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## Detection of antibiotic residue in raw milk in Mashad by Delvo-test

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### ABSTRACT

Drug residue is an important aspect in food safety. Also antibiotics are one of the most important bioactive and chemotherapeutic groups of compounds made by microbiological synthesis. Use of antibiotics may produce residues in milk, and subsequently induce allergic reactions in humans. In addition, antibiotics give rise to an increase in the antibiotic resistance of pathogenic bacteria, which may result in health problems. In many countries, governmental authorities have established monitoring programs to determine a maximum residual level (MRL) for them. So the aim of this study was to determine antibiotic presence in milk samples in Khorasan province in Iran. During 4 months (September to December 2013), one hundred milk samples were collected from seven dairies. Antibiotic presence was determined using Delvo test, a broad-spectrum test capable of detecting different antibiotics. Results showed that Copan milk test was positive for 9% of the samples (8 samples). In the other samples the antibiotic content has been below the detection limit of kit.

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### KEYWORDS

Antibiotic;  
Milk;  
Food safety.

### INTRODUCTION

Also antibiotics are one of the most important bioactive and chemotherapeutic groups of compounds made by microbiological synthesis<sup>[1]</sup>. Use of antibiotics may produce residues in milk, and subsequently induce allergic reactions in humans<sup>[2]</sup>. In addition, antibiotics give rise to an increase in the antibiotic resistance of pathogenic bacteria, which may result in health problems<sup>[3]</sup>. In many countries, governmental authorities have established monitoring programs to determine a maximum residual level (MRL) for them<sup>[4]</sup>.

Nowadays Amino glycoside (Streptomycin, Neomycin, etc), Tetracycline (Oxytetracyclin, etc) and Beta

lactam antibiotics (Penicillin G, etc), antibiotics are widely used for antimicrobial purposes, e.g. for treatment of mastitis in dairy cows, and consequently are most commonly type of residues found in milk<sup>[5]</sup>. B-lactams, which had been approved for the treatment of mastitis by the food and drug administration, belongs to primary class of antibiotics. It is considered that the beta lactam group of antibiotics probably may cause 95% of milk antibiotic contamination, and its residues may cause hygienic as well as industrial problems<sup>[6]</sup>. It is important to regulate antibiotic residues in milk and to fix the residues limits to minimum. Therefore, National monitoring programs are present in different countries like Turkey, while

## FULL PAPER

their primary purpose is to regulate and control the antibiotics residues in milk<sup>[7,8]</sup>. Quantity of antibiotic residues in milk higher than MRLs (maximum residues limits) are illegal. So the aim of this study was to determine antibiotic presence in milk samples in Khorasan province in Iran. During 4 months (September to December 2013)

### MATERIALS AND METHODS

Ninety raw milk samples were taken from retail stores in Mashad-Iran. Samples were analyzed by Delvo test kit for qualitative analysis of different antibiotic according to TABLE 1. The Delvotest (Gist-brocades BV, Delft, The Netherlands) is one of the best known microbial inhibitor tests. The first version to be developed, in the 1970s, was the Delvotest P, designed to detect b-lactams. This test is also based on *B. stearothermophilus*, which is encapsulated in an agar medium containing a pH indicator, a nutrient tablet and the milk sample dispensed onto the agar surface<sup>[9,10]</sup>. The ampoule version is designed for individual tests or small-scale testing, whilst a micro-titer plate version is designed for mass testing, and allows up to 96 tests to be undertaken simultaneously. A color change from purple to yellow, due to acid development during incubation at 64°C for 2½ hours, indicates a negative result. The Delvotest P has been used throughout the world and has a sensitivity to penicillin G of 0.005 IU/ml.

### RESULTS AND DISCUSSION

Ninety raw milk samples were analyzed by Delvo test kit for qualitative analysis of different antibiotic. Positive results were shown in 9 (8%) samples. This is a suitable finding from the food safety view point. Different studies have been done in different countries which show a risk for antibiotic residues. Mohamadi sani et al., (2010) showed 40.8% contamination in raw milk samples by Copan test in Mashad-Iran<sup>[11]</sup>. Abedi Shirazi (1983) reported 57.2% contamination in raw milk samples in Shiraz-Iran<sup>[12]</sup>. Moarefi<sup>[13]</sup> indicated to 32.5% contamination in milk samples in Tehran-Iran<sup>[13]</sup>. Karim & Navabpour<sup>[14]</sup> analyzed milk samples in Kerman-Iran by Delvo test and reported 67.5% contamination to antibiotic residues<sup>[14]</sup>.

**TABLE 1 : Detection limit of antibiotics in delvo test kit (ppm)**

Antibiotic Group	MRL <sup>2</sup>	Detection range
B –lactams		
penicillin	4	1-2
penethamate	4	4
ampicillin	4	4
amoxicillin	4	2-3
nafcillin	30	5
cloxacillin	30	20
dicloxacillin	30	10
oxacillin	30	10
cefecetri	125	20
cefslexin	100	50
cefalonium	20	5-10
cefoperazon	50	40
ceftiofur	100	25-50
cefquinome	20	75-100
cephapirin	60	5
cephazolin	50	25
cefuroxime		30-80
Tetracyclines(sum)	100	200
Chlortetracyclines(sum)	(0)	100-150
Doxycycline	100	250-500
Oxytetracycline(sum)	100	250-500
tetracycline		
Sulfonamides(sum)	100	
Sulfadimidine	100	100
Sulfadimethoxine	100	100a
Sulfamerazine	100	50-100
Sulfathiazole	100	50
Sulfadiazine	100	25-50
Sulphamethazine	100	50
Suplhadoxine	-	100-200
suplhamethaxole	-	<50
Macrolides		
Erythromycin	40	40-80
Spiramycin	200	400-600
Tilmicosin	50	50-100
tylosin	50	30
Aminoglycosides		
Gentamycin	100	50
Kanamycin	150	5000
Neomycin	1500	100-200
Param omycin	(0)	5000
Spectinomycin	200	>300

Antibiotic Group	MRL <sup>2</sup>	Detection range
Streptomycin	200	>1000
DH streptomycin	200	>1000
Quinolones		
Enrofloxacin(sum)	100	1000-1500
Danofloxacin	30	1000-2000
Difloxacin	(0)	
Flumequin	(0)	
Marbofloxacin	75	
Oxilinic acid	(0)	>1000
Various		
Bacitracin	100	1000-2000
Baquiloprim	30	50-75
Chloramphenicol	0	2500
Clavulanic acid	200	
Colistin	50	1000-5000
Dapsone	0	0.5-1
Florfenicol	(0)	
Lincomycin	150	200
Novobiocin	50	1000
Pirlimycin	100	20-100
Rifaximin	60	>25
Thiamphenicol	50	>1000
Trimethoprim	50	50-100
tulathromcin	(0)	

**\*Maximum residual limits**

Also, different quantitative researches have been done in this matter. Dimitrieska-Stojkovic<sup>[15]</sup> studied 497 raw milk samples from eight regions from Macedonia. Immunoassay methods were used for the determination of SAs. Measured range of concentrations (in µg/kg) was 13.5-147.9 for sulfonamides<sup>[15]</sup>. In another study Abdullah Siddiqui (2010) analyzed 147 cow and camel milk samples which were collected from 23 different stations on different occasion from different farms of UAE. He observed that approximately 71% of the tested milk samples were found positive with low level traces of sulfonamides. No targeted antibiotic residues of sulfonamides were found above the MRL of 100 µg/lit<sup>[16]</sup>. Sauvé et al. (2010) indicated reported the amount of SAs in milk samples between 2 and 14 µg L<sup>-1</sup><sup>[17]</sup>. In a research by Rong-wei HAN (2013), 180 samples of ultra high temperature (UHT) milk of four top Chinese dairy brands collected in the 25 cities in China in June 2011, and SAs residue was assessed using the ELISA method. Results showed that

16.7% contamination in the samples. The maximum concentrations of the sulfonamides and sulfamethazine were 26.2 and 22.6 µg kg<sup>-1</sup>, respectively<sup>[18]</sup>.

It is concluded that the results seems to be good, but the need for continues monitoring is felt. Also it is needed to control the antibiotic residues in milk and different dairy products quantitatively by different techniques such as ELISA or HPLC methods.

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**FULL PAPER**

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