THE USE OF RAPID SCREENING TESTS FOR THE DETECTION OF ANTIBIOTICS IN MILK

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Abstract

Drugs used in animal farms can affect the public health, because of their trace in edible animal tissues. Antibiotics are an integral part of treatment in many infectious diseases. They are widely used therapeutically, as growth promoters and to prevent infection in animals. After systemic or intramammary administration of antibiotics in animal, antibiotic residues in milk can be detected. In fact, consumers can inadvertently be exposed to these chemical compounds. Antibiotic residues are important for three major reasons. First, they can cause antibiotic resistance in microorganisms. Second, antibiotics have side effects therefore; feeding of edible products containing antibiotic residues can cause similar complications. The possible complications can vary from slight and transient changes in the body's natural flora to severe allergic reaction. Third, the bactericidal and bacteriostatic activity of these compounds are interfering with the production process of some dairy products. In the current study that was evaluated, the contamination level of antibiotic residues in 75 raw cow milk samples from small farms in the region of Fier, Lushnja and Kavaja. The detection of inhibitory substances in milk was carried out using a rapid diagnostic system, Beta-lactam AuroFlow ™ kit. 8/75 (10.6%) milk samples resulted strongly positive, while other slightly positive 3/75 (4%) samples.

Keywords*: Milk, Beta-lactam, rapid test, public health*

1. Introduction

Veterinary drugs are pharmacologically and biologically active chemical agents especially for the treatment and prevention of animal diseases. Currently, veterinary drugs are widely used to protect animal health. This is associated with tremendous growth and intensification of animal production, (Heeschen W. H, 1993).
Antibiotics and other medications are used to combat various diseases. The most important use and most frequently used group with veterinary drugs is that antimicrobial agents (Fischer W.J., Tritscher A.M., Schilter B., Stadler R.H., 2003). In some cases these are also used as supplementary food. A crucial change in the use of complementary agents designated

as antibiotic growth stimulators has been brought by Regulation of the EU, imposing a ban on further use of these agents for animal nutrition as from 1-st January 2006 (EC, No 1662/2006). In lactating cows, microbial agents are used primarily for the treatment of mastitis but also to other diseases (respiratory diseases, metritis etc.). Prolonged use of antimicrobial is used in the treatment of dry cows, (Herrman J.L, 1995). Antibiotics used to cow during the lactation can pass into breast milk at different level. A source for contamination of milk is intramammary infection and different antibiotics used for treatment. Detecting violative levels of antimicrobial residues in commingled milk through the use of residue screening tests can help prevent contaminated milk from entering the human food supply. Testing at this level, however, does not protect the dairy producer from the loss of milk and financial penalties due to a contaminated bulk tank (Andrew, 2000).

Although milk is not regulated at the individual cow basis, a study conducted by McEwen et al. (1991) determined that the use of antibiotic screening tests for milk from individual cows was associated with reduced risk of bulk milk residue incidence when used under field conditions. A frequents and prevailing source of the milk contamination is the intramammary administration of a specific antibiotic. Other pathways for the milk contamination are coetaneous, subcutaneous, intramuscular and intravenous, drugs administration (Heeschen W. H., Blüthgen A., 1991).

In many countries, veterinary medicine is allowed to use only those agents that are officially registered and approved. In drugs which are registered and approved for use with food-producing animals, protection periods are prescribed during which the quantity of residues in foodstuffs of animal origin (meet, eggs, and milk) should be reduced to a level not threaten the consummator's health. Countries worldwide rely on regulatory agencies and international committees in evaluating the safety of all drugs used with food animals for potential human health risk as an integral part of the drug registration process.

Codex Alimentary and joint FAO / WHO program have been developing the standards concerning residues in food since 1985. These standards are based upon scientific assessment performed by joint FAO / WHO Expert Committee on food. Additives (JECFA) determining the acceptable daily limits (ADIS) and making recommendations for maximum residue limits (MRLs), (Heeschen W.H., Blüthgen A., 1991; Honkanen-Buzalski T., Reybroeck W. (1997). Consequently, in EEC countries the approval of an antibiotic or a sulfonamide drug for treatment of farm animals will require, (Heeschen W.H., 1993), fixation of Noel / ADI, development of suitable deductive method and fixation of the withholding period on the basis of residues detected (ADI / MRL).

For the international registration of veterinary drugs to the EU, the Committee for Medicinal Products for Veterinary Use (CVMP) has been established. CVMP based on toxicological assessment sets the MRL levels for the pharmacologically active chemical agents of the veterinary medicinal products occurring in foodstuff. The establishing of MRL level in EU is regulated by the Council Regulation, (EEC. No 2377/90). All veterinary drugs at the European market, specified for food animals must be toxicologically assessed and categorized in Annexes I-IV, depending on the type of MRL.MRLs plated represents international acknowledged limits which specify maximum residues that may be found in foodstuffs of animal origin.

According to (Council Regulation, EEC., No 2377/90), business operators must introduce procedures ensuring that raw milk will not be marketed if it contains the residues of antibiotics in quantities exceeding the levels for any of the substances authorized in the Annexes I and III, (Council Regulation, EEC., No 2377/90), or if the overall content of all antibiotic residues exceeds the maximum residue limits. Currently, however, there is no method which can detect all these agents at the level of established MRL values. In order to

provide for the high technological of raw milk and the same time the safety of milk and milk products for the consumer, IDF has developed a so called integrated system of checking veterinary drugs in milk and milk production to meet in control of drugs in milk and milk products milk. The system recommends various methods for the detection of antibiotics and defines responsibilities for the health safety of milk and milk products of particular subjects in the whole of the technological producing and processing milk (Honkanen- Buzalski T., Suhren G., 1999). The reasons for monitoring veterinary drugs residues in foodstuffs and food of animal origin include the ethnical ones (preventing undesired exposition of healthy consumers to therapeutically doses of drugs in food), hygienic (protection against possible harmful effects of the residues on the consumer's health), technological (preventing the disruption of fermentation processes) and ecological (Mäyrä Mäkinen A., 1995).

2. Material and Methods

2.1 Regions where the study was conducte

The study was conducted in 13 small farms in the region of Lushnja, Fier and Kavaja during the period of March – June 2015. In this region there are also major national issues in the livestock industry and milk production.

2.2 Sampling

Sampling was carried out according to standardized criteria (SSH ISO 707: 1999 method, "The method of sampling for milk and milk-based products"). Samples of milk milked at night and in the morning, were transported in cooling boxes (at 4-6° C), to the Laboratory of Control of Animal Origin Products, Faculty of Veterinary Medicine, where they tested.

2.3 Control laboratory

The milk samples were tested first for determining the number of somatic cells. The detection of inhibitory substances in milk was carried out using a rapid diagnostic system, Beta-lactam AuroFlow ™ kit, whose sensitivity is in line with EU standards for the detection of 13 antibiotic beta-lactam group and a core group of tetracycline. The features of the kit are rapid strip test method – 7 minutes, novel binding protein that quires no heating step, high sensitivity and high reproducibility. Because this test requires no other additional equipment, it is more practical and refined at the collection points, farms etc.

2.3 Sample preparation.

Using scissors, carefully cut the number of reaction wells and remove test strips appropriate for the number of samples that will be tested and allow reagents to reach ambient temperature (25˚C +/- 5). Ensure that unused wells remain firmly capped. Promptly re-store the remaining components at 4˚C.

Before testing milk samples were left at room temperature. After homogenization in the vortex and temperature values measurement, the milk samples underwent testing.

3.4 Strip Testing Procedure.

Milk was mixed vigorously to ensure homogeneity of the sample. Each well shall used was identified with a sample number. With the micropipette, 200 µl milk carefully disposed inside the well. Using the same pipette aspirating through up and down 10 times the content was mixed so that reagent containing lyophilized gold pass entirely in milk suspension. This is

done carefully avoiding the formation of bubbles. The sample should turn a uniform pink colour. Sample mixed with the reagent is left to incubate at ambient temperature (25C +/- 5) for 3 minutes. The strip should be inserted such that the arrows are pointing down. Be sure the strip is oriented vertically. After 3 minutes a strip is dipped for 4 minutes in wells which contains sample. After 4 min, remove the strip and place it on a horizontal surface with the unmarked side facing up. Immediately visually examine the intensity of the signal at each test line and compare the intensity to the C-line, figure 1.

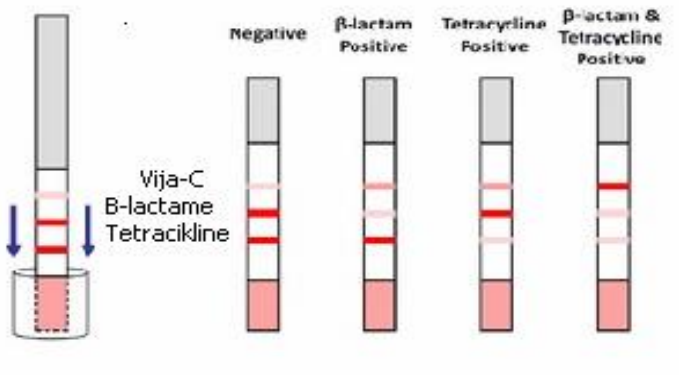


Figure 1. Representative Results.

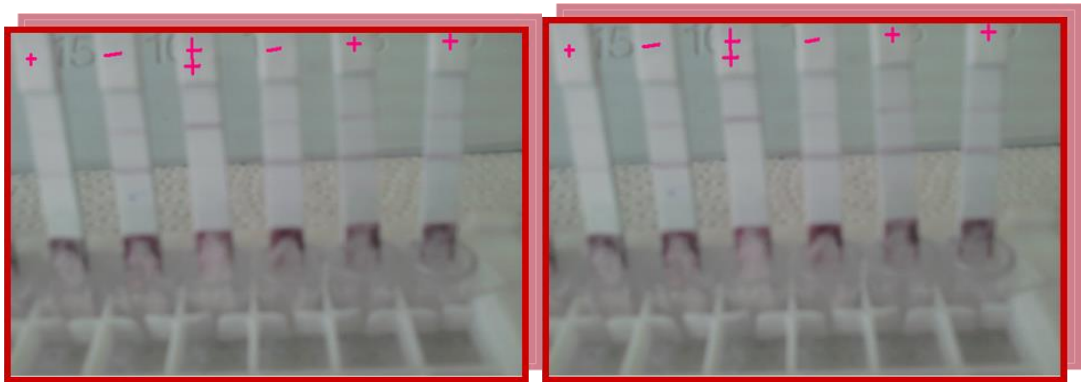
3. Results and Discussion

Our result test for detectives of antibiotic in raw milk was: 8/75 (10.6%) of milk samples resulted strongly positive, while slightly positive 3/75 (4%) samples and 64/75 (84.5%) are negative. Signal at a test line which is more intense than or equal to the signal at the C-line indicates the milk sample is negative for presence of that category of antibiotics, table1.

Table1. Results of AuroFlow™ kit.

Result	Samples 75
Negative	64/75, (85.4%)
Slightly positive	3/75, (4%).
Strongly positive	8/75, (10.6%).

Signal at a test line which is clearly less intense than the signal at the C-line indicates the presence of that respective category of antibiotics in the milk sample. If the signal in C is the same intense or more intense than the T line then milk samples containing these groups, (positive, negative, positive and strong cases), figure 2.



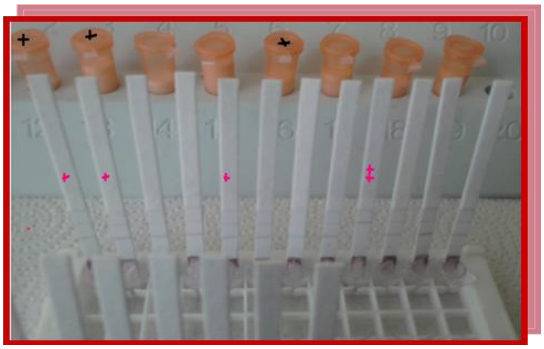


Figure 2. Samples and Results from antibiotics in raw milk.

4. Conclusions

In general, monitoring of antimicrobial agent residues, microbial growth inhibition methods and rapid tests are used. Microbial inhibitor screening methods are easy to perform and enable the detection of a wide spectrum of agents.

The microbial inhibitor screening methods do not attain with some antibiotics the sensitivity at the levels specified by the MRLs. For the determination of these agents, other methods should be used (immunochemical, receptor).

Another disadvantage is a long period needed to perform the test and the occurrence of falsely-positive results if the tests are used for the analysis of individual samples containing higher levels of naturally occurring antimicrobial agents.

Through this method of rapid testified to the presence of antibiotics in milk. The risk to the presence of antibiotics in milk should be considered important and should be done occasionally monitoring of milk, according to the regulations and standards of BE. Veterinary drug residues in milk represent a health risk to the consumer.

The purpose and strategy of the antibiotics and sulfonamide detection in milk include two different aspects: the ability to sell the milk depending on its quality (technological safety).

Monitoring the quality of milk in dairy farms under study, we believe that will be a subsidiary (the study is ongoing..) for the competent authorities to be able to act in preventing the entry, into the food chain, of products harmful to health by respecting the customer and applicable legislation (Instruction 5 dt.25.3.2011 III / 8 and Dir. Cee 92/23 EEC.

At the same time the milk in this region, will be able to avoid the economic damage caused by such products, which have a negative impact on public health, as well as the dairy processing industry.

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