

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

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Key words

Raw milk • Pasteurized milk • Milk contamination

Summary

Introduction. The quality of milk is influenced by different bacteria present in milk. This study was undertaken to investigate the bacterial contamination of raw and pasteurized milk in Sari Township, Iran, 2011.

Methods. In this investigation, 100 pasteurized milk samples were collected randomly from the super markets in the city and 100 raw milk samples from 4 dairy farms from suburb areas and evaluated for the presence of coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* by culture methods and biochemical tests. Data analysis was performed by SPSS software using χ^2 test and described in percentage.

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.

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].

Considering the geological location of Mazandaran province and being a major milk and milk products center of Iran, and knowing that during processing, milk gets contamination with different types of microorganisms from a wide variety of sources, such as, infected cow's udder, feces, milk containers, dust in barns, workers' hand and lack of the workers' knowledge towards hygienic practice in keeping the quality of milk constant during storage and transportation. This study was aimed to evaluate the rate of contamination to different microorganisms of *E. coli*, coliforms, *S. aureus* and *L. monocytogenes* in the pasteurized and raw milk in Sari Township.

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 College laboratory.

In all, 200 pasteurized and raw milk samples were studied and cultured based on the standard methods for the iden-

Tab. I. Grade of raw milk contamination by referring to the National Standard of Iran, code number 2406.

Colony Forming Unit	$< 3 \times 10^4$	$3 \times 10^4 - 1 \times 10^5$	$1 \times 10^5 - 5 \times 10^5$	$5 \times 10^5 - 1 \times 10^6$	$> 1 \times 10^6$
Quality	Excellent	First grade	Second grade	Third grade	The unstandard raw milk
Number (%)	30 (30%)	33 (33%)	17 (17%)	12 (12%)	8%

tification of bacteria in the dairy products [6]. According to the recommended procedures (pour plate method) colony counting of the aerobic and meso-aerophilic 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 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. For isolation of *L. monocytogenes* the samples were kept at 4°C for 7 days and identification was done according to the routine procedure. 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 shaken 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 [5, 6].

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 (National Standard of Iran 2002). 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. Also colony characteristics, catalase test and staining of the colony as well as manitol test were performed, the conformatory tests such as aerobic, anaerobic fermentation of glucose, sensitivity to lysostaphin and coagulase test were performed, too.

The obtained data were analyzed by SPSS software, using X^2 test, and recorded in percentage, mean and distribution rate.

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 ($X^2 = 1.30$, $df = 6$ 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

Tab. II. 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 (%)
<i>Escherichia coli</i>	42 (42)	9 (9)
<i>Coliforms</i>	36 (36)	2 (2)
<i>Staphylococcus aureus</i>	22 (22)	2 (2)

Tab. III. The frequency distribution of the isolated organisms from 100 pasteurized milk samples, distributed from supermarkets of the Sari Township.

Dairy company	Samples number	Escherichia coli		Coliforms		Staphylococcus aureus		Total	
		No.	%	No.	%	No.	%	No.	%
Khaz shir	25	2	8	1	4	0	0	3	12
Maz shir	25	2	8	1	4	1	4	4	16
Kal shir	25	2	8	0	0	0	0	2	8
Pak shir	25	3	12	0	0	1	4	4	16

Tab. IV. Frequency distribution organisms from 100 raw cow's milk samples collected at 4 Township dairy farms.

Dairy farm	Sample number	Escherichia coli		Coliforms		Staphylococcus aureus	
		No.	%	No.	%	No.	%
1- Dimtron	20	6	30	6	30	8	40
2- Dinek	23	18	78.3	5	21.7	0	0
3- Magham	17	6	35.3	10	58.8	1	5.8
4- Daryek	40	12	30	15	37.5	13	32.5

Tab. V. Rate of contamination of the raw milk in different seasons of the year in 2011

Organisms	Spring number (%)	Summer number (%)	Fall number (%)	Winter number (%)	Total %
<i>Escherichia coli</i>	11 (26.2)	24 (57.1)	4 (9.5)	3 (7.2)	42
<i>Coliforms</i>	9 (25)	19 (52.8)	4 (11.1)	4 (11.1)	36
<i>Staphylococcus aureus</i>	6 (27.4)	10 (45.4)	3 (13.6)	3 (13.6)	22

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 Malayer 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 measurements, 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 high-

er 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 *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].

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.

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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 Escherichia 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 bacteri grown at 30°C*.
- [6] National Standard of Iran, the food and fodder microbiology. *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, Iran, Tehran 2006;30-50. (Persian language).
- [8] Sadeghi-Fard N, Azizi-Jalillean F, Seyed-Khani-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 Ma-layer 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 Burduo*. 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 cull 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, Rojumbokan 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 milch animals*. Pak J Nutr 2004;3:161-2.
- [21] Rall VL, Vieira FP, Rall V, et al. *PCR detection of staphylococcal 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.

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