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Chromatographic Qualification and Quantification of Antibiotic Residue in Poultry Meat

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ABSTRACT

Background and Objective: Antibiotic residues in food-producing animals create a substantial threat to public health. Antibiotic residues in food items are attributable to the misuse of antibiotics in veterinary medications. Earlier research in Bangladesh has found a significant incidence of antibiotic residues in animal-based foods, including chicken tissue. The objective of the study screening of antibiotic residues in broiler and native chicken liver, kidney and breast muscle and to estimate Amoxicillin residues from broiler liver and kidney from local markets in Bangladesh. **Materials and Methods:** Six months research on antibiotic residue has been conducted to examine the risk to human health. A total of 120 poultry specimens (60 broiler and 60 native chicken) were obtained from several marketplaces around the Chittagong Metropolitan Area for this study, including liver, kidney and breast muscles. Thin layer chromatography (TLC) has been used to test the specimens for Tetracycline, Amoxicillin, Sulfanilamide and Ciprofloxacin accompanied by Ultra-High-Performance Liquid Chromatography (UHPLC) on selected positive tested cases. **Results:** The TLC results showed that 48.3% of broiler and 23.3% of native chicken samples were positive. In the research, broiler and native chicken liver had the most antibiotic residues respectively 70 and 35%. There was a significant ($p<0.05$) difference between the Tetracycline, Amoxicillin, Sulfanilamide and Ciprofloxacin residues of native chicken and the Tetracycline, Amoxicillin, Sulfanilamide and Ciprofloxacin residues of broiler chicken. Additionally, Amoxicillin levels were found to be 16.9-149.9 g g^{-1} in the liver and 45.3-60.5 g g^{-1} in the kidney using UHPLC. The greatest and shortest peak times were 4.7 and 5.1 min, correspondingly. **Conclusion:** It is concluded that antibiotics are administered to animals without the need for a prescription and the right dose is not maintained due to withdrawal symptoms. For the sake of public health, the research suggests that a withdrawal time be implemented, as well as good surveillance and careful drug usage in animals.

KEYWORDS

Antibiotic residues, broiler, native chicken, thin-layer chromatography (TLC), ultra-high-performance liquid chromatography

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INTRODUCTION

Antibiotics are small to medium-sized compounds having a variety of biochemical properties[1]. Antibiotics are natural molecules produced by microorganisms, equivalent manufactured commodities or analogous semi-synthetic agents that inhibit or eradicate the growth of microbes[2]. Antibiotics are "Chemical substances produced by one organism which are toxic to other organisms"[3]. Antibiotics are largely used in the poultry sector for the following three components: Therapeutic use in treating sick poultry, preventive use to prevent infections in poultry and even as feed additives to increase feed intake and output according to their growth-promoting properties. In sub-therapeutic dosages, they're often employed as chicken feed additives[4]. Antibiotics leave the body in a variety of ways and at various periods[5]. However, uncontrolled and illogical antibiotic use leaves antibiotic remnants in foods, which can jeopardize human health by causing antibiotic intolerance, allergic reactions and intestinal microbiota instability[6]. It is generally considered one of the leading health challenges of the contemporary era by the major international health agencies, although some of the factors are widely accepted, including the usage of antibiotics as growth stimulants in cattle or poultry feedstuffs, the incorrect use of antibiotics, especially for non-bacterial infections and inadequate or inappropriate antibiotic usage in the clinical field[7]. Antimicrobial growth promoters (AGP) can increase feed conversion ratio by 17% in beef cattle, 10% in lambs, 15% in poultry and 15% in swine when administered as a feed supplement[8]. Antibiotic usage in chicken, in particular, has increased efficiency while simultaneously increasing poultry health and very well by reducing sickness incidence[9]. Antibiotics are still utilized in food-producing animals, leaving residues in meat, milk and eggs. Chemical residues are either the original molecule or its byproducts which can be accumulated, stored or otherwise maintained inside animal cells, tissues, organs or consumables even after they've been employed to prevent, regulate or treat animal sickness or improve production[10]. Violators residue is defined as a remnant level exceeding the Maximum Residue Level (MRL) and it is responsible for significant public health problems and also the development of resistance to antibiotics[11]. Consumption of a trace amount of antibiotic remnant from food animals disrupts the human gut microbiota, which acts as a barrier against dangerous bacteria invasion[12]. Antibiotics that are used in poultry and animal products have been mostly reported to accumulate in the liver, kidney, muscle and bones, surpassing the maximum permissible limit in several investigations[13]. Antibiotic residues in animal-based foods have been linked to a variety of human health issues and include allergic reactions (e.g., penicillin), immune diseases, mutagenicity, carcinogenic effects (e.g., sulfamethazine, oxytetracycline and furazolidone), the mutation in a cell, nephropathy (e.g., gentamicin), toxicity, hepatotoxicity, bone marrow toxicity (e.g., chloramphenicol), reproductive disorders, imbalance of intestinal microbiotaand destruction of beneficial bacteria (e.g., sulfamethazine, oxytetracycline and furazolidone) present in the gastrointestinal tract, especially in children resulting in dyspepsia[8,14,15]. The existence of xenobiotics, specifically antibiotic remnants in animal-derived foods, is one of the most important markers of food safety standards[16]. Animals bred for food, notably poultry, are often treated in flocks and roughly 80% of all food-producing animals are nowadays treated for some or maybe all of their lives. Antibiotic medication use in food-producing animals may also result in antibiotic remnants in meat and offal and protecting the health of the public from the possibly hazardous implications of veterinary medicine residues is an important problem[17]. Concerns about antibiotic residues prompted the creation of Maximum Drug Residue Limits (MRLs), which appear to be the maximum amount of residues that can legally be found in a food product without causing harm to consumers[18,19]. Monitoring antibiotic residues are critical for assuring the safety of human-consumed food[20]. In recent times, many analytical techniques for detecting tetracycline in foodstuffs have been reported[21,22]. The two different types of analytical methods accessible include screening methods and immunoassay-based methods, as well as confirmatory procedures utilizing gas chromatography or liquid chromatography[23]. Poultry plays a crucial role in addressing the protein gap caused by animal-origin protein in most parts of the world. Because of indiscriminate use and a lack of information about drug withdrawal intervals, the medicated birds are known to also have remained in their meat. Such meat is unfit for human consumption and poses a

serious health risk to the public. Given the significance of the study, the following goals were set: Detection methods of frequently used antibiotics (Tetracycline, Amoxicillin, Ciprofloxacin and Sulfanilamide) residues in poultry meat using thin layer chromatography (TLC), estimation of Amoxicillin residue in poultry using Ultra-High-Performance Liquid Chromatography (UHPLC), notifying the general public regarding antibiotic residues probably found in a retail location, identification of possible hazards to public health.

MATERIALS AND METHODS

Sample collection: A total of 120 samples (60 broilers and 60 native chicken) were purchased from retail outlets in different parts of the Chittagong Metropolitan area (Jhautola Bazar, Reajuddin Bazar, Kornafuly Market and Veterinary Hospital, Chittagong) during January to June, 2021. Samples were taken from the breast muscle (40), liver (40) and kidney (40) of broiler and native chickens separately. These samples were screened and detected for antibiotic residue in tissues by thin layer chromatography (TLC). Then, quantitative determination of Amoxicillin residue in the broiler was done by Ultra-High-Performance Liquid Chromatography (UHPLC).

Solution preparation

Preparation of phosphate buffer (pH 6.5): To make a phosphate buffer solution, combine all of the ingredients in a mixing bowl and mix well. With the aid of an electric balance, 1.76 g disodium hydrogen phosphate (Merck, Germany) and 2.46 g sodium dihydrogen phosphate (Merck, Germany) were weighted according to the manual's instructions. These ingredients were then placed in a 1000 mL volumetric flask and distilled water was carefully added to get the solution up to the 1000 mL mark. Following that, a pH meter was used to check the pH.

Preparation of 30% trichloroacetic acid: Trichloracetic acid was packaged as a solid crustal wrapped with polyethylene and contained within a plastic bottle. It was weighted down and 30 g of crystal trichloracetic acid was diluted with distilled water in a 100 mL volumetric flask up to the 100 mL mark level with caution.

Silica plates for sample running: Before usage, TLC Silica plates with a thickness of 0.25 mm (Merck, Germany) were activated at 120°C for 2 hrs.

Standard preparation and selected antibiotics: Four antibiotics, Tetracycline, Amoxicillin, Ciprofloxacin and Sulfanilamide, were made by dissolving 0.1 g of powder/0.1 mL of solution in 4 mL methanol for comparison of retrieved residues with commonly used antibiotics.

Sample preparation and antibiotic extraction: These samples were preserved in deep freezers at -20°C until additional sophisticated methods such as thin layer chromatography could be undertaken to detect antibiotics. A food mixer (Macro Food Processor) was used to adequately combine the samples (muscle tissue, liver and kidney). The blender was run for one minute, then paused for 5-8 sec before running again. This procedure was continued until the tissues were suitably integrated. These mashed/blended samples were carefully placed in Petri-dishes that had been cleaned and sanitized, as well as covered. With the use of a weighing balance and a spatula, 4 g of aliquoted material was transferred to a beaker. After that, 10 mL of phosphate was added to homogenize the mixture (pH 6.5). Following adequate mixing, the protein content of these samples was precipitated with the addition of 2 mL Trichloroacetic acid (30%) while paying close attention to the process. The materials were then centrifuged in test tubes that had been thoroughly cleaned and sanitized. Then, using an automated time-regulated centrifuged equipment, centrifugation was done/conducted at 3000 rpm for 25 min

(Labofuge, 200). For 45 min at 100°C in a water bath, 2 mL supernatant was combined with 100 L formaldehyde. To accomplish defamation, the supernatant was extracted with an equal volume of diethyl ether and well mixed. After that, the mixture was allowed to sit for 10 min to separate into two layers: An upper oily layer and a bottom one. These mixtures were then separated from one another using a cleaned and sanitized separating funnel, with the upper greasy layer being discarded and just the bottom layer being collected. Diethyl ether was used to extract the supernatant twice more. The extracts were then evaporated until they were completely dry. The dried material was reconstituted in 2 mL of mobile phase methanol, acetone was used as the control (1:1). After that, the extracts were carefully collected into screw cap vials and stored in the refrigerator for further advanced investigation. The entire process was completed as the reference cited by Popelka *et al*.[24].

Preparation of solvent system: A mobile phase or solvent preparation had been done as specified in the references to execute thin layer chromatography (TLC) together with the stationary phase. The mobile phase was made up of 50 mL of methanol and 50 mL of acetone, which were mixed appropriately.

Determination of RF (Retardation Factor) value: The Retardation factor is used to calculate the relative migration rate of compounds under different situations. It's the proportion of the substance's movement to the solvent's movement. The distance covered from the starting position by each spot was recorded in centimetres, measured from the spot's centre. The solvent distance was also measured from the starting position instead of the bottom of the chromatogram. Then the calculation of RF values was done using the following equation:

$$\text{RF} = \frac{\text{Distance moved by the substance}}{\text{Distance moved by the solvent}}$$

The results of all RF values were tabulated and documented on paper.

Statistical Analysis: The collected data were input into MS Excel-2007 before being exported to STATA 9.0 for analysis. For each outcome variable, we ran an analysis to find the frequency, percentage, mean and standard error.

RESULTS

Thin layer chromatography (TLC): A total of 120 samples of various poultry tissues (60 broilers and 60 native chicken) were evaluated for antibiotic residues in the TLC investigation. Antibiotic residues were found in 60 samples from 20 broilers (20 liver, 20 kidney and 20 breast muscles). After collecting samples from several Chittagong City Corporation wet markets, they were analyzed for every type of antibiotic and antimicrobials and antibiotic residues were found using a thin layer plate. The presence of antibacterial compounds was detected in 29 (48.3%) of the poultry (broiler) samples when exposed to UV light as shown in Table 1. Antibiotic detection was highest in the liver (70%) followed by the kidney (9%) and breast muscle (6 and 30%). There were 60 samples in all, with 29 of them being positive. Ciprofloxacin 5 (25%) had the highest incidence in liver tissue, trailed by Tetracycline 4 (20%), Sulfanilamide 3 (15%) and Amoxicillin 2 (30%), respectively. Three kidney samples (15%) tested positive for tetracycline, three (15%) tested positive for Ciprofloxacin, two (10%) tested positive for sulfanilamide and one (5%) tested positive for Amoxicillin. Two (10%) of the breast muscle samples tested positive for tetracycline and two (10%) tested positive for Ciprofloxacin. The 1 (5%) and 1 (5%), tested positive for Amoxicillin and sulfanilamide, respectively. Ciprofloxacin was perhaps the most common antibiotic found in broiler meat, accounting for 10 (16.7%) of the four antibiotics tested, followed by Tetracycline 9 (15%), Sulfanilamide (6%) and Amoxicillin (4.7%).

Table 1: Positive percentage (%) of studied samples for four antibiotics in broiler meat samples

Antibiotics	Liver (N = 20)	Kidney (N = 20)	Breast muscle (N = 20)	Total sample (N = 60)
Tetracycline	4 (20%)	3 (15%)	2 (10%)	9 (15.0%)
Amoxicillin	2 (10%)	1 (5%)	1 (5%)	4 (6.7%)
Sulfanilamide	3 (15%)	2 (10%)	1 (5%)	6 (10.0%)
Ciprofloxacin	5 (25%)	3 (15%)	2 (10%)	10 (16.7%)
Total	14 (70%)	9 (45%)	6 (30%)	29 (48.3%)

Table 2: Positive percentage (%) of studied samples for four antibiotics in native chicken meat samples

Antibiotics	Liver (N = 20)	Kidney (N = 20)	Breast muscle (N = 20)	Total sample (N = 60)
Tetracycline	1 (5%)	1 (5%)	2 (10%)	4 (6.7%)
Amoxicillin	1 (5%)	1 (5%)	1 (5%)	3 (5.0%)
Sulfanilamide	2 (10%)	0 (0%)	0 (5%)	2 (3.3%)
Ciprofloxacin	3 (15%)	2 (10%)	1 (5%)	6 (10.0%)
Total	7 (35%)	4 (20%)	3 (15%)	14 (23.3%)

According to Table 2, 14 (23.3%) of native chicken flesh samples tested positive for antibacterial compounds using this approach. The largest proportion of residue for Ciprofloxacin 3 (15%) is found in the native chicken liver, followed by Sulfanilamide 2 (10%), Tetracycline 1 (5%) and Amoxicillin 1 (5%), respectively. Ciprofloxacin 2 (20%) was discovered in the greatest proportion in the native chicken kidney, followed by Tetracycline 1 (5%) and Amoxicillin 1 (5%), respectively. Tetracycline 2 (10%) was found to have the greatest incidence in the native chicken breast muscle, followed by Amoxicillin 1 (5%) and Ciprofloxacin 1 (5%), respectively. Sulfanilamide residue is absent in kidney and breast muscle tissues of native chickens. Ciprofloxacin was perhaps the most prevalent antibiotic identified 6 (10%) in native chicken meat such as broiler meat, followed by Tetracycline 4 (6.7%), Amoxicillin 3 (5%) and Sulfanilamide 2 (3.3%). Antibiotic residues were detected in the native chicken liver at a rate of 7 (35%), followed by the kidneys 4 (20%) and the breast muscle 3 (15%).

The antibiotic residue sequences in broiler meat were greatest in the liver, followed by kidney and breast muscle as shown in Fig. 1. Similarly, the antibiotic residue in the native chicken was greatest in the liver, followed by the breast muscle and kidney, respectively, as shown in Fig. 2.

The greatest amount of tetracycline residue was found in broiler liver 4 (20%), followed by kidney 3 (15%) and breast muscle 2 (2%). (10%). Tetracycline residues were found in multiple broiler organs with a weakly significant ($p \leq 0.05$) significance. In comparison to the liver 1 (5%) and kidney 1 (1%), the native chicken breast muscle 2 (10%) had the greatest level of tetracycline residues (5%). Tetracycline residue was found in considerable amounts in several native chicken organs ($p < 0.05$). The existence of Amoxicillin residue in various broiler and native chicken organs was statistically significant ($p < 0.05$). Sulfanilamide residue was found in several organs of broiler and native chickens in a statistically significant ($p < 0.05$) manner. In comparison to kidney 3 (15%) and breast muscle 2, the broiler liver 5 (25%) had the greatest quantity of Ciprofloxacin residue (10%). The existence of Ciprofloxacin remnant in several broiler organs was not statistically significant ($p > 0.05$). In comparison to kidney 2 (10%) and breast muscle 1 (5%), the native chicken liver 3 (15%) had the greatest quantity of Ciprofloxacin residue (5%). Ciprofloxacin residue was found in significant amounts in multiple organs of native chicken ($p < 0.05$) (Table 3, 4).

Results of amoxicillin antibiotic residue in poultry (broiler and native chicken) by Ultra Performance Liquid Chromatography (UPLC): The concentrations of amoxicillin residues in six organ samples (liver and kidney) taken from different market places in the Chittagong City Corporation Region were shown in Table 5. The greatest (149.9 g g^{-1} liver) and lowest (16.9 g g^{-1} liver) amoxicillin concentrations were observed in the liver. The liver has an average of 65.353 µg of Amoxicillin/g of

sample. The greatest (60.5 μg g^{-1}) and lowest (45.4 μg g^{-1}) Amoxicillin concentrations were reported in the kidney. The average concentration of Amoxicillin in the kidney is 53 μg g^{-1} of the sample. Peak retention times of separate samples ranged from 4.7-5.1 min, with peak areas ranging from 2413165-6208358.

Table 3: Antibiotic residues in the liver, kidney and breast muscles of broiler and native chicken were acquired from different wet markets in the Chittagong Metropolitan Area

		Broiler chicken			Native chicken		
Antibiotics	Types of sample	Yes (%)	No	p-value	Yes (%)	No	p-value
Tetracycline	Liver	4 (20)	16	0.05	1 (5)	19	0.03
	Kidney	3 (15)	17		1 (5)	19	
	Breast muscle	2 (10)	18		2 (10)	18	
Amoxicillin	Liver	2 (10)	18	0.03	1 (5)	19	0.03
	Kidney	1 (5)	19		1 (5)	19	
	Breast muscle	1 (5)	19		1 (5)	19	
Sulfanilamide	Liver	3 (15)	17	0.04	2 (10)	18	0.03
	Kidney	2 (10)	18		0 (0)	20	
	Breast muscle	1 (5)	19		0 (0)	20	
Ciprofloxacin	Liver	5 (25)	15	0.06	3 (15)	17	0.04
	Kidney	3 (15)	17		2 (10)	18	
	Breast muscle	2 (10)	18		1 (5)	19	

Between broiler and native chicken meat, there was no significant difference in antibiotic residue in different organs (p>0.05)

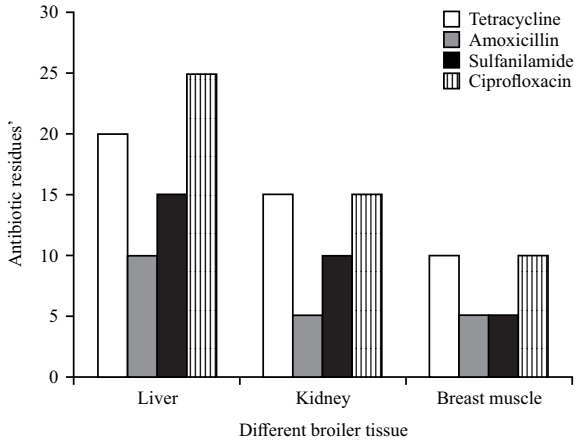

Fig. 1: Comparison of different antibiotic residues in different broiler tissues

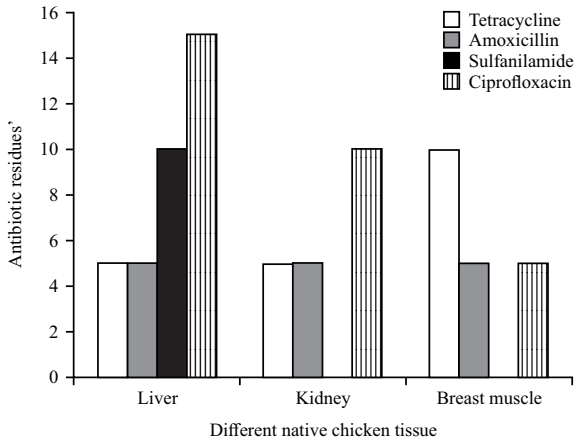

Fig. 2: Comparison of different antibiotic residues in different native chicken tissues

Table 4: Comparison of the antibiotics residue between different organs of broiler and native chicken

Antibiotics	Organs samples	Broiler chicken	Native chicken	p-value
Tetracycline	Liver	4 (20%)	1 (5%)	0.193
	Kidney	3 (15%)	1 (5%)	
	Breast muscle	2 (10%)	2 (10%)	
Amoxicillin	Liver	2 (10%)	1 (5%)	0.584
	Kidney	1 (5%)	1 (5%)	
	Breast muscle	1 (5%)	1 (5%)	
Sulfanilamide	Liver	3 (15%)	2 (10%)	0.153
	Kidney	2 (10%)	0 (0%)	
	Breast muscle	1 (5%)	0 (0%)	
Ciprofloxacin	Liver	5 (25%)	3 (15%)	0.373
	Kidney	3 (15%)	2 (10%)	
	Breast muscle	2 (10%)	1 (5%)	

Table 5: Amoxicillin residues in different organ samples of poultry

Peak area of standard	Concentration of standard (mg/50 mL of solution) to the peak area	Peak area of samples	Concentration of samples (mg mL^{-1} of solution) to the peak area	Concentration of antibiotics (µg g^{-1} of tissue) to the peak area	Mean± Standard	Min-Max (Peak time) retention time (min)	Types of sample
12421069	0.6 mg mL^{-1}	6208358	0.2998	149.9	65.3±73.5	4.7-5.1 (4.9)	Liver (1)
		2413165	0.1166	29.1		4.7-5.0 (4.9)	Liver (2)
		2801971	0.1398	16.9		4.7-5.1 (4.9)	Liver (3)
		4483527	0.2166	54.1	53.4±7.6	4.7-5.05 (4.9)	Kidney (1)
		3758133	0.1816	45.4		4.7-5.1 (4.9)	Kidney (2)
		5013572	0.2422	60.5		4.7-5.1 (4.8)	Kidney (3)

DISCUSSION

From a total of 120 tissue samples (broiler and native chicken) screened for antibiotic residues, 35.83% of the samples tested positive for residue while 64.17% were negative. Similarly, the current finding in Maharjan *et al.*[25], from a total of 300 broiler samples tested for antibiotic residues 24.66% were found to be positive and 75.33% were found to be negative. In many developing nations, the issue of veterinary medication residues in poultry-derived products has grown more critical. This study used the TLC technique to determine antibiotic residues (Tetracycline, Amoxicillin, Sulfanilamide and Ciprofloxacin) in different types of poultry tissue. Tetracycline, Amoxicillin, Enrofloxacin and Ciprofloxacin were extracted from the liver, kidney, breast and thigh muscle of broiler and layer chickens[26] and Tetracycline, Streptomycin, Sulphanilamide and Ciprofloxacin were extracted from the liver and muscle of chickens[27]. According to thin layer chromatography (TLC), 48.33% of broiler meat samples were found positive for antibiotic residues in all investigated samples, which is consistent with Shahid *et al.*[28], who reported 44.8% antibiotic residue positive in chicken meat samples. Islam *et al.*[29] found that more than half of the samples tested were positive (52%). Tajik *et al.*[23] in Iran discovered that more than half of the poultry meat samples they tested contained detectable antibiotic residue. Adla and Nada[30] reported that 77.5% of the sample were at least contaminated with antibiotics residues within 80 chicken muscles samples. In another research, Alla *et al.*[31] differed with this conclusion, reporting that 52 (17.3%) of 300 broilers evaluated for antibiotic residues were found to be positive, indicating antibiotic overuse by veterinarians and owners, as well as a lack of antibiotic residue monitoring in the market. Al-Ghamdi *et al.*[32] disputed current findings, claiming that 69% of broiler meat samples tested positive for antibiotic residue. Antibiotic residues were identified more in liver samples than in kidney and breast muscle samples, indicating that the liver is the organ that detoxifies numerous compounds, including antibiotics, as reported by Kabir *et al.*[33], Naeem *et al.*[34] and Islam *et al.*[35]. Khan *et al.*[36] found that the liver (100%) contains more antibiotic residue than muscle (20%). Almashhadany[37] disputed the current finding of the highest rate of antibiotic residue present in the liver than thigh and beast muscle of poultry in Iraq. However, Hassan *et al.*[38] and Ezenduka[39] found that antibiotics were more prevalent in the kidney than in the liver, breast muscle and gizzard. This might mean that the majority of the samples were obtained when the

medications were still being metabolized in the liver and not yet cleared by the kidney. Antibiotics are almost always given right before slaughter, according to Oyedyji *et al.*[40]. Tasneem *et al.*[41] found a significant number of positive samples in muscle and liver (29.2 and 28.3%, respectively) but a lesser rate in kidneys (21.4%) in their earlier investigation. Sapkota *et al.*[42] found that muscle has the highest percentage of positive results than the liver by the Disc assay method. Ciprofloxacin residues were found in greater abundance in broiler and native chicken tissue than in other antibiotic residues. This finding resembles that of Heller *et al.*[43]. Ciprofloxacin was found in varying percentages and amounts in the liver, kidney, breast and thigh muscles, according to Islam *et al.*[44] and Faten *et al.*[45]. Ciprofloxacin was detected at the highest concentrations in both muscle and liver, according to Ciprofloxacin's abusive usage and prescription pattern in Bangladesh[43]. Another reason for the high levels of Ciprofloxacin in Banglanative chicken meat is that alterations in the chemical properties of quinolones have a major impact on their antibacterial action. Demethylation of enrofloxacin following therapy is known to convert it to Ciprofloxacin[46,47]. Another explanation for Ciprofloxacin's greater frequency and Enrofloxacin's lower frequency is because of this. Ciprofloxacin may also be degraded into additional degradation products[48]. The residue of Enrofloxacin appeared higher than Ciprofloxacin and Amoxicillin in the poultry of meat market[36]. Tetracycline residues were found in different percentages in broiler and native chicken liver, kidney and breast muscle. The findings of the study were substantially identical to those of Islam *et al.*[29], who found that 28% of liver samples examined were positive. The findings were comparable to those of Cetinkaya *et al.*[49], who found that 11 (18.3%) of 60 chicken flesh samples had tetracycline residues. However, this percentage differs significantly from the findings of Salehzadeh *et al.*[50], who found that oxy-tetracycline positive samples above MRL were 27.8, 95.6 and 18.9% in muscles, liver and kidney samples, respectively. In contrast to the current investigation, Al-Ghamdi *et al.*[32] found that 87 and 100% of muscle and liver samples were positive for oxy-tetracycline. Tetracycline residues were found in broiler and layer livers, kidneys, breasts and thighs, according to Sattar *et al.*[26]. Tetracycline residues were found in varying percentages in the liver and muscle, according to Nkechi and Abiodun[51].

Tetracycline residues were found to be higher in the liver among the antibiotics studied in the previous study[26,51]. These results are consistent with those of research done in Lebanon on 80 chicken samples harbouring tetracycline residues[30]. To cut costs, the farmer's self-administered antimicrobial medicines, mostly tetracycline since it is relatively cheap, were acquired from feed shops without the inspection of veterinarians and most significantly, failed to adhere to withdrawal periods[51]. In varying percentages, Amoxicillin residues were found in broiler and native chicken liver, kidney and breast muscle, which corresponds with Popelka *et al.*[24]. According to Satter *et al.*[26] and Islam *et al.*[44], amoxicillin residues were found in the liver, kidney, breast and thigh muscles of chickens as well. According to Youcef *et al.*[52], amoxicillin residues were found in the liver, breast and thigh muscles of chickens. The chicken liver had a greater sulfanilamide residue than the other samples (kidney and breast muscle). In the liver, kidney and breast muscles of broiler and DSH chicken samples, sulfanilamide residues were found in varying percentages. Kabir *et al.*[33] investigated the presence of sulfanilamide in the liver at the greatest percentage. According to Satter *et al.*[26], Sulfonamides in the liver are detected in 33.1% of samples. According to Maharjan *et al.*[25], There is significant antibiotic residue in the marketed broiler meat of Nepal. Saiful *et al.*[53] and Chowdhury *et al.*[15] reported that antibiotic residues found in chicken carcasses pose a serious health concern to consumers. Antibiotics can be transferred into the human food chain via drug residues in animal source foods as a result of their extensive use in commercial chicken in Bangladesh. The issue of antibiotic residues is still a major concern in Germany, Poland and Lithuania's poultry sectors. As a result, the poultry business is constantly looking for an innovative alternative to antibiotics Khan *et al.*[36]. However, the problem of antibiotic residues is a threat to the people of Bangladesh. In this study, only a qualitative test has been done in a specific area with a comparatively less sample. Therefore, a comprehensive study requires qualitative and quantitative testing and finding at the MRLs level correctly and developing public awareness among the people that antibiotic residues are not in the food.

After TLC identification of liver and kidney, further analysis by UHPLC was performed. The quantity of Amoxicillin residual in the liver was measured and this result was pretty comparable to Sattar *et al.*[26], who found that the Amoxicillin concentration in breast muscle ranged from 45.38-60.55 μg kg^{-1}. Peak retention periods of distinct samples were from 4.7-5.1 min and peak areas ranged from 2413165-6208358, which agrees with Sattar *et al.*[26]. These compounds' residues may develop at unacceptable amounts in edible tissues during slaughter, posing a risk of adverse consequences in sensitive persons, such as poisoning, allergy and so on.

CONCLUSION

Antibiotic residues were identified in a small proportion of native chicken samples, indicating that antimicrobials from commercial farms were overused in domestic farms. These antibiotic residues have the potential to infect the environment and food animals. Antibiotic residues contribute to antimicrobial resistance and, as a result, to major health issues. Antibiotic residue detection and estimate in food animals and environmental resources will require further research in the future.

SIGNIFICANCE STATEMENT

This study showed a significant amount of antibiotic residue was present in both broiler and native chicken tissues. The results of this study will help to make public awareness and the use of antibiotics.

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