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*Original Article*

# Simultaneous estimation of multiple antibiotic residues from chicken samples using High Performance Liquid Chromatography (HPLC)

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**A B S T R A C T**

This study was designed to quantify the residual concentrations of some antibiotics such as oxytetracycline (tetracycline), ciprofloxacin (quinolones group), enrofloxacin (quinolones group) and levofloxacin (quinolones group) in broiler production systems. For the quantification of antibiotic residues (ARs), a rapid, simple and accurate method has been developed and validated for simultaneous identification and quantification of oxytetracycline, ciprofloxacin, enrofloxacin and levofloxacin residues in chicken muscles and livers by using reverse phase high performance liquid chromatography (RP-HPLC). The proposed method showed good linearity with the determination of coefficient, $r^2$=0.999 for each antibiotic and $r^2$=1.0 for ciprofloxacin in the range of 1.25 – 15.00 µg/mL with high sensitivity with detection limit (LOD) of 25 ppb, 34 ppb, 27 ppb and 29 ppb and quantitation limit (LOQ) of 83 ppb, 110 ppb, 89 ppb and 96 ppb for levofloxacin, oxytetracycline, enrofloxacin and ciprofloxacin, respectively. A total of 20 chickens (muscle and liver) were examined using high performance liquid chromatography (HPLC). Studied result revealed that oxytetracycline and levofloxacin were detected in 100% chicken samples whereas ciprofloxacin and enrofloxacin were completely not detected in all the chicken samples. Oxytetracycline residues levels were above the Maximum Residue Limit (MRL) of 55% in chicken muscle and 75% in liver and levofloxacin was detected below Maximum Residue Limit (MRL) of 85% in muscle and 75% in liver in chicken samples. This study revealed that chicken samples contain antibiotic residues. The method was successfully applied to the simultaneous quantitation of multiple antibiotic residues in pharmaceutical formulations or other sectors for the routine analysis.



## Introduction

Poultry meat is very important as it is the second most widely eaten type of meat globally due to its affordability to fulfill the dietary protein needs of the overgrowing human population in Bangladesh. Here, a large number of people are engaged in the business of poultry especially broiler chicken which is commercially grown for meat but the financial investment of this sector is too much low. Most of the people who are engaged in this sector maximum are uneducated and have no idea about the use of antibiotic or rarely observe drug withdrawal periods. Therefore, abuse or over uses of antibiotics, contributing to drug residues in food products. Antibiotics are natural products of a microorganism or identical synthetic products or similar semi-synthetic products that inhibit the growth of or destroy microorganisms (Kirbis, 2007) and therapeutic use to treat sick chickens, prophylactic use to prevent infection in chicken and as growth promoters to improve feed utilization and production (WHO, 2011). The widespread use of antibiotics, the failure to follow label directions or inappropriate withdrawal period of time before slaughtering of animals may cause residuals in foodstuff of animal origins. Antibiotic drug residues may persist in foods derived from poultry, which may pose adverse health effects for the consumer (Chanda *et al.*, 2014 and Riviere & Papich, 2018). Human exposure to significant levels of antibiotic residues from animal products may aggravate immunological responses in susceptible individuals and negatively affect

intestinal microbiota (Normanno *et al.,* 2007). The presence of antibiotics in human food is associated with several adverse public health effects, including hypersensitivity, gastrointestinal disturbance, tissue damage, and neurological disorders (Babapour *et al*., 2012). In recent years most of the studies have shown antibiotics administered to the poultry and livestock accumulated in liver, muscle (thigh and breast), bone and other edible tissues exceeding the maximum residue limits (MRL). MRL means the maximum concentration of residue resulting from the use of a veterinary medicinal product (expressed in mg/kg or µg/kg or µg/mL or µg/L on a fresh weight basis) which may be accepted by the community to be legally permitted or recognized as acceptable in or on a food (McGlinchey *et al.*, 2008). Withdrawal period is the time that allows the veterinary drugs and its residues to levels below the established MRL. Until the withdrawal period has elapsed the animal or its products are not fit for human consumption. Depending upon the drug, products, dosage, and route of administration, it varies from a day to several days or weeks (Lee *et al*., 2001).

Antibiotic residues are detected by chemical, biological and immunological methods. Detection methods can be classified by their degree of quantification into qualitative, semi-quantitative and quantitative methods. In this study, advanced analytical methods like HPLC are required to monitor antibiotic drug residues in human foods of animal origin because it contributes to fulfill the need for protein constituents in the human body.

Therefore, the objective of this study was to determine the residue levels of commonly used antibiotics in chicken collected from different farm of Dhaka district. However, all antibiotics were tested individually and we find that there is a necessity to estimate simultaneously three antibiotics is less laborious and cost effective and save time, which is the requirement of regulatory agencies and the industries too. Thus, a new method has been developed by which four antibiotics can be detected by one method saving time, money and labor.

## Methods and Materials

### Techniques for detection of residues

Diversified techniques have been applied for detection of ARs in food, which are classified broadly as chromatographic, immunological, microbiological, and miscellaneous. The highest quantification capability (51.34%) is based on chromatography, followed by immunological, (25.89%), microbiological, (16.96%), and miscellaneous, (8.04%) (Hollstein *et al*., 1981; Khanal *et al*., 2018; Moghadam *et al*., 2016; Chen Y *et al*., 2017; Rama A, *et al*., 2017). The chromatographic technique is increasingly being used over others, especially the rate is much higher in recent times, due to higher sensitivity and specificity, higher quantification capability. On the other hand, various immunological and microbiological techniques can be applied at a cheaper rate, rapidly with lesser efficiency, though the quantification and detection are not satisfactory.

### Surveillance study

No authentic data has been made available from the government and other non-government agencies on therapeutic and growth promoting uses of antibiotics in poultry. The antibiotics which are more frequently used, or at least which are detected most often in the meat, information was gathered by conducting a surveillance study pertaining to use of antibiotic in various markets located in Dhaka District. The information was also gathered to shortlist most commonly and frequently used four antibiotics in chickens for further investigations and hence oxytetracycline, enrofloxacin, ciprofloxacin and levofloxacin were short listed for determination of their residual concentration in samples of muscle and liver of broiler chickens.

### Collection of samples

A total of 20 broiler chickens were collected from four different markets of Dhaka district of Bangladesh (Jatrabari bazar, Palasi bazar, Karwan bazar and Hatirpool bazar). Then the chicken samples were cut into specific parts to collect the muscle and liver. Each sample was placed into a separate plastic zipper bag and transferred to laboratory using ice bag plastic container and stored at -20°C until extraction. Samples were collected from January 2020 to June 2020.

### Chromatographic equipments

HPLC was performed on SIL 20 series Prominence HPLC (Shimadzu, Japan) equipped with an auto sampler (Model SIL-20 AC), dual pumps (Model 20 AD), column oven (Model CTO-20A), vacuum degasser (Model DGU-20A), UV-visible detector (Model SPD-20A), and LC solution software was used. Analytical reversed phase C-18, Luna 5µ, 250 x 4.6 mm, Phenomenex, Inc., Japan was used.

### Chromatographic conditions

Mobile phase: Acetic acid (10%): Acetonitrile (90: 10)
UV detection: 280 nm
Run time: 15 mins
Flow rate: 1.0 mL/min
Column temperature: Room temperature
Injection volume: 20 µL
Elution: Isocratic

### Chemicals and Reagents

The chemicals and techniques used for extraction, detection and quantification of residual concentration of ciprofloxacin, enrofloxacin, levofloxacin and oxytetracycline were gifted from Incepta pharmaceutical ltd. Methanol: HPLC grade, DUKSAN, Korea; Acetonitrile: HPLC grade, DUKSAN, Korea; Deionized water, Phosphate Buffer Saline (PH 6.5) Trichloro acetic acid AR., 98% analytical grade, Loba Chemie and Research-Lab Fine Chem Industries. Purity of all standard chemicals and reagents was at least 98%.

### Sample preparation and antibiotic extraction

Sample extraction was performed according to Popelka *et al.,* (2005) with some required adjustments. The meat sample was cut into small pieces and then it was grinded properly with a grinder. 4g sample were weighted with weight box and taken into a falcon tube. Then 10mL phosphate buffered saline (pH-6.5) was added and mixed by vortexing. Then 2mL 30% trichloroacetic acid was added and mixed properly by vortexing. Then it was centrifuged (Hettich MIKRO 220R, Germany) at 4000 rpm for 20 minutes. Supernatant was collected and filtered by 0.45 µm nylon filter. Inject sample into HPLC.

### Preparation of standard

To prepare standard solution, 10 mg of ciprofloxacin, oxytetracycline, levofloxacin, and enrofloxacin were weighed and diluted with 10ml 5% TCA in a volumetric

flask containing a concentration of 1000ppm. Then this solution was mixed by vortexing for 5 mins and was filtrated by 0.45 µm nylon filter. Serial dilution was done to get concentration 1250ppb-15,000ppb i.e., 1.25-15.00 µg/mL with TCA solution. The supernatant was filtrated by 0.45 µm nylon filter and then sample was injected into HPLC.

**Results and discussion**

To ensure whether this method for determination of antibiotic residues was applicable to real samples, validation parameters were evaluated through several basic analytical parameters, including specificity, linearity, LOD, LOQ, precision (intra-day and inter-day repeatability) (RSD%) and accuracy (% recovery). All these parameters are describing below:

Under the optimized chromatographic conditions [mobile phase acetic acid (10%): Acetonitrile (90: 10) on C-18 column at flow rate of 1.0 mL/min and detection at 280nm], chromatograms which were observed are shown in figure 1 (A-F). The chromatogram of four antibiotics OTC, LEVO, ENRO and CIP were detected at about 6.01 ± 0.01 min, 7.01 ± 0.02, 9.03 ± 0.01 and 10.51 ± 0.01 min, respectively without any interference. It may be said that the method is specific. System suitability was examined by injecting five replicates of 100% test concentration of omeprazole into the system. Excellent resolution for four antibiotics was obtained as shown in table 1.

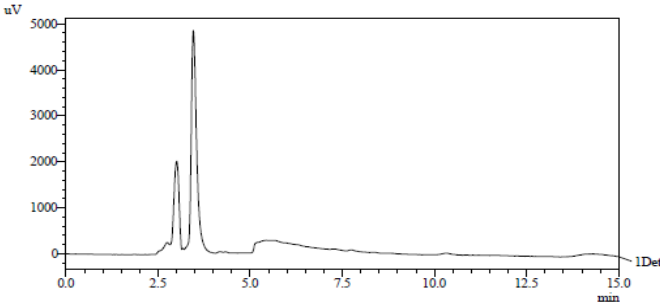


**Figure 1 (A): Chromatogram for blank sample.**

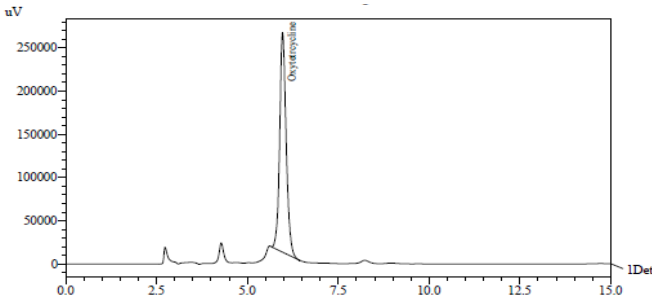


**Figure 1 (B): Chromatogram for reference (Oxytetracycline)**

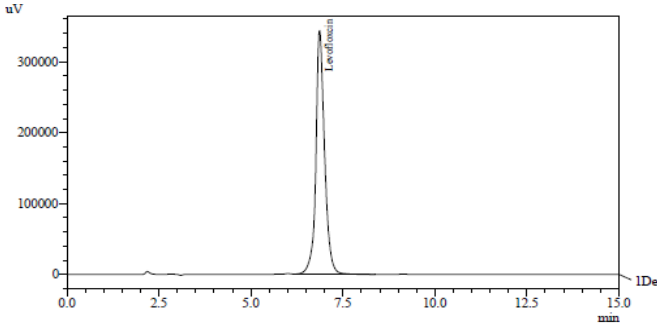


**Figure 1 (C): Chromatogram for reference (Levofloxacin).**

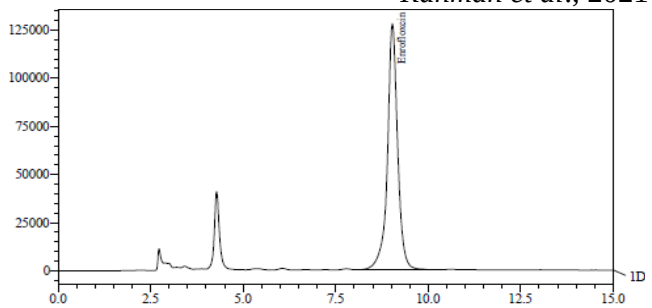


**Figure 1 (D): Chromatogram for reference (Enrofloxacin)**

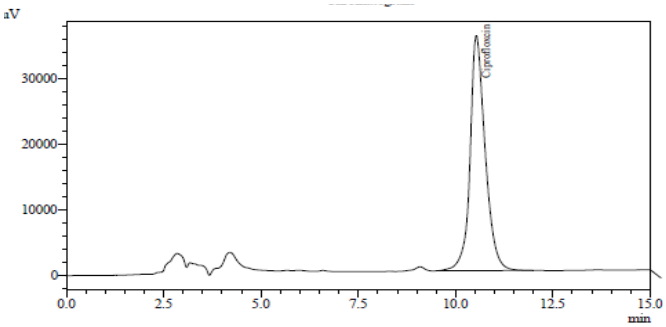


**Figure 1 (E): Chromatogram for reference (Ciprofloxacin)**

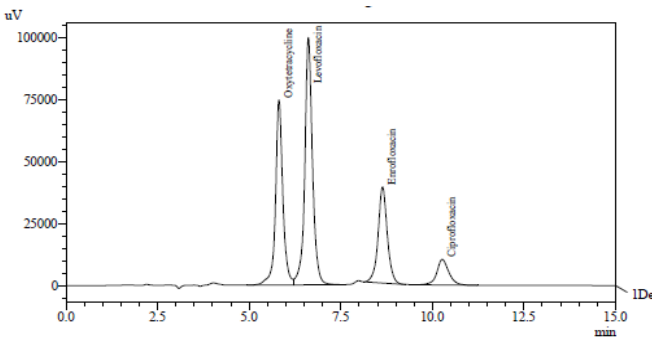


**Figure 1 (F): Chromatogram for mixture references (Oxytetracycline, Levofloxacin, Enrofloxacin and Ciprofloxacin).**

This method resulted in symmetric peak shape with tailing 1.07, 10.4, 1.04 and 1.08 and good no. of theoretical plates with 31590, 35674, 40337 and 34795 for OTC, LEVO, ENRO and CIP, respectively. It showed insignificant resolution (Rs) and no. of theoretical plates (N). The proposed method showed good linearity with the determination of coefficient, $r^2$=0.999 for LEVO, OTC and ENRO and $r^2$=1.0 for CIP in the range of 1.25 – 15.00 µg/mL concentrations of four antibiotics in the limit of ($r^2$>0.995) indicating good linearity of calibration curve. The linearity curves are shown in figure 2 and the parameters are given in table 1.

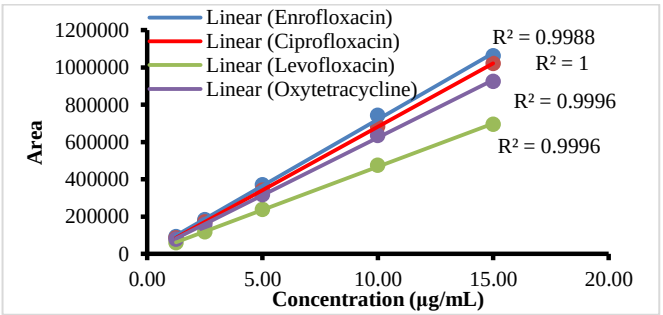


**Figure 2: Linear curve for enrofloxacin, ciprofloxacin, levofloxacin and oxytetracycline**

The limits of detection (LOD) were found to be 25 ppb, 34 ppb, 27 ppb and 29 ppb and quantitation limit (LOQ) of 83 ppb, 110 ppb, 89 ppb and 96 ppb for LEVO, OTC, ENRO and CIP, respectively. The average percentage of recovery was calculated and it was found to be 100.54%, 100.85%, 100.46% and 100.28% for LEVO, OTC, ENRO and CIP, respectively. The proposed method was found to be precise (intra-day) and reproducible (inter-day) with % RSD of 0.37%, 0.33%, 0.38% and 0.25% for LEVO, OTC, ENRO and CIP and 0.17, 0.03%, 0.28 and 0.41% for LEVO, OTC, ENRO and CIP, respectively. All the validation parameters are shown in table 1, table 2 and table 3.

**Table 1. Results of method validation parameters**

| Parameters | LEVO | OTC | ENRO | CIP |
|---|---|---|---|---|
| Linear equation | y= 79329x+1827.1 | y= 59663x+841.06 | y= 71485x+6615.7 | y= 68081x+872.48 |
| Coefficient of determination (r2>0.995) | 0.999 | 0.999 | 0.999 | 1.0 |
| Linearity range | 1.25-15 µg/mL | | | |
| Resolution (≥2) (n=5) | 2.30 | | 5.02 | 3.22 |
| Theoretical plates (≥2000) (n=5) | 35674 | 31590 | 40337 | 34795 |
| Tailing factor (≤2) (n=5) | 1.04 | 1.06 | 1.04 | 1.08 |
| Precision (intra-day, n=6) (% RSD≤2) | 0.37% | 0.33% | 0.38% | 0.25% |
| Precision (inter-day, n=6) (% RSD≤2) | 0.17% | 0.03% | 0.28% | 0.41% |
| LOD (µg/mL) | 0.025 | 0.034 | 0.027 | 0.029 |
| LOQ (µg/mL) | 0.083 | 0.110 | 0.089 | 0.096 |
| n = number of determinations | | | | |

**Table 2. Accuracy of OTC (% Recovery)**

| OTC | | |
|---|---|---|
| **Accuracy (n=3) (avg. % recovery) Standard+sample (µg/mL)** | **Recovered mean concentration (µg/mL)** | **% Recovery [± SD (*n*=3)]** |
| (1.25+1.25)= 2.5 | 2.55 | 101.87% ± 0.61 |
| (2.5+1.25)= 3.75 | 3.73 | 99.55% ± 0.48 |
| (5.0+1.25)= 6.25 | 6.24 | 99.76% ± 0.15 |
| Avg. | | 100.39% ± 0.41 |

**Table 3. Accuracy of LEVO (% Recovery)**

| LEVO | | |
|---|---|---|
| **Accuracy (n=3) (avg. % recovery) Standard+sample (µg/mL)** | **Recovered mean concentration (µg/mL)** | **% Recovery [± SD (*n*=3)]** |
| (1.25+0.50)= 1.75 | 1.74 | 100.09% ± 0.58 |
| (2.50+0.50)= 3.0 | 2.98 | 99.35% ± 0.27 |
| (5.0+0.50)= 5.5 | 5.54 | 101.21% ± 0.17 |
| Avg. | | 100.22% ± 0.34 |

Laboratory experiments were performed to detect the antibiotic residues in different tissues of broiler chicken applying the above method. HPLC was used for determining the concentration of antibiotics residues of four target antibiotics named as ciprofloxacin (CIP), enrofloxacin (ENR), levofloxacin (LEVO) and oxytetracycline (OTC). A total of 20 chickens (muscle and liver) were examined using high performance liquid chromatography (HPLC). Studied result showed OTC and LEVO were detected in 100% chicken samples whereas CIPRO and ENRO were completely not detected in all the chicken samples. OTC residues levels were above the Maximum Residue Limit (MRL) of 55% in chicken muscle and 75% in liver recommended limit according to the Bangladesh Food Safety Authority (BFSA) and LEVO was above the Maximum Residue Limit (MRL) of 15% in chicken muscle and 25% in liver according to Commission Regulation (Eu) No 37/2010 Of 22 December 2009 (table 5). Chromatograms of chicken samples were represented in figure 3. Results were showed in table 4 and graphical representation represented in figure 4. A similar trend of the present study was observed by Al-Ghamdi *et al.*, 2000 conducted in Saudi Arabia where a higher percentage of oxytetracycline residues was reported by examining 33 broiler farms and 100% of samples showed positive results for Oxytetracycline in liver tissues.

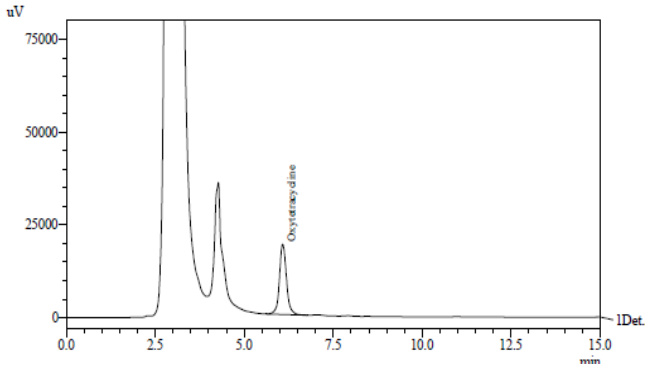


**Figure 3 (A): Chromatogram of Oxytetracycline in chicken sample**

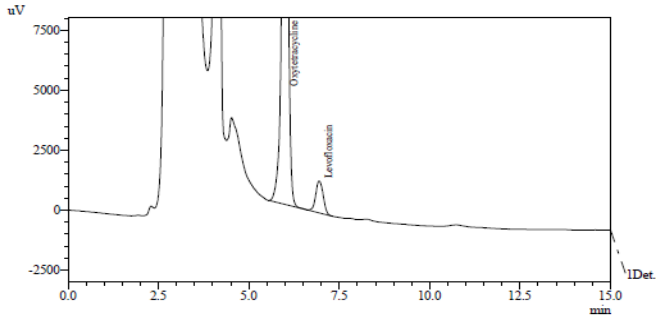


**Figure 3 (B): Chromatogram of Oxytetracycline and levofloxacin in chicken sample**

**Table 4. OTC and LEVO MRLs in chicken muscle and liver**

| Name of Antibiotic residues | Muscle | | Liver | | Detected |
|---|---|---|---|---|---|
| | Below MRL | Above MRL | Below MRL | Above MRL | |
| Oxytetracycline | 45% | 55% | 25% | 75% | 100% |
| Levofloxacin | 85% | 15% | 75% | 25% | 100% |

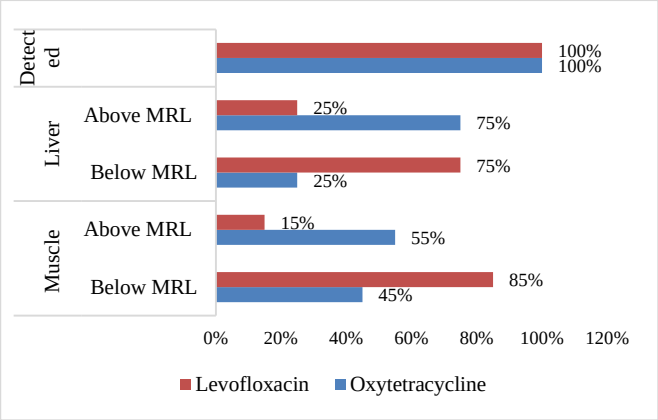


**Figure 4: Qualitative results of target antibiotics**

**Table 5. Regarding maximum residue limits (MRLs) in foodstuffs of animal origin**

| Name of regulatory affairs | Oxytetracycline (µg/kg)/ ppb | | Enrofloxacin (µg/kg)/ ppb | | Ciprofloxacin (µg/kg)/ ppb | |
|---|---|---|---|---|---|---|
| Bangladesh Food Safety Authority (BFSA) regulation, 2013 | 200 (µg/kg)<br>600 (µg/kg) | Muscle<br>Liver | No entry | | No entry | |
| MRLs in poultry | 200 (µg/kg) | Muscle | 100 (µg/kg) | Muscle | 200 (µg/kg) | Muscle |
| European Commission Regulation (EU) No 37/2010) | 600 (µg/kg) | Liver | 100 (µg/kg) | Liver | 200 (µg/kg) | Liver |

**Oxytetracycline residues in different tissues of broiler chicken**

Oxytetracycline is a natural tetracycline compound that is derived from the fungus *Streptomyces rimosus*. It is a wide-spectrum antibiotic with bacteriostatic activity against both gram-positive and gram-negative bacteria such as the species of Spiroethete, Antinomies, Rickettsia and Mycoplasma (Slana & Dolenc, 2013). It is poorly metabolized in target animals and excreted in its parent form due to high water solubility. The residues of this antibiotic can cause allergic reactions in some hypersensitive individuals if consumed for a long time. However, overuse and insufficient lengths of withdrawal of oxytetracycline antibiotics in poultry production resulted in the presence of residues that may endanger human health and reach to teratogenic malformation to the fetus, hypoplasia in developing teeth when administered to infants (Cetinkaya *et al.*, 2012).

The residual mean concentration of oxytetracycline was shown in table 6 and graphical representation showed in figure 5. The highest concentration was found of 0.24 µg/mL at Karwan bazar and 0.68 µg/mL at Palashi bazar in muscle and liver, respectively. The lowest concentration was found 0.19 µg/mL at Hatirpool bazar and 0.64µg/mL at Karwan and Hatirpool bazar in muscle and liver, respectively. Concentration ranges in muscles were 0.17-0.31 µg/mL, 0.13-0.27 µg/ml, 0.19-0.32 µg/mL and 0.17-0.23 µg/mL at Jatrabari, Palashi, Karwan and Hatirpool bazar, respectively. Concentration ranges in liver were 0.53-0.76 µg/mL, 0.57-0.76 µg/mL, 0.57-0.69 µg/mL and 0.57-0.67 µg/mL at Jatrabari, Palashi, Karwan and Hatirpool bazar, respectively. The oxytetracycline concentration in the studied samples were in the order of liver> muscles. The level of antibiotic residues may vary among different tissues.

The residual concentration of oxytetracycline found 55% above MRL in muscle and 75% above MRL in liver approved by Bangladesh Food Safety Authority (BFSA), European Union (EU) for muscle [200 µg/kg (0.2 µg/g)] and for liver tissue [600 µg/kg (0.6 µg/g)] (EC, 2010). The concentration result of oxytetracycline in a study conducted by Ahmed and Gareib (2016) was in line with our findings where chicken breast muscle showed higher concentration (2.5µg/g) than MRL (200 µg/kg) approved by EU. On the other hand, R. widiastuti and Y. Anastasia (2015) detected OTC in broiler chicken meat marketed in different cities in Java Island.

**Table 6. Concentrations (Mean ± SD) and range of Oxytetracycline residues in different tissues of broiler chicken(µg/mL)**

| Twenty Chicken tissues | Study Area | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Jatrabari Bazar | | Palashi Bazar | | Karwan Bazar | | Hatirpool Bazar | |
| | Mean (µg/mL) ± SD | Range (µg/mL) | Mean (µg/mL) ± SD | Range (µg/mL) | Mean (µg/mL) ± SD | Range (µg/mL) | Mean (µg/mL) ± SD | Range (µg/mL) |
| Muscle | 0.22 ± 0.06 | 0.17-0.31 | 0.21±0.06 | 0.13-0.27 | 0.24±0.06 | 0.19-0.32 | 0.19±0.03 | 0.17-0.23 |
| Liver | 0.62 ±0.09 | 0.53-0.76 | 0.68±0.08 | 0.57-0.76 | 0.64±0.05 | 0.57-0.69 | 0.64±0.04 | 0.57-0.67 |

Shalezadeh *et al*. (2006) revealed that all chicken samples collected from 90 broiler farm in Iran, showed OTC residues which was detected by HPLC exceeding the MPL. Shahid et al. (2007) also found that 4 out of 7 samples of chicken meat in Pakistan positive for OTC. They confirmed widespread misuses of OTC in farms may be the main reason of the presence of OTC in maximum samples. In a separate research conducted by Cetinkaya et al. (2012) in Turkey with the use of LCMS/MS, did not find any OTC residue on 60 tested samples. Therefore, stricter regulations for the use of antibiotics in the poultry industry and the monitoring of drug residues in chicken meat in Turkey prior to marketing were maintained.

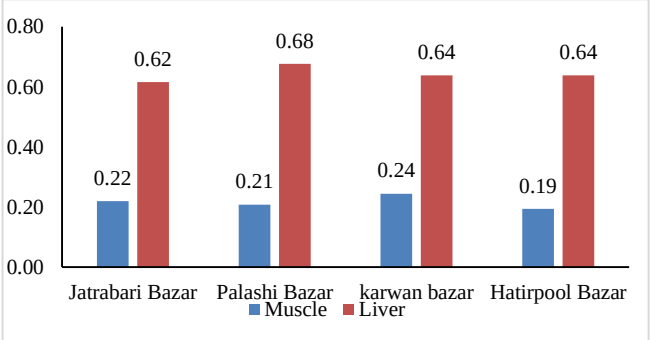


**Figure 5: Average concentrations (µg/mL) of OTC in chicken muscle and liver tissues**

**Levofloxacin residues in different tissues of broiler chicken**

Levofloxacin is a third-generation fluoroquinolone, an optical isomer of ofloxacin having two-fold higher antimicrobial activity than the parent compound. Currently, it is successfully used in human medicine in the treatment of infections of upper and lower respiratory tract, genitourinary system, skin and soft tissue (Gary J. Noel, 2009). It is also known as a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe life-threatening bacterial infection caused by species of Staphylococci, Streptococci, Enterobacteriaceae, Escherichia, Klebsiella, Proteus, Pseudomonas, Bacteroides, Clostridium, Haemophilus, Moraxella, Mycoplasma and Chlamydia (Dumka and Srivastava, 2007). The average concentration results of LEVO residues were presented in figure 4. Muscles and liver showed a positive response for levofloxacin residues but 85% in chicken muscle showed below MRL and in liver 75% of chicken samples showed below the MRL. The residual mean concentration of levofloxacin was shown in table 7 and graphical representation showed in figure 6. The highest concentration was found of 0.13 µg/mL at Karwan bazar and 0.21 µg/mL at Jatrabari bazar in muscle and liver, respectively. The lowest concentration was found 0.02 µg/mL at Palashi bazar and 0.05 µg/mL at Jatrabari bazar in muscle and liver, respectively. Concentration ranges in muscles were 0.05-0.11 µg/mL, 0.02-0.11 µg/mL, 0.06-0.13 µg/mL and 0.06-0.08 µg/mL at Jatrabari, Palashi, Karwan and Hatirpool bazar, respectively. Concentration ranges in liver were 0.05-0.21 µg/mL, 0.08-0.22 µg/mL, 0.07-0.23 µg/mL and 0.06-0.26 µg/mL at Jatrabari, Palashi, Karwan and Hatirpool bazar, respectively.

**Table 7. Concentrations (Mean ± SD) and range of Levofloxacin residues in different tissues of broiler chicken (µg/mL)**

| Twenty Chicken tissues | Study Area | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Jatrabari Bazar | | Palashi Bazar | | Karwan Bazar | | Hatirpool Bazar | |
| | Mean (µg/mL) ± SD | Range (µg/mL) | Mean (µg/mL) ± SD | Range (µg/mL) | Mean (µg/mL) ± SD | Range (µg/mL) | Mean (µg/mL) ± SD | Range (µg/mL) |
| Muscle | 0.08 ± 0.02 | 0.05-0.11 | 0.06±0.04 | 0.02-0.11 | 0.08±0.03 | 0.06-0.13 | 0.07±0.01 | 0.06-0.08 |
| Liver | 0.11 ±0.07 | 0.05-0.21 | 0.12±0.06 | 0.08-0.22 | 0.14±0.08 | 0.07-0.23 | 0.15±0.08 | 0.06-0.26 |

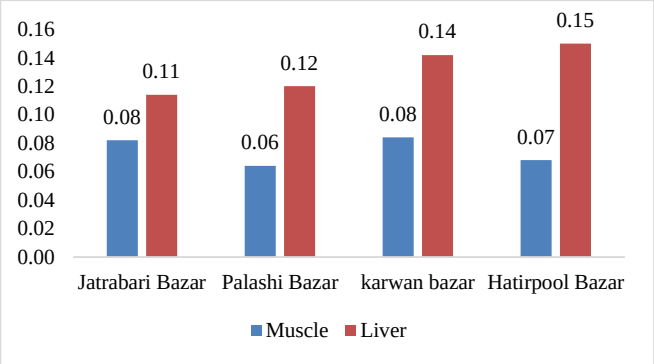


**Figure 6: Average concentrations (µg/mL) of LEVO in chicken muscle and liver tissues.**

There were few studies for assessing the residual status of levofloxacin in muscle tissues of broiler chicken (Kyuchukova *et al.*, 2013). On the contrary, there is no MRL level and established withdrawal period for the Levofloxacin by European Economic Community (EEC) and the European Commission (EC). As Levofloxacin follows the same group of ciprofloxacin and enrofloxacin which is fall in the group of fluoroquinolones, the present study considers the MRLs of ciprofloxacin and enrofloxacin which was 100 µg/kg (0.1 µg/g) for muscle tissue and was 200 µg/kg (0.2 µg/g) for liver tissue.

**Conclusion**

The present study showed that there are no ciprofloxacin and enrofloxacin residues in the collected broiler chicken tissues. On the other hand, oxytetracycline was the predominant antibiotics (100%) detected in 20 chicken tissue samples where OTC was detected 55% above MRL in muscle and 75% above MRL in liver. Though levofloxacin showed 100% detection, 85% below MRL in muscle and 75% below MRL in liver detected in 20 chicken samples. It may be stated that the chicken meat producers do not follow the regulations about the withdrawal periods of the respective veterinary drugs. After a certain time, inappropriate and non-judicious use of these drugs results in an accumulation of toxic and harmful residues in meat and eggs of treated birds which affect consumer health by triggering allergic reactions and transmitting antibiotic-resistant microbial infections. Therefore, in this regard community-based awareness programs, trainings, proper monitoring, enforcing legal rules and compelled farmers to follow it, authorized by the government teams can play a vital role to reduce veterinary drug residues in broiler chicken meat for ensuring food safety of general people.

**Conflict of interests**

There is no conflict of interest as declared by the authors.

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