

ORIGINAL ARTICLE

**Detection of oxytetracycline and doxycycline residue in different growth stages of commercial broiler**

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**Abstract**

**Background:** Indiscriminate use of antibiotic in broiler farms is responsible for residual deposition in broiler's edible tissue that can possess health hazard including antibiotic resistance to human being by entering in food chain. This study was designed to detect and compare two common antibiotics residue in broiler at three different growth stages.

**Methods:** A questionnaire survey was conducted to understand the farmers' perspective regarding antibiotic residue in four upazila of Mymensingh district. Samples were collected from eight poultry farms of each upazila namely Fulbaria, Trishal, Nakla and Mymensingh sadar. From each farm, nine samples were collected at the three different ages of 9-17 days, 19-22 days and 29-32 days, respectively. Thin layer chromatography (TLC) was used for the qualitative detection of oxytetracycline and doxycycline residues in broiler meat. Data was analyzed by SPSS IBM 20.

**Results:** Most of the farmers are educated up to secondary level. Only 9.37% farmers have residual knowledge but all are unaware about withdrawal period. No one is practicing good management system for their flock. A total of 288 meat samples were analyzed from four upazila. Oxytetracycline residue was detected in >50% samples: in Fulbaria (55.56%), Trisal (66.66%) and Nakla (55.55%) upazila and slightly lower in Mymensingh sadar (44.44%). Similar observation was obtained for doxycycline: Fulbaria (44.44%), Mymensingh sadar (66.67%), Nakla (55.55%) and in Trishal (44.44%). The residual level detected positive in first, second and third collections were 66.67%, 25% and 58.33% for doxycycline and 50%, 25% and 91.66% for oxytetracycline, respectively.

**Conclusions:** These result indicates that doxycycline and oxytetracycline are being misused in poultry industry especially in first and last quarter of broiler life and withdrawal periods are not maintained when broiler are marketed. Measures are needed to ensure human safety regarding antibiotics use in poultry industry.

**Keywords:** Antibiotic residue, Questionnaire, Broiler farm, TLC

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## Introduction

Antibiotics are widely used in poultry production not only to treat diseases but also to maintain health, promote growth and enhance feed efficiency (Okerman *et al.*, 2007). However, this practice may lead to deposit drug residue in poultry meat and products that is related with adverse health hazard to the consumers (Sarker *et al.*, 2018). These hazard include toxic effects, immune-pathological effects, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, bone marrow toxicity and allergy. Moreover, indiscriminate use of antibiotics could lead to the emergence of antibiotic resistance bacteria in the environment and living system (Nisha *et al.*, 2008; Hasan *et al.*, 2014).

Indiscriminate use of antibiotics, lack of guidance and failure to notice drug withdrawal period, lack of consumer awareness are some primary reasons for occurrence of antibiotic residues in poultry edible tissue (Singh *et al.*, 2014).

Tetracycline, a broad spectrum antibiotic is commonly used in poultry industry for its antibacterial and growth promotion effects. (Sarker *et al.*, 2018). Oxytetracycline (OTC) a member of tetracycline family is difficult to be metabolized and partly excreted in the environment in the form of parent compounds due to its high solubility in water (Widiastuti *et al.*, 2015). Higher OTC are being used due to its spectrum, availability, relatively cheaper price, and easy oral administration through drinking water or feed (Slana and Dolenc, 2013). Doxycycline (DC), a semi-synthetic, second-generation tetracycline is also used in poultry farm due to its wide coverage of organisms like Rickettsia, Chlamydia, Mycoplasma etc. and bacteriostatic activity (Hsiao *et al.*, 2016; Prats *et al.*, 2016).

Therefore, these two antibiotics have public health significances. Excessive use of these antibiotics in poultry farms causes accumulation of residue in meat that could be transferred to humans through the food chain. Limited studies have been performed about detection of

oxytetracycline and Doxycycline residue in poultry meat in Mymensingh division (Sarker *et al.*, 2018) but no study is designed to access the residual presence in broiler meat at different growth stages to understand when and why farmers mostly prescribe oxytetracycline and doxycycline. The study was undertaken to determine the presence of oxytetracycline and doxycycline residue in raw broiler meat at different growth stages at Mymensingh division.

## Materials and Methods

### Experimental design

The research project was divided into two contexts: Firstly, assessing farmers using questionnaire survey. The survey was conducted by a semi-structured questionnaire for assessing (i) the farmers' educational level, (ii) Knowledge of residue, (iii) Storage of drugs and (iv) the purpose of antibiotic usage. And secondly, screening of antibiotic residue: oxytetracycline and doxycycline residue in poultry meat were identified and detected by TLC for screening.

### Sample collection

A total of 288 broiler samples were collected from thirty-two broiler farms in Fulbaria, Mymensingh sadar, Trishal and Nakla upazila. Three meat samples were collected at three different ages from each farm. We have collected 1<sup>st</sup> batch of sample at the age of (9-17) days, 2<sup>nd</sup> batch at (19-22) days and 3<sup>rd</sup> batch at (29-32) days. Each sample was placed into a separate plastic zipper bag transferred to laboratory maintaining cold chain and stored at -20°C until extraction.

### Chemical and standard drugs

HPLC grade methanol (Merck-Germany), trichloroacetic acid (TCA), diethyl ether and acetone (RCI Labscan-Thailand) was used. Oxytetracycline (OTC) and doxycycline (DOX) were obtained from Sigma-Aldrich via Renata Limited, Bangladesh. The standard for the OTC and DOX were prepared by dissolving 0.1 gm of powder in 4 mL solution of methanol as cited by (Hussain *et al.*, 2005). Standard solution was stored in -4°C and fresh solution was prepared for every month.

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### **Sample preparation**

The experiment was conducted as described by Popelka *et al.*, 2005. In brief, Four grams of each sample was grinded and blended after cutting into small pieces. 10 mL Phosphate Buffer Saline (pH-6.5) was added and mixed by vortex (Vortex- XHC, Wincom, China). Then the sample was centrifuged (Hettich D-78532, Germany) @ 60000 rpm for 20 minutes after mixing with 2 mL 30% TCA. We have used Whatman filter paper and funnel for filtration of Supernatant. Filtrate was collected in another falcon tube and it was compounded with same amount of diethyl ether and left for 10 min in room temperature. Supernatant extraction was repeated twice after collection of the bottom layer using diethyl ether. Final extracts were transferred carefully into screw cap vial and preserved into refrigerator for further analysis.

### **Thin Layer Chromatography (TLC)**

#### **TLC apparatus**

TLC plate (MN-Germany), TLC tank and UV detection box (UV light: F18W-Germany) were used. TLC was performed according to (Tajick and Shohrel, 2006) with some modification. TLC plate was cut into a size of 4x5 cm from 20x20 cm. A straight line was put across the plate approximately 2 cm from the bottom by a pencil. Similar line was drawn across the plate below 1 cm from the upper edge of the plate. A spot were marked on the bottom line to drop the analytes. A volume of 50 µl drops were put on the spot of the plate using thin capillary glass pipettes. Then we placed Plate in TLC tank (contained mobile phase; Acetone and Methanol: 1:1) and covered by lid and it was left until the mobile phase reached the upper line. UV detection box at 256 nm was used to visualize the spots. Spots marking were done by pencil for calculation of retention factor (Rf).

#### **Calculation of Rf values**

The Rf values are calculated with the distance travelled by the solvent, and the distance travelled by individual sample spots. Same Rf value of standard and sample considered similar compound. (Sarker *et al.*, 2018).

### **Data Analysis**

Microsoft Excel-2010 was used to store data. Results were analyzed statistically using SPSS IMB 20 for descriptive statistics (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20, Armonk, New York USA: IBM Corp) and graphs were prepared by Graph Pad Prism 6.

### **Results**

#### **Questionnaire Survey to assess farmers' perspective**

Survey covered different geographical location of Mymensingh division. A total of thirty-two farmers were surveyed from four upazilas (eight farms from each upazila). We have selected the farms that were using oxytetracycline and doxycycline at the time of survey.

The questionnaire was written both in English and Bangla. The surveyors were enough competent to explain the questions to the farmers properly. All the farms were small scale broiler farms having flock size 500-2000 (Islam *et al.*, 2010). 21.88% farmers had primary education, where as a large proportion of farmers (46.87%) were secondary, 18.75% were higher secondary and 12.5% were Diploma/Graduate level educated. All the farmers use antibiotic for both prophylactic and therapeutic purposes. 75% farmers preserve the antibiotic in their poultry shed and the rest (25%) have storage room for the drugs. Only 9.37% farmers possess the knowledge of residual deposition. But no none of them practice the withdrawal period. Every farmer collects their feed and chicks from renowned companies. But they don't practice proper farm management and hygiene.

#### **Assessment of antibiotic residue**

The highest percentage of oxytetracycline and doxycycline residue was detected in Trishal (Figure 1) and Mymensingh Sadar (Figure 2) whereas the lowest in Mymensingh sadar and Trishal respectively (44.44%). The usage pattern of both oxytetracycline and doxycycline are similar in Nakla (55.55%) and 10%-20% variations in other three upazilas.

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Farmers use more doxycycline (67%) in 9-17 days than oxytetracycline (50%); however, they tend to use more oxytetracycline in 29-32 days. During 19-22 days of age, farmers use similar amount of oxytetracycline and doxycycline (Figure 3 & 4).

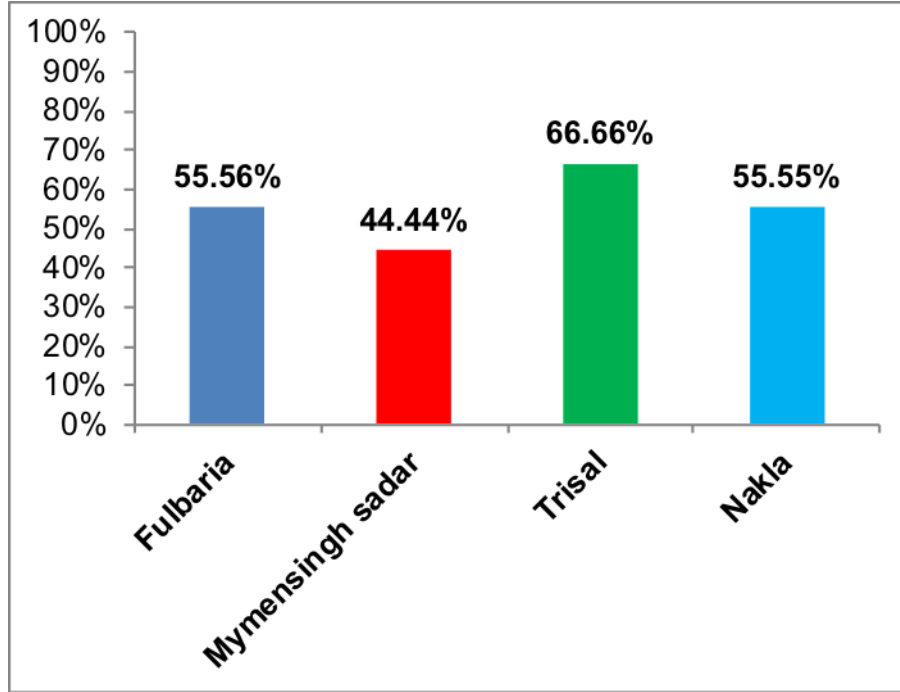


Fig 1. Presence of oxytetracycline residue in broiler samples in three different growth stages in four upazilas

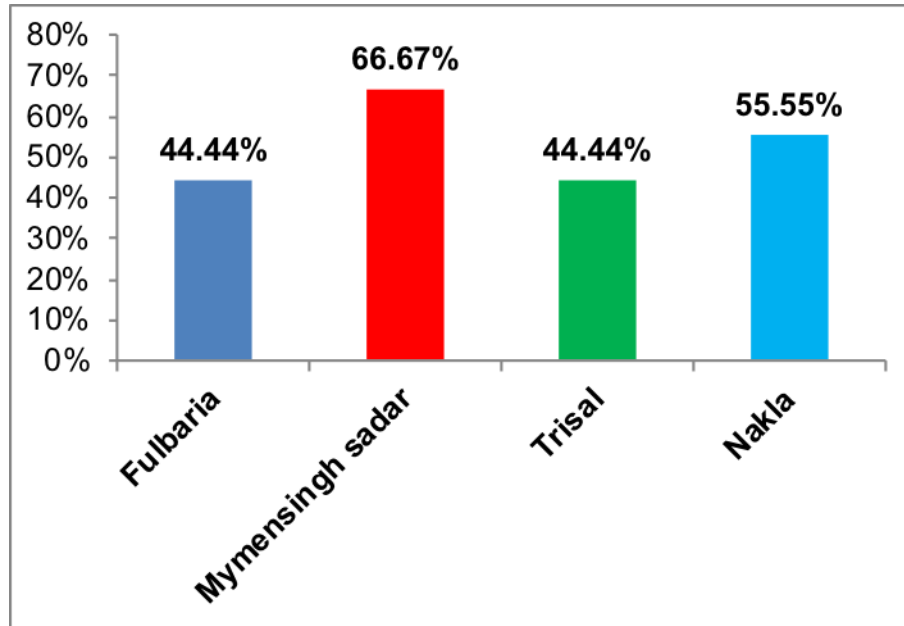


Fig 2. Presence of doxycycline residue in broiler samples in different growth stages in four upazilas

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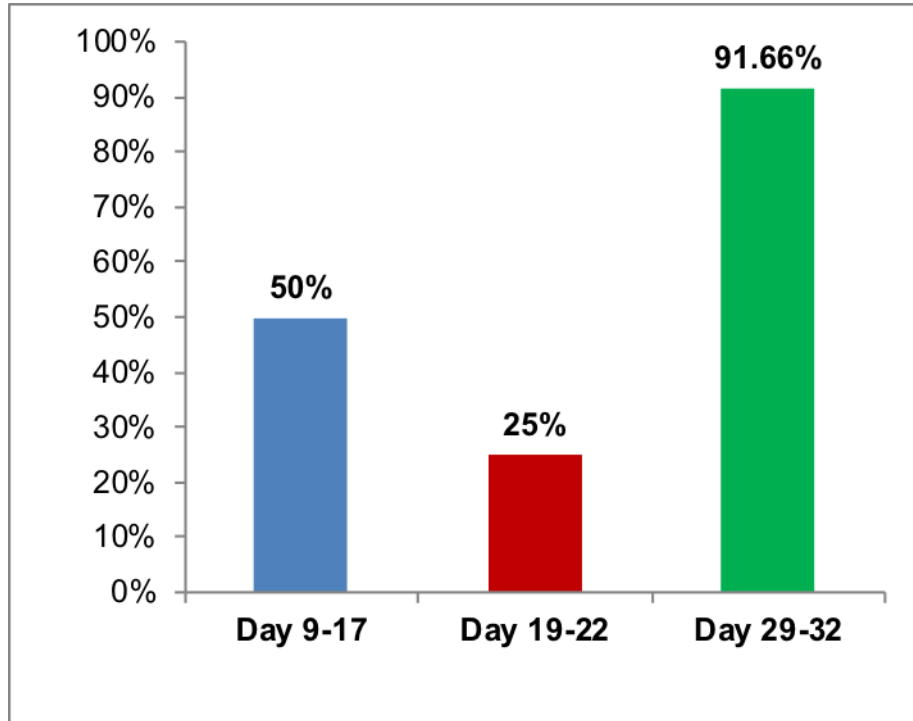


Fig 3. Presence of Oxytetracycline residue in different growing stages

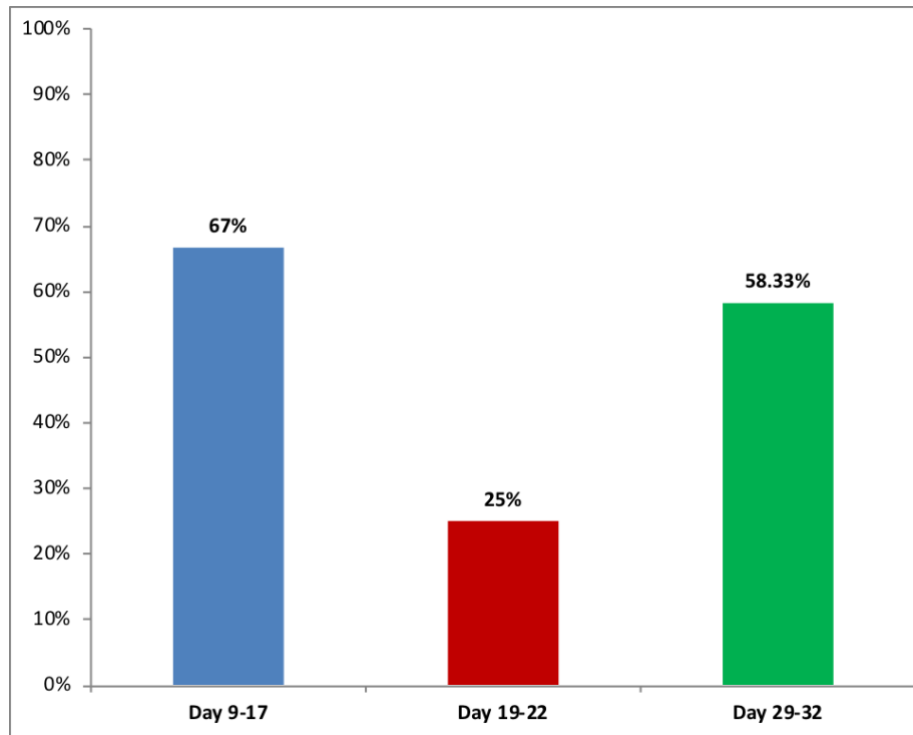


Fig 4. Presence of Doxycycline residue in different growing stages

### Discussion

Questionnaire survey was conducted to assess the usage of oxytetracycline and doxycycline in broiler farming system in Mymensingh region of Bangladesh. The majority of farmers are formally educated and some with tertiary education. However, educational background doesn't reflect with enforcing farm hygiene and marketing informed decisions on choice, administration, storage and withdrawal periods for antibiotics upon veterinarian advice and prescriptions. Similar findings were described by (Basharun and Odoch, 2015; Krishnasamy *et al.*, 2015; Guetiya *et al.*, 2016). We have observed that antibiotics are being used in 100 percent poultry farms in Mymensingh division. This finding is comparable with the observations of other studies (Sirder *et al.*, 2012; Oluwasile *et al.*, 2014).

Different scientists have revealed the presence of antibiotic residue in chicken meat and offal by different methods including TLC. These chickens were receiving antibiotic either by prophylactically or therapeutically in respective of withdrawal period (Hind *et al.*, 2014). We have detected oxytetracycline and doxycycline through TLC that are comparable with some other studies some studies (Hind *et al.*, 2014; Hussain *et al.*, 2013; Salehzadeh *et al.*, 2006).

In TLC analysis, oxytetracycline residue was highest in the sample of Trisal upazila and lowest in Mymensingh sadar upazila. In case of doxycycline the highest residual percentage was in Mymensingh sadar upazila and lowest in Fulbaria upazila comparison of usage pattern between oxytetracycline and doxycycline is scanty but studies in porcine stomach tissue observed variations exist (Jayalakshmi *et al.*, 2017).

We have observed those residues are mostly found during 7-19 days and after 28 days. This is probably due to reduce the stress mortality during the early stage of life and to reduce mortality before marketing of broiler. Due to treatment interruption and metabolism of drug in the body, the residue become gradually decreased and balanced in the edible tissue (Lindquist *et al.*,

2014, Crivineanu *et al.*, 2007). This observation justify why high level of residue was observed again in third collection of samples.

Indiscriminate use of antibiotic is a possible cause to find residue in broiler meat that can exceed MRL. No withdrawal period maintenance, improper dosing and selection of antibiotic, low quality drugs are the related parameters with the indiscriminate use (Sarker *et al.*, 2018). Though research related with antibiotic residue is limited in Bangladesh but many countries have detected residue in food chain. (Hussain *et al.*, 2013; Wijayanti *et al.*, 2012; Zhao *et al.*, 2009; Pent *et al.*, 2010; Sattar *et al.*, 2014). This study assures the presence of oxytetracycline and doxycycline residue in broiler meat. It is necessary to investigate the pathways of residue entrance in human food chain directly or indirectly to combat with antibiotic resistance.

### Conclusions

Our study concludes that oxytetracycline and doxycycline are misused in the poultry industries leading unexpected residues in animal food that can cause serious health hazards to consumers. The data generated from this research will help the policy makers and scientists to tackle antibiotic residue and resistance successfully by implementing necessary steps that would eventually help the well-being of human through consumption of safe poultry meat. Such data are vital for the understanding of and response to the problem of antimicrobial overuse and the loss of drugs due to resistance.

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### Competing Interest

The authors declare that they have no competing interests.

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