Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 28 issue 1, 2018, pp 22-28

DIRECT SCREENING OF ANTIBIOTIC RESIDUES IN PASTEURIZED, STERILIZED AND RAW MILK SUPPLIED IN ZANJAN MARKET, IRAN

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ABSTRACT: The aim of this study was to determine the presence of antibiotic residues in raw and pasteurized milk supplied in Zanjan market, Iran. 160 milk samples including packed industrial samples of different brands and local raw milk samples were collected from Zanjan market. Antibiotic Trisensor Kit was used to determine presence of beta-lactam, tetracycline and sulfonamide antibiotics in the samples, according to the method provided by the kit manufacturer. In this study, 20 (31.25%) and 9 (9.38%) samples of packed (heated) industrial and local raw milk samples had antibiotic residue over than MRL, respectively. Antibiotic residues of heated (pasteurized and sterilized) milk samples were sulfonamide antibiotics (31.25%) and in local raw milk samples were beta-lactam (8.33%) and tetracycline (1 %) antibiotics respectively. Special measures and effective monitoring program should be taken to reduce these chemical hazards in the Zanjan province.

Keywords: Antibiotic; β-lactams; Tetracycline; Sulfanamide; Raw milk; Pasteurized Milk; Zanjan; IRAN

INTRODUCTION:

One of the most important threats to human health are chemical and microbial contamination of foodstuffs especially foods with animal origin. Nowadays, veterinary drugs are widely used to improve the health and prevention diseases of the livestock through the world (Doyle 2006; Turnipseed and Andersen 2008; Manafi et al. 2011). Among the commonly used drugs in veterinary medicine, antibiotics are used to control and treat infectious diseases such as mastitis, as well as to promote growth efficiency by increasing nutrients absorption in the digestive tract (Doyle, 2006; Turnipseed and Andersen, 2008; Manafi et al., 2011; Ali, 2015).

Milk and dairy products have special place in the diet plan for all people with different ages particularly children, due to nutritional and essential ingredients for the body. Therefore, presence of drug residues including antibiotics in milk and dairy products is very important (Turnipseed and Andersen, 2008; Ghanavi et al., 2013; Mahmoudi et al., 2014a). Four major classes of antibiotics are used to treat infectious diseases in animals, especially mastitis in dairy cows including β-lactams (penicillin G, etc.), aminoglycosides (streptomycin, neomycin, etc.), tetracyclines (oxytetracycline, etc.) and sulfanamides (Sulfadimidine and etc.). As a result, mostly antibiotic residues in milk belong to these classes of antibiotics (Table 1). Maximum Residue Limits (MRL) for some common antibiotics in milk samples proposed by Codex Alimentarius Commission (CAC) and accepted in Iran national standard legislation was showed in table 2.

Remnants of veterinary medicines such as antibiotics in animal products can have side effects on the health of consumers of these products (Doyle, 2006; Turnipseed and Andersen, 2008; Movasagh, 2012). Several studies have reported adverse effects of long-term exposing to antibiotic residues in humans, including allergic reactions in susceptible individuals, gastrointestinal disorders and disturbances in the intestinal normal flora, drug resistance and producing resistant foodborne pathogens (Doyle, 2006; Turnipseed and Andersen, 2008; Mahmoudi et al., 2014b). Even some studies have been emphasized on carcinogenicity of antibiotic residues (Khaskheli et al., 2008; Won et al., 2011; Al-Waili et al., 2012). Sherri et al. reported that 0.06 μg/ml of penicillin, as one of the most commonly used antibiotic of beta-lactam class, can cause allergic reactions in very sensitive individuals (Doyle 2006). Toxicity of sulfonamides is based on the effect on the hypothalamic-pituitary-thyroid interactions (Doyle, 2006).

Tab. 1.
Maximum Residue Limits (MRL) for some common antibiotics in milk used in veterinary medicine (CAC,2012)

Antibiotics (mg/L*)					
β-lactams		**Tetracyclines**	**Sulfanamides**	**Macrolids**	**Aminoglycosides**
Penicillins	**Cephalosporins**				

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Penicillin	Ceftiofur	Tetracycline	Oxytetracycline	Chlortetracycline	Sulfadimidine	Tilmicosin	Spiramycin	Streptomycin	Spectinomycin	Neomycin	Gentamycin
4	100	100	100	100	25	50	100	200	200	500	200

*Part Per Million (mg/L)

Economic losses are other concerns of antibiotic residues in dairy industry due to their negative effects on the growth and activity of starter cultures and fermentation disruption in fermented dairy products (Ruegg and Tabone, 2000; Mahmoudi et al., 2014b). Therefore, there is no doubt that evaluation and effective monitoring of antibiotic residues in milk and dairy products are very important that enforce national monitoring programs to assess the amount of veterinary drug residues. As a result, regulatory agencies in each country determine the maximum levels for these drugs in foods, especially in milk and its products. The residue limit of some commonly used veterinary antibiotics in milk was shown in Table 1, according to the latest changes in the Codex Alimentarius Commission (CAC 2012). Different methods have been developed for the determination of drug residues including microbiological, chromatographic, enzymatic methods such as ELISA and immunochemical methods (Table 2). As showed in Table 2, different methods have been used for detection and to determine antibiotic level in dairy products which commercial screening tests (Delvotest SP-NT and Copan Milk Test) plays a key role among these methods (Mahmoudi et al., 2014a; Movasagh, 2012; Mahmoudi et al., 2012).

Growing of production and consumption of livestock products in the Middle East, especially in Iran, has increased the use of antibiotics to increase livestock productivity in recent years (Zeina et al., 2013). A few studies have been implemented on the antibiotic residues in milk in Iran. Some of these studies are shown in Table 2 which shows high contamination rate and presence of antibiotics in raw milk and dairy products in Iran.

Tab. 2.
Some different methods used for determination of Antibiotics in milk

Sample	Method	Contamination rate (%)	Sampling region of Iran	Reference
Raw milk	Copan Test	10	Tabriz-Ilikhchi	(Movasagh 2012)
Raw milk Pasteurized milk	Delvo Test	21 30	East Azerbaijan	(Manafi et al. 2011)
Raw milk	ELISA, Delvo Test	43	Ghazvin	(Mahmoudi et al. 2013)
Raw milk Pasteurized milk	Copan Test	30.76 22.2	Ilam	(Mahmoudi et al. 2014a)
Pasteurized milk	Delvo test, HPLC	33.39	Tehran	(Dabbagh Moghaddam et al. 2014)
Dried milk	β-star kit	30	Tehran	(Noori et al. 2013)
Pasteurized & Sterilized milk	HPLC	34.75	Iran	(Aalipour et al. 2015)
Raw milk	Four Plate Test (FPT)	33	Maragheh-Bonab	(Zarangush and Mahdavi 2016)

Given to increasing consumption of antibiotics in animals and their adverse effects on the health of their products consumers, surveys on antibiotics residues are the important issues in quality control of raw milk and other food with animal origin. Therefore, the aim of this study was to determine the presence of antibiotic residues in raw and pasteurized milk supplied in Zanjan, Iran.

MATERIALS AND METHODS:

Materials

Antibiotic's diagnostic Kit, *Trisensor KIT035* (Unisensor, Liege, Belgium) with detection ability of beta-lactam, tetracycline and sulfonamide antibiotics (The first component of kit is a microwell containing predetermined amounts of both receptors and antibodies linked to gold particles. The second is a dipstick made up of a set of membranes with specific capture lines). Block Heater with a temperature of 40 ° C to sample incubation.

Sampling

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In this cross-sectional study, 160 milk samples including 64 industrial samples of different brands (34 and 30 packed pasteurized and sterilization samples, respectively) and 96 samples of local raw milk were collected from market of Zanjan city during summer in 2016. All samples were transmitted under cold storage to laboratory of school of health of Zanjan University of Medical Sciences. Antibiotic Trisensor Kit (Unisensor, Liege, Belgium) was used to determine the presence of beta-lactam, tetracycline and sulfonamide antibiotics in the samples, according to the method provided by the kit manufacturer.

Test Method

Trisensor as *a* commercial screening test is a competitive test involving two receptors and generic monoclonal antibodies in one single operation. At the first step, put the microwell containing receptor and antibodies linked to gold particles into a block heater well with a constant temperature of 40 °C, and add 200 µl of the sample to microwell and wait 3 minutes to reaction between antibiotics and antibodies if antibiotics residue be present in the sample. In the second step, after the first three minutes, drop the dipstick into the microwell and wait again for three minutes. Then remove the dipstick and read the results of the kit according to the guide provided by the manufacturer of the kit. According to the kit manual, if the test is negative with no antibiotic, the dipstick color bands is formed for each antibiotic and in the presence of antibiotics, color band related to each antibiotic is not formed. A positive sample containing Penicillin G, sulfadimidine, Oxytetracycline antibiotics was used to evaluate this method. The detection limit (LOD) for each antibiotic of these three groups showed in Table 3, according to kit brochure. The LOD for each antibiotic is close to the maximum residue limit (MRL) for each antibiotic of these groups.

Tab. 3.
Limit of detection of Trisensor Kit (mg/L)

β- Lactames		**Sulfanamides**		**Tetracycline**	
Penicillines		Sulfadiazine	8-10	Tetracycline	80-100
Penicilline G	2.5-3.5	Sulfapyridine	0.5-1	Oxytetracycline	60-70
Ampicilin	3-4	Sulfathiazole	7.5-8.5	Chlortetrcycline	50-60
Amoxicillin	3-4	Sulfamethoxazole	320-360	Doxycycline	20-30
Oxacillin	13-15	Sulfamethazine	1-2		
Cloxacillin	7-9	Sulfamethoxypyridazine	2-3		
Dicloxacillin	4-6	Sulfadimethoxine(Sdm)	10-15		
Nafcillin	50-70	Sulfacetamide	300-600		
Cefalosporines					
Ceftiofur	8-12	Sulfamerazine	2-3		
Cefazolin	15-19	Sulfamonomethoxine	8-12		
Cefapirine	4-6	Sulfaguanidine	15-25		
Cefacitrile	16-20	Sulfamethizole	220-260		
Cefalexin	700-800	Sulfachloropyridazine	5-10		
Cefalonium	3-5	Sulfasalazine	250-350		

RESULTS AND DISCUSSION:

In this study, 20 (31.25%) and 9 (9.38%) samples of 64 and 96 packed (heated) industrial and local raw milk samples had antibiotic residues over than MRL, respectively (Table 4). Industrial and local raw milk sample's contamination was higher than the kit LOD. Sulfonamide and β-lactam groups were main antibiotic residue in packed industrial (heated) milk samples, whereas in local raw samples, main antibiotic residue belonged to β-lactams and tetracycline antibiotic was found only in one sample.

Tab. 4.
Results of antibiotic residues in supplied Raw and pasteurized/sterilized milk samples in Zanjan, Iran

Milk sample	**Total**	**β-lactams positive**	**Sulfanamides Positive**	**Tetracycline Positive**	**Total positive samples**	**Contamination rate (%)**
Pasteurized & Sterilized milk	64	0	20	0	20	31.25
Raw milk	96	8	0	1	9	9.38

In this study, antibiotic residues were detected in 29 samples (18.1%) of a total 160 milk samples taken from Zanjan city during summer in 2016. Contamination in pasteurized and sterilized milk samples (12.5% of samples) and local raw milk samples (5.6% of samples) were related to the presence of sulfonamide antibiotics (31.25%), beta-lactam (8.33%) and tetracycline (1%) antibiotics respectively (Table 4). Several studies were conducted to detect high and low contamination rates of milk samples with beta-lactam and tetracycline antibiotics in Iran and through the world. Noori *et al.* (2013), Ghanavi *et al.* (2013), Movassagh and Karami (2011), Hosseinzadeh *et al.* (2013) were reported contamination rate of milk samples with beta-lactam antibiotics, 30%, 2.66%, 5% and 20.17%, respectively. In the studies of Dabbagh

Moghadam *et al.* (2013), Noori *et al*. (2013) and *Aalipour et al.* (2015), contamination rate of milk samples with tetracyclines has been reported 33.39%, 17.5% and 34.75%, respectively. Rama *et al.* (2017), Ibraimi *et al.* (2013) and Ahlberg *et al.* (2016) were reported chemical contamination rates due to beta-lactam residues in milk samples 0.5%, 50.4%, and 5%, respectively. Another studies conducted by Navrátilová *et al.* (2009), Orwa *et al.* (2017) and Gaurav *et al.* (2014) contamination rate of milk samples with tetracyclines residues were reported 50.6%, 31.4% and 13.5%, respectively. Mahmudi *et al.* (2014) and Ahlberg *et al.* (2016) reported 25.39% and 2.5% contamination rate of milk samples with sulfonamides.

Antibiotics were decomposed during milk storage and in the presence of high microbial population, due to the activity of degrading enzymes such as beta-lactams and etc. secreted by milk microflora. Decomposition is higher in stored milk at ambient temperature and ready for spoilage samples (Ahlberg et al., 2016). Therefore, one or more antibiotic groups may have been decomposed and did not detectable in the milk samples in this study due to high storage time and probably high microbial populations especially in local raw milk samples. In other word, while antibiotics used in livestock farms but are not detectable in milk samples. Difference in the type of antibiotic residues between industrial and local raw milk samples in this study can be due to the easy access to an antibiotic group or use of more specialized drugs such as sulfanamides in the treatment of animal diseases by livestock breeders.

There are many factors involved in antibiotics presence in milk including: administration incorrect dose of antibiotics particularly in mastitis treatment, not consider to antibiotics withdrawal time of the animal body, inability and no competence of livestock breeders to use antibiotics and no record presence of their consumption, as well as intentional addition of antibiotics in milk, to delay milk spoilage (Trevisi et al., 2014; Ali, 2015). An increase in the milk contamination rate with antibiotics may occur in packed milk samples in dairy industry which mostly their raw material was provided of industrial livestock, especially when farms hygiene and milking conditions are poor and antibiotics withdrawal time is not considered during sick cattle treatment period. Obtained results of present and other studies confirm this issue (Table 2) (Manafi et al., 2011; Movasagh, 2012; Noori et al., 2013; Dabbagh Moghaddam et al., 2014; Aalipour et al., 2015). Availability, easy access and uncontrolled use of antibiotics make it difficult to select the appropriate technique for evaluating their residual levels (Ahlberg et al., 2016). It is recommended that the sensitivity of the used laboratory methods should be high to determine MRL and maintain the consumer's health (Movasagh, 2012). Screening tests like the *Trisensor* KIT are qualitative tests and are very useful to prevent consumption and unintentional sale of antibiotic-contaminated milk products because of their ease and low cost (Noori et al., 2013). However, all screening tests have a functional limitation. Based on the kit's compounds, only the presence or absence of antibiotic residues is shown within the kit's diagnostic scope and not able to determine the quantity amounts of residues. In other words, the positive outcome of a screening kit may not being approved by quantitative methods or other screening tests.

Side effects of synthetic drugs have led peoples to use natural antimicrobial compounds and make decisions to prevent entrance of these substances into food (Aminzare et al., 2017a; Aminzare et al., 2017b; Bahmani et al., 2016). The most important advices to reduce antibiotic residues in milk and dairy products are: Identify drastic corrective actions to reduce infectious diseases, provide an accurate and reliable monitoring system for distribution and use of antimicrobial drugs including antibiotics, careful considering to withdrawal time of drugs in suspicious dairy animals before milk supply, continuous control of antibiotic residues in industrial and raw milk samples, training livestock breeders about correct use of antibiotics and legal dealing with breeders when antibiotics are detected in milk samples.

CONCLUSIONS:

This study showed the presence of antibiotic residues in milk samples that has risks to health of milk consumers. Hence, special measures should be taken by regulatory agencies, veterinarians, livestock breeders and consumers to reduce these chemical hazards. Continuous monitoring of antibiotic residues in milk and dairy products with qualitative and quantitative tests is very effective in this issue.

REFERENCES:

Aalipour F, Mirlohi M, Jalali M, Azadbakht L, Dietary exposure to tetracycline residues through milk consumption in Iran. Journal of Environmental Health Science and Engineering, 13(1): 2-7, 2015.

Ahlberg S, Korhonen H, Lindfors E, Kang'ethe E, Analysis of Antibiotic Residues in milk from Smallholder farms in Kenya. Aferican Journal of Dairy Farming and Milk Production, 3(4): 151-158, 2016.

Ali R, Residual Antibiotics In Marketable Milk In Khartoum State: UOFK., 2015.

Aminzare M, Abbasi Z, Amiri E, Hashemi M, Raeisi M, Mousavi N, Hassanzadazar H, Colibacillosis Phytotherapy: An Overview on the most important world medicinal plants effective on Escherichia coli. Journal of Pharmaceutical Sciences and Research, 9(5): 629-636, 2017a.

Aminzare M, Hashemi M, Hassanzadazar H, Amiri E,. Abbasi Z. Antibacterial activity of corn starch films incorporated with *Zataria multiflora* and *Bonium persicum* essential oils. Annual

Research and Review in Biology, 19(1): 1-9, 2017b.

Al-Waili N, Salom K, Al-Ghamdi A, Ansari MJ, Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. The Scientific World Journal, 2012: 1-9, 2012.

Bahmani M, Rafieian-Kopaei M, Hassanzadazar H, Taherikalani M. Pseudomoniasis phytotherapy: A review on most important Iranian medicinal plants effective on Pseudomonas aeruginosa. Iranian Journal of Microbiology, 8(5): 347-350, 2016.

[CAC] Codex Alimentarius Commission. Codex Veterinary Drug Residue in Food Online Database. Updated as at the 35th Session of the Codex Alimentarius Commission. July 2012.

Dabbagh Moghaddam A, Tayebi L, Falahatpisheh H, Mahmoudian M, Kowsari N, Akbarein H, Sabzikar A, Evaluation of the tetracycline residues in pasteurized milks distributed in Tehran by HPLC method. Journal of Army University of Medical Sciences, 11(4): 318-323, 2014.

Doyle M, Veterinary drug residues in processed meats-potential health risk. Food Research Institute, University of Wisconsin-Madison Briefings. FRI Briefings: 1-11, 2006.

Ghanavi Z, Mollayi S, Eslami Z, Comparison Between the Amount of Penicillin G Residue in Raw and Pasteurized Milk in Iran. Jundishapur Journal of Microbiology, 6(7): 1-5, 2013.

Gaurav A, Gill J, Aulakh R, Bedi J, ELISA based monitoring and analysis of tetracycline residues in cattle milk in various districts of Punjab. Veterinary World, 7(1): 26-29, 2014.

Hoseinzadeh H, Hanifi A, Farzamfard E, Heidary M, Talebi F. comparative study on Antibiotic Residue in Raw milk of Gilan Farms During the Various seasons. The 13th Iranian and the 2nd International congress of Microbiology Ardabil, Iran., 2012.

Ibraimi Z, Shehi A, Hajrulai Z, Mata E, Murtezani A, Detection and risk assessment of beta-lactam residues in Kosovo's milk using Elisa method. International Journal of Pharmacy and Pharmaceutical Sciences, 5(4): 446-450, 2013.

Khaskheli M, Malik R, Arain M, Soomro A, Arain H, Detection of ß-lactam antibiotic residues in market milk. Pakistan Journal of Nutrition, 7(5): 682-685, 2008.

Mahmoudi R, Amini K, Vahabzade M, Mir H, Vagef R, Antibiotic residues in raw and pasteurized milk, Iran. . Journal of Research Health, 4(4):884-889, 2014a.

Mahmoudi R, Golchin A, Farhoodi A, A Review on Antibiotic Residues in Animal-derived Foods in Iran over the Last Thirty Years. Journal of Mazandaran University of Medical Sciences, 24(119): 213-222, 2014b.

Mahmoudi R, Norian R, Gajarbeygi P, Survey of antibiotic residues in raw milk samples in Qazvin (2012). Journal of Qazvin University of Medical Sciences, 18(1): 45-52, 2013.

Manafi M, Hesari J, Rafat SA, Monitoring of Antibiotic Residue in Raw and Pasteurised Milk in East Azerbaijan of Iran by Delvotest Method. Journal Food Science and Technology, 20(2): 125-131, 2011..

Movasagh MH, Detection of antibiotic residues in raw cow's milk in Eilikhchi (Southwest of Tabriz). Journal of Food Technology and nutrotion of Iran, 9(3): 214-221, 2012. .

Movassagh MH, Karami AR, Beta-lactam antibiotics residues in pasteurized milk by beta star test in the North West Region of Iran. ARPN Journal of Agricultural and Biological Science, 6(11): 7-10, 2011.

Navratilova P, Borkovcova I, Dračkova M, Janštova B, Vorlova L, Occurrence of tetracycline, chlortetracyclin, and oxytetracycline residues in raw cow's milk. Czech Journal of Food Sciences, 27(5): 379-385, 2009.

Noori N, Karim G, Raeesian M, Khaneghahi Abyaneh H, Bahonar A, Akhondzadeh Basti A, Ghadami F, Antibiotic residues and aflatoxin M1 contamination in milk powder used in Tehran dairy factories, Iran. Iranian Journal Veternary Medicine, 7(3): 221-226, 2013.

Orwa JD, Matofari JW, Muliro PS, Lamuka P, Assessment of sulphonamides and tetracyclines antibiotic residue contaminants in rural and peri urban dairy value chains in Kenya. International Journal of Food Contamination, 4(1) :5, 2017.

Rama A, Lucatello L, Benetti C, Galina G, Bajraktari D, Assessment of antibacterial drug residues in milk for consumption in Kosovo. Journal of Food and Drug Analysis, 25(3): 525-532, 2017.

Ruegg P, Tabone T, The relationship between antibiotic residue violations and somatic cell counts in Wisconsin dairy herds. Journal of dairy science, 83(12): 2805-2809, 2000.

Trevisi E, Zecconi A, Cogrossi S, Razzuoli E, Grossi P, Amadori M, Strategies for reduced antibiotic usage in dairy cattle farms. Research in Veterinary Science, 96(2): 229-233, 2014.

Turnipseed SB, Andersen WC, Veterinary drug residues. Comprehensive Analytic Chemistry, 51: 307-338, 2008.

Won SY, Lee CH, Chang HS, Kim SO, Lee SH, Kim DS, Monitoring of 14 sulfonamide antibiotic residues in marine products using HPLC-PDA and LC-MS/MS. Food Control, 2(7): 1101-1107, 2011.

Zarangush Z, Mahdavi S, Determination of Antibiotic Residues in Pasteurized and Raw Milk in Maragheh and Bonab Counties by Four Plate test (FPT) Method Scientific. Journal of Ilam University of Medical Sciences, 24(5): 48-54, 2016.

Zeina K, Pamela AK, Fawwak S, Quantification of antibiotic residues and determination of antimicrobial resistance profiles of microorganisms isolated from bovine milk in Lebanon. Food Nutritin Sciences, 4(07): 1-9, 2013. http://khartoumspace.uofk.edu/handle/123456789/8637 .