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Prevalence of antibiotic residues in commercial milk and its variation by season and thermal processing methods

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ABSTRACT

Aims: In this study, the prevalence of antibiotic residues in pasteurized and sterilized commercial milk available in Shahre-kourd, Iran, was investigated. In addition, the influence of seasonal temperature changes on the prevalence of contamination was studied.

Materials and Methods: Commercial milk samples of 187, including 154 pasteurized and 33 sterilized, milk samples were collected from the market between early January 2012 and late July of the same year. The presence of antibiotic residues was detected using the microbiological detection test kit, Eclipse 100, as a semi-quantitative method.

Results: The results showed that 37 of the samples (19.8%) have contained antibiotic residues above the European Union Maximum Residues Limits (EU-MRLs), of which 28 samples (14.97%) were found to be contaminated but at the concentrations below the EU-MRLs. There was no significant difference between the contamination rate of pasteurized and Ultra High Temperature (UHT)-sterilized samples. Similarly, variation of weather temperature with seasons had no effect on the contamination prevalence of milk samples ($P > 0.05$).

Conclusion: Based on the result of this study, antibiotics residues were present in the majority of milk samples. Neither the season nor the type of thermal processing of the commercial milks had noticeable impact on the prevalence level of the milk samples. However, an increasing trend of prevalence level for antibiotic residues was observed with increasing the temperature through the warm season.

Key words: Antibiotic residues, milk, prevalence, season, thermal processing

INTRODUCTION

Antibiotics are commonly used in animal husbandry. These substances are widely used at high dosage due to medicinal purposes for terms of therapy. They are also added to the dairy cattle's feed at low dosage among a long period for prophylactic and growth promotion purposes. In either cases, the antibiotic residue can be found in milk that is considered as a human health hazard in terms of allergic reactions and development of bacterial resistance, and causes failure in dairy fermentation industry.[1,2] Moreover, long-term antibiotic residue intake through food consumption may pose some specific adverse health effects such as gastrointestinal and liver implications.

Antibiotic residues in milk as well as other animal-originated foods are regulated by safety legislation and standard

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agencies in developed countries. Safe limits have been established by international organizations such as the Food and Agriculture Organization, World Health Organization, Codex Alimentarious and Scientific Committee of Food of European Union (EU). The antibiotics residue concentration in milk should not exceed the maximum residue levels suggested by the above-mentioned organizations.[3]

In Iran, despite the lack of monitoring program for detecting and determining the antibiotic residues in milk, several research studies were conducted on this issue in different regions of the country at both the farm and the market level. Based on these researches, a violation rate of 5-24% and 4.7% was reported for raw and thermal processed milk samples, respectively.[4-6] The appearance of antibiotics in milk has been attributed to season, type of market agent and location, in which the last one has been considered as the most influential factor.[7,8] It has been reported that weather conditions, specifically temperature, had a great impact on antibiotic residues in milk. Hence, season was considered as an independent variant when antibiotic detection in milk was of interest.[7,8]

Besides, it is suggested that antibiotic substances tend to be destroyed in thermal processing.[9] Due to this, application of thermal processing and the intensity and severity of heat applied to milk during processing have been supposed to be influential parameters reducing the concentration of the antibiotic residues in milk.

In the urban society of Iran, distribution of raw milk is not legislated and processed milk in the forms of pasteurized and sterilized is quiet prevalent. The purpose of this study was to detect the prevalence of antibiotic contamination of commercially available milk and to assess the impact of season and different thermal processing on the prevalence of contaminated milk samples that carry an unacceptable level of antibiotic residues.

MATERIALS AND METHODS

Collection of milk samples

Commercial milk samples (n = 187) were collected from the market during the cold season, from January to March 2012 (n = 90; 19 sterilized and 71 pasteurized) and warm season, from April to July of the same year (n = 97; 14 sterilized and 83 pasteurized). All the collected samples were produced in less than 1 week of the sampling date. The number of pasteurized products picked up in each sampling time were planned to be three to four times more than the UHT-sterilized samples. It was due to the distribution frequency and the sales ratio of 3-4 for pasteurized versus UHT-sterilized commercial products. Random sampling was conducted among the 24 various brands distributed in the Shahre-kord outlet from January to July 2012. Samples were sent to the food laboratory of the Shahre-kourd University of Medical Science, where the experiments were performed in less than 24 h from the sampling time.

Detection of antibiotic residues

The Eclipse 100-kit (Zeu-Inmunotec, Zaragoza, Spain) containing 96 wells micro- titer plate was used to detect the presence of antimicrobial residues. Detection is based on the microbial activity of *Geobacillus stearothermophilus* var. *calidolactis*, which can be inhibited in the presence of antibiotics. Sensitivity and specificity of the used detection kit for permitted level of different antibiotics in milk are shown in Table 1.[8,10,11]

Sample preparation and test

Each well of the kits was filled with 0.10 mL of the milk sample. The wells were left at room temperature (approximately 20-24°C) for 1 h, allowing the milk to diffuse through the well. The wells were washed using 0.3 mL of distilled water for about three to four times. The wells were carefully sealed with an adhesive sheet and incubated at 65 ± 1°C for 150 min. At the end of the incubation period, the results were recorded. Based on the instruction of the kit, when the purple color of the media, which was filled in the wells completely, changed to yellow, the test was considered negative. However, if the color remained purple, it was counted as positive for antibiotic residue. However, when the color was slightly changed into yellow-purple color, the test result was recorded as partially positive, meaning that the concentration of the inhibitory substances was below the detection limit of the test.

Statistical analysis

The prevalence of antibiotic residues in milk in two different sampling times (winter and summer) and in two different groups of thermally processed commercial milk (pasteurized and UHT sterilized) was compared using the Statistical Package for Social Science software version-18. Chi-square test was conducted for comparison of the variants. All statistical tests for significance were performed two-tailed and interpreted at an alfa level of 0.05. Microsoft Office Excel version 2003 was used to provide diagrams.

Table 1: Limits of detection for antibiotic residues in milk described for Eclipse test

Inhibitor	Negative* µg/kg	Positive* µg/kg	MRLs (µg/kg)	
			EU	US
Penicicilins	2	4	4	5
Sulfonamids	<30	50	25	10
Tetracyclins	50	150	100	300
Streptomycin	<200	300	200	-
Lyncomicin	100	300	150	150
Erythromycin	100	500	40	50
Tylosin	40	80	100	50
Ceftifours	50	75	100	50
Gentamycin	100	300	200	30
Neomycin	100	500	1500	150

MRLs: Maximum residues limits set by US (United state of America) and EU (European union), *Data was provided by the Eclipse kit instruction manual

RESULTS

The prevalence of antimicrobial residues in the different target groups of milk samples is presented in Table 2 and Figure 1. Sixty-four of a total of 187 examined milk samples (34.8%) were tested positive. The prevalence rate of the contaminated samples in pasteurized and UHT-sterilized milk was almost the same (33 vs. 34). It is noteworthy that there was no significant difference between the contamination rates of samples in both types of thermal processed milk when compared in terms of high or low antibiotic residue levels. Comparison of the contamination rate between the milk samples regarding the seasons, which they were produced in cold and warm season, revealed that there was no significant difference in the contamination rate. Thus, season had no impact in this regard ($P > 0.05$). However, distribution of the contamination rate during the total sampling time period within 7 months of the study showed that the highest contamination rate occurred in February (45%), followed by July (41%) and June (40%) [Figure 2]. Instead, a noticeable decline in contamination was observed during March to May.

DISCUSSION

Milk is one of the most notorious articles of our diet, which is designed to be just a food in nature. Because the most sensitive age groups of human beings, children, are always advised to have plenty of milk during a day, it is far more important to provide a safe supply of milk in the society as regards chemicals as well as microbiological aspects. Several studies conducted in different parts of the world showed that antibiotic residues could be found in cow milk, which is the first choice of milk for human use worldwide. However, the prevalence of contamination was reported to be widely different based on the location, season and milk type. The results of this study showed that 34% of the processed milk marketed in Shahre-kord was contaminated with antibiotic residues. Among them, 20% were shown to carry concentrations above the safe limit. This is the highest prevalence rate of such contamination compared with the reports of similar studies in different parts of the country.

Previously, a study conducted in Parsbad, Iran, from March to May 2009 showed that 14% of the raw milk samples was positive in antibiotic residues.[6] Based on the results of the present study, a contamination rate of 11.6% was observed during March-May. A higher contamination rate (24%) was stated by another study in Bostanabad, Iran, in which raw milk samples were tested for antibiotic residue from

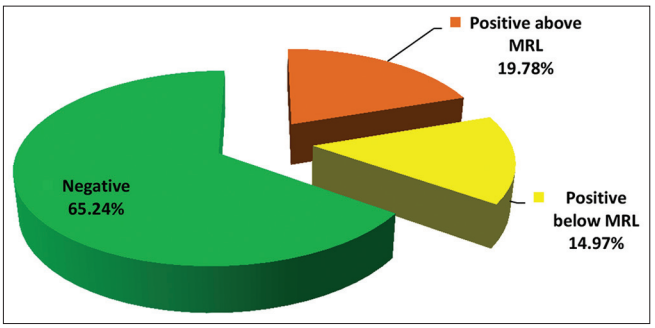

Figure 1: Antibiotic contamination rate among the thermally processed milk samples marketed in Iran in the year 2012

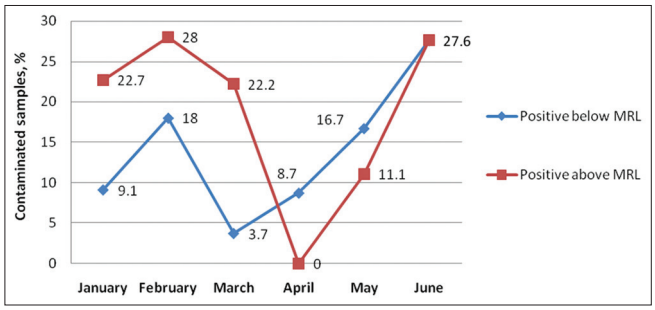

Figure 2: Contamination percentage of the processed milk samples with antibiotic residues during the study period (6 months-2012)

Table 2: Prevalence of antibiotic residues in processed milk

Milk brand	*n*	Fat content		Above MRL[a]		Below MRL[b]		Negative samples	
		>2.5%	≤2.5%	*n*	%	*n*	%	*n*	%
Pasteurizes	154	35	119	31	20.1	23	14.9	100	64.9
Sterilized	33	19	14	6	18.2	5	15.1	22	66.7
Total	187	54	133	37	19.8	28	14.97	122	65.24
Winter	99	73	26	25	25.3	12	12.1	62	62.6
Spring	88	61	27	12	13.6	16	18.2	60	68.2
Brands									
Pak pay	63	+	-	15	23.8	12	19	36	57.1
Damdaran	18	-	+	4	22.2	2	11.1	12	66.7
Koohrang	13	+	-	3	23.1	1	7.9	9	69.2
Sheembar	15	+	-	2	13,3	2	13,3	11	73.3
Apada	12	-	+	3	25	1	8.3	8	66.6
Pak	10	-	+	1	10	3	30	6	60
Mihan	10	-	+	1	10	1	10	8	80
Pegah	5	+	+	1	20	0	0	4	80
Other	40	+	+	7	17.5	6	15	27	67.5
Total	187	49	16		65		34.75	122	65.25

MRLs: Maximum residues limits. [a]Positive samples showed clear color change, [b]Samples presented relative color change

April to September 2010.[5] Consistently, in the present study, the prevalence of contamination during the latter period (April-June) was revealed to be 18.5%, which was more than that of the former period. It could be concluded that despite the insignificant effect of season, in the warm season of the year, along with increase in weather temperature, the prevalence of contaminated samples was raised. It is probably due to the higher incidences of diarrheal diseases in the chattels, which result in the enhancement of antibiotic administration to the cattle.

In a Kenyan study, difference between the dry and wet season had a great effect on the prevalence of antibacterial residues in milk in a way that contamination frequency during the dry season was found to be 1.6 times more than that of the wet season. The higher incidence of diarrheal diseases in the dry season was referred to as the cause of this effect.[7] Considering the results of two other studies carried out in African countries, a very different rate of prevalence (3.1% and 36%) was seen in this part of the world. Nevertheless, season was not considered in any of them as an influential parameter.[12,13] However, in a single Ghanaian study, it was shown that being in a dry or wet season, there was no significant difference in the prevalence of raw milk samples with antibiotic residues. In that study, during a whole study period, a prevalence rate of 35.5% was found among the tested samples.[8]

Some researchers have reported the rate of antibiotic prevalence in the countries nearby Iran (with almost climate condition). In Turkey, a contamination rate of 1.25-44% was found by several studies. Although seasonal difference was not focused in these pieces of research studies, they mostly presented a lower contamination rate than that reported in Iranian studies.[14] In Pakistan, a study showed that among 137 unprocessed market milk samples, 36.50% had ß-lactam antibiotic residues at the levels above EU maximum residues limits.[15]

It has been known that there is a slight decrease in the concentration of antimicrobial agents during thermal processing, especially in low-temperature–long-time treatment (conventional sterilization).[16-19] In a field area of antibiotic residues in milk, some studies assessed the prevalence of contaminated samples between raw and thermal processed.[20-22] In Iran, raw milk has not been offered in the formal market and milk is just supplied as pasteurized or UHT sterilized. Based on the results of this study, there was no significant difference between the contamination rates of these products, which implies that antibiotic compounds used in veterinary practices in Iran resist the sever temperature of UHT processing. In line with these results, another Iranian study reported on the almost equal contamination rate of 4.7% and 5% for ultra-high temperature and raw milk samples with β-lactam antibiotics.[4]

The degree of contamination of milk and dairy products with antibiotics residues differs, depending on the level of legislation and effectiveness of the methods in different countries.[23] It is well known that milk containing antimicrobial residues poses several health hazards to consumers from which resistance to antimicrobials is regarded as a serious problem, with increasing evidences in recent studies in Iran.[24] Based on the results of a study in which *Escherichia coli* and Streptococcus strains involved in mastitis infection were examined for antibiotic resistance, 52-84% of *E. coli* isolates and 13-20% of Streptococcus strains were found to be resistant to penicillin, oxy-tetracycline, streptomycin, erythromycin, and colistin.[24] Moreover, there are substantial evidences on the antibiotic resistance bacteria among human isolates in Iran. This, partly, could be due to the chronic antibiotic consumption via animal products.[25,26]

Unfortunately, in Iran, there is no national program to ensure that milk is free of antibiotic residue and monitoring of milk safety concerning chemical residues has been restricted to research studies. It seems that the contamination rate of milk by antimicrobials is being increased during the recent years relying upon the results of these studies. This results show that a control program for this issue needs to be developed.

Based on the results of this study, neither the season nor the type of thermal processing of the commercially marketed milks had a significant impact on the prevalence level of contaminated samples in the food outlet. However, a trend of increasing the prevalence level for residues can be observed with increasing the temperature through the warm seasons.

As a limitation of this study, visual justification of the test results might not provide a precise border of detection, in particular when sight color changes took place. Using the spectrophotometric devices would have a more accurate result. Furthermore, the possible interference of naturally occurring antimicrobial agents can be pointed out, which may impose falsely positive results. While the microbial inhibition method or some immunological methods are used for detection of drug, residues of such biases are inevitable. However, the advantage of such methods is that the presence of a wide spectrum of antibiotic agents in milk can be monitored.

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