), *Staphylococcus aureus*, coliform, and *E. coli*.

Results: Out of 300 samples of raw milk, contamination with coliforms, *E. coli*, and *S. aureus* was observed in 237 (79%), 207 (69%) and 125 (41.66%) samples, respectively. The highest rate of contamination was in the samples from southwest region with coliforms, *E. coli*, and *S. aureus* were present in 30 (100%), 29 (96.66%), and19 (63.33%) samples, respectively ($p<0.05$).

Conclusions: Considering the high rate of raw milk contamination with *S. aureus*, *E. Coli*, and coliforms, sanitary practice during collecting, transporting, and storage especially in the summer season is recommended.

Keywords: bacteriological quality, contamination, raw milk, sanitary measures.

Introduction

Milk and other dairy products from cows, goats, and sheep are important components of the people diet. Milk do have distinct physical, chemical and biological characteristics and its colour, odour, taste, consistency, freezing point (-0.55°C), pH (6.6) and specific gravity (1,032) are characteristics that remain particularly constant [1]. The bacterial contamination of milk not only reduces the nutritional quality but also consumption of such milk threatens health of the society [2]. Milk is also an excellent medium for the growth of different microorganisms, which may cause various food borne diseases.

The disease causing bacteria in the milk are *Salmonella spp., Mycobacterium bovis, Corynebacterium spp., Clostridium perfringens, Yersinia enterocolitica, Coxiella burnetii, Brucella, Staphylococcus, Campylobacter jejuni, Mycobacterium avium, Listeria spp., Escherichia coli,* and coliforms. Many bacteria could get an easy access to milk and milk products such as *E. coli* and coliforms; they are often used as indicator organisms to confirm the bacterial contamination of milk. Most common pathogens that have been involved in milk borne diseases include *Salmonella spp., Staphylococcus aureus,* and *E. coli*. In recent years, there are several studies related to raw milk contamination including: infection with *C. jejuni*, *Listeria monocytogenes*, and *E. coli* strain O157 [3, 4], *Campylobacter spp., Salmonella spp.* and *E. coli* [5], *C. jejuni* [6], *E. coli, coliforms, S. aureus* [4]. The quality and safety of raw milk can be evaluated by assessing hygiene indicator microorganisms. Total coliform, *E. coli* and *S. aureus* are used as hygienic parameters for milk production, as they indicate the conditions of raw milk obtaining and storage, and inadequate handling during the manufacturing process. These microorganisms are usually associated with food borne diseases and outbreaks, as recorded by official health organizations [7].

The presence of these pathogenic bacteria in milk appeared as main public health concerns, especially for those people who still drink raw milk [8]. It therefore became the aim of the study to determine the presence of contaminating micro-organisms in the milk produced by small scale dairy farmers in a typical in Shahrekord, Southwest Iran South area where milking is done by hand.

Materials and Methods

Samples: A total of 300 samples of raw milk (milk collection station) were collected from different places (North-east, East, Southeast, South, and South-west) of Shahrekord city. At each location, samples of approximately 300 ml milk were taken aseptically from the bulk milk container into sterile glass bottles.

Procedure: According to the recommended procedures (pour plate method) colony counting of the bacteria was done. The Eosine Methylene Blue (EMB) and blood agar media were inoculated and kept at 37 °C for 24-48 hours. For identification of coliforms, and *E. coli*, the differential media, such as TSI, urea, and Simmon citrate were inoculated. For the isolation and

Table-1: Contamination of raw cow milk samples by coliform, *E. coli* and *S. aureus*

Seasons	Region	Sample	No. of positive sample (%)		
			Coliform	*E. coli*	*S. aureus*
Summer	Northeast	30	21(70)	19(63.33)	14(46.66)
	East	30	29(96.66)	20(66.66)	11(36.66)
	Southeast	30	22(73.33)	21(70)	5(16.66)
	South	30	28(93.33)	24(80)	7(23.33)
	Southwest	30	30(100)	29(96.66)	19(63.33)
winter	Northeast	30	17(56.66)	11(36.66)	8(26.66)
	East	30	22(73.33)	18(60)	18(60)
	Southeast	30	23(76.66)	21(70)	15(50)
	South	30	19(63.33)	19(63.33)	10(33.33)
	Southwest	30	26(86.66)	25(83.33)	18(60)
Total	-	300	237(79)	207(69)	125(41.66)

identification of *E. coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37ºC for 24 h. Morphologically, typical colonies (at least 4/plate) producing metallic sheen were taken into nutrient broth for further identification. Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, Urease production, Simmon's citrate agar and various sugar fermentation tests. For isolation and colony counting of *S. aureus* the medium blood agar and DNase media were inoculated and incubated at 35°C for 24 hours, the catalase and coagulase test, were performed too. The colonies were counted using colony counter and the number was recorded as colony forming unit/ml (CFU). For testing, 5 dilutions of milk samples, 1, 0.1, 0.01, 0.001 and 0.0001 ml were used. Aseptically, 1 ml of milk was added to the sterile test tube containing 9 ml of sterile distilled water, mixed properly by cyclometer, 15 ml of medium was poured in the plate containing 0.1 ml of sample and shake to mix thoroughly and uniformly with the agar medium. The agar was allowed to be solidified and the petri-dishes were incubated at 37°C for 48 hours. A negative control was prepared using plate count agar only. The plates were placed on a colony counter and the number of bacterial colonies was recorded. The blood agar and DNase test media were inoculated and incubated at 37°C for 24 hours [9, 10]. On the pasteurized samples, the lactose broth with dilution of 1, 0.1, 0.01 was prepared and incubated at 37°C for 24 hours. For identification of *S. aureus*, on each blood agar and EMB media, 0.1 ml of milk sample was inoculated. In case of observing any colony, identification of *S. aureus* was intended.

Statistical analysis: Data were analyzed using descriptive statistics and Chi-square,Mann-Whitney and Kruskal-Wallis.

Results and Discussion

In this research, total of 300 raw milk sample was studied. In the raw milk samples, contamination with coliforms, *E. coli*, and *S. aureus* was observed in 237 (79%), 207 (69%) and 125 (41.66%), respectively (Table-1). Report given by Nihar in India indicated, that of the 144 samples, Staphylococcus was found in 47%, whereas Streptococcus and E. coli in 32% and 21%of the samples respectively [11].Another report revealed that out of 135 samples, 25 samples were found to be contaminated with *Staphylococcus* 14 and *E.coli* 11 [12]. Report on contamination of the raw milk samples in Malayer city of Iran was as follow: *E. coli* 75%, *Enterobacter* 42%, *Klebsiella* 36% and *S. aureus* 52% [13]. The most rate of contamination was in the samples from southwest region with coliforms, *E. coli*, and *S. aureus* 30 (100%),29 (96.66%) and 19(63.33%), respectively ($p<0.05$) (Table-1). The rate of contamination of raw milk prepared during summer with coliforms, *E. coli*, and *S. aureus* with 30 (100%), 29 (96.66%) and19 (63.33%), respectively. Indeed, they were more than those prepared in winter with coliforms, *E. coli*, and *S. aureus* with 26 (86.66%), 25 (83.33%) and 18 (60%), respectively ($p<0.05$).In Nowary and Italay 55%,43% samples of cow milk contaminated with *S. aureus*, respectively [14,15]. In Malaysia, Thaker and colleagues [16] indicated that 90% of the examined raw milk were contaminated by coliform bacteria and 65% were *E. coli.* When the number of isolates in the raw milk are compared in two seasons of the year, it is noticed that numbers of the detected organisms in the summer is higher than the winter season. The reason could be that in the summer the ambient temperature is high and lacking of refrigeration in the situation of long distance milk transportation helps the situation [17, 18].

Table-2 shows the highest rate of isolates in the raw milk orderly as follow: coliforms, *E. coli*, and *S. aureus*. The highest mean value of Total Plate Count was found in milk from the southwest region with $1.91\times107\pm2.8\times10^3$ cfu/ml^{-1}, while the lowest mean value of $1.1\times104\pm2.8\times10^3$ cfu/ml^{-1} was detected in milk obtained from the southeast region. The results for coliforms, *E. coli*, and *S. aureus* contamination in raw milk are shown in Table-2. Counts for coliforms, *E. coli*, and *S. aureus* were an average count of $1.4\times105\pm 2.2\times10^3$, $2.1\times10^3\pm101$, and $1.3\times10^3\pm137$ cfu/ml^{-1}, respectively. Detection of *E. coli* in milk often reflects manure contamination, soil and contaminated water, and poor hygienic practices in dairy environment although environmental coliforms have also been detected in milk. *E. coli* and coliform bacteria are often used as indicator microorganisms, and the presence of *E. coli* a risk that other pathogens may be present in the

Table-2: Mean counts of total plate counts, coliform, *E. coli* and *S. aureus* in raw cow milk sample collected from region of Shahrekord city.

Seasons	Region	Sample	Mean ± SD (cfu/ml)			
			Total plate count	*Coliform*	*E. coli*	*S. aureus*
Summer	North-east	30	$7.5\times10^{4}\pm2.5\times10^{3}$	$7.7\times10^{4}\pm2.1\times10^{3}$	$2.0\times10^{2}\pm44$	$1.5\times10^{2}\pm45$
	East	30	$1.9\times10^{5}\pm2.7\times10^{3}$	$1.7\times10^{4}\pm2.7\times10^{3}$	$2.1\times10^{3}\pm120$	$1.4\times10^{2}\pm78$
	South-east	30	$1.5\times10^{5}\pm2.5\times10^{3}$	$1.5\times10^{4}\pm2.05\times10^{3}$	$2.2\times10^{3}\pm400$	$1.2\times10^{3}\pm111$
	South	30	$2.11\times10^{5}\pm2.9\times10^{3}$	$1.11\times10^{4}\pm2.9\times10^{3}$	$2.0\times10^{3}\pm560$	$1.1\times10^{2}\pm66$
	South-west	30	$1.91\times10^{7}\pm2.8\times10^{3}$	$2.8\times10^{7}\pm3.8\times10^{3}$	$3.8\times10^{3}\pm89$	$2.3\times10^{3}\pm109$
Winter	North-east	30	$6.5\times10^{4}\pm2.1\times10^{3}$	$2.5\times10^{4}\pm2.1\times10^{3}$	$1.9\times10^{3}\pm89$	$1.2\times10^{3}\pm121$
	East	30	$1.7\times10^{4}\pm2.3\times10^{3}$	$1.1\times10^{4}\pm2.3\times10^{3}$	$2.5\times10^{3}\pm359$	$1.3\times10^{3}\pm102$
	South-east	30	$1.1\times10^{4}\pm2.8\times10^{3}$	$1.1\times10^{4}\pm3.8\times10^{3}$	$2.3\times10^{3}\pm149$	$1.1\times10^{3}\pm89$
	South	30	$1.9\times10^{5}\pm2.61\times10^{3}$	$1.9\times10^{4}\pm2.61\times10^{3}$	$2.2\times10^{2}\pm30$	$2.3\times10^{3}\pm113$
	South-west	30	$1.01\times10^{6}\pm2.6\times10^{3}$	$1.5\times10^{6}\pm3.6\times10^{3}$	$2.7\times10^{3}\pm167$	$1.7\times10^{3}\pm220$
Total	-	300	$1.03\times10^{6}\pm2.9\times10^{3}$	$1.4\times10^{5}\pm2.2\times10^{3}$	$2.1\times10^{3}\pm101$	$1.3\times10^{3}\pm137$

Table-3: Microbial load in milk samples collected from five region of Shahrekord city, Iran

Milk ranking	Cfu/ml	%
Excellent	<30000	11
First-grade	30000-100000	31.6
Second- grade	100000-500000	33.9
Third-grade	500000-1000000	15
Nonstandard	>1000000	8.5

samples. Presence of *S. aureus* in milk may originate from mastitic animals [19], or human sources. The total plate count, coliforms, *E. coli* and *S. aureus* counts for raw milk significantly differed ($P<0.05$) amongst the study regions. In similar studies, report on contamination of the raw milk samples in Zimbabwe was similar to this study [20]. Report of the raw milk samples in Greece show that From the 240 milk samples tested, only 5% were E. coli positive, with mean counts ranged from2.4×10^{4} cfu/ml. *S. aureus* was isolated from 24% of the samples [21]. In Italy, Foschino *et al* isolated [15] *E. coli* (2.9×10^{3} cfu/ml), coliforms (9.1×10^{3} cfu/ml) and *E. coli* in 1.7% of the samples. Hill *et al* reported that in New Zealand, [22] *E. coli*, 99% of samples tested had counts $<10^{2}$ cfu/ml and only 0.7% were $>10^{3}$ cfu/ml and *S. aureus*, the results show that 60% of the raw milk samples contained $<10^{2}$ cfu/ml and 30% contained between 10^{2} and 10^{3} cfu/ml. Overall, contamination of very high number of milk samples could be due to insanitary farms, failing to wash the udders before milking, no mastitis investigates, unhealthy milking vessels and milk containers or tanks, lengthy delivery time, lack of education, and poor staff's hygiene.

By referring to the grading of raw milk contamination of national standard of Iran which is given in the Table-3 and considering the microbial load, the quality of raw milk was determined as follow: 11% had very good quality, 65.5% with first and second grades, that is, with good quality and the rest were of poor quality. Report on contamination of the raw milk samples in Markazi province of Iran was 100% poor quality [23, 24].

Conclusion

Results from this study showed that raw milk sampled from five different regions in Shahrekord township contained identified pathogens. The prevalence and concentration of the pathogens included in the study were relatively high. Recognition rates for *E. coli*, coliforms, and *S. aureus* were generally higher than those found from in other countries. Recommendations for solving this problem includes i) Educating the farmers on general hygienic practices, quickening the delivery of milk to collection centres, or availing cooling facilities on-farm to improve the microbiological quality of milk, ii) Improve animal health, iii) Improved milking hygiene, iv) pasteurization or boiling of milk prior to consumption. Overall, on farm production of milk must ensure that the milk is produced by healthy animals under generally accepted conditions such as: animal's health, milking health, animals feeding and water, animal's welfare, and environment.

Acknowledgements

This research was financially supported by Shahrekord University of Medical Sciences (project number 560), Iran.

Competing interests

The author declare that they have no competing interests.

References

1. Lues, J. F. R., De Beer, H., Jacoby, A., Jansen, K. E. and Shale, K. (2010) Microbial quality of milk, produced by small scale farmers in a peri-urban area in South Africa, *Afr. J. Microbiol. Res*. 4(17):1823-1830.
2. Nanu, E., Latha, C., Sunil, B., Prejit, Thomas M. and Venon, K.V. (2007) Quality assurance and public health safety of raw milk at the production point, *Am. J. Food Technol*. 2: 145-152.
3. LeJeune, J. T. and Rajala-Schultz, P. J. (2009) Food safety: unpasteurized milk: a continued public health threat, *Clin. Infect. Dis* 48:93-100.

4. Vahedi, M., Nasrolahei, M., Sharif, M. and Mirabi, A.M. (2013) Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011, *J Prev Med Hyg* . 54: 120-123.
5. Griffiths, M. W. (2010) Milk production and processing in improving the safety and quality of milk. Guelph: Wood head Publishing Limited. 1 : 520.
6. Heuvelink, A.E., van Heerwaarden, C., Zwartkruis-Nahuis, A., Tilburg, J.J., Bos, M.H.and Heilmann, F.G., Hofhuis, A., Hoekstra, T. and de Boer, E. (2009) Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk, *Int J Food Microbiol*. 134(1-2): 70–74.
7. Bouazza, F., Hassikou, R., Ohmani, F., Hmmamouchi, J., Ennadir, J., Qasmaoui, A., Mennane, Z., Reda Charof, R. and Khedid, K. (2012) Hygienic quality of raw milk at Sardi breed of sheep in Morocco, *Afr. J Microbiol. Res*. 6(11): 2768-2772.
8. Claeys, W.L., Cardoen, S., Daube, G., Block, J.D., Dewettinck, K., Katelijne Dierick, K., Zutter, L.D. , Huyghe baert, A., Imberechts, H., Thiange, P., Vandenplas, Y. and Lieve Herman,L. (2013) Herman Raw or heated cow milk consumption: Review of risks and benefits, Food Control, 31: 251-262.
9. National Standard of Iran (2002) The milk and milk products. Method of colony counting of bacteria grown at 30°C.First edition. Institute of standards and industrial research of Iran (ISIRI), Tehran, Iran., p3-20.
10 National Standard of Iran (2006) The food and fodder microbiology. The method for counting of coagulase positive *staphylococcus* (*S. aureus* and the other *Staph. spp*.) The 3rd section (the most probable number MPN). p24-30.
11. Mohanty, N.N., Das, P., Pany, S.S., Sarangi, L.N., Ranabijuli, S., and Panda, H.K. (2013) Isolation and antibiogram of *Staphylococcus, Streptococcus and E. coli* isolates from clinical and subclinicalcases of bovine mastitis, *Vet. World* 6(10): 739-743.
12. Kumar, R. and Prasad, A. (2010) Detection of *E.coli* and *Staphylococcus* in milk and milk Products in and around Pantnagar, *Vet. World*.3 (11):495-496.
13. Pourhassan, M., and Taravat-Najafabadi, A.R.T. (2011) The spatial distribution of bacterial pathogens in raw milk consumption on Malayer City, *Iran. Shiraz E Med J*.12:2-10.
14. Jorgensen, H.J., Mork, T., Hogasen, H.R.,and Rorvik, L.M. (2005) Enterotoxigenic Staphylococcus aureus in bulk milk in Norway, *J Appl Microbiol*. 99:158-166.
15. Foschino, R., Invernizzi, A., Barucco, R.,and Stradiotto, K. (2007) Microbial composition, including the incidence of pathogens, of goat milk from the bergamo region of Italy during a lactation year, *J Dairy Res*.69 (2):213-225.
16. Thaker, H.C., Brahmbhatt, M.N.,and Nayak, J.B. (2012) Study on occurrence and antibiogram pattern of Escherichia coli fromrawmilk samples in Anand, Gujarat, India, *Vet. World*. 5(9): 556-559.
17. Przysucha, T., Grodzki, H., and Zdziarski, K. (2003)The influence of delivery system monthly milk supply and season on TBC in raw milk qualified to the highest quality classes. *J Polish Agr Univ*; 68:115-22.
18. Bradley, A. J. (2002) Bovine mastitis an evolving disease, London. *Vet. J*.164: 116-128.
19. Jeykumar, M., Vinodkumar, G., Bashir, B.P. and Krovvidi,S. (2013) Antibiogram of mastitis pathogens in the milk of crossbred cows in Namakkal district, Tamil Nadu. *Vet. World* . 6(6): 354-356.
20. Mhone, T.A., Matope, G. and Saidi, P.T. (2011) Aerobic bacterial, coliform, Escherichia coli and Staphylococcus aureus counts of raw and processed milk from selected smallholder dairy farms of Zimbabwe. *Int J Food Microbiol* 151: 223–228.
21. Fotou, K., Tzora, A., Voidarou, C.h., Alexopoulos, A., Plessas, S., Avgeris, I., Bezirtzoglou, E., Akrida-Demertz, K. and Demertzis, P.G. (2011) Isolation of microbial pathogens of subclinical mastitis from raw sheep's milk of Epirus (Greece) and their role in its hygiene. *Anaerobe*. 17: 315-319.
22. Hill, B., Smythe, B., Lindsay, D. and Shepherd, J. (2012) Microbiology of raw milk in New Zealand. *Int J Food Microbiol*. 157: 305–308.
23. Rezaei, M., Karimi, F., Yahyaei, M., Javdani, H., Shahabi, A. and Farahi, A. (2013) The survey of microbial total count and prevalence of Escherichia coli in raw milk in Markazi Province, Iran. *Res. Opin. Anim. Vet. Sci*. 3 (12):474-477.
24. ISIRI (2008) Microbiology of milk and its products. Institute of Standards and Industrial Research of Iran, p2406.

****