JRH
www.jrh.gmu.ac.ir

Journal of Research & Health
Social Development & Health Promotion Research Center
Vol. 4, No.4, Winter 2014
Pages: 884-889
Original Article

Antibiotic residues in raw and pasteurized milk, Iran

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Received: 18 Aug 2013
Accepted: 30 Oct 2013

How to cite this article: Mahmoudi R, Amini K, Vahabzade M, Mir H, Vagef R. Antibiotic residues in raw and pasteurized milk, Iran. *J Research Health*2014; 4(4): 884-889.

Abstract

Consumption of food products of animal origin with residual antibiotic has become a major concern of public health and dairy industry. In the study, antibiotic residue in raw and pasteurized milk was investigated. Of the 9 semi-industrial farm units, random on 3 occasions sampling was carried out from 4 milk collection stations and 3 pasteurized milk producing companies, and antibiotic residue was analyzed using Copan Milk Test method. The results showed that of the 96 samples collected, 28 (29.16%) were positive in terms of presence of antibiotics residue. Analysis of milk samples taken in spring and in summer showed a significant difference in antibiotic residue content, such that samples taken in summer contained higher percentage of antibiotic residue (37.5%) compared to samples taken in spring (20.83%). Contamination of raw milk samples (30.76%) with antibiotic residue was significantly higher than that in pasteurized milk samples (22.2%). Given the adverse effects of consumption of contaminated milk with antibiotic residues and huge losses to dairy industry, it seems more comprehensive studies are needed to ascertain precise types of antibiotic residues in milk consumed in this field.

Keywords: Anti-Bacterial, Residue, Milk, Copan Milk

Introduction

Milk is one of the best and most balanced food compositions that provide a variety of proteins, fats, minerals, calcium, phosphorus, potassium, and a variety of vitamins (A, B2, B12, and D), lactose, and all necessary amino acids for maintenance, growth and development of body tissues. It is therefore an essential part of daily diet of all age groups, particularly children and the elderly [1]. Food for human consumption should be free of all types of compounds of adverse quality to human health. These harmful compounds are not restricted to microorganisms and biological factors and toxins excreted from them, but all types of chemicals that directly or indirectly contaminate food stuff are also highly important. Unlike microbial infections and poisoning, complications of chemical poisonings often take effect in the long term. Through extensive use of chemical compositions such as medicinal compositions in farm animals, and subsequent production of residue and its accumulation in food matter with animal origins, the chances of people's constant exposure to life-long complications are fully obvious and clear [2]. Since livestock, as the only source of milk production are exposed to risk of infectious diseases, especially mastitis, antibiotics are commonly used in them to control and treat diseases, and

also to stimulate growth. Indiscriminate use of antibiotics and failure to observe required time for expulsion of drug in farms, lead to antibiotics finding their way into their products such as milk and meat. Entry of antibiotics to the food chain causes allergic reactions in humans and a variety of bacterial resistances as well as impaired natural microflora in gastrointestinal system, carcinogenesis, mutagenesis, and deformed births. Moreover, the presence of antibiotic residue in milk used in dairy industry can adversely affect production of fermented dairy products such as yoghurt and cheese [3,4,5].

For many years, food health research has solely focused on food microbiology and microbial contamination, and most standards were developed in connection with these factors. In the recent two decades, scientists and researchers attention has been drawn toward chemical causes of diseases and lesions and chemical poisonings, and standards have been developed and implemented for various substances that more or less contaminated food (especially food of animal origin) and caused health risks to consumers. In most European countries, to further protect consumers, regular measures have been taken to restrict use of antibiotics in food products of animal origin, especially in milk. For instance, permitted antibiotic residues have been reported for gentamicin (200µg/L), enrofloxacin (100µg/L), tetracycline (100µg/L), sulfonamides (25µg/L), and tylosin (100µg/L) [6]. Examination of raw milk samples from sheep and cattle in Spain and Italy showed negligible contamination in sheep milk samples (1.7%) and beta-lactam antibiotic contamination in cattle milk samples, respectively [7,8]. Unfortunately, less attention is paid to the above issues in food quality control in Iran. Accordingly, some recent studies can be cited: Movasaq et al. (2010), evaluated antibiotic residue in samples of raw cattle milk in Ilkhchi area of Tabriz city using Copan Milk Test, and showed that over 10% of samples contained antibiotic residue [3]. Also, examination of raw and pasteurized milk samples in Tabriz showed significant antibiotic residue contamination, especially in raw milk [9]. Given the harmful effects of consumption of food of animal origin with antibiotic residue, quality control of food products in terms of antibiotic residue is highly necessary. Amid, the high per capita consumption of milk as a major nutrient, and its special place in human diet, especially in children's diet, makes milk more important. Thus, this study aimed to investigate antibiotic residue in pasteurized and raw milk (collected from semi-industrialized farms and milk-collection centers) in Ilam province in spring and summer of 2012.

Method

This was a descriptive cross-sectional study, conducted in 2012 to determine antibiotic residue in raw (78 samples) and pasteurized milk (18 samples), as study population. Random cluster sampling method [9] was used, and sample size was calculated according to the formula:

In the present study, proportion of P (presence of antibiotic residue in milk samples) was estimated at around 21% in accordance with many existing reports from various milk samples across Iran, showing value of P between 15% and 26%, and with a 6% error, sample size was determined 96. However, using the lower value of 15% and 5% error, sample size was still be 96.

To determine the presence or absence of antibiotic residue in milk, Copan Milk Test method was used (quality kit from Hansen Company, Denmark). Milk is added to the kit, which is then incubated at 64 °C for 3 hours. In the absence of antibiotic, indicator microorganism in milk samples (Bacillus Stearothermophilus, a variety of Caledo Lactis) feeding from nutrients, grows and through fermentation of lactose and production of acid turns the culture medium yellow in the presence of bromocresol purple. In the presence of antibiotic residue in milk sample, growth of Bacillus Stearothermophilus is inhibited, and thus no color change occurs in the culture medium, and it remains purple (Figure 1).

In this study, variables included sampling seasons (warm and cold), and sampling sites (semi-industrial farms, milk collection centers, and pasteurized milk producing companies), and affected by these variables, the presence or absence of antibiotic residue in milk samples

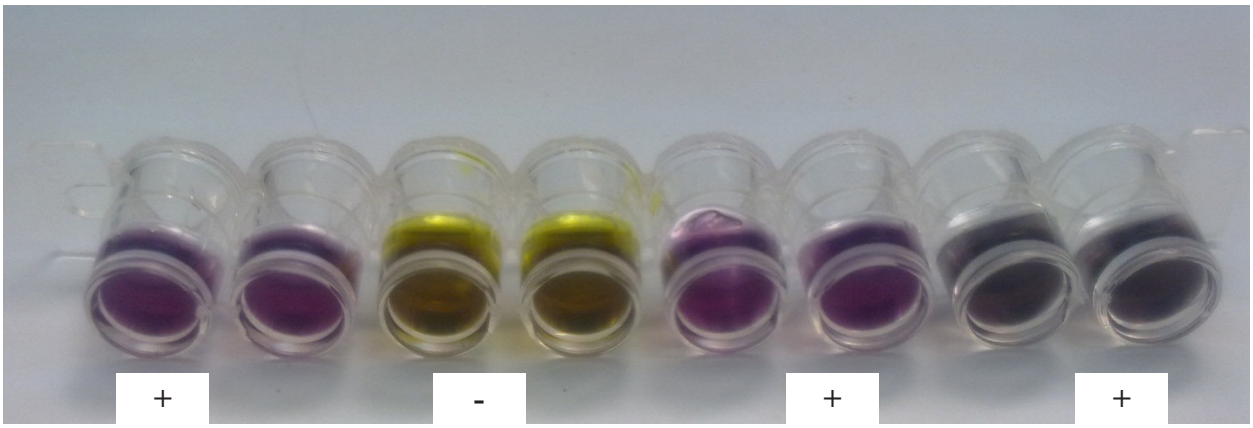

Figure 1 *Evaluation of presence or absence of antibiotic residues in milk using Copan Milk Test [+ve: presence of residues in milk (no color change), -ve: absence of residues in milk (color change from purple to yellow)].*

was investigated. Statistical analysis was carried out using chi-square test with SPSS software. Significant level was considered $P<0.05$.

Results

The results obtained from evaluation of presence of antibiotic residue in milk from semi-industrial cattle farms, milk collection centers, and pasteurized milk samples are presented in Table 1. According to the results, 28 (29.16%) out of 96 samples were positive in terms of antibiotic residue. Moreover, in terms of antibiotic residue contamination, a significant difference was found between raw and pasteurized milks ($P<0.05$), such that the highest antibiotic contamination (30.76%) was found in raw milk samples.

Table 1 *presence or absence of antibiotic residue in difference milk sample during spring and summer seasons*

Type of milk samples	Sample size(n)	Positive samples (n)	Percentage Positive samples (%)
Semi indutrial farms	54	17	31.51
Milk collection Centers	24	7	29.15
Pasteurized milk	18	4	22.20
Total	96	28	29.16

Also, in terms of sampling seasons, a significant difference was found between summer and spring samples in the amount of antibiotic residue, and results of evaluation of antibiotic residue, using Copan Milk Test, showed significantly higher contamination in summer samples compared to that in spring ($P<0.05$), such that, of the 48 different samples taken in spring, 10 (20.83%), and of the 48 different samples taken in summer, 18 (37.5%) were positive for antibiotic residue (Figure 1).

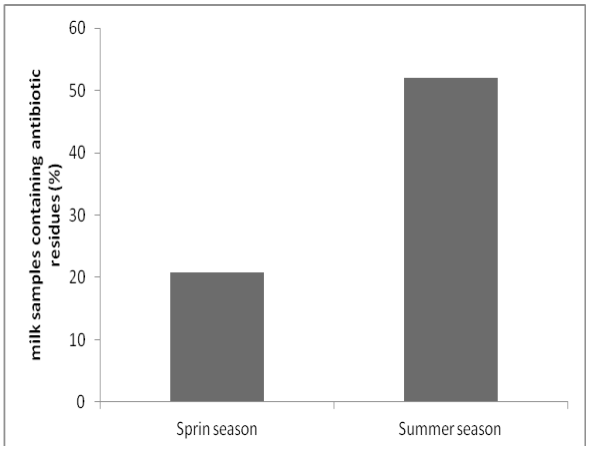

Figure 2 *Comparison of different samples of milk contaminated with antibiotic residues during spring and summer*

On the other hand, according to data in table 2, there is a significant difference between amount of antibiotic residue found in milk samples taken from different sources in warm and cold seasons, such that samples of raw milk taken from milk collection centers

Table 2 *Antibiotic residue contamination in difference milk samples during spring and summer seasons*

Type of milk samples	Spring season			Sumer season		
	Sample size(n)	Positive samples (n)	Positive samples (%)	Sample size(n)	Positive samples (n)	Positive samples (%)
Semi indutrial farms	27	5	18.50	27	12	44.50
Milk collection Centers	12	3	25.00	12	4	33.35
Pasteurized milk	9	2	20.20	9	2	22.20

(25%) showed the highest contamination of antibiotic residue in spring, compared to raw milk samples from semi-industrialized farms (18.5%) and pasteurized milk samples (20.2%). Meanwhile, raw milk samples taken from semi-industrialized farms in summer contained the highest antibiotic residue contamination (44.44%) compared to other milk samples (Table 2).

Discussion

The results showed that 30.75% of raw milk samples (collected from farms and milk collection centers) taken during spring and summer 2012 in Ilam province were contaminated with antibiotic residues. , Little is known regarding status of raw milk in other parts of the country. Manafi et al. (2010), using Delve method, investigated antibiotic residue in raw milk in Tabriz city, and found that 26% of raw milk samples collected from semi-industrialized farms and 16% collected from milk collection centers were contaminated with a variety of antibiotic residues, and in pasteurized milk samples, 30% were found to be contaminated. The results showed insignificant differences between raw milk samples collected from semi-industrial farms and milk-collection centers [9]. Identifying antibiotic residue in raw cattle milk in Ilkhchi region of Tabriz, using Copan Milk Test showed that out of 50 raw milk samples, 10% contained antibiotic residue [3]. The difference between results found in above studies and the present study could be attributed to infectious diseases (especially, mastitis) among milking cattle in Ilam, as well as non-compliance with withdrawal period. Evaluation of raw and pasteurized milk samples in Mashhad showed that over 11.6% of all samples analyzed were contaminated with gentamicin [10]. The results of a study by Rassoli et al. (2005), investigating residues of tetracycline and oxytetracycline in pasteurized milk samples in Tehran showed the presence of these antibiotics residues in 7.8% of pasteurized milk samples [11]. According to the results obtained in the above and the present studies, there is a significant and worrying amount of antibiotic contamination in raw and pasteurized milk samples produced in Iran. The results of a study by Yamaki et al. (2004) in Spain showed that out of a total of 2686 samples of raw sheep milk, 1.7% contained antibiotic residue [7]. In a study by Gidini et al. (2002) in Italy, beta-lactam antibiotic residue was found in 49% of cattle milk samples [8]. Results of a study by Addison & Webb (1997) showed that antibiotic residue only existed in 10.8% of samples. They also argued that non-compliance with withdrawal period in cattle treated with antibiotics could be the main reason for antibiotic residue [12]. Generally, in developed countries, antibiotic residues are often found in cattle treated with higher doses than permitted, while in developing countries, antibiotic residues are found in most samples due to non-compliance with withdrawal period, which is one of the major reasons for high contamination of food products with animal origin, especially contamination of milk with veterinary drugs in many parts of Iran, and also the area investigated in this study compared to existing

reports from developed countries [6, 13]. Because people consider milk and other dairy products complete and healthy foods, thus, such an attitude should be preserved and reinforced. Therefore, quality and health control of milk in terms of antibiotic residue, and correct use of antibiotics seems necessary. Results of present study also showed significant contamination with antibiotic residue of pasteurized milk samples, such that 22.2% of these samples were positive in terms of contamination. To justify the high rates of contamination in pasteurized milk, it could be asserted that in dairy factories, usually healthy raw milk without antibiotics and preservatives are used to produce fermented dairy products such as yoghurt, Dooq, and cheese. In preparation of sterile milks (UHT), high quality raw milks are also used, so as to make possible high temperature process. As a result, low quality and contaminated milks are directed toward pasteurized milk production lines, which is a highly consumed basic dairy product [9].

The high rates of contamination with antibiotic residues in milk samples taken in summer (37.5%), compared to similar samples in spring (20.83%) could have been caused by higher prevalence of such diseases as mastitis during summer. As a result, treatment with antibiotics, and non-compliance with withdrawal period has led to high contamination of milk samples with antibiotic residue taken during summer.

Many studies confirm the efficacy of Copan and Delve tests in detecting a variety of antibiotics in milk. Lebriton et al. (2006) investigated the efficacy of Copan and Delve tests in detecting milk-specific antibiotics, and reported both tests were capable of detecting penicillin, cloxacillin, sulfamethazine, cephalexin, and gentamicin, equal to or less than residues permitted by the European Union standards. They described both tests as simple, practical, and efficient in meeting European Union conditions [14].

In Copan and Delve tests, antibiotics are evaluated together, which could be more useful in practical and nutritional terms. In other words, there may be several types of antibiotics and other inhibitors in milk, yet, concentration of each may be less than the maximum permitted, but their combination can be harmful to health. The above cases can be evaluated using Copan and Delve tests, and kits are so designed to enable detection of antibiotic residues at permitted level [3, 9].

Conclusion

The present study indicates significant contamination of raw and pasteurized milks in Ilam province, which could have been caused by extensive use of antibiotics in milking cattle farms and non-compliance with withdrawal period. It is recommended that a permanent continuous control program replace temporary control of cases to detect microbial growth inhibitors and antibiotics in all milk collecting centers, farms, and dairy factories, along with training and awareness-raising of livestock farmers about harms caused by drug residues in food products with animal origin, especially in milk and dairy products, and also about compliance with exact withdrawal period.

Considering that the present study was a part of Master's Degree thesis, limitations were imposed in relation to sample size in assessment of other parameters such as aflatoxin due to high costs of Copan and aflatoxin test kits.

Acknowledgements

This paper has been performed to present the results of a Master's degree (thesis) in agricultural engineering, nutrition discipline, School of Agriculture, Islamic Azad University, Saveh division). We thank all who helped us to perform it, as well.

Contributions

Study design: MR, AK
Data collection and analysis: MR, VR, VM
Manuscript preparation: MR

Conflict of interest

"The authors declare that they have no competing interests."

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