

Investigation of Enrofloxacin and Chloramphenicol Residues in Broiler Chickens Carcasses Collected From Local Markets of Tabriz, Northwestern Iran

Vahideh Ebrahimzadeh Attari ¹, Mehran Mesgari Abbasi ², Nasim Abedimanesh ¹,
*Alireza Ostadrahimi ³, Abolfazl Gorbani ⁴

¹ Department of Nutrition & Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran

² Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

³ Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁴ Department of Animal Science, Islamic Azad University, Shabestar Branch, Shabestar, Iran

ARTICLE INFO

Article type:

Original Article

Article history:

Received: Apr 09 2014

Accepted: Jul 27 2014

e-published: Dec 30 2014

Keywords:

Antibiotic residues,
Broiler chicken,
MRLs,
Enrofloxacin,
Chloramphenicol

*Corresponding Author:

Alireza Ostadrahimi

Tel: +98 41 33357582;

e-mail:

ostadrahimi@tbzmed.ac.ir

ABSTRACT

Background: The present study was aimed to determine the residual amounts of chloramphenicol and enrofloxacin in broiler chickens muscle and liver samples gathered from local markets of Tabriz City, northwestern Iran.

Methods: Ninety broiler chickens carcasses were collected from different local markets of Tabriz, during July/August 2013. Random samples of thigh and breast muscle and liver were gathered and kept at -80°C until analyzed. The samples were then assayed using enzyme-linked immunosorbent assay (ELISA) according to the protocol of each antibiotic kit. Data were statistically analyzed using the computer program SAS 9.1.

Results: Eighty two samples (91/1 %) contained residues of enrofloxacin, although mean (\pm SD) of enrofloxacin concentration was lower than the European Union maximum residue limits (MRLs) value ($P < 0.001$). Moreover, 28 (31/1 %) had detectable concentrations of chloramphenicol while it was not defined any MRLs value for chloramphenicol because its using has been forbidden in food animals.

Conclusion: The frequency of contamination with enrofloxacin was considerable for the analyzed samples. Furthermore, the existence of chloramphenicol in almost one third of samples seems to be a public health threat due to its illegal use in food animals including poultry.

Citation: Ebrahimzadeh Attari V, Mesgari Abbasi M, Abedimanesh A, Ostadrahimi A, Gorbani A. Investigation of Enrofloxacin and Chloramphenicol Residues in Broiler Chickens Carcasses Collected From Local Markets of Tabriz, Northwestern Iran. Health Promot Perspect 2014; 4(2):151-157

Introduction

Nowadays antibiotics have become an integral part of poultry industry. Often they can be used for some targets such as treatment and prevention of several diseases, as well as improvement of feed efficiency, growth promotion and also preventing economic losses to growers.¹⁻³ Antibiotics can penetrate and remain in an animal's tissues

especially if the antibiotic initial dosage is high or withdrawal time is not sufficient before slaughtering.⁴ Antibiotic residues in foodstuffs are harmful for consumer's health because they may cause allergic reactions, gastrointestinal disorders, resistance to pathogenic bacteria, development of resistant bacterial strains and some other problems.⁵⁻⁷

Enrofloxacin, a kind of amphoteric Quinolones (fluoroquinolones) antimicrobial family, is a potent antibiotic with a broad spectrum of activity against gram-positive and negative micro-organisms.¹ Quinolones may have direct effect through inhibiting bacterial DNA-gyrase and topoisomerase IV enzyme activities or lead to the emergence of drug-resistant bacteria predicating a potential risk to human health.^{8,9} In order to deal with pulmonary and digestive infections among poultry, this antibiotic was indicated as veterinary agent in Iran since 1990.¹⁰ To ensure that humans are not exposed to residues at potentially harmful concentrations, maximum residue limits (MRLs) for residues of antibiotics in food animal tissues and products have been established.

Chloramphenicol (CAP) is another antibiotic with extensive spectrum antimicrobial activity as well as its remarkable penetration into the tissues which has lethal effect in humans.^{11,12} CAP is a cytotoxic, genotoxic and hemotoxic compound that can cause bone-marrow depression, as well as serious and irreversible aplastic anemia that can result in leukemia.¹² Food and Drug Administration (FDA) regulations have forbidden chloramphenicol to be used in food animals.¹³ It could be found in some samples taken from all European countries and third Countries.¹⁴ Besides, it is widely used in poultry farms in Iran despite legal prohibition in domestic animals.¹⁵

It seems necessary to determine and monitor antibiotics residues in poultry tissues in Iran because of increased microbial resistance and other health threatening hazards observed for these agents and to control the safety of foods for human consumption. Hence the objective of this study was to investigate the presence of two agents: enrofloxacin and CAP in broiler chickens meat and liver samples gathered from local markets of Tabriz.

Materials and Methods

Samples

Ninety broiler chickens carcasses were collected from different local markets of Ta-

briz City, northwestern Iran during July/August 2013. Samples from liver, breast and thigh muscle were randomly selected and at least, 50 g of each sample was placed in a sterile polyethylene container and kept in a -80°C deep freezer until analyzes.

Sample preparation

The samples were cut into fine pieces then thawed at 4°C for homogenization (10 g of sample). The homogenized samples were then prepared according to the protocol suggested by ELISA kit supplier (Euro-Proxima, The Netherlands).

ELISA Analyzes

To measure the amount of enrofloxacin in chicken samples a commercial ELISA kit (EFRX ELISA kit, EuroProxima, Netherlands; Lot nr: ON 5906) was used. The kit had a specificity of 100% for enrofloxacin. The detection limit (LOD) of test was 4ng/ml and the mean recovery rate was between 75% and 130% for all samples. The ELISA technique was performed according to manufacturer's instructions. After homogenization of samples, 0.5 g of the homogenized samples was transferred into a test tube and 1.5 mL of 80% methanol was added as dilution buffer. The suspension was vortexed for 30 min and centrifuged at $2,000 \times g$ for 10 min at room temperature (20–25°C). Following centrifugation, 100 μ L of supernatant was transferred into a new centrifuge tube and 900 μ L of sample dilution buffer was added and ultimately 50 μ L of the aqueous (upper) layer was used for analyzes. The absorbance was measured at 450 nm using an ELISA plate reader (Awareness, stat fax-2100, USA).

To measure the amount of CAP a commercial ELISA kit (CAP ELISA kit, Euro-Proxima, Netherland; Lot nr: ON5901) was used. The kit had a specificity of 100% for CAP. The assay was performed according to the procedure suggested by the kit. After homogenization of samples, 3 g of the homogenized samples was transferred into a glass tube and 6 mL of ethyl acetate was added. The suspension was vortexed for 10

min and centrifuged at $2,000 \times g$ for 10 min. Following centrifugation, 4 mL of ethyl acetate is pipetted into a glass tube and the ethyl acetate was evaporated at 50°C under a mild stream of nitrogen. The fatty residue was dissolved in 1 ml of n-hexane and 1 ml of sample dilution buffer was added. The whole was mixed and centrifuged at 2000 g for 10 min. Finally, 50 μL portions of the upper layer were used for analyzes. The LOD of test was 0.02 ng/ml and the mean recovery rate was about 80% for all samples. The absorbance was measured at 450 nm using an ELISA plate reader (Awareness, stat fax-2100, USA).

Ethical Considerations

This proposal of this study was approved by ethics committee of Tabriz University of medical sciences.

Statistical Analyses

Statistical analyzes were performed using the SAS 9.1 statistical package (SAS Institute Inc.). The data were described using fre-

quencies, percentages, means, and standard deviations as descriptive statistics. The tissue distribution of antibiotics was compared by general linear model (GLM) and Duncan's post hoc test. Moreover one sample *t* test was used to compare results with Maximum residue limits (MRLs) of each antibiotic. Statistical difference with $P < 0.05$ was considered as significant.

Results

From 90 samples, including 35 thigh meats, 35 breast meats and 20 liver tissues of different broiler chicken gathered from Tabriz markets, 28 (31.1%) and 82 (91.1%) contained the residues of CAP and enrofloxacin antibiotics, respectively. Six breast, 14 thigh and 8 liver samples were positive for CAP and 31 breasts, 32 thigh and 18 liver samples were positive for enrofloxacin. The mean \pm SD of these two antibiotics concentration is demonstrated in Table 1 separately for different tissues.

Table 1: Concentrations of Chloramphenicol (CAP) and Enrofloxacin antibiotics in different tissues

Tissues	Antibiotics	Samples with antibiotic residuals (%)	Mean (SD) ($\mu\text{g}/\text{kg}$)	Minimum ($\mu\text{g}/\text{kg}$)	Maximum ($\mu\text{g}/\text{kg}$)
Breast (n=35)	CAP#	17.14	0.008 (0.004)	0.002	0.013
	Enrofloxacin	85.57	23.92 (12.31)	5.30	80.00
Thigh (n=35)	CAP	40.00	0.021 (0.02)	0.001	0.026
	Enrofloxacin	91.43	21.17 (32.62)	10.70	122.70
Liver (n=20)	CAP	40.00	0.018 (0.008)	0.001	0.024
	Enrofloxacin	85.00	39.54 (18.99)	5.30	90.70

#CAP: Chloramphenicol

Assessment of tissue distribution of antibiotics represented that enrofloxacin concentration in liver was significantly higher compared to other tissues ($P=0.023$) while the difference between thigh and breast muscle was not considerable ($P>0.05$). The CAP concentration among three tissue samples was not significantly different ($P=0.362$), however it was to some extent higher in thigh sample in comparison to other tissue samples ($P=0.271$).

Mean detectable concentrations of enrofloxacin in 14.4% all samples were higher

in comparison with the recommended MRL according to FDA regulations ($30\mu\text{g}/\text{kg}$). Of course, comparison of enrofloxacin mean concentration in three tissues with MRLs values of FDA indicated that just liver concentration ($39.54\mu\text{g}/\text{kg}$) of this antibiotic was above the MRL allowed concentration ($P=0.245$) (Fig. 1). Comparison of mean concentration of enrofloxacin in different tissues with MRLs according to European Union ($100\mu\text{g}/\text{kg}$ for muscles and $200\mu\text{g}/\text{kg}$ for liver tissue) showed significant difference ($P<0.001$).

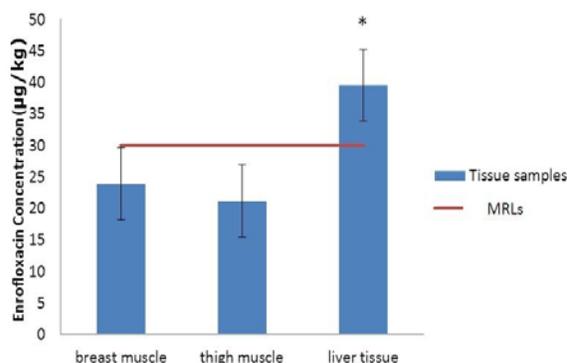


Fig. 1: The residue levels of enrofloxacin in the homogenates of breast, thigh and liver tissue of chickens. The asterisk indicates statistical significance ($P= 0.023$) compared to the values of two other tissues. Thick line indicates the MRL according to FDA. MRLs: Maximum Residue Limits

Discussion

The results of present study revealed that the different samples of broiler chickens in Tabriz were positive for CAP and enrofloxacin. Mean detectable concentrations of enrofloxacin in some of samples were higher in comparison with the recommended MRL according to FDA regulations ($30\mu\text{g}/\text{kg}$).

Screening of antibiotic residues in food-producing animals has received enormous worldwide attention from local and international regulatory and public health agencies. This is owing to the importance of the issue and its possible significant impact on public health. Human exposure to animal products containing considerable levels of antibiotic residues may induce and transfer resistance to human pathogens and also cause several problems including disturbed immunological response and disorder of intestinal flora in susceptible individuals.^{2,16}

Limited studies on antibiotic residues in poultry animals have been performed in Iran. In the present study we examined muscle and liver samples of chicken for presence of enrofloxacin and CAP residues. The results showed that 82 from 90 samples from the investigated broiler farms had detectable levels of enrofloxacin at the time of mar-

keting that is consistent with results from other studies in Iran.^{3,17,18} These results confirmed that enrofloxacin was abundantly used in poultry farms under research. They also suggest that the recommended withdrawal time was either not strictly applied or may be insufficient for this drug. An investigation in Saudi Arabia has indicated extensive abuse of norfloxacin (NFX) in the local poultry industry.¹⁹ Recently Er et al.²⁰ determined the quinolone residues in chicken meat and beef samples collected from Ankara region local markets. According to the results the mean levels of quinolone antibiotic residue were $30.81 \pm 0.45 \mu\text{g}/\text{kg}$ in positive chicken samples investigated in Ankara which was partially consistent to our results (mean levels of enrofloxacin for our results was $28.93 \pm 24.24 \mu\text{g}/\text{kg}$). These fluoroquinolones residual rates in chicken samples of several countries were lower in comparison with values reported in this study.²¹⁻²³

Several organizations like the food and agriculture organization (FAO) and Food and Drug Administration of USA have set tolerance or Maximum Residue Limits for antibiotic residues in animal derived foodstuffs. According to the supervision commission report of European Union, the maximum residue levels of the enrofloxacin should not exceed $100 \mu\text{g}/\text{kg}$ in chicken muscle, fat tissue and skin, $200 \mu\text{g}/\text{kg}$ in liver and $300 \mu\text{g}/\text{kg}$ in kidney while according to FDA the safe residue levels in different poultry tissues is 30 micrograms per kilogram body weight.^{24,25} The result of the present investigation indicated that liver samples had enrofloxacin residues higher than the recommended MRLs (Fig. 1) while in Rokni et al.¹⁷ study three different samples of liver, kidney and muscle had enrofloxacin residues above the recommended MRLs with highest rate in kidney tissues. Quinolones disturb DNA replication and transcription process and have several other toxic effects in animals and humans including damage to the juvenile joint, the kidney, the eye, and the central nervous system previously reported by animal experiments.²⁶⁻²⁹ Some antibiotic-induced allergic reactions

have also been reported, in relation to quinolones.³⁰

Given the harmful effects of quinolones on human health, it is necessary to monitor and analyzes frequently consumed animal products with high nutritional value for the presence of residual quinolones. Like other countries using quinolone for the prophylaxis and treatment of chicken and beef is legal, but unfortunately there is not a limit and strict surveillance in the applied dose and pre-slaughter withdrawal times.

Chloramphenicol is a prohibited antibiotic according to FDA regulations in food animal industry because of several hazardous toxic and carcinogenic effects on human health.^{11,12} Therefore, there are no withdrawal times and no safe residue levels in foods with animal origin.¹⁶ The most serious and lethal effect of CAP is aplastic anemia which is not dose dependent and can occur with even extremely low doses.³¹ There is evidence suggesting CAP may be widely used in poultry production systems in Iran despite its legal prohibition.¹⁵ According to investigations conducted recently in Iran the number of CAP positive samples were inconsistent. Until know, the percent of CAP positive samples was higher in Mehdizadeh et al.¹⁵ study (54.8%) while it was 17.5% in Tajik et al.³² study and 31.1% in ours. In Turkey, 8.3% presented the residues of this antibiotic.³³ Maximum levels of 73.34 ± 88.60 ng/kg was detected in the breast muscle while the highest concentrations of immunoreactive CAP were detected in kidney and liver tissues in Mehdizadeh et al.¹⁵ and liver in Tajik et al.³² study. The CAP concentration among three tissue samples was not significantly different in this study however it was higher in thigh sample to some extent in comparison to other tissue samples.

As the limitations of the present study it should be mentioned that: 1) assessment of kidney samples was not done in present study. 2) Evaluation of antibiotic residues in current study was performed by ELISA which is a fast, not expensive method with high sensitivity, but there may be false positive results with this method and that sus-

pected results should be confirmed with chromatographic mass spectrometric detection.³⁴ Limited sample size and not measuring the other quinolones may be accounted as the other limitations of this study.

These results are valid for the obtained samples during the study. Results may change periodically. Therefore, the steps in food processing should be kept under continuous monitoring for preventing over expressed levels of drug residues.

Conclusion

Our study indicates that illegal uses of CAP in broiler farms is still in continue despite the international prohibition so it should be taken into account seriously. On the other hand, the frequency of contamination with enrofloxacin was considerable for the analyzed samples. In terms of preventing antibiotic resistance in humans and other health related hazards, the approximately high amount of enrofloxacin residue levels observed in this study represents a negative result for local food control. Therefore, there is further need to strict supervision and following of antibiotics usage in broiler farms in Iran.

Acknowledgments

This work was financially supported by Nutrition Research Center and Drug Applied Research Center, Tabriz University of Medical Sciences.

Competing interests

The authors declare that there is no conflict of interests.

References

1. Cinquina AL, Longo F, Anastasi G, Giannetti L, Cozzani R. Validation of a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine

- milk and muscle. *J Chromatogr A* 2003;987:227-233.
2. Linton AH. Antibiotics, animals and man, An appraisal of a contentious subject. In: Woodbine M, editor. *Antibiotics and Antibiosis in agriculture*. London: Butterworths, Inc; 1977.
 3. Salehzadeh F, Madani R, Salehzadeh A, Rokni N, Golchinefar F. Oxytetracycline residue in chicken tissues from Tehran slaughterhouses in Iran. *Pakistan J Nutr* 2006;5:377-381.
 4. Droumev D. Review of antimicrobial growth promoting agents available. *Vet Res Commun* 1983;7:85-99.
 5. Holt D, Harvey D, Hurley R. Chloramphenicol toxicity. *Adverse Drug React Toxicol Rev* 1993;12:83-95.
 6. Schenck FJ, Callery PS. Chromatographic methods of analyzes of antibiotics in milk. *J Chromatogr A* 1998;812:99-109.
 7. Nisha A. Antibiotic residues-a global health hazard. *Vet World* 2008;1:375-377.
 8. Zechiedrich EL, Cozzarelli NR. Roles of topoisomerase IV and DNA gyrase in DNA unlinking during replication in *Escherichia coli*. *Genes Dev* 1995;9:2859-2869.
 9. Juan-García A, Font G, Pico Y. Determination of quinolone residues in chicken and fish by capillary electrophoresis-mass spectrometry. *Electrophoresis* 2006;27:2240-2249.
 10. Salehzadeh F, Salehzadeh A, Rokni N, Madani R, Golchinefar F. Enrofloxacin residue in chicken tissues from Tehran slaughterhouses in Iran. *Pakistan J Nutr* 2007;6:409-413.
 11. Papich MG, Riviere JE. Chloramphenicol and derivatives, macrolides, lincosamides, and miscellaneous antimicrobials. In: HR Adams, editor. *Veterinary Pharmacology and Therapeutics*. Iowa: Iowa State university Press; 2001.
 12. Wareham DW, Wilson P. Chloramphenicol in the 21st century. *Hosp Med* 2002;63:157-161.
 13. FDA (Food and Drug Administration) [Internet]; 1997. Available from: <http://www.fda.gov>.
 14. World Health Organisations. Toxicological evaluation of certain veterinary drug residues in food. Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva: Switzerland; 2004.
 15. Mehdizadeh S, Kazerani HR, Jamshidi A. Screening of chloramphenicol residues in broiler chickens slaughtered in an industrial poultry abattoir in Mashhad, Iran. *Iran J Vet Sci Technol* 2010;2:25-32.
 16. Sogaard H. Incidence of drug resistance and transmissible R factors in strains of *E. coli* isolated from faeces of healthy pigs. *Acta Vet Scand* 1973;14:381-391.
 17. Rokni N, Kamkar A, Salehzadeh F, Madani R. Study on the enrofloxacin residue in chicken tissues by HPLC. *Iran J Food Sci Technol* 2007;4:11-17.
 18. Amjad H, Naeem M, Iqbal J, Khan K. Estimation of selected residual antibiotics in poultry products available in local markets during summer. *J Chem Soc Pakistan* 2005;27:637-642.
 19. Al-Mustafa ZH, Al-Ghamdi MS. Use of norfloxacin in poultry production in the eastern province of Saudi Arabia and its possible impact on public health. *Int J Environ Health Res* 2000;10:291-299.
 20. Er B, Onurdag FK, Demirhan B, Ozgacar SO, Oktem AB, Abbasoglu U. Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. *Poult Sci* 2013;92:2212-2215.
 21. Pena A, Silva LJ, Pereira A, Meisel L, Lino CM. Determination of fluoroquinolone residues in poultry muscle in Portugal. *Anal Bioanal Chem* 2010;397:2615-2621.
 22. Zhao S, Li X, Ra Y, Li C, Jiang H, Li J, et al. Developing and optimizing an immunoaffinity cleanup technique for determination of quinolones from chicken muscle. *J Agric Food Chem* 2009;57:365-371.
 23. Weiss C, Conte A, Milandri C, Scortichini G, Semprini P, Usberti R, et al. Veterinary drugs residue monitoring in Italian poultry: Current strategies and possible developments. *Food Control* 2007;18:1068-1076.
 24. The European Commission. Commission regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Union* 2010;15:L15/1-72.
 25. San Martín B, Comejo J, Lapierre L, Iragüen D, Pérez F, Hidalgo H, et al. Withdrawal time of four pharmaceutical formulations of enrofloxacin in poultry according to different maximum residues limits. *J Vet Pharmacol Ther* 2010;33:246-251.
 26. Suto MJ, Domagala JM, Roland GE, Mailloux GB, Cohen MA. Fluoroquinolones: relationships between structural variations, mammalian cell cytotoxicity, and antimicrobial activity. *J Med Chem* 1992;35:4745-4750.
 27. Shimoda K. Mechanisms of quinolone phototoxicity. *Toxicol Lett* 1998;102-103:369-373.

28. Khadra A, Pinelli E, Lacroix MZ, Bousquet-Melou A, Hamdi H, Merlina G, et al. Assessment of the genotoxicity of quinolone and fluoroquinolones contaminated soil with the *Vicia faba* micronucleus test. *Ecotoxicol Environ Saf* 2012;76:187-192.
29. Patterson DR. Quinolone toxicity: Methods of assessment. *Am J Med* 1991;91:35S-37S.
30. Gruchalla RS, Pirmohamed M. Clinical practice. Antibiotic allergy. *N Engl J Med* 2006;354:601-609.
31. Lam RF, Lai JS, Ng JS, Rao SK, Law RW, Lam DS. Topical chloramphenicol for eye infections. *Hong Kong Med J* 2002;8:44-47.
32. Tajik H, Malekinejad H, Razavi-Rouhani SM, Pajouhi MR, Mahmoudi R, Haghazari A. Chloramphenicol residues in chicken liver, kidney and muscle: a comparison among the antibacterial residues monitoring methods of Four Plate Test, ELISA and HPLC. *Food Chem Toxicol* 2010;48:2464-2468.
33. Yibar A, Cetinkaya F, Soyutemiz GE. ELISA screening and liquid chromatography-tandem mass spectrometry confirmation of chloramphenicol residues in chicken muscle, and the validation of a confirmatory method by liquid chromatography-tandem mass spectrometry. *Poult Sci* 2011;90:2619-2626.
34. Impens S, Reybroeck W, Vercammen J, Courtheyn D, Ooghe S, De Wasch K, et al. Screening and confirmation of chloramphenicol in shrimp tissue using ELISA in combination with GC-MS2 and LC-MS2. *Anal Chim Acta* 2003;483:153-163.