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Screening of quinolone antibiotic residues in beef sold in Kosovo

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Abstract

This study aimed to find the effects of quinolone antibiotics in beef used in three regions of Kosovo. Total numbers of 89 beef meat samples were collected randomly from local meat shops for analysis. Extraction and determination of quinolones were made by ELISA procedure. Among the beef samples, 14 (15.7%) of beef meat samples were positive for quinolones. The mean levels (±SE) of quinolones were found to be in average of 28.22 ± 1.11 µg/kg in samples respectively for enrofloxacin, ciprofloxacin and flumequin. This study indicated that some beef meat sold in Kosovo contains residues of quinolone antibiotics. From the evaluation of tested samples is found positive the presence of enrofloxacin in 6 (6.7%) beef meat samples and respectively for ciprofloxacin and fumequin in 3 (3, 35%) and in 5(5, 6%) beef meat samples. Study results confirmed quinolone residues in beef sold in Kosovo as constitute and serious risk for public health. Use of quinolones in treatment of cattle diseases in Kosovo remain an effective method of diseases control but are considered a common way of residues in beef produced and sold in Kosovo.

Key words: residue, quinolone, beef, meat, cattle, Kosovo.

1. Introduction

Fluoroquinolones are antibiotics used in both medical and veterinary applications. Use of these antibiotics in production of animal products generated f the presence of these residues in food increasing microbial resistance in humans [3, 4, 14, 17]. On this contex, efficient methods are needed for the analysis of a variety of fluoroquinolone residues in meat and other animal products. Fluoroquinolones are well absorbed after oral administration and having a long elimination half-life and widespread distribution throughout the animal body cousing diffrent changes in biochemical pathways[6, 9]. There are also geographical differences in the proportion of resistance to antimicrobials, including fluoroquinolones, among common human pathogens [1, 7, 9]. As is reported by many studies fluoroquinolone resistance is increasing among major nosocomial pathogens such as *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae* and *Neisseria* [1. 7]. The resistance is also an increasing problem for enteric zoonotic agents (*Salmonella*, *Campylobacter*) and for those infections, the link to veterinary use of fuoroquinolones [7, 12]. Fluoroquinolones inhibit the activity of the DNA gyrase and in most bacterial species resistance is caused due to mutations in the gyrase or topoisomerase genes [12, 15, 16]. Resistance to quinolones in Enterobacteriaceae is most commonly casued by mutations in two steps. Firstly, mutation in the gyrA gene is responsible for fullresistance of first generation quinolones such as nalidixic acid and flumequin [12]. Secondaly, mutation in either gyrA or gyrB genes mediates 'fullresistance' to fluoroquinolones [5, 11, 16]. For fluoroquinolones authors reported that giving enrofloxacin to poultry would select for fluoroquinolones resistance in Campylobacter. Similarly, others researchers found that administration of 50 ppm enrofloxacin in drinking water led to the immediate emergence of fluoroquinolone resistant *Campylobacter jejuni* isolates [3, 4]. As a consequence, residual amounts of quinolones remain in the different tissues of medicated animals and increase the risk of adverse effects or antibiotic resistance on people consuming them.

2. Materials and method

Meat samples were collected from slaughtered animals in three regions of Kosovo as following:

Podujevo, Pristina and Prizeren. From each carcass was taken 500g meat. All samples were ket in refrigerated temperature (0-8^{o}C) during the transport to Veterinary Laboratory in Pristina. After registration were performed analytical control for detection of quinolones residues; enrofloxacin, ciprofloxacin and fumequine. The AuroFlow Fluoroquinolone Strip Test Kit as much easier, rapid, and cost-effective screening solution is used for fluoroquinolone detection. This kit, can detect fluoroquinolone contamination in meat in less than 20 minutes. It should be kept room temperature (20-23℃) before opening any vials and starting the assay avoiding also the contamination during manipulation in laboratory**.** The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat has the capacity for 96 determinations. The positive control vial contains 1 mL of PBS spiked with 10 ppb norfloxacin. The positive control is provided solely to assure performance of the kit. Since there is no dilution of the positive control, it should not be used as a comparison with potential positive meat samples. The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat is a qualitative and rapid lateral flow assay designed to detect fluoroquinolone antibiotic residues in meat samples such as chicken and beef. This state-of-the-art test uses a broad-spectrum antibody capable of specifically detecting a range of fluoroquinolone residues, and is designed for rapid field use or reference lab settings and requires no expensive lab equipment such as heaters and centrifuges. The assay uses a competitive colloidal gold based format. The extracted meat sample (200 µL) is added to a clear plastic reaction vessel, and used to resuspend the lyophilized reagents to a uniform pink color in the bottom of the microtiter wells. The sample is incubated briefly (3 min) to allow the fluoroquinolone antibody on the gold particles to engage with any fluoroquinolone antibiotic residues present [6]. The test strip is then inserted into the sample well with the arrows pointing downward initiating capillary flow up the strip. Any gold particles that are not complexed with antibiotics present in the sample will bind to the fluoroquinolone antibiotic imprinted at the Test line (T-line), forming a signal (red line) at that position. If the fluoroquinolone antibody on the gold particle has engaged with antibiotic present in the sample, the gold particle will flow past the T-line and reach the Control line (C-line). For visual interpretation of the test results, T-line signal intensity that is stronger than the signal at the C-line indicates a negative result. Signal at the T-line which is equal or less intense compared to the C-line indicates the presence of fluoroquinolone antibiotics in the meat [6, 9]. The greater the reduction in signal intensity at the T-line, the greater the concentration of fluoroquinolone antibiotic residues present in the sample.The resulting color intensity, afteraddition of substrate, has an inverse relationship with the target concentration in the sample. Quantity evaluation is achived by reading theabsorbance at 450 nm and 630 nm using a microplate ELISA spectrophotometer within 5 minutes after the addition of the stopping solution. The detailed steps of are explained in instruction manual of "AuroFlow™ Fluoroquinolone Strip Test Kit for Meat".

3. Results discusion

In 2013 were analyzed for detection of fluoroquinolone residues 89 beef samples collected from different slaughterhouses of Kosovo (Pristina, Prizeren and Podujeve). Samples were muscle tissues which are tested by The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat which used for rapid detection of quinolones. In the following table (1) are shown positive resultes confirmed by this kit. The MRLs established in the Commission Regulation (EU) No 37/2010 depend of the substances and on the matrix. Quinolones range between 100 and 400µg/kg in beef muscle. The most of quinolones have the MRLs 100µg/kg meat [2, 3].

Table 1. Control for fuoroquinolones; enrofloxacin, ciprofloxacin and fumequine in beef samples collected from sloughterhouses in Kosovo (Podujevo, Pristina and Prizeren).

Regions	*Number of beef samples*	*Residues of fluoroquinolones above MRLs*		
		enrofloxacin	ciprofloxacin	fumequine
Podujevo	30	1/30	1/30	0/30
Pristina	32	3/32	2/32	1/32
Prizeren	27	1/27	0/27	1/27
Total	89	6/89 (6.7%)	5/89 (5.6%)	3/62 (3.4%)

The fumequine MRL is fixed to value 50µg/kg meat. There are developed difrrent ELISA kits for detection of quinolon es in food samples with diffrent detection limits. In our study we have chosen "The

AuroFlow™ Fluoroquinolone Strip Test Kit for Meat" because of having detection limit less than MRLs of enrofloxacin (100), Ciprofloxacin (100), Flumequine (50) [4, 5, 17]. In the table 2 are given the specific detection limits for each flouroqinolone residues selected in our study. Detection limit of quinolones using recommended 8-fold sample dilution factor AuroFlow™ is as following: Enrofloxacin; 10-15 ppb/kg, ciprofloxacin; 8-12 ppb/kg and flumequine 30-40 ppb/kg [2, 4, 8]. The detection limits of "The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat" for each floroquinolones is lower tha MRLs.

4. Discusion

The control for detection of fluoroquinolone is performed in 89 beef samples collected from slougterhouses in Kosovo. The overall prevalence of quinolones residues in beef was 15.7%. The prevalence rates of quinolones varied by the substances planed to be controlled (Table 1). In beef prevalence rates were 6.7% for enrofloxacin, 5.6 % for ciproloxacin and 3.4% for fumequine. Significant differences were observed in the prevalence rates of quinolons residue of different sampling places. All samples are tested by "The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat". Some authors declared in their studies the incidence of flouroquinolones residues until 30% of meat samples [4, 5, 8, 7, 12]. The highest overall food contamination rate of 22.8% which was associated with beef translates into an average risk of exposure one in every four time a consumer takes in the commodity (beef). Studies carried out in Europe reported the values of incidence in diffrent values in checked food comodities. [1, 2, 5, 9, 10]. The higher values of incidence quinlones was confirmed in beef samples. The significant association of extensive farming with quinolones contamination of food is difficult to explain. However, it is likely that in extensive farming, reduced control of animals makes it difficult to efficiently monitor animals after drug administration [1, 2, 4]. The control of use of quinolones as group of antibiotics in animal treatmen is remainig difficult. This fact and others are indicating the high risk exposure of quinolnes to human body. A study published recently that around half of the chicken and beef sold in Ankara contains residues of quinolone antibiotics . About 57% of beef meat samples were positive for quinolones [13]. The incidece of enrofloxacin was 6.7%, higher than ciprofloxacin and fumequine. This study has exposed a potentially serious public health problem for consumers of locally produced beef in Kosovo.

5. Conclusions

Analytical check of tested samples for three fluoroquinolones resulted with 14 positive cases or 15.7% of total samples. The prevalence of enrofloxacin in meat samples was 5.6%. "The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat" determined the presence of ciprofloxacine in 5.6% of beef samples. Positive samples with fumequine residues were confirmed in 3.4% of beef samples. Continuing analytical controls are necessary to assess the quinolons residues level in different food matrices produced in Kosovo.

6. References

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