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Detection of Antibiotic Residues in Edible Poultry Tissues by Thin-Layer Chromatography (TLC) in Sher-e-Bangla Nagar, Dhaka

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ABSTRACT

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The use of antibiotics in poultry production has become a common practice worldwide to promote growth and prevent diseases. However, indiscriminate and excessive use of these drugs often leads to residues remaining in poultry meat and eggs, posing serious public health risks such as antimicrobial resistance, allergic reactions, and disruption of gut microbiota in consumers. In Bangladesh, particularly in urban areas like Sher-e-Bangla Nagar, poultry products are a major source of animal protein, making the issue of antibiotic residues highly relevant. Lack of awareness among farmers, improper withdrawal periods, and weak regulatory enforcement further aggravate the problem. Therefore, investigating the presence of antibiotic residues in poultry and identifying possible mitigation strategies are essential steps toward ensuring food safety, protecting consumer health, and promoting sustainable poultry production. The study was undertaken to determine antibiotic residues in poultry edible tissues and to identify possible mitigation approaches. The study conducted in Sher-e-Bangla Nagar, Dhaka, Bangladesh, analyzed 144 samples (liver, kidney, breast, and thigh muscle) from nine agro-markets. The primary detection method used was Thin Layer Chromatography (TLC). The study found a 15.28% prevalence of antibiotic residues across all samples, with breast muscle at 19.44%, thigh muscle at 11.11%, liver at 16.67%, and kidney at 13.89%. The most detected antibiotics were ciprofloxacin (7.64%), followed by oxytetracycline (6.94%), levofloxacin (0.7%), and enrofloxacin, which was not detected. Mohammadpur Krishi Market had the highest residue (31.25%). The presence of antibiotic residues in poultry poses significant risks to food safety and public health. Effective monitoring, farmer awareness, and strict enforcement of withdrawal periods are essential to reduce residue levels. Adoption of good farming practices and alternative disease control measures can ensure safer poultry production and protect consumers.

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Introduction

The poultry industry in Bangladesh stands as a cornerstone of the nation's food security, economic growth, and employment. As the country's population grows and urbanization accelerates, demand for chicken and eggs is rising, offering significant opportunities for industry expansion. The annual per capita consumption of poultry is likely to range from 6.3 to 8.5 kg. It is projected to have a 17% increase by the year 2025 (Hashem et al. 2024). Initially, only the positive aspects of antibiotic use in the poultry industry were noted, and thus it was considered a great achievement in the poultry sector (Thapa et al., 2021). The use of veterinary drugs in food-producing animals includes preventing and controlling animal diseases, increasing animal growth, and improving feed conversion. These drugs can leave residue in their original compound form or their metabolites in food products (Pratiwi et al. 2023). However, this practice may lead to depositing drug residue in poultry meat and products, which is related to adverse health hazards to consumers (Islam et al. 2023). These hazards include toxic effects, immune-pathological effects, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, bone marrow toxicity, and allergy (Barros et al. 2021; Kamouh et al. 2024). Fluoroquinolones, including ciprofloxacin, enrofloxacin, nalidixic acid, etc. have been widely used for treatment and prophylaxis (Er et al. 2013). Ciprofloxacin is found effective where microorganisms are resistant to aminoglycosides, tetracyclines, macrolides, and β -lactams (Sultan I, 2014). Tetracycline, a broad-spectrum antibiotic, is used to treat infections and is a growth promoter in animals. About 60% of an ingested dose of oxytetracycline is absorbed from the gastrointestinal tract and widely spread in the body (Mund et al. 2017). Some reviews about veterinary drug residues in poultry tissues have been published in previous years. Ferdous et al., 2019; Matubber et al., 2025; Sani et al., 2023 reviewed analysis of veterinary drug residues in edible poultry tissues at Chittagong, Mymensingh, and the southwest region of Bangladesh, respectively. But no work has been done in this regard in a densely populated area of Sher e Bangla Nagar, Dhaka, yet. Several screening methods are available for detecting the presence of antibiotic residues in poultry tissues or other food animals (Premarathne et al. 2018; Bhuiyan et al. 2021). The present study aims to detect antibiotic residues in edible poultry tissues collected from the Sher-e-Bangla Nagar region using the Thin Layer Chromatographic (TLC) method. Findings from this research will contribute to a better understanding of the current residue status, support evidence-based regulation of antibiotic use in poultry production, and promote safe, residue-free poultry consumption in Bangladesh.

Materials and methods

Sample Collection

Samples were collected from nine agro markets near Sher-e-Bangla Nagar. Such as: Krishi market, Bihari camp, Taltola Bazar, Songgit College Bazar, SAU Mini Bazar, Amtola Bazar, Agargaon Bazar, BNP Bazar, Farmgate Bazar. Four broilers were collected from each market. A Total of 144 samples, including 36 livers, 36 kidneys, 36 thigh muscles, and 36 breast muscles, were collected for laboratory investigations. Immediately after collection, samples were transported through an ice box from the markets and stored at 4°C until analysis.

Materials and Reagents

Beaker, Pipette, Micropipette, Falcon tube, Capillary tube, Mortar and Pestle, Measuring tube, Digital weigh machine, Vortex mixer, Grinder machine, Eppendorf tube, Centrifuge machine, Tips, Scissors, pencil, Scale, Methanol, Acetonitrile, Trichloroacetic acid, Diethyl ether, Phosphate-Buffered saline, Distilled water.

Sample Preparation & Antibiotic Extraction. At first, the samples were cut into small pieces and thoroughly homogenized using a mechanical grinder. 4g of the homogenized tissue was accurately weighed in a digital weight balance and transferred into a sterile Falcon tube. Subsequently, 10mL of Phosphate-Buffered Saline (PBS) was added. The mixture was vortexed by a vortex mixer machine to obtain a uniform suspension. Following this, 2mL of 30% trichloroacetic acid (TCA) was added, and the mixture was vortexed to form a homogenized mixture. Then, the mixture was centrifuged at 6000rpm for 20min, and 2ml of the resulting supernatant was carefully collected using filter paper. An equal volume of diethyl ether was added to the supernatant, and the mixture was gently mixed. After mixing, the mixture was allowed to stand at room temperature for 8-10minutes. After separation, 1mL of the lower aqueous phase was withdrawn using a micropipette. This extract was spotted on a TLC plate for chromatographic analysis (Islam et al., 2024).

Thin Layer Chromatography (TLC) (Islam et al., 2024).

TLC is a precise, accurate, sensitive, and dependable technique for tracking trace concentrations of various biological substances. UV light serves as a straightforward detector for the antibiotics. More than half of thin-layer chromatography (TLC) applications are in pharmaceutical, medical, and biological clinical or research. One of the significant uses of TLC is the detection of drug residues in food.

TLC Apparatus

TLC plate (TLC Silica gel 60F₂₅₄), TLC tank, UV detection box were utilized. TLC was performed, and the same R_f values for the sample and standard indicated that the compounds were similar.

TLC Plate Preparation

- TLC plate was cut into an appropriate size (5x4 cm)
- A straight line was drawn across the plate approximately 2cm from the bottom with a pencil. Another straight line was drawn across the plate, 1cm below its upper edge.
- Desired spots marking was marked on the bottom line where analytes were dropped.
- Spots were applied to the plate using thin capillary glass pipettes. A volume of 10µl was used for spotting.

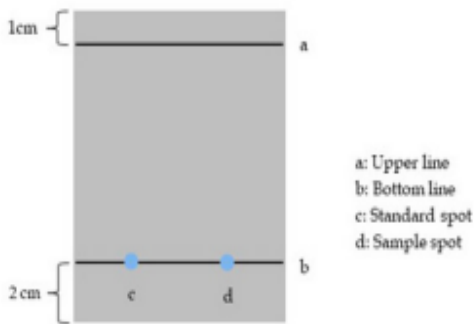
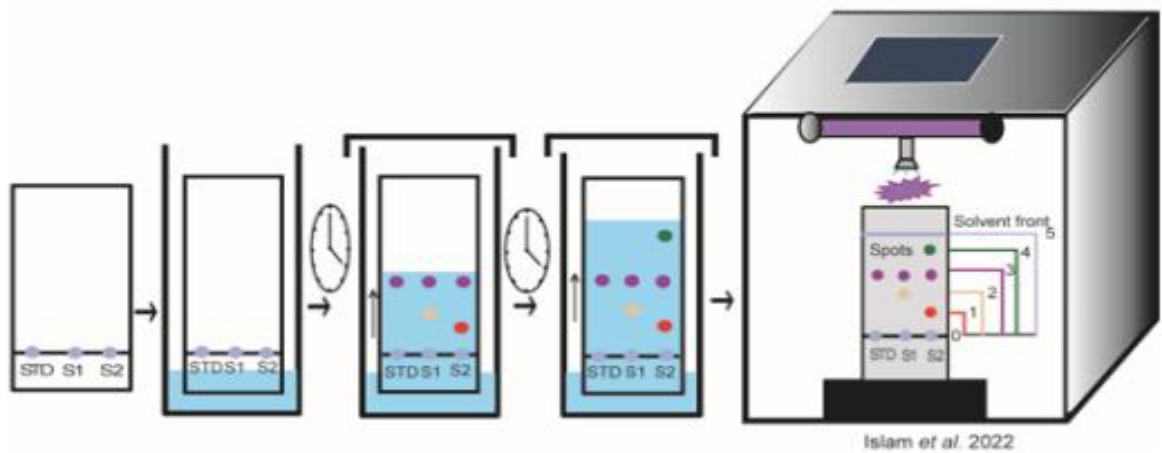


Figure 1. TLC plate

- Plate was placed in TLC tank (contained mobile phase: Acetonitrile and Methanol; 1:1) and covered by lid, and it was left until the mobile phase reached the upper line.
- Spots were visualized in UV detection box at 256 nm
- Spots marking was done by pencil for the calculation of the retention factor (Rf) (Islam et al., 2021)

A Thin layer chromatography



Thin layer chromatographic analysis

Figure 2. Thin Layer Chromatography analysis procedure

Mobile Phases

Using the two best types of mobile phases in this experiment to identify four types of antibiotic residues resulted in satisfactory outcomes (Islam et al., 2024).

Antibiotic residue	Chemical substances of the mobile phase	Ratio
Ciprofloxacin	Butanol: Distilled Water: Acetic Acid	6:2:2
Oxytetracycline	Butanol: Distilled Water: Acetic Acid	6:2:2
Enrofloxacin	Hexane: Ethyl Acetate	1:1
Levofloxacin	Hexane: Ethyl Acetate	1:1

Calculation of Rf Value

To identify the substances present, measurements are conducted regularly. These estimates represent the solvent's travel distance and the distance covered by an individual spot. Before the solvent has evaporated, the plate is removed from the beaker, and the location of the solvent is noted with a separate line when the solvent approaches the plate's highest point (Islam et al., 2021).

$$R_f = \text{Distance travelled by the sample (a)} / \text{Distance travelled by the solvent (b)}$$

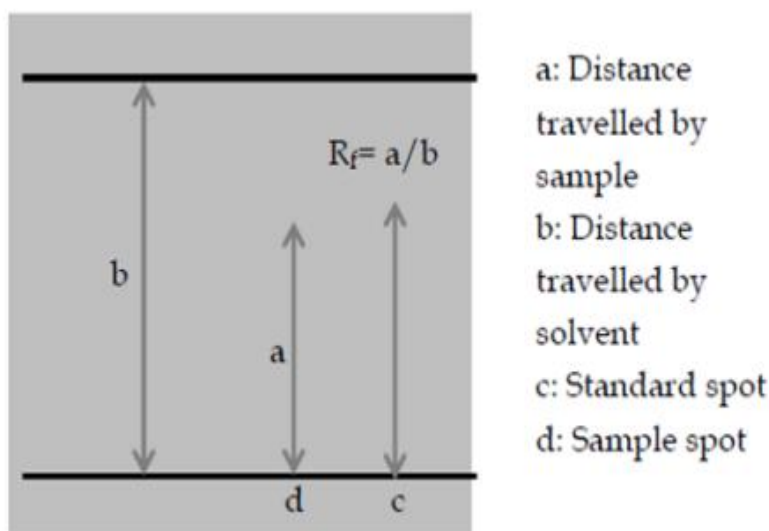


Figure 3. Calculation of Rf value

Interpretation of Results

Three repeated examinations using the standard solution were performed to determine the first standard setting with reference chemicals, to aid in interpreting the results. When a compound in the unknown solution behaved exactly like the reference substance, it was positively identified. That is, following a comparison of two substances (standard & unknown), a sample was positively identified based on the following criteria:

- The same Rf value as the reference sample.
- The same color under UV light (Islam et al., 2021)

Data analysis

Data from the experiments and analysis were analyzed using standard statistical techniques.

Results and Discussion

Assessment of antibiotic residues

A total of thirty-six livers, thirty-six kidneys, thirty-six thigh muscles, and thirty-six breast muscles were analyzed in the laboratory of Pharmacology & Toxicology at Sher-e- Bangla Agricultural University, Dhaka, Bangladesh. The antibiotic screening test was performed using the most commonly used antibiotics: ciprofloxacin, tetracycline, enrofloxacin, and levofloxacin. Among 144 samples, 22 (15.28%) were found positive: 7 breast muscle, 4 thigh muscle, 6 liver, and 5 kidney samples, respectively (Figure 4). These findings are comparable with the findings of Sarker et al., 2018.

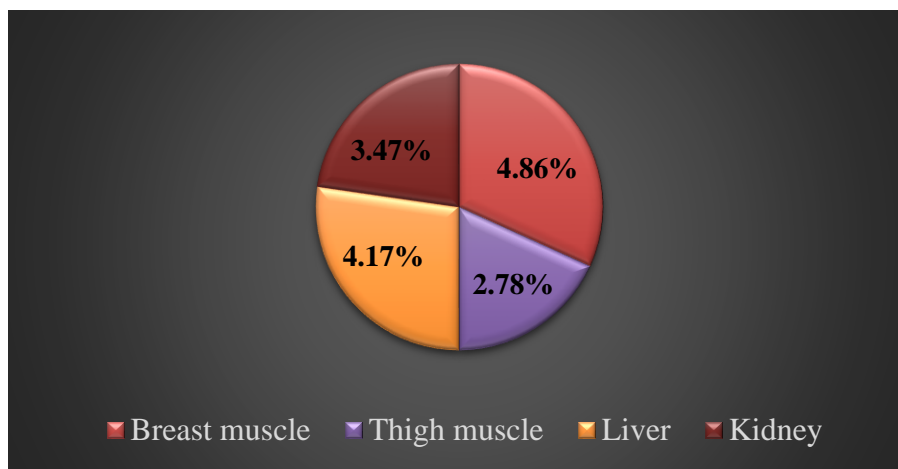


Figure 4. Percentage of total positive samples

Prevalence of antibiotic residues in different edible poultry tissues

The results of the prevalence of antibiotic residues in different edible poultry tissues are presented in Figure 5. Among the different edible poultry tissues (36 samples of each), residue was found in breast muscle (19.44%), followed by thigh muscle (11.11%), liver (16.67%), and kidney (13.89%). These findings are consistent with previous reports indicating that antibiotic residues can accumulate differentially in tissues due to variations in blood flow, metabolic activity, and tissue composition. For example, the liver and the kidneys are metabolically active organs responsible for drug detoxification and excretion, which can lead to higher residue accumulation compared to muscle tissues (Lee et al. 2001; Sarker et al. 2018). The presence of residues in breast and thigh muscles is particularly concerning since these are commonly consumed parts of poultry, posing a direct risk to public health (Nisha 2008).

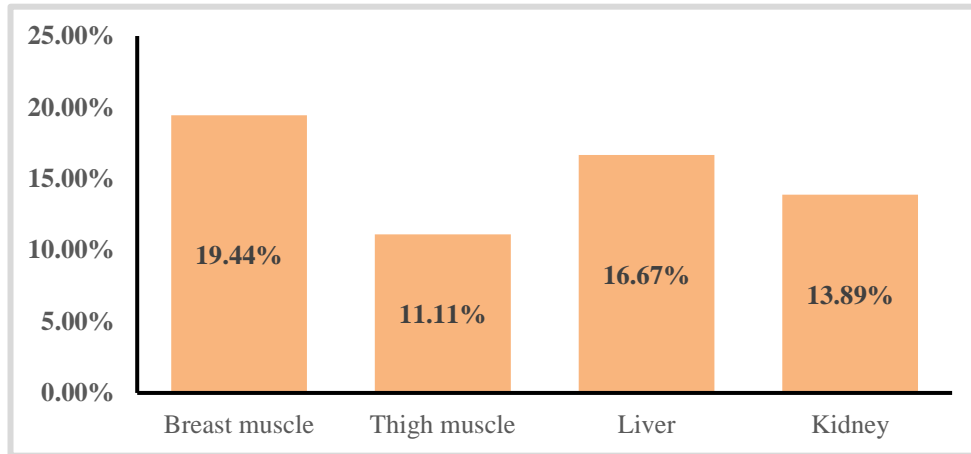


Figure 5. Prevalence of antibiotic residues in different edible poultry tissues

Antibiotic residues in breast muscle

TLC analysis revealed that of 36 breast muscle samples, only 4 were positive for ciprofloxacin, 3 for tetracycline, and enrofloxacin & levofloxacin were negative in all samples (Figure 6). Ciprofloxacin and tetracycline are among the most frequently used antibiotics in poultry due to their broad-spectrum activity, which aligns with reports by Bayou & Haile, (2017).

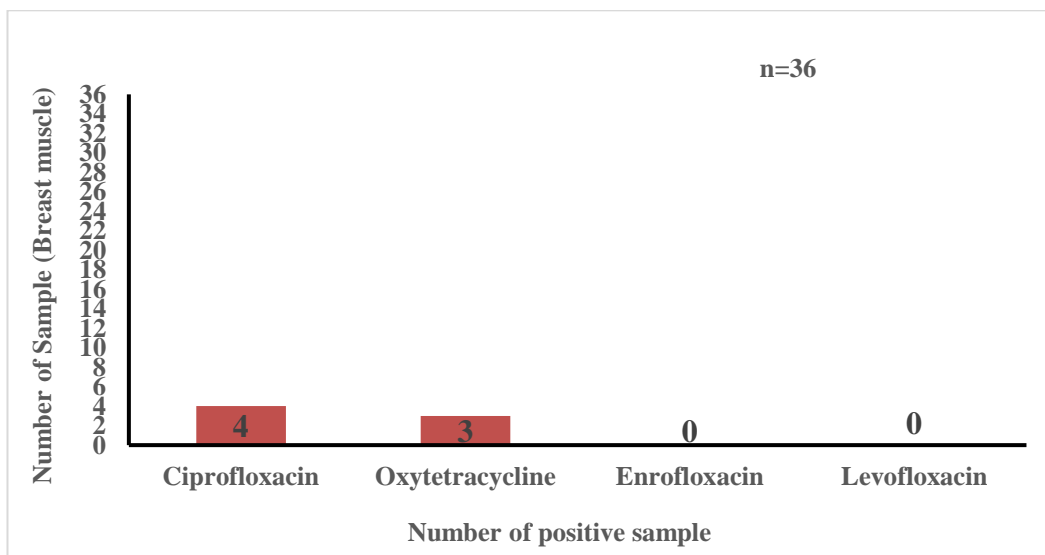


Figure 6. Antibiotic residues in breast muscle

Antibiotic residues in thigh muscles

In the thigh muscles, of 36 samples, 3 were positive for Tetracycline, 1 for ciprofloxacin, and enrofloxacin & levofloxacin were negative in all samples (Figure 7).

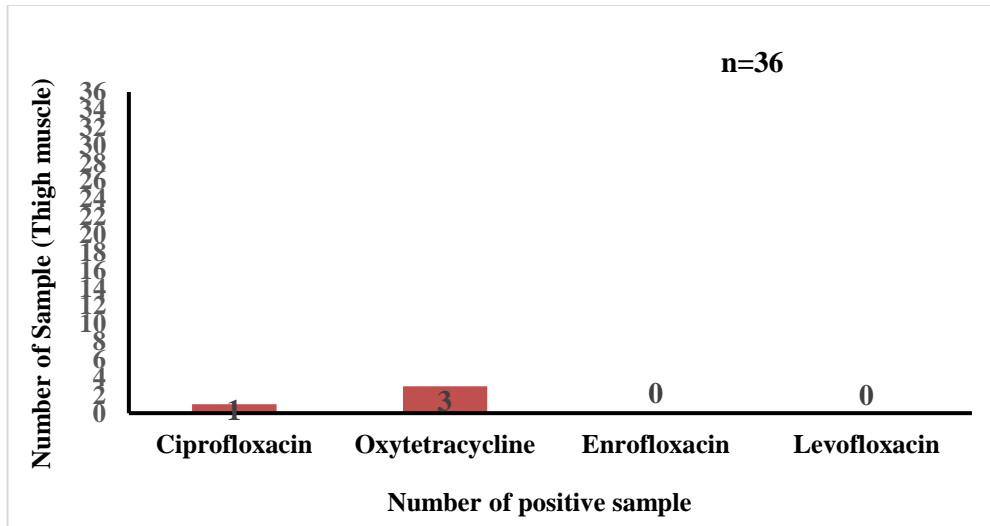


Figure 7. Antibiotic residues in the thigh muscle

Antibiotic residues in the liver

In the case of liver, 4 of 36 samples were positive for ciprofloxacin, 1 for tetracycline, and 1 for levofloxacin. Enrofloxacin was found to be negative for all samples (Figure 8). These findings highlight the importance of monitoring liver tissues, as they are often consumed by humans and can serve as a significant source of exposure to antibiotic residues, potentially contributing to antimicrobial resistance and other health risks (Nisha 2008).

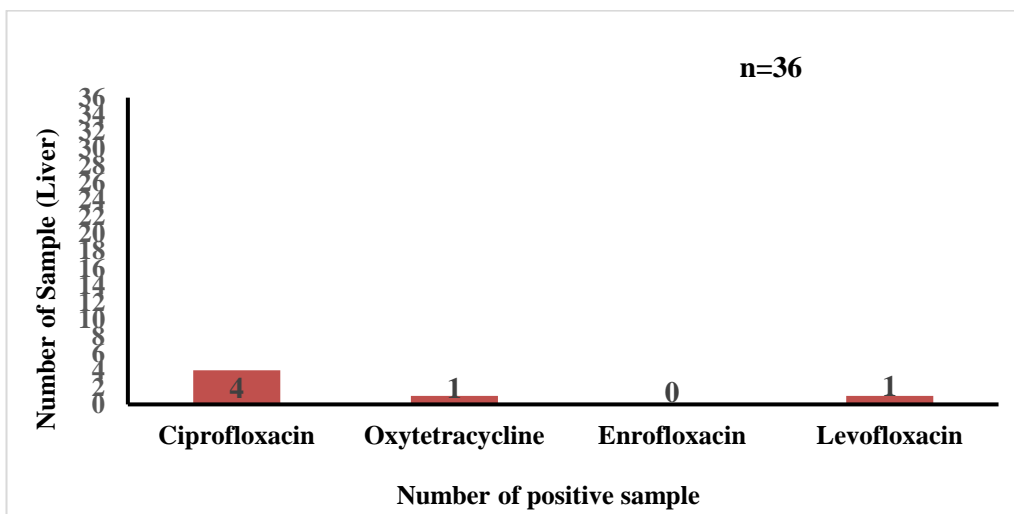


Figure 8. Antibiotic residues in the liver

Antibiotic residues in the kidney

In the kidney, of 36 samples, 2 were positive for ciprofloxacin and 3 for tetracycline. Enrofloxacin and levofloxacin were found to be negative for any samples (Figure 9).

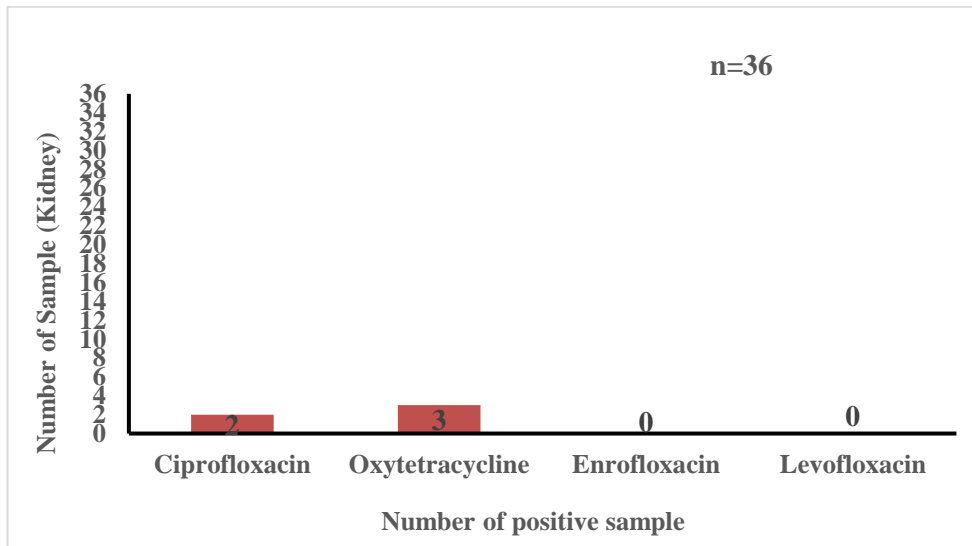


Figure 9. Antibiotic residues in the kidney

Prevalence of antibiotic residues in different agro markets

Out of total one hundred forty-four samples (sixteen samples from each market); five samples (31.25%) were positive in Mohammadpur Krishi market, four samples (25%) were positive in Bihari camp and Taltola Bazar; three samples (18.75%) were positive in Songit College Bazar; two samples (12.5%) were positive in Sher-e- Bangla Agricultural University Mini Bazar and Amtola Bazar; one sample (6.25%) was positive both Agargaon Bazar and BNP Bazar. No sample was found positive in the Farmgate Bazar (Figure 10). The differences in residue prevalence may reflect variations in farming practices, antibiotic usage patterns, and adherence to withdrawal periods by suppliers servicing these markets (Nisha 2008; Sarker et al. 2018). Higher contamination in some urban markets suggests limited monitoring and awareness among vendors and producers, emphasizing the need for systematic surveillance, stricter regulation, and public awareness campaigns to ensure food safety.

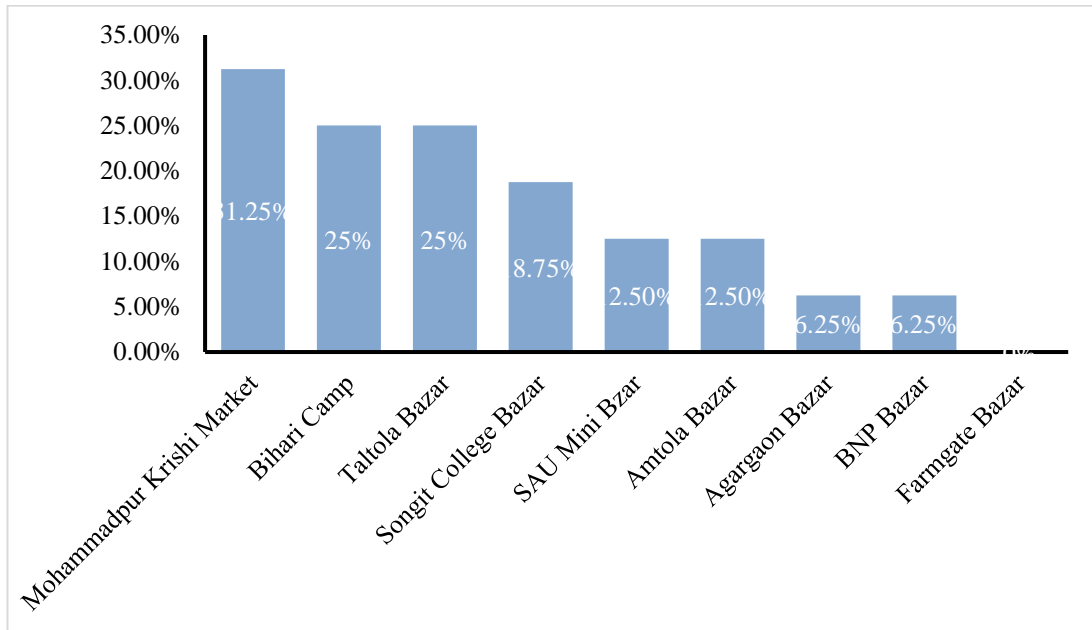


Figure 10. Prevalence of antibiotic residues in different agro markets

Prevalence of antibiotic residues

Out of a total of one hundred forty-four samples, eleven samples (7.64%) were ciprofloxacin, ten samples (6.94%) were tetracycline, and one sample (0.7%) was levofloxacin positive. No sample was found to be positive for enrofloxacin (Figure 11). This overall prevalence indicates that ciprofloxacin and tetracycline are the most used antibiotics in poultry production in the study area, reflecting both their broad-spectrum activity and accessibility (Bayou and Haile 2017).

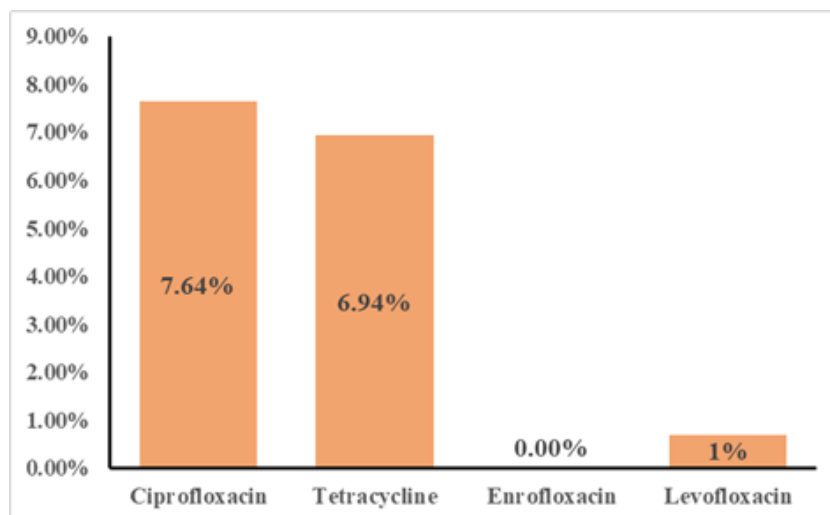


Figure 11. Prevalence of antibiotic residues

Conclusion

This study confirmed the presence of antibiotic residues in poultry edible tissues at Sher-e-Bangla Nagar, Dhaka, indicating widespread misuse of antibiotics and poor adherence to withdrawal periods. Such residues pose serious health risks, including antimicrobial resistance, allergic reactions, and long-term toxic effects, while also threatening food safety and the potential of the poultry trade. To address this issue, regular monitoring programs should be established, and strict enforcement of residue limits is needed. Farmers must be trained on responsible antibiotic use, withdrawal periods, and biosecurity practices, while alternatives such as probiotics, vaccination, and improved hygiene should be promoted. Public awareness campaigns are essential to reduce consumer risk, and affordable detection methods like TLC can strengthen local monitoring capacity.

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Conflict of Interest

There are no conflicts of interest declared by the authors.

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