

# Raw cow milk quality: Relationship between antibiotic residue and somatic cell count

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# <u>Abstract</u>

The aim of this study was to evaluate the relationship between antibiotic residues and somatic cell count (SCC) value in raw cow milk. The milk samples (n = 200) were randomly collected during summer and winter seasons from dairy farms in Tabriz. In this study presence of antibiotic residues in raw cow milk, evaluate SCC value, and relationship between milk SCC and antibiotic residue value in raw milk were investigated. All samples were examined by Copan milk test and somatic cell counter, also relationship between SCC and the risk of antibiotic residue violation was determined. Out of 115 samples were positive for antibiotic residues. Antibiotic residue in winter season was significantly higher than in summer season (P < 0.05). Also SCC rate was considerably high during winter season compared with summer samples (P < 0.05). The SCC value was significantly higher for milk samples with positive antibiotic residue and SCC (r<sup>2</sup> = 0.305, P = 0.002). According to adverse effects of antibiotic residues, which can be considered an important factor threatening human health and numerous losses that would caused in the milk industry, this seems to be a need for more studies to determine the exact type of antibiotic residue in milk consumption in this province.

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#### Introduction

Milk is one of the most nutritious and complete food. It is rich in high quality protein providing all ten essential amino acids, fat especially essential fatty acids, most of the mineral and vitamins. Meanwhile milk as a nutrient has the main role in human diet, especially for children (Hassan, 2005; Enb et al., 2009). Concerns about food safety, with animal source foods are increasing in developing countries where urbanization, increasing incomes and changing of life-styles are associated with greater dependence on marketed foods by an increasing number of people (Delgado et al., 1999). The safety of food is threatened by various agents including pathogenic microorganisms, aflatoxins, pesticides and antimicrobial agents. Pathogenic microorganisms constitute the most important food related to threat public health. Little information about level of the antimicrobial residues in food is found in developing countries. While pasteurization and other forms of heat treatment eliminate pathogenic microorganisms from animal source food. These procedures have

\*Corresponding author. Email: *mahmodi@tabrizu.ac.ir* Tel: +98 (0) 411 3392378 Fax: +98 (0) 411 3357834 limited or variable effects have on drug residues in animal originated food (Moa, 1988; Wang *et al.*, 2006).

Dairy cattle are susceptible to many diseases particularly mastitis, in this regard, somatic cell count (SCC) is used as a key indicator of milk quality and reflects the prevalence of subclinical mastitis in a dairy herd. The SCC is an indirect measure of the overall amount of mastitis and herds with high SCC have been reported to have higher rates of clinical mastitis (Rodrigues et al., 2004). Farms experiencing consistently high SCC have considerable motivation to reduce the number of infected quarters. Treatment of infected quarters using antibiotics is one practice used to control mastitis. The use of antibiotics introduces the risk of having an antibiotic residue. Indiscriminate use of antibiotics and mistakes regarding withholding periods causing antibiotic residues in milk and meat. The occurrence of antibiotic residues in milk intended for human consumption is undesirable for a number of reasons such as: cause allergic reactions, incidence of bacterial resistance, disrupting of the balance of gut microflora, carcinogenesis, mutagenesis and



Figure 1. Guidance of the using Copan milk test kit (yellow ampoule after incubation indicates a negative samples & purple ampoule indicates positive samples)

malformation risks. In addition, the presence of antibiotic residues in milk be used in dairy industry can have adverse effects on production of fermented dairy products such as yogurt and cheese (Suhren, 2002; Tikofsky *et al.*, 2003; Erskine *et al.*, 2004; Mohsenzadeh and Bahrainpour, 2008; Movassagh and Karami, 2010). The objectives of this study were to investigate the occurrence of antibiotic residues in raw cow milk, evaluate SCC value, and survey the relationship between milk SCC and antibiotic residue value in raw milk.

# **Material and Methods**

#### Sampling

A total of two hundred raw cow milk samples were randomly collected from Tabriz suburb dairy farms (25 milk samples every month during July 2012 to January 2013).

#### Antibiotic residue analysis

The Copan milk test (CHR. Hansen, Denmark) was used to detection antibiotic residue in milk samples. This test can detect a wide range of antimicrobial substances at different concentration levels. Copan Milk Test is inhibition assays and consist a microplate containing spores of Bacillus stearothermophillus and nutrient. With addition of milk and subsequent incubation (3 h at 64°C), spores germinate and produce carbonic acid. This acid causes the bromocresol purple indicator in the ampoule to change the color from purple to yellow. Therefore, a yellow ampoule after incubation indicates a negative sample. The presence of antimicrobials inhibits the growth of bacteria and the color of the agar in the ampoule remains purple, indicating a positive result (Figure 1) (Le Breton et al., 2007).

#### Somatic Cell Count

SCC analysis was done electronically by flux cytometer (Fossomatic 90), also results of SCC was expressed 10<sup>3</sup>/ml.

## Statistical analysis

All experiments were conducted in triplicate, and statistical analysis was performed using SPSS 17.0

(SPSS 17.0 for Windows; SPSS Inc.). Data were subjected to analysis of variance (ANOVA). Results were considered statically significant when P < 0.05.

## **Results and Discussion**

#### Antibiotic residue

The results of monitoring antibiotic residues in milk samples are presented in table 1. Based on the results, 115 samples (57.70%) had antibiotic residue (Table 1). In general, the winter milk samples (32.50%) had significantly the most contamination with antibiotic residues compared to summer milk samples (25.00%) (P < 0.05) (Figure 2). Today, the daily consumption of milk and dairy products make up an important part of diet, suggested the accuracy and sensitivity of laboratory methods for detection and assessment of pharmaceutical residues to be so that an acceptable level in order to protect the health of consumers.

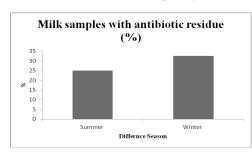
The results of many researchers have showed Delvo test and Copan milk test are that both proper to detect of antibiotic in milk samples. Le Breton et al. (2007) investigated performance of the Copan milk test and Delvo test to detection of specific antibiotics residue in milk. They reported, both experiments are capable to detect penicillin, cloxacillin, sulfamethazine, sulfadiazine, cephalexin and gentamicin at or below the EU maximum residue limits, also both tests were found easy to use, robust and fulfilled EU requirements. In the other hand, it is possible that several types of antibiotics and other inhibitors in milk exist and values of each these compounds less than the maximum tolerance, but there is a limit combined together, they can be harmful to human health (Le Breton et al., 2007).

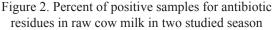
Manafi *et al.* (2010) evaluated antibiotic residues in raw and pasteurized cow milk in Tabriz using the Delvo test, their findings showed that 26% of raw milk samples from dairy farms and 16% of raw milk samples from milk collection centers contained a variety of antibiotics residue. Detection of antibiotic residues in raw cow's milk in Tabriz using Copan milk test showed 5 milk samples (10%) contaminated with antibiotic residues (Movasegh, 2011).

In the other study, evaluation of raw and pasteurized milk samples in Mashhad province showed that more than 76.11% of evaluated milk samples had gentamicin antibiotic residue (Fallah *et al.*, 2005). The results of the evaluation of tetracycline and oxytetracycline residues in milk samples in Tehran province indicated that above 8.7% of milk samples were positive for these antibiotic residues

Table1. Mean values of SCC in raw cow milk samples and relationship with antibiotic residue

Season	<b>Sample size</b>	Somatic cell Count (1000cells/ml)		Positive antibiotic residue N (%)
		Mean ± SD	Min-Max	Fositive antibiotic residue IN (%)
Summer	100	890.71±250.73ª	5-15195	50 (25.00) <sup>a</sup>
Winter	100	1052.00±321.26 <sup>b</sup>	9-25195	65 (32.50) <sup>b</sup>
Total	200	971.35±301.68	5-25195	115 (57.50)
Means $\pm$ SD in the column with different letters are significantly different (P < 0.05).				





(Rasouli et al., 2005).

#### Milk SSC

The mean SCC of summer raw milk was 890.71  $\pm$  250.73  $\times 10^3$  and the winter milk samples had the highest SCC with 1052.00  $\pm$  321.265  $\times 10^3$  (Figure 2).

# Relationship between antibiotic residue and SCC value

In many regions, the SCC is used to define financial incentives paid for high quality milk and herds shipping milk containing high levels of somatic cells may have a significant financial disadvantage (Rodrigues *et al.*, 2004). Many studies have consistently identified a relationship between SCC and the level of antibiotic residue (Sargeant *et al.*, 1998; Ruegg and Tabone, 2000; Saville *et al.*, 2000; Van Schaik, 2002). The results of the study in Wisconsin on determination of relationship between SCC and the risk of antibiotic residue in milk samples showed that SCC value was significantly high for milk samples with positive antibiotic residue (Ruegg and Tabone, 2000).

The SCC can be an indicator to recognize mastitis (Poutrel, 1981). Also, the SCC can be high in health udder in some conditions such as: milk yield, flock management, colostral period, and the end of lactation periods, cow age, season and stress, (Fruganti *et al.*, 1985; Konig *et al.*, 1985). Our results indicated that the antibiotic residue in milk samples was in a statistically highly significant correlation ( $r^2 = 0.305$ ) with SCC in milk samples, Also SCC rate (Table 1) was considerably high during winter season compared with summer samples (P < 0.05). The SCC value was significantly higher for milk samples with positive antibiotic residue (P < 0.05).

#### Conclusion

Adulteration of milk supplies with antibiotics is clearly undesirable and the regulation of milk supplies to prohibit antibiotic residues is useful to protect public health. Researchers have identified a consistent relationship between SCC and the occurrence of antibiotic residues. Interventions that reduce the prevalence of subclinical mastitis and therefore reduce the need for antibiotics may have an added benefit of further reducing the risk of antibiotic residues.

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