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Prediction of Antibiotics Residues in Raw Milk by Using Binary Logistic Regression Model

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Abstract

medical compounds, especially antibiotics, in which remains in milk and dairy products on the one hand causes health problems such as allergic reactions and Development of bacterial resistance to antibiotics are a serious threat to the health of consumers , and on the other hand, industrial troubles such as failure to produce fermented products can cause the milk back to the rancher. The purpose of this study is to determine the presence of antibiotic residues in raw milk ,milk in a timely manner after receiving a detailed mathematical model. 120 samples of bulk tank milk were randomly selected from 10 collection centers and farms Industrial in the Province of Khorasan Razavi (Iran). The presence of antibiotic residues and microbial and physicochemical properties were evaluated. Then based on different variables , binary logistic regression model to predict the presence or absence of antibiotic residues in milk were determined using the software SPSS Statistics ver.22.0. Somatic cells and electrical conductivity was the main determined factor of model in summer season equation. Also in autumn season equation, somatic cells and acidity were the main factors. The results are indicative of the relationship between chemical and microbial antibiotic residues in milk.

Key words: Raw milk, Modeling, Antibiotic, Prediction.

Introduction

Milk has a major role in human nutrition at different ages and monitoring and supervising on health standards of it in process of production, transportation, storage and supply is essential. Today, different drugs are used for controlling diseases or accelerating growth that residues may transfer to the milk. The presence of antibiotics in milk and dairy products of are not acceptable in term of international standards because pharmaceutical compounds of the residues, specially antibiotics, in milk and dairy products cause health problems such as allergic reactions, digestive problems and spread of antibiotic- resistant strains of bacteria and drug resistance is a serious threat to the health of consumers. On the other hand, by industrial

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complications such as disruption in production of fermented dairy products can cause returning of milk to ranchers and its financial damages. All antibiotics which have been used appear in the milk after a while. So presence of antibiotics in raw milk should be controlled by dairy manufacturers (1). Antibiotics which are used to treat livestock include five main groups: Aminoglycosides (Gentamicin), Macrolides (Erythromycin), Quinolones, Sulfonamides (Trimethoprim), Penicillin and Cephalexin (β - lactam) (2, 3). According to a study by association of Illinois North West dairy farmers in America, it is clear that the lack of statistics and records about the treatment of clinical mastitis or inaccuracy of statistics, not observing of pouring milk away time after using antibiotics and also not observing medication instructions correctly are major reasons for existence of antibiotic in milk (4). The aim of this study is to determine the presence of antibiotic residues in raw milk in a short time after receiving milk by using precise mathematical models that leads to savings in expensive and time consuming microbiological tastings of quality control. In addition, such models can be very affordable and reasonable estimate of the quality of raw milk.

Materials and Methods

Sampling

According to the National Standards No. 326, 120 samples were randomly collected from 10 Khorasan Razavi milk centers and industrial farms in 4 repetitions and rate of 100 ml in the summer and fall (5). Random sampling was disciplined. Cold chain to preserve samples of raw milk and to avoid any changes in the measured parameters was applied during storage of raw milk to testing. In this study, it was tried to lower the time from sampling to testing.

Measuring Parameters

1. Measuring the somatic cells count

Somatic cell of whole samples were counted by fluoro opto electronic counters-Fossmatic 5000 basic, Denmark Foss Electric Company, based on the international standard Iso-13366 (6).

2. The total count of mesophilic microorganism

The total count of mesophilic microorganisms were measured based on 5484 national standards and according to international standard ISO 6610 (7).

3. Acidity

The acidity of raw milk was measured base on per grams of lactic acid percent according to the national standards NO.2852 (8).

4. PH

PH was measured by PH meter- model 720 inolab, made in Wilhelm of Germany at 20°c according to the national standard NO.2852 (8).

5. Electrical conductivity

EC was measured by EC meter - model720 inolab Cond, made in Germany Wilhelm Company at 25°c according to the method recommended by the manufacture.

6. Lactose

Polari metric assessment of raw milk lactose was done based on national standard NO.4449 (9).

7. Fat and Protein

Milk samples were evaluated in terms of protein and fat using Milk-scan Model 134 made in Foss Electric Company, Denmark, in accordance with ISO 1996: B 141IDF standards (10).

8. Identifying antibiotic residues

In this study, the test methods used for monitoring antibiotic residues in milk was Eclipse 50 kit which is made in Spain ZEU Company. This kit acts based on inhibiting the growth of microorganisms indicator of Bacillus stearothermophilus due to presence of antibiotics in milk. In the absence of antibiotics, after adding 50 ml of each sample to the kit and incubate in 65 ± 1 °C for 2.15 to 2.45 hours, Bacillus stearothermophilus grow by nutrients and with lactose fermentation and acid production in the presence of Bromocresol purple reagent causes a yellow color. While in the case of antibiotics, Bacillus stearothermophilus cannot grow and therefore the medium, without any changes in color, remains purple (Fig. 1, 2). According to the manufacturer's instructions of kits, formation of a yellow-violet indicates the presence of antibiotic residues in samples but inhibit microbial growth rate is less than the sensitivity of the kit (Table 1).

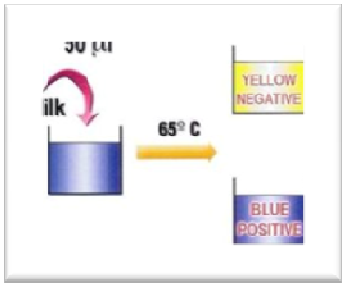


Fig. 1: Test Method

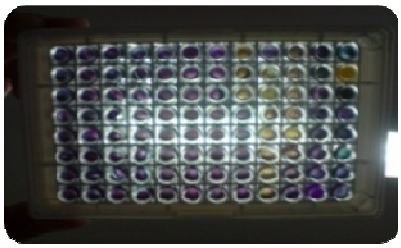


Fig. 2: Antibiotics Kit in raw milk samples

Table 1: Detection limit of the ECLIPSE test for several inhibitors (mg/ml) in cow´s milk

ECLIPSE 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
CEPHAPIRINE	0.005	0.008
SULFATHIAZOLE	0.02	0.075
SULFAMETHAZINE	0.1	0.2
SULFANILAMIDE	0.1	0.6
OXYTETRACYCLIN	0.05	0.15

TETRACYCLIN	0.05	0.15
ERYTHROMYCIN	0.2	0.4
TYLOSIN	0.02	0.1
NEOMYCIN	<0.500	0.80

Statistical Plan

After examination and getting results with 4 repetitions, possible regression equations between antibiotics residues parameters and chemical and microbial parameters of raw milk have been studied. The software SPSS Statistics ver.22.0 has been used to evaluate the regression equations and finding the best model, and drawing diagrams to predict.

Results

Achieving the best binary logistic regression model

After accomplishing of tests and collecting data in summer and autumn, binary logistic regression model has been chosen to describe and predict the presence or absence of antibiotic residues in raw milk and some tests have been done for achieving the best equation based on the dependent variable (antibiotic residues) and independent variables (somatic cell count, total count of mesophilic microorganisms, protein, fat, lactose, acidity, PH and electrical conductivity). In analysis by this regression, the code one means presence and the code zero means the absence of antibiotic residues in raw milk. One of the greatest methods to achieve the best logistic regression model is to use Backward Conditional Technique. The basis of this method is that with the help of software SPSS Statistics ver.22.0 once all the independent variables and their interactions with the dependent variable are considered as models. Then the software changes the variables from model based on probability ratio and this process goes on as far as the variables are not deleted because of the importance (Table 2). By using the mentioned technique, the following equations were obtained based on logistic regression, respectively, for summer and autumn:

- $\text{Logit}(\theta) = -81.267 + (7.731 \ \log \text{Somatic cell}) + (6.778 \ \text{EC})$
- $\text{Logit}(\theta) = -3.868 + (6.719 \ \log \text{Somatic cell}) - (245.439 \ \text{Acidity})$

Table 2. The variables in equations

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C. I. for EXP(B)	
								Lower	Upper
Summer	Log Somatic cell	7.731	3.260	5.623	1	.018	2277.317	3.824	1356389.426
	EC	6.778	3.691	3.373	1	.046	878.530	.634	1216988.365
	Constant	-81.267	32.096	6.411	1	.011	.000		
Autumn	Log Somatic cell	6.719	2.422	7.698	1	.006	828.397	7.189	95451.644
	Acidity	-245.439	98.242	6.242	1	.012	.000	.000	.000
	Constant	-3.868	13.534	.082	1	.775	.021		

To achieve the best model, parameters such as the coefficient of the Cox and Snell and correlation coefficient of Nadgelkrke should be considered and also there should be no correlation between the independent variables in the final model. In achieving of mentioned models, the these indicators have been considered. The linear regression, the value R^2 shows the amount of similar variance of the dependent variable. The two correlation coefficient Cox & snell and Nadgelkrke are the same as squared correlation coefficient of linear regression. The minimum rate of Cox & snell is zero, but the maximum is unknown, due to this reason it is difficult to interpret. However the coefficient of Nadgelkrke like Pearson correlation coefficient has values between zero and one that the interpretation is very simple. The results are shown in Table 3.

Table 3. Summary of Model

	Chi-square	df	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
Summer	9.571	8	19.086	0.595	0.843
Autumn	9.580	8	19.468	0.492	0.777

Base on models analysis, there are a similar variance about 3.84 and 77.7 percent between the independent variables and the dependent variable in the summer and autumn. Thus it can be argued that there is almost a good correlation between the independent variables and the dependent variable in two seasons. According to Table 4 in the summer, all 42 samples were not contaminated with antibiotic residues that were predicted correctly by the model. But among the 18 samples, only 14 were correctly predicted as contaminated with residues of antibiotics. In other words, predicting samples with no contamination and samples contaminated with antibiotic residues, is respectively, 100% and 77.8 percent. Overall, the model has predictive power about 3.93 percent.

Table 4. Classifying the proposed model in summer

observed		predicted		
		Antibiotic		Percentage correct
		0	1	
Antibiotic	0	42	0	100
	1	4	14	77.8
Overall percentage				93.3

Table 5. Classifying the proposed model in autumn

observed		predicted		
		Antibiotic		Percentage correct
		0	1	
Antibiotic	0	46	2	95.8
	1	0	12	100
Overall percentage				96.7

As can be seen in Table 5, in autumn, the binary logistic regression model has correctly predicted 100% of samples contaminated with antibiotic residues and 95.8% samples that were not contaminated with antibiotic residues. In total, the model has predictive power for about 7.96 percent. Diagrams of predicted and observed probability distribution of raw milk samples (Dig. 1) and also Case wise List tables, the samples which were not classified correctly in the related model, are as follow (Table 6 , 7).

Table 6. Case wise list of summer model

Case	Selected Status [a]	Observed	Predicted	Predicted Group	Temporary Variable	
		antibiotic			Resid	ZResid
23	S	1**	.340	0	.660	1.392
30	S	1**	.077	0	.923	3.470
31	S	1**	.346	0	.654	1.374
54	S	1**	.152	0	.842	2.317

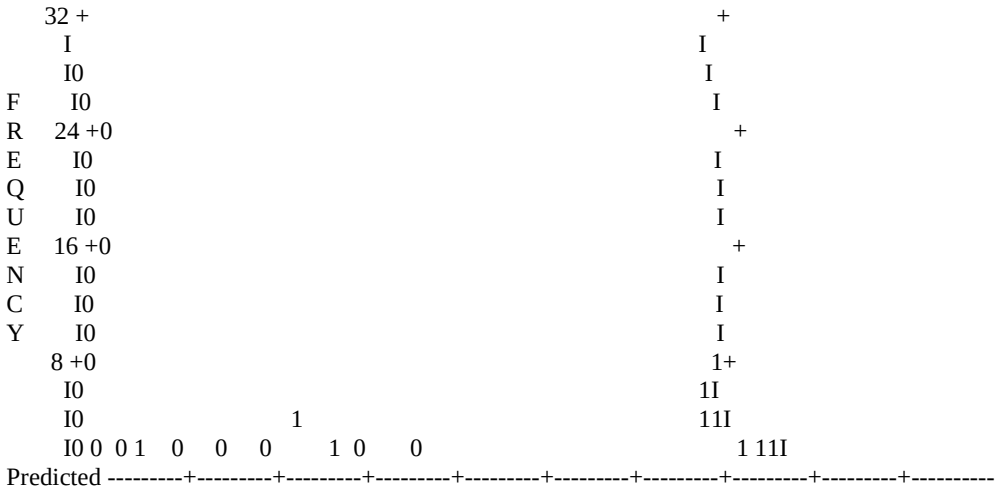
Table 7. Case wise list of autumn model

Case	Selected Status [a]	Observed	Predicted	Predicted Group	Temporary Variable	
		antibiotic			Resid	ZResid
26	S	0**	.938	1	-.638	-1.297
57	S	0**	.725	1	-.838	-3.892

a. S = Selected, U = Unselected cases, and ** = Misclassified cases.

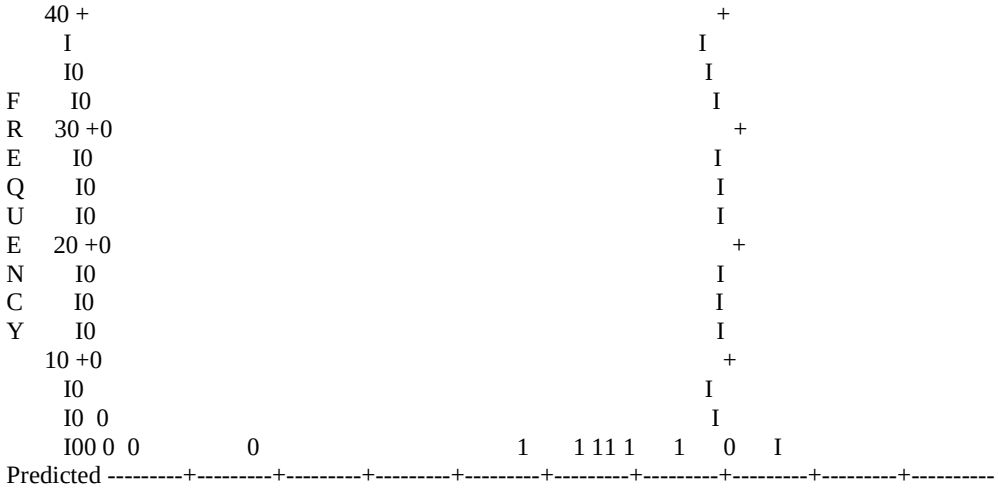
Cases with studentized residuals greater than 2.000 are listed.

Observed Groups and Predicted Probabilities (Summer)



Each Symbol Represents 2 Cases.

Observed Groups and Predicted Probabilities (Autumn)



Predicted Probability is of Membership for 1.00

The Cut Value is .50 / Each Symbol Represents 2.5 Cases.

Diagram 1: Diagrams of predicted and observed probability distribution of raw milk samples in summer and autumn

Considering the presence of antibiotic residues in industrial farms and milk collection centers

Statistical analysis showed that in the summer from the total samples, 70% were without contamination and 30% were with antibiotic residues. From 42 samples without contamination to antibiotics, 28 samples were related to industrial farms and 14 samples were related to the milk collection centers that the percentage of cases, respectively, were 66.67% and 33.33%. While from the 60 samples of raw milk in autumn, 80% (48 samples) were without contamination and 20% (12 samples) had antibiotic residues and all 12 samples contained residues of antibiotics have been related to milk collection centers. The study showed that the number of samples contaminated with antibiotic residues in autumn were 10% less than in summer (according to the results of Fig. 3).

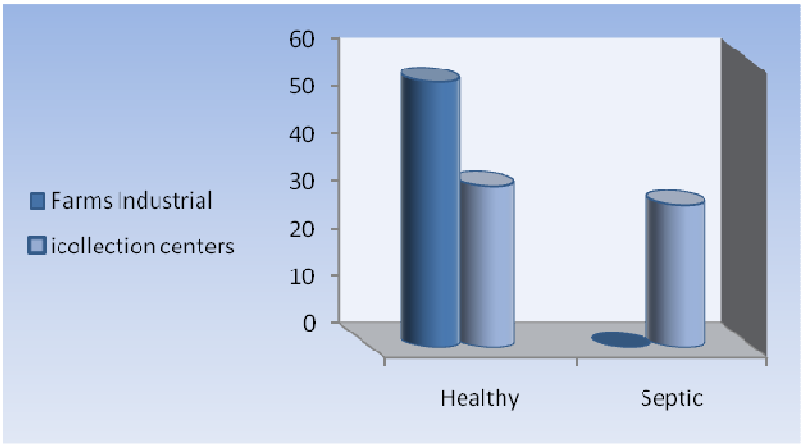


Fig. 3: Comparison of healthy and antibiotic residues samples of milk in industrial farms and milk collection centers

Statistical analysis showed that among the samples of raw milk from industrial farms and province milk collection centers there are significant differences (P < 0/01) in term of contamination to antibiotics.

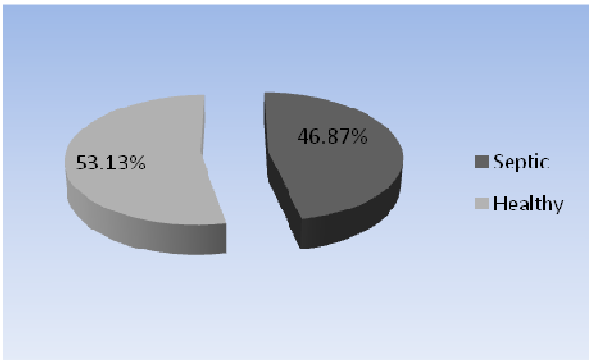


Fig. 4: The contamination rate of raw milk collection centers to antibiotic residues

Discussion

The amount of Nadgelkrke correlation coefficients obtained during the two studied seasons shows a good compatibility of predicted values and experimental observations. The results of this study indicate a relationship between chemical and microbial variables with antibiotic residues in raw milk. This correlation is so impressive that based on the variables and conditions on milk, prediction models of antibiotic residues can be generalized. The results of this study showed that the increase in the number of somatic cells in raw milk cause problems of antibiotic residues on more number of raw milk samples. Norberg and et al (2005) and Ogola and et al (2007) declared in their studies that by increasing somatic cells, changes in amount of mineral of raw milk occur. The changes will affect in the type and amount of milk minerals, acidity and electrical conductivity. So that by increasing sodium and chlorine and decreasing potassium and calcium, conductivity increases and acidity decreases to less than 0/14 percent. Due to the high correlation between these chemical variables and antibiotics residues in raw milk, based on those, the presence or absence of antibiotic residues in milk can be predictable (11, 12).

According to the obtained statistical results, it is shown that the rate of drug residues in raw milk of collection centers is more. In traditional farms, due to the absence of health authorities that should monitor health issues, disease prevention and treat sick animals, antibiotics are given to livestock inappropriately and do not observe waiting time for sure. Research results of Habibi (2010) also showed that antibiotic residues in milk of Sanandaj (City) industrial farms were more than semi-industrial farms and milk collection centers. The result was that 103 samples were negative and 64 samples were positive in terms of presence of antibiotic (13). Also Manafi and et al (2010) in a similar study for evaluation of antibiotic residues in raw and pasteurized milk in East Azarbayejan province, 20 samples of raw milk and 6 samples of pasteurized milk have been tested by Delvotest SP-NT method. 46% of raw milk samples and 30% of pasteurized milk samples were detected to contaminate with antibiotics.

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