Bacteriological study of raw and unexpired pasteurized cow’s milk collected at the dairy farms and super markets in Sari city in 2011

M. VAHEDI1, M. NASROLAHEI1, M. SHARIF2, A.M. MIRABI3
1 Department of Microbiology, 2 Department of Parasitology and Mycology, 3 Department of Immunology, Sari Medical School, Mazandaran University of Medical Sciences, Sari, Iran

Key words
Raw milk • Pasteurized milk • Milk contamination

Summary

Introduction
Milk is an excellent high quality food providing major nutritional requirement to man at any age [1] and extremely susceptible to spoilage by microbes [2]. Unhygienic production of milk and milk products and improper storage, cause the early spoilage with microorganisms [3]. Bacteria present in the milk cause unpleasant effect on the taste and physical properties and disease. The bacterial contamination of milk not only reduces the nutritional quality but also consumption of such milk threatens health of the society [4].

Total number of organism in milk as disease causative agent in relation to its proper evaluation for consumption is important. The notable disease causing bacteria in milk are Salmonella, Brucella, Staphylococcus (S.), Listeria (L.), E. coli and coliforms. Coliforms and E. coli are normal inhabitants of the large intestine and their presence in milk could indicate fecal contamination.

Presence of organisms in the pasteurized milk is indicative of unhygienic for consumption. It has been shown that contamination of milk to E. coli in the milk distributing centers is increasing, which is indicative of the unhygienic conditions in preparing, distribution and transportation [5].

Results
In the raw milk, contamination with E. coli, coliforms and Staphylococcus aureus was observed in 42 (42%), 36 (36%) and 22 (22%) of samples respectively, and the same for the pasteurized milk samples was 9 (9%), 2 (2%) and 2 (2%), respectively. Listeria monocytogenes was not detected in any sample.

Presence of E. coli in the milk could be due to contamination with waste water and fecal materials.

Conclusions
Considering the contamination of raw and pasteurized milk with E. coli and coliforms, sanitary practice during collecting and transporting, particularly in the summer season is recommended.

Materials and methods
In this descriptive study, 100 samples, each in 5 ml were collected during the spring, summer, fall and winter seasons from 4 dairy farms of Sari Township (only 4 dairy farms permitted). Also 100 pasteurized milk samples were collected from the retail dealers (those dealers who had milk at the time of referring). The samples were preserved in ice container and transferred to the Sari Medical Collage laboratory.

In all, 200 pasteurized and raw milk samples were studied and cultured based on the standard methods for the iden-
Bacteriological study of raw and unexpired pasteurized cow’s milk

The frequency distribution of the organisms in 100 raw and 100 pasteurized milk samples in Sari Township in 2011.

| Bacteria             | Raw milk number (%) | Pasteurized milk number (%) |
|----------------------|---------------------|-----------------------------|
| Escherichia coli     | 42 (42)             | 9 (9)                       |
| Coliforms            | 36 (36)             | 2 (2)                       |
| Staphylococcus aureus| 22 (22)             | 2 (2)                       |

Results

In this investigation, total of 200 samples (100 pasteurized and 100 raw milk) were studied. In the raw milk samples, contamination with E. coli, coliforms and S. aureus was observed in 42 (42%), 36 (36%) and 22 (22%), respectively. In the pasteurized samples, contamination with E. coli, coliforms and S. aureus was observed in 9 (9%), 2 (2%), and 2 (2%) samples, respectively.

By referring to the grading of raw milk contamination of national standard of Iran which is given in the Table I and considering the total bacterial count, the quality of raw milk was determined as follow: 30% had very good quality, 50% with first and second grades, that is, with good quality and the rest were of poor quality.

Table II shows the highest rate of isolates in the raw and pasteurized milk orderly as follow: E. coli, coliforms and S. aureus. From the statistical point of view, insignificant relationship was observed between the seasons of the year and the isolated organisms (X² = 1.30, df = 6 and P = 0.97). Listeria could not be isolated in any of the samples. The highest number of isolates were observed in the raw cow’s milk. Isolated organisms from the pasteurized milk samples collected from the dairy companies indicate the higher rate of E. coli (Tab. III).

Table IV indicates the frequency of the isolates from the raw cows milk samples collected from the dairy farms under study, here also presence of E. coli and coliforms is significant, which is due to unhygienic production of milk.

The frequency distribution of the isolates from the pasteurized milk samples based on the seasons of the year is depicted in the Table V.

Discussion

In the present study, 200 (100 pasteurized and 100 raw) milk samples were collected and in the pasteurized milk, E. coli, coliforms and S. aureus were observed in 9%, 2% and 2%, respectively and in the raw milk 42%, 36% and 22%, respectively. L. monocytogenes was not isolated in any of the samples under study. Data on 739
pasteurized milk samples in Iran showed contamination of 8.68% with higher than the standard level, in a way that, in 15 samples, coliforms count was 20 to 40 per ml of sample [7].

A study in Iran, comparing the concurrent contamination of E. coli and S. aureus revealed that in milking 19.7%, transporting 49% and in milk selling centers 58.4% of samples were contaminated with 2 organisms [8]. Report on contamination of the raw milk samples in Maayer city of Iran was as follow: E. coli 75%, Enterobacter 42%, Klebsiella 36% and S. aureus 52% [9]. Also higher count of E. coli followed by S. aureus was reported too, which refers to the improper public health measures, and poor cleaning, in addition to the primitive system of transportation. Report given by Asmahan in Khortoum indicated, 63% of raw cow’s milk samples contaminated with E. coli [10].

Fulya revealed that 10% of the raw milk samples under study were contaminated with E. coli [11]. Crump et al., studied on 216 raw milk samples and found that 28 (13%) of them were contaminated with E. coli [12]. The reason could be due to the animals and their living environment. Another study showed that 1.46% of the milk samples in the milk storage tankers were contaminated with E. coli [13]. The reason was expressed as contamination of the milk with animal feces.

Zelalem and Bernard in Ethiopia found higher Coliform count in raw milk samples under study which could be due to the initial contamination of the milk samples from the cow’s milk, the milkers, milk containers and the milking environment [14]. Chye et al., stated that the lower counts of bacteria may be due to good cleaning system and good handling from farms to the plant [15]. The higher percentage of E. coli could be due to the fact that E. coli may grow in raw milk and reaches higher number in tropical countries or in the absence of cooling system. The isolation of Coliform and other food pathogens from dairy products poses a serious threat to food safety [16].

High contamination of raw milk to Escherichia and Coliform, particularly in the summer was reported [17]. Presence of S. aureus in milk may originate from mastitic animals [18], or human sources, which is in agreement with our findings. S. aureus in milk and milk products is an indicator of the spoilage [19].

Ekici et al., in their study detected S. aureus in 75% of the raw cow’s milk but no E. coli was isolated [20]. Findings showed that 38% of raw milk and 11% of pasteurized milk contaminated with S. aureus [21].

A study on 366 raw milk samples, revealed 25.3% contaminated with to L. monocytogenes and 9.2% to Salmonella typhi [22].

When the frequency distribution of the detected organisms in the raw and pasteurized milk samples are compared, it is noticed that the number of the isolates are higher in the raw cow’s milk. It is because, during pasteurization process some organisms are killed, as is indicated from our findings which are given in the Table III and IV. When the number of isolates in the raw milk are compared in the different seasons of the year, it is noticed that number of the detected organisms in the summer is higher than the winter season (Tab. V). 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. It agrees with the report documenting difficulty in obtaining high quality milk during summer season [23].
Bacteriological study of raw and unexpired pasteurized cow’s milk

Considering the results of this study and the similar investigations, it could be concluded that the presence of coliforms bacteria may not necessarily indicate a direct fecal contamination of milk, but it is a precise indicator of poor sanitary practices during milking and further handling process. In this condition, awareness about the source of contamination is very important. Considering the rate of raw milk contamination to E. coli and coliforms, in the Sari Township, practice of hygienic condition and also supervision to the milk processing, transportation and preservation, particularly during the summer season is recommended.

ACKNOWLEDGEMENTS

This study was supported by the research deputy of Mazandaran University of Medical Sciences, and conducted at the laboratory of Sari Medical College. Thanks to the laboratory staff who helped us while performing this study.

References

[1] Theresa A, Nicklas, Dr PH, LN. Calcium intake trends and Health Consequences from childhood through Adulthood. J Am Coll Nutr 2003;22: 340-56.
[2] Soomro AH, Arian MA, Khasheli M, et al. Isolation of Enterichia coli from raw milk and milk products in relation to public health sold under market conditions at Tandojam, Pakistan. J Nutr 2002;1:151-2.
[3] Nanu E, Latha C, Sunil B, et al. Quality Assurance and public health safety of raw milk at the production point. Am J Food Tech 2007; 2: 145-52.
[4] Karmen GT, Slavica GT. The microbiological quality of raw milk after introducing the two day’s milk collecting system. Acta Agr Slovenica 2008;92:61-74.
[5] National Standard of Iran, 2002. The milk and milk products. Method of colony counting of bacterial grown at 30°C.
[6] National Standard of Iran, 2002. The milk and milk products. The method for counting of coagulase positive staphylococcus (Staph. aureus and the other Staph. spp.) 2006. The 3rd section (the most probable number MPN).
[7] Karimi G. Milk and milk products. Sepehr Pub, Tehran 2006;30-50. (Persian language).
[8] Sadeghi-Fard N, Azizi-Jalilzadeh F, Seyed-Khania-Nahal A. Evaluation of contamination of raw milk for E.coli and Staphylococcus aureus in Eilam University of Medical Sciences. J Eilam Univ Med Sci 2006;14:44-9.
[9] Pourhassan M, Taravat-Najafabadi ART. The spatial distribution of bacterial pathogens in raw milk consumption on Malay- City, Iran. Shiraz E Med J 2011;12:2-10.
[10] Asmahan AA, and Warda SA. The incidence of escherichia coli in raw cow’s milk in Khartoum State. Br J Dairy Sci 2011;2:23-6.
[11] Fulya T. Micrological and chemical properties of raw milk consumed in Barduo. J Anim Vet Adv 2011;10: 635-41.
[12] Crump JA, Sulka AC, Langer AJ, et al. An outbreak of E.coli O157:H7 infections among visitors to a dairy farm. N Engl J Med 2002;347:555-60.
[13] Murinda SE, Nguyen LT, Ivey SJ, et al. Prevalence and molecular characterization of E.coli O157:H7 in bulk tank milk and fecal samples from cul cows: a 12 month survey of dairy farms in east Tennessee. J Food Prod 2002;65:752-9.
[14] Zelalem Y, Bernard F. Handling and microbial load of cow’s milk and irgo-fermented milk collected from different shops and producers in central highlands of Ethiopia. Eth J Anim Prod 2006:6:67-82.
[15] Chye FY, Abdullah A, Ayob MK. Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol 2004;21:535-41.
[16] Uzeh RE, Ohenhen RE, Rojugbokan AK. Microbiological and nutritional qualities of dairy products. Nono and Wara. Nature and Science 2006;4:37-40.
[17] Fadaee A, Jamshidi, E, Kheyri S. Estimation of low bacterial concentration: Listeria monocytogenes in raw milk. J Shahrekord Univ Med Sci (Iran) 2008;10:37-44.
[18] Adesiyun AA, Stoute S, David B. Pre-processed bovine milk quality in Trinidad: Prevalence and characteristics of bacterial pathogens and occurrence of antimicrobial residues in milk from collection centers. Food Control 2007;18: 312-20.
[19] Chambers H. Methicillin resistance in Staphylococcus: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 1997;10:781-91.
[20] Ekici K, Bozkurt H, Isleyici O. Isolation of some pathogens from Raw milk of different milk animals. Pak J Nutr 2004;3:161-2.
[21] Rall VL, Vieira FP, Rall V, et al. PCR detection of staphyloccocal enterotoxin genes in Staphylococcus aureus strains isolated from raw and pasteurized milk. Vet Microbiol 2008;132:408-13.
[22] Nero LA, Mattos MR, Barros Mde AM, et al. Listeria monocytogenes and salmonella spp in raw milk produced in Brazil: occurrence and interference of indigenous microbiota in their isolation and development. Zoonoses Public Health 2008;55:299-305.
[23] Przysucha T, Grodzki H, Zdziarski K. The influence of delivery system monthly milk supply and season on TBC in raw milk qualified to the highest quality classes. E J Polish Agr Univ 2003;68:115-22.

Received on January 13, 2013. Accepted on February 27, 2013.

Correspondence: Mohtaram Nasrolahei, Department of Microbiology, Sari Medical School, Mazandaran University of Medical Sciences, 18th km of Khazar Abad Road, Sari, Iran - E-mail: mnasrolahei@yahoo.ca