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Review Article

Control for Quinolnes Residues in Cattle Meat in Kosovo

Veterinary Medicine

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Abstract

In 2014, 102 samples of cattle meat collected from 8 slaughterhouses of Kosovo (Pristina, Prizren and Podujeve) are checked for level of quinolones residues. Muscle tissues were collected randomly from cattle carcasses and were transported to laboratory in refrigerating condition at temperature 0-4oC. For detection of quinolones was performed the analytical control using commercial product the AuroFlow™ Fluoroquinolone Strip Test Kit for Meat. This kit determinates these quinolones; enrofloxacin, ciprofloxacin and fumequin. The detection limits for checked quinolens are: 10-15 ppb/kg for enrofloxacin, 8-12 ppb/kg for ciprofloxacin and 30-40 ppb/kg for fumequin. The analytical results confirmed that 8/102 or 7.8% of meat samples were found positive for the presence enrofloxacin. Ciprofloxacin was found in 5/102 or 4.9% of collected samples. There was confirmed only 1 positive case (0.98%) for fumequin residue. These study result are demonstrating use of quinolones in cattle treatment in Kosovo without any pharmacological vigilance procedure for reducing of residues in beef produced in Kosovo.

1. Introduction

Quinolones and fluoroquinolones are important antibiotics used in human and veterinary medicine and they may cause directly toxic effect or lead to the emergence of drug-resistant bacteria predicating a potential risk to human health. These residues can produce allergic hypersensitivity reactions or toxic effects. In nowdays, several quinolones are used for treatment of beef, poultry, and fish in many countries all over the world. Quinolones promote decreasing activity of the immune system and reducing waste of nutrients and toxin formation (Anonymous, 2002; Biedenbach & Jones 2000). They are used commonly in veterinary medicine and mainly as effective drug in respiratory and intestinal infections of animals. Fluorqouinolons are widely used in treatment of human infection such as: prostatis, gastrointestinal tract infections, respiratory infections, intra-abdominal infections, shigellosis and treatment of sexually transmitted diseases ect (Barron et al., 2001; Kittl et al., 2011). Fluoroquinolones are very effective drug on treatement of campylobacteriosis and salmonellosis. The main concern on public health is realated to possible transfers resistant zoonotic bacteria in veterinary medicine especially *Salmonella spp* and *Campylobacter spp* from animals to man through direct contact with animals or through the food chain (Ganchingco et al., 2012). The resistant foodborne pathogens caused by use of fluoroquinolones affects therapeutic options and perhaps the effectiveness of antimicrobial therapy in human medicine increasing morbidity and mortality in human and animals because of not properly treatment. In such cases, increased risk caused from invasive infections and bacteremia as well as for various complications and consequences. Modification of the various 4-quinolone ring structures produced many broad-spectrum of quinolones. Chromosomal mutational resistance to the original fluoroquinolones is low in frequency and plasmid-mediated resistance nonexistent. In gram-negative bacteria quinolones commonly target DNA gyrase; emerging resistance more often associated with changes in the GyrA compared to the GyrB subunit.

The resistance mechanisms are dedicated changes in DNA gyrase (Anonymous, 2001; Barron et al., 2001; Ganchingco et al., 2012). Comparing with the older quinolones third and fourth-generation fluorinated quinolones are characterized by an effective anaerobic spectrum. Biotransformation of fluoroquinolones in anmals is producing active metabolites. So, use of in treatment produce its active metabolite ciprofloxacin, danofloxacin, difloxacin, norfloxacin, pipemidic acid, ofloxacin, benofloxacin and others (Bucknall et al., 2003; Dalhoff & Shalit, 2003). Enrofloxacin, which was the first fluoroquinolone antimicrobial used in veterinary infections caused by *E. coli, Salmonella, Pasteurella, Mycoplasma* and *Hemophilus* species. The MRLs of enrofloxacin and its active metabolite ciprofloxacin is fixed at 100 ppb for muscle tissue and fat between quinolones and β-lactams, aminoglycosides, clindamycin, and other metabolites (Arts et al., 2000; Biedenbach & Jones, 2000). Flouroquinolones residues in food with animal origin considered to be commonly possible risk of consumer health.

2. Materials and method

As study materials are used meat samples collected from slaughtered animals in three regions of Kosovo as following: Podujevo, Pristina and Prizeren. Meat samples were ensured taking 500g meat from each carcass. Meat samples are kept in refrigerated temperature (0-4^{o}C) during the transport to Food Safety and Veterinary Institute in Tirana. All samples are registered and proceeded for analytical control for detection of quinolones residues; enrofloxacin, ciprofloxacin and fumequine. For detection of these quinolones is used "AuroFlow™ Fluoroquinolone Strip Test Kit for Meat". This commercial kit is being kept at 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 32 determinations. The shelf life is 24 months when the kit is properly stored. 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 (Bucknall et al., 2003, Ganchingco et al., 2012). 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 photometer within 5minutes 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 and discusion

The control for detection of fluoroquinolones in cattle meat samples collected from 8 slaughterhouses of Kosovo located in Pristina, in Prizeren and in Podujeve was carried out in 2014. As material for analytical check are used 102 beef samples represented by muscle tissues tested by The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat used for rapid detection. In the following table (2) are shown positive results. The most of MRLs for fluoroquinolone residues in meat samples are set at value 100μg/kg meat. The MRL of fumequine is 50μg/kg meat. For detection of quinolones residues in food are developed rapit tests consiting mainly on different ELISA kits with diffrent detection limits. The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat" has detection limit less than MRLs of enrofloxacin (100μg/kg), Ciprofloxacin (100μg/kg), Flumequine (50μg/kg).

Table 1. Detection limit by visual interpretation method for different fluoroquinolone antibiotics by AuroFlow™ Fluoroquinolone Strip Test Kit. The lower limit of detection will yield weak positive samples by visual interpretation.

Fluoroquinolones (using recommended 8-fold sample dilution factor)	
Antibiotic (MRL μg/kg)	Detection limit (μg/kg)
Enrofloxacin (100)	10-15 ppb
Ciprofloxacin (100)	8-12 ppb
Flumequine (50)	30-40 ppb

This kit is recommendet to be used in reference laboratories which are performing a large number of meat samples. In some studies is recommended to be firstly used the ELISA test for quantitaive detection and then quantitative detection carried out by instrumental method as HPLC and GC-MS.The detection limits of "The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat" for each floroquinolones is lower tha MRLs. Regarding to this there are many study reports confirming as well the benefits of using it in detection of fluoroquinolone residues in meat samples. However, there are developed diffrent ELISA kits used for qualitive and quatitative detection [5, 8]. In this case the slected ELISA should have low detection limit than fixed MRLs of chosen antibiotics. In EU are aproved many ELISA kits for detction of anibiotics residues in food samples as well as for flouroquinolones.

Table 2. Analytical chek for detection of fuoroquinolones; enrofloxacin, ciprofloxacin and fumequine in beef samples collected from 5 sloughterhouses in Kosovo (Podujevo, Prishtina and Prizeren).

Regions	Number of beef samples	Residues of fluoroquinolones above MRLs		
		enrofloxacin	ciprofloxacin	fumequine
Podujevo	30	2/ 30	2/30	0/30
Pristina	37	2/ 37	2/37	1/37
Prizeren	35	4/35	1/35	0/35
Total	102	8/102 (7.8%)	5/102 (4.9%)	1/102 (0.98%)

The analytical check for fluoroquinolone residues carried out in 102 beef samples orginated from sloughterhouses in Kosovo resulted with positivity 7.8%. As is showed in table 2 enorfloxacine is detected in 2 samples or 6.6% in meat samples collected in Podujevo. Beef samples collected in Pristina region showed positivity for enrofloxacin in value 5.4%. The hightest value of incidence for ennrofloxacin is found in Prizeren areas claculated in value of 11.4%. Regading to the positive cases of ciprofloxacin residues is confirmed that 4.9% of analyzed samples were positive. The higher incidence for ciprofloxain residues is detected in beef samples collected from Prizeren region. There are some studies in EU confirmin the incidence of flouroquinolones residues to veried from 1.8-17% (Barron et al., 2001; Duan & Yuan, 2001; Dalhoff & Shalit, 2003).

Diffrent results are reported for incidence of quinlone residues. I some region of Europe as Turkey in 2007, are reported the values of incidence until 40% of meat samples checked for quinlones residues [Anonymous, 2002]. Other studies in Bulgaria reported that quinolones residues are found in 13% of beef samples. The values of incidence are related to animal health situation in cattle farms. Tracebility of positive cases showed common infections as mastitis, genital infections, urinary tract infections, diarrea and respiratory infections in cattle farms (Arts et al., 2000; Bucknall et al., 2003; Dalhoff & Shalit 2003; Torres & Rello, 2009). In such cases, quinolones are used frequently so the residues level after treatment remain higher. The use of quinolones in animal treatment without any control or surveillance is causing the phenomenon of antibiotic resistance both to animal and people in Kosovo.

4. Conclusions

This situation of quinolones residues in beef samples collected in three region of Kosovo (Podujeveo, Pristina and Prizeren) remain problematic because of widely use of quinolones in animal treatment. The analytical control carried out for three fluoroquinolones in 2014 confirmed the incidence 12.7%. The enrofloxacin residues were detected in 7.8% of beef samples. Use of "The AuroFlow™ Fluoroquinolone Strip Test Kit for Meat" was conluded that two beef samples or 4.9% resulted positive for the presence of ciprofloxacine. Low incidence of fumequine residues is confirmed. Residues of fluoroquinolones in bovine meat in Kosovo threat the health of consumers.

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