Journal of Coastal Life Medicine

Journal homepage: www.jclmm.com

Document heading doi: 10.12980/JCLM.2.201414J8

Determination of antibiotic residues in the pasteurized milk produced in West Azerbaijan province, North West of Iran

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PEER REVIEW

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Comments

The information presented in the study indicates the relevance of antibiotic contamination. The design of the study was good and the method used for investigation was appropriate, easy to follow and was able to achieve the intended goals.
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ABSTRACT

Objective: To monitor antibiotic residues in pasteurized milk in West Azerbaijan province, North West of Iran.
Methods: In this study, 848 pasteurized milk samples were collected from factories and tested for the presence of antibiotic residues using the Copan test kit based on the manufacturer's instructions.
Results: Results indicated that 30.14% of samples were contaminated with a variety of antibiotics based on the detection of associated residues and 3.19% of these samples were suspected. Given the current rise of antimicrobial resistance among microbial pathogens, these findings amplify the need to ensure continuous monitoring of pasteurized milk that intended for human consumption.
Conclusions: Continuous monitoring of pasteurized milk may improve human health but also limit the development and transmission of antibiotic resistant strains in the environment.

KEYWORDS
Antibiotic residue, Pasteurized milk, Monitoring, West Azerbaijan province, Iran

1. Introduction

Antibiotics are antimicrobial substances which are produced by microorganisms as secondary metabolites and low concentration of these substances can destroy bacteria or preventing their growth by affecting some metabolic or structural elements that required for survival[1]. Unlike primary metabolites, these are compounds that are not essential for growth and reproduction and they are produced by microorganisms due to variety of reasons[1]. Therefore, antibiotics are commonly used for treatment, control of infectious diseases and also stimulating growth in animal species[1]. Mastitis is an infection that affects cow in dairy farms worldwide and poses a lot of financial burden to the farmer. According to studies performed, approximately 40% of cattle herds are affected by mastitis yearly. However, this disease is commonly controlled using antibiotics[2].

In animals the application of antibiotics in less than therapeutic concentrations (1-10 mg/kg of feed) for long periods may cause an increase of animal growth rates[3]. It is suggested that this results in an improvement in the efficiency in which feed is digested and absorbed in the digestive system. Consequently, animal weight may increase and this may result in changes in the composition

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Foundation Project: Supported by Food and Drug, Urmia University of Medical Sciences, Urmia, Iran (Grant number of this work research is 324-6543-92).

Article history:
Received 30 Jan 2014
Received in revised form 7 Feb, 2nd revised form 26 Feb, 3rd revised form 14 Mar 2014
Accepted 10 Apr 2014
Available online 28 Apr 2014

of microbial flora in the digestive system[3]. Therefore, uncontrolled use of antibiotics for treatment and prevention, do not only compromise the time needed for these drugs to be excreted in faeces by the animal but may also facilitate the progression of antibiotics to milk. The presence of antibiotic residues in milk may result in allergies in humans, and in the long run may facilitate the development of resistant bacteria pathogens[4]. Moreover, the presence of resistant strains usually poses severe health consequences on humans and huge burden to medical profession[4].

Methods used in the detection of antibiotic residues in milk usually depend on the type of antibiotic targeted, the expected time limitations, selectivity and its cost. These methods that are used for the measurement of antibiotics may be qualitative and quantitative[5,6]. Overall, three methods are identified including immunological, microbiological and analytical techniques. The Copan test methods are known to be the best microbial inhibitor tests for detecting antibiotic residues[7]. The test is performed by culturing milk samples on solidified agar plates and plates are incubated for 3 h at 64 °C. The detection of color change from purple to yellow resulting from the production of acid due to bacterial activity is considered a negative test. This test can be used to detect a wide variety of antibiotics that include sulfonamides, tyluzyn, erythromycin, neomycin, gentamycin and trymptuprym[7]. A study has been conducted to compare the performance of antibiotics using diagnostic tests such as high performance liquid chromatography (HPLC) and expressed that it is highly sensitive results obtained using the disk diffusion method[8].

The Copan test is an appropriate technique for detecting antibiotic residues in milk and results obtained have been reported to show comparable sensitivity with the HPLC. However, the Copan test is a microbial test that is highly sensitive against beta-lactam antibiotics, tetracycline and sulfonamides. The principle behind the test is based on the fact that microbial growth is prevented. Considering that antibiotic residues in food can cause detrimental effects on consumers, quality control of all food products for these residues is highly imperative. This is even more important in milk that is known to constitute a special part of human diet based on its nutrient composition. Unfortunately, in Iran food quality control measures are given very little attention and it is suggested the implications on consumers may be alarming. The objective of the current study was to monitor antibiotic residues in pasteurized milk obtained from factories in West Azerbaijan province, Iran.

2. Materials and methods

A total of 848 of pasteurized milk samples produced in West Azerbaijan province of Iran during year 2010 were collected randomly by inspectors of Food Control Laboratories of Urmia University of Medical Sciences. The sampling was performed according to standard procedures set by the Institute of Standards and Industrial Research of Iran-2836. Samples were transported on ice to the laboratory for the control of food. They were analysed for the presence of antibiotic residues.

2.1. Search method for antibiotic resisdues

The Copan test kit of Denmark Christian Hansen Company was used to monitor antibiotic residues in milk. Aliquots of 100 μL of milk samples were poured into the kit that contained *Bacillus stearothermophilus* var. *calidolactis* using a micro-pipette and the kits were incubated at 64.5 °C. Results were recorded after 3 h. In the absence of antibiotic residues, microbial growth is identified. During growth, the microorganism uses nutrients, ferments lactose and produces lactic acid which results in a change in the colour of the purple compound bromocresol to yellow. On the contrary, if there are antibiotic residues *Bathymodiolus thermophilus* is unable to grow and the purple colour of the medium remains unchanged.

The color of suspected samples would be between purple and yellowish; hence, determination of the presence of antibiotics in these samples was not conclusive. Kit reading guide is specified in Figure 1.

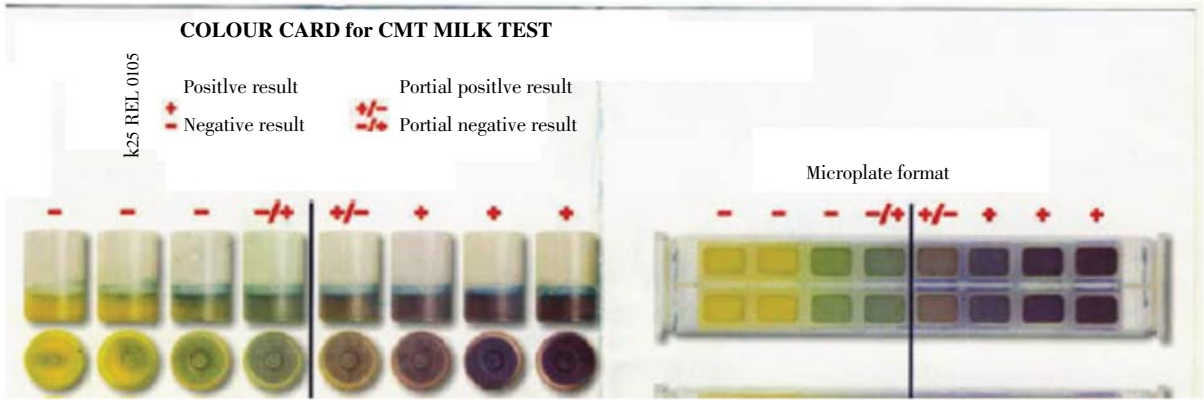

Figure 1. Kit reading guide.

2.2. Statistical analysis

In order to evaluate the data obtained, the SPSS 16 software was used to determine significant differences between data with One-way ANOVA. The Tukey test was used in comparing treatment samples.

3. Results

In this study, 848 pasteurized milk samples collected from the Urmia city were examined for the presence of antibiotic residues. The amount of positive, suspected and negative cases of antibiotic residues were 255, 27 and 566, respectively. In 30% of cases antibiotic residues were observed, which was a high percentage. Milk consumption is high, so drug residues will impact on the health of society. Of total 848 milk samples, 30% cases were eligible for antibiotic residues.

4. Discussion

Unfortunately, in Iran many studies havn't been performed in conjunction with antibiotic residues in milk.

Copan test is a rapid, specific and applicable method that is useful in regulatory and production centers. Although it doesn't show the level of contamination with the different antibiotics in milk, it provides indication of the presence of the antibiotics by inhibiting microbial growth. However, the high sensitivity ensures that even trace amounts of contaminating antibiotic residues are detected. It is been shown that even 2-3 mg/L penicillin, 6-7 mg/L ampicillin and 3-5 mg/L amoxicillin residues have been detected in milk[9].

On the other hand, the remaining authorized amount of antibiotics according to credible standard, is characterized that detection limit of this test is comparable with international standards such as CODEX. For example, the limit rate of penicillin residues in milk according to Europe Union and CODEX is 4 mg/L and in the American standard is 5 mg/L while detection limit of Copan test for penicillin is less than or equal to this amount. The ampicillin detection limit of the test is identical with the Europe Standard and this is similar with the other antibiotics.

Copan test is validated in scientifically reports that it is able to detect these compounds within the maximum residue limit with very small quantity[9].

The results of conducted researches about the efficiency of Copan test in the diagnosis of various antibiotics in milk have also confirmed the above mentioned. In a study which investigated the performance of Copan and Delve tests in the diagnosis of specific antibiotics in milk, it was reported that both tests can recognize penicillin, keloksasillin, sulfomethazin, cephalexin and gentamicin in equal or less than the detection limit of Europe Unions standard[10]. They introduced both experiments, simple, practical and efficient to provide Europe Unions conditions. The results of this study showed that on average 30.14 percent of the samples of pasteurized milk produced in the West Azerbaijan province were including antibiotic residue and 3.19 percent of them are suspected.

Copan test will be able to evaluate all of the antibiotics together, which can be useful from nutritional and functional view point. It is possible that several specific types of antibiotics and other inhibitors exist in milk. However, the concentration of each of these compounds in less than the maximum amount is allowed, but when combined together they can be harmful to health. In this case, antibiotics may be assessed by Copan test and some manufacturers have designed the kits that can detect the presence of residual antibiotics in maximum residue limit[10].

Some studies have been conducted in Iran to determine the level of antibiotic residues in milk[11]. The results of a previous study indicated that 1.7% of sheep milk contained antibiotic residues and this was considered to be low[11].

Study of Addison and Webb in Trinidad showed that 10.8 percent of sheep's milk were containing antibiotic residues[12].

In a study by Ghidini *et al.* in Italy on residual beta-lactam antibiotics in cow's milk, lactam antibiotic residues were detected in the samples[13]. In general, antibiotic residues in developed countries are often found in the milk of animals that have been treated with excessive doses, while in developing countries and third world countries due to lack of milk and meat withholding period after the use of antibiotics and other animal drugs, in most cases the antibiotics residues and animal drugs are observed[14].

Justification in high presence of the contaminated samples can be expressed in the dairy industry. Healthy raw milk without antibiotics and preservatives are usually used to produce fermented dairy products like yogurt, butter milk and cheese.

Studies by Žvirdauskienė and Šalomskienė compared the the tests such as LPT, MaI-1, Copan, Valio T 101, Penzym S, β-STAR, SNAP and ROSA tests showed that the Copan test was simple to use and interpret, had a long shelf life and was sensitive to all β-lactams[15]. Since the dairy factories rely on milk for production of yogurt and cream and dairy from the very first, thus milk containing antibiotics can cause economic losses. Our results show that Copan test is a rapid test and can be applied to detect antibiotic contamination in milk.

Milk is an important food and existing of antibiotic residues in milk in long-term will bring many disadvantages, especially the rapid rise of resistant pathogens. Also some investigated milk is the majority brand in the market.

Nowadays, food safety is given little attention. Concerns should be arised in the Food Safety Management Department regarding the presence of antibiotics in livestocks and pasteurized milk production.

The use of antimicrobials is to prevent the creation of a solution of the chemical residues of antibiotics[16-27].

In developed countries, antibiotics residues are often seen in dairy cattle that are treated with excessive doses while in developing and third world countries, in most instances, the remains can be seen in animal drugs due to lack of time to avoid milk and meat consumption of antibiotics and other animal drugs.

Nowadays, it is recommended that the sensitivity of laboratory methods used must be enough to protect the health of consumers with determining the upper limit of acceptable level of antibiotics. In our study, we found Copan test has high sensitivity and it is a reliable method for monitoring antibiotic residues in milk.

The recommended continuous controls instead of temporary and case control are enforced for detecting microbial growth inhibitors and antibiotics in milk collecting centers and in livestock and dairy factories.

Given the importance of monitoring antibiotic residues in milk, Copan test is suitable method for the detection of antibiotic residues in pasteurized milk.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the Deputy for Food and Drug, Urmia University of Medical Sciences, Urmia, Iran for their financial supports. Grant number of this work research is 324-6543-92.

Comments

Background

Antibiotics are agents which are produced by microorganisms as secondary metabolites and low concentrations of these substances can destroy bacteria by affecting some metabolic or structural elements that required for survival. Uncontrolled use of antibiotics for treatment and prevention of diseases, do not only compromise the time needed for these drugs to be excreted in faeces by the animal but may also facilitate the progression of antibiotics to milk and the environment. The presence of antibiotic residues in milk may result in allergies in humans, and in the long run may facilitate the development of resistant bacteria pathogens. Moreover, the presence of resistant strains usually poses severe health consequences on humans and huge burden to medical profession. It is therefore important to determine the occurrence of these metabolites in food products and the environment.

Research frontiers

This study was designed to determine the occurrence of antibiotic residues in milk and therefore indirectly evaluate the potential of these products in developing and transmitting resistant isolates to humans. This usually has huge medical and therapeutic implications. Despite the fact that a lot of effort is put into screening bacterial pathogens for antibiotic resistant genes, little is done to screen both food products and water for the presence of antibiotic residues.

Related reports

The Materials and methods section was well addressed and any other suggestions have been introduced. However, the authors showed adequate link between their work and previous studies.

Innovations and breakthroughs

The method used was a simple test that was straight and easy to follow. I strongly suggest that in future organisms should be cultured from these samples and their antibiotic resistant profiles be determined. The identities of the isolates can later be determined using some molecular methods.

Applications

It is known that uncontrolled use of antibiotics results in contamination with residues in the environment. This is evident from information obtained in literature. Given the fact that waste water from hospitals and farmlands in which antibiotics are used as growth promoters, considerable efforts have been made to demonstrate the occurrence of antibiotic residues in milk and therefore indicate the health risks associated with the consumption of these products. Although this serves a baseline study, it is suggested that the investigation should be improved to cover a larger sample scale and should involve a number of detection methods.

Peer review

The information presented in the study indicates the relevance of antibiotic contamination. The design of the study was good and the method used for investigation was appropriate, easy to follow and was able to achieve the intended goals.

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