Advances in Bioresearch
Adv. Biores., Vol 9 (1) January 2018: 135-139

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.1.135139

Advances in Bioresearch

ORIGINAL ARTICLE

Studies on Antibiotic Residues in Chicken and its Public health Significance

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ABSTRACT
The widespread use of antibiotics in poultry industry as therapeutic and prophylactic agent may results in occurrence of their residues in poultry products. The present study was conducted to determine antibiotic residues in chicken, to evaluate effect of various processing techniques on these residues and to access the human health risk associated with them. Chicken muscle samples were collected in and around the Ludhiana city and examined by high performance liquid chromatography. Amongst 150 samples examined for tetracycline residues 14, 2, and 6% samples were found positive for oxytetracycline, tetracyclines, and chlortetracycline residues, respectively. Similarly, residues of enrofloxacin and ciprofloxacin were detected in 22.66 and 11.33% samples, respectively when other 150 samples were examined. Out of positive samples 4.66, 0.66, 1.33, 12 and 1.33% samples were above maximum residual limit for oxytetracycline, tetracyclines, chlortetracycline, enrofloxacin and ciprofloxacin, respectively. Heat treatment on antibiotic residues indicated that sufficient cooking temperature and time can have a significant effect on the residual losses and based on Hazard Quotient values it can be concluded that risk assessed is negligible since the calculated Hazard Quotient values are potentially safe.
Key Words: fluoroquinolones, hazard quotient, heat treatment, HPLC, tetracycline

Received 14.07.2017 Revised 24.08.2017 Accepted 30.12.2017

How to cite this article:
S L Moon, J Singh Bedi, J Paul Singh Gill and R Singh Aulakh.Studies on Antibiotic Residues in Chicken and its Public health Significance. Adv. Biores., Vol 9 [1] January 2018.135-139.

INTRODUCTION

The use of antibiotics in chicken meat production is usually meant for therapeutic and prophylactic purpose. The antibiotics commonly used in poultry are β-lactams (penicillin), tetracyclines, aminoglycosides, fluoroquinolone and sulfonamides [1]. However, indiscriminate and injudicious use like incorrect or extra labelled dosage, wrong route of administration, not respecting recommended withdrawal period, improper use of licensed product or through illegal use of unlicensed substances and use of drugs which have not been approved for certain species results in the form of residues in chicken meat [2]. Therefore, internationally recognized organizations such as World Health Organization, Food and Agriculture Organization, Veterinary Medicines Directorate of the European Union as well as Food and Drug administration of the USA have set tolerance or maximum residue limits (MRLs), acceptable daily intakes (ADI) for humans and withholding times for pharmacologically active substances including antimicrobial agents prior to marketing [3, 4]. In India, Food Safety Standard Authority of India (FSSAI) has set the tolerance limit for antibiotics and other pharmacologically active substances only for sea foods but no tolerance limit has been set for antibiotics and other pharmacologically active substances in poultry meat and meat products. There are no regulations for domestic consumption of chicken, while for export EU standards are followed by the Export Inspection Council (EIC) of India [5].

In present study two antibiotic groups have been targeted; tetracyclines (TCs) and fluoroquinolones (FQs). TCs are administered to chicken for bacterial enteritis, fowl cholera, chronic respiratory disease and infectious sinusitis etc. their residues in chicken may cause possible allergic reactions, liver damage, yellowing of teeth and gastrointestinal disturbances etc. in consumers [6]. FQs are extensively used in our

country in poultry production and the transfer of fluoroquinolone- resistant campylobacter species and *Salmonella Typhimurium*-type DT-104 from animals to humans is a major concern [7]. Therefore, keeping in view the public health significance of antibiotic residues in chicken and consumer safety, the present study was envisaged for, monitoring and evaluation of these residues in chicken samples, to study the effect of thermal processing techniques on the levels of these residues and to perform risk analysis in humans for intake of chicken meat having residues.

MATERIALS AND METHODS

One hundred and fifty chicken muscle samples were collected from freshly slaughtered birds from various retail markets and butcher shops located around Ludhiana city and were stored at -18^{0}C to -20^{0}C till further analysis. The standard antimicrobial powders were procured from Sigma Aldrich, Co, USA. Stock standard solutions (100 µg/mL) of TCs i.e. tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC) and Doxycycline (DC) were prepared in methanol and FQs standards i.e., enrofloxacin (EFX) and ciprofloxacin (CFX) were prepared in acetonitrile and HPLC water respectively and stored at 4-7^{o}C. The working solutions were prepared daily.

2.1 Residue extraction and analysis

Extraction of TCs residues was done according to method proposed by Cinquina et al. (2003a) [8]. In five mg homogenized chicken 2 ml of 20% trichloroacetic acid (TCA) was added followed by 20 ml of Mcllvaine-EDTA (Ethylene Diamine Tetra Aceticacid). Mixture was vortexed and centrifuged at 4000 rpm for 20 min. The supernatant was filtered through filter paper and processed for further cleanup on Oasis hydrophilic lipophilic base (HLB) cartridge preconditioned with 3 ml methanol and 2 ml of water. Sample was loaded and cartridges were washed with 2 ml of 5% methanol. The putative tetracycline residues were finally eluted with 3 ml of methanol. The solvent was removed by evaporation and the residues were re-dissolved in 1 ml mobile phase.

To extract FQs residues were extracted according to method proposed by Cinquina et al. (2003b) [9]. In four grams of homogenized muscle 4 ml of 20% TCA in methanol was added. After shaking for 5 minutes and sonication for 10 minutes, the mixture was centrifuge at 3500 g for 10 minutes. Then 10 ml of phosphate buffer (pH 7.4) was added the sample was vortexed and subsequently centrifuged at 3000 rpm for 15 minutes. The supernatant was combined and clean up by Sep-Pak Vac cartridge preconditioned with 2 ml methanol and 2 ml of water under gravity. After loading the sample cartridge was washed with 2 ml of water. The FQs residues were finally eluted with 2 ml of 1% trifluroacetic acid in acetonitrile. The solvent was removed residues were re-dissolved in 1 ml of 20% acetonitrile.

High Performance Liquid Chromatography (HPLC) of Varian Inc. (Varian 920-LC, Liquid Chromatography) with auto-sampler and Diode Array Detector (DAD) was used in the study for quantitative estimation of residues. To remove specific interferences form the samples 20 port solid phase extractor manufactured by 'Waters' was used. Oasis Hydrophilic–lipophilic-balanced (HLB) (60 mg, 3 cc): reversed phase sorbent for acids, bases and neutrals and Sep-Pak Vac 3cc (200mg) C18 cartridge were used for cleanup of the extraction solutions of TCs and FQs respectively.

For TCs residues mobile phase of Methanol: Acetonitrile: 0.01 M oxalic acid (1:1, 5:2.5, v/v) was used. The flow rate was kept at 0.75 ml/ min. Chromatography was performed at 27^{o}C, 365 nm and run time was kept at 10 min**.** Similarly for FQs residues mobile phase of 0.1% formic acid in methanol was used. The flow rate was kept 1 ml/min. Chromatography was performed at 27^0 C, 270 nm and run time was kept at 10 min.

2.2 Effect of processing methods on antibiotic residue levels

To study the effect of chicken processing techniques 10 gm of two chicken samples which were not having any residues were spiked at two concentrations i.e. 100 µg/kg and 150 µg/kg, allowed to stand for at least 30 min at room temperature and exposed to various heat treatments viz., boiling (100^{0}C, 20 min) microwave heating (2450 MHz, 5 min) and roasting (180^{0}C, 10 min) further processed and analyzed.

2.3 Estimation of Hazard Quotient (HQ) and risk assessment

The risk assessment in this study was estimated based on amount of TCs and FQs in chicken samples examined for the presence of antibiotic residues. The mean level of antibiotic concentration in chicken sample was evaluated. Average daily consumption of chicken based on 60 kg body weight was taken into account. Risk analysis was done by using suitable models like Hazard Quotient (HQ). Numerically the hazard of antibiotic residues was assessed by calculating the HQ by the formula, HQ= EDI/ ADI (Acceptable Daily Intake). The estimated daily intake (EDI) was calculated by equation given by Juan et al. [10]. EDI= (Mean of mg of antibiotic per Kg of food) × (Daily Intake of food) / (Adult body weight 60 kg).

RESULTS

High performance liquid chromatography method used in this study was found to be sensitive, precise, specific and convenient analytical method for detection and quantification of TCs and FQs residues. The recovery analysis was done by using 5 mg of blank chicken sample spiked with the antibiotic standards at three different concentrations of 50, 100, 150 ppb. The retention time of OTC, TC, CTC and DC were found to be 3.3, 3.4, 4.2, 5.5 minutes with recoveries of 87.36, 89.36, 70.2 and 74.83%, respectively. The retention time of EFX and CFX were 2.16 and 1.83 minutes with recoveries 93.2 and 92.6%, respectively. A standard calibration curve was obtained by plotting concentration against averages of the peak areas obtained. The method was validated at 6 different concentrations (0.025, 0.25, 0.5, 1, 2 and 4 ppm).

Out of 150 samples analyzed 33 (22%) chicken muscle samples contained TCs residues, including 21 (14%) violated by OTC, 3 (2%) contained TC and 9 (6%) contained CTC residues (Table 1). The levels of residues in some chicken samples were more than the international levels set by EU MRL (100µg/kg). Out of positive samples for TCs residues, 7 (4.66%), 1 (0.66%) and 2 (1.33%) samples were above MRL for OTC, TC and CTC, respectively. Presence of all three TCs in this study indicates that these were continuously used throughout the production cycle. With regard to 150 samples examined for FQs residues, 34 (22.66%) and 17 (11.33%) samples were found positive for EFX and CFX, respectively (Table 1). Out of positive samples 18 (12%) and 2 (0.14%) samples were above MRL for EFX and CFX, respectively. These results have suggested that EFX has been heavily used and the recommended withdrawal time has not strictly followed.

Table 1: Tetracycline and fluoroquinolone residues detected in chicken samples

Antibiotics	Positive samples	% positive	Mean (µg/kg)	SD (µg/kg)	Min (µg/kg)	Max (µg/kg)	Samples above MRL
OTC	21	14%	108.9	76.89	36.2	317.4	7
TC	3	2%	93.33	27.68	62.3	115.5	1
CTC	9	6%	87.16	54.01	38.4	215.6	2
EFX	34	22.66%	101.80	82.23	19.1	341.5	18
CFX	17	11.33%	75.91	28.53	19.8	142.6	2

OTC- Oxytetracycline; TC- Tetracycline
CTC- Chlortetracycline EFX- Enrofloxacin
CFX- Ciprofloxacin; SD- standard Deviation
Min- Minimum; Max- Maximum
MRL- Maximum Residual Limit

This study aimed to present the changes by different cooking processes on TCs and FQs in chicken meat which will help to determine the average cooking temperature and time required to make the cooked sample safer for consumption. The cooking methods used in the present study were similar to those widely applied before consumption during household cooking. In present study, the mean percentage reduction of OTC, TC, CTC, EFX and CFX in spiked chicken samples after boiling were 23.5, 40.7, 34.3, 27.1 and 26.0%, respectively. After microwave cooking reduction was 33.87, 42.95, 44.9, 14.9 and 14.55%, respectively and after roasting it was 28.14, 34.6, 39.7, 9.8 and 7.9%, respectively. The result showed that amount of residue in the sample has been significantly affected by cooking, which could be due to denaturation of proteins [11].

In the present study based on the mean values of antibiotic residues, the HQ was evaluated for OTC, TC, CTC and EFX & CFX combined with value of 0.0006, 0.0005, 0.0004 and 0.0025, respectively (Table 2).

Table 2: Estimation of risk assessment based on HQ for residues (mean concentration)

Antibiotic	EDI (µg/kg/day)	ADI (µg/kg/day)	Hazard Quotient
OTC	0.018	30	0.0006
TC	0.015	30	0.0005
CTC	0.014	30	0.0004
EFX & CFX	0.015	6.2	0.0024

OTC- Oxytetracycline; TC- Tetracycline
CTC- Chlortetracycline; EFX- Enrofloxacin
CFX- Ciprofloxacin; EDI- Estimated Daily Intake
ADI- Acceptable Daily Intake

DISCUSSION

Very few studies on antibiotic residues in poultry birds have been performed in India. However, the literature available on the occurrence of antibiotics in poultry samples indicates higher rates of antibiotic

residues. In present study 33 (22%) chicken muscle samples contained TCs residues. However, Al-Ghamdi et al. [3] detected TCs residues in 69.7% broiler poultry farm, Salehzadeh *et al*. [12] reported highest percentage of OTC (95.55%) residues in broiler muscle, liver and kidney samples, Shahid et al. [13] detected OTC (20.7%) residues in chicken meat, Salama et al. [14] also detected TCs residues in 44% chicken samples including 42% breast, 38% thigh and 52% liver samples, Elnasri *et al.* [15] detected residues in 27% of chicken tissue samples (liver, kidney and muscle). Based on all these studies it can be concluded that TCs residues are widely distributed and can be found in high concentration in poultry meat products. While some other studies indicated higher rates of FQs residues. Salehzadeh *et al.* [12] detected EFX residues in all samples from 90 broiler farm at the time of marketing. Similarly, Naeem et al. (2006) [16] analyzed poultry products in Pakistan and reported that 58 to 85% of the samples contained CFX and 55 to 92% of the samples contained EFX residues. In another report Er *et al.* [17] detected 45.7% FQs residues in chicken meat samples. Therefore, it is necessary to frequently monitor chicken products of high nutritional value for the presence of antibiotic residues before marketing.

In the present study effect of cooking on antibiotic residues was also investigated. The results showed that sufficient cooking temperature and time can reduce the antibiotic concentration. The reported results are in consistent with the previously reported studies. Moats *et al*. [18] stated that prolong cooking of meat might inactivate the antibiotics. The same results with different values were recorded for TCs residues by many researchers. Abou-Raya *et al*. [19] in their studies reported the time required to destroy 90% of the initial TC level was 23.9, 53.2 and 101.6 min for microwaving, boiling and roasting, respectively. There are very few references in the literature to study the effect of cooking on FQs residues. Lolo *et al.* [20] explained in their study that there was an apparent decrease in quinolones concentration in tissue because some was lost by exudation into liquid used for cooking; conversely, for a cooking procedure with water loss, there was an apparent increase in residue concentration. From this study it can be concluded that cooking process cannot eliminate the total concentration of the residues but it can only decrease their concentration [21]. These findings show an additional advantage of cooking as a food processing method from the safety and toxicological point of view.

HQ is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected. If the Hazard Quotient is calculated to be less than 1, then no adverse health effects are expected as a result of exposure. If the Hazard Quotient is greater than 1, then adverse health effects are possible. Since, all the HQ values were less than one thus the risk assessment based on HQ shows these residues were within the safe level.

It is evident that antibiotic residues are present in broiler chicken samples collected from Ludhiana suggesting lack of implementation of withdrawal period for antibiotics when used in poultry industry. Although the cooking can have residual losses upto some extent and calculated HQ values are potentially within safe limit but there is a need of stricter regulations for the use of antimicrobial drugs in the poultry industry as well as inspection of chicken for drug residues prior to marketing. The presence of these residues can be prevented at producer level by educating poultry farmers and making them aware to follow best poultry practices to prevent infection and follow up of withdrawal period.

CONFLICT OF INTEREST

No financial or personal relationship between the authors and other people of organisations have inappropriately influenced (bias) this work.

ACKNOWLEDGEMENTS

The author sincerely acknowledges the School of Public Health and Zoonoses, GADVASU, Ludhiana 141004 (Punjab) for providing laboratory and other facilities to conduct this study.

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