ORIGINAL ARTICLE

Comparative Evaluation of Antibiotic Residues in Raw Milk Samples by ECLIPS 50 and TWINE SENSOR kits in Sepidan and Beyza, Iran

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Abstract

Background: This research was conducted to evaluate antibiotic residues in raw milk samples in Sepidan, using ECLIPS 50 kit and TWINE SENSOR kit.

Methods: In this cross-sectional study, one hundred raw cow milk samples were randomly collected from different farms and milk factories in Sepidan and Beyza townships from winter 2017 to spring 2018. The ECLIPS 50 and TWINE SENSOR kits were used to monitor antibiotic residues in milk samples. The data were analyzed employing Chi-square test, using SPSS software version 20. The significance level was considered $P<0.05$.

Results: In total, 100 raw milk samples were collected, of which 60 (60%) were from Beyza and 40 (40%) from Sepidan. A total of 95 samples (95%) were antibiotic-free and 5 (5%) contained antibiotic residual. 5 samples (5%) of ECLIPS 50 kit, 5 samples (5%) of TWINE SENSOR kit were shown to be positive, using both kits.

Conclusion: There was no difference between ECLIPS 50 kit and TWINE SENSOR kits in detecting antibiotics residue in raw milk samples. The positive samples in the two sets of kits were identical. Furthermore, there was no significant difference between the two types of kits regarding the season and region.

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Keywords: Sepidan, Milk, Antibiotic residues, TWINE SENSOR kit, ECLIPS 50 kit

Introduction

Milk and dairy products contain nutrients that are important for growth.[1] Antibiotics are used to control and prevent livestock infections.[2] Livestock is exposed to various infections, including mastitis,[3] and antibiotics are being used to control and treat infections.[4] The presence of antibiotics in milk is due to lack of attention to the excretion time of drugs and a high doses of drug administration.[5] The presence of antibiotic residues may prevent the growth of the starter bacteria and the production of fermentative dairy products.[2] The antibiotic residues can contaminate the food[6] and cause serious problems in humans,[7] including antibiotic resistance, allergy, hypersensitivity (itching, rash, nausea, vomiting, anaphylaxis shock),[8] and imbalance in micro-flora in the digestive tract in high concentrations.[9] These chemicals are also human carcinogenic and can impair the production of fermented dairy products.[10] It is possible to provide healthier and more quality products to consumers with more accurate monitoring and control. Different microbial, immunochemical

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and physicochemical methods have been developed to screen, detect and determine the amount of antibiotics remaining in foods by animal origin.[11]

Eclipse 50 kit (Zollab, Spain) can distinguish a wide range of antibiotics in milk. (Table 1). This kit can determine the presence of antibiotics in milk based on inhibition of the growth of the Geobacillus stearothermophilus. The microorganism grows in the sample as a desirable environment and produces acid, which changes the color of the kit from violet to yellow (purple means the test is positive and yellow means the test is negative). Towine sensor kit (Uni sensor, Belgian) can detect the antibiotics in a shorter time, but it detects less range of antibiotics compared to Eclipse 50 kit (Table 2). Eclipse 50 kit is more expensive and can detect the antibiotics after a long time, but the advantage is that it can detect a wide range of antibiotics compared to Towine Sensor kit.

Table 1: The detection limit of the Eclipse kit test for several inhibitors (µ g/ml) in milk samples

LIPSE 50	Negative	Positive
Penicillin G	0.002	0.004
Ampicillin	0.003	0.005
Amoxicillin	0.003	0.005
Oxacillin	0.005	0.025
Cloxacillin	0.025	0.04
Cephalexin	0.025	0.075
Cephapirin	0.005	0.008
Sulfathiazole	0.02	0.075
Sulfamethazine	0.1	0.2
Sulfanilamide	0.1	0.6
Oxytetracycline	0.05	0.15
Tetracycline	0.05	0.15
Erythromycin	0.2	0.4
Tylosin	0.02	0.1
Neomycin	<0.500	0. 800

Table 2: The limit of dDetection (LOD) of Towine kit

Antibiotic type	Limit of Detection (ppb)
Ampicillin	3-5
Amoxicillin	3-5
Benzathine Penicillin	2-3
Cefazolin	20-25
Cefoperazone	2-3
Ceftiofur	10-15
Cephapirin	4-8
Coloxacillin	4-8
Naficilin	40-50
Chloro-tetracycline	25-30
Doxycycline	10-20
Oxacillin	30-40
Tetracycline	40-50

In developing countries, especially Iran, antibiotics are widely used. Thus, extensive studies have been done to investigate and determine the antibiotic residues in dairy products. Sadeghi et al. evaluated the contamination and antibiotics residue on raw milk samples in milk tanks in Garmsar, Semnan in 2003. The results demonstrated that 19% of raw milk samples were contaminated with antibiotic residues.[12] Movasegh et al. studied 50 milk samples from milk collection centers of Ilkhchi district in East Azerbaijani and the antibiotic residues were determined in 10% of the samples.[13]

Due to the antibiotic residue adverse health effects on humans and economy, this study aimed to evaluate the antibiotic residue in raw milk samples in Fars province (Sepidan and Beyza), using two common kits of Eclipse 50 and Towin sensor.

Materials and Methods

Sample Collection

This cross-sectional study was conducted from winter 2017 to spring 2018 in Fars province. In this study, 100 samples were randomly collected from 100 industrial dairy farms with a health license in Sepidan and Beyza in the north west of Fars province, Iran. Sixty samples were collected from Beyaz and 40 from Sepidan. 40- 50 mL of raw milk samples was taken with 5 replicates (the sample was taken once, and analyzed for 5 times) according to the protocol recommended by Codex Alimentarius and then stored at 4 to 10˚C for one day. Finally, the samples were transferred to the Pishro milk collection laboratory for analysis.

Sample Analysis and Antibiotic Determination

In this way, 50µl of raw milk was added to the kit and incubated at 65°C. Then, the results were recorded after 2 hours and 15 minutes.

For Towin sensor kit (Uni sensor, Belgian), the incubator temperature was set at 40°C and about 200µl of raw milk was poured into microwells containing the reagent and mixed it to get pink. Then, the samples were incubated for 3 minutes. After that, a twin sensor tape was placed into each microwell, so that the arrow was pointing downward. Then, the mixture was incubated for 3 minutes again. Finally, the tapes were getting out from microwell. One, 2 or 3 red lines were visible on the tape in this step. An optical interpretation was performed by comparing the intensity of the color between the test lines and the control line. (the test line could appear above the control line (tetracyclines) or under the control line (beta-lactams)). The test was negative for samples when the test line was more obvious than the control line and the test was positive if the intensity of the test line was similar or less specific than the control line or

Table 3: The results of Eclipse 50 and Towine sensor kits in different seasons

		Positive sample		Negative sample		P value
		Number	Percent	Number	Percent	
Kit Type	TWINE SENSOR kit	5	5%	95	95%	0.626
	ECLIPSE 50 kit	5	5%	95	95%	
	Total	-	5%	95	95%	
Season	Winter	3	6%	47	94%	0.646
	Spring	2	4%	48	96%	
	Total	5	5%	95	95%	
City	Beyza	3	6%	47	94%	0.646
	Sepidan	2	4%	48	96%	
	Total	5	5%	95	95%	

the test line was not appearing. The limit of detection of the Eclipse kit and Towin sensor kit is presented in Tables 1 and 2, respectively.

Results

The results of the two kits used in this study are shown in Table 3.

The results of this study showed that 5 samples were positive for antibiotic residues. It is worth mentioning that the positive samples were higher in winter, which was not significant. The reason for the presence of antibiotic residues could be due to lack of adequate information on excretion time of antibiotics from milk, which means that milk should be removed from the human nutrition cycle for a time by considering the type of antibiotics and their excretion time. Since the presence of antibiotics in the samples was 5% and by comparison with other studies, the obtained result was desirable, more training and more scientific usage of antibiotics amongst farmers seem to be necessary.

In this study, Eclipse 50 kits and a Towine Sensor were used. The results of the two kits were the same, so it can be concluded that both have the same efficacy.

Discussion

In this study, results for the presence of antibiotic residues in milk showed that milk samples collected from 2 different collection points in Fars were contaminated with antibiotic residues. Out of 100 analyzed raw milk samples from winter 2017 to spring 2018, 55%)) were identified with antibiotic residues. Results obtained showed no significant diffrences between the seasons during the research. Identification of positive samples (5%) in different areas of Fars is a concern that must be taken into consideration to determine the reason for the high percentage of positive samples.

Many studies were conducted around the world regarding antibiotic residue in milk samples. In a study conducted by Manafi et al. in East Azerbaijan in 2008, 26% and 16% of raw and pasteurized milk samples were positive, respectively.[14] The difference in the results and concentration of antibiotics in different studies can be related to differences in the screening methods. Ghanavi et al. examined 200 milk samples in 2003 and concluded that 5% and 27% of pasteurized and raw milk samples were contaminated by antibiotics that could be possibly due to non-compliance of health standards in the past, which imposes irreparable losses given that raw milk is used for other dairy products and fermentation products.[15] Also, in a study by Mahmoudi et al. in Tabriz, 2012, about 57.5% of raw milk samples were contaminated with antibiotic residues, which indicates excessive use of antibiotics in this city. The higher percentage of contamination by antibiotic residue was probably due to lack of- compliance with health codes in livestock farms and improper training of the ranchers.[16] Another study conducted by Mahmoudi et al. using the coupon method on pasteurized milk and raw milk samples in 2011 in Ilam showed that 29.1% of raw milk samples and 22.2% of pasteurized milk samples contained antibiotic residues.[17] Their higher contamination compared to the present study could be due to the fact that in recent years there is more monitoring of veterinary centers. Abedini et al. examined 800 raw and pasteurized milk samples using Delvo test in 1973 in Shiraz and reported that about 2.7% of the samples were contaminated, which could be possibly due to lower accuracy of this method than other methods.[11] In a study in Qazvin in 2012, using ELISA method, about 46% of the collected samples were positive. The percentage of contaminated samples was higher in the warmer season as a result of more livestock diseases during this season.[1] Yamaki et al. examined 2686 raw milk samples in Spain and concluded that only 7.1% of the samples contained antibiotic residues,[18] which illustrates the strict observance of EU rules by the ranchers. A study conducted by Gonzales et al. in Brazil (2009) showed that about 10.68% of the total samples were contaminated,[19] which illustrates the strict observance of EU rules by the ranchers. Also, a study conducted by Gonzales et al. in Brazil (2009) showed about 10.68% of the total samples were contaminated,20 which may be related to the differences in laboratory procedures. A study in Pakistan (2006)

demonstrated that 36.5% of samples were contaminated with beta-lactam antibiotics,21 which may be due to poor health conditions. Ram et al. (2016), using Delvo test SP on 1734 samples, reported that more than 6.11% of the samples were contaminated which may be due to the difference in the method and tests.

The mentioned study showed that high use of antibiotics in livestock was due to the antibiotic residues in raw milk samples. The important note is that there are not enough control measures in the milk collection centers to determine the antibiotic residues in delivery of milk. On the other hand, poor livestock management, low control actions on the livestock, no sanitary conditions, and insufficient physical environments can make the animals sick, while the farmers have no choice except taking antibiotics. Therefore, a training program can be effective on the farmers' attitude; also, a suitable physical environment seems to be an effective way in prevention of contaminated milk. Moreover, there is a need to a rapid and accurate method to check if milk contains antibiotic, so that we can protect the consumer's health. Finally, the present study showed that antibiotic residues were observed in raw milk samples collected from Fars Province (Sepidan and Beyza). Given the budget constraints and the low number of samples, it is suggested that more studies should be conducted elsewhere. With regards to budget constraints and the low number of samples, further studies with large samples are recommended.

Conclusion

The findings of the present study indicated that raw milk samples contained antibiotics in Sepidan and Beyza, which were fewer compared to other studies and by considering the health and cultural conditions due to antibiotic adverse health effects on humans, it seems necessary to educate the ranchers, by preventing the sale of non-prescription antibiotics to dairy farmers, applying enforcement deterrent rules and also training the farmworkers, who may sometimes misuse antibiotics due to lack of information.

In this study, Eclipse 50 kits and a Towine Sensor were used. The results of the two kits were the same. Milk contamination with the antibiotic residue is considered as a serious concern. Based on the present results, strict and careful monitoring of the animal products is necessary. Further studies with larger sample size are recommended. Also, it was better to confirm some positive and negative samples with HPLC method. In these kits, there is the possibility of a false positive or negative result.

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