



Study on indiscriminate use of antibiotics in poultry feed and residues in broilers of Mymensingh city in Bangladesh

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Abstract

Knowledge, attitude and practice of broiler sellers about the indiscriminate use of antibiotic in poultry feed and antibiotic residue status of broiler in the live bird market of Mymensingh city was investigated. Twenty live poultry selling shops were randomly interviewed and six broilers from each market (5x6= 30) were randomly selected for detection of residual antibiotics in the liver and breast muscle. All the broiler meat sellers were male and the number of participants appeared higher in 21-30 year age group. The proportion of different age groups found to vary significantly ($P<0.01$). Among them, 10% sellers were illiterate, 60% sellers merely cross primary education, 25% sellers had secondary education and 5% sellers reached level of higher education. All sellers were familiar with antibiotics and vitamins. About 57.5% and 22.5% of the broiler sellers had knowledge onto the use of antibiotic as growth promoter. Only 7.5% sellers understood the term antibiotic resistance and 12% had little knowledge on human health hazard following indiscriminate use of antibiotics. All broiler sellers provided commercial feed and water throughout selling period, whereas, 77.5% and 17.5% of them used mixed vitamins and antibiotics in water to prevent unwanted mortality. In terms of adding extra antibiotics in feed, 32.5% of the sellers depended on feed sellers and only 20% of them used prescription provided by the registered veterinarian. The residue of ampicillin, ciprofloxacin and enrofloxacin in liver and meat of broilers were evaluated by using thin layer chromatography (TLC). The highest percentages of antibiotic residues were detected in the birds of Kachijhuli bazar (26.67%) and lowest in Shankipara bazar (13.33%). The highest percentages of antibiotics used in poultry feed was enrofloxacin (46.67%) followed by ciprofloxacin (30.00%) and amoxicillin (23.33%). In addition, amoxicillin plus ciprofloxacin (30%) and ciprofloxacin plus enrofloxacin (43.33%) were commonly found in the liver of broilers. All the broilers had antibiotic residue in their liver and breast meat containing antibiotic residues in 20% cases. Amoxicillin, ciprofloxacin and enrofloxacin were routinely used in poultry feed and the meat sellers had little knowledge about the indiscriminate use of antibiotics in poultry sector. Therefore, community base awareness regarding the discriminate and indiscriminate use of antibiotics in poultry sector is essential, and to evaluate the effect of residual antibiotics in the broilers onto the resistance of common bacterial pathogens living in the gut & systems.

Key words: KAP, antibiotic residues, broiler feed & meat

Introduction

Annually more than 50 billion chickens are raised as a source of protein food, while the present meat and egg production in Bangladesh can meet only 68% and 64% of the national demand (Rahman *et al.*, 2017). To meet the current need, farmers and stakeholders rely greatly on intensive poultry farming which brings usage of vaccines, vitamins and minerals and mostly antibiotics. Antibiotic usage has facilitated the efficient production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality meat and egg. Currently, approximately 80% of all food-producing animals and birds receive medication for part or most of their lives (Lee *et al.*, 2001). Though factors related to farm management help to minimize the use of antibiotics, however, even if well managed, the increased density of livestock or poultry in intensive rearing operations requires an aggressive approach to disease control, which can lead to heavy prophylactic and therapeutic antibiotic use (Tollefson and Miller, 2011). Although these usages have value in terms of more production, unfortunately edible poultry tissues are being contaminated with harmful concentration of drug residues (Donoghue, 1993). In true sense, indiscriminate and injudicious use of drugs make poultry meat unsafe for human consumption if withdrawal period is not maintained accordingly (Lee *et al.*, 2001). The use of antibiotics in food producing animals may result in the presence of residues in foodstuffs of animal origin. Protection of public health against possible harmful effects of veterinary drug residues is a relatively recent preoccupation. The initial intention for adequate consumer protection led to the desire to achieve complete elimination of all traces of drug residues in food commodities. Therefore, animal drugs were initially approved based on a “no residue” tolerance policy, but actually the “zero” tolerance represented the sensitivity of the analytical method used to monitor for drug residues. As analytical methods improved, the “no residue” tolerance was continually being lowered. Ultimately, a policy of

negligible tolerance, based on toxicology data, was developed (Boisseau, 1993).

The earliest screening methods used for detecting antibiotic residues in foods, including milk, were based on the detection of growth inhibition of various bacterial strains. Such methods were based on microbial agar diffusion tests or on the inhibition of acid production by starter organisms. From the 1950s, assays were developed for the testing of tissues, primarily from the existing milk testing procedures (Huber *et al.*, 1969). In 1970 a microbiological test for both detection and identification of antibiotics in meat was described. Few studies have reported antimicrobial residues in livestock products in Bangladesh, (Chowdhury *et al.*, 2015; Sattar *et al.*, 2014), rigorous attempt for screening of drugs residue in marketed animal products is too limited. Besides, there is a lack of field data regarding various knowledge, attitude, and practices of farmers and live bird sellers in wet market that could provide valuable information given the availability of the study. Thus awareness building program regarding discriminate and indiscriminate use of antibiotics, and related health hazard could be outlined. Based on above scenario, our current research is focused on Knowledge, attitude and practice of broiler sellers about the indiscriminate use of antibiotic in poultry feed and antibiotic residue status of broiler in the live bird market.

Materials and Methods

A cross-sectional study was carried out for a period of 1 month at 5 different wet markets in Mymensingh Sadar from 1st October to 30th October 2017 namely Swadeshi Bazar, Notun Bazar, Kachijhuli Bazar, Shankipara Bazar, and Pouroshova Bazar. A total of 20 shops were selected randomly for questionnaire study and sample collection. Each live bird sellers were questioned with a close ended questionnaire. The survey was performed at night around 9.00pm when they were free of customers. During interview, workers

in the live bird market was questioned targeting age of the meat seller, level of education, experience of antibiotic use in poultry feeds, consequences of antibiotics abuses, left over antibiotics in poultry meat and antibiotic resistance microbes etc.

Collection of bird and sample: Altogether 30 live chickens (06 from each market) were collected randomly for this study. Chickens were collected only from those shops whose owners agreed to participate in the survey willingly. After slaughtering, liver and breast muscle were collected in plastic zipper bag from each chicken and stored at -20°C until proceed to extraction procedure.

Antimicrobials extraction: Total extraction procedure was performed following the standard procedure (Popelka et al., 2005). Meat and liver samples from each bird were blend with mortar and pestle. Aliquot sample of 4gm was taken in a falcon tube with phosphate buffer saline (PBS) and homogenized by vortex and protein was precipitated by using 2ml of 30% trichloroacetic acid. (The mixture was centrifuged at 6000rpm for 20mins, supernatant was collected and filtered. Then the supernatant was taken into falcon tube and added equal amount of diethyl ether. The mixture was kept steady in room temperature for 10mins and separated through separating funnel. The upper oily layer was discarded and bottom layer was collected into screw capped vial. Then antibiotic residue detection was done by thin layer chromatography (TLC).

Pointing on TLC plate: Initially for pointing pre-coated TLC plates were cut with scissors according to the shape of TLC tank and pointing was done with the pencil. A line was drawn on plate that was sufficiently high up to touch the solvent. Then with capillary tube pointing of standard solution was done on this line about 2.00cm distances from each other. For multiple spotting (usually three), first spot was dried before adding another one. After drying of spots the plates was then placed in the TLC tank and allowed for running.

Running of TLC: For For better performance continuous development of chromatogram was done. The duration of mobile phase to run was 25mins for each plate placed in the TLC tank. Thus a continuous flow of the mobile phase maintained along with the stationary phase. This technique was performed as used to separate antibiotics from solution (Thangadu et al., 2002).

Examination of chromatogram: The chromatogram was examined under the ultraviolet lamp at 256nm for spots i.e., spot that fluorescence. The outline of the spot was mark with a series of dots using a sharp pencil. The colour of each fluorescent spot was recorded on a separate paper (Thangadu et al., 2002; Abdul-Ghani, 2005).

Detection of Specific Antibiotic Using Standard: Each UV positive sample was compared using known standard drug in the same TLC plate and confirmed by measuring R_f value according to the standard procedure (Al-Ghamdi et al., 2000).

Determination of R_f (Retardation factor) value: To define the relative migration rate of substances under various conditions retardation factor was determined. It is the ratio of distance moved by the substance and distance move by solvent (Abdul-Ghani, 2005). For this, the distance that each spot had travelled from the start line was measured in cm, taken from the center of the spot. Also the distance of the solvent was measured from the start line not from the bottom of the chromatogram. Then calculation of R_f values was carried out using the following equation:

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

Results of all R_f values were recorded on a paper in tabular form.

Interpretation of results: For interpretation, first setting of standard with reference/ pure substances was determined with three repeated times of examination by standard solution. A substance was positively identified in the unknown solution when it behaved

identically as the reference substance (Abdul-Ghani, 2005). Following comparison of two substances (standard & unknown) a sample was identified based on the color (same colour under UV light) and R_f value (same R_f value as those of the reference sample).

Results and Discussion

Results of this study mostly focused onto the level of academics, knowledge, awareness and practice of the meat sealers of live bird market about the use and abuse of antibiotics (Table 1 and 2).

Table 1. Different age group of seller participated in the survey.

Age group (Years)	Frequency	Percentage	P value
<20	8	20	0.001
21-30	14	35	
31-40	11	27.5	
41-50	5	12.5	
50<	2	5	

The highest relative frequency was 14 in age group of 21-30 years, and the lowest was 2 in more than 50 years of age respectively. As shown in Figure 1, about 60, 25, 5, and 10 percentages of participants have had their primary, secondary, higher secondary level of education and illiterate respectively.

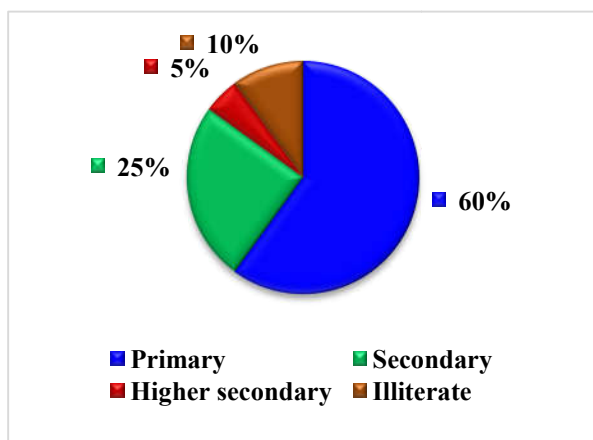


Figure 1. The educational qualification of the meat seller of live bird market of Mymensingh district.

All the participants had knowledge about the use of antibiotics and vitamins in feed but none of them had knowledge about the antibiotic abuse or residues. After explaining the fact, 12.5% could understand about hazard of antibiotics misuse to some extent and 7.5% about antibiotic resistance.

Table 2. Meat seller’s knowledge and awareness regarding the use of antibiotics (N=40) in poultry feeds.

Regarding knowledge	Frequency (percentage)
Antibiotics	40 (100)
Growth promoter	23 (57.5)
Withdrawal period	9 (22.5)
Antibiotics residue	0 (0)
Antibiotic resistance	3 (7.5)
Human health hazards of antibiotics misuse	5 (12.5)
Vitamins	40 (100)
Measures taken to prevent bird mortality	
Antibiotics in water	7 (17.5)
Vitamin supplement	31 (77.5)
Only feed and water	40(100)
Selling in local restaurant at lower cost	36(90)
Purchasing of antibiotics	
Using prescription (Vet)	8 (20)
Without prescription (Feed seller, self)	13 (32.5)
No response	19 (47.5)

The response rate was lower (52.5%) when asked about purchasing of antibiotics. Most of them (32.5%) were purchased antibiotics motivated by feed sellers and self-knowledge. Only 20% of them took assistance from registered veterinarians. The percentage of respondents who mixed antibiotics and vitamins in water respectively was 17.5% and 77.5% while all of them provided commercial broiler feed and water throughout the selling period. The antibiotics residue in

meat and liver was, therefore, evaluated in the live bird market of Mymensingh city (Table 3).

Table 3: Area-wise sampling of Mymensingh district.

Area	Source	Positive	Negative
Swadeshi Bazar	Liver	6	0
	Muscle	1	5
Notun Bazar	Liver	6	0
	Muscle	1	5
Kachijhuli Bazar	Liver	6	0
	Muscle	1	5
Shankipara Bazar	Liver	6	0
	Muscle	2	4
Pouroshova Bazar	Liver	6	0
	Muscle	1	5

The table 3 represents the frequencies of positive and negative samples from different areas (6 samples from each area). Antibiotic residues either in meat or liver was seen all the live bird market investigated.

As shown in Figure 2 and Table 4, The residue of enrofloxacin appeared higher (47%) in the birds of meat market followed by ciprofloxacin (30.00%) and amoxicillin (23.33%). One sample t test showed the p value was significant ($p > 0.017$) among groups of antibiotics tested.

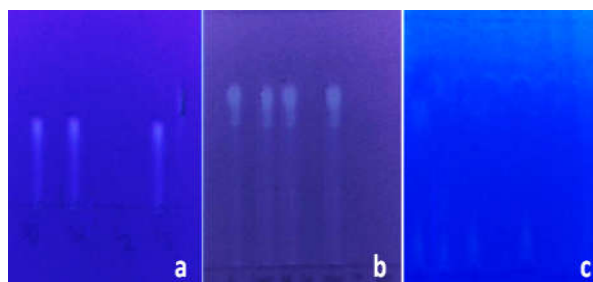


Figure 2. Thin layer chromatography (TLC) of the extracts from liver/ meat on silica gel to identify the residue of Enrofloxacin (a), Amoxicillin (b) and Ciprofloxacin (c). The antibiotic residue in liver appeared higher than the muscle samples (Table 4).

Table 4. Various antibiotic residues found in the liver and muscle samples obtained from live bird market of Mymensingh city.

Antibiotics	Positive Sample	Percentages +ve	Remarks
Amoxicillin	7	23.33	None of the samples of live bird market appeared free from antibiotics as investigated
Ciprofloxacin	9	30.00	
Enrofloxacin	14	47.00	
Amoxicillin plus Ciprofloxacin	9	30.00	
Enrofloxacin plus Ciprofloxacin	13	43.33	

About 100 % of liver and 20 % of muscle samples found to contain antibiotic residue. The unpaired one sample t test revealed that the difference was statistically significant ($p > 0.005$) between liver and muscle samples (Table 5).

Table 5. Proportion of antibiotic residue positive samples from different sources.

Organ	Positive (%)	Negative (%)	Remarks
Liver (30)	30 (100)	00 (0.00)	Liver containing more antibiotic residue than muscle
Muscle (30)	6 (20)	24 (80)	

All the six live bird markets of Mymensingh city containing antibiotic residues in their liver as presented in Table 6. The highest percentages (26.67%) of liver containing antibiotic residue of the birds of Kachijhuli Bazar and least percentages (13.33%) in the broilers of Shankipara bazaar was seen. All the liver samples containing antibiotic residues along or in combination of antibiotics in the broilers of live bird market, Mymensingh city.

Table 6. Proportion of positive and negative samples investigated to analyze the antibiotic residue in the liver of live bird markets of Mymensingh city.

Areas (6 birds from each location)	Single antibiotic residue detected (percentages) in the liver			
	Amoxicillin	Ciprofloxacin	Enrofloxacin	Over all
Swadeshi Bazar	2 (6.67)	2 (6.67)	2 (6.67)	6 (20.00)
Notun Bazar	1 (3.33)	2 (6.67)	3 (10.00)	6 (20.00)
Kachijhuli Bazar	1 (3.33)	3 (10.00)	4 (13.33)	8 (26.67)
Shankipara Bazar	1 (3.33)	1 (3.33)	2 (6.67)	4 (13.33)
Pouroshova Bazar	2 (6.67)	1 (6.67)	3 (10.00)	6 (20.00)
Total	7 (23.33)	9 (30.00)	14 (46.67)	30 (100)

Discussion

Indiscriminate use of antibiotics in poultry production now-a-days imposing potential health risks for the consumers. The live bird sellers has been playing pivotal role towards injudicious use of antibiotics in the poultry feed and water to reduce morbidity and mortality of broilers thus bringing harmful consequences for consumers. Majority of the sellers were in middle aged group while a small percentage of them were in older age group. That means middle aged male are more involved in this profession than any other. On the other hand, majority of them had received only primary education whereas a little proportion had studied up to higher secondary. Unfortunately, a considerable portion was illiterate also. Altogether, these scenarios reveal that they are more prone to take uneducated guess and rely on mere advice and inclination rather seeking help from a registered veterinarian.

This picture was also portrayed with the higher percentage of ignorance of the meat sellers, consumers and market regulators in terms of antibiotic abuse, antibiotic residue, antibiotic resistance, withdrawal period, and health hazard related to antibiotic misuse. In contrast, they were well aware of the terms like antibiotics and vitamins. Besides there are also some circumstances arise when poultry birds remain unsold for 2 days, then to prevent mortality they add

antibiotics powder with the feed and water. Their extent of ignorance and uneducated perception was also pictured in case of lower percentage of prescription by registered veterinarian. Besides, ignorantly or intentionally, the mixing of antibiotics with water was revealed by some sellers but they were unable to realize it unnecessary and harmful. Above all, it is at least justified that they supply feed and water throughout selling period and sometimes sell the sick birds at lower price in local market as the profit is too little to sacrifice a smaller portion of live birds due to morbidity and mortality. Almost Similar findings were described elsewhere (Lee *et al.*, 2001).

It is well observed that the middle age group of people is the most prevalent in live bird markets. Because they are energetic, enthusiastic, and inquisitive learner they can be easily trained and made aware of the harmful effects of antibiotics misuse and their consequences to the environment. This big challenge can be overcome by an implementing a well-structured awareness building program targeting the root level uneducated sellers. It is not wise to just only give importance to the registered veterinarians. The knowledge is an important factor to overcome this problem as described earlier (Chang *et al.*, 2015).

The Kachijhuli bazar of Kotoali Thana, Mymensingh, found to contain highest percentages of antibiotics (26.67%) in their liver and lowest percentages

(13.33%) of birds displayed antibiotic residue in broilers of Shankipara bazar. Broilers of all other markets containing antibiotic residues in their liver at closer range (about 20%). Mainly these markets were considered as the concentration zone of the live birds coming from all around the district. The competition is higher among the sellers and the price is also higher than other markets. So, the sellers might injudiciously mix the antibiotics purchased from the local pharmacy in order to exhibit their bird more healthy than others. Besides, they might fear the reputation loss if the birds morbidity or mortality is regular. The least positive samples was observed in the distant market of the district which is situated just beside the government quarter where there is a perception of the sellers about selling birds to the government officials. The price was also moderate and fixed from shop to shop. So, the placement of the market also plays an important role in this aspect.

Out of three commonly used antibiotics, enrofloxacin found in half of the samples tested (n=14) which is higher than some published articles, where 17 to 45% samples reported to contain Enrofloxacin (Elamanama and Albayoumi, 2016). Beside enrofloxacin, ciprofloxacin was also found in majority of the samples (n=09); these two antibiotics appeared as the most prevalent antibiotic residue in the poultry. Earlier reports (Habib *et al.*, 2006; Chowdhury *et al.*, 2015) described similar antibiotic residues in liver and muscle samples of live bird market and ciprofloxacin, and enrofloxacin were the most reportable antibiotics. The reason behind the excessive use of these two antibiotics is the effectiveness against bacterial infectivity. Amoxicillin residue was the least antibiotic found in tested samples. The result was compatible with other studies (Krishnasamy, 2015) who reported 27.40%, 38.90% and 28.15% positivity for amoxicillin, ciprofloxacin, and enrofloxacin respectively. The percentage of individual antibiotic residues found in the liver and meat is surprising. The admixture of antibiotics found in the liver and meat were much higher than individual antibiotics, indicated that more

than one antibiotic are used by the meat seller to prevent regular morbidity and mortality of broilers. All of the liver found to contain antibiotic residues but muscle tissues containing antibiotic in 20% cases. Liver is the main metabolic organ where drugs are metabolized. So, where or not the residues are found in other organs given that recommended doses of antibiotics is provided, liver will surely deposit the residue. Similar results were reported earlier by Gouvea *et al.*, (2015); Chowdhury *et al.*, (2015); Sattar *et al.*, (2014). However, antibiotic residues are not recommended to produce safe food of bird's origin (Mund *et al.*, 2017).

Conclusions

In Bangladesh significant amount of antibiotics used in poultry farming but concerns have been raised that tissues of food animals contaminated with antimicrobial residues may cause adverse side effects in consumers. Our study confirmed the presence of antibiotic residues in poultry meat samples collected from different Union of Mymensingh Sadar. This may pose potential hazard to public health. Thus, it is recommended that rules should be taken to ensure maintaining proper withdrawal periods before marketing and drug control in veterinary use. The respective authorities should take necessary steps to increase awareness among respective community. It needs to train up poultry meat sellers about the abuse of antibiotics in poultry feeds and its consequences on consumers. A large number of samples from wider sources need to be analyses to know the actual concentration of residual antibiotics with identify drug resistance bacterial load in the boilers if any to design future strategy to provide safe food from broilers origin.

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