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Detection of quinolones residues in beef and chicken meat in hypermarkets of Urmia, Iran using ELISA

Z. Mashak¹, A. MojaddarLangroodi^{2*}, T. Mehdizadeh², A. Ebadi Fathabad², A. HoomanAsadi³

¹Department of Food Hygiene; Karaj Branch; Islamic Azad University, Karaj; Karaj, I. R. Iran

²Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, I. R. Iran

³Graduated from Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj, I. R. Iran

* Corresponding Author: drali_ml2@yahoo.com

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ABSTRACT-The aim of this study was to determine the quinolone residues in beef and chicken meat samples collected from Urmia region local markets. A total of 395 beef and chicken meat samples varied in three various brands were analyzed in Urmia, Iran, by using a sensitive and reliable analytic method based on ELISA detection, for quantification of quinolone antibiotics residues. Samples preparation was performed according to the instructions of the Ridascreen kit (RBIopharm AG, Darmstadt, Germany). Two hundred seventeen of 395 (54.9%) examined chicken meat and beef samples were found to be positive for quinolone antibiotic residue. Seventy-nine number (48.7%) of beef samples and 138 number (59.2%) of chicken meat samples were contaminated to quinolone residues. The mean levels of quinolone antibiotic residue found to be 37.86 ± 0.57 $\mu\text{g}/\text{kg}$ in positive chicken samples and the mean levels of quinolone residues were as low as 5.51 ± 1.17 $\mu\text{g}/\text{kg}$ in positive beef samples. Present study indicated that 395 samples of beef and chicken meat sold in Urmia contained residues of quinolone antibiotics. In terms of preventing antibiotic resistance in humans, the low level of quinolone residue levels observed in this study represents a positive result for local food control.

INTRODUCTION

Antibiotics are used by the poultry industry to raise growth and reduce diseases. Also, these agents are widely used for the prevention of diseases in farm animals. The use of veterinary drugs has played an important role in the field of animal husbandry and agro-industry to prevent and treat diseases and as growth promoting agents, but they have the potential to generate residues in animal derived products (meat, milk, eggs and honey) and cause a health hazard to the consumer. Contamination of food in low-levels may not generate a serious problem on public health. However, extensive use and not observing the withdrawal time of drugs may increase the risk of occurrence of microbial drug resistance, hypersensitivity reaction and disruption of normal intestinal flora (Beyene et al., 2016; Cotter et al., 2012). Quinolones and fluoroquinolones are significant antibiotics used in human and animals medicine (Velissariou, 2006; Andreu et al., 2007; Chafer-Pericas et al., 2010). Quinolones (flumequine, enrofloxacin) are among the most widely used antibiotics in veterinary medicine for treatment and prevention of diseases. Unfortunately, consumers' perceptions are that edible

poultry tissues are contaminated with harmful concentrations of drug residues; so, investigation about antibiotics residues and their health risk factors are essential (Somasundaram and Manivannan, 2013; Gouvea et al, 2015). The antibacterial effects of the quinolone family are that they are active against a great range of Gram-negative organisms (Barrett and Fuchs, 1997). There is an annoying worldwide trend of increased resistance to these drugs among bacteria responsible for both hospital and community acquired infection including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratiamarcescens*, *Escherichia coli*, *Salmonella* spp, and *Campylobacter* spp (Johnston, 1998). The World Health Organization (WHO) and Food and Drug Administration (FDA) have placed severe restrictions on veterinary use of FQs (Fluoroquinolones) given the concerns about drug-resistant bacteria and the possible failure of human antibiotic therapy. Fluoroquinolones were introduced for veterinary use in Iran at 1987 and have been available since 1991 in pure powder and solution form for addition to poultry drinking water, prophylaxis or treatment of infection due to Gram-

negative microorganisms. Antibiotic residues in foodstuffs are harmful for consumers' health because they may cause gastrointestinal disorders, development of resistant bacterial strains and some other problems (Holt et al, 1993; Nisha et al, 2008). Therefore, quantification and determination of even low levels of these residuals is crucial for food safety. ELISA is a powerful verifying technique; it is, however, an expensive method which is not available to all laboratories. In the case of fluorescent drugs such as FQs, because of its selectivity and sensitivity, ELISA is a very good detection method. In Iran, marbofloxacin, enrofloxacin, ciprofloxacin, difloxacin and flumequin are approved quinolones for treatment in animal production. To safeguard human health, the EU (European Union) has established safe maximum residue levels (MRLs) for veterinary medicinal products in foodstuffs of animal origin at the Community level under Council Regulation 2377/90 EEC and its later modifications (2002). According to this regulation and the US FDA administration and WHO, MRLs of quinolones in food products of animal origin are usually at the level of 100 µg/ kg and 500-3000 µg/ kg in different species, respectively (FDA, 2006). The aim of this study was to determine the quinolone residues in beef and chicken meat samples collected from Urmia region local markets.

MATERIALS AND METHODS

Sample Collection

In the present study, 162 beef round meat and 233 chicken breast meat samples were obtained from hyper markets of Urmia (Iran) in three different brands (A, B, and C) of packaging. Sample collection was done between March 2014 and November 2014. All samples were randomly purchased, transported to the laboratory and kept at 4°C until analysis.

Sample Preparation and ELISA Analysis

In this study, quinolone residues were determined with an ELISA using the Rid a screen Quinolones (RBIopharm AG, Darmstadt, Germany). The assay was tested according to the guidelines of the manufacturer. The detection limit of kit was reported 5 µg/kg, as the lowest value for beef and chicken meat in the test kit. Sample preparation was done based on Rid a screen Kit instructions. In brief, 5 g of each sample was weighed and homogenized with mixer Ultraturrax (Ikea, Germany); then, the homogenized samples were mixed with methanol/water (70:30, vol/vol). Later, the suspension was vortexed for 10 min and centrifuged at 4,000 × g for 10 min in room temperature. Fifty microliters of the standard solution and each of the samples were added to 96-well ELISA microplate. Fifty microliters of enzyme conjugate and 50 µL of antibody

were added to each well, respectively, ELISA microplate was incubated for 1 h at 4°C. At the end of the incubation, the liquid phase was poured from the wells and washed twice with the washing buffer and vortexed intensively for approximately 1 min. One hundred microliters of the substrate/chromogen were added to the wells and incubated at ambient temperature for 15 min. Finally, hundred microliters of the stop solution were added to each well and the absorbance of the samples was read at 450 nm in ELISA plate reader (Versa Max Tunable). Calibration curves were plotted as semi-logarithmic concentration versus the ratio of the mean absorbance at each concentration divided by the mean absorbance of the zero standard. Quinolones concentration was calculated through the guidelines of the Rid a screen chloramphenicol test which was 0.05 ng/g and the recovery rates were >80% for all samples.

Statistical Analysis

Data analysis was performed using SPSS 18 (IBM, PASW Statistics 18.0, USA). ANOVA and Tukey's test with a significance set at $P < 0.05$ were used to compare means of the groups.

RESULTS AND DISCUSSION

The results of this study indicated that quinolone antibiotics were detectable in some samples by ELISA. Two hundred and seventeen of 395 (54.9%) examined chicken meat and beef samples were found positive for quinolone antibiotic residue. One hundred and eight (59.2%) of the chicken meat samples were found to contain quinolone antibiotics, and 79 (48.7%) of beef meat samples were positive for quinolones, distinctively. The mean levels of quinolone antibiotic residues were found to be 37.86 ± 0.57 µg/kg in positive chicken samples. However, quinolone residues were found in lower levels in beef samples. The mean levels of quinolone residues were as low as 5.51 ± 1.17 µg/kg in positive beef samples. Results of this study revealed that quinolone mean levels in beef and chicken meat samples were lower than 100-120 µg/kg, which is the lowest residue value mentioned in standard administration of Iran. The result of ANOVA showed that there was a significant difference between A-C brands compared to B brand and no significant difference was found between A and C brands of chicken and beef meat samples ($P < 0.05$). This study indicated that some chicken and beef meat sold in Urmia contains residues of quinolone antibiotic although detected levels were lower than the national standard limit. In seven of chicken samples, the levels of quinolone residues were determined to be higher than 100-120 µg/kg. The concerning values are shown in Tables 1 and 2.

Table 1. Statistical analyses for levels of quinolone in positive samples of chicken meat

| Brands | Sample tested, n | Positive sample, n (%) | Concentration of positive samples, µg/kg (mean ± SE) | Mean value of samples |
|--------|------------------|------------------------|--|-----------------------|
| A | 82 | 51(62.1%) | 17.41±3.2 | 17.12 ^a |
| B | 70 | 49(70%) | 42.37±5.03 | 41.09 ^b |
| C | 81 | 38(46.9%) | 31.42±6.32 | 19.51 ^a |
| Total | 233 | 138(59.2%) | 36.71±4.09 | - |

^{a,b}For an attribute, means within a group in a column (between different brands) not having a common superscript letter are different ($P < 0.05$).

Table 2. Statistical analyses for levels of quinolone in positive samples of beef meat

| Brands | Sample tested, n | Positive sample, n (%) | Concentration of positive samples, µg/kg (mean ± SE) | Mean value of samples |
|--------|------------------|------------------------|--|-----------------------|
| A | 75 | 24(32%) | 21.47±2.34 | 22.51 ^a |
| B | 63 | 42(66.6%) | 43.12±4.07 | 39.16 ^b |
| C | 24 | 13(54.1%) | 38.31±6.02 | 31.53 ^a |
| Total | 162 | 79(48.7%) | 27.38±3.64 | - |

^{a,b}For an attribute, means within a group in a column (between different brands) not having a common superscript letter are different ($P < 0.05$).

Akar (1994) analyzed 175 chicken meat samples and found 5.7% antibiotic residues (chloramphenicol, erythromycin, and tylosin) in Turkey. Weiss et al. (2007) assayed 299 samples of chicken meat and did not find enrofloxacin antibiotic residue in Italy. These contamination rates were lower in comparison with values reported in the present study. Many reports indicated that microbial resistance to antibiotics may be intensified as animals are exposed to these agents, which leads to resistance of human pathogens. Clearly, drug residues in milk and meat are great problems for human safety issues in some countries (Bertini et al., 2003; Gustavson et al., 2002; Levy, 1998). Omotoso and Andrew (2014) assayed fluoroquinolone residues in broiler chicken meat in Ibadan. Their results showed that most frozen chicken products imported to Nigeria at the time of that study contained higher levels of residual fluoroquinolones than the locally produced chicken. Antibiotic residues have been known as a public health concern due to potent inhibitors of DNA gyrase enzyme, which is critical for DNA replication and transcription. The side effects of quinolones are well appointed in animals and humans (Christian, 1996; Makinen et al., 1997; Shimoda, 1998; Khadra et al., 2012). Alla et al. (2011) analyzed beef samples in Sudan and reported that just 3% of the muscles contained antibiotic residues. Masztis (1984) analyzed 487 bovine carcasses in Canada and found that 12 samples were positive. Salehzadeh et al. (2007) examined 270 chicken muscle, liver, and kidney samples from 90 broiler farms in Tehran, Iran, and mentioned enrofloxacin mean concentrations as 18.32 ± 32.29 , 18.34 ± 12.36 , and 26.06 ± 19.52 ng/g in muscle, liver, and kidney samples, respectively. In Iran, there are several studies related to monitoring antibiotics in cattle. Manafi et al. (2010) found 26% antibiotic

residues contamination in raw milk and 30% in total samples. Kaya and Filazi (2010) found 1.25% antibiotic residue contamination rates in milk samples. Some allergic reactions have also been reported, in relation to quinolones (Gruchalla and Pirmohamed, 2006). These results in poultry samples show compatibility with the results of the present study. There are no major differences between beef and chicken quinolone residues. Pena et al. (2010) assayed poultry muscle in Portugal and found 44.2 % fluoroquinolone residues contamination in chicken samples and 37.8% in turkey samples. Yuksek (2001) did not discover residue of oxy tetracycline and chloramphenicol in chicken meat in Turkey. Koc (2006) assayed cattle and sheep meat samples in Ankara and did not find any antibiotic residue. Given the adverse effects of quinolones on human health, residues in food and other environmental contaminants are also expected to affect human health. Therefore, it is necessary to frequently monitor consumed meat and meat products with high nutritional value for the presence of residual quinolones.

CONCLUSIONS

The intensive use of antibiotics in meat has raised questions about the impact of veterinary medicines on organisms in the environment and on human health. Resistance in human microbes' antibiotics may induce resistance in avian and livestock bacterial pathogens. To alleviate these problems, basic and advanced education of poultry and livestock farm workers is necessary. This study stresses the need for stricter regulations for the use of antimicrobial drugs in poultry and beef industry as well as the inspection of chicken for residues prior to marketing in Iran.

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باقیمانده کینولون در گوشت مرغ و گوساله در فروشگاه‌های ارومیه، با استفاده از روش الایزا

زهره مشاک^۱، علی مجدر لنگرودی^{۲*}، تورج مهدیزاده^۲، ایوب عبادی فتح آباد^۲، امیر هومان اسدی^۳

^۱گروه بهداشت مواد غذایی، دانشگاه آزاد اسلامی، واحد کرج، کرج، ج. ا. ایران.
^۲گروه بهداشت و کنترل کیفی مواد غذایی، دانشکده دامپزشکی، دانشگاه ارومیه، ارومیه، ج. ا. ایران.
^۳فارغ التحصیل دکتری دامپزشکی، دانشگاه آزاد اسلامی، واحد کرج، کرج، ج. ا. ایران.

*نویسنده مسئول

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چکیده- هدف از این مطالعه بررسی باقیمانده‌های کینولون در گوشت مرغ و گوساله که از سوپرمارکت‌های شهر ارومیه نمونه‌برداری گردیده شده می‌باشد. در مجموع تعداد ۳۹۵ نمونه از گوشت مرغ و گوشت گوساله در سه برند متفاوت به وسیله آزمون حساس و قابل اعتماد الایزا جهت ارزیابی میزان کینولون در شهر ارومیه مورد بررسی قرار گرفتند. آماده‌سازی نمونه‌ها بر اساس روش ارائه شده در ریدالاسکرین کیت انجام شد. از مجموع ۳۹۵ نمونه، ۲۱۷ نمونه (۵۴/۹٪) از گوشت مرغ و گوشت گوساله از جهت وجود باقیمانده آنتی‌بیوتیکی مثبت ارزیابی گردید. ۷۹ نمونه از گوشت گوساله (۴۸/۷٪) و ۱۳۸ نمونه (۵۹/۲٪) از گوشت مرغ به باقیمانده آنتی‌بیوتیک کینولون آلوده بودند. میانگین باقیمانده آنتی‌بیوتیک کینولون در نمونه‌های مثبت گوشت مرغ $1/57 \pm 37/86$ و در نمونه‌های مثبت گوشت گوساله $1/17 \pm 5/51$ میکروگرم/کیلوگرم بوده است. مطالعه حاضر نشان می‌دهد که ۳۹۵ نمونه خریداری شده گوشت مرغ و گوساله در شهر ارومیه حاوی باقیمانده آنتی‌بیوتیک می‌باشند. از جهت مقاومت آنتی‌بیوتیکی در انسان، میزان پایین باقیمانده آنتی‌بیوتیک در نمونه‌های مطالعه حاضر نشان دهنده‌ی شرایط مثبت کنترل مواد غذایی در این منطقه است.