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Article

Heavy metal contamination and antibiotic residues in poultry feed and meat in Bangladesh

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Abstract: Presence of harmful contaminants and residues in poultry feed and meat have serious public health consequence. This study was carried out to identify and quantify antibiotic residues, heavy metals and toxins in poultry feed and meat in the two selected poultry production belts of Bangladesh. A total of 94 broiler feed samples and 60 broiler meat samples were collected and tested by Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Enzyme-linked Immunosorbent Assay (ELISA) for identification and quantification of the parameters. Antibiotic residues were detected in 18.89% of the feed samples, whereas, there were no toxin (Aflatoxin) positive samples. Among the antibiotic positive samples, Oxytetracycline (OTC) was found predominant and detected in 12.22% cases. The mean concentrations of Cadmium (Cd), Lead (Pb), Chromium (Cr) were found as 0.04 mg/kg, 1.28 mg/kg and 2.55 mg/kg respectively in feed samples. In the case of meat samples, the mean concentration of OTC, Ciprofloxacin (CIP), and Tetracycline (TCL) residues were found 8.67 ppb, 7.18 ppb and 0.81 ppb accordingly. The highest mean concentration of Oxytetracycline (OTC) (10.15 ppb) was found in samples collected from local poultry sellers, whereas, the highest mean concentration of Tetracycline (TCL) (1.35 ppb) and Ciprofloxacin (CIP) (10.62 ppb) were observed in the samples obtained from local farm. The highest percentage of TCL and CIP (64% and 48% respectively) were found in samples collected from local farm. Chlortetracycline (CTC) was found predominant (70%) in samples collected from Contract farms. On the other hand, out of 60 meat samples, Cd and Cr were detected in only one meat sample with concentration of 56.41 mg/kg and 14.44 mg/kg respectively. Lead was not detected in any of the meat samples.

Keywords: antibiotic residue; food safety; heavy metal; meat; poultry feed; public health; toxin

1. Introduction

Poultry is one of the fastest, easiest and cheapest sources of protein (meat and egg) for human consumption in Bangladesh and sometimes it's referred as poor man's animal protein. Adulteration of poultry meat and products

is a serious public health concern which have detrimental effects for human life and health. Poultry meat and poultry feed can be contaminated by variety of contaminates (Sarker *et al.*, 2020; Altherwi *et al*., 2018). Among them principle contaminants are heavy metals, antibiotics, metabolites, microorganisms, mycotoxins, hormones, polychlorinated biphenyls, genetically modified organisms, nitrates and nitrites, toxic pigments, pesticide residues, dioxins, , melamine and so one (Sarker *et al.*, 2018; Hu *et al*., 2017).

Heavy metals are growing concern among principle contaminants of poultry meat, feed and other poultry products. Therefore, contamination of heavy metal in food and water is a major concern at national and international level due to food safety issues and human health risk through the food chain (Aktar *et al.*, 2020; Yeasmin *et al*., 2017). Accumulations of heavy metals that occur through meat or other food materials eventually make their course into the body tissue (Islam *et al.*, 2016; Baykov *et al.*, 1996). The high degree toxicity of arsenic, cadmium, chromium, lead, and mercury has ranked them among the priority metals that are of public health significance. These are the systemic toxicants which cause multiple organ damage, even at lower levels of exposure. According to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer, these metallic elements are also identified as human carcinogenic agents (Tchounwou *et al*., 2012).

Antibiotics are the chemical agents which were formulated for saving the life of people and livestock from infections of bacteria (Hyung *et al*., 2017). The imprudent use of antibiotics both for prophylactic and therapeutic purposes has considered as global concern due to its residual effect, and subsequent adverse health hazards of consumers (Ferdous *et al.*, 2019; Chanda *et al*., 2014). More importantly, in unauthorized veterinary practice, antibiotics are used illegally as growth promoter in the livestock and poultry instead of therapeutic (Altherwi *et al*., 2018; Talukder *et al.*, 2017). Thus the wide spread use of antibiotics in poultry industry resulted in the presence of residuals in foodstuffs leading to a potential health hazards for consumers which include; carcinogenicity, mutagenicity, bone marrow toxicity, allergy (Paul *et al.*, 2020; Nisha, 2008) as well as appearance of a resistant strains of pathogenic bacteria (Hussein and Khalil, 2013).

The poultry sub-sector of livestock sector is an essential part of fostering agricultural growth and reduces malnutrition for the people of Bangladesh (Hamid *et al*., 2017). It is an integral part of farming system in Bangladesh which created direct and indirect employment opportunity including support services near about six million people (Hamid *et al*., 2017; Raihan and Mahmud, 2008). Unfortunately, the research findings from Bangladesh showed presence of antibiotic residues and heavy metals in poultry feed, meat and eggs at public health concerning level (Bhuiyan *et al*., 2021, Bristy *et al*., 2019; Islam *et al*., 2019; Rashid *et. al.,* 2018). Currently, the status of commonly used poultry feed quality ensuring standard nutrition values is very little known. Therefore, this research was undertaken to understand the current status of the presence of antibiotic residue (Tetracycline (TCL), Chlortetracycline (CTC), Oxytetracycline (OTC), Ciprofloxacin (CIP), Azithromycin (ATM)); heavy metals (Cadmium (Cd), Chromium (Cr), Lead (Pb) and Arsenic (As)); and toxins (Aflatoxin) in poultry feed and meat for recommending implementable food safety measures in Bangladesh. However, CIP and ATM tested only for meat samples and CTC, As and aflatoxins tested only for feed samples.

2. Materials and Methods

2.1. Study area

The two poultry production belt districts of Bangladesh (Gazipur and Rajshahi) (Figure 1) were selected considering the number of poultry farms, number of hatcheries, and findings of previous field work of different organizations.

2.2. Sampling design

A cross-sectional study was carried out in the study areas in order to identify and quantify the presence of heavy metals, antibiotic residues, and toxin in broiler feed and meat. A total of 94 broiler feed samples and 60 broiler meat samples were collected and necessary subsets of the samples have been produced for laboratory investigation outlined in Table 1.

2.2.1. Feed samples

Among the 94 feed samples, 20 samples were collected from farm level which were prepared or mixed by farmer him/herself, 33 were from local and non-registered feed mills, and 41 were from the registered feed mills. Ready feed samples were collected from the retailer shops. Every sample (500gm) was collected aseptically for each of the subsets (3 subsets/sample), and placed into a sterile zipper-type plastic bag, and leveled with necessary information. To facilitate the absorption of additional moisture from feed, small silica gel packet was placed in each of the bag. A cautious approach was taken during the collection process of samples

for Aflatoxin analysis to avoid moisture contamination. All the samples were transported in plastic box to the laboratory. All samples were stored in dry and cool place before analysis.

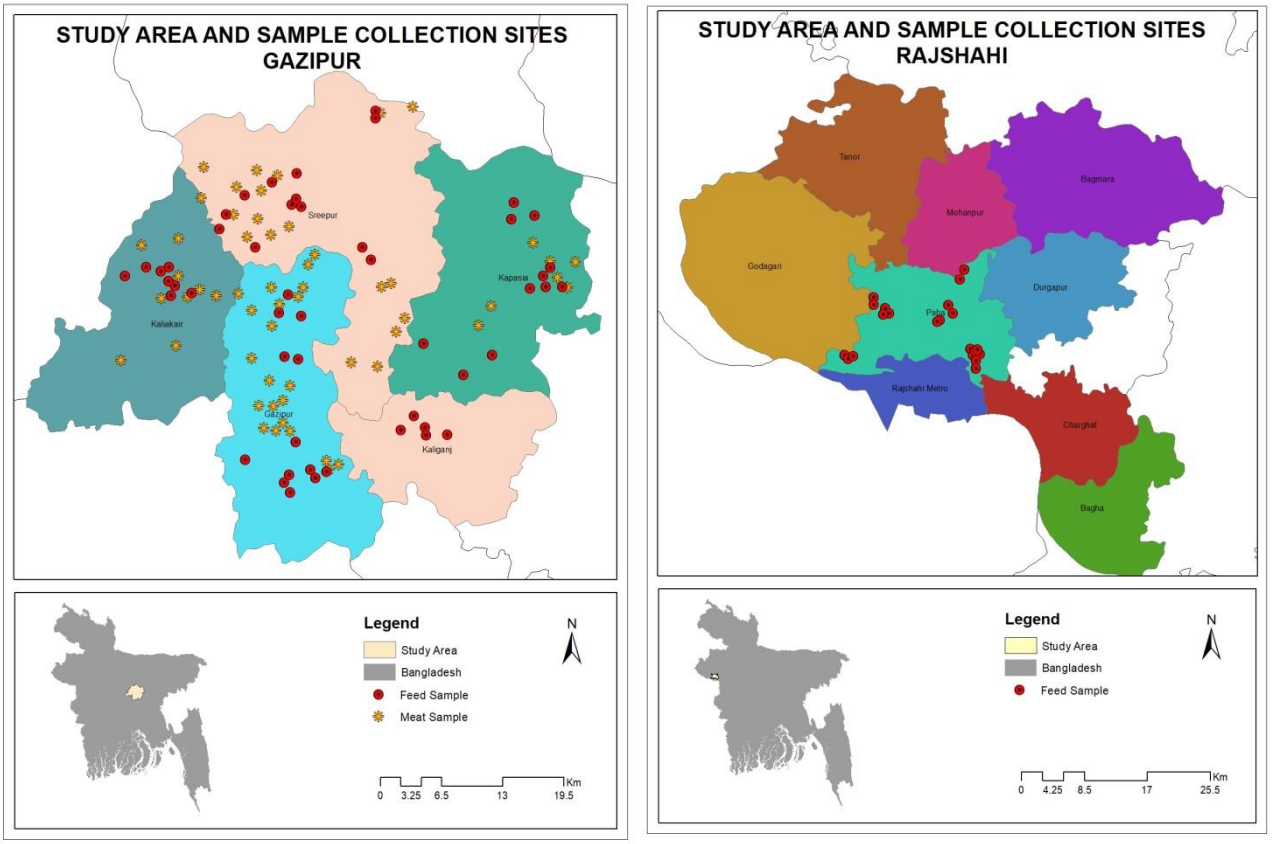

Figure 1. Study area maps of Gazipur and Rajshahi district based on Geographical Information System (GIS) created with ArcMap 10.7 software (ESRI, Redlands, California, USA).

2.2.2. Meat samples

Thigh and breast muscles of 5-6 weeks old broiler chicken were collected for this study. Out of 60 broiler meat samples, 10 were from contract farms, 25 from local broiler farms, and 25 from local poultry sellers. About 500gm of thigh and breast muscle was collected aseptically for each of the subsets (three subsets/sample), and leveled with necessary information. The sample was minced and mixed properly, and placed into a sterile zipper-type plastic bag. All the samples were transported in an icebox to the laboratory, and stored in -20 ^{0}C freezer before analysis.

2.3. Laboratory Test

A capacity assessment was conducted by a Technical Advisory Committee and they provided necessary guidance to identify and select the laboratories to perform necessary laboratory investigations for this study. For the identification and quantification of antibiotic residue, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) was used. To verify the result found through HPLC, same subset of the samples were also analyzed using Enzyme-linked Immunosorbent Assay (ELISA) technique.

The laboratory test techniques are outlined in Table 2. All the lab tests were performed by the laboratories following their own standardized and optimized protocols.

3. Results and Discussion

3.1. Antibiotic residue in poultry feed

A total of 90 feed samples were tested to identify and quantify the concentration of TCL, CTC, OTC residues. Overall, antibiotic residues were detected in 18.89% of the feed samples. Among them, OTC found predominant and was detected in 12.22% cases, whereas TCL found in 4.44% and CTC found in 2.22% samples (Table 3). Similar study conducted by Bangladesh Agricultural Research Council (BARC) and Patuakhali Science and

Technology University (PSTU) and they found antibiotic residue in 50% of the collected samples (Molla, 2019). It was also observed that the samples obtained from registered feed companies having the highest percentage of antibiotic residues these were 0.81%, 27.03% and 5.41% respectively for TCL, OTC and CTC. In addition to that, the concentrations of these antibiotics were 171.3 ppb, 40 ppb and 100.27 ppb accordingly (not shown in table). Nonetheless, no antibiotic was detected in the sample obtained from non-registered or local feed companies.

Uncontrolled use of antibiotic growth promoter has been criticized by scientists, public health practitioners and food safety activists due to developing antibiotic resistance and treatment failure both in animal and human (Hyung *et al*., 2017). Studies found that antibiotic registrant gene can be transferred from animal to human pathogens. Also, it has public health significance for the possibility of horizontal transmission of Antibiotic Resistant Gene (ARG) in human and animal (Lerminiaux *et al*., 2019). To avoid the public health hazards, the Government of Bangladesh (GoB) prohibited the use of antibiotics in animal feed by establishing 'Fish Feed and Animal Feed Act 2010'.

3.2. Antibiotic residue in poultry meat

A total of 60 samples were collected to identify and quantify four different types of antibiotics (TCL, OTC, CIP and ATM). Overall screening result is showing that CIP residue ranked top and no ATM residues in the samples collected from all the three sources. Mean concentration of OTC was 8.67 ppb whereas CIP, TCL and ATM were 7.18 ppb, 0.81 ppb and 0 ppb consequently. In addition, the highest concentration of OCT, CIP, TCL and ATM were 152 ppb, 88 ppb, 20 ppb, and zero ppb respectively (Table 4). Importantly, OCT found as common antibiotic used in poultry farms. However, one good thing is that all the revealed antibiotic residual concentration were below Maximum Residue Limit (MRL) recommended by Food Safety (Contaminants, Toxins and Harmful Residues) Regulations, 2017 (Table 4).

The highest percentage of positive samples of antibiotic residue was found in samples of Local Farms (data not shown. Among them TCL was the highest and it was 64%, whereas, CIP was found 48%. In the case of contact farming, CIP was predominant in 70% meat samples (Table 5). On the other hand, the highest mean concentration (10.15 ppb) of antibiotic residue found in samples collected from local poultry sellers and the antibiotic was OTC (Table 5).

Nonetheless, though all the revealed concentration of the antibiotic residues under below the MRL, still the findings of this research is a very good indication of widespread misuse of antibiotics in the farm level and live bird market. It also indicates lack of awareness among the poultry producers or disrespect to the regulations about the withdrawal periods of antibiotics in food animals and birds. Importantly, scientific evidence already proved that the unnecessary exposure of antibiotic even with a low dose for a long period has its drawbacks on consumer health and can lead to develop antibiotic resistance (Khan *et al*., 2015). Antimicrobial resistant bacteria in food animal can play serious consequence in human health system when they enter into the food (Founou *et al*., 2016).

3.3. Heavy metal contamination in poultry feed

There were 94 poultry feed samples was assessed for heavy metal contamination. Over all, all feed samples of all sources were positive for heavy metal contamination. The mean concentrations of Cd, Pb, and Cr were 0.04 mg/kg, 1.28 mg/kg and 2.55 mg/kg respectively (Table 6). It was found that both the mean and highest concentrations of Cd were below the MRL (0.5 mg/kg) as prescribed by European Union in Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, 2003. On the other hand, the mean concentration of Pb was also found below the MRL (5 mg/kg), whereas, the highest concentrated Pb positive sample was around three times higher (14.65 mg/kg) than the MRL (Table 6). However, earlier studies in Bangladesh found up to 30.27 ppm lead in their analyzed poultry feed samples (Rashid *et al*., 2018). In addition, the highest mean concentration of Cd (0.054 mg/kg) and Pb (2.48 mg/kg) found in the feed samples collected from Non Registered Companies. In contrast, the highest concentration (2.78) of Cr found in the samples obtained from Registered Companies (Table 7).

According to European Union (EU, 2003), there are no maximum allowed concentrations of Cr for poultry feed. In this study, chromium was detected in all feed samples. However, the MRL for Cr concentrations in human food lies between 0.1 and 0.5 mg/kg (Alkhalaf *et al*., 2010). The major source of Cr in poultry feed samples could be from tannery solid waste and its effluent (Tariq, 2009). Accumulation of Pb in human body might be occurred from animal and poultry feed. As non-essential nutrients, Cd and Pb have no or very limited requirement to human and livestock health, and may accumulate in the body, particularly in the kidney, liver, and to a lesser extent in the muscle (Li *et al*., 2005). Musculo-skeletal, renal, ocular, neurological,

immunological, reproductive and developmental disorders are common outcomes of Pb toxicity in human and animal health (Ambedkar and Muniyan, 2012). In spite of the evidence of carcinogenic potency of Cd in experimental animals, its carcinogenicity in humans is not well defined (Costa, 2000). However, Cd has been classified as 'Group I' human carcinogen by 'International Agency for Research on Cancer (IARC, 1993). The effects of acute cadmium poisoning in humans are very serious. Among them are high blood pressure, kidney damage, destruction of testicular tissue, and destruction of red blood cells (Altherwi *et al*., 2018).

3.4. Heavy metal contamination in poultry meat

Among the tested 60 poultry meat samples, there was only one sample found positive for Cd and Cr and concentration were 56.41 mg/kg and 14.44 mg/kg respectively. Moreover, Pb was not detected in any of the meat samples.

3.5. Aflatoxin in poultry feed

Aflatoxins are classified as poisonous carcinogens that are produced by certain fungi which grow in agricultural crops, decaying vegetation, hay, and grains. Quality of feed and feed ingredients can be affected by the presence of Aflatoxin, and this can subsequently create health hazards to animals and humans. In this study, a total of 50 feed samples were tested to identify and quantify Aflatoxin (B1, B2, G1, G2) using HPLC with fluorescence detector. The detection limit was 0.5µg/kg. However, Aflatoxin was not detected in any of the feed samples. This indicates the good stranded of manufacturing and storing practice of feed ingredients by the commercial poultry feed producer.

Table 1. Subset of samples for laboratory analysis of heavy metal, antibiotic residue and toxin.

Lab investigation	**Subset of the samples**		**Total**
	Meat	**Feed**	
Heavy metal	60	94	154
Toxin (Aflatoxin)	0	50	50
Antibiotic residue	120	90	210
Total	**180**	**234**	**414**

Table 2. Laboratory tests and associated methods.

Type of Sample	**Name of the Test**	**Name of the Laboratory and Country**	**Method**	**Number of the Sample Subset**
Meat	Antibiotic residue test (TCL, OCT, CIP, ATM)	BAU*, Mymensignh, Bangladesh	TLC, HPLC and ELISA	60
	Heavy metal test (Cd, Cr, Pb)	SGS*, India	S0-IN-MUL-TE-063 by ICP-MS	60
Feed	Antibiotic residue test (TCL, CTC, OCT)	BCSIR*, Bangladesh	HPLC-FLD	92
	Heavy metal test (Cd, Cr, Pb)	SGS*, India	AOAC-ICP-OES/MS	94
	Toxin test (Aflatoxin)	BCSIR*, Bangladesh	HPLC-FLD	50

*BAU, Bangladesh Agricultural University; SGS, Standard Global Services; BCSIR, Bangladesh Council of Scientific and Industrial Research

Table 3. Percentages of positive feed samples having antibiotic residues using HPLC-FLD.

Source of Samples	Positive feed samples for antibiotic residues			
	TCL	**OTC**	**CTC**	**Total Positive**
	n (%)	**n (%)**	**n (%)**	**n (%)**
Registered Companies (n=37)	4 (10.81)	10 (27.03)	2 (5.41)	**16 (43.25)**
Non Registered Companies/Local poultry feed mills (n=33)	0 (0.00)	0 (0.00)	0 (0.00)	**0 (0.00)**
Farmer Produced Feed (n=20)	0 (0.00)	1 (5.00)	0 (0.00)	**1 (5.00)**
Total (N=90)	**4 (4.44)**	**11 (12.22)**	**2 (2.22)**	**17 (18.89)**

TCL-Tetracycline; OTC- Oxy-tetracycline and CTC- Chlortetracycline

Table 4. Positive sample percentages and concentrations of antibiotic residue in poultry meat samples by HPLC (N=60).

HPLC	Positive Sample (%)	Lowest Concentration (ppb)	Highest Concentration (ppb)	Mean (ppb)	MRL[1]* (ppb)
Tetracycline (TCL)	43.33	0	20	0.81	200
Oxytetracycline (OTC)	33.33	0	152	8.67	200
Ciprofloxacin (CIP)	50	0	88	7.18	100
Azythromycin (ATM)	0	0	0	0	0

*MRL, Maximal Residue Limit
[1]Food Safety (Contaminants, Toxins and Harmful Residues) Regulations, 2017

Table 5. Percentages and concentration of positive poultry meat samples having antibiotic residues using HPLC-FLD.

Source of Samples (N=60)	Positive feed samples for antibiotic residues							
	TCL		**OTC**		**CTC**		**ATM**	
	%	**Mean (ppb)**	**%**	**Mean (ppb)**	**%**	**Mean (ppb)**	**%**	**Mean (ppb)**
Contract Farm (n=10)	0	0	10	5.47	70	8.91	0	0
Local Farm (n=25)	64	1.35	48	8.46	52	10.62	0	0
Local Poultry Seller (n=25)	40	0.58	28	10.15	40	2.76	0	0

TCL, Tetracycline; OTC, Oxy-tetracycline and CTC, Chlortetracycline; ATM, Azithromycin

Table 6. Concentrations of Cd, Pb and Cr in poultry feed samples.

Heavy Metals	Positive Sample (%)	Lowest Concentration (mg/kg)	Highest Concentration (mg/kg)	Mean (mg/kg)	MRL[2] (mg/kg)
Cadmium (Cd)	100	0.01	0.21	0.04	0.50
Lead (Pb)	100	0.03	14.65	1.28	5.00
Chromium (Cr)	100	0.05	49.22	2.55	0.00

[2]European Union, Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, 2003

Table 7. Concentrations of Cd, Pb, and Cr in poultry feed samples obtained from different sources.

Source of Feed Samples	Cd		Pb		Cr	
	Positive Sample (%)	Mean (mg/kg)	Positive Sample (%)	Mean (mg/kg)	Positive Sample (%)	Mean (mg/kg)
Registered Feed Companies (n=41)	100	0.033	100	0.46	100	2.78
Non Registered Feed Companies/Local poultry feed mills (n=33)	100	0.053	100	2.47	100	2.61
Farmer Produced (n=20)	100	0.05	100	0.99	100	2.16

4. Conclusions

In this study found that only 18.89% collected poultry feed sample had antibiotic residues which were below the MRL and majority of the positive samples sourced from commercial poultry feed. However, only presence of residues is more than enough for public health concern and increased AMR risk. According to the Govt. law of Bangladesh, indiscriminate use of antibiotics in commercial poultry feed production is very unethical, illegal and punishable crime. In addition, this study also revealed that the mean concentration of heavy metals (Cd and Cr) below the MRL except Pd. There were 6% feed samples had Pd which exceeded MRL and it can be hazardous both for animal and human health. Therefore, this study recommended for the proper maintenance of the antibiotic withdrawal period, increased food safety concern of the civil society and come forward activities of the government to mitigate the food safety challenges.

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Conflict of interest

None to declare.

Authors' contribution

A Atiq Rahman and Dwijendra Lal Mallick planned and deigned the study. Eftesum helped in the collection of samples. Farhana Rahman prepared the map of the study areas. Md Mahbubul Alam and S. M. Lutful Kabir were involved to analyze the samples in the laboratory. Md Murshidul Ahsan wrote the manuscript. AHM Taslima Akhter and S. M. Lutful Kabir finalized the manuscript. All authors have read and approved the final manuscript.

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