

**ANTIMICROBIAL RESIDUES IN BROILER POULTRY AND FISH AND ITS SIGNIFICANCE IN PUBLIC HEALTH IN CHITTAGONG**

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Roll No. 0213/02

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**A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Pharmacology**

**Department of Physiology, Biochemistry and Pharmacology**

**Faculty of Veterinary Medicine**

**Chittagong Veterinary and Animal Sciences University**

**Chittagong-4225, Bangladesh**

**December 2014**

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**This is to certify that we have examined the above Master’s thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made**

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**December 2014**



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# List of abbreviation

|  |  |
| --- | --- |
| **Abbreviation** | **Elaboration** |
| **%** | Percentage |
| **°C** | Degree centigrade |
| **ADI** | Acceptable Daily Intake |
| **β-lactam** | Beta-lactam |
| **CMC** | Chittagong metropolitan city |
| **CI** | Confidence interval |
| **CVASU** | Chittagong Veterinary and Animal Sciences University |
| **EC** | European Commission |
| **EU** | European Union |
| **FDA** | Food and Drug Administration |
| **g** | Gram |
| **HPLC** | High Performance Liquid Chromatography |
| **kg** | Kilogram |
| **km** | Kilo Meter |
| **MRL** | Maximum Residue Limit |
| **mg** | Milligram |
| **µg** | Microgram |
| **ml** | Mille Liter |
| **µl** | Micro liter |
| **µm** | Micrometer |
| **ng** | Nanogram |
| **NO(A)EL** | No Observed (Adverse) Effect Level |
| **OTC** | Oxytetracycline |
| **TLC** | Thin Layer Chromatography |
| **UV** | Ultra Violet |
| **UHPLC**  **VRC** | Ultra High Performance Liquid Chromatography  Veterinary Residue Committee |
| **WHO** | World Health Organization |

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# Summary

Food safety with regard to human health is a great concern all over the world. The existence of antimicrobial residues at variable concentrations in edible tissues of poultry and fish has been reported to be due to indiscriminate and overuses, not maintaining withdrawal periods etc and consequences of antimicrobial residues on human body are enormous as reported such as multi-drug resistant, hypersensitivity reactions, suppression of immunity or even cancer. However, a systematic study of antimicrobial residues in broiler chickens and fishes has not been attempted earlier in Bangladesh. Therefore, the present repeated cross-sectional study in 2014 was carried out to assess the status of antimicrobial residues in broiler chickens and fishes in Chittagong and evaluate the potential public health risk. 82 broiler chickens and 232 fish flesh (September 41 and 116; November 41 and 116) were purchased and sampled. Outlets (poultry and fish) belonging to five different markets were randomly selected before purchasing and sampling broiler chickens and fishes.

A total of 478 samples (246 poultry organs and 232 fleshes of fishes) were evaluated by the Thin Layer Chromatography (TLC) and 18 TLC positive samples were further evaluated by Ultra High Performance Liquid Chromatography (UHPLC) methods for quantifying concentration of selective antimicrobial residues. Descriptive, summary statistics and Fisher’s exact test was performed on the data generated from the study.

The overall prevalence regardless of antimicrobial types was 84% in broiler chickens (43.9% in September and 37% in November). The prevalence of antimicrobial residues in poultry organs was 39.4% and liver had significantly higher prevalence of residues (54.5%) than that of thigh (24.2%) or breast muscle (21.2%). The prevalence of antimicrobial residues in broiler chicken in September and in November was 26.8% and 22.0% respectively, for ciprofloxacin (*p*>0.05).

The overall prevalence of antimicrobial residues in fishes was 13.8% (8.6% in September and 5.2% in November). Residues of oxytetracycline and amoxicillin were detected (10.3% and 6.9% respectively). Irrespective of months Rui had the highest prevalence of residues (10.3-13.8% oxytetracycline; 3.4-10.3% amoxicillin). Regardless of antimicrobial types poultry vendor categories (wholesaler: 50%, retailer: 83.3%, and both: 92.9%) varied significantly (*p*=0.002).

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Average concentration of amoxicillin residues ranged from 75.8-111.8 µg/kg in poultry organs and 95.4-95.9 µg/kg in fish (Rui and Koi), whereas average concentration of ciprofloxacin residues ranged from 133-269.2 µg/kg in poultry organs in this study. These values crossed the maximum reference values (amoxicillin 50 µg/kg and ciprofloxacin 100 µg/kg), indicating potential public health risk, however, washing and cooking treatment may reduce the public health risk. Indiscriminate use of antimicrobials with their withdrawal period in poultry and fish should strictly be maintained before human consumption.

***Key words****: Antimicrobials, Residues, Broiler chicken, Fish, Chittagong*

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# Chapter-1: Introduction

Antimicrobial residues in animal-derived foodstuffs with regard to human health are great concern across the world (Chowdhury et al., 2009). Antimicrobial residue is the small amount of an antimicrobial drug or its breakdown product(s) that remains in or on an agricultural product following treatment with that antimicrobial (Botsoglou and Fletouris, 2001). After administration some drugs are quickly excreted from the animal or fish body, whereas others are not readily metabolized or excreted, for example streptomycin and therefore these drugs or their metabolites remain in different organs of animal body or fish as residues for a long time are termed as residues (Booth, 1973; Dipeolu and Alonge, 2002; Olatoye and Basiru, 2013). Several classes of antimicrobials are commonly used in large quantity in fish industry especially in developing countries; some of them like oxytetracycline, sulfonamide etc are often non-biodegradable (Rasul, 2012) and persist in the aquatic environment as residues. When they are mixed with fish feeds, residues may be deposited in meat (Cabello, 2006; Shamsuzzaman and Biswas, 2012).

Poultry farmers in Bangladesh are very much inclined in using antimicrobials to their flocks to prevent infectious diseases and mortality, and other purposes with or without consultation of registered veterinarians as drugs are easily accessible to them without vet prescription from different drug stores. Fish farmers, in particular, are very much reluctant to have professional advice on use of antimicrobials for fish farming, rather they rely on feed sellers, and veterinary representatives etc. and sometimes choose by their own (Anka et al., 2013). Neither trained professionals (Veterinarians and Fishery Officers) nor poultry and fish farmers are well-aware about the knowledge of drug withdrawal period and the importance of maintaining drug withdrawal period in this country. Furthermore, only limited pharmaceutical companies (national and international) mention the withdrawal period of antimicrobials or other drugs in the drug handout and packet (Personal observation). Therefore, those indiscriminate and overuses of antimicrobials, violations of withdrawal periods, improper maintenance of treatment records, and failure to identify treated animals and fishes prior to human consumption could leave high concentration of residues in edible tissues of poultry and fishes (Mumtazet al.,2000; Dipeolu and Alonge, 2002; Faruk et al., 2008; Nisha, 2008; Chowdhury et al., 2009).

Fishes in open water bodies are continuously exposed to antimicrobials or its active metabolites through medical and veterinary effluents, as well as livestock farm effluents in this country, supported by number of earlier studies (Milstein, 1996; Schulman et al., 2002; Sanderson et al., 2003; Hossain et al., 2013), which can develop multi-drug resistant organisms and also leave antimicrobial residues in tissues of fishes, which in turn are transmitted to humans through fish consumption and thereby posing public health threat (Shamsuzzaman and Biswas, 2012). Human beings that consume animal products or fishes with high concentration or continuous exposure of low dose of antimicrobial residue make common organisms to be multi-drug resistant, hypersensitivity reactions, allergic reactions, suppression of immunity and cancer in human beings (Sutiak et al.,2000; [Tendencia and De La Pena, 2001](http://scialert.net/fulltext/?doi=ajava.2013.401.408&org=10#204745_ja); Holmstrom et al., 2003; Nonga et al., 2009; Olatoye and Basiru, 2013). Hence, a thorough investigation of antimicrobial residues in commercial poultry and fishes was needed to explore the true estimate of antimicrobial residues for an effective intervention to save the poultry and fish, and public health.

Estimation of the prevalence of antimicrobial residues in livestock and poultry products, as well as fish has been documented across the world and overall prevalence estimates in poultry include 14% for penicillin, 8-52% for tetracycline, 28% for oxytetracycline, 4% for streptomycin, 12.5-50% for sulfonamide, 28% for sulphadiazine in poultry products (Diez et al., 2002; Salem, 2004; Reyes-Herrera et al., 2005; Sareef et al., 2009; Salama et al., 2011). The overall prevalence estimates of antimicrobial residues include 34.4% for oxytetracycline in African catfish in Nigeria (Olatoye and Basiru, 2013), 30% for tetracycline in rainbow trout, 19% for sulfonamide and 7% for chloramphenicol in rainbow trout in Iran (Mahmoudi et al., 2014) and 5.8% for antibiotics as qualitative analysis using rapid microbial test kit (Euroclone, Italy) in farm fish (red tilapia *Oreachromis sp*. red hybrids, keli *Clarias spp*. and patin *Pangasius sutchii*) (Bakar et al., 2010).

Some non-systematic studies in Bangladesh estimated prevalence of antimicrobial residues as follows 37.5% for tetracycline in liver samples of poultry followed by 7.5% for ciprofloxacin and 5% for sulfonamide (in poultry, Mahmud, 2012; Karim, 2013). Literature on prevalence estimates of antimicrobial residues in fish is not readily available in this country. However, a sporadic study conducted in Dhaka fish market revealed 87% positive samples to oxytetracycline residues in different fish samples (pangas, rui and tilapia fish) (Rasul, 2012). It is therefore, a repeated cross-sectional and systematic study was required to estimate true prevalence of antimicrobial residues in commercial broiler chickens and fishes. Considering the above background the present study was performed to meet the following specific objectives:

* To measure the selective antimicrobial residues in broiler chickens and fishes of retail and wholesale poultry meat and fish markets belonging to Chittagong Metropolitan City (CMC)
* To determine the concentration of antimicrobial residues in broiler chickens and fishes of retail and wholesale poultry meat and fish markets of CMC

**Anticipated outcomes**

1. Determination of commonly used antimicrobial residues in broiler poultry and fish by TLC
2. Estimation of the concentration of antimicrobial residues in broiler chicken and fish by UHPLC
3. Identifying the seasonal pattern of antimicrobial residues in broiler chicken and fish
4. Awareness about the potential impact of antimicrobial residues in broiler chicken and fish on public health

# Chapter-2: Review of Literature

Pertinent literatures on reasons of antimicrobial residues in animal products and associated factors, importance of drug withdrawal period, qualitative and quantitative status of antimicrobial residues in animal products and possible public health risks have been reviewed. The main purpose of this chapter is to provide up-to-date information concerning the research work which is addressed here. Important information related to the present study is presented below under the following headings and sub-headings.

## 2.1. Antimicrobial residues

The term “Antimicrobial residue” is the small amount of an antimicrobial drug or its breakdown product(s) that remains in or on an agricultural product following treatment with that antimicrobial (Botsoglou and Fletouris, 2001).

## 2.2. Reasons for existence of antimicrobial residues in edible organs of poultry and fish

Commercial poultry industry and fish farming are the growing industry in Bangladesh and the use of antimicrobials for different purposes is a common practice in this industry (Hasan et al., 2011; Hossain et al., 2013; Parvej, 2013). Globally antimicrobials are commonly used for the treatment, prevention of infectious diseases and for promotion of growth in food-producing livestock, poultry and fish since their discovery (Donoghue, 2003; Olatoye and Basiru, 2013). However, many industrial based livestock producing countries for example, India, China and South Africa have been using antimicrobials in a limited scale (Darwish et al., 2013, Nonga et al., 2009; Nisha, 2008; Sarmah et al., 2006). Antimicrobials are also used to improve performance in growth and feed efficiency, to synchronize or control reproductive cycle and breeding performance in poultry (Gaudin et al., 2004; Nisha, 2008). In particular, broiler chickens are often grown actively with antimicrobials in feed to attain maximum weight within a short period of time (Nonga et al., 2009).

Indiscriminate and overuses of antimicrobials, violations of withdrawal periods, improper maintenance of treatment records, and failure to identify treated animals before slaughtered could leave high concentrations of residues in edible tissues and tissue by-products both in poultry and fish (Mumtazet al.,2000; Dipeolu and Alonge, 2002; Pena et al., 2007; Nisha, 2008; Chowdhury et al, 2009; Olatoye and Basiru, 2013).

Drug residues can also occur as a result of improper use of a licensed product or through the illegal use of unlicensed substances. Extra label dosages and use of drugs which have not been approved for the species in question may lead to violative residues (Papich et al., 1993; Kaneene and Miller, 1997; Higgins et al., 1999). Locally produced drugs with easy availability and the affordable price rate such as ampicillin, amoxicillin, erythromycin, quinolone, neomycin, kanamycin, pefloxacin and sulfamethoxazole could encourage using them more commonly than older drugs (Parvej, 2013). Even retailers use additional antimicrobials with feed in the vendor of poultry market.

### 2.2.1. Description and importance of drug withdrawal period

The term “Drug withdrawal period” is often used more broadly to describe the time needed after drug administration to any food producing animal where drug residue may be found in marketed meats, eggs, organs, or other edible products (Eiichi et al., 2006).

To ensure that drug residues have declined to a safe concentration following the use of drugs in animals, a specified period of drug withdrawal must be observed prior to providing any products for human consumption. It is the time which passes between the last dose given to the animal and the time when the concentration of residues in the tissues: muscle, liver, kidney, skin/fat or products milk, eggs, honey was lower than or equal to the MRL (Cholas, 1976; Nouws and Ziv, 1978; Jackson, 1980).

Table 1. Withdrawal periods of different antimicrobials in poultry (Rana, 1988; Maqbool, 1988; Calnek et al., 1991; Nawaz et al, 1996; Mumtaz et al., 2000; Bell and Weaver, 2002)

|  |  |
| --- | --- |
| **Antimicrobial** **types** | **Withdrawal period (Days)** |
| Amoxicillin | 5 |
| Oxytetracycline | 7 |
| Ciprofloxacin | 6 |
| Trimethoprim | 10 |
| Sulphaquinoxaline | 10 |
| Sulphachloropyrazine | 5 |
| Sulphadimethoxine | 5 |
| Erythromycin | 2 |
| Enrofloxacin | 2 |

## 2.3. Prevalence of antimicrobial residues (in poultry and fish)

The overall prevalence estimates in poultry all over the world include 14% for penicillin, 8-52% for tetracycline, 28% for oxytetracycline, 4% for streptomycin, 12.5-50% for sulfonamide, 28% for sulphadiazine in poultry products (Diez et al., 2002; Salem, 2004; Reyes-Herrera et al., 2005; Sareef et al., 2009; Salama et al., 2011). The overall prevalence estimates of antimicrobial residues include 34.4% for oxytetracycline in African catfish in Nigeria (Olatoye and Basiru, 2013), 30% for tetracycline in rainbow trout, 19% for sulfonamide and 7% for chloramphenicol in rainbow trout in Iran (Mahmoudi et al., 2014) and 5.8% for antibiotics as qualitative analysis using rapid microbial test kit (Euroclone, Italy) in farm fish (red tilapia *Oreachromis sp*. red hybrids, keli *Clarias spp*. and patin *Pangasius sutchii*) (Bakar et al., 2010).

In Bangladesh some non-systematic and cross-sectional studies estimated prevalence of antimicrobial residues as follows 37.5% for tetracycline in liver samples of poultry followed by 7.5% for ciprofloxacin and 5% for sulfonamide (in poultry) (Mahmud, 2012; Karim, 2013). Literature on prevalence estimates of antimicrobial residues in fish is not readily available in Bangladesh. However, a sporadic study conducted in Dhaka fish market revealed 87% positive samples to oxytetracycline residues in different fish samples (pangus *Pangasius hyphthalamus*, ruhit *Labeo rohita* and tilapia *Orechromis nilotica*) (Rasul, 2012).

Table 2. Prevalence of antimicrobial residue in according to previous studies in broiler chickens in Chittagong, Bangladesh

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Authors** | **Antimicrobials** | **Raw tissue sample** | | |
| **Liver**  **N (%)** | **Thigh muscle**  **N (%)** | **Breast muscle**  **N (%)** |
| Islam (2009) | Tetracycline | 24 (48) | 10 (20) | 13 (26) |
| Ciprofloxacin | 23 (46) | 17 (34) | 15 (30) |
| Enrofloxacin | 20 (40) | 12 (24) | 10 (20) |
| Amoxicillin | 21 (42) | 13 (26) | 11 (22) |
| Sattar et al. (2014) | Tetracycline | 24 (48) | 10 (20) | 12 (24) |
| Ciprofloxacin | 22 (44) | 17 (34) | 15 (30) |
| Enrofloxacin | 20 (40) | 11 (22) | 9 (18) |
| Amoxicillin | 21 (42) | 13 (26) | 11 (22) |
| Mahmud (2013) | Tetracycline | 15 (38) | 15 (38) | 15 (38) |
| Ciprofloxacin | 3 (8) | 3 (8) | 3 (8) |
| Sulfonamide | 2 (5) | 2 (5) | 2 (5) |
| Amoxicillin | 4 (10) | 4 (10) | 4 (8) |
| Karim (2013) | Ciprofloxacin | 4 (3.3) | 2 (1.6) | 2 (1.6) |
| Amoxicillin | 1 (0.8) | 1 (0.8) | 1 (0.8) |
| Sulfonamide | 1 (0.8) | 1 (0.8) | 1 (0.8) |
| Oxytetracycline | 1 (0.8) | 2 (1.6) | 2 (1.6) |
| Parvej (2013) | Ciprofloxacin | 7 (18) | 3 (8) | 5 (13) |
| Amoxicillin | 3 (8) | 3 (8) | 2 (5) |
| Sulfonamide | 3 (8) | 1 (3) | 2 (5) |
| Tetracycline | 4 (10) | 2 (5) | 1 (3) |
| Doxycycline | 1 (3) | 0 | 1 (3) |

## 2.4. Concentration of antimicrobial residues (including residue limits and acceptable dietary intake of residues) (Poultry and fish)

Concentration levels of antimicrobial residues in livestock and poultry products, as well as in fish has also been studied worldwide including Bangladesh, and generally there is evidence that concentrations of amoxicillin (50 µg/kg), ampicillin (50 µg/kg), tetracycline (100-148 µg/kg), sulfonamides (100 µg/kg) and gentamycin (750 µg/kg) routinely exceed upper threshold levels in muscle tissues of poultry **(**EC, 1998; Diez et al., 2002; Salem, 2004; Parvej, 2013). A study in Pakistan found the seasonal variation of concentration of quinolone in poultry products (89 µg/kg in liver, 95 µg/kg in kidney and 54 µg/kg in muscle during summer versus 70µg/kg in liver, 57 µg/kg in kidney and 50 µg/kg in muscle during winter) (Naeem et al., 2006). This seasonal variation is supported by another study of qualitative assessment of antimicrobial residues in poultry product in Bulgaria (33% in kidney and 6% in liver during summer versus 11% in kidney and 3% in liver during winter) (Pavlov et al., 2008).

Similar to poultry, oxytetracycline at concentration range between 234.3-987.5 μg/kg in the liver and 22.5-553.2 μg/kg in fillet of African catfish respectively, have been reported in Nigeria (Olatoye and Basiru, 2013). The estimated concentration of oxytetracycline, chloramphenicol and sulfonamide residues in muscle samples of rainbow trough fish have been reported to be average 8.4 ng/g, average 0.2 ng/g, average 3.9 ng/g, respectively in Iran (Mahmoudi et al., 2014).

In pangas (*Pangasius hyphthalamus*), chloramphenicol found in lowest concentration (0.1 μg/kg) than other fish and highest concentration (0.5 μg/kg) was recorded in Rui (*Labeo rohita*) fish in Bangladesh (Bakar et al., 2013).

Some non systemic cross-sectional studies in Chittagong (Bangladesh) conducted for the estimation of concentration level of antimicrobial residues in poultry and evident that ciprofloxacin regardless of organ type ranges from 193-681 μg/kg (Khan, 2014) and 140-520 μg/kg (Parvej, 2013). Similar to ciprofloxacin, concentration of amoxicillin found 170-670 μg/kg (Mahmud, 2012; Karim, 2013; Khan, 2014), 16.9-820 μg/kg (Parvej, 2013; Sattar et al., 2014). Cross-sectional investigations of antimicrobial residues in poultry and livestock have been performed in Bangladesh, but no systematic investigations have been conducted yet. No study with proper sampling calculation has previously been performed.

## 2.5. Determinants that considered as safety margin of antimicrobial residues

No observed (adverse) effect level [NO(A)EL] is the marker of safety evaluation of veterinary drugs. This level is the basis for calculating an acceptable daily intake (ADI). After an ADI has been determined, MRLs are determined for various drugs in food products.

Table 3. Acceptable dietary limits of antimicrobial residues in livestock and poultry products (Fitzpatrick et al., 1995; EC, 2001; EMEA, 2004)

|  |  |  |
| --- | --- | --- |
| **Antimicrobial types** | **Minimum (µg/kg)** | **Maximum (µg/kg)** |
| Amoxicillin | 4 | 40 |
| Tetracycline | 15 | 100 |
| Oxytetracycline | 15 | 100 |
| Chlortetracycline | 15 | 100 |
| Sulfonamides | 25 | 100 |
| Trimethoprim | 8 | 50 |
| Erythromycin | 12 | 40 |
| Quinolones | 47 | 147 |

**Table 4. Maximum Residue Limit for different antimicrobials in food producing animals (including fish) (**Commission Regulation (EU) No 37/2010)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Pharmacologically active substances** | **Animal species** | **MRL**  **(µg/kg)** | **Target tissue** | **Pharmacologically active substances** | **Animal species** | **MRL**  **(µg/kg)** | **Target tissue** |
| Amoxicillin and Ampicillin | All food producing species | 50 | Muscle | Enrofloxacin | All food producing species | 100 | Muscle |
| 50 | Fat | 100 | Fat |
| 50 | Liver | 200 | Liver |
| 50 | Kidney | 200 | Kidney |
| 4 | Milk | 100 | Milk |
| Chlortetracycline,  Oxytetracycline and Tetracycline | Do | 100 | Muscle | Erythromycin | Do | 200 | Muscle |
| 300 | Liver | 200 | Fat |
| 600 | Kidney | 200 | Liver |
| 100 | Milk | 200 | Kidney |
| 200 | Eggs | 40 | Milk |
| Trimethoprim | Do | 50 | Muscle | 150 | Eggs |
| 50 | Fat | Streptomycin | All ruminants, porcine, rabbit etc | 500 | Muscle |
| 50 | Liver | 500 | Fat |
| 50 | Kidney | 500 | Liver |
| 50 | Milk | 1000 | Kidney |

MRL: Maximum Residue Limit

## 2.6. Public health consequences of antimicrobial residues

There are two major areas of concern over the presence of residues of antimicrobials in animal-derived foodstuffs with regard to human health. The first is allergic reaction that has been evidenced by the residues of β-lactams, aminoglycosides, sulfonamides and tetracycline (Darwish et al., 2013) and the second is development of antibiotic resistance in the gut bacteria of human (Chowdhury et al.,2009). There is evidence that resistance in some human enteric pathogens has arisen because of transfer of resistant bacteria or resistance genes from animals and fish to people via the food chain (Barton, 2000; Olatoye and Basiru, 2013). Antimicrobial resistance has been recognized as a global health problem. It has now been escalated by major world health organizations to one of the top health challenges facing the twenty-first century (FDA, 2000; CDC, 2010).

Indiscriminate use of aqua drugs and chemicals causes problems like drug resistance, tissue residues, adverse effect on species biodiversity etc ultimately affect the cultured species, wild local species, human and environment (Tendencia and De La Pena, 2001; Hossain et al., 2013).

Some antibiotics are directly toxic, e.g. chloramphenicol which cause fatal aplastic anemia, while allergic reactions and toxic side effects may have fatal consequences in human (Popelka et al., 2005). Consumer of animal products and fish with high concentration or continuous exposures of low dose of antimicrobial residues are subjected to multi-drug resistance, hypersensitivity reactions, immune suppression, toxic effects or even cancer (Woodward, 1991; Donoghue, 2003; WHO, 2006; Nonga et al., 2009, Olatoye and Basiru, 2013).

## 2.7. Types of studies of antimicrobial residues conducted in poultry and fish so far

The studies so far conducted in different parts of the world and Bangladesh to assess the status of antimicrobial residues in poultry have predominantly been cross-sectional and sporadic in nature (Diez et al., 2002; Salem, 2004; Reyes-Herrera et al., 2005; Sareef et al., 2009; Salama et al., 2011; Bakar et al., 2010; Olatoye and Basiru, 2013; Mahmoudi et al., 2014) and in Bangladesh (Rasul, 2012; Bakar et al., 2013; Karim, 2013; Mahmud, 2013; Parvej, 2013; Sattar et al., 2014). It is therefore necessary to conduct a systematic repeated cross-sectional study to estimate status of antimicrobial residues in broiler chicken and fish.

## 2.8. Diagnostic techniques to detect antimicrobial residues

Microbiological, immuno-enzymatic and analytic organo-chemical methods, including TLC and HPLC are commonly used techniques to detect antimicrobial residues in tissues of food producing animals (Shareef et al., 2009). However, performance in terms of specificity and sensitivity of different diagnostic techniques in detecting residues is somewhat variable (Diez et al., 2002; Pena et al., 2007). Therefore, this study has been used both TLC and UHPLC for the estimation of antimicrobial residues in poultry and fish.

## 2.9. Conclusion

According to literature cited in this chapter it is clearly apparent that the high concentration of antimicrobial residues in edible tissues and tissue by-products both in poultry and fish exists due to indiscriminate and overuses of antimicrobials, and not maintaining withdrawal periods before consumption. Prevalence of antimicrobial residues such as penicillin (14%), oxytetracycline (28%), streptomycin (4%) and sulfonamide (12.5-50%) in poultry all over the world whereas in Bangladesh tetracycline (37.5%), ciprofloxacin (7.5%), sulfonamide (5%) were estimated. The overall prevalence regardless of type of fish were detected in different antibiotics such as oxytetracycline (34.4%), tetracycline (30%), sulfonamide (19%) and chloramphenicol (7%) in different countries whereas only oxytetracycline (87%) in Bangladesh. In poultry concentration of different antibiotics such as amoxicillin (50 µg/kg), ampicillin (50 µg/kg), tetracycline (100-148 µg/kg), sulfonamides (100 µg/kg) and gentamycin (750 µg/kg) were found all over the world including Bangladesh. Similar to poultry, in fish has also estimated oxytetracycline (234.3-987.5 µg/kg), sulfonamide (3.9 ng/g) and chloramphenicol (0.5 µg/kg). Allergic reaction, multi-drug resistance, immune suppression, fatal aplastic anemia, or even cancer is the public health concern of antimicrobial residues. Studies so far conducted all over the world including Bangladesh are cross-sectional and non systemic in nature.

# Chapter-3: Materials and Methods

# 3. Materials and Methods

## 3.1. Description of study area and markets

Chittagong district is located in the south-eastern region of [Bangladesh](http://en.wikipedia.org/wiki/Bangladesh). Chittagong is the second most populous city and main [seaport](http://en.wikipedia.org/wiki/Seaport) of Bangladesh. Chittagong Metropolitan city (CMC) has a total area of 168.07 square kilometers. The city is located at [22°22′0″N and 91°48′0″E](http://tools.wmflabs.org/geohack/geohack.php?pagename=Chittagong&params=22_22_0_N_91_48_0_E_type:city_region:BD) on the banks of the [Karnaphuli River](http://en.wikipedia.org/wiki/Karnaphuli_River). The CMC has a population of four million peoples. It has a [tropical monsoon climate](http://en.wikipedia.org/wiki/Tropical_monsoon_climate) (<http://en.wikipedia.org/wiki/Chittagong>).

The city wet markets are the gateway of poultry and fish from different parts of the country. There are 42 wet bird markets in Chittagong metro that also provide raw fish supply to the consumer (Personal Communication).The wholesale, retail and both types of vendors do business in those markets. Among those markets five (Pahartali, Jhautala, Kazir Dewri, Karnafulli and Riazuddin bazar) were randomly selected for the investigation of antimicrobial residues in commercial broiler chickens and fishes.

## 3.2. Study design and period

A repeated cross-sectional study was conducted on commercial broiler and fish retailer outlets belonging to five different live bird markets of CMC during August to November, 2014.

## 3.3. Reference population

Broiler chickens and fishes under retailer outlets belonging to live bird markets of CMC were treated as reference population for the present study.

## 3.4. Source population

Broiler chickens and fishes of the outlets of five selected live bird markets and fish markets of CMC were considered as source population for the study.

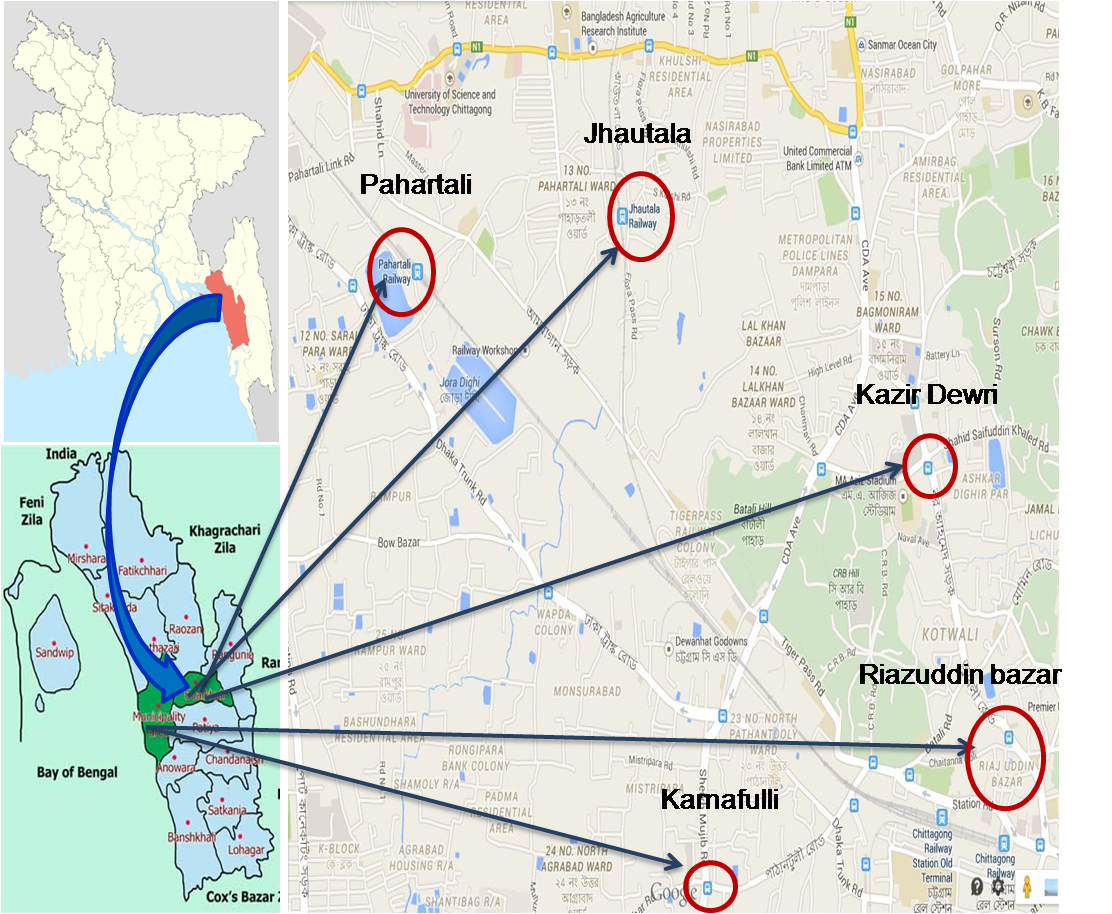
****

Figure 1. The map indicating the selected wet markets at Chittagong Metro

Table 5. Distribution of outlets of selected live bird markets of CMC and population range according to outlet owner

|  |  |  |  |
| --- | --- | --- | --- |
| Name of Live Bird Markets | No of outlets | Population range /outlet/day | No of outlets (according to ≥ 100 birds/outlet/day) |
| Pahartali | 15 | 40-1000 | 11 |
| Jhautala | 12 | 40-250 | 7 |
| Riazuddin bazar | 15 | 50-4000 | 12 |
| Karnafulli | 12 | 20-700 | 7 |
| Kazir Dewri | 7 | 30-250 | 4 |

Table 6. Distribution of outlets of selected fish markets and amount of fish per day sold according to outlet owner

|  |  |  |  |
| --- | --- | --- | --- |
| Name of wet fish markets | No of outlet | kg/outlet/day | No of outlets (according to 50 kg fish sold out/outlet/day) |
| Pahartali | 13 | 30-200 | 7 |
| Jhautala | 11 | 20-100 | 6 |
| Riazuddin bazar | 16 | 20-250 | 7 |
| Karnafulli | 10 | 30-100 | 5 |
| Kazir Dewri | 8 | 10-75 | 4 |

## 3.5. Sampling frame

A complete list of broiler chicken and fish retailer outlets (≥100 birds/outlet; 50 kg fish/outlet) of the selected markets were developed separately and used them as sampling frame.

## 

## 3.6. Sample size calculation

Fifty percent outlets (bird and fish) per market were randomly selected. Accordingly, a total of 41 poultry and 29 fish outlets were sampled twice: one was done in September (summer) and other was done in November (winter).

## 3.7. Sampling strategy and sample collection and transportation

One bird per outlet and one fish of each type (Rui, Tilapia, Koi and Pangas) per outlet were purchased and sampled. Liver, breast muscle and thigh muscle of individual bird (N=82 birds, 41 each month; n=246 organs) were collected. Flesh sample of individual fish was also collected (N=232, 116 each month). Samples were taken in sterile zip bags and given unique identity numbers and sample names. Samples obtained were promptly transferred to laboratory at the Department of Physiology, Biochemistry and Pharmacology of Chittagong Veterinary and Animal Sciences University and stored in -20°C for further analysis.

## 3.8. Data collection

Name of the markets, number of retailer outlets, population size, sold per day, surplus, drug used at shop (for poultry only) and surplus birds and fish and contact details of owner were collected through administration of face-to-face questionnaire interview.

## 3.9. Laboratory evaluation

### 3.9.1. Extraction of antimicrobials residues

Propelka et al. (2005) described the protocol of extraction procedure of antimicrobial residues was used for the present study. Briefly, 10 gm of each organ sample were blended and meshed using a food processor (Model No. KFP1333). Four gm of meshed sample then was added to 10 ml of phosphate buffer (pH 6.5) within a 50 ml beaker for homogenization. After homogenization 2 ml of 30% trichloroacetic acid was added to the mixture to precipitate proteins and then transferred to a 15 ml falcon tube for centrifugation at 7000 rpm for 15 minutes. The supernatant was collected and filtered through Whatman filter paper by using sterile funnel. To remove lipids, an equal volume of diethyl ether was added to the solution and mixed, and incubated on the bench at room temperature for 10 minutes to separate an upper oily layer and the extraction layer. The oily layer was discarded and the extracted layer was collected using a sterile funnel. The extraction process was repeated twice with diethyl ether and then placed in a sterile vial at 4°C until analysis being performed. .

### 3.9.2. Thin Layer Chromatography

A total of 478 extracted samples (246 poultry organs and 232 fleshes of fishes) were evaluated by the TLC (Sareef et al., 2009 and Popelka et al., 2005). Ciprofloxacin, Oxytetracycline, Amoxicillin, Enrofloxacin, Doxycycline, Erythromycin and Sulfachlorpyradazine were evaluated for antimicrobial residues. Antimicrobials were selected for TLC evaluation was based on previous literature and with consultation of professionals in the respective fields of poultry and fish. Representative TLC positive samples was subjected to UHPLC for quantification of ciprofloxacin (N=6; liver 3, thigh muscle 1 and breast muscle 2), amoxicillin (N=6; liver 2, thigh muscle 2 and breast muscle 2) and oxytetracycline (N=2; liver 1 and breast muscle 1) for poultry organs and for quantification of amoxocilin (N=3; rui 1, tilapia 1 and koi 1) and oxytetracycline (N=1; koi 1) for fish flesh (Parvej, 2013; Sattar et al., 2014). The detail procedures of TLC is given in annex-I.

### 3.9.3. Ultra high performance liquid chromatography

A previously described (Wang et al., 2009) the UHPLC procedure was applied in this study. Briefly, a stainless steel column C18 (2μm) P/N 891-5002, 2mm ID×100ml No. 22G2C-001 was used for chromatography with a wavelength of the spectrophotometric detector set at 254 nm. Mobile phase (A and B) was run at a specific flow rate for different antimicrobials. Mobile phase A consisted of 1 part of acetonitrile R (Phillipsburg, NJ, USA) and 99 parts of buffer solution (pH 5.0) and mobile phase B consisted of 20 parts of acetonitrile and 80 parts of buffer solution (2M sodium hydroxide added to 0.2M potassium dihydrogen phosphate (Sigma Corp., St. Louis, MO, USA pH 5.0). Twenty μl sample extracts was injected through an auto sampler and the amoxicillin standard was diluted in mobile phase A (after being filtered through a 0.2 micron filter) at the rate of 0.2 ml/min. The column was equilibrated with a mobile phase A to B at a ratio of 98: 8. Twenty μl sample extracts was injected through an auto sampler and the Ciprofloxacin standard was diluted in mobile phase A (after being filtered through a 0.2 micron filter) at the rate of 1ml/min. The column was equilibrated with a mobile phase A to B at a ratio of 90: 10. Twenty μl sample extracts were injected through an auto sampler and the oxytetracycline standard was diluted in mobile phase A (after being filtered through a 0.2 micron filter) at the rate of 1.5ml/min. The column was equilibrated with a mobile phase A to B at a ratio of 85: 15. The calibration curves prepared with sample standards was used to calculate the amoxicillin/ciprofloxacin/oxytetracycline concentrations in unknown samples. The spiked sample was processed and analyzed using this procedure.

## 3.10. Data entry and statistical evaluation

Field and laboratory data obtained were entered into spread sheets of the Microsoft (MS) Excel-2007 programme. Data were sorted and cleaned in the MS Excel programme before exporting to STATA-13 (STATA Corp, USA). Descriptive and summary statistics was used on the results of antimicrobial residues according to time, markets, broiler chicken, fish, and organs and types of antimicrobial. A Fisher’s exact test was used to detect the difference between the proportion of positive and negative antimicrobial residues according to time, organ, types of fishes and types of antimicrobials. The *p* value of ≤ 0.05 was considered as significant. The results were presented in frequency number, percentage, mean, standard deviation, 95% confidence interval and *p* value.

# Chapter-4: Results

## 

# 4. Results

## 4.1. Prevalence of antimicrobial residues in broiler chickens in Chittagong

On TLC evaluation the overall prevalence of antimicrobial residues in broiler chicken was 84.1% (95% CI 74.4-91.3%; N=82) of which September contributed 43.9% and November contributed 37%. No significant difference was observed for the prevalence of antimicrobial residues in broiler chickens between months (87.8%; N=41 versus 73.2%; N=41; *p*=0.162).

The prevalence of antimicrobial residues in broiler chicken in September and November was 26.8% and 22.0%, respectively for ciprofloxacin (*p*=0.796); 12.2% and 7.3%, respectively for oxytetracycline (*p*=0.712); 19.5% and 7.3%, respectively for amoxicillin (*p*=0.194); 12.2% and 9.8%, respectively for enrofloxacin (*p*=1.00); 9.8% and 7.3%, respectively for doxycycline (*p*=1.00); 4.9% and 4.9%, respectively for erythromycin (*p*=1.00); 2.4% and 14.6%, respectively for sulfachlorpyradazine (*p*=0.109).

The prevalence of antimicrobial residues in broiler chicken varied significantly among different types in September (*p*=0.016) where ciprofloxacin had the highest prevalence (26.8%) followed by amoxicillin (19.5%), oxytetracycline (12.2%), enrofloxacin (12.2%), doxycycline (9.8%), erythromycin (4.9%) and sulfachlorpyradazine (2.4%). Although the prevalence of antimicrobial residues was statistically equal among different types in November (*p*=0.240), the highest prevalence encountered for ciprofloxacin (22.0%) followed by sulfachlorpyradazine (14.6%), enrofloxacin (9.3%), amoxicillin (7.3%), oxytetracycline (7.3%), doxycycline (7.3%) and erythromycin (4.9%).

No significant difference of antimicrobial residues was observed for any of the antimicrobial types among markets by months (*p*≥0.1235) (Table 7).

The prevalence of antimicrobial residues of any type in broiler chickens varied significantly among types of poultry vendor categories (*p*=0.002) where wholesaler had 50% (N=32); retailer had 83.3% (N=36); both had 92.9% (N=14).

## 4.2. Prevalence of antimicrobial residues in organs of broiler chickens in Chittagong

The overall prevalence estimate of antimicrobial residues in broiler chicken organs was 39.4% (95CI 33.3-45.8%; N=246) of which 23.2% was for September and 16.3% for November. The prevalence was significantly higher (46.3%; N=123) in broiler chicken organs in September than in November (32.5%; N=123) (*p*=0.036).

No statistical difference was evidenced for the prevalence of antimicrobial residues in any of the organ samples evaluated between months (Liver 73.2% versus 56.1%; *p*=0.165; Thigh 34.1% versus 24.4%; *p*=0.467; Breast 31.7% versus 17.1; *p*=0.198).

The prevalence of antimicrobial residues among broiler chicken organs within a month varied significantly (September: Liver-thigh-breast 73-34.1-31.7%; *p*<0.001 and November: Liver-thigh-breast 56.1-24.4-17.1%; *p*<0.001).

No significant difference of antimicrobial residues was evidenced for any of the antimicrobial types among organs by months except ciprofloxacin in September (*p*=0.015) (Table 8).

In most of the cases ciprofloxacin residues had the highest prevalence among different antimicrobials and however, no statistical difference was observed between months for each type of antimicrobials (Table 9).

Table 7. Frequency distribution of antimicrobial residues in poultry sampled from different wet markets of Chittagong metro city by sub-sites, month and types of antimicrobials (Samples were evaluated by Thin Layer Chromatography)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Markets (N)** | **September** | | | | | | | **November** | | | | | | | **% Difference between months**  **(*p* of Fisher’s exact test)** | | | | | | |
|  | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Dox  n (%) | Ery  n (%) | Sul  n (%) | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Dox  n (%) | Ery  n (%) | Sul  n (%) | Cip | OTC | Amx | Enr | Dox | Ery | Sul |
| Jhautala  (7\*) | 2  (28.5) | 1  (14.3) | 3  (42.9) | 0 | 0 | 0 | 0 | 1  (14.3) | 1  (14.3) | 0 | 0 | 0 | 0 | 2  (28.5) | (1.000) | 0 | (0.192) | 0 | 0 | 0 | (0.461) |
| Pahartali  (11\*) | 4  (36.4) | 1  (9.1) | 1  (9.1) | 2  (18.2) | 1  (9.1) | 1  (9.1) | 0 | 1  (9.1) | 1(9.1) | 1  (9.1) | 0 | 2  (18.2) | 1  (9.1) | 2  (18.2) | (0.311) | 0 | 0 | (0.476) | (1.000) | 0 | (0.461) |
| Karnafulli  (7\*) | 0 | 1  (14.3) | 1  (14.3) | 1  (14.3) | 2  (28.5) | 1  (14.3) | 1  (14.3) | 3  (42.9) | 0 | 0 | 2  (28.5) | 0 | 0 | 2  (28.5) | (0.192) | (1.000) | (1.000) | (1.000) | (0.461) | (1.000) | (1.000) |
| Riazuddin  (12\*) | 4  (33.3) | 2  (16.7) | 2  (16.7) | 1  (8.3) | 0 | 0 | 0 | 2  (16.7) | 2(16.7) | 2  (16.7) | 1  (8.3) | 1  (8.3) | 0 | 0 | (0.641) | 0 | 0 | 0 | (1.000) | 0 | 0 |
| Kazir Dewri  (4\*) | 1  (25.0) | 0 | 1  (25.0) | 1  (25.0) | 1  (25.0) | 0 | 0 | 2  (50.0) | 0 | 0 | 1  (25.0) | 0 | 1  (25.0) | 0 | (1.000) | 0 | (1.000) | 0 | (1.000) | (1.000) | 0 |
| ***p***  **(Fisher’s exact test)** | 0.476 | 1.000 | 0.517 | 0.701 | 0.123 | 0.634 | 0.439 | 0.269 | 0.936 | 0.827 | 0.143 | 0.759 | 0.180 | 0.222 |  |  |  |  |  |  |  |

*N=Total number of birds; n=Number of positive birds; p=probability value of significant test; Cip: Ciprofloxacin; OTC: Oxytetracycline; Amx: Amoxicillin; Enr: Enrofloxacin; Dox: Doxycycline; Ery: Erythromycin; Sul: Sulfachlorpyradazine; \*Number for each month*

**Table 8. Frequency distribution of antimicrobial residues in poultry sampled from different wet markets of Chittagong metro city by organ types and month** (Samples were evaluated by Thin Layer Chromatography)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Organs**  **(N)** | **September** | | | | | | | **November** | | | | | | | **% Difference between months**  **(*p* of Fisher’s exact test)** | | | | | | |
|  | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Dox  n (%) | Ery  n (%) | Sul  n (%) | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Doxy  n (%) | Ery  n (%) | Sul  n (%) | Cip | OTC | Amx | Enr | Dox | Ery | Sulfa |
| Liver  ( 41\*) | 11 (28.8) | 4  (9.8) | 7  (17.1) | 2  (4.8) | 3  (7.3) | 2  (4.8) | 1  (2.4) | 7  (17.1) | 4  (9.8) | 3  (7.3) | 2  (4.8) | 1  (2.4) | 1  (2.4) | 5 (12.2) | (0.424) | 0 | (0.311) | 0 | (0.615) | (1.000) | (0.201) |
| Thigh  ( 41\*) | 2  (4.8) | 1  (2.4) | 5  (12.2) | 3  (7.3) | 2  (4.8) | 1  (2.4) | 0 | 2  (4.8) | 2  (4.8) | 2  (4.8) | 1  (2.4) | 0 | 1  (2.4) | 2  (4.8) | 0 | (1.000) | (0.432) | (0.615) | (0.493) | 0 | (0.493) |
| Breast  ( 41\*) | 4  (9.8) | 2  (4.8) | 2  (4.8) | 1  (2.4) | 3  (7.3) | 0 | 1  (2.4) | 2  (4.8) | 1  (2.4) | 1  (2.4) | 0 | 1  (2.4) | 0 | 2  (4.8) | (0.675) | (1.000) | (1.000) | (1.000) | (0.615) | 0 | (1.000) |
| ***p***  **(Fisher’s Exact test)** | 0.015 | 0.500 | 0.254 | 0.870 | 1.000 | 0.772 | 1.000 | 0.109 | 0.500 | 0.870 | 0.772 | 1.000 | 1.000 | 0.504 |  |  |  |  |  |  |  |

*N=Total number of birds; n=Number of positive birds; p=probability value of significant test; Cip: Ciprofloxacin; OTC: Oxytetracycline; Amx: Amoxicillin; Enr: Enrofloxacin; Dox: Doxycycline; Ery: Erythromycin; Sul: Sulfachlorpyradazine; \*Number for each month*

Table 9. Frequency distribution of antimicrobial residues by site and antimicrobial types in organ samples of broiler chickens in different wet markets of Chittagong metropolitan areas

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** |  |  | **September** | | **November** | |  |
| **Class of antimicrobials** | **Type of antimicrobials** | **No of positive (%)** | **No of organs tested** | **No of positive (%)** | **No of organs tested** | **p (Fisher’s exact)** |
| Jhautala |  |  |  | **21** |  | **21** |  |
| Fluroquinolone | *Ciprofloxacin* | 2 (9.5) |  | 1 (4.8) |  | 1.000 |
| Tetracycline | *Oxytetracycline* | 1 (4.8) |  | 1 (4.8) |  | 1.000 |
| β-Lactam | *Amoxicillin* | 3 (14.3) |  | 0 |  | 0.231 |
| Sulfonamide | *Sulfachlorpyradazine* | 0 |  | 2 (9.5) |  | 0.487 |
|  | **Total** |  | **6 (28.6)** |  | **4 (19.0)** |  | **0.718** |
| Pahartali |  |  |  | **33** |  | **33** |  |
| Fluroquinolone | *Ciprofloxacin* | 4 (12.1) |  | 1 (3.0) |  | 0.355 |
| *Enrofloxacin* | 2 (6.0) |  | 0 |  | 0.492 |
| Tetracycline | *Oxytetracycline* | 1 (3.0) |  | 1 (3.0) |  |  |
| *Doxycycline* | 1(3.0) |  | 2 (6.0) |  | 1.000 |
| β-Lactam | *Amoxicillin* | 1 (3.0) |  | 1 (3.0) |  |  |
| Macrolide | *Erythromycin* | 1 (3.0) |  | 1 (3.0) |  |  |
| Sulfonamide | *Sulfachlorpyradazine* | 0 |  | 2 (6.0) |  | 0.492 |
|  | **Total** |  | **10 (30.3)** |  | **8 (24.2)** |  | **0.782** |
| Karnafulli |  |  |  | **21** |  | **21** |  |
| Fluroquinolone | *Ciprofloxacin* | 0 |  | 3 (14.2) |  | 0.231 |
| *Enrofloxacin* | 1 (4.8) |  | 2 (9.5) |  | 1.000 |
| Tetracycline | *Oxytetracycline* | 1 (4.8) |  | 0 |  |  |
| *Doxycycline* | 2 (9.5) |  | 0 |  |  |
| β-Lactam | *Amoxicillin* | 1 (4.8) |  | 0 |  |  |
| Macrolide | *Erythromycin* | 1 (4.8) |  | 0 |  |  |
| Sulfonamide | *Sulfachlorpyradazine* | 1 (4.8) |  | 2 (9.5) |  | 1.000 |
|  | **Total** |  | **7 (33.3)** |  | **7 (33.3)** |  | **1.000** |
| Riazuddin |  |  |  | **36** |  | **36** |  |
| Fluroquinolone | *Ciprofloxacin* | 4 (11.1) |  | 2(5.5) |  | 0.673 |
| *Enrofloxacin* | 1 (2.8) |  | 1 (2.8) |  |  |
| Tetracycline | *Oxytetracycline* | 2 (5.5) |  | 2(5.5) |  |  |
| *Doxycycline* | 0 |  | 1 (2.8) |  |  |
| β-Lactam | *Amoxicillin* | 2 (5.5) |  | 2 (5.5) |  |  |
|  | **Total** |  | **9 (25.0)** |  | **8 (22.2)** |  |  |
| Kazir Dewri |  |  |  | **12** |  | **12** |  |
| Fluroquinolone | *Ciprofloxacin* | 1 (8.3) |  | 2 (16.7) |  | 1.000 |
| *Enrofloxacin* | 1 (8.3) |  | 1 (8.3) |  |  |
| Tetracycline | *Doxycycline* | 1 (8.3) |  | 0 |  |  |
| β-Lactam | *Amoxicillin* | 1 (8.3) |  | 0 |  |  |
| Macrolide | *Erythromycin* | 0 |  | 1 (8.3) |  |  |
|  | **Total** |  | **4 (33.3)** |  | **4 (33.3)** |  |  |

## 4.3. Prevalence of antimicrobial residues in fishes in Chittagong

The overall prevalence of antimicrobial residues in fishes was 13.8% (95% CI 9.6-18.9%; N=232) (September 8.6% and November 5.2%). Residues of oxytetracycline and amoxicillin were detected as 10.3% and 6.9%, respectively in September (*p*=0.482) and 7.8% and 3.4%, respectively in November (*p*=0.138). No statistical difference of prevalence of residue between months was observed either for oxytetracycline (*p*=0.647) or amoxicillin (*p*=0.216). The prevalence of oxytetracycline or amoxicillin residues was statistically equal among markets (*p*≥0.105) (Table 10).

The prevalence of oxytetracycline residues in September and November was 13.8% and 10.3%, respectively in Rui; 10.3% and 6.9%, respectively in Tilapia; 0% and 6.9%, respectively in Koi; 10.3% and 6.9%, respectively in Pangas. The prevalence of amoxicillin residues in September and November was 10.3% and 3.4%, respectively in Rui; 3.4% and 3.4%, respectively in Tilapia, 6.9% and 0%, respectively in Koi and 6.9% and 3.4%, respectively in Pangas. No differences were observed among types of fish within each month or between the months for the prevalence of oxytetracycline or amoxicillin (*p*≥0.218) (Table 11).

No difference of prevalence of residues between oxytetracycline and amoxicillin was evidenced within each market (Table 12).

Table 10. Frequency distribution of antimicrobial residues in fishes sampled from different wet markets of Chittagong metro city by sub-sites, month and types of antimicrobials (Samples were evaluated by Thin Layer Chromatography)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Markets**  **(N)** | **September** | | | | | | | **November** | | | | | | | **% Difference between months**  **(*p* of Fisher’s exact test)** | | | | | | |
|  | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Dox  n (%) | Ery  n (%) | Sul  n (%) | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Dox  n (%) | Ery  n (%) | Sul  n (%) | Cip | OTC | Amx | Enr | Dox | Ery | Sul |
| Jhautala (24\*) | 0 | 0 | 2  (8.3) | 0 | 0 | 0 | 0 | 0 | 1  (4.2) | 0 | 0 | 0 | 0 | 0 | 0 | (1.000) | (0.489) | 0 | 0 | 0 | 0 |
| Pahartali  (28\*) | 0 | 4  (14.3) | 1  (3.6) | 0 | 0 | 0 | 0 | 0 | 3  (10.7) | 1  (3.6) | 0 | 0 | 0 | 0 | 0 | (1.000) | 0 | 0 | 0 | 0 | 0 |
| Karnafulli  (20\*) | 0 | 2  (10.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (0.489) | 0 | 0 | 0 | 0 | 0 |
| Riazuddin  (28\*) | 0 | 5  (17.9) | 5  (17.9) | 0 | 0 | 0 | 0 | 0 | 5  (17.9) | 2  (7.2) | 0 | 0 | 0 | 0 | 0 | 0 | (0.415) | 0 | 0 | 0 | 0 |
| Kazir Dewri  (16\*) | 0 | 1  (6.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | (1.000) | 0 | 0 | 0 | 0 | 0 |
| ***p***  **(Fisher’s exact test)** |  | 0.224 | 0.105 |  |  |  |  |  | 0.363 | 0.624 |  |  |  |  |  |  |  |  |  |  |  |

*N=Total number of birds; n=Number of positive birds; p=probability value of significant test; Cip: Ciprofloxacin; OTC: Oxytetracycline; Amx: Amoxicillin; Enr: Enrofloxacin; Dox: Doxycycline; Ery: Erythromycin; Sul: Sulfachlorpyradazine; \*Number for each month*

Table 11. Frequency distribution of antimicrobial residues in fishes sampled from different wet markets of Chittagong metro city by fish types and month (Samples were evaluated by Thin Layer Chromatography)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Fish**  **(N)** | **September** | | | | | | | **November** | | | | | | | **% Difference between months**  **(*p* of Fisher’s exact test)** | | | | | | |
|  | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Dox  n (%) | Ery  n (%) | Sul  n (%) | Cip  n (%) | OTC  n (%) | Amx  n (%) | Enr  n (%) | Dox  n (%) | Ery  n (%) | Sul  n (%) | Cip | OTC | Amx | Enr | Dox | Ery | Sul |
| Rui  (29\*) | 0 | 4  (13.8) | 3  (10.3) | 0 | 0 | 0 | 0 | 0 | 3  (10.3) | 1  (3.4) | 0 | 0 | 0 | 0 | 0 | (1.000) | (0.611) | 0 | 0 | 0 | 0 |
| Tilapia  (29\*) | 0 | 3  (10.3) | 1  (3.4) | 0 | 0 | 0 | 0 | 0 | 2  (6.9) | 1  (3.4) | 0 | 0 | 0 | 0 | 0 | (1.000) | 0 | 0 | 0 | 0 | 0 |
| Koi  (29\*) | 0 | 0 | 2  (6.9) | 0 | 0 | 0 | 0 | 0 | 2  (6.9) | 0 | 0 | 0 | 0 | 0 | 0 | (0.491) | (0.491) | 0 | 0 | 0 | 0 |
| Pangas  (29\*) | 0 | 3  (10.3) | 2  (6.9) | 0 | 0 | 0 | 0 | 0 | 2  (6.9) | 1  (3.4) | 0 | 0 | 0 | 0 | 0 | (1.000) | (1.000) | 0 | 0 | 0 | 0 |
| ***p***  **(Fisher’s Exact test)** |  | 0.218 | 0.957 |  |  |  |  |  | 1.000 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |

*N=Total number of birds; n=Number of positive birds; p=probability value of significant test; Cip: Ciprofloxacin; OTC: Oxytetracycline; Amx: Amoxycillin; Enr: Enrofloxacin; Dox: Doxycycline; Ery: Erythromycin; Sul: Sulfachlorpyradazine; Rui, Rohu (Labeo rohita);* *Tilapia*, *Nile Tilapia (Orechromis nilotica); Koi, Climbing perch (Anabas testudineus);Pangas, Yellowtail catfish* (*Pangasius hyphthalamus*); *\*Number for each month*

Table 12. Frequency distribution of antimicrobial residues by site and antimicrobial types in fish samples obtained from different wet markets of Chittagong metropolitan areas

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Class of antimicrobials** | **Type of antimicrobials** | **September** | | **November** | |
| **No of positive (%)** | **No of fish samples tested** | **No of positive (%)** | **No of fish samples tested** |
| Jhautala |  |  |  | 24 |  | 24 |
|  | Tetracycline | *Oxytetracycline* | 0 |  | 1 (4.6) |  |
| β-Lactam | *Amoxicillin* | 2 (8.3) | 0 |
| Pahartali |  |  |  | 28 |  | 28 |
|  | Tetracycline | *Oxytetracycline* | 4 (14.2) |  | 3 (10.1) |  |
| β-Lactam | *Amoxicillin* | 1 (3.5) | 1 (3.7) |
| Karnafulli |  |  |  | 20 |  | 20 |
|  | Tetracycline | *Oxytetracycline* | 2 (10.0) |  | 0 |  |
| β-Lactam | *Amoxicillin* | 0 | 0 |
| Riazuddin |  |  |  | 28 |  | 28 |
|  | Tetracycline | *Oxytetracycline* | 5 (17.8) |  | 5 (17.5) |  |
| β-Lactam | *Amoxicillin* | 5 (17.8) | 2 (7.4) |
| Kazir Dewri |  |  |  | 16 |  | 16 |
|  | Tetracycline | *Oxytetracycline* | 1 (6.2) |  | 0 |  |
| β-Lactam | *Amoxicillin* | 0 | 0 |

## Estimation of concentration of antimicrobial residues in broiler chickens and fishes in Chittagong

The concentration (µg/kg) of amoxicillin, ciprofloxacin and oxytetracycline was average 106.6, 269 and 35.56, respectively in liver; average 111.8, 133 and 0, respectively in thigh muscle and average 75.8, 396.9 and 57.2, respectively in breast muscle of broiler chickens (Table 13).

The concentration (µg/kg) of amoxicillin in Rui, Tilapia and Koi was 95.4, 36.5 and 95.9, respectively. The concentration (µg/kg) of oxytetracycline in Koi was 60.7 (Table 13).

Table 13. Estimation of concentration of antimicrobial residues in different organs of broiler chickens and fish by UHPLC

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Antimicrobials** | **Organ**  **(Broiler chicken)** | **n** | **Concentration**  **(µg/kg)** | **Reference concentration MRL(µg/kg)\*** | **Fish** | **Concentration**  **(µg/kg)** | **Reference concentration MRL(µg/kg)\*** |
|  | **Liver (mean)** | **2** | **106.6** | **50** |  |  | **50** |
| Amoxicillin | Liver 1 |  | 106.3 |  | Rui | 95.4 |  |
| Liver 2 |  | 111 |  | Tilapia | 36.5 |  |
| **Thigh (mean)** | **2** | **111.8** | **50** |  |  |  |
| Thigh 1 |  | 81.4 |  | Koi | 95.9 |  |
| Thigh 2 |  | 142.2 |  |  |  |  |
| **Breast (mean)** | **2** | **75.8** | **50** |  |  |  |
| Breast 1 |  | 96.8 |  |  |  |  |
| Breast 2 |  | 54.7 |  |  |  |  |
| Ciprofloxacin | **Liver (mean)** | **3** | **269.2** | **200** |  |  |  |
| Liver 1 |  | 18 |  |  |  |  |
| Liver 2 |  | 724.2 |  |  |  |  |
| Liver 3 |  | 65.3 |  |  |  |  |
| **Breast (mean)** | **2** | **396.9** | **100** |  |  |  |
| Breast 1 |  | 762.5 |  |  |  |  |
| Breast 2 |  | 31.3 |  |  |  |  |
| Thigh | 1 | 133 | 100 |  |  |  |
| Oxytetracycline | Liver | 1 | 35.56 | 300 | Koi | 60.7 | 100 |
| Breast | 1 | 57.2 | 100 |  |  |  |

\* Commission Regulation (EU) No 37/2010

# Chapter-5: Discussion

To best of my knowledge this is the first repeated cross-sectional study to assess the status of antimicrobial residues in commercial broiler poultry and fish at wet markets in Chittagong metropolitan city in Bangladesh.

The overall prevalence of antimicrobial residues, regardless of the types of antimicrobials was 84% in commercial poultry in the present study. In contrast lower prevalence of antimicrobial residues (52.5%) has been reported in commercial poultry by many earlier workers in Chittagong (Hossain et al., 2011; Mahmud, 2012; Karim, 2013; Parvej, 2013).

The current study has not observed any statistical difference of prevalence of antimicrobial residues between summer (September) and winter (November) (43.9% versus 37%) and this finding is supported by a study conducted in Pakistan (summer 47% versus winter 36%) (Naeem et al., 2006). Higher prevalence has also been reported in winter (14%) than in summer (8.9%) (Pavlov et al., 2008). However, it is difficult to say the true season effect on antimicrobial status unless disease prevalence in broiler poultry was too high during summer; rather it could be due to how close antimicrobial use prior to selling out the broiler poultry or whether birds were provided antimicrobials on stall in the market.

The overall prevalence of antimicrobial residues in organs of broiler poultry was 39.4%, is supported by number of earlier studies in broiler poultry such as 16-56% in Chittagong of Bangladesh (Mahmud, 2012; Karim, 2013; Parvej, 2013; Sattar et al., 2014), 43% in Pakistan (Mehtabuddin et al., 2012) and 50% in Iran (Tajick and Shohreh, 2006). On the other hand, lower prevalence of antimicrobial residues in broiler poultry has been documented by other studies such as 21% in Ghana (Donkor et al., 2011) and 26.8% in Nigeria (Ezenduka et al., 2014).

The prevalence of antimicrobial residues was significantly higher in liver (54.5%) than that of thigh (24.2%) or breast muscle (21.2%), and these findings are corroborated with previous published works (Muriuki et al., 2001; Dipeolu and Alonge, 2002; Karim, 2013; Parvej, 2013). Those studies reported a prevalence of antimicrobial residues of 30-45% in liver, 15-27% in breast muscle and 12-23% in thigh muscle. Higher prevalence in liver in the present study along with the cited studies (Amjad et al., 2005; Naeem et al., 2006; Pavlov et al., 2008) is because liver is the principal drug metabolism site in the body (Amjad et al., 2005; Naeem et al., 2006; Pavlov et al., 2008).

All antimicrobials under residual investigation were detected in broiler poultry in the current study and the prevalence significantly varied among antimicrobial types of which ciprofloxacin and amoxicillin residues were more frequent detected (19.5-26.8%) than the others (2.4-12.2%) (Table 8). Similar type-specific prevalence of antimicrobial residues has been reported by many other cross-sectional works in Chittagong of Bangladesh (Mahmud, 2012; Karim, 2013; Parvej, 2013). They documented the prevalence of antimicrobial residues of 6.7-7.5% for ciprofloxacin, 4.2-7.3% for oxytetracycline, 2.5-8.5% for amoxicillin and 2.5-5% for sulfonamides.

Significant differences were observed among poultry vendor types (wholesaler: 50%, retailer: 83.3%, and both: 92.2%) among different markets in this study. Identical results have been published by (Karim, 2013; Sattar, 2012). Assessing the effect of vendor types for the occurrence of antimicrobial residues is probably the first kind investigation in Bangladesh.

The overall prevalence of antimicrobial residues in fishes in the present study was 13.8% (regardless of types of fishes: Rui, Tilapia, Pangas and Koi). However, a variable prevalence of antimicrobial residues in fishes was reported previously in number of countries such as 5.8% (regardless of types of fishes: red tilapia *Oreachromis spp*. red hybrids, keli *Clarias spp*. and patin *Pangasius sutchii*) in Malaysia (Bakar et al., 2010), 8% (regardless of types of fishes: tiger shrimp *Penaeus monodon*) in Thailand (Holmstrom et al., 2003), 30% (African catfish) in Nigeria (Olatoye and Basiru, 2013), 56% (Rainbow trout) in Iran (Mahmoudi et al., 2014). Contrarily, a sporadic study conducted in Dhaka of Bangladesh has been reported 87% of antimicrobial residues in fishes (regardless of fish types: Rui, Pangas and Tilapia) (Rasul, 2012).

Oxytetracycline, Chlorotetracyclin, Amoxicillin, Co-trimoxazole, Sulphadiazine, Sulphamethoxazole are commonly used antimicrobials in aquaculture in Bangladesh (Hossain et al., 2013; Faruk et al., 2008; Rasul, 2012).

Only the residues of oxytetracycline (10.3%) and amoxicillin (6.9%) were detected in this study and the results varied significantly between antimicrobial types. Moderate to very high prevalence of oxytetracycline residue has also been detected in fishes (african catfish, rainbow trough fish) across the world, for example 30% in Iran (Mahmoudi et al., 2014), 34.4% in Nigeria (Olatoye and Basiru, 2013) and 87% in Bangladesh (Rasul, 2012).

The prevalence of oxytetracycline and amoxicillin residues in September was significantly higher (13.8% versus 10.3%) than in November (10.3% versus 3.4%) but, no relevant literature have been available to discuss temporal variability of prevalence of antimicrobial residues in fishes in this study. However, a possible explanation is that the rain during September can move out the left-over antimicrobials from agriculture fields (if antimicrobials are used), effluents of medical and veterinary hospital etc (Martinez, 2009; Obasohan, 2009) to ponds and rivers.

Irrespective of month of sampling, Rui had the highest prevalence of antimicrobial residues (10.3-13.8% oxytetracycline and 3.4-10.3% amoxicillin) followed by Koi (0-6.9% oxytetracycline and 0-6.9% amoxicillin), Tilapia (6.9-10.3% oxytetracycline and 3.4% amoxicillin) and Pangas (6.9-10.3% oxytetracycline and 3.4-6.9% amoxicillin) in the present study. These findings are in-lined with the reports of others for instance, the prevalence of oxytetracycline residue of 38% detected in Pangas, 31% in Rui and 31% in tilapia in Bangladesh (Rasul, 2012). As a cross reference the residue has been detected 30% for tetracycline in rainbow trout, 19% for sulfonamide and 7% for chloramphenicol in rainbow trout in Iran (Mahmoudi et al., 2014) and 34.4% for oxytetracycline in African catfish in Nigeria (Olatoye and Basiru, 2013).

An average concentration of amoxicillin, ciprofloxacin and oxytetracycline residues in organ of broiler poultry was detected as 106.6, 269 and 396.9 µg/kg, respectively in liver; 111.8, 133 and 0 µg/kg, respectively in thigh muscle; 75.8, 396.9 and 57.2 µg/kg, respectively in breast muscle. Similar kind of concentration has been quantified in organs of broiler poultry in many earlier studies in Bangladesh (270 µg/kg amoxicillin and 350 µg/kg ciprofloxacin residues in liver, 120 µg/kg amoxicillin and 400 µg/kg ciprofloxacin residues in thigh muscle and 100 µg/kg amoxicillin and 120 µg/kg ciprofloxacin residues in breast muscle) (Mahmud, 2012; Karim, 2013; Parvej, 2013, Sattar et al., 2014).

Similar to the aforementioned cited Bangladeshi studies along with the present investigation high average concentration of different antimicrobial residues in organs of broiler poultry has been estimated in Pakistan and other countries (Naeem et al., 2006; Yibar et al., 2011; Mehtabuddin et al., 2012) such as 180 µg/kg ciprofloxacin and 807 µg/kg enrofloxacin in liver, 150 µg/kg ciprofloxacin and 1157 µg/kg enrofloxacin in kidney and 89 µg/kg ciprofloxacin and 400 µg/kg enrofloxacin in muscle (Amjad et al., 2005; Naeem et al., 2006), 0.02 to 0.8 µg/g sulphonamide in muscle (Mehtabuddin et al., 2012).

An average concentration of amoxicillin residues in liver, thigh muscle in the present study (Table 13) crossed the maximum threshold values of 50 µg/kg (Commission Regulation (EU) No 37/2010). The average concentration of ciprofloxacin residues in liver, breast and thigh muscle also crossed the maximum threshold value (Table 13) 200 µg/kg, 100 µg/kg and 100 µg/kg, respectively (Commission Regulation (EU) No 37/2010). Crossing the maximum limits of concentration of antimicrobials generally pose public health risk of multi-drug resistance, mutagenic effects, hallucination, twitching, headache, seizures, neurotoxicity, hypersensitivity reactions, immune suppression and toxic effects or even cancer (Woodward, 1991; Ferhan and Aydin, 2000; Donoghue, 2003; VRC, 2005; WHO, 2006; Nonga et al., 2009). However, heat, uses of sausages and other treatment can reduce the level of concentration of antimicrobial residues (ciprofloxacin, amoxicillin, oxytetracycline, sulfonamides) in poultry meat as observed in many earlier studies (Furusawa and Hanabusa, 2002; Javadi et al., 2009; Karim, 2013; Parvej, 2013) and thereby reduce public health risk a bit (Hossain et al., 2011; Mahmud, 2012; Parvej, 2013).

The concentration of amoxicillin residue in Rui and Koi exceeded the maximum threshold value (50µg/kg) in the current study (Table 13). Similar level of concentration that crossed the maximum limit have been reported in Rui (oxytetracycline: 100 µg/kg; chloramphenicol: 0 µg/kg) in Tilapia (oxytetracycline: 100 µg/kg; chloramphenicol: 0 µg/kg) and in Pangas oxytetracycline: 100 µg/kg; chloramphenicol: 0 µg/kg) (Rasul, 2012; Bakar et al., 2013). The concentration of antimicrobials above the maximum limits in the fishes in this study also certainly pose public health as discussed earlier.

# Chapter-6: Conclusions, Limitations and Recommendations

# 6.1. Conclusions

The overall prevalence of antimicrobial residues was 84% in birds, 13.8% in fishes and 39.4% in poultry organs. No significant seasonal, spatial (markets) variation was observed for the prevalence of antimicrobial residues either in broiler chicken or fishes except among poultry vendor types (wholesaler: 50%, retailer: 83.3% and both: 92.9%). Antimicrobial residues were more commonly detected in liver followed by breast and thigh muscle in birds. In case of fishes antimicrobial residues were more commonly detected in Rui followed by Pangas, Tilapia and Koi. Ciprofloxacin and amoxicillin residues were more prevalent than other antimicrobial residues in bird and organs. On the other hand, only oxytetracycline and amoxicillin residues were found in fishes among seven types of antimicrobials tested. Ciprofloxacin and amoxicillin crossed the average maximum threshold values in organs of poultry whereas amoxicillin crossed the average maximum threshold values in Rui and Koi.

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## 6.2. Limitations

Firstly, only limited number of TLC positive samples was evaluated for quantifying concentration of antimicrobial residues. Secondly, it has not been possible to conduct an intervention trial as originally planned to assess the effect of different treatments on reduction of antimicrobial concentration. Thirdly, a comprehensive temporal effect (year round) on the occurrence of antimicrobial residues in poultry and fish has not also been possible. These limitations have been evolved because of financial constraints and shortage of time.

## 

## 6.3. Recommendations

* Drug withdrawal periods should strictly be maintained before human consumption of broilers and fishes.
* Veterinarians and Fishery Officers are advised to be more rigorous when prescribing veterinary medicinal products or antimicrobial in fishery and to become aware of the rules for the prudent use of antimicrobials. Owners should respect the prescribed withdrawal periods of drugs. It is also necessary to organize seminars on the risk of the excess use of antimicrobial substances in food animals for public health for relevant stakeholders.
* Indiscriminate or excessive use of drugs should be restricted through education and motivation of broiler farmers, fish farmers, vendor owners and practicing veterinarians.
* A year round study should be planned in future to evaluate temporal pattern of prevalence of antimicrobial residues and concentration in broiler poultry.
* A country wide study should be focused in future to estimate overall status of antimicrobial residues in broiler poultry and fishes in Bangladesh.

# References

Amjad H, Iqbal J, Naeem M. 2005. Analysis of some residual antibiotics in muscle, kidney and liver of broiler chicken by various methods. Pakistan Academy of Science. 42(2): 223-231.

Anka IZ, Faruk MAR, Hasan MM, Azad MAK. 2013. Environmental issues of emerging pangas (*Pangasianodon hypophthalmus*) farming in Bangladesh. Progress Agriculture. 24(1-2): 159-170.

Anonymous. 2015. Chittagong district. [cited 2015 Feb 20]. Available from: <http://en.wikipedia.org/wiki/Chittagong>.

Bakar IA, Ayub MK, Yatim AM, Sani NA. 2010. Pesticide and antibiotic residues in freshwater aquaculture fish: Chemical assessment from farm to table. Asian Journal of Food and Agro-Industry. 3(3): 328-334.

Bakar MA, Morshed AJM, Islam F, Karim R. 2013. Screening of chloramphenicol residues in chickens and fish in Chittagong city of Bangladesh. Bangadeshl. Journal of Veterinary Medicine. 11(2): 173-175.

Barton MD. 2000. Antibiotic use in animal feed and its impact on human health. Nutrition Research Review. 13(2): 279-299.

Bell DD, Weaver WD. 2002. Medication for prevention and treatment of diseases. In: Commercial Chicken meat and egg production. 5th ed. p.468. [cited 2015 Mar 22]. Available from: <https://books.google.com.bd>.

Booth NH. 1973. Development of a regulatory research program in Veterinary medical toxicology. Veterinary Toxicology. 15: 100-101.

Botsoglou NA, Fletouris DJ. 2001. Drug residues in foods. Pharmacology, Food Safety and Analysis, (1st ed). Marcel Dekker, Inc., ISBN: 0-8247-8959-8, New York, USA.

Cabello FC. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental Microbiology. 8(7): 1137-1144.

Calnek BW, Barnes HJ, Beard CW, Reid WM, Yoder HW. 1991. Diseases of Poultry*,* 9th Edn. Iowa State Univ. Press, Ames, Iowa, USA. pp. 784–789.

CDC. 2010. Get smart: know when antibiotics work. Centers for Disease Control, Atlanta, GA. [cited 2014 Nov 22]. Available from: [www.cdc.gov/Features/GetSmart](http://www.cdc.gov/Features/GetSmart).

Cholas G. 1976. Withdrawl times and limitations for use of animal drugs and certifiable antibiotics used in food-producing animals*.* Journal of Southwest Veterinarian. 29: 144-158.

Chowdhury R, Haque MN, Islam KMS, Khaleduzzaman ABM. 2009. A review on antibiotics in an animal feed. Bangladesh Journal of Animal Sciences. 38(1-2): 22-32.

Commission Regulation (EU) No 37/ 2010.Regulationon pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of the European Union. 15: 1-72.

Darwish WS, Eldaly EA, El-Abbasy MT, Ikenaka Y, Nakayama S, Ishizuka M. 2013. Antibiotic residues in food: the African scenario.Japanese Journal of Veterinary Research. 61(Suppl): S13-S22.

Diez P, Medina E, Martin M, Calderon V. 2002. Validation of the five-plate screening test for the detection of antibiotic residues in food. Estimation of the limit of detection. Revista del Comite Cientifico de la AESAN. 16: 109-130.

Dipeolu MA, Alonge DO. 2002. Residues of streptomycin antibiotic in meat sold for human consumption in some states of SW Nigeria. Archivos de zootecnia. 51(196): 477-480.

Donkor ES, Newman MJ, Tay SCK, Dayie NTKD, Bannerman E, Olu-Taiwo M. 2011. Investigation into the risk of exposure to antibiotic residues contaminating meat and egg in Ghana. Food Control. 22(6): 869-873.

Donoghue DJ. 2003. Antibiotic residues in poultry tissues and eggs: human health concerns? Poultry Science. 82(4): 618-621.

Eiichi K. 2006. On a new withdrawal time of veterinary drugs under positive list system. Journal of Livestock Medicine. 516: 363-365.

EMEA. 2004. MRL summary reports on the scientific evaluations carried out by the Committee for Veterinary Medicinal Products (CVMP). [cited 2014 Sep 14]. Available from: <http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2012/02/WC500123138.pdf>.

European Commission (EC). 2001. Notice to applicants and note for guidance. Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin. [cited 2014 Nov 12]. Available from: <http://ec.europa.eu/health/files/eudralex/vol-8/pdf/vol8_10-2005_en.pdf>.

European Community (EC). 1998. Regulation ń 2316/98, October 26, 1998. [cited 2014 Mar 16]. Available from: <https://estudogeral.sib.uc.pt/bitstream/10316/10604/1/Determination%20of%20Tetracycline%20Antibiotic%20Residues%20in%20Edible%20Swine%20Tissues.pdf>.

Ezenduka EV, Ike OS, Anaelom NJ. 2014. Rapid detection of antimicrobial residues in poultry: A consequence of non-productive use of antimicrobials. Health. 6(2): 149-152.

Faruk MAR, Ali MM, Patwary ZP. 2008. Evaluation of the status of use of chemicals and antibiotics in freshwater aquaculture activities with special emphasis to fish health management. Journal of Bangladesh Agricultural University. 6(2): 381-390.

FDA. 2000. FDA Task Force on Antimicrobial Resistance: key recommendations and report, Washington, DC. FDA, Washington, DC. [cited 2014 Apr 20]. Available from : <http://www.fda.gov/downloads/forconsumers/consumerupdates/ucm143458.pdf>.

Ferhan N, Aydin H. 2000. Quinolone antibiotic residues in raw milk and chicken liver in Kenya, Eurasian. Journal of Veterinary Science. [cited 2014 Sep 20]. Available from: [www.eurasianjvetsci.org](http://www.eurasianjvetsci.org).

Fitzpatrick SC, Brynes SD, Guest GB. 1995. Dietary intake estimates as a means to the harmonization of maximum residue levels for veterinary drugs. Journal of Veterinary Pharmacology and Therapeutics. 18: 325-327.

Furusawa N, Hanabusa R. 2002. Cooking effects on sulfonamide residues in chicken thigh muscle. Food Research International. 35: 37-42.

Gaudin V, Maris P, Fusetier R, Ribouchon C, Cadieu N, Rault A. 2004. Validation of a microbiological method: The Star protocol, a five plate test for screening of antibiotic residues in milk*.* Food Additives and Contaminants. 21(5): 422-433.

Ghani A. 2005. Practical Phytochemistry. 4th ed. Prakash Publication, Dhaka, Bangladesh. pp. 55-69.

Hasan B, Faruque R, Drobni M, Waldenstrom J, Sadique A, Ahmed KU, Islam Z, Parvez MBH, Olsen B, Alam M. 2011. High prevalence of antibiotic resistance in pathogenic *Escherichia coli* from large and small scale poultry farms in Bangladesh. Avian diseases. 55(4): 689-692.

Higgins HC, McEvoy JDG, Lynas L, Fagan NP. 1999. Evaluation of a single plate microbiological growth inhibition assay as a screening test for the presence of antimicrobial agents I compound animal feeding stuffs at therapeutic and contaminating concentration. Food Additive and Contaminants. 16: 543- 554.

Holmstrom K, Graslund S, Wahlstrom A, Poungshompoo S, Bengtsson BE, Kautsky N. 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food Science and Technology. 38(3): 255-266.

Hossain MB, Amin SMN, Shamsuddin M, Minar MH. 2013. Use of aqua-chemicals in the hatcheries and fish farms of greater Noakhali, Bangladesh. Asian Journal of Animal and Veterinary Advances. 8(2): 401-408.

Hossain MZ, Saifuddin AKM, Islam SKM, Islam S, Uddin MI, Hoque MA. 2011. Prevalence of antimicrobial residue in livestock meat using microbiological and chromatographic techniques in Bangladesh. Proceedings of the 9th Annual Scientific Conference, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. 7-8 March 2012, Chittagong, Bangladesh. Abstract book, p.51.

Islam A. 2009. Determination of antibiotics residues in layer hen using microbial inhibition test and thin layer chromatography at Chittagong, Bangladesh. M.Sc.Thesis, Department of Physiology, Biochemistry and Pharmacology. Chittagong Veterinary and Animal Sciences University, Chittagong. [cited 2014 Dec 8]. Available from: <http://www.cvasu.ac.bd/>

Jackson GS. 1980. Safety assessment of drug residues. Journal of American Veterinary Medical Association. 176: 1141-1144.

Javadi A, Mirzaei H and Khatibi SA. 2009. Effect of roasting process on antibiotic residues in edible tissues of poultry by FPT method. Journal of Animal and Veterinary Advances. 8: 2468-2472.

Kaneene JB, Miller R. 1997. Problem associated with drug residues in beef from feeds and therapy. Revue Scientific-Technique Office of International Epizootic. 16: 694- 708.

Karim MR. 2013. Determination of selective antibiotics residues in chicken meat (broiler and deshi) by thin layer chromatography and ultra high performance liquid chromatography. M.Sc.Thesis, Department of Physiology, Biochemistry and Pharmacology. Chittagong Veterinary and Animal Sciences University, Chittagong. [cited 2014 Nov 8]. Available from: <http://www.cvasu.ac.bd/>

Khan MM. 2014.Detection of antibiotic residue from raw meat and restaurant meat from broiler at Chittagong Metropolitan Area. M.Sc.Thesis, Department of Physiology, Biochemistry and Pharmacology. Chittagong Veterinary and Animal Sciences University, Chittagong. [cited 2014 Dec 9]. Available from: <http://www.cvasu.ac.bd/>

Mahmoudi R, Gajarbeygi P, Norian R, Farhoodi K. 2014. Chloramphenicol, sulfonamide and tetracycline residues in cultured rainbow trout meat (*Oncorhynchus mykiss*). Bulgarian Journal of Veterinary Medicine. 17(2): 147-152.

Mahmud K. 2012. Determination of antibiotic residue in commercial poultry meat and assess the heat tolerance level of that residues at cooking and autoclaving. (MS Thesis). Department of Physiology, Biochemistry and Pharmacology. Chittagong Veterinary and Animal Sciences University. [cited 2014 Dec 8]. Available from: <http://www.cvasu.ac.bd/>

Maqbool J. 1988. Residues of slulfachloropyradazin in poultry products.M.Sc. Thesis, Department of Veterinary Pharmacology, University of Agriculture,Faisalabad. [cited 2014 Sep 20]. Available from: <http://www.uaf.edu.pk/>

Martinez JL. 2009. Environmental pollution by antibiotics and by antibiotic resistance determinations (review). Environment Pollution. 157: 2839-2902.

Mehtabuddin A, Mian A, Ahmad T, Nadeem S, Tanveer ZI, Arshad J. 2012. Sulfonamide residue determination in commercial poultry meat and eggs. The Journal of Animal and Plant Sciences. 22(2): 473-478.

Milstein A. 1996. Do management procedures affect the ecology of warm water polyculture ponds? World Aquaculture. 28(3): 12-19.

Mumtaz A, Awan JA, Athar M. 2000. Rational use of drugs in broiler meat production. International Journal of Agriculture and Biology. 2(3): 269-272.

Muriuki FK, Ogara WO, Njeruh FM, Mitema ES. 2001. Tetracycline residue level in poultry meat from Nairobi slaughter house in Kenya. Journal of Veterinary Sciences. 2(2): 97-101.

Naeem M, Khan K, Rafiq S. 2006. Determination of residues of quinolones in poultry products by high pressure liquid chromatography. Journal of Applied Sciences. 6(2): 373-379.

Nawaz R, Ahmed R, Akter P, Nawaz M, 1996. Residues of sulfadimethoxine in blood, eggs and tissues of poultry birds. Pakistan Veterinary Journal. 16: 181–185.

Nisha AR. 2008. Antibiotic residues-A global health hazard. Veterinary World. 1(12): 375-377.

Nonga HE, Mariki M, Karimuribo ED, Mdegela RH. 2009. Assessment of antimicrobial usage and antimicrobial residues in broiler chickens in Morogoro Municipality, Tanzania. Pakistan Journal of Nutrition. 8(3): 203-207.

Nouws JFM, Ziv G. 1978. Pre-slaughter withdrawal times for drugs in dairy cows. Journal of Veterinary Pharmacology and Therapeutics. 1: 47-56.

Obasohan EE. 2009. Pollution in aquaculture: Chemical health concerns of aquaculture system in Nigeria. African Scientist. 10(2): 117-122.

Olatoye IO, Basiru A. 2013. Antibiotic usage and oxytetracycline residue in African Catfish (*Clarias gariepinus* in Ibadan, Nigeria). World Journal of Fish and Marine Sciences. 5(3): 302-309.

Papich MG, Korsrud GO, Yates WD, MacNeil JD, Janzen ED, Cohen RD, Lardy DA. 1993. A study of the disposition of procaine penicillin G in feedlot steers following intramuscular and subcutaneous injection. Journal of Veterinary Pharmacology and Therapeutics. 16: 317- 327.

Parvej M. 2013. Intensity of use of antimicrobial drugs and their residues in broiler chicken, Chittagong, Bangladesh. M.Sc.Thesis, Department of Physiology, Biochemistry and Pharmacology. Chittagong Veterinary and Animal Sciences University, Chittagong. [cited 2014 Dec 8]. Available from: <http://www.cvasu.ac.bd/>

Pavlov AI, Lashev L, Vachin I, Rushev V. 2008. Residue of antimicrobial drug in chicken meat.Trakia Journal of Sciences. 6(1): 23-25.

Pena A, Lino CM, Alonso R, Barcelo D, 2007. Determination of tetracycline antibiotic residues in edible swine tissues by Liquid Chromatography with spectrofluorometric detection and confirmation by mass spectrometry. Journal of Agricultural and Food Chemistry.55: 4973-4979.

Popelka P, Nagy J, Germuska R, Marcincak S, Jevinova P, Rijk DA. 2005. Comparison of various assays used for detection of β-lactam antibiotics in poultry meat. Food Additive and Contaminants. 22(6): 557-562.

Rana R. 1988. Residues of sulphaquinoxaline in poultry product. M.Sc. Thesis. Department of Veterinary Pharmacology, University of Agriculture,Faisalabad. [cited 2014 Apr 20]. Available from: <http://www.uaf.edu.pk/>

Rasul A. 2012. Quantitation of oxytetracycline residue in the common cultured fishes of Bangladesh by analytical high performance liquid chromatography. MSc. Thesis. Department of Pharmacy, East-West University, Bangladesh.

Reyes-Herrera I, Schneider MJ, Cole K, Farnell MB, Blore PJ, Donoghue DJ. 2005. Concentrations of antibiotic residues vary between different edible muscle tissues in poultry. Journal of Food Protection. 68: 2217-2219.

Salama NA, Abou-Raya SH, Shalaby AR, Emam WH, Mehaya FM. 2011. Incidence of tetracycline residues in chicken meat and liver retailed to consumers. Food Additives and Contaminants: Part B. 4(2): 88-93.

Salem DA. 2004. Monitoring of some antimicrobial residues in chicken from Assiut, Egypt. Environmental Encyclopedia of Assiut University. [cited 2015 Mar 19]. Available from: <http://www.aun.edu.eg/env_enc/ee2004/139-192%28end_p%29.pdf>.

Sanderson MG, Jones CD, Collins WJ, Johnson CE, Derwent RG. 2003. Effect of Climate Change on Isoprene Emissions and Surface Ozone Levels. Geophysical Research Letters. 30(18): 1936.

Sarmah AK, Meyer MT, Boxall AB. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and eﬀects of veterinary antibiotics (VAs) in the environment. Chemosphere. 65(5): 725-759.

Sattar S, Hassan MM, Islam SKMA, Alam M, Faruk MSA, Chowdhury S, Saifuddin AKM. 2014. Antibiotic residues in broiler and layer meat in Chittagong district of Banglades. Veterinary World. 7(9): 738-743.

Sattar S. 2012. Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. M.Sc.Thesis, Department of Physiology, Biochemistry and Pharmacology. Chittagong Veterinary and Animal Sciences University, Chittagong. [cited 2014 Dec 12]. Available from: <http://www.cvasu.ac.bd/>

Schulman LJ, Sorgent EV, Naumann BD, Faria EC, Dolan DG, Waego JP. 2002. A human health risk assessment of pharmaceuticals in aquatic environment. Human and Ecological Risk Assessment. 8(4): 657-680.

Shamsuzzaman MM, Biswas TK. 2012. Aqua chemicals in shrimp farm: A study from south-west coast of Bangladesh. Egyptian Journal of Aquatic Research. 38(4): 275-285.

Shareef AM, Jamel ZT, Yonis KM. 2009. Detection of antibiotic residues in stored poultry products. Iraqi Journal of Veterinary Sciences. 23(1): 45-48.

Sutiak V, Sutiakova I, Korenek M, Krokavec P, Kozak M, Saly J, Neuschl J. 2000. Current problems with drug use and the need for their solution in poultry and some other animals. In: Proceedings of Lectures and Posters of the International Conference. Hygiena Alimentorum. XXI: 113-115.

Tajick MA, Shohreh B. 2006. Detection of antibiotics residue in chicken meat using TLC. International Journal of Poultry Science. 5(7): 611-612.

Tendencia EA, de la Pena LD. 2001. Antibiotic resistance of bacteria from shrimp ponds. Aquaculture. 195(3): 193-204.

Thangadurai S, Shukla SK and Anjaneyulu Y. 2002. Separation and detection of certain beta- Lactam and fluroquinolone antibiotic drugs by thin layer chromatography. Analytical Sciences. 18:97-101.

Veterinary Residues Committee (VRC). 2005. Annual Report on surveillance for veterinary residues in Food in the UK. 11pp. [cited 2014 May 20]. Available from:<http://collections.europarchive.org/tna/20100907111047/vmd.gov.uk/vrc/reports/vrcar2005.pdf>.

Wang JH, Chao MR, Chang MH, Kuo TF. 2009. Liquid chromatographic determination of amoxicillin residues in grouper muscle following oral administration of the veterinary drug. Taiwan Veterinary Journal. 35: 21-28.

Woodward KN. 1991. Hypersensitivity in humans and exposure to veterinary drugs. Veterinary and Human Toxicology. 33(2): 168-172.

World Health Organization (WHO). 2006. Antimicrobial use in aquaculture and antimicrobial resistance. [cited 2014 May 20]. Available from: <http://www.who.int/topics/foodborne_diseases/aquaculture_rep_13_16june2006%20.pdf>.

Yibar A. Centinkaya F. Soyutemiz GE. 2011. ELISA screening and liquid chromatography-tandem mass spectrometry confirmation of chloramphenicol residues in chicken muscle, and the validation of a confirmatory method by liquid chromatography-tandem mass spectrometry. Poultry Science. 90: 2619-2626.

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# Annex-I: Procedure of Thin Layer Chromatography (TLC)

## Procedure of Thin Layer Chromatography:

## 1. Preparation of Trichloroacetic acid (30%)

Thirty gm of solid crystal trichloroacetic acid was weighed and diluted with distilled water in 100 ml volumetric flask up to 100 ml mark level with proper care.

## 2. Preparation of TLC silica plates

Commercially available pre-coated TLC plates (Silica gel 60 F254) which are 20X20 cm in size (Merck, Germany) were used because these plates are ideal for research, easy to handle and free from hazards for preparation. The TLC plate cuts into 05X10 cm in size.

## 3. Standard preparation

Antimicrobial extracted residues with routinely used seven antimicrobials such asCiprofloxacin, Oxytetracycline, Amoxicillin, Enrofloxacin, Doxycycline, Erythromycin and Sulfachlorpyradazine were prepared by dissolving 0.1 g of powder in 4 ml methanol.

## 4. Preparation of solvent

In order to perform TLC along with stationary phase or adsorbents, a mobile phase or solvent preparation was prepared as directed in the references (Thangadurai et al.,2002). For this, 50ml of methanol and 50 ml of acetone were mixed properly to form a mobile phase.

## 5. Sample processing

The sample processing procedure has been given in the section of Methods and Materials (3.9.1)

## 6. Pointing of sample or standard on TLC plate

In order to point pre-coated TLC plates were cut according to the shape of TLC tank with scissors and pointing was done as follows:

* At first a line was drawn on TLC plate 1 inch above the edge of the plate and then
* With capillary tube standard solution was pointed on this line about 2 cm distance from each other with proper care about 2-3 mm in diameter. Sample was spotted with capillary tube on the drawn line of TLC plate. After drying of spot sample, the plate was placed in TLC tank and allowed for running.

## 7. Running of TLC

The duration of mobile phase to run was 25 minutes for each plate placed in the TLC tank, thus, maintaining a continuous flow of the mobile phase along the stationary phase to separate antimicrobials (Thangadurai et al.,2002).

## 8. Examination of chromatogram under UV detector

In this TLC, the chromatogram was examined under the ultraviolet lamp at 256 nm for spots i.e., spot that fluorescence. The outline of the spot was marked with a series of dots using a sharp pencil. The colour of each fluorescent spot was recorded on a separate paper.

## 9. Determination of Retardation factor (RF) value

To define the relative migration rate of substances under various conditions retardation factor is determined. It is the ratio of distance moved by the substance and distance moved by solvent (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 and calculation of Rf values was done using the following equation:

Distance moved by the substance

Rf =

Distance moved by solvent

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

## 10. Interpretation of results

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 (Ghani, 2005). That is, after comparison of two substances (standard and unknown sample) based on following criteria, a sample was positively identified such as:

* Same colour under UV light
* Same colour with the spray reagent
* Same Rf value as those of the reference sample

# Annex-II: Questionnaire of baseline information on wet market commercial broiler poultry and drugs used at different markets of Chittagong metro city

1. Date of data collection :…………………..…………………………………..….
2. Name of the market:…………………………………; Longitude………..……. Latitude………………………
3. Identity no of broiler outlet:………Name of broiler outlets(If given)…………
4. Owner’s name:………………………………Address:………………………….

…………………………………………………. Cell no:……………………..

1. Total no of broiler:………………; Strain:-------------; Age of birds:--------------;
2. No. of birds sold out per day:…………No of surplus unsold birds:-------------;
3. Name of the farm or area from where the birds are supplied from: Farm:…………………………; Area:---------------
4. Any drugs used during the stay (day and night) at outlet? Yes / No

If yes, what are those drugs? Drug details:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SI No** | **Name of drug** | **Company name** | **Dose and route** | **Frequency** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

1. Additional information:………………………………………………………

Name of interviewer:

Signature:

# Annex-III: Questionnaire of baseline information on wet market fish at different markets of Chittagong metro city

1. Date of data collection:……………………………….…………………………
2. Name of the Market:…………………………………………..………………… Longitude……………………; Latitude……………………
3. Identity no. of fish outlet:………………Name of fish outlets(If)………………
4. Owner name:……………………………………. Address:……………………

……………………………………………….. Cell no:………………..………

5. Types of fishes at outlet:…………………………………………………………

6. Total amount of fish (kg):………kg of fish sold out per day:…………Surplus:...

7. Name of the farm or area from where the fishes one supplied from……………

8. Additional information:………………………………………………………….

Name of interviewer:

Signature:

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# Annex-VI: Graphical presentation of concentration of antimicrobial residues by UHPLC



Figure 2. Graphical presentation of concentration of oxytetracycline residue by UHPLC

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Figure 3. Graphical presentation of concentration of amoxicillin residue by UHPLC

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Figure 4. Graphical presentation of concentration of ciprofloxacin residue by UHPLC

# Brief biography

Farzana Rabbi passed the Secondary School Certificate Examination in 2004 followed by Higher Secondary Certificate Examination in 2006. She obtained her Doctor of Veterinary Medicine Degree in 2011 (held in 2013) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Pharmacology under the Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, CVASU. She published one scientific article in national journal. She has immense interest to work in mass spectrometry based antimicrobial residues in poultry and fish.