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Detection of Antibiotic Residues in Broiler Meat by Thin Layer Chromatography

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Abstract

Antibiotic usage has played a significant role in the growth of poultry industry all across the globe. But because of indiscriminate use of antibiotics, several incidences related to occurrence of antibiotic residues in broiler meat have been reported. In the context of Nepal, only limited studies have been attempted to assess the presence of antibiotic residues in poultry meat and thus this study aims to determine the same in Dharan. A questionnaire survey was conducted among poultry farmers and veterinary shops in Dharan where 44% of respondents had training on poultry farming but only 4 % had knowledge on antimicrobial resistance in microorganism. The survey report showed maximum usage of doxycycline and tetracycline in poultry farms. Four types of broiler meat, namely, liver, breast muscle, kidney and gizzard were collected and screening of antibiotic residues (ciprofloxacin, doxycycline, enrofloxacin, gentamycin and tetracycline) in them was performed by thin layer chromatography. On thin layer chromatography, 9%, 17%, 8%, 3% and 21% of samples were detected with ciprofloxacin, doxycycline, enrofloxacin, gentamycin and tetracycline residues respectively. Highest occurrence of ciprofloxacin, doxycycline, enrofloxacin and tetracycline residues were found in kidney (16%), gizzard (32%), liver (12%) and kidney (36%) samples respectively.

Keywords: Antimicrobial agents, tetracycline, doxycycline, ciprofloxacin, enrofloxacin, gentamycin, meat safety

Introduction

With the growing population, income and urbanization, the demand for animal derived foods is estimated to grow by 70% between 2005 and 2050 (Alexandratos & Bruinsma, 2012). Among such foods, demand for poultry meat is expected to increase at the highest rate, by 121% (Mottet & Tempio, 2017). To meet this growing demand, poultry industry is also growing rapidly throughout the world. The situation is no different in Nepal and it is growing at a rapid rate of around 17-18% (FAO, 2014). Nepal is one of the best places for poultry rearing due to its rich biodiversity (Dhakal et al., 2019). Nepal lies at the 112th position for chicken meat production of the world (FAO, 2014). In Nepal, production of chicken has been found to be increased from 16,662 metric tons to 60,122 metric tons within a decade from fiscal year 2008/09 to fiscal year 2017/18 (MOALD, 2022). Poultry industry has become a major attraction to Nepalese farmers. It may be because of a higher success rate and better profitability than other sectors. More than 75% of the poultry farms in Nepal are in profit (CBS, 2015). For this tremendous growth of poultry industry, use of antibiotics has played a significant role (FAO, 2014).

In commercial poultry industry, antibiotics are used not only for therapeutic purposes but also as feed additives to promote growth, improve feed efficiency, and breeding performance, and enhance feed acceptability (Chowdhury et al., 2009). However, severe problems may arise if the farmers fail to follow the guidelines related to withdrawal times, dosage level, etc. for the antibiotics (Ezenduka, 2019). It may lead to occurrence of antibiotic residues in meat produced (Beyene, 2016) and occurrence of antibiotic resistance in poultry pathogens (Grane, 2000) and consumption of such contaminated meat can affect the consumers by causing imbalance in intestinal microflora, antibiotic resistance in human gut bacteria, immunological effects, and also many allergic reactions (Shareef et al., 2009). In Nepal, most of the farmers have been using common antibiotics based on their individual judgment and analysis of disease and flock condition rather than consulting with veterinary doctors (Osti et al., 2017). Such carelessness has led to occurrence of antibiotic residues in poultry meat sold in markets all over the nation (Gwachha, 2017; Maharjan et al., 2020; Pandey et al., 2009; Prajapati et al., 2018; Raut et al., 2017; Rawal & Manandhar, n.d.; Sapkota et al., 2019; Shrestha, 2017). Some workers have even reported residue levels to be above the MRLs (Maharjan et al., 2020; Raut et al., 2017). This study is thus aimed at assessing the antimicrobial usage habit of local farmers of Dharan and also to determine the prevalence of antibiotic residues in poultry meat samples collected from the retail outlets within the city.

Methods and Procedures

Research Design

This study used the survey design of quantitative research method. A total of 25 poultry farms situated in Dharan were surveyed to collect information regarding their education level, training level, antibiotics used in the farm, knowledge regarding occurrence of drug residues, maximum residue limits, withdrawal periods and antibiotic resistance. Similarly, 10 veterinary doctors working within Dharan Sub-Metropolitan city were surveyed to find out the common diseases in poultry in Dharan.

Sample Collection

A total of 100 samples (25 samples of each tissue variety) were randomly collected from broiler meat shops of Dharan. The tissue samples (liver, breast muscle, kidney and gizzard) were collected from randomly selected poultry meat shops within Dharan Sub-Metropolitan city. Each sample was kept separately in sterile plastic bag with proper labeling and transported to the Research Laboratory of Central Campus of Technology, Hattisar, Dharan in an ice box. These samples were stored in refrigerator at -20°C until further analysis (Sarker et al., 2018).

Antibiotic Standard Preparation

In brief, 0.05 g of ciprofloxacin, doxycycline, enrofloxacin, gentamycin and tetracycline were dissolved in 5 ml of dilute acetic acid, methanol, dilute acetic acid, distilled water and methanol respectively (Hossain, 2010). Working concentration for each antibiotic was determined by experiments such that a discrete spot without any tailing was obtained after chromatography.

Selection of Suitable Solvent System

A variety of solvent systems and TLC plate pretreatments were tested which are shown in Table 1 and the most suitable solvent system was selected among them which were ultimately used during TLC analysis of the meat samples.

Table 1

List of Preliminary Trials for Thin Layer Chromatography

Trial	TLC plate predevelopment	Mobile phase	Reference
A	No	Methanol: acetone (1:1)	(Sarker et al., 2018)
B	No	Water: methanol: dichloromethane (6:35:59)	(Bečić et al., 2018)

C	No	n-butanol: oxalic acid: water (100ml: 5g: 100ml)	(Kapadia & Rao, 1964)
D	No	Methanol: acetone: 1% aq. ammonia (4:4:1)	-
E	No	Water: methanol: dichloromethane: 1% ammonia (6:35:59:5)	-
F	No	Chloroform: methanol: conc. NH_4OH: H_2O (1:4:2:1)	(Claes & Vanderhaeghe, 1982)
G	Pretreatment by saturated EDTA solution	Chloroform: methanol: 5% aq. Na_2EDTA (65:20:5), lower layer	(Oka et al., 1983)
H	Pretreatment by saturated EDTA solution	Chloroform: methanol: acetone: 1% aq. NH_4OH (10:22:50:18)	(Xie et al., 1997)
I	Predevelopment in aq. Na_2EDTA solution (100g/l conc. and pH 8) and dried at 120°C/1 hr	Methanol: acetone (1:1)	-
J	Predevelopment in aq. Na_2EDTA solution (100g/l conc. and pH 8) and dried at 120°C/1 hr	Water: methanol: dichloromethane (6:35:59)	-
K	Plates washed with methanol and predevelopment in aq. Na_2EDTA solution (100g/l conc. and pH 8) and dried at 120°C/1 hr	Chloroform: methanol: 25% NH_4OH (60:35:5)	(Chen & Schwack, 2013)

Sample Preparation

Extraction of antibiotics from samples was done by using phosphate buffer followed by protein precipitation and defatation (Tazrin, 2014)with slight modifications. Briefly, samples were grinded separately in mortar pestle. Then, 10g of sample was taken in a centrifuge tube and 7 mL phosphate buffer (pH 6.5) was added to it. Then 3 mL aq. EDTA solution (0.1 mol/L and pH 8.0) was added to it (Chen & Schwack, 2013). They were mixed well using vortex mixture followed by the addition of 2 mL 30% trichloroacetic acid for protein precipitation. The mixture was centrifuged at 7000 rpm for 15 min. The supernatant was filtered and defatted with equal volume of diethyl ether. The upper oily layer was discarded and defatation of bottom layer was further done twice with equal volumes of diethyl ether. It has been found that concentrating the extract made detection easier while performing TLC (Tajick & Shohreh, 2006). So the defatted extract was then concentrated to about 2 mL in a rotary vacuum evaporator at a temp of 50 °C. The concentrate was collected in screw capped tubes and stored in refrigerator until TLC analysis.

Pointing, Running and Detection

A straight line was drawn on EDTA treated TLC plates using a pencil 1.5 cm above the lower end of TLC plate. The line was sufficiently high up the plate so that when it was placed in the solvent, the spotted samples remained above the level of solvent. Then each antibiotic standard solutions and concentrated sample extracts were spotted on the line 1 cm apart by using a micropipette. Proper care was taken to ensure that the spot was as small as possible as and not greater than 2-3 mm in diameter. After spotting, the spots were left to dry properly (Tazrin, 2014). Before placing the spotted TLC plates in TLC tank, 200 mL of mobile phase was poured into TLC tank lined with blotting paper and left for saturation for about an hour. The plates were then immersed carefully in the TLC tank. Before the mobile phase exceeds the upper end of TLC plate, the plates were taken out and solvent front was marked with a pencil. The plates were then left to dry for 30 min at room temperature. The TLC plates were observed under UV light 254 nm in a UV chamber (Ramatla et al., 2017). Dark or blue fluorescent spots seen against the green fluorescent background were circled and retention factor (Rf) values for the spots were calculated (Table 2).

Table 2

R_f Value of Spots Seen on Performing Thin Layer Chromatography of Positive Samples

Sample	Retention factors							
				First run				
Ciprofloxacin						0.36		
Doxycycline			0.17					
Enrofloxacin								0.50
Gentamycin							0.42	
Tetracycline				0.24				
L1	0.06				0.31			
K1		0.09		0.24	0.31			
G1		0.09		0.24	0.31			0.51
L2		0.09			0.31			0.50

M2		0.09		0.24		0.50			
K2		0.10		0.24	0.31	0.50			
G2		0.09		0.24		0.50			
L3	0.06	0.10			0.31	0.51			
M3		0.10		0.24					
G4		0.09			0.31				
L5		0.09			0.31				
M5		0.09		0.23		0.50			
K5		0.09		0.23	0.31				
G22		0.09	0.17		0.31				
Second run									
Ciprofloxacin								0.54	
Doxycycline				0.22					
Enrofloxacin									0.62
Gentamycin							0.49		
Tetracycline					0.29				
L6	0.04	0.08		0.22	0.28		0.49		
L7	0.05		0.10	0.22		0.34			
G7			0.10		0.28				
L8			0.11					0.54	
K8					0.28				
G8			0.11	0.22					
M9			0.11						
K9			0.12		0.29				
G9			0.11	0.22		0.34			
L10		0.08							
M10		0.08	0.11	0.23		0.35			
G10			0.11	0.22					
Third run									
Ciprofloxacin					0.47				
Doxycycline		0.16							
Enrofloxacin						0.51			
Gentamycin				0.41					
Tetracycline		0.20							
L11	0.07		0.27						
M11	0.07	0.20							
K11	0.07	0.17	0.27	0.41					
L12	0.07	0.20	0.28						
K12	0.07	0.17	0.28						
G12	0.08		0.27						

L13	0.08	0.17	0.28				
K13	0.07	0.20	0.28				0.48
L14	0.07	0.20	0.28				
K14	0.08	0.20					
G14	0.08	0.21					
K15		0.21					0.49
G15	0.08	0.21					
Fourth run							
Ciprofloxacin						0.50	
Doxycycline	0.22						
Enrofloxacin							0.57
Gentamycin					0.45		
Tetracycline		0.26					
L16			0.30				
K16	0.22			0.33		0.50	
G16	0.23		0.31			0.50	
G17	0.23						
L18				0.34			
K18	0.23			0.34			
G18	0.23		0.30		0.45	0.50	
K19						0.50	
L20				0.34		0.50	
K20	0.23			0.33		0.50	
G20	0.23		0.30			0.50	
L21			0.31				
K21		0.25		0.33		0.50	
L22							0.58

*L, M, K and G represent liver, muscle, kidney and gizzard sample respectively and numbers following the alphabets represent the sample position.

Statistical Analysis

Experimental data were introduced and well tabulated in Microsoft Excel 2016. Results were analyzed statistically for the test of significance using IBM SPSS Statistics 20 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20, Armonk, New York USA: IBM Corp). Tests were performed for descriptive statistics using Chi-Square test at 5% level of significance.

Results and Discussion

Education Level of Poultry Farm Owners

Education level of poultry farm owners was found satisfactory. Significantly high ($p<0.05$) percentage of poultry farm owners reported to have obtained an education up to school level (76%) while 16%, and 8% of farmers had higher level education up to college level, and bachelors level respectively while none have graduated and masters level. According to a report by Central Bureau of Statistics (CBS), Nepal, 84.7%, 10.4%, 3.5% and 1.4% of poultry farmers in Sunsari district had an education level up to school level, certificate level, bachelors level and masters level respectively (CBS, 2015). It was found that there's been a slight increase in level of education of owners. Further survey information on knowledge related to poultry farming and use of antibiotics are shown in Table 3. Central Bureau of Statistics, Nepal has reported that 23% of poultry farm owners in Sunsari district are trained (CBS, 2015). The present study revealed higher percentages of poultry farm owners to have received such trainings at least once. Almost half of the respondents have a general concept regarding withdrawal period and reported to have stopped providing antibiotics to the birds for prophylactic purpose after they were above 30 days old. Almost none of the respondents have knowledge regarding antimicrobial resistance development in microorganisms.

Table 3

Knowledge Levels of Poultry Farm Owners on Several Aspects Related to Poultry Farming and Antibiotic Usage

S.N.	Particulars	Number of respondents (Percentages)
1.	Acquirement of trainings on poultry farming	11 (44)
2.	Knowledge regarding occurrence of antibiotic residues in meat	9 (36)
3.	Knowledge on withdrawal period of antibiotics	13 (52)
4.	Knowledge on maximum residue limit of antibiotics in meat	5 (20)
5.	Knowledge on antimicrobial resistance in microorganisms	1 (4)

Antibiotics Usage in Poultry Farms

The percentage of poultry farms that reported the use of different antibiotics is shown in Table 4. It was found that the most common groups of antibiotics to be used are tetracyclines (doxycycline, tetracycline), followed by quinolones (ciprofloxacin, enrofloxacin, levofloxacin), β-Lactams (amoxicillin), sulfonamides (sulfamethoxazole), aminoglycosides (neomycin) and macrolides (tylosin).

Table 4

Antibiotics Usage in Poultry Farms

Antibiotics	% of poultry farms	Antibiotics	% of poultry farms
Ciprofloxacin	16	Tylosin	24
Doxycycline	60	Sulphamethoxazole	16
Enrofloxacin	28	Trimethoprim	16
Levofloxacin	36	Colistin	20
Tetracycline	44	Neomycin	36
Amoxicillin	36		

Poultry Diseases

According to veterinary doctors working in Dharan, the common poultry diseases in Dharan are chronic respiratory disease (CRD), E. coli infections, coccidiosis and foulpox. They also mentioned that enrofloxacin, ciprofloxacin, levofloxacin and tylosin are prescribed in case of CRD, tetracycline groups such as chlortetracycline against foulpox, amoxicillin, levofloxacin and colistin against E. coli infections and amoxicillin against coccidiosis. A report back in 2015 mentioned colibacillosis, CRD and coccidiosis as some major poultry diseases diagnosed at Regional Veterinary Laboratory, Biratnagar and Regional Veterinary Laboratory, Janakpur(GARP-Nepal, 2015).

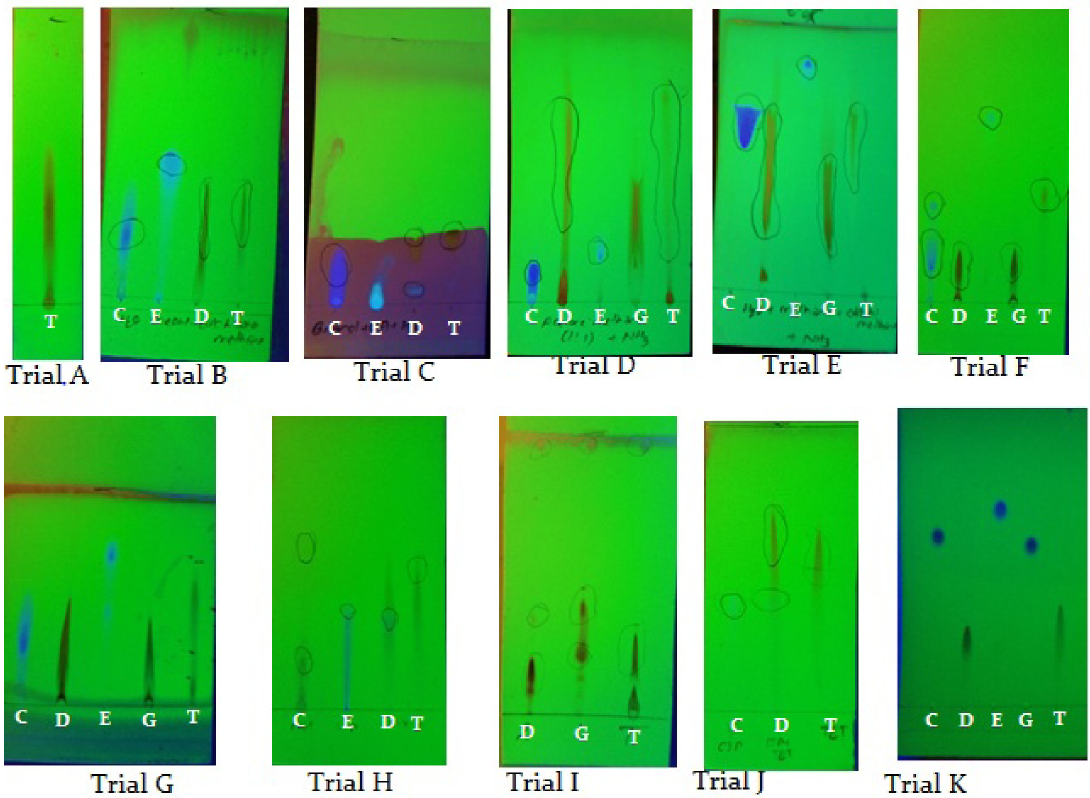
Suitable Solvent System for TLC

Almost all of the works regarding detection of antibiotic residues in meat tissues using TLC have been performed using acetone: methanol (1:1) as the mobile phase (Ramatla et al., 2017; Sarker et al., 2018). But it couldn't be implied in this study due to very unsatisfactory results and thus a number of solvent systems were tested to determine the best one. Among different types of solvent systemsand TLC plate pretreatments used (Table 1), trial K was found to be the optimum solvent system for TLC analysis of the test antibiotics, (Fig. 1) in this study. One of the major problems encountered during TLC was excessive tailing of doxycycline and tetracycyline antibiotics even though very small concentration of these antibiotic standards (as small as 0.1mg/ml) was spotted on the plates. Such tailing was observed in trials A and B for all of the antibiotics tested. In order to minimize the problem, mobile phases added with a few amount of liquid ammonia were tried as in trials D, E and F. But no any significant improvement was observed. Oka et al.(1983) described n-butanol as a suitable developing solvent for TLC of tetracycline and thus trial C containing greater fractions of n-butanol was tested. But very unsatisfactory results were obtained that covered the entire TLC plate with a dark patch when visualized under UV light. The reason for this couldn't be explained. The possibility is that some form of interaction might have taken place between the fluorescent material of the TLC plate and the component of solvent system used.

Chen and Schwack (2013) reported that the analytes (especially tetracyclines) displayed a strong tendency to form chelate complexes with alkaline earth and transition metal ions present in the silica plate, leading to serious tailing effects. So trials G, H, I and J were conducted which involved predevelopment of TLC plates in saturated Na_2EDTA solution prior to running the antibiotic standards. This technique was found to improve the results to some extent but still the results were not satisfactory as the antibiotic standards incurred similar retention factors. Finally, trial K was found to be the most satisfactory one with better resolution of the spots as well as minimum occurrence of tailing effect.Another major problem encountered during TLC analysis was the occurrence of a dark band on the TLC plate just below the solvent front after the solvent was run through the plate. It was initially suspected to be because of the impurities that may have been present in the solvent. But even on running distilled water through the plate, the dark band was formed. It indicated that the band might have been formed due to impurities in the TLC plate itself. So to avoid the band, the plates were first pre-developed in methanol.So, predevelopment of silica plates in aq. Na_2EDTA solution (100 g/L conc. and pH 8) and drying the plates at 120°C/1 hr followed by the use of Chloroform: methanol: 25% NH_4OH (60:35:5) as mobile phase was finally found to alleviate the problem.

Figure 1

TLC plates for different mobile phases with antibiotics (Preliminary trials for selecting mobile phase: The denotations C, D, E, G and T represent spotted points for ciprofloxacin, doxycycline, enrofloxacin, gentamycin and tetracycline respectively)



Detection of Antibiotic Residues

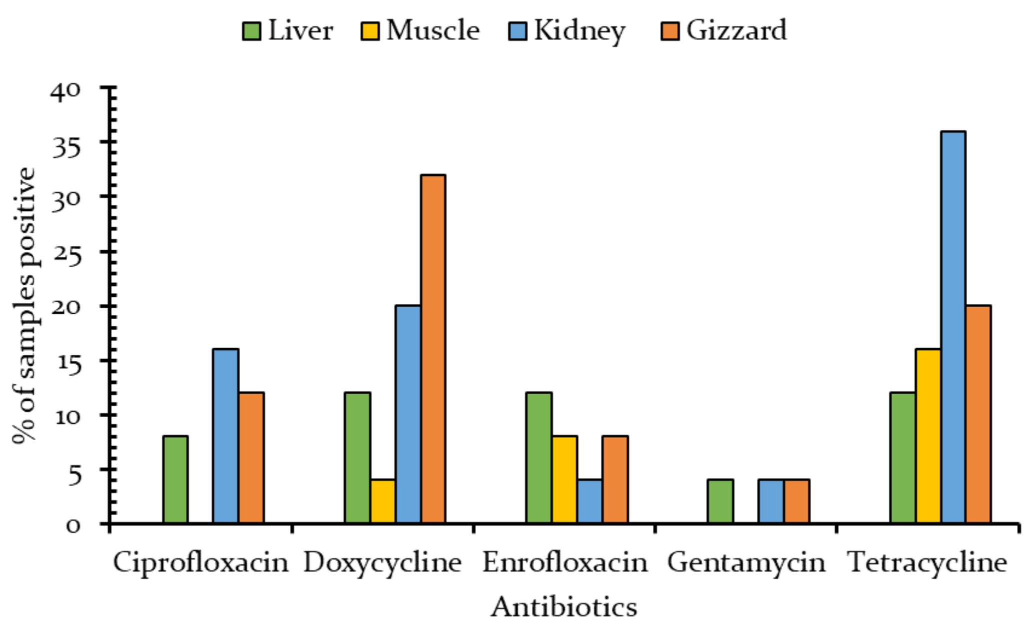
On TLC of meat samples, 9%, 17%, 8%, 3% and 21% of samples were detected with ciprofloxacin, doxycycline, enrofloxacin, gentamycin and tetracycline residues respectively. Prevalence of different antibiotics in the meat samples was found to differ significantly ($p<0.05$). Tetracycline and doxycycline were found to be the most common antibiotics detected followed by ciprofloxacin and enrofloxacin. The results obtained are also justified by the survey report in which tetracycline and doxycycline are found to be the most commonly used antibiotics. Such a high incidence of tetracycline and doxycycline residues in meat can also be attributed to their usage in poultry feed. Previous study has shown doxycycline, chlortetracycline and tetracycline as the major antibiotics added to the feed and they are each added at the rate of 500g to 1kg per ton of feed during feed preparation (Ramdam, 2015).

Ciprofloxacin Residues

According to our findings, ciprofloxacin was detected in 9% of the samples. A study back in 2018 found higher occurrence of ciprofloxacin residues (15.21%) in broiler meat from Kathmandu, Kaski and Chitwan (Prajapati et al., 2018). Similarly, residues of ciprofloxacin have been detected in varying percentage of chicken meat samples, 44.37% of samples in Bangladesh (Sarker et al., 2018), 40.7% of samples in Bangladesh (Sattar et al., 2014), 21.4% of samples in South Africa (Ramatla et al., 2017) and 3% of samples collected from Bangladesh (Tazrin, 2014). Although no significant difference (p>0.05) in occurrence of ciprofloxacin among different tissues was observed, highest incidence was found in kidneys (16%) followed by gizzard (12%), liver (8%) and nil in breast muscles (Figure 2). Analogous results have been reported in Bangladesh where maximum occurrence of ciprofloxacin was found in broiler kidneys (48.57%) followed by liver (42.85%) and breast muscles (31.42%) (Sattar et al., 2014). Similarly, another study from Bangladesh also reported 52% liver samples and 39% breast meat samples to contain ciprofloxacin residues (Sarker et al., 2018). Kidney is the most important organ for excretion of antibiotics(Ezenduka, 2019), due to which it has relatively higher prevalence of ciprofloxacin residues.

Figure 2

Prevalence of Antibiotics in Different Tissues on TLC Analysis



Doxycycline Residues

Residues of doxycycline were detected in 17% of the samples inspected. Prevalence of doxycycline as high as 32.3% was reported in chicken meat of Bangladesh (Sarker et al., 2018).

Occurrence of doxycycline residues in gizzard and breast muscles was found to differ significantly ($p<0.05$). In contrast to other antibiotics, doxycycline was found to be the most prevalent in gizzard samples (32%) followed by kidney (20%), liver (12%) and breast muscles (4%) which is presented in Fig. 2. In a study regarding tissue depletion of doxycycline administered orally at high dosage to broiler chickens via drinking water, it was found that doxycycline concentrations are higher in gizzard followed by kidney, liver and breast muscles after the third day of administration. But on the fifth day, the concentrations in gizzard depleted at a higher rate than in kidney and liver (Hsiao et al., 2016). It may be because of 5 to 10 times higher liphophilicity of doxycycline, resulting in higher tissue penetration, larger volume of distribution and longer elimination times (Papich & Riviere, 2017). Our results suggest that doxycycline in fed to the birds even till the day of selling the birds and no withdrawal times are followed.

Residues of Enrofloxacin

Residues of enrofloxacin were found in 8% of the collected broiler meat samples. Our findings were much smaller than the findings in Bangladesh where 26.8% (Sarker et al., 2018), and 27.85% (Sattar et al., 2014) of the samples were detected with enrofloxacin residues. Similarly, enrofloxacin residues have been detected in 2.5% samples collected from Bangladesh (Tazrin, 2014).Occurrence of enrofloxacin among different tissues was not found to differ significantly ($p>0.05$). Highest occurrence of enrofloxacin was found in liver (12%) followed by breast muscles (8%), gizzard (8%) and 4% in kidney (Figure 2). Highest occurrence of enrofloxacin in liver among liver, kidney and breast muscle samples have also been reported in samples collected from Bangladesh (Sattar et al., 2014). Similar values of enrofloxacin residues in breast muscles (8.7%) was found in samples collected from Kathmandu, Kaski and Chitwan (Prajapati et al., 2018). In contrast to other antibiotics, least number of kidney samples were detected with enrofloxacin residues. Higher prevalence of enrofloxacin residues in chicken liver than in kidney samples was also reported in another study from Bangladesh (Islam et al., 2016).

Residues of Gentamycin

Gentamycin residues were detected in the least number of samples (3%). None of the chicken meat samples collected from Iraq were found to contain gentamycin residues (Shareef et al., 2009). Occurrence of gentamycin residues in different tissues was not found to differ significantly ($p>0.05$). 4% of each of liver, kidney and gizzard samples was found to contain gentamycin residues whereas no residues of gentamycin were detected in breast meat samples (Figure 2).

Residues of Tetracycline

Tetracycline was found to be the most prevalent antibiotic in poultry meat of Dharan and was found in 21% of the meat samples. Other studies have also shown occurrence of tetracycline in 30% samples(Sattar et al., 2014) and 11.83% samples (Hossain, 2010) from Bangladesh and in 14.6% samples from South Africa (Ramatla et al., 2017). Using rapid test kits, tetracycline residues were detected in 33.33% samples collected from Kathmandu valley (Prajapati et al., 2018) and in 29.09% of chicken meat samples from Kavre and Kailali (Raut et al., 2017).Among different tissues, tetracycline residues were the most prevalent in kidney samples (36%), followed by gizzard (20%), breast muscles (16%) and 12 % in liver (Fig. 2) Occurrence of tetracycl.ine residues between liver and kidney was found to differ significantly ($p<0.05$). Tetracycline residues were detected in 48% livers, 24% kidneys and 24% breast muscles collected from Bangladesh (Sattar et al., 2014). Using rapid test kit, tetracycline residues were detected in 40% liver and 10.66% muscle samples from Kathmandu valley (Maharjan et al., 2020). Kidney is the most important organ for excretion of antibiotics (Ezenduka, 2019) which may be the reason for higher prevalence of tetracyclines in kidneys.

Conclusion

This study aimed at assessing the prevalence of antibiotic residues in poultry meat sold in Dharan. Although the education level of farmers was satisfactory, knowledge regarding safety aspects of antibiotic usage such as prevalence of drug residues in meat and occurrence of antimicrobial resistance in microorganisms was found to be still insufficient. Tetracycline and quinolones group of antibiotics were the most commonly used ones. On TLC analysis, tetracycline and doxycycline were the highest detected antibiotics. Almost all of the samples were found to contain multiple drug residues. The highest prevalence of tetracycline and ciprofloxacin was detected in kidneys whereas highest number of gizzard samples contained doxycycline residues. Occurrence of antibiotic residues in such a large number of samples indicates that farmers are not using these antibiotics sedulously. Such malpractice may not only affect the birds but also affect the consumers as it enters the food chain. Antibiotics have become an inevitable part of modern poultry farming and the only way out is to use them wisely. Concerned authorities should also encourage farmers to lessen the use of such antibiotics and should routinely monitor the meat sold in market to ensure consumer safety.

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