Assessment of temporal pattern of antimicrobial usage in commercial broiler farms in Cumilla, Bangladesh

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A Thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Epidemiology

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List of Abbreviations

Abbreviations	Elaborations
%	Percentage
<	Less than
>	Greater than
ADD	Animal Daily Dose
AM	Antimicrobials
AMR	Antimicrobial Resistance
AMU	Antimicrobial Usage
ATC	Anatomical Therapeutic Chemical
ATD	Animal Treatment Days
BDT	Bangladeshi Taka
BPICC	Bangladesh Poultry Industries Central Committee
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CRD	Chronic Respiratory Disease.
DCD	Defined Coursed Dose
DCD_{vet}	Defined Course Doses in animal
DDD	Defined Daily Dose
DDD_{vet}	Defined Daily Doses in animal
DLS	Department of Livestock Services
DURG	Drug Utilisation Research Group
ECDC	European Centre for Disease Control
EMA	European Medicines Agency
ESFA	European Food Safety Agency
ESVAC	European Surveillance for Veterinary Antimicrobial Consumption
EU	European Union
FAO	Food and Animal Organization
GDP	Gross Domestic Product
IBD	Infectious Bursal Disease
IBH	Inclusion Body Hepatitis
IEDCR	Epidemiology Disease Control and Research
Kg	Kilograms
LMIC	Low and Middle Income Countries

Abbreviation	Elaboration
MDR	Multi-drug Resistant
Mg	Milligrams
N/A	Non-applicable
N/D	Non-descriptive
ND	Newcastle Disease
nDCD	Number of Defined Course Dose
nDDD	Number of Defined Daily Dose
nDDD	Number of Defined Daily Dose
nDDDvet	Number of Defined Daily Dose in animals
NMD	Norwegian Medicinal Depot
OIE	Office International des Epizooties/World Organisation for Animal Health
PCU	Population Correction Unit
PDD	Prescribed Daily Dose
PHAC	Public Health Agency of Canada
QS	Qualität und Sicherheit GmbH
SDG	Sustainable Development Goal
TF	Treatment Frequency
UDD	Used Daily Dose
US CDC	United States Centers for Disease Control and Prevention
USA	United States of America
VCIA	Veterinary Critically Important Antimicrobial Agents
VHIA	Veterinary Highly Important Antimicrobial Agents
VIA	Veterinary Important Antimicrobial Agents
WHO	World Health Organization

Abstract

Occurrence of infectious poultry disease is one of the main constraints of Bangladesh's growing commercial poultry sector which leads to misuse of antimicrobials for prophylactic and therapeutic purposes. Antimicrobial usage (AMU) in food-producing animals is a possible factor promoting antimicrobial resistance in both veterinary and human health sectors. A longitudinal study was therefore conducted to evaluate antimicrobial usage in the broiler production period on 40 commercial exotic small- to medium-scale broiler farms in eight sub-districts (Upazilla) of Cumilla, Bangladesh from January to March 2020. Farms were chosen purposively. Antimicrobial drug usage data was collected through over phone communication (every day) and repeated farm visit (at 3 days interval). Demographic and management data were obtained through a structured questionnaire for farmer as well as through farm observation. Descriptive analysis was carried out by using STATA-14. Flock-level AMU was characterized using qualitative and quantitative approach. All broiler farms used antimicrobials (AMs) and diverse AMU patterns were identified as 154 treatment courses (median 4; 95% CI: 3.4-4.5) of AM were administered in the studied broiler farms. AMs were used for prevention of disease or disease conditions by all but one farm. AMs were administered in the farms for prophylaxis (n=74, 48.1%), therapeutics (n=68, 44.2%), growth promotion (n=1, 0.7%), prophylaxis and growth promotion (n=7, 4.6%) and both prophylaxis and therapeutics (n=4, 2.6%). Despite government rules, AMs were suggested and used without any veterinary consultation, mostly by dealers and farmers. Descriptive AMU analysis provided the following results: 1) of the 154 total antimicrobial courses, fluoroquinolones were the most commonly (n=44, 28.6%) used AM group; followed by penicillins (n=29, 18.8%), tetracyclines (n=12, 7.8%) and sulfonamide-coccidiostats (n= 12, 7.8%). Chosen quantitative metrics yielded the following results: 1) calculated total AMU (according to milligrams per population correction unit (mg/PCU) metric) was 130.9 mg/PCU. Farm level median mg/PCU was 98.2 mg/PCU (range 4.4mg/PCU to 618.9 mg/PCU). Penicillins (29.5mg/PCU), fluoroquinolones (24.8mg/PCU) and sulfonamide-coccidiostat mixed preparations (13.5mg/PCU) were the most commonly used AM according to this metrics. 2) Total 221.4 number of defined daily dose per population correction unit (nDDD/PCU) (farm level median 3.1, range 0.3 to 21.7, 95% CI: 3.7-7.3) and 49 number of defined course dose per population correction unit (nDCD/PCU) (median 1.1, range

0.5 to 4.6, 95% CI: 0.8-1.6) of AM were used in the studied farms. Depending on the metrics chosen, variations were observed in temporal trends (change of mg/PCU in different weeks of production period was evidently steadier in curves than crude amount) and relative ranking (of descriptive, weight-based and dose-based-metrics). When using frequency measures, top three AMs were amoxicillin, enrofloxacin and doxycycline. Dose-based metrics, nDDD/PCU and nDCD/PCU showed the same top three AM patterns. However, while using mg/PCU metrics, the top three AMs used were amoxicillin, tylosin and neomycin. The choice of the AMU metric is an important consideration for any AMU reporting. Understanding the effects of parameters used in AMU reporting would help in a better reporting and would allow stakeholders to understand better the impacts of AMU and formation and evaluation of AMU reduction strategies.

This study implied a high level of AMU, especially medically important AMs in broiler farms, and it requires immediate intervention. Rules and regulations should be strictly followed in trade of AMs. Continuous research works are needed to improve stewardship and provide better monitoring of AMU and subsequent antimicrobial resistance (AMR) situation. Awareness programs should be arranged for the farmers and relevant stakeholders on risk of indiscriminate AMU and AMR.

Keywords: Antimicrobial usage, broiler, Cumilla, Bangladesh.

Chapter I: Introduction

Bangladesh government sets a target to attain Sustainable Development Goals (SDG) by 2030 and to promote to be a developed country by 2041 (UN, 2020). Achieving food security, ending hunger, improving nutrition and promoting sustainable agriculture are required to fulfill those targets. Poultry sector as a subsector of livestock production can provide the nation with an excellent low-cost source of good quality nutritious animal protein by means of meat and egg; and can play a vital role in economic development and subsequently provides employment opportunities (Das et al., 2008). Current poultry population in Bangladesh is 365.9 million, in which chicken contributes to 304.1 million (DLS, 2021). Also, 2.1 billion eggs were produced in 2020-21, which ensured 126 egg/person/year (DLS, 2021). Approximately poultry supplies 20% of consumable protein in Bangladesh (Das et al., 2008). With a GDP value worth of 503.0 billion taka, livestock sector along with poultry sector provides 1.4% annual GDP (with 3.8% GDP improvement) (DLS, 2021). Livestock contributes 13.1% of total agricultural GDP of the country (DLS, 2021). 20% of total populations are directly and 50% is indirectly dependent on livestock production (DLS, 2021). Investment on poultry sector in 2019 was equivalent to 3.7 billion Euros (RVO, 2020).

Poultry sector in Bangladesh can be divided into four segments i.e. broiler, sonali, layer and backyard deshi (indigenous); among which broiler is most reared (RVO, 2020). There are 3 different scales of commercial broiler chickens: i) small-scale (≤500), ii) medium-scale (501-5000) and large-scale (>5000) (Rahman et al., 2019).

There are about 206 breeder farms and hatcheries, 16 grandparent stock farms, 65-70 thousand commercial layer and broiler farms, 198 registered feed mills with 5.5 million metric tons of annual feed production and 500 animal health companies in this country (WPSA, 2020).

The biggest challenge for commercial chicken producers is the occurrence of diseases; reported both in Bangladesh (FAO, 2022) and worldwide (FAO, 2009). Common poultry diseases that affect the broiler poultry sector in Bangladesh are Colibacillosis, Salmonellosis, Newcastle Disease (ND), Infectious Bursal Disease (IBD), Infectious

Bronchitis, Chronic Respiratory Disease (CRD), Coccidiosis, Infectious Coryza etc. (Roy et al., 2012, Islam at al., 2014b; Badruzzaman, et al., 2015; Rahman et al., 2019). To check disease and disease conditions farmers opt to use antimicrobials.

Antimicrobials in commercial chickens in Bangladesh and other countries are used for different purposes: i) as prophylaxis, ii) to treat infections and iii) as growth-promoter agents (Molla et al., 2003; Oyekunle et al., 2003; Paintsil et al., 2021), because of intensive production system like other food-producing animals which require mass medication (Timmerman et al., 2006). Poultry production is going to increase in the near future to meet rising demand for animal proteins, causing a rapid raise in antimicrobial usage (Van Boeckel et al., 2015).

Antimicrobial usage (AMU) monitoring is a critical step in controlling emergence and dissemination of antimicrobial resistance (AMR) (Mevius et al., 1999; Gyssens, 2001), because AMU in animal can lead to AMR situation in human (Marshall et al., 2011). The world health organization (WHO) recommended a unified approach for AMU surveillance (WHO, 2016), which was supported by OIE (OIE, 2021). As of now, OIE surveillance system is not fully integrated, as 30 of 182 OIE member countries yet to respond about AMU in the food producing animal production (OIE, 2021b). AMU surveillance unit was formed by different countries as USA, Germany, Canada, Belgium and Morocco (Rahamatullah et al., 2018). In Bangladesh, Institute of Epidemiology Disease Control and Research (IEDCR) in collaboration with the United States Centers for Disease Control and Prevention (US-CDC) works on AMR surveillance on human. Department of livestock services (DLS) in collaboration with the Fleming fund Bangladesh has started working on AMU and AMR surveillance on two districts in Bangladesh from 2022. But there is no established nationwide AMU and AMR surveillance for human and animal in Bangladesh.

Two types of approaches are used to calculate AMU worldwide, i.e. i) descriptive approach and ii) quantification methods. Quantification methods include financial units (cost analysis), commercial units (sales data), weight indicators and dose metrics (farm and individual level) (Chauvin et al., 2001). However, there are not many published studies that focused on AMU and consumption in Bangladesh. Studies mainly focused on cross-sectional one off approach and calculated frequency distributions and factors affecting AMU. Commonly used antimicrobials in commercial broiler chicken in Bangladesh were amoxicillin, oxytetracycline, ciprofloxacin, enrofloxacin, doxycycline, erythromycin, neomycin, tiamulin, colistin sulfate and sulfa drugs. (Islam et al., 2016; Rahman et al., 2017; Ferdous et al., 2019; Islam et al., 2020; Imam et al., 2021). Farmers tend to use antimicrobials on veterinarians prescription as well as based on self-medication, veterinary field assistant, technicians and local vendors suggestion (Islam et al., 2016; Tasmim et al., 2020).

Quantitative approach to compute AMU requires suitable numerators (weight measures, treated animals, treatment courses etc.) and denominators (weight, defined doses, animal at risks etc.) and based on the denominator chosen, weight-based and defined-dose based metrics were used in previous studies worldwide (Jensen et al., 2004; Cuong et al., 2018). Animal at risk and treatment frequencies are also counted in previous studies (Agunos et al., 2017; Kasabova et al., 2021).

Data in low and low middle income countries (LMIC) are very scarce. A systematic review showed that only 17 out of 89 published research papers containing AMU data was from LMICs, among which only 7 have quantitative evaluation (Cuong et al., 2018). No publication from Bangladesh on quantitative approach is found, and the descriptive studies followed cross-sectional and often retrospective approach. With the aforementioned background, this longitudinal study on 40 commercial exotic broiler farms was designed with these following objectives:

1. Asses the pattern of antimicrobials usage during a production cycle of broiler farms of Cumilla, Bangladesh
2. Estimate antimicrobial usage by weight based and dose based metrics in broiler farms of Cumilla, Bangladesh

1.1. Outcomes

1. Identified antimicrobial usage pattern during a production cycle of broiler farms of Cumilla, Bangladesh
2. Quantified the consumption level of antimicrobials in broiler chickens during a production cycle of broiler farms in Cumilla, Bangladesh

Chapter II: Review of Literature

The overall goal of this chapter was to review past relevant research findings related to the Master's project **"Assessment of temporal pattern of antimicrobial usage in commercial broiler farms in Cumilla, Bangladesh"** to identify the gaps and justify the present research. Published literatures were obtained by searching online sources like PubMed, Google Scholar and Web of Science. This chapter is arranged in a series of sections including a review of literatures on Bangladesh poultry production, challenges of broiler farming, antimicrobial usage, and antimicrobial quantification metrics.

2.1. Poultry production in Bangladesh

Bangladesh is a densely populated country, ranks within top 10 of the most populous countries in the world. As about 71% of the population lives in rural areas (BBS, 2020), its economy heavily relies on agriculture. The majority of people are engaged in agricultural operations as crop farming, livestock and poultry rearing and fish farming. Poultry production has become popular due to its quick economic benefit, generation of employment and production of cheaper animal protein (Raihan et al., 2008; Rahman et al., 2017). A study shows that 28% of Bangladeshi households raise chicken as their main animal (FAO, 2022). Poultry production sub-sector also plays a vital role in serving a prominent part of human nutrition. Poultry meat and eggs supplies approximately 20% of the protein consumed in developing countries (Alders and Pym, 2009). Poultry meat contributes 37% of total meat production of livestock origin in Bangladesh (WPSA, 2020). The poultry products provide 22–27% of the total human protein demand in Bangladesh (Prabakaran, 2003).

Bangladeshi poultry rearing system can be divided into four segments: i) backyard deshi, ii) broiler, iii) layer and iv) sonali production. In most low-income and food deficit countries, rural household poultry production contributes 70% of total production (Branckaert et al., 2000). Backyard poultry production is confined in the rural households depending on scavenging feed resources and can survive in low-nutrition and harsh environmental conditions (Barua et al., 1997; Chowdhury, 2013). Backyard poultry had a low level of productivity (Sazzad et al., 1990); yet was the sole medium of meeting producers' family demand before industrialization (Ahmed and Islam, 1985).

Introduction of high yielding breeds/varieties/strains, adoption of modern and scientific housing and technologies, gradually improving marketing system, increasing government supports, involvement of national and international organizations and entrepreneurs, the change in socioeconomic status, raising income and urbanization of the country had helped in a great shift in poultry sector (Raha, 2013; Islam et al., 2014a; Howlader et al., 2022); and commercial poultry production had proceeded to industrialization in last decade (Das et al., 2008). Broiler meat has become popular due to its tenderness, palatability and digestibility. Due to shorter production cycle (30-35 days), low capital investment and quick return, commercial broiler farming has become a ready source of income and has provided self-employment for educated unemployed youth (Bhende, 2006; Rahaman et al., 2006; Kawser et al., 2013; Chowdhury et al., 2015). Bangladesh Poultry Industries Central Committee (BPICC) stated that the poultry sector had an investment worth about BDT 35,000 crore and had generated employment for over 6.0 million people, majority of them being unemployed youth and women (Saleque et al., 2020). Commonly available broiler chicken strains in Bangladesh are Cobb500, Ross 308, Habbard, Indian River meat, Tiger Sasso and Arber acre. And Hy-line Brown/White, ISA Brown, Novogen Brown/White, Shaver 579, HiSex Brown/White, and Bovine White are the commonly available layer strains. These strains are produced in 206 registered breeder and hatcheries from the parent stocks imported or produced from 16 grandparent stocks by 8 companies (WPSA, 2020). The commercial broilers and layers are reared in around 65-70 thousand commercial farms, and the feed supplies are ensured by 198 registered feed mills with 5.5 million tons annual feed production. There are 500 animal health companies in the country (WPSA, 2020).

2.2. Challenges of poultry farming

After two decades of exponential growth (15-20% annually), poultry sector in Bangladesh faced extreme loss due to avian influenza outbreak in 2007-08. With 60% of the commercial farms and 70% of hatcheries being closed in 2007-08 (Mamun, 2019), the industry suffered a loss worth 700 crores in that year (Islam et al., 2014a). The consequence of the outbreak carried through several years as studies reported approximately 35,000 small and medium scale farms were closed within 2011 (Chand et al., 2009; Chowdhury, 2013).

Like many other countries, disease and disease outbreaks are the most common challenges in poultry sector in Bangladesh. In previous studies, 31% of the respondents from Khulna and Rangpur (FAO, 2022) and 72.2% Sonali farmers from Barishal (Howlader et al., 2022) reported disease as a constraint for poultry. Commonly reported poultry diseases in Bangladesh were salmonellosis, colibacillosis, mycoplasmosis, infectious coryza, fowl cholera, necrotic enteritis, infectious bursal disease, Newcastle disease, avian influenza, infectious bronchitis, avian leucosis and fowl pox (Roy et al., 2012, Islam at al., 2014b; Badruzzaman, et al., 2015; Rahman et al., 2019).

In overall, current poultry sector development in Bangladesh hindered by constraints include disease outbreaks (because of insufficient veterinary, technology and laboratory services; unorganized and poor animal health and disease control policies; lack of policy implementation; poor biosecurity standards at farm level and seasonal fluctuation of temperature and humidity), excessive production costs (due to strong dependency on imported ingredients, lack of quality feed ingredients, lack of control and monitoring monopoly business), an underdeveloped marketing chain resulting in unstable meat and egg price (scarcity of modern slaughtering and cool chain maintenance facilities, lack of control over live bird market) and absence of access to finance and credits (particularly for small and medium-sized enterprises; which are heavily full or partial credit based and feed and chick dealer dependent) (Raihan et al., 2008; Kawsar et al., 2013; Rahman et al., 2015; Masud et al., 2020; RVO, 2020; Howlader et al., 2022).

To overcome those problems, especially disease and disease outbreaks, out of fear of economic losses, and to satisfy growing demand, farmers often rely on excessive use of AMs as growth promoter (Molla et al., 2003; Kusiluka et al., 2005; Islam et al., 2016), preventive as well as treatment measures, often without consulting veterinarians (Kusiluka et al., 2005; Nonga et al., 2008; Islam et al., 2016; Rahman et al., 2019).

2.3. Antimicrobial usage and antimicrobial resistance

Antimicrobials (further termed as AM in this literature) are used worldwide both in humans and in animals for the prevention and treatment of infectious diseases (O'Neil et al., 2014), and sometimes as growth promoters (Page and Gautier, 2013). In 1999, Europe scheduled a total ban on antimicrobials use as growth promoters by January 2006 (Persoons et al., 2012). Previous studies established that there was a correlation between antimicrobial usage (AMU) and antimicrobial resistance (AMR) in animal production (Asai et al., 2005; Burow et al., 2014, Chantziaras et al., 2014; Simoneit et al., 2015). AMR emergence affects livestock production as the animals become more prone to multi-drug resistant (MDR) bacteria and thus reduces productivity and increases treatment cost (Tang et al., 2017).

Transmission of antimicrobial resistant microbes (both pathogenic and non-pathogenic) from animal to animal or animal to human can be occurred through direct contact with animals and animal wastages or through contaminated foods (Marshal et al., 2011; Hoelzer el al., 2017). Fomites and dusts can be a good media for bacterial transmission (Gerd et al., 2003; Shawn et al., 2006). Even farm-to-farm spread of *Salmonella* through shared farm equipment was reported in Denmark (Holzbauer and Gautier, 2006). Remnants of antimicrobial residues in manure/wastage and the use of contaminated litter in fertilization permits the storage of drugs in soil, and the transmission to ponds, wells and the surface water (Phuong Hua et al. 2011; Meyer et al. 2013). Human consumption of livestock product and by-products rich in antimicrobial residues may lead to AMR in humans. Anaphylactic reaction due to consumption of penicillin-treated chicken was reported in UK (Teh and Rigg, 1992). Microbial resistance to veterinary drugs are becoming common and posing public health threats day by day (Wegener, 2003; Ferri et al., 2017). However, evidences of reduction of prevalence of AM resistant bacteria in both human (24%) and animals (15%) due to reduction of AMU are found (Tang et al., 2017).

World Organization for Animal Health (OIE: Office International de Epizooties) recommended a list of antimicrobials for veterinary use. Antimicrobial agents are classified into three categories; Veterinary Critically Important Antimicrobial Agents (VCIA), Veterinary Highly Important Antimicrobial Agents (VHIA), and Veterinary Important Antimicrobial Agents (VIA) (OIE, 2015). OIE also recommended avoiding antimicrobial for prophylactic purposes in the absence of clinical signs in the animals (OIE, 2020). WHO set up the classification of AMs used human medicine based on prioritization as highest priority critically important antimicrobials (as 3rd, 4th and 5th generation cephalosporin, macrolides, polymixins quinolones and fluoroquinolones), high priority critically important antimicrobials (as aminoglycosides, aminopenicillins), highly important antimicrobials (1st and 2nd generation cephalosporin, sulfonamides, tetracyclines) and important antimicrobials (nitroimidazole) (WHO, 2018). The European Medicines Agency (EMA) classified antibiotics into four categories considering AMR spreading from animals to humans. Category A (Avoid group; AMs authorized for human medicine, no veterinary authorization; as carbapenems, fosfomycin). Category B (Restrict group; critically important for human health; as quinolones and fluoroquinolones, the 3rd and 4thgeneration cephalosporins and polymyxins). Category B should be prescribed when no other alternative antibiotics in Categories C (Caution group; as aminoglycosides, macrolids) or D (Prudence group; as imidazole, tetracylines, narrow spectrum penicillins) are found effective (EMA, 2019). Unnecessary use and unnecessarily long treatment periods were suggested to be avoided, and group treatment should be restricted to situations where individual treatment is not feasible (EMA, 2019).

An Food and Agriculture Organization (FAO)-World health Organization (WHO) joint report has projected a global increase in annual meat production from 218 million tons in 1997-1999 to 376 million tons by 2030 (FAO and WHO, 2003). AMU in animal production is estimated to be increased 67% in between 2010 and 2030 in low- and middle-income countries (LMICs) due to a shift in intensive production practices and increased animal protein demand (Van Boeckel et al., 2015).

Considering the imminent threat and risk of AMR on human health, scientific consensus to study and evaluate the impact of the AMU/AMR in livestock production and the probable counter-measures has emerged in recent years. Any endeavor to reduce AMR must include the containment policy to reduce AMU, and hence AMU quantification is necessary.

2.4. Antimicrobial usage calculation- overview

To fight the emergence of AMR situation, the WHO's 'Global action plan on antimicrobial resistance' recommended coordinated monitoring and harmonized surveillance system as well as setting up internationally standardized data collection and reporting system for human, medical, veterinary and agricultural sectors (WHO, 2016). OIE also recommended a consolidated single programme to facilitate comparative risk analysis among medical, food-producing animal, agricultural and other AMU data, which would promote optimal AMU in all sectors (OIE, 2021).

Global data collection and reporting system on veterinary AMU are not established yet. But works are on progress in different part of the world. European Medical Agency (EMA) established a project named European Surveillance for Veterinary Antimicrobial Consumption (ESVAC). Several members of European Union (31 members in 2019-20) routinely report total annual AM sales data as milligrams of active ingredients adjusted by population correction unit (PCU) to ESVAC (EMA, 2021). Government of Canada developed a federal action plan in 2015. Public Health Agency of Canada (PHAC) established 'Canadian Integrated Program for Antimicrobial Resistance Surveillance' (CIPARS) which worked in coordination with federal action plan as well as followed WHO and OIE guideline (Agunos et al., 2017). Other organizations actively working on AMR and AMU data surveillance are National Animal Health Monitoring System (NAHMS, established in 2016) in the United States, Danish Programme for surveillance of antimicrobial consumption and resistance in bacteria from animals (DANMAP, established in 2016), German programme for monitoring the consumption of antimicrobials and the extent of resistances against antimicrobials in human and veterinary medicine (GERMAP, established in 2016) and Qualität und Sicherheit GmbH

(QS, established in 2012), and Office National de Sécurité Sanitaire des Produits Alimentaires (ONSSA, established in 2015) in Morocco (Rahamatullah et al., 2018).

The total amount of AMs used globally for animal production has been estimated to be 63 thousand tons per year (Van Boeckel et al., 2015). European Centre for Disease Control (ECDC), European Food Safety Agency (ESFA) and European Medicines Agency (EMA) joint surveillance report (2021) stated that overall total AM consumption in food-producing animals (in tons of active ingredients) in 29 EU/EEA member countries is three times higher than the humans. AMU in food-producing animal production accounted for 70% of total annual AM consumption in 2014 in the USA (O'neill, 2014).

2.5. Antimicrobial usage calculation

Different drug consumption and usage quantifying units have been described in previous studies, including financial units (cost analysis), commercial units (sales data), weight indicators, dose metrics and descriptive units (Merlo et al., 1996; Chauvin et al., 2001).

2.5.1. Descriptive calculation

The magnitude of AMU in livestock production in Bangladesh is unknown (Khatun et al., 2016). Study on AMU is inadequate and available studies showed that majority (close to 100%) small and medium scale broiler farms used AMs for treatment and prevention, often with a multi-drug approach (Islam et al., 2016; Chowdhury et al., 2021; Tasmim et al., 2021). Commonly used AMs in broilers in Bangladesh were amoxicillin (5-33%), oxytetracycline (11-63%), ciprofloxacin (19-55%), enrofloxacin (18-55%), doxycycline (15-26%), erythromycin (26-38%), neomycin (38%), tiamulin (32%), colistin sulfate (15-65%), sulfa drugs (14-16.6%), sulfa-trimethoprim (26-41%) (Islam et al., 2016; Rahman et al., 2018; Ferdous et al., 2019; Islam et al., 2020; Imam et al., 2021). Farmers tend to use AMs on veterinarians (25.7- 38.4%) prescription as well as based on self-medication (16.4%), veterinary field assistant (24.7%), technicians (11%) and local vendors (9.6%) suggestion (Islam et al., 2016; Tasmim et al., 2021). **Table 2.1** shows descriptive AMU findings from previous studies in Bangladesh and other nations.

Farmer's knowledge and acceptance to withdrawal period is limited in Bangladesh. In a study, 94.2% of the layer farmers in Mymensigh area showed no interest to maintain withdrawal period (Ferdous et al., 2019). Farmers non-compliance to withdrawal period was also described in Tanzania (Nonga et al., 2008); Nigeria (Kabir et al., 2004) and Ghana (Boamah et al., 2016).

It also appears that antibiotics were prescribed solely on the experience of the veterinarian rather than using an established treatment protocol for each poultry disease at the hospital (Rahman et al., 2019).

Above-mentioned information showed a few studies attempted to calculate AMU in farm and veterinary hospital level with a cross-sectional descriptive approach which may cause recall bias due to retrospective nature. Hence, we designed this study to check farm-level AMU in broiler farms in Cumilla with a prospective longitudinal approach.

Table 2. 1: Descriptive antimicrobial usage findings from previous studies

Antimicrobials	Bangladesh	Tanzania	Nepal	Cameroon	China	Pakistan
Amoxicillin	5.5%-33%	--	--	5%	76%	--
Ampicilin	1.4%	--	--	--	--	--
Doxycycline	15%-26%	20%	15%-33%	25%	--	100%
Oxytetracycline	10%-83%	75%-90%	--	25%	38.6%	13%
Ciprofloxacin	19.2%-55%	--	3%	25%	--	--
Levofloxacin	12%	--	--	--	--	--
Azithromycin	1.4%	--	--	--	--	--
Cephalosporin	1.4%	--	--	--	--	--
Erythromycin	26-29%	--	--	5%	25%	
Enrofloxacin	17.5%-55%	--	4%-15%	25%	--	100%
Norfloxacin	2.8%	--	--	25%	47.7%	--
Pefloxacin	2.8%	--	--	--	--	--
Sulfa-drugs	14%-16.6%	20-85%	17%	40%	6-11%	--
Trimethoprim	26%	55%	--	20%	--	--
Sulfa-Trimethoprim	26%-41%	--	--	--	--	--
Gentamicin	7%	--	4%-13%	--	--	--
Neomycin	38%	25%	15%-33%	5%	--	63%
Colistin	15-67%	--	47%	15%	--	100%
Tylosin	13.7%	--	47%	--	--	100%

Antimicrobials	Bangladesh	Tanzania	Nepal	Cameroon	China	Pakistan
Flumequine	2.8%	10%-15%	--	25%	--	--
Tiamulin	32%	--	--	--	--	--
Metronidazole	4-36%	--	--	--	--	--
Cephasporins	1%	--	--	--	18.2%	--
Amprolium	--	35%-85%	--	--	--	--
Chlortetracycline	--	10%	--	--	3.4%	--
Chloramphenicol	--	10%	--	--	8%	--
Reference	(Islam et al., 2016; Rahman et al., 2018; Ferdous et al., 2019;Sabuj et al., 2019;Islam et al., 2020; Hassan et al., 2021; Imam et al., 2021)	(Nonga et al., 2008; Nonga et al., 2009)	(Koirala et al., 2021)	(Kamini et al., 2016)	(Xu et al., 2020)	(Mohsin et al., 2019)

2.5.2. Antimicrobial usage calculation- quantification metrics

Measuring AMU in animal production may look for different targets: AMU surveillance over time, benchmarks setting to control AMU, and investigating AMU-AMR correlation. Monitoring should aim:

- enabling intervention and control to guarantee compliance to established AMU policies and regulations;
- ensuring AMs are used responsibly;
- assisting in the data analysis from resistance-surveillance (Chauvin et al., 2006);
- producing data for studies on the usage conditions that control the selection and spread of AMR microorganisms and meeting consumers demand for transparency (Wadman, 2001); and
- providing important data for population-level resistance developing risk assessment (Nicholls et al., 2001)

Researchers often choose weight indicators, e.g. amount of active compound (gm), the defined daily dose (DDD) and the prescribed daily dose (PDD) (Jesnsen et al., 2004). Different quantification metrics considering terms, ***numerator*** (‘quantities/amounts of active ingredients used’ or ‘number of animals treated’ or ‘numbers of treatment courses/daily doses’ etc.) and ***denominators*** (population at risk as ‘numbers of animals grown/produced/present’ or ‘slaughter/sold/treatment/standardized body weight’ or ‘animal-time’) were used in previous studies (Cuong et al., 2018). For international human drug usage studies, the WHO has suggested the use of the anatomical therapeutic chemical classification system (ATC) and the defined daily dose system (WHO, 2001). International comparisons are possible with statistics based on total amount of the active ingredient and population size as denominator. When evaluating the overall weight usage of a therapeutic class, the assumption is made that each active compound has the same potency. However the daily dose metrics has a wide range depending on the active ingredient used (Jesnsen et al., 2004).

We didn’t find any Bangladeshi study dealing with farm-level AM quantification data till to date.

2.5.2.1. Defined daily dose and defined daily dose in animal

The WHO’s Drug Utilization Research Group (DURG) and Norwegian Medicinal Depot (NMD) developed a unique system called Anatomical Therapeutic Chemical (ATC) classification system for all the drugs, which was applicable internationally in drug utilization research. From there the measurement in DDD (Defined daily dose) was developed and WHO recommended using ATC/DDD system for drug utilization research (MacKenzie et al., 2005). DDD is termed as the assumed average maintenance dose per day for a drug, mainly in adults (when referred to body weight, an adult person at 70 kg) (Grave et al., 2004).

DDD is formulated based on a review of available information: recommended dosages by drug catalogues, published in scientific journals or major international textbooks; data on PDD (if available); established indication; or other DDDs within the same chemical subgroup (Jensen et al., 2004; Agunos et al., 2017). DDDs are often identical; however may vary based on route of administration (as parenteral versus oral) due to bioavailability. DDDs are revised every three years, and are not changed unless the difference is at least 50%. However, small amounts of change are often accounted in some very important drugs (Jensen et al., 2004). DDDs cannot be used to calculate drug use prevalence (patients’ number or population size) because the measure is influenced by various factors (Mantel-Teuweisse et al., 2001). Other dose dependent metrics are evaluated against DDD values to compute the assistance between defined dose and the used measure.

In veterinary sector EMA proposed a herd/flock level national surveillance framework which included census and multiple sampling surveys for the collection of AMU data from the member countries (EMA, 2016). EMA developed Defined Daily Doses in animal (DDD_{vet}) and Defined Course Doses in animal (DCD_{vet}) standards through ESVAC (EMA, 2016) which provides necessary guidance to estimate AMU over time, and it was suggested to calculate AMU for every species, though most of the reporting contained overall data for food-producing animals (Cuong et al., 2018). DDD_{vet} was also calculated and assigned in Canada (Agunos et al., 2017), Morocco (Rahamatullah et al., 2018), Norway (Grave et al., 2004), Belgium (Persoons et al., 2012) and Japan (Fujimoto et al., 2021); and often showed deviation from DDD_{vet} assigned by EMA as environmental, technological and other factors might influence daily dose.

2.5.2.1.1. Number of defined daily dose in animal

A formula of Number of Defined Daily Dose (nDDD) in human medication calculation was described by MacKenzie et al. (2005) which was later adapted and adjusted to compute $nDDD_{vet}$ in veterinary medicine (Collineau et al., 2017; Mills et al., 2018)

$$nDDD_{vet} = \frac{(Active\ antimicrobial\ ingredient(mg))}{(\text{Daily dose } (\frac{\text{mg}}{\text{kg}}) \times (\text{total weight})}$$

In modifications of this equation, DDD_{vet} is used as daily dose (mg/kg) and PCU standardized value suggested by ESVAC is used to calculate total weight (Firth et al., 2017; Abe et al., 2020; Ferroni et al., 2020; Merle et al., 2020; Khan et al., 2021). nDDD metric takes into animal weight and number of cattle in account. Availability of country or region wise dose (DDD_{vet} value) and weight (PCU value) would help to get more accuracy and compatibility in AMU computation.

2.5.2.2. Animal defined daily doses

The median maintenance dose for the main indication in a given species is defined as the Animal defined daily doses (ADD). The ADD is computed by multiplying the median value of the recommended dosage range by the frequency per day. ADD is a benchmark set by the Danish VetStat database (Jensen et al., 2004) and often defined as per kg body weight (ADD_{kg}), calculated by multiplication with a defined standard animal body weight (1 kg for poultry) for each the age-group. The VetStat developed ADDs for each species, considering animal weight. To verify the authenticity of those recommendations, PDD information were gathered from a group of experts and the results showed a minimal (≤10%) deviation of PDD from ADD in bovine and porcine and no deviation in poultry practice (Jensen et al., 2004). ADD approach is also used by Danish Integrated

Antimicrobial Resistance Monitoring and Research Programme (DANMAP) and Danish Veterinary and Food Administration (DVFA).

A previous study used the concept of treatment courses and ADDs to analyze the relation between disease occurrence (mastitis) and treatment courses in dairy cattle. However, the concept is not applicable in fast-growing animals (as poultry, fattening pigs) due to use of grouped and combined medication and variable dosing regimen and treatment course (Chauvin et al., 2001). The average ADD-per-animal can be used as a measure of a selective importance imposed on the herd (herd-exposure; in case of poultry, flock-exposure). As dose and course duration of treatment are not related and can vary considerably between practitioners, farmers and flocks; and over time (Chauvin et al., 2001), herd-exposure might be an alternative indicator to the number of animals treated (Jensen et al., 2004).

2.5.2.2.1. The number of animal defined daily doses

To calculate number of ADDs the following must be known: quantity of product, dosage of product per kg body weight and the weight of the animal at treatment. Number of ADDs can be calculated by this formula suggested by VetStat and used by VetStat, DANMAP, DVFA and other studies (Carsons et al., 2008; Trauffler et al., 2014; Dupont et al., 2015)

$$\text{nADD}=\frac{(Amount\ of\ product\ used)}{(Dose\ per\ kg\ body\ weight)\times(Standard\ body\ weight)}$$

Amount of product used= Total active ingredient used

Dose per kg body weight= ADD described

Standard body weight= Standard value provided by VetStat

2.5.2.3. Prescribed daily dose

This unit has been particularly useful in studies to determine prescribing patterns or when data is collected at the prescription stage. PDD is variable in species, among strains/verities, across countries. However, this variability is not reflected by the total weight or the DDD. The difference may be minimal in national level or within country; significant differences are observed when comparing among individual prescriptions and also with other countries (Harris et al., 1994). Given the fact that it is not a standard measurement, the PDD can be used appropriately in a second step to describe differences identified by the DDD (Merlo et al., 1996). Knowledge of the PDD:DDD ratio allows for the adjustment of DDD in individual studies (for a species/ strain/certain geographic area) (Harris et al., 1994).

The PDD exemplifies doctors' habits, not experts' opinions, and may differ for reasons such as a poor evaluation of the patient's weight, a default adaptation to packaging, or the use of a different therapeutic regimen, among many others. Calculating PDD is usually difficult because determining the treatment duration is complicated. Dosage regimens described on prescription data are not always precise even in human medicine. It is more difficult to ensure dosage regimens prescribed are followed when whole the flock is treated. A drug change in a treatment period may have an impact on the estimated number of patients, particularly for prescription medications used in short-term medications as AMs (Tamblyn et al., 1995). Only when the timeframe of treatment is known, number of treatment courses can be estimated. A combined analysis of average duration of the treatment course and PDDs can provide more reliable information about AMU and can be a better indicator for drug prevalence (Friis et al., 1987; Resi et al., 2001).

2.5.2.4. Used daily dose

The used daily dose (UDD) is a metric which describes the amount of active ingredients actually administered to the animals in mg/kg (Grave et al. 2004). UDD can be computed by dividing the amount of AM ingredient used (mg) by the number of animals multiplied by their average weight at treatment to define a standard treated bird. The UDD/DDD or UDD/ADD ratios are calculated to check the dosage correction. Ratios ranged from 0.8 to 1.2 (in case of UDD/ADD) and 1 (in case of UDD/ADD) are denoted as correct dosing. Values beyond or lesser than those limits are considered to be under-dose and overdose, respectively (Jensen et al., 2004; Timmerman et al. 2006; Camini et al., 2016).

$$\text{UDD}=\frac{(Active\ antimicrobial\ ingredient(mg))}{(Total\ number\ of\ animals\ treated)\times(average\ weight)}$$

Another formula to calculate used daily dose was discussed and used by other studies (Gonzalez et al., 2010) and defined as UDD_{kg}. UDD_{kg} is calculated using this formula:

$$\text{UDD}_{\text{kg}}(\text{mg/kg/day})=\frac{(Active\ antimicrobial\ ingredient(mg))}{(Standard\ weight\ (kg))\times(treatment\ period(days))}$$

UDD is a metric that is very useful in describing herd or flock level AM consumption data. UDD depends on actual substances used in farm or animal level, hence not prone to non-compliance prescription dose like PDD (Persoons et al., 2012).

2.5.2.4.1. Number of used daily dose

Consumption of AMs is often expressed as number of UDD (nUDD). The amount of active AM ingredients is divided by calculated median UDD_{kg} to calculate nUDD (Gonzalez et al., 2010). nUDD is sometimes adjusted with PCU (Merle et al., 2020).

$$\text{nUDD}=\frac{(Active\ antimicrobial\ ingredient(mg))}{(UDDkg)}$$

2.5.2.5. Course dose metrics

Course dose metrics endeavor to designate the number of courses an animal receives while taking the daily dose and course length into account. Common used Course Dose metrics are defined course dose (DCD), used coursed dose (UCD) UK also has a cow calculated course (CCC) metric which is not applicable and adaptable in poultry.

2.5.2.5.1. Defined Course Dose

ESVAC group had also formulated a defined course dose for animals (DCD_{vet}) as a suitable metric for EU monitoring (EMA, 2016). DCD_{vet} is similar to DDD_{vet}, other than that it has used fixed course dose definitions rather than fixed daily dose definitions (based on the same nine European countries as DDD_{vet}) and assumes a standard weight (i.e., PCU, 1 kg for broiler) (Mills et al., 2018). The defined course dose (DCD) of a drug can be defined as the used dose per treatment course per animal. The course dose enables to correct not the variation in potency but also for the length of the treatment period, which may vary among AM drugs, among farms/individuals and among countries (Chauvin et al., 2001)

2.5.2.5.2. Number of Defined Course Dose

nDCD calculation is similar to nDDD calculation, the dosage used in a certain course is taken in consideration instead of a daily dose (Collineau et al., 2017; Mills et al., 2018). The formula of calculating nDCD is as follows:

$$\text{nDCD}= \frac{(Active\ antimicrobial\ ingredient(mg))}{(\text{Daily dose }(\frac{\text{mg}}{\text{kg}})\times(\text{total weight})}$$

In modifications of this equation, DCD is used as daily dose (mg/kg) and PCU standardized value suggested by ESVAC is used to calculate the total weight (Ferroni et al., 2020; Merle et al., 2020; Khan et al., 2021). Like nDDD, nDCD metric also takes into animal weight and number of cattle in account. Availability of country or region wise dose (DCD value) and weight (PCU value) would help get more accuracy and compatibility in AMU computation.

2.5.2.5.3. Used course dose

The used course dose per kg (UCD_{kg}) of a drug can be defined as the used dose per treatment course per kg animal (Gonzalez et al., 2010).

$$\text{UCD}_{\text{kg}}\text{(mg/kg/course)}=\frac{(Active\ antimicrobial\ ingredient(mg))}{(Standard\ weight\ (kg))}$$

2.5.2.5.4. Number of used course dose

nUCD is calculated by following this formula (Gonzalez et al., 2010).

$$\text{nUCD}=\frac{(Active\ antimicrobial\ ingredient(mg))}{(UCDkg)}$$

2.5.2.6. Treatment incidences

The frequency of treatments can be quantified by calculating treatment incidences. Treatment incidences can be calculated based on DDD or UDD or ADD (Timmerman et al., 2006; Persoons et al., 2012; Sjölund et al., 2015; Joosten et al., 2019); or DCD (Joosten et al., 2019) or UCD (Gonzalez et al., 2010). This formula can be used to calculate treatment incidence

$$\text{TI}=\frac{(\text{Total amount of antimicrobials administered})}{\left(\text{UDD or UCD or DDD or DCD or ADD}\left(\frac{\text{mg}}{\text{kg}}\right)\right)\times(\text{Number of days at risk})\ \times(\text{kg chicken})}$$

In this equation, total amounts of AM substances administered are needed. The number of days at risk is the time chicken is at risk of exposure to AMs, which is in broiler farming, the whole life expectancy period (Persoons et al., 2012) or the average duration of rearing period (Joosten et al., 2019). Kg-chicken is calculated as the number of chickens multiplied by their mean weight (Persoons et al., 2012) or population correction unit (PCU) (Joosten et al., 2019). The TI for chickens is thus defined as the number of chickens that is treated daily with one DDD or UDD or DCD or ADD (Timmerman et al., 2006; Persoons et al., 2012; Joosten et al., 2019; Juliani et al., 2019; Garber et al., 2021) or UCD (Gonzalez et al., 2010); and often expressed for 100 or 1000 animals or birds.

2.5.2.7. Animal defined dosage per year

An ADDD/Y of 1 means that animals in the population were exposed to an AM for one day per year. ADDD/Y can be calculated by dividing total treatable animal weight (actual biomass) times day treated and mean total weight (Bos et al., 2013).

$$\text{ADDD/Y}=\frac{(Total\ treatable\ weight)\times(day)}{Mean\ total\ weight}$$

2.5.2.8. Animal treatment days and animal treatment days per year

Animal treatment days/cycle doesn’t depend on standardized or actual animal biomass, AM dosage or efficacy. The method of calculation was described by Bos et al. (2013) for a year of poultry production. The method was modified by Agunos et al. (2017) for a cycle. Days at risk indicates mean number of production length.

$$\text{ATD}=\frac{(Flock\ population)\times(Number\ of\ treatment\ days\ in\ specific\ flock)\times(days\ at\ risk)}{(Flock\ population)\times(Production\ length\ in\ specific\ flock)}$$

The method was modified (Agunos et al., 2017) for a single cycle and the modifications correlate with our study design. Days at risk indicates mean number of production length.

2.5.2.9. Treatment frequency

The treatment frequency is a farm-level measure of AMU in livestock and shows how many days on average an animal in the observed population is treated, for example, how many used daily doses (UDDs) on average were given to one animal within a specific time frame (Van Rennings et al., 2013; Kasabova et al., 2021). This calculation includes the actual number of animals treated and the duration of treatment duration (treatment days) in the numerator and the estimate of the population under risk in the denominator. The number of day old chicks (DOC) is used to quantify the population at risk. However, broiler chickens are treated flock-wise, so the number of animals treated is equal to the number of animals in the population in most cases. The number of animals housed is not adjusted for losses due to mortality or selling during the production period. The formula of treatment frequency is described by Kasabova et al. (2021)

$$\text{TF}=\frac{\sum(Animals\ tretaed)\times(Treatmetnt\ days)}{(Animals\ in\ the\ population)}$$

2.5.2.10. Total amount/weight (expressed as total mg)

Total crude amount of AMU is calculated by multiplying total number of animals present, present of animals treated, amount of feed/water consumed and average dosage (Mellon et al., 2001; Krishnaswamy et al., 2015). In case of injectable preparations, amount of feed/water consumed isn’t needed. In Europe, total consumption or sales were expressed in terms of active substance weight, in kilograms (kg) or tons, and at a national or regional level (Chauvin et al., 2001). Total mg of active substance is easy to calculate and demonstrate. It does, however, disregard variation in doses rates across AMs (e.g.

highest priority critically important drugs have lower dose regime compared to other groups) as well as individual differences between farms and veterinarians. This metric is not suitable for farm level comparison due to variations (weight, age, production length etc.) (Mills et al., 2018). So this metric doesn’t provide a clear insight on actual AMU. So, total amounts are adjusted against weight and total number of animals in various studies.

2.5.2.11. Milligrams per kilogram live weight

Dividing the total mass of the medicine used by total animal mass at risk of treatment helps improve on ‘total mg’ accounting for fluctuation in animal numbers and weights across farms. However, using this metric may encourage use of HP-CIA’s due to their lower dosage requirement. HP-CIA use is recommended to be reduced to tackle AMR emergence (O’Neill, 2014). To prevent a shift towards HP-CIA, a separate calculation is used in some reports (VMD, 2015), and a standardized biomass (mg/PCU) metric is commonly used (Mills et al., 2018).

2.5.2.12. Milligrams per population correction unit

Actual total live weight of treated animal and birds are not known, and calculations depend on estimated weights. Using an incorrect estimation for live weight of the animals at risk of treatment on a farm may lead in any of the 'per kg' metrics to under- or over-representing actual AM use. To avoid this problem, standard animal weights are used. The population correction unit (PCU) is a standardized animal weight measure suggested by ESVAC and commonly used in calculating sales and farm use data (Agunos et al., 2017; Mills et al, 2018; Mohsin et al., 2019; Reese et al., 2019; Merle et al., 2020)

2.5.2.12.1. Population correction unit

The biomass, or PCU pertains to the total number of birds surveyed multiplied by standard weight at treatment for a broiler chicken (1 kg according to ESVAC guideline) (EMA, 2018). One PCU is equivalent to 1 kg broiler chicken. The PCU is a theoretical unit of measurement developed by the European Medicines Agency (EMA) in 2009 and adopted across Europe (VMD, 2016). However some recent studies approached to adjust the PCU taking recently reported animal body weight and actual length of life in consideration and hence adjustments may be needed (Sanders et al., 2021). CIPRAS calculates PCU for Canada. An adjusted population correction unit (APCU) is described and showed marked deviation (81-89% in case of broilers) from PCU (Radke, 2017).

2.5.2.12.2. mg/PCU calculation

A 50 mg/PCU for food producing animals would mean that 50 mg of antibiotic active ingredient was used for every kg of bodyweight at time of treatment.

$$\text{mg/PCU}= \frac{\text{Total amount (mg.) of antimicrobials used in cycle}}{\text{PCU}}$$

2.5.2.13. Antibiotic consumption index

The weight ratio of all consumed antibiotics to all generated animal feed in a certain society is characterized as the "antibiotic consumption index" when doing exposure evaluation using a deterministic approach (Sahoo et al., 2010). The antibiotic consumption index was reported to be 26 and 100 mg/kg in animal products in Australia and the USA, respectively (Kools et al., 2008; Sahoo et al., 2010). To calculate consumption unit, the following formula (Aalipour et al., 2014) is used

$$E=n \times \frac{\sum(C \times V)}{N} \times 10^3$$

E= the total amount of antibiotic active ingredient for each dosage form (kg),

N= the number of each packaged antibiotic dosage form

C= the concentration of antibiotic active ingredient (%) for each type of antibiotic, included in each dosage form,

V= the net weight or volume of the package (g or ml),

N= the number of antibiotic types that were offered through the given dosage form

All of the administration formats are then summed up.

2.5.2.14. Usage per chicken per time unit (or 'intensity' of usage)

Usage per week per chicken ($U_{wc\ milligrams}$) was calculated by dividing in each farm the amount of each AM used ($U_{r\ milligrams}$) by the length of the reporting period for that farm (t_{weeks}), and then by the number of chickens present in the farm ($N_{chickens}$) on the visit date (Carrique-mas et al., 2014).

2.5.2.15. Usage related to production output (usage per 1000 chickens produced)

The 'amount of each AM used to produce 1000 chickens' (in grams) (U_{1000c} grams) is dependent on the length of production cycle in each farm. Therefore chicken output and AM usage were estimated in each study farm over 1 year.

Afore-mentioned information stated the methods and importance of quantification of farm-level AMU and its implication on veterinary and public health sector. Till-date to our best knowledge, no studies have been performed with an approach to quantify AMU in Bangladesh. Therefore, this study aimed to calculate quantitative AMU calculation using appropriate weight and dose-based metrics in selected broiler chicken farms in Cumilla, Bangladesh.

2.6. Summary of the review

This review indicates the importance of AMU quantification. AMU in animals has public health importance. AMU in farm level influences AMR in animal level. Studies show that AMR in animal leads to AMR in humans. A few Bangladeshi published papers were found to deal with AMU, but none of them took quantitative computation in concern. Therefore this study aimed to assess AMU in broiler farm level in Cumilla, Bangladesh and set AMU quantification benchmarking in Bangladesh.

Chapter III: Materials and methods

3.1. Study area description

Cumilla is located in south-eastern part of Bangladesh and a district under Chattogram Division (between 23º01' to 23º47'36" north and 90º39' to 91º22' east) (GoB, 2022). Cumilla is bordered by Brahmanbaria and Narayanganj districts to the north, Noakhali and Feni districts to the south, Tripura state of India to the east and Munshiganj and Chandpur districts to the west. It has a total area of 3087.3 sq. km (106 km international border with India) with a total population of 5,387,288, among which 84.4% resides in rural areas. The population density is 1712 per sqkm. The district consists of 17 Upazillas (sub-districts). Cumilla is mostly plain land with a small hilly area in Lalmai upazilla. Major rivers passing through Cumilla include the Gumti, Dakatia and the Little Feni (GOB, 2022). Economy of Cumilla is mainly based on agriculture, though cottage industries especially homegrown 'Khadi' textile and sweetmeat manufacturing leads the industrialization which is also favored by the export processing zone (Wikipedia, 2022a). The tropic of Cancer (a line of latitude approximately 23°27′ north of Earth's Equator, most northern place on earth where sun can be directly over-head) went past Cumilla, which impacts the district to have a tropical savanna climate (Wikipedia, 2022b). The climate of Cumilla is marked with monsoons, moderate temperature (average 25.5^0C) with considerable humidity and heavy rainfall (2295 mm annual precipitation). The summer starts usually early in April and continues till August. The winter is marked with dry, almost rain-less season with average 19.7^0C temperature (GoB, 2022; Wikipedia, 2022a). Those geographical factors have made Cumilla a suitable zone for poultry development.

3.2. Study design and period

The prospective longitudinal study was carried out between January 2020 to March 2020.

3.3. Population

3.3.1. Reference population

All small- (≤500) and medium-scale (501-2500) commercial broiler chicken farms under Cumilla District were considered as the reference population of the study.

3.3.2. Source population

Poultry farms from eight upazillas of Cumilla district were chosen as the source population for the present study. They included Adarsha Sadar, Sadar Dakkhin, Laksam, Monoharganj, Lalmai, Nangalkot, Barura and Chauddogram. These upazillas were chosen based on the highest poultry population and density.

3.3.3. Epidemiological unit and sampling frame

Small (≤500) and medium (501-2500) scale farms were considered as the epidemiological unit of the study.

Table 3. 1: Farms distribution in Cumilla, Bangladesh.

Upazilla	No of small scale farms	Min-Max (Flock size)	No of medium scale farms	Min-Max (Flock size)
AdarshaSadar			9	700-2500
Sadar Dakkhin	1	500-500	5	1000-2500
Laksam	1	500-500	8	700-2100
Monoharganj			3	1000-2500
Lalmai	1	500-500	5	1000-1200
Nangalkot			2	2500-2500
Barura			2	1000-1800
Chauddogram			3	800-2500
Total	3		37	

3.4. Sample size calculation

A manageable number of 40 farms (3 small scales and 37 medium scales) along with the farmers' willingness were considered for this longitudinal study. Distribution of farms is presented in **Table 3.1.** Though the study aimed to collect equal number of small and medium scale farms, during study period enough small scale farms weren't found in the study area.

3.5. Sampling technique

A total of 40 farms were selected purposively from the sampling frame with the cooperation of feed and pharmaceutical company veterinarians, feed and chick dealers and farmers. Dealers provided the farmers' contact numbers, addresses and tentative date of starting a new batch with day old chicks. All studied farms were entered in to the study within one month.

3.6. Data collection

3.6.1. Questionnaire

Two sets of questionnaires were developed per objectives: i) Questionnaire for antimicrobial usage and ii) Farm biosecurity questionnaire

Questionnaire for AMU was drafted and peer-reviewed and piloted on 2 farms to identify gaps and timing. Accordingly, the questionnaire was updated. This questionnaire contained the following information: name of antimicrobials, dosage and way of administration, amount of water supplied, source and prescriber of drugs, cause of drugs, disease symptoms and diagnosis (tentative/lab confirmatory) and death count.

The questionnaire for assessing farm bio-security was drafted and peer-reviewed and piloted as described above to identify gaps and timing and mitigate the gaps accordingly. The questionnaire contained i) demographic information as farmers' age, gender, education status and economic status; ii) farm and biosecurity information; iii) disease history and iv) knowledge on AMU and AMR.

The full questionnaires are given as **Appendix I:** Farm biosecurity questionnaire and **Appendix II**: Antimicrobial Usage data collection sheet.

3.6.2. Farm visit plan and recording data

To collect AMU data, each farm was visited in every 3 days. A set of number tags and a stapler was provided to the farmers to tag the used packaging of drugs. The farmers were prior trained on how to fill-up the questionnaire (if literate) and to mark the used packages with the date tags (available in **Appendix III**) and stapler provided and store all the tagged packets in a plastic bin bag. To ensure data quality, each of the farmers was contacted every day over phone to collect information on drug usage. The data from questionnaire were later rechecked in corporation with the packaging stored in the bin bag. Farm biosecurity data were collected at the first day of AMU data collection. It took approximately 20 minutes to fill-up the questionnaire.

3.7. Sample collection, transportation, preservation, storage and lab testing

Environmental samples were collected by wearing sterile plastic boot cover and walking on the litter in ‘Z’ fashion in each poultry farms. The boot cover containing samples were later kept in plastic zipper bag containing Buffered Peptone Water (BPW). The bags were then kept in an insulated cool box containing ice packs and later stored in deep freeze temperature prior transferring to the laboratory where the samples were kept in -20°C until further analysis. Standard bacteriological culture (to isolate *Salmonella*), cultural sensitivity test (to assess antibiogram) and molecular testing (to characterize AMR genes) were performed at bacteriology lab of Department of Microbiology and Veterinary Public Health, Chattogram Veterinary and Animal Sciences University. However, the results of laboratory tests were not used for this thesis.

3.8. Statistical evaluation

3.8.1. Data entry and cleaning

Field data were entered in Microsoft excel 2016. Data were cleaned, coded and recorded. Consistency of data was checked for validation, and then exported to STATA SE-16

(StataCrop, 4905, Lakeway Drive, College Station, Texas 77845, USA) for epidemiological analysis and AMU calculations.

3.8.2. Descriptive analysis

Numbers of antimicrobial courses were counted in all 40 farms in the production length. Frequency of courses per AM class was calculated by dividing the frequency of each class by total number of treatment courses. Combined preparations were kept as combined class (i.e. sulfadimidine-trimethoprim combined drugs were listed as sulfadimidine-trimethoprim class).

Purpose of antimicrobial usage was calculated by dividing the frequency of each purpose by total number of AM courses. Purposes of using AM preparations were computed by the frequency of AM course of each antimicrobial preparation divided by total AM course numbers.

Prescriber of antimicrobial drugs according to purpose of use was calculated by dividing the frequency of each prescriber by total AM courses. Descriptive results were expressed frequency number, percentage and 95% confidence interval.

3.8.2. Quantitative analysis (antimicrobial usage calculation metrics)

Details of the formula used to calculate different metrics are enlisted in **Appendix IV.**

3.8.2.1. Weight-based metrics

3.8.2.1.1. Total amount of antimicrobial usage

Total amount of antimicrobials usage was expressed in milligram (Total mg) using equation 1.

$$\text{Total mg} = \sum (\text{Total volume or amount of antimicrobials used} \times \text{mg of active ingredients in the drug}) \quad \ldots\ldots (1)$$

3.8.2.1.2. Total mg/population correction unit

Population correction unit (PCU) is the standard of biomass suggested by ESVAC to calculate antimicrobial usage. Standard biomass for broiler bird is 1 kg (EMA, 2016)

PCU= (Total number of birds) × (Standard weight, 1 kg)…………………………….(2)

We considered total DOC numbers entered in the production as total number of birds, hence, total number of birds=56890

So, overall PCU in 40 broiler farms in Cumilla=56890kg

Total mg/PCU is calculated by dividing total mg by PCU value.

Total mg/PCU= $\frac{\text{Total mg}}{\text{PCU (kg)}}$………………………………………………………….(3)

3.8.2.2. Dose based metrics

3.8.2.2.1. Number of defined daily dose per population correction unit and number of defined course dose per population correction unit

Defined daily dose (DDD) for animals and defined course dose (DCD) were defined by ESVAC for European countries. As no DDD or DCD values were suggested in Bangladesh or in any neighboring countries, we used DDD and DCD values set up by EMA to calculate nDDD/PCU and nDCD/PCU using these formulas (4 and 5),

nDDD/PCU= $\frac{\text{Total mg}}{\text{DDDesvac x PCU (kg)}}$……………………………………………….(4)

nDCD/PCU= $\frac{\text{Total mg}}{\text{DCDesvac x PCU (kg)}}$……………………………………………….(5)

3.8.2.3. Treatment frequency and treatment frequency per day

Treatment frequency is calculated by dividing animal treated times treatment days by animals in population (Formula 6). Birds present during the time of a specific treatment were considered as ‘animals treated’, whereas ‘animals in the population’ was total DOC at the start of the batch and was not adjusted for losses due to mortality during the production period. In case of combination drugs, all the antimicrobials were calculated separately, i.e. if a trade preparation has two antimicrobial combinations, we calculated the treatment twice (Kasabova et al., 2021). All the values were then added to calculate overall TF. Median values and 25% and 75% percentiles were calculated.

$$TF=\sum\frac{(Animals\ treated)\ x\ (Treatment\ days)}{(Animals\ in\ the\ population)}\ldots\ldots(6)$$

TF/day in a farm was calculated using TF value (from formula 6) divided by average production cycle, 31 days.

$$TF\ per\ day=\frac{TF}{Average\ Production\ Cycle}\ldots\ldots(7)$$

3.8.2.4. Animal treatment days per cycle

Average animal treatment day per cycle was calculated by using a formula (8) suggested in a previous study (Agunos et al., 2017).

$$ATD=\frac{(Flock\ population)*(Number\ of\ treatment\ days\ in\ specific\ flock)*(Days\ at\ risk)}{(Flock\ population)*(Production\ length\ in\ specific\ flock))}\ldots\ldots(8)$$

Days at risk were defined as the time a farm remained at risk of antimicrobial usages. For broiler, a bird remains at risk of antimicrobials in its production length. Hence, days at risk were equal to average production length.

Chapter IV: Results

4.1. Farm description and farmers demography

Farms' and farmers' demography have been presented in **Table 4.1.** Of all the broiler farms (N=40), medium scale (501-2500) farms (92.5%) dominated over small scales (≤500). Total flock size 54890 (range 500-2500; median 1120, 1st quartile 1000, 2nd quartile 1120, and 3rd quartile 1750). All the farmers were male. Most of the farmers completed at least secondary or higher education (62.5%). Farmer's main income source was poultry farming (52.5%), followed by business (30%) and agriculture (5%). Most of the farmers had one year minimum to five years' experience (55%), whereas 30% of them had an experience range of more than 10 years. Only 7.5% of the farms had more than one poultry sheds. Average production length of the broilers was 30.2 days (95% CI: 29.5-30.9; smallest 24 days, largest 35 days).

Table 4. 1: Demographic characteristics of commercial broiler farms in Cumilla, Bangladesh.

Criteria	**Category**	**Farmers (N=40) n (%)**
Flock Size	≤500	3 (7.5%)
	501-2500	37 (92.5%)
Gender	Male	40 (100%)
	Female	--
Education	Illiterate or primary education	15 (37.5%)
	Secondary or higher education	25 (62.5%)
Main income source	Poultry	21 (52.5%)
	Business	12 (30%)
	Agriculture	2 (5%)
	Others	5 (12.5%)
Farming experience (years)	0-5	22 (55%)
	6-10	6 (15%)
	>10	12 (30%)

Criteria	**Category**	**Farmers (N=40)** **n (%)**
Number of poultry sheds	One	37 (92.5%)
	More than one	3 (7.5%)
Vaccination (number of vaccines administered)	1	16 (40%)
	More than one	24 (60%)

4.2. Antimicrobial usage

4.2.1. Descriptive results

Farmers used 26 different AMs, 20 in single form and 12 in combined. Those 20 single forms belonged to 11 antimicrobial classes; and 8 different classes were present in the combination medications. 56 different trade preparations were administered; among them 55 were veterinary preparation and one metronidazole was dedicated for human medication.

4.2.1.1. Numbers and frequency of antimicrobial courses

All of the farmers used AM within the production length, starting from day 1. Antimicrobials were administered through drinking water. Farmers used a wide range of AM, which has been featured in **Table 4.2.** All the farms used at least one course of antimicrobials. A total of 154 courses (median course 4; range 1 to 5; 95% CI 3.4-4.5) of AM were used among the 40 farms in the production cycle. Median course period was 3 days (range 1 to 7 days).

Fluoroquinolones were the most common (n=44 courses, 28.6%) AM used, followed by penicillins (n= 29 courses, 18.8%), tetracyclines (n=12 courses, 7.8%) and sulfonamide-coccidiostats (n= 12 courses, 7.8%).

Table 4. 2: Antimicrobial groups used in broiler farms in Cumilla, Bangladesh (N=40 farms, no of AM course=154, range 1-5)

Antimicrobial group	**Treatment courses n (%)**
Fluoroquinolones	44 (28.6%)
Penicillins	29 (18.8%)
Tetracyclines	12 (7.8%)
Sulfonamides-coccidiostats	12 (7.8%)
Coccidiostats	11 (7.2%)
First-generation-cephalosporins	6 (3.9%)
Macrolide	5 (3.3%)
Sulfonamides-trimethoprim	5 (3.3%)
Aminoglycosides	4 (2.6%)
Quinolones	4 (2.6%)
Macrolide-tetracycline	4 (2.6%)
Polymyxins	3 (1.9%)
Polymixin-trimethoprim	3 (1.9%)
Sulfonamides	3 (1.9%)
Tetracyclines-tetracyclines	3 (1.9%)
Tetracyclines-aminoglycosides	2 (1.3%)
Imidazoles	2 (1.3%)
Penicillins-polymyxins	1 (0.7%)
Tetracyclines-polymyxins	1 (0.7%)

4.2.1.2. Purpose of antimicrobial usage

Antimicrobials were used for prevention of diseases by 97.5% (n=39) of the farmers; 25% of them used AM solely to prevent disease, whereas 70% (n=28) of the farmers used AM to treat diseases with 1 farm (2.5%) used AM solely to treat disease. 7.5% (n=3) of the farmers used antibiotics as growth promoters. Course-wise purpose of the AM usage has been shown in **Table 4.3**. Amoxicillin was the most administered (18.8% of treatment courses) AM preparation in farms (Appendix V), followed by enrofloxacin (16.9%), toltrazuril (5.8%) and oxytetracycline (4.6%). Enrofloxacin was the most common drug used as for preventive purpose (27%), whereas amoxicillin was most used to treat (17.6%) disease conditions. Amoxicillin was also used as for growth promotion (100%).

Table 4. 3: Number of antimicrobial courses according to purpose of antimicrobial usage in broiler farms in Cumilla, Bangladesh

Antimicrobial usage purpose	Treatment courses(N=154) n (%)
Prevention	74 (48.1%)
Treatment	68 (44.2%)
Growth promotion	1 (0.7%)
Prevention and growth promotion	7 (4.6%)
Prevention and treatment	4 (2.6%)

4.2.1.3. Disease prevalence and antimicrobial usage against diseases

Antimicrobials were used for therapeutic and prophylactic purposes as well as growth promoters. List of conditions that were either treated or prevented using AM has been shown in **Table 4.4**. Omphalitis was the most common condition against which AM was used as a preventive measure (27.9% of total AM courses). Coccidiosis and chronic respiratory disease (CRD) were the most prevalent diseases/conditions where antimicrobials were used as both preventive (8.2% and 5.2% respectively) and therapeutic (11.7% and 10.4%, respectively) measure. AMs were used in viral and mixed conditions with probable viral disease, but upon further inspection, no antiviral drug use was found in the farms. All of the diagnoses were based on clinical signs and or post-mortem lesions by registered veterinarian, dealer or the farmer.

Table 4. 4: Purpose of antimicrobial usage against disease or disease conditions in broiler farms in Cumilla, Bangladesh (N=40, Total AM course=154)

	Disease/ condition	Purpose of use	n (%)	Prescriber	n (%)
Bacterial	Omphalitis	Prevention	43 (27.9%)	Vet	1 (2.3%)
				Non-Vet	42 (97.7%)
	Omphalitis + others	Prevention	4 (2.6%)	Vet	2 (50%)
				Non-Vet	2 (50%)
		Prevention and Treatment	4 (2.6%)	Vet	--
				Non-Vet	4 (100%)
	Colibacillosis	Treatment	1 (0.6%)	Vet	--
				Non-Vet	1 (100%)
	CRD	Prevention	5 (3.2%)	Vet	1 (20%)
				Non-Vet	4 (80%)
		Treatment	16 (10.4%)	Vet	6 (37.5%)
				Non-Vet	10 (62.5%)
Protozoan	Coccidiosis	Prevention	8 (5.2%)	Vet	--
				Non-Vet	8 (100%)
		Treatment	18 (11.7%)	Vet	7 (38.9%)
				Non-Vet	11 (61.1%)
Viral	ND	Treatment	6 (3.9%)	Vet	3 (50%)
				Non-Vet	3 (50%
	Gumboro	Treatment	8 (5.2%)	Vet	2 (25%)
				Non-Vet	6 (75%)
	Gumboro + ND	Prevention and Growth promotion	3 (1.9%)	Vet	--
				Non-Vet	3 (100%)
	IBH	Treatment	1 (0.6%)	Vet	--
				Non-Vet	1 (100%)
Mixed	ND + Ascites	Treatment	2 (1.3%)	Vet	1 (50%)
				Non-Vet	1 (50%)
	Coccidiosis + CRD	Prevention	10 (6.5%)	Vet	--
				Non-Vet	10 (100%)
		Treatment	2 (1.3%)	Vet	2 (100%)
				Non-Vet	--
	Coccidiosis + Gumboro	Prevention	2 (1.3%)	Vet	--
				Non-Vet	2 (100%)
Non specific	Cold	Prevention	2 (1.3%)	Vet	--
				Non-Vet	2 (100%)
		Treatment	2 (1.3%)	Vet	--
				Non-Vet	2 (100%)
	Diarrhea	Treatment	4 (2.6%)	Vet	--
				Non-Vet	4 (100%)
	Gout	Treatment	3 (1.9%)	Vet	3 (100%)
				Non-Vet	--
	Respiratory Problems	Treatment	5 (3.2%)	Vet	3 (60%)
				Non-Vet	2 (40%)

Disease/ condition	Purpose of use	n (%)	Prescriber	n (%)
Non descriptive	Growth promotion	1 (0.6%)	Vet	
			Non-Vet	1 (100%)
	Prevention and Growth promotion	4 (2.6%)	Vet	
			Non-Vet	4 (100%)

CRD- Chronic respiratory disease, **ND**- Newcastle Disease, **IBH**- Inclusion Body Hepatitis.

4.2.1.4. Prescriber of antimicrobials

Farmers showed tendency of using AM without consulting veterinarian (**Table 4.5**). Of the 154 different AM courses, 5.4% (n=4) of the preventive courses (n=74) were used with prior veterinary consultation. 60.3% (n=41) of the therapeutic courses were used either by the farmer themselves or with suggestion of either feed dealers and other farmer or drug traders.

Table 4. 5: Prescriber of antimicrobials in broiler farms in Cumilla, Bangladesh (N=40, Total AM course=154)

	Prevention (n=74)*	Treatment (n=68)*	Growth promotion (n=1)*	Prevention and treatment (n=4)*	Prevention and growth promotion (n=7)*
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Self-medication	19 (25.7%)	12 (17.7%)	1 (100%)	1 (25%)	1 (14.3%)
Feed dealer	51 (68.9%)	27 (39.7%)	--	2 (50%)	3 (42.9%)
Veterinarian	4 (5.4%)	27 (39.7%)	--	--	--
Others*	--	2 (2.9%)	--	1 (25%	3 (42.9%)

* Includes other farmers and drug traders

4.2.2. Quantitative analysis

4.2.2.1. Weight based metrics

4.2.2.1.1. Total amount of antimicrobial usage in 40 farms

A total of 54890 DOC were introduced in the studied 40 farms in Cumilla district during the study period. Including single, mixed antibiotics, coccidiostats and antibiotic-coccidiostat preparations, total usage of AM in the entire production period of 40 broiler farms were 7183014 mg (≈7.2 kg; average 0.18 kg per farm). All of those AM were administered with drinking water. No evidence of drug administration through other routes was found.

4.2.2.1.2. Total mg/population correction unit

Calculated total mg/PCU was 130.9 mg/PCU; which indicate to produce 1 kg broiler, 130.9 mg of antimicrobials were used. Farm level AMU varied from 4.4mg/PCU to 618.9mg/PCU (median 98.27 mg/PCU; 95% CI- 97.5 to 189.9). Farm level mg/PCU is shown in **Figure 4.1**. Global indicator for food-producing animals was reported 117.48mg/PCU reported by OIE, whereas in Asia, Far East and Oceanian subsector reported 198.89mg/PCU (Khan et al., 2021; OIE, 2021). A previous study estimated global AMU in chicken in 2013 were 148 mg/PCU, and in Bangladesh for food producing animal the AMU value was estimated were 40 mg/PCU (Van Boeckel et al., 2015; Ritchie, 2017; CDDEP, 2022).

Figure 4. 1: Milligram/population correction unit of antimicrobials used in broiler farms of Cumilla, Bangladesh.

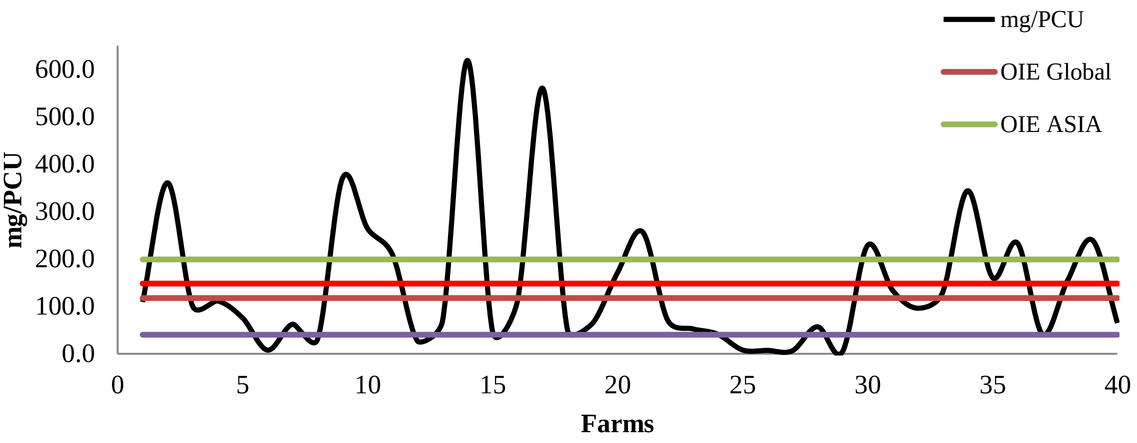
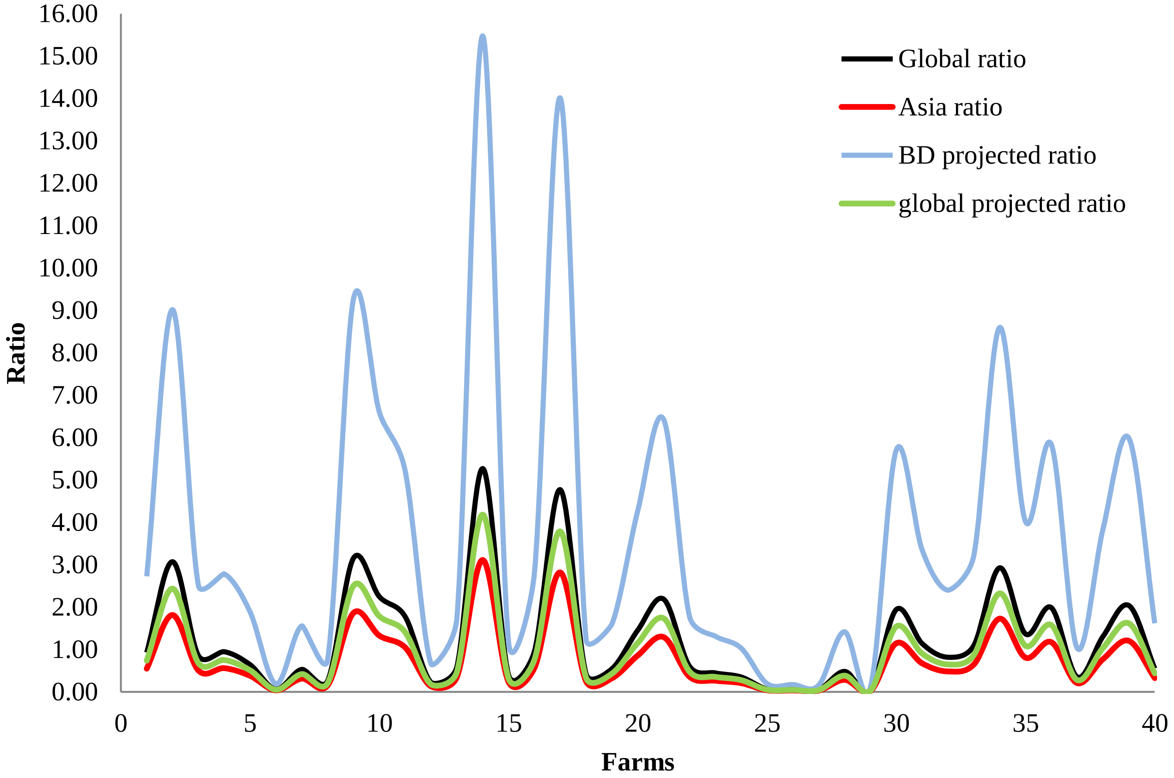


Figure 4. 2 showed the farm AMU vs Asia, far-east and Ocenian AMU ratio, farm AMU vs global AMU ratio, farm AMU vs Bangladesh estimated ratio and Farm AMU vs global estimated. Of the 40 farms, 27.5% (n=11), 40% (n=16), 82.5% (n=33) and 35% (n=14) farms used antimicrobials higher than Asian level (range 0.02 to 3.11), global level (range 0.04 to 5.25), Bangladesh estimated level (range 0.11 to 15.47) and global estimated level (range 0.03 to 4.18), respectively.

Figure 4. 2: Antimicrobial usage comparison



Mg per population correction unit (mg/PCU) according to AM class was presented in **Table 4.6**. Penicillins had the highest mg/PCU (29.5mg/PCU) followed by fluoroquinolones (24.8mg/PCU), sulfonamide-coccidiostats mixed preparation (13.5mg/PCU), aminoglycosides (12.3mg/PCU) and macrolide-tetracycline mixed preparations (11.0mg/PCU).

Table 4. 6: Milligram/population correction unit per antimicrobial classes used in broiler farms

Antimicrobial class	Total mg	mg/PCU
Penicillins	1617405.0	29.5
Fluoroquinolones	1363045.0	24.8
Sulfonamide-coccidiostats*	738579.0	13.5

Aminoglycosides	673120.0	12.3
Macrolide-tetracyclines*	605700.0	11.0
Tetracyclines	512600.0	9.3
Macrolide	480750.0	8.8
Sulfonamide	480000.0	8.7
Tetracyclines-tetracyclines*	241185.0	4.4
Coccidiostats*	150051.3	2.7
Polymyxins-trimethoprim*	93074.4	1.7
First-generation-cephalosporins	58946.3	1.1
Tetracyclines-aminoglycosides*	41250.0	0.8
Polymyxins	31218.8	0.6
Sulfonamide-trimethoprim*	26820.0	0.5
Penicillins-polymyxins*	26693.1	0.5
Quinolones	15100.0	0.3
Imidazoles	14415.0	0.3
Tetracyclines-polymyxins*	13061.0	0.2

* Combined preparations

4.2.2.2. Dose based metrics

4.2.2.2.1.: Number of defined daily dose per population correction unit (nDDD/PCU) and Number of defined course dose per population correction unit (nDCD/PCU)

Number of defined daily dose per population correction unit (nDDD/PCU) and Number of defined course dose per population correction unit (nDCD/PCU) values were calculated using ESVAC standard. DDD and DCD value of some antimicrobials (including coccidiostats) were not defined by ESVAC. Those were classified as non-defined. Total nDDD and nDCD used by the broiler farmers were 221.4 and 49 respectively. Median nDDD/PCU and nDCD/PCU in our study was 3.1 (range 0.3 to 21.7, 95% CI: 3.7-7.3) and 1.1 (range 0.5 to 4.6, 95% CI: 0.8-1.6), respectively. **Table 4.6** shows the nDDD and nDCD values calculated from farms in Cumilla. **Table 4.7** shows that to grow 1 kg standard broiler, amoxicillin was used most (1.8 daily dose, 0.4 course dose), followed by enrofloxacin and doxycycline.

Table 4.7: Calculated number of defined daily doses and number of defined course doses per population correction unit values in broiler farms in Cumilla, Bangladesh

Active AM substance	nDDD/PCU	nDCD/PCU
Amoxicillin	1.882	0.407
Colistin Sulphate	0.405	0.076
Chlortetracyclin	0.041	0.006
Doxycyclin	0.535	0.131
Enrofloxacin	0.611	0.149
Flumequine	0.020	0.005
Neomycin	0.467	0.098
Oxytetracyclin	0.254	0.047
Sulfaclozine	0.125	0.029
Sulfaquinoxaline sodium	0.061	0.013
Sulphachloropyridazine	0.007	0.002
Sulphadiazine	0.005	0.001

Active AM substance	nDDD/PCU	nDCD/PCU
Sulphadimethoxine	0.015	0.001
Sulphadimidine	0.026	0.007
Tilmicosin	0.110	0.037
Trimethoprim	0.073	0.013
Tylosin	0.160	0.038
Amprolium	N/D	N/D
Cephalexin	N/D	N/D
Ciprofloxacin	N/D	N/D
Gentamicin	N/D	N/D
Levofloxacin	N/D	N/D
Metronidazol	N/D	N/D
Norfloxacin	N/D	N/D
Pefloxacin	N/D	N/D
Toltrazuril	N/D	N/D

N/D=Non-defined, **nDDD/PCU**- Number of defined daily dose per population correction unit, **nDCD/PCU**- Number of defined course dose per population correction unit

4.2.3. Overall treatment frequency and treatment frequency per day

Overall median TF was 9.9 and TF/day was 0.3 (**Table 4.8).** Each broiler under this study received 9.9 (≈10) dose of antimicrobial in production cycle (av. 30.2 days)

Table 4. 8: Median treatment frequency and treatment frequency per day in broiler farms Cumilla, Bangladesh

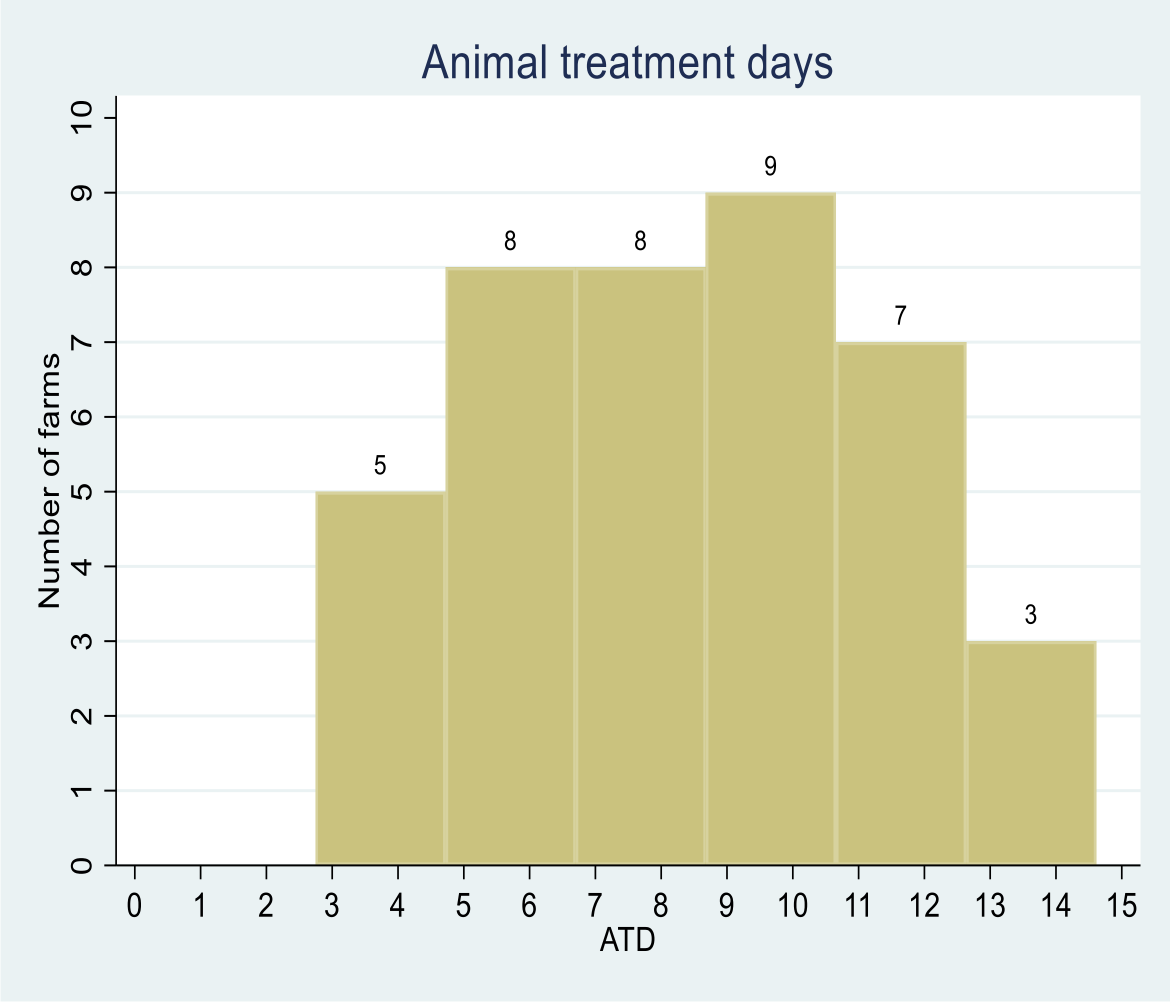
	Minimum	25%-quantile	Median	75%-quantile	Maximum
TF	3	6.8	9.9	14.4	26.7
TF/day	0.1	0.2	0.3	0.5	0.9

*TF-treatment frequency

4.2.4. Animal treatment days (ATD) per cycle

The frequency distribution of ATD/cycle is shown in **Figure 4.3**. Broiler farms in Cumilla had an arithmetic mean/median ATD/cycle of 8.3/8.1 (P25 =3.9, P75 = 11.9).

Figure 4. 3: Animal treatment days (ATD) in cycle



4.3. Quantification ranking

Descriptive AMU and quantification metrics (mg/PCU, nDDD and nDCD) were ranked and compared (**Table 4.9**). To compare, combined antimicrobials active compounds were separated as nDDD and nDCD vet only accounted for individual active ingredients. Amoxicillin ranked top in every category, whereas Enrofloxacin ranked 2nd in frequency, nDDD and nDCD measure but 8th in mg/PCU measure.

Table 4. 9: Comparative ranking of AMU in broiler farms in Cumilla, Bangladesh.

Active AM substance	**Frequency** n (%)**	**Ranking (Freq)**	**mg/PCU**	**Ranking (mg/ PCU)**	**nDDD/ PCU**	**Ranking (nDDD/ PCU)**	**nDCD/ PCU**	**Ranking (nDCD/ PCU)**
Amoxicillin	30 (16.22%)	1	30.117	1	1.882	1	0.407	1
Colistin Sulphate	8 (4.32%)	6	2.064	14	0.405	5	0.076	5
Chlortetracyclin	1 (0.54%)	24	1.239	18	0.041	11	0.006	12
Doxycyclin	14 (7.57%)	3	8.026	7	0.535	3	0.131	3
Enrofloxacin	26 (14.05%)	2	6.107	8	0.611	2	0.149	2
Flumequine	4 (2.16%)	17	0.275	23	0.020	13	0.005	13
Neomycin	5 (2.7%)	16	11.216	3	0.467	4	0.098	4
Oxytetracyclin	10 (5.41%)	4	9.925	5	0.254	6	0.047	6
Sulfaclozine	4 (2.16%)	17	8.745	6	0.125	8	0.029	9
Sulfaquinoxaline Sodium	6 (3.24%)	11	3.646	12	0.061	15	0.013	10
Sulphachloropyridazine	4 (2.16%)	17	0.225	25	0.007	16	0.002	14
Sulphadiazine	1 (0.6%)	24	0.182	26	0.005	17	0.001	15

Active AM substance	**Frequency** n (%)**	**Ranking (Freq)**	**mg/PCU**	**Ranking (mg/ PCU)**	**nDDD/ PCU**	**Ranking (nDDD/ PCU)**	**nDCD/ PCU**	**Ranking (nDCD/ PCU)**
Sulphadimethoxine	6 (3.24%)	11	0.467	21	0.015	14	0.001	15
Sulphadimidine	6 (3.24%)	11	4.669	10	0.026	12	0.007	11
Tilmicosin	2 (1.08%)	20	1.981	15	0.110	9	0.037	8
Trimethoprim	8 (4.32%)	6	0.465	22	0.073	10	0.013	10
Tylosin	7 (3.78%)	9	12.922	2	0.160	7	0.038	7
Amprolium	8 (4.32%)	9	3.763	11	N/D	N/A	N/D	N/A
Cephalexin	6 (3.24%)	11	1.074	19	N/D	N/A	N/D	N/A
Ciprofloxacin	8 (4.32%)	6	11.122	4	N/D	N/A	N/D	N/A
Gentamicin	1 (0.54%)	24	1.423	17	N/D	N/A	N/D	N/A
Levofloxacin	6 (3.24%)	11	4.946	9	N/D	N/A	N/D	N/A
Metronidazol	2 (1.08%)	20	0.263	24	N/D	N/A	N/D	N/A
Norfloxacin	2 (1.08%)	20	0.911	20	N/D	N/A	N/D	N/A
Pefloxacin	2 (1.08%)	20	1.746	16	N/D	N/A	N/D	N/A
Toltrazuril	9 (4.86%)	5	2.643	13	N/D	N/A	N/D	N/A

*__AM-__ Antimicrobials, **mg/PCU**- Milligram per population correction unit, **nDDD/PCU**- Number of defined daily dose per population correction unit, **nDCD/PCU**- Number of defined course dose per population correction unit.

**Combined drugs were separated to compare with DDD and DCD

4.4. Week-wise (temporal) antimicrobial usage pattern

4.4.1. According to frequency distribution

Farms commonly used AM till the 4th week of production. One farms used AM till 5th week of production. AMU pattern in farms across weeks has been featured in **Table 4.10.** Numbers of farms that used in week one, two, three, four and fifth week of production cycle was 38, 21, 26, 21 and 1 respectively**.** Number of farms used AM declined in second week, but the spectrum of AM became wider which declined in later weeks. Both AM usage and spectrum declined in following weeks.

Enrofloxacin was used most in first week (50%), declined in the next weeks (10%, 7.5%, 5% in 2nd, 3rd and 4th week, respectively). Amoxicillin was used in 35% farms in 1st week, declined in the 2nd (10%) and then raised in 3rd (17.5%) and 4th (20%) weeks. Neither of ciprofloxacin and toltrazuril was used in first week, but both of them were used from 2nd to 5th week.

Table 4. 10: Weekly descriptive antimicrobial usage pattern in broiler farms in Cumilla, Bangladesh.

AM Name*$^{\#}$	**Week 1 n (%)**	**Week 2 n (%)**	**Week 3 n (%)**	**Week 4 n (%)**	**Week 5 n (%)**
Amoxicillin	**14 (35%)**	**4 (10%)**	**7 (17.5%)**	**8 (20%)**	
Amprolium		1 (2.5%)			
Cephalexin	4(10%)	1 (2.5%)	1 (2.5%)		
Ciprofloxacin		1 (2.5%)	1 (2.5%)	**6 (15%)**	1 (2.5%)
Colistin sulphate	2 (5%)	1 (2.5%)			
Chlortetracycline			1 (2.5%)	1 (2.5%)	
Doxycycline		1 (2.5%)			
Enrofloxacin	**20(50%)**	**4 (10%)**	3 (7.5%)	2 (5%)	
Gentamicin				1 (2.5%)	
Levofloxacin		1 (2.5%)	1 (2.5%)	3 (7.5%)	
Flumequine	4 (10%)				
Metronidazole		1 (2.5%)	1 (2.5%)		

AM Name*$^{\#}$	**Week 1** **n (%)**	**Week 2** **n (%)**	**Week 3** **n (%)**	**Week 4** **n (%)**	**Week 5** **n (%)**
Neomycin sulphate		1 (2.5%)	2 (5%)		
Norfloxacin	2 (5%)	1 (2.5%)			
Oxytetracycline	1 (2.5%)		**4 (10%)**	3 (7.5%)	
Pefloxacin		1 (2.5%)	1 (2.5%)	1 (2.5%)	
Sulphaclozine			3 (7.5%)		
Tilmicosin			1 (2.5%)	1 (2.5%)	
Toltrazuril		2 (5%)	**5 (12.5%)**	2 (5%)	**1 (2.5%)**
Tylosin		2 (5%)		1 (2.5%)	
Amoxicillin MP$^{\alpha}$	1 (2.5%)				
Amprolium MP2$^{\gamma}$			2 (5%)		
Amprolium MP2$^{\gamma}$		**4 (10%)**	3 (7.5%)		
Sulphachlorpyridazine MP$^{\theta}$	2 (5%)	2 (5%)			
Sulphadiazine MP$^{\kappa}$		1 (2.5%)			
Colistin Sulphate MP$^{\delta}$	3 (7.5%)	1 (2.5%)			
Doxycycline MP1$^{\varepsilon}$	1 (2.5%)				
Doxycycline MP2$^{\zeta}$	2 (5%)	2 (5%)			
Doxycycline MP3$^{\eta}$	1 (2.5%)	3 (7.5%)	1 (2.5%)		
Tylosin MP1$^{\mu}$		1 (2.5%)			
Tylosin MP2$^{\rho}$	1 (2.5%)		2 (5%)		
Sulphadimethoxine MP$^{\lambda}$	1 (2.5%)	2 (5%)	3 (7.5%)		

*Farms sometimes used more than one preparation in a week. Hence, the total exceeds N=40 farms.

AM- Antimicobial, #- In case of mixed preparations, main preparation is written; **MP**- Mixed preparation

α- Amoxicillin + colistin sulphate, **β-** Amprolium + Sulfaquinoxaline sodium; **γ-** Amprolium + sulfaquinoxaline sodium + vitamin K; **δ-** Colistin sulphate + trimethoprim; **ε-** Doxycycline + colistin sulphate; **ζ-** Doxycycline + neomycin; **η-** Doxycycline +

oxytetracycline; **θ-** Sulphachlor-pyridazine + trimethoprim; **κ-** Sulphadiazine+ trimethoprim; **λ-** Sulphadimethoxine + sulphadimidine + diaverdine + nicotinamide + vitamin K3; **μ-** Tylosin + doxycycline; **ρ-** Tylosin + doxycycline + bromhexin HCL

4.4.2. According to usage quantification

Weekly AMU pattern according to three metrics calculated had been shown in **Figure 4.4, 4.5 and 4.6**. A steadier rise in third week was observed after adjusting the amount of active ingredient with population correction unit (**Figure 4.4 and 4.5**). Significant changes in pattern of nDDD and nDCD values were observed across weeks. This might be occurred due to farmers' reluctance to completion of course period of AMs.

Table 4.11 showed the weekly quantitative pattern of AMU according to antimicrobial groups. In first week, amoxicillin was most used (4.9 mg/PCU) according to mg/PCU metrics, followed by enrofloxacin (2.7 mg/PCU) and colistin sulphate (1.6 mg/PCU). However, 0.3 of defined daily dose of amoxicillin, enrofloxacin and colistin was used. In second week, tylosin was most used (4.2 mg/PCU), followed by doxycycline (3.7mg/kg) and amoxicillin (2.6 mg/PCU), whereas amoxicillin and doxycycline (0.2 nDDD/PCU) dominated the defined dose metrics. In third week, neomycin was most used (10.3 mg/PCU) followed by amoxicillin (9.7 mg/PCU) and oxytetracycline (7.1 mg/PCU). Ciprofloxacin (1.5 mg/PCU) and toltrazuril (0.2mg/PCU) was used in fifth week.

Figure 4.4: Weekly antimicrobial usage pattern according to total weight used.

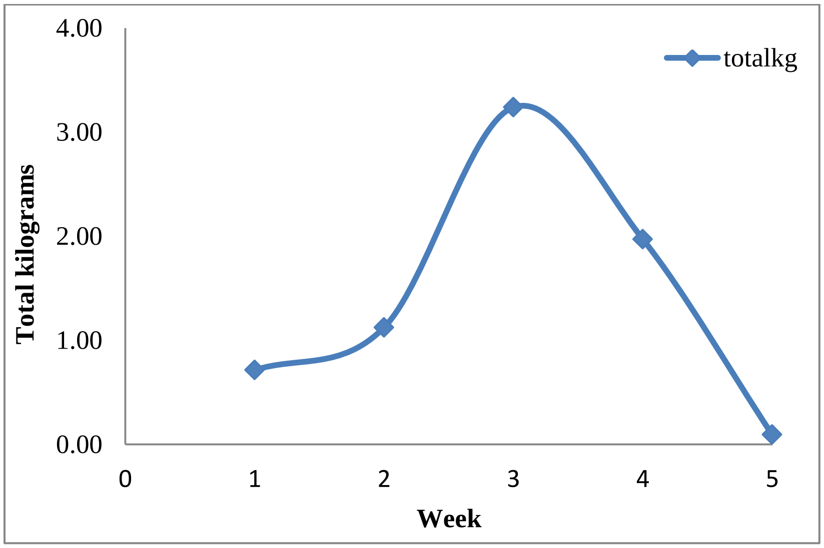


Figure 4. 5: Weekly antimicrobial usage pattern according to milligram per population correction unit metric.

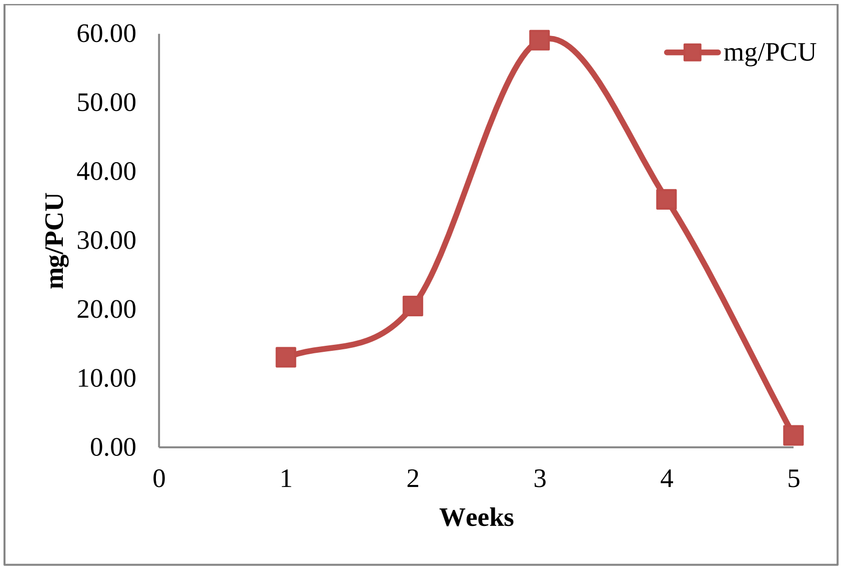


Figure 4. 6: Weekly antimicrobial usage pattern according to dose-based metrics.

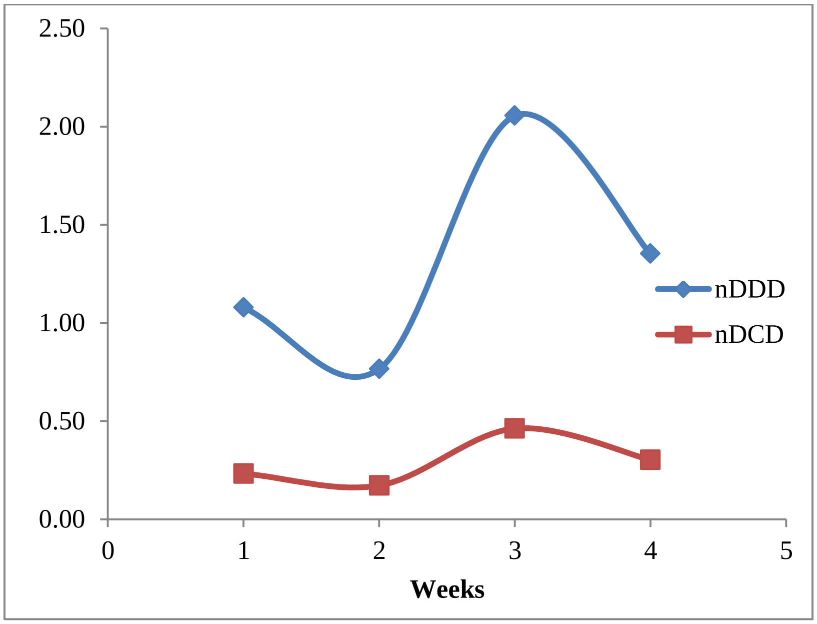


Table 4. 11: Weekly quantitative antimicrobial usage pattern in broiler farms in Cumilla, Bangladesh.

Antimicrobials	Week one			Week two			Week three			Week four			Week five		
	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU
Amoxicillin	4.974	0.310	0.067	2.563	0.160	0.035	9.745	0.609	0.132	12.832	0.802	0.172			
Amprolium				0.880	-	-	2.881								
Cephalexin	0.219	-	-	0.547	-	-	0.307								
Ciprofloxacin	-	-	-	0.528	-	-	2.769			6.367			1.457		
Chlortetracycline							0.619	0.021	0.003	0.619	0.021	0.003			
Colistin Sulphate	1.644	0.322	0.061	0.419	0.082	0.016									
Doxycycline	1.134	0.076	0.019	3.692	0.246	0.061	3.199	0.213	0.052						
Enrofloxacin	2.664	0.267	0.065	0.43	0.04	0.011	2.228	0.223	0.054	0.783	0.078	0.019			
Flumequine	0.275	0.019	0.005	-	-	-									

Antimicrobials	Week one			Week two			Week three			Week four			Week five		
	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU
Gentamicin										1.423	0.102	0.024			
Levofloxacin				0.729	0.052	0.012	1.321	0.094	0.022	2.897	0.207	0.408			
Metronidazol				0.109			0.153								
Neomycin	0.376	0.016	0.003	0.547	0.023	0.005	10.293	0.429	0.091						
Norfloxacin	0.510			0.401											
Oxytetracycline	0.443	0.011	0.002	0.919	0.024	0.004	7.067	0.181	0.031	1.494	0.038	0.007			
Pefloxacin				0.066			0.222			1.457					
Sulphachloro-pyridazine	0.134	0.004	0.001	0.091	0.003	0.001									
Sulfaclozine				2.132	0.030	0.007	6.613	0.094	0.022						

Antimicrobials	Week one			Week two			Week three			Week four			Week five		
	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU	mg/PCU	nDDD/PCU	nDCD/PCU
Sulfaquinoxaline Sodium				0.774	0.012	0.003	2.872	0.048	0.010						
Sulphadiazine				0.182	0.005	0.001									
Sulphadimidine				0.838	0.004	0.001	3.777	0.021	0.006						
Sulphadimethoxine	0.005	0.0001	0.00001				0.378	0.012	0.001						
Tylosin				4.196	0.052	0.012	1.677	0.021	0.005	6.777	0.084	0.020			
Tilmicosin							1.594	0.089	0.030	0.387	0.022	0.007			
Trimethoprim				0.156	0.024	0.004									
Toltrazuril				0.204			1.300			0.891			0.248		

Chapter V: Discussion

The impact of antimicrobial usage on the emergence of antimicrobial resistance (AMR) in both animal and human has become the global public health concern in recent times. But study on indiscriminate antimicrobial usage (AMU) in poultry sector with a quantitative approach was rarely performed in Bangladesh. To deal with this knowledge gap, this investigation attempted to estimate the level of AMU in exotic broiler farms in Cumilla in Bangladesh using both descriptive and quantitative approaches. In this chapter, significant findings, their implications, limitations, have been discussed under different sub-heading as follows.

5.1. Antimicrobial usage in broiler farms

The indiscriminate use of asntimicrobials in poultry production is a main driver of antimicrobial resistance in the food chain globally (Bamidele et al., 2022). Our study recorded that AMU is very common in the commercial broiler rearing in Cumilla district as all broiler farmers administered antimicrobials (AMs) for different purposes to their flocks in the study period. A few cross-sectional, retrospective and qualitative studies were previously conducted on AMU in food producing animals in south Asian and Bangladesh which had limitations of data authenticity because of being conducted those snapshot studies (Imam et al., 2020, Chowdhury et al., 2021). As stated by Pinto Ferreira et al (2017) collecting actual farm-level AMU data are the most accurate way to monitor AMU, because only recorded actual usage data enable to avoid approximations and resulting data distortion. Therefore, our longitudinal study filled the gaps and ensured farm-level AMU data authenticity and the first attempt, to best of our knowledge, to evaluate the farm-level AMU in Bangladesh.

This study revealed that broiler farms in Cumilla region were highly dependent on medications with AMs, as all of the farmers under the study used at least one AM in their production period. All the farms used AMs for therapeutic, prophylactic, and, to a limited extent, growth promotion purposes which are supported by earlier studies (Islam et al., 2015; Imam et al., 2020). Multi-drug administration was also common. Farmers used 26 different AMs, in single (20) or combined (12) form, 56 different trade preparations (55 veterinary and 1 human preparations) in 154 treatment courses; median course period 3 was days. These findings are similar to a previous study conducted in Vietnam (Carrique-Mas et al., 2015). Also administration of single dose or single day was observed in this study which might lead to possible AMR situation (Alhaji et al., 2010).

Farmers in this study administered AMs orally through water and likewise high administration of AM through water was reported in previous studies (Alhaji et al., 2010; Imam et al., 2021). Blanket medication through water (and feed) has its own demerits as dosage can't be controlled, which may lead to AMR emergence (Love et al., 2011) and excretion of AM residues through wastage (Iglesias et al., 2012).

AMU metrics allowed to benchmark level of AMU in different countries (Agunos et al., 2020; Kasabova et al., 2021). The mg/PCU metric is used in Europe as a measure of distinction among users (EMA, 2016), in Netherlands to monitor legislative changes (NethMap, 2021), in Canada as a benchmark for CIPARS (Agunos et al., 2017). Adjustment of AMs for population and weight provides critical context for interpreting the AMU quantities. We used the ESVAC standard weight of 1 kg/bird to adjust the average weight at treatment (EMA, 2016; Agunos et al., 2017; Mills et al., 2018; Khan et al., 2021).

Total 7.2 kilograms of AMs were used in the studied farms and penicillin, fluroquinolone, sulfonamide-coccidiostat mixed preparations and aminoglycosides were used most as per weight metrics. High amount of these drugs were reported in other studies (Agunos et al., 2017, Khan et al., 2021; Koirala et al., 2021).

When compared to other studies, mg/PCU (130.9 mg/PCU) of AMU in this study is higher (1.2 times) than global average for food producing animals (117.48 mg/PCU), Africa (30.35mg/PCU), America (90.50 mg/PCU), Europe (59.55 mg/PCU) (OIE, 2021b); but lower than Asia, Far East and Ocenia (198.89 mg/PCU) (OIE, 2021b).. When compared with other studies focused on broilers, mg/PCU of this study is slightly lower (0.98 times) than 2013-2015 data from Canada (134mg/PCU) (Agunos et al., 2017; Agunos et al., 2020) and almost 11 times higher than Fiji 12mg/PCU (Khan et al., 2021).

A previous study estimated AMU around the world in 2010, later updated in 2013, which showed global AMU for chicken would be 148 mg/PCU and for Bangladesh the estimated value was 40 mg/PCU (Van Boeckel et al., 2015; Ritchi et al., 2017; Khan et al., 2021; CDDEP, 2022). In comparison with that, our study findings are slightly lower (0.88 times) than the cited global estimates , more than 3 times higher than the Bangladesh estimate and similar with estimated level in Iran (131 mg/PCU) (Van Boeckel et al., 2015). Forty percent of the farms had AMU level higher than global level, which is higher than the findings of a previous study (Khan et al., 2021). The deviation in comparison with other areas of the world is probably occurred due to the inclusion of other food-producing animal species and also the geographical and temporal variations

Penicillins (29.5 mg/PCU; 18.8% of total treatment courses) were among the most used AMs in this study; in spite of high AMR reported in Bangladesh (Al-amin et al., 2020). High amount of AMU of Penicillin class was reported in previous study (Agunos et al., 2020). Fluoroquinolones (24.8 mg/PCU; 28.6% of total treatment courses) was another commonly used drugs in this study. Coccidiostats in single (2.7mg/PCU; 9.1% of total treatment courses) or combined preparations with sulfonamides (13.5mg/PCU; 7.8% of total treatment courses), sulfonamides alone (8.7mg/PCU; 1.9% of total treatment course) or with trimethoprim (0.5mg/PCU; 3.3% of total treatment course) were administered as prophylactic and therapeutic measure which are agreed by Agunos et al.(2017) due to lower price and of broad spectrum of activity (Pastor-Navarro et al., 2009; Iglesias et al., 2012). Though coccidiostats are not medically important, but sulfonamides are frequently used against non-typhoidal *Salmonella* infections and other Enterobacteriaceae (Collignon et al., 2016).

The nDDD/PCU and the nDCD/PCU allowed accounting for the average daily and average course dose (potency) of available poultry products in Bangladesh. But absence of national and regional DDD and DCD values didn't allow us to fully explore the extent of AMU as previous studies showed significant change of the values from ESVAC standard around the world (Bosman et al., 2019; Abe et al., 2020). Calculation of nDDD and nDCD also permitted detection of shifts in dosage due to changing the route of administration (Agunos et al., 2017). However, farmers under this study only used oral antibiotic through water. EMA draft guideline suggested reporting and communicating the number of DDD/PCU value along with mg/PCU which allowed harmonization in reporting over time (EMA, 2017). A difference in the number of defined daily dose and defined course dose with other countries is observed in the present study. Median nDDD/PCU calculated in our study was 3.1, which is almost 5.5 times lower than previous study in Canada (17 nDDD/PCU) (Agunos et al., 2020) and almost 6 times higher than a previous study in Fiji (0.5 nDDD/PCU) (Khan et al., 2021). Calculated median nDCD/PCU(1.1) in our study is 11 times higher than Fijian data (0.1 nDCD/PCU) (Khan et al., 2021). This difference may be caused by lower production period in Bangladesh, well organized DDD and DCD values in other countries and unavailable DDD and DCD values of used AM in ESVAC standard (EMA, 2016). Therefore, development of national reference values are strongly recommended to conduct further AMU assessment in future.

Another metric used was animal treatment days. The metric did not account for biomass or potency of the antimicrobial, rather allowed to compare length of treatment days in production length (AMU exposure length) among farmers and could easily accessible to vets and technical authority for benchmarking AMU (Bos et al., 2013; Agunos et al., 2017). Median ATD per cycle reported in this study (8.1 ATD/cycle) was lower than previous studies in Canada (Agunos et al., 2017) and Netherlands where they took over the year approach (Bos et al., 2013).

The median TF in our study population was ten, which means during the production period, each broiler received ten UDD on average. Median TF in our study is around 1.7 times higher than broiler flocks in Germany (Kasabova et al., 2021), 2 times higher than Belgian broiler study (Persoons et al., 2012) and higher than QS findings (QS, 2019). However, Belgian and QS study counted mixed antimicrobial preparations as one, whereas we counted all the ingredients separately, which may lead to the difference in TF calculation (Kasabova et al., 2021). In Germany, TF are calculated twice a year in a farm and production length are different from us (up to 6 week per flock), which may lead to a lower TF (Kasabova et al., 2021). Median TF/day in our study was 0.3, which higher than the Fijian study (<0.1) (Khan et al., 2021).

Amynoglycosides (gentamicin, neomycin), penicillins (amoxicillin), polymixin (colistin), quinolone (flumequin), fluoroquinolones (ciprofloxacin, pefloxacin, levofloxacin, norfloxacin) drugs were also recorded to be administered in this study. All of those drugs were WHO listed 'critically important antimicrobials used in human medicine' (Collignon et al., 2016; WHO, 2019). Veterinary clinically important drugs as gentamicin, tilmicosin and tylosin were administerd in the studied farms (OIE, 2015). A recent study from our study area showed significant resistance against ciprofloxacin, colistin, enrofloxacin and tetracyclines in *E. coli* in both broiler and human (Fazal et al., 2022). Colistin is an incredibly valuable antimicrobial agent for treating serious nosocomial infections in humans caused by multidrug-resistant gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Kadar et al., 2013).

When the AMU pattern changed across weeks and production periods, change in total quantity was observed, but when the quantity was adjusted for population and weight, the resultant change in mg/PCU was relatively higher. Number of farms using in AMU was higher in first week as most of the farmers used preventive medication in brooding period. In second week, number of farms using AMs significantly declined, but the spectrum of AMs increased. Number of farms again increased in third weeks and declined in fourth week with a declining trend of AM spectrum in both weeks. Only one farm used AM in fifth week of production. However, ciprofloxacin (clinically important medication for human) and tylosin (veterinary clinically important antimicrobial) was used in that farm (OIE, 2015; Collignon et al., 2016), and may lead to possible public and veterinary health issues as the deposited residue may transmit to human through meats and other byproducts (Menkem et al., 2019) and cause health effects in animals (Bacanlı et al., 2019).

Most of the farms administered AMs at the earlier stage of production to prevent Omphalitis (Dutil et al., 2010; Boulianne et al., 2016) which could cause early chick mortality (Walker et al., 2002). Coccidiosis was another economically important disease in poultry and chemoprophylaxis and vaccination to prevent coccidiosis was a common practice (McDonald and Shirley, 2009). Till date, no vaccine is available for coccidiosis in Bangladesh. So, farmers depend on using antimicrobials to prevent and treat coccidiosis. Chronic respiratory disease (CRD) was another disease where AMs were used as both preventive and treatment measures. However, in our observation, we found a trend of using herbal and non-AM preparation in both preventive and early stage treatment. AMs were also used to treat viral diseases also, which may be due to control secondary bacterial infection followed by viral diseases and in fear of economic loss.

The majority of farmers used AMs for prophylactic purposes without veterinary consultation. Prophylactic administration of AMs may be conducted as a risk-minimization measure to mitigate sub-standard farm management, low and mixed-quality day-old-chicks and relatively weaker bio-security protocol which might lead to high prevalence of poultry diseases (Okeke et al., 2005; Begum el al., 2013; Roess et al., 2013; Imam et al., 2020; Masud et al., 2020; Casabova et al., 2021). AMU as growth promoter was reported by relatively a lower number of farmers without veterinary consultation and also a limited extent, which might be due to extreme cost of AMs required for continuous use (Islam et al., 2015). Most of the therapeutic AMU weren’t prescribed by veterinarians. AM administration by non-veterinarians (like poultry feed dealers) were reported in earlier studies (Carrique-Mas et al., 2015; Boamah et al., 2016; Islam et al., 2016; Imam et al., 2020) and could lead to under-dosing or over-dosing of drugs and thus emergence of AMR.

5.2. Effects of using different antimicrobial usage calculation metrics

Results from the study have highlighted important distinction between qualitative and quantitative estimates of AMU. For example, fluoroquinolones, penicillins, tetracyclines, sulfonamides-coccidiostat mixed preparation and coccidiostats were the most commonly used AMs in terms of frequency of usage by farms; however, penicillins, fluoroquinolones, sulfonamide-coccidiostats mixed preparation, aminoglycosides and macrolide-tetracyclines mixed preparation were used more in quantitative weight-based metrics. These differences may be explained by differences in the doses and concentrations of active principles used by the various antimicrobials. Similar discrepancy was reported elsewhere (Carrique-Mas et al., 2015).

Widespread use across weeks of production cycle of amoxicillin and enrofloxacin in farms lead to rank top in different metrics except for weight based metric in case of enrofloxacin. Tetracyclines (doxycycline, oxytetracyline and chlortetracycline) and sulfonamides had a larger relative distribution when measured by both active ingredient and dose metrics, which partially contradicted with a previous study in Japan (Abe et al., 2020). Probable cause of the deviation might be the availability in lower packaging size and lower price of aforementioned drugs in Bangladesh. High potency AMs such as cephalosporins (cephalexin), macrolides (tilmicosin) and quinolons (flumequine) presented a larger relative distribution when a dosage-based metric was used (Abe et al., 2020). This change caused by the use of dosage-based indicator instead of using a weight-based indicator of active ingredient has been highlighted in other studies (Arnold et al., 2004; Aarestrup, 2005; Chauvin et al., 2008; Agunos et al., 2017; Abe et al., 2020). However, a moderate correlation between our used weight-based indicator and dose-based indicator was shown in a previous study and it was suggested and used by CIPARS to calculate at least one weight-based and one dose-based metric to better characterization (Agunos et al., 2020).

5.3. Limitations

Main objective of the study was to calculate AMU in broiler farms and a longitudinal approach was taken. However, due to absence of well-defined sampling frame and shorter study period, a purposive sampling strategy with a manageable sample size was adopted.

To avoid common biases in a longitudinal study as attrition, conditioning and information bias, the study design was adjusted based on piloting. The farmers were prior trained properly. Daily phone calls, regular repeated farm visits to collect used packaging and verification with the respective feed dealers was done to ensure farmers’ participation as well as data authenticity. During field visit, the investigator didn’t comment on any management issues or take part in any activities that might lead to AMU.

There is no set standard of DDD and DCD values nationally or regionally from Bangladesh. Reliance on DDD and DCD values from ESVAC standard allowed misinterpretation as previous studies showed significant change in DDD and DCD values when geographical and other studies were taken into consideration (Augunos et al., 2017; Bosman et al., 2019; Abe et al., 2020). Therefore, the present study should be interpreted cautiously.

Chapter VI: Conclusion, Recommendations and Future Directions

6.1. Conclusion

Antimicrobial usage (AMU) calculation with a special focus on quantitative methods was conducted in broiler farms in Cumilla. Antimicrobial (AM) administration was common in all the farms for prophylaxis, treatment as well as growth promotion purpose. Multi-drug AM administration was recorded. Multiple combination of AM was used in the farms. Despite legal restrictions, farmers could buy and use AMs without prior veterinary consultation.

Common AM classes according to all the used metrics in the present study were penicillins, fluroquinolone, amynoglycosides, tetracyclines and several combinations as sulfonamide-coccidiostats and macrolide-tetracyclines. Total AMU in the studied broiler farms were 130.9 mg/PCU, 221 nDDD/PCU and 49 nDCD/PCU according to our used quantification factors, respectively. However, farm-level variation in all three metrics was observed.

Temporal variation was observed according to our chosen metrics. Choice of quantification metrics also influenced the relative ranking of AMs used. Top three AMs according to descriptive use, number of Defined Daily Dose (nDDD) and number of Defined Course Dose (nDCD) metrics were amoxicillin, enrofloxacin and doxycycline. Amoxicillin was also most used according to the mg/PCU metric but tylosin and neomycin stood next two positions. Hence, choice of quantification metrics is important in reporting AMU.

AMU in food-producing animals is a potential factor to influence the emergence of AMR in both animals and human. High level of AMU hence poses a potential public threat. Fear of economic losses due to high prevalence of disease, poor biosecurity, poor day-old-chick quality, low price and easy access to AMs may play a role in high level AMU. Immediate intervention as well as awareness programme is very much needed to tackle the situation.

6.2. Recommendations

Multiple ‘critically important’ (as colistin, ciprofloxacin, neomycin) and ‘highly important’ (cephalexin, sulfa drugs) human and veterinary (tylosin) AM preparation was used in broiler production. Used AMs as colistin, multiple doxycycline combinations, ciprofloxacin combinations and amoxicillin-bromhexin combination are banned in Bangladesh. Therefore, farmers and veterinarians should be cautious in selection of AMs. Respective authorities should take necessary action enforce the regulations regarding AM sales and the availability of banned veterinary drugs.

Farm biosecurity status should be improved. Farmers should be encouraged to seek veterinary advice before administering AMs. Disease diagnosis in this study was based on symptomatic and often done by non-vets (farmer, feed dealer and other farmers). Diagnostic and lab facilities should be improved so that the veterinarians could prescribe based on AMR info as well as immunization based on titer level could be ensured.

These findings should be discussed with related stakeholders as government and private veterinarians, feed and drug dealers and the farmers. AMU and AMR stewardship programme should be improved.

6.3. Future directions

- We need to depend on European standards to quantify AMU because of absence of national standards. Study on determination of DDD and DCD values for food-producing animal should be designed and conducted to set up our own standard dose metrics in future.
- A longer version of this longitudinal study should be conducted in future in all types of food-producing animal production to evaluate AMU change across time, place and species.
- Intervention studies and economic evaluations to reduce AMU and subsequent AMR could be considered.

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Appendix I

Assessment of different practices (including antibiotic usages) at broiler farms in Cumilla, Bangladesh

Objectives:

1) To know the extent of antimicrobial usage (AMU) during a production cycle of broiler farms in Cumilla, Bangladesh
2) To assess the current practices (including antibiotic usage) at broiler farms.
3) To assess farmers' view on AMU and antimicrobial resistance (AMR).

Interviewee Information

1. Date of interview	
2. Farm identity	
3. Farm name	
4. Name of the interviewee	
5. Status of interviewee	1=Owner 2=Manager 3=Worker 4=Family member of the owner?_____________________
5.1. If not owner, who is the owner?	1=Company (Mention Name)_______________________________ 2=Relative (Mention type)______________________
6. Mobile number:	
7. Address	a) Village ………………………………………………… b) Street………………………………………………… c)Ward………………………………………………… d) Thana………………………………………………… e) Latitude…………………Longitude…………………
8. Age (year)	
9. Gender	1= Male 2= Female
10. Education	1= Illiterate 2= Primary 3= Secondary 4= Higher; 5=Any other ___________________________
11. What is your main source of income?	1= Poultry Farming 2= Agriculture 3= Business 4= Public/Private Service 5= Others______________________
12. How much does poultry rearing	

contribute to your income? (amount in taka yearly)	
13. How much crop land do you have? (In Decimal)	
14. Area of farm land (In Decimal)	
15. Other properties	a. Ponds 1=Yes 0=No b. Trees 1=Yes 0=No

Farm Info (Interviewer would ask and put tick marks)

1. How many years of poultry rearing does the farmer have?	
2. What is the farm type?	1= Broiler (Exotic) 2= Broiler (Sonali) 3=Layer, 4=All
3. How many broilers are there in the current shed?	
4. When did the current batch (the batch under study) start?	
5. How many batches are there?	1= One 2= More than one______
6. Floor type	1= Floor 2= Cage 3= Both
7. How many batches of broiler did the farmer rear in previous one year? (Mention the number)	
8. Does the farmer keep record on various aspects of farms?	1=Yes 0=No
9. If yes, what records do they maintain?	1= Feed usage record 2= Drug usage record 3= Vaccine record 4= Others (please mention)
10. Is there any assigned Veterinarian	1= Present 2= Absent
10.1. If absent, where do they get poultry health care support from last year?	1= Govt. Vet 2= Feed company Vet 3= Medicine company Vet 4= Other farmers 5= Company representatives 6= Self experience 7= Other_________________
11. Are there workers in the	1= Yes 0=No

farm?	
11.1.If yes, are they permanent?	1= Yes 0=No
11.2.How many?	

Shed and building related questions (Interviewer would observe and fill these)

1. What is the roof type?	1= Concrete 2=Tin-shed 3=Others
2. Is there a ceiling under roof?	1= Yes 2=No
3. Rate the ventilation in farm	1=Excellent 2=Good 3= Fair 4=Poor
4. What is the floor type?	1=Earthen floor 2=Cemented floor
5. Which litter materials are used?	1=Sand 2=Rice husk 3=Saw dust 4=Others
6. Is there a wall on surroundings?	1=Yes 0=No
6.1. If yes, height of surrounding wall (feet)	
7. Where is the farm located?	1= Near the locality 2= Middle of the locality 3= Far from locality
8. Where is the residence of workers?	1= Inside the farm 2= Outside the farm

Chick Information (Current Batch)

1. From where did the farmer collect the DOC?	1= From dealer 2= Directly from hatchery
1.1. In case of dealer, name and contact address of dealer	
1.2. In case of direct collection from hatchery. Name and address of hatchery	
2. Which company?	
3. Which strain?	1=__________________________(Mention the Name) 2=Unknown

Feed and water information (for current batch)

1. What type of feed does the farmer use?	1= Commercial 2= Hand mixed
1.1.In case of commercial feeds, which company?	
1.2.Name and address of the	

dealer	
1.3. In case of hand mixed feed, name and combination of the ingredients	
2. Where is the feed storage?	1= Inside the farm house 2= Outside
3. Is the storage directly accessible by the birds?	1= Yes 0= No
4. Where are the feed bags kept?	1= On floor 2= On a platform
5. Which types of drinkers are being used?	1= Nipple drinker 2= Water troughs
6. What is the water source?	1= Tube well 2= Pond 3= Other__________________
7. Were the water and water source analyzed for bacterial contamination?	1= Yes 0= No
8. Is the water treated for any reagents?	1= Yes 0= No
8.1. If yes, which reagent?	
9. Is the water source accessible to wild life?	1= Yes 0= No

<u>Bird selling related questions</u>

1. To whom does the farmer sell bird?	1= Dealer 2= Middleman 3= Traders 4= Direct to the consumers
2. Who is the owner of the transport vehicle taking poultry for selling?	1= Farmer 2= Dealer 3= Traders
3. Is the transport vehicle taking the poultry to the slaughterhouses/traders/individuals always empty on arrival at the farm?	1= Yes, always empty 2= Sometimes empty 3= No, never empty
4. Is the transport vehicle for poultry always cleaned and disinfected on arrival at the farm?	1= Yes 0= No
5. Are individuals and traders allowed to enter the farm where direct contact is possible with the birds?	1= Yes 2= Sometimes 3= No

Materials cleaning, farm access and removal of wastages and dead birds

1. Is there easy access of wild birds to the farm premise?	1=Yes 0=No
2. Is there easy access of rodents to shed?	1=Yes 0=No
3. Is there easy access of house hold poultry to the farm and shed?	1=Yes 0=No
4. Is there access of visitors?	1=Yes 0=No
5. If yes, are the visitors who visited other farms or LBM at same day allowed?	1=Yes 0=No
6. Are proper measures taken before entry in the farm?	1=Yes 0=No
7. If yes, which measures?	1= Feet and shoe washing 2= Using shoe cover 3= Dress changing 4=Others
8. Is there a footbath at entrance?	1=Yes 0=No
9. If yes, with what?	1= Only water 2= With disinfectant (Types:----------) 3=Disinfectant spray (Ingredient)
10. Is there a vehicle wash?	1=Yes 0=No
11. If yes, with what?	1= Only water 2= With disinfectant 3=Disinfectant spray (Ingredient)
12. Is there a wheel wash?	1=Yes 0=No
13. If yes, with what?	1= Only Water 2= With disinfectant 3=Disinfectant spray (Ingredient)
14. What protective measures are available for the workers while working?	1= Mask 4= Others_________________ 2= Gloves 5= None 3= Wash hands after handling of birds
15. How the sheds are cleaned at batch interval?	1= Sweeping 2=Drenching with water 3= With water and other reagents (Please mention the reagent)______________________
16. What is the frequency of cleaning the materials (feeder/drinker etc.)	1= Daily 2= Weekly 3= Monthly 4= Batch interval
17. How are the materials cleaned?	1= Water only 4= Sweep/ dusting only 2= Soap and water 5= Other …………………… 3= Disinfectant

18. Does the farmer change litters?	1= Yes, all at once (at batch interval) 2= Yes, partially (Mid batch) 0= No
19. How do farmers dispose litters?	1= Dry and re-use later 2= Throw in open place 3= Throw in river/canal 4= Personal use as fish feed 5= Selling to the fish project 6= Others: Mention:------------- ________________
20. How do farmers dispose dead birds?	1= Throw in open place 3= Incineration 2= Pitting 4= Selling 5=Others__________________
21. Distance of disposal area (in Meter)	
22. Is there any Ditch/ponds/canal/river near the disposal area?	1=Yes________(Distance in Meter) 0=No

Disease related

Diseases in other batch(es) at the same period of study time

1. Are there diseases in other batches at the time of the study?	1= Yes 0= No	
If no, skip rest of this section		
2. If Yes, What type of disease?	0=Undiagnosed 1= Salmonellosis 2= Colibacillosis 3= Avian influenza 4= Coccidiosis 5 =Infectious bronchitis (IB) 6 = Necrotic enteritis 7= Egg peritonitis 8=Nutritional deficiency	9= Fowl cholera 10= Newcastle 11= Avian leucosis 12= Infectious bursal disease (IBD) 13= Mycoplasmosis/ CRD 14 = Helminth parasitic infection 15= Others (specify)_________
3. If, undiagnosed, what were the symptoms?		
4. If diagnosed, basis of diagnosis?	1= Symptomatic 2= lab diagnosed	
5. If symptomatic diagnosed, by		

whom?	
6. If l ab diagnosed, by whom?	1=FDIL 2=Feed company (Mention name)
7. Did the farmer use vaccines in other batches?	1= Yes 0= No
7.1. If yes, name and day of vaccines given	

Diagnostic facilities and others

1. Is diagnostic facility available nearby?	1= Yes 0= No
1.1. If yes, provided by whom	1= DLS 2= University 3= Private diagnostic center 4= Diagnostic facility by feed/medicine company
2. Does the farmer get any training on poultry disease?	1= Yes 0= No
2.1. If Yes, Training provided by whom?	1= DLS 2=NGO 3= University 4= Others______________
2.2. Does the farmer get any training on poultry management	1=Yes 0=No
2.3. If yes, training provided by whom?	1= DLS 2=NGO 3= University 4= Others______________
2.4. Does the farmer get any training on poultry biosecurity	1=Yes 0=No
2.5. If yes, training provided by whom?	1= DLS 2=NGO 3= University 4= Others______________

About AMU and AMR

1. Do you use antibiotics in farm?	1= Yes 0= No
2. Do you administer same dose of antibiotic in all sheds?	1= Yes 0= No
2.1. If no, why?	

2.2. Which shed gets highest amount of antibiotic?	
3. Do you fulfill AM course suggested by vets?	1= Yes 0= No
4. Do you stop usingdrugs (AM/Mineral/Vitamin/Prebiotic/Probiotic before selling the bird?	1= Yes 0= No
4.1. If yes, how many days prior selling?	
4.2. Who provide you the AM?	1= Pharmacy 2= Dealers 3= Vets 4= Company MR's 5= Others_____________
4.3. Providers name and contact	
5. Did you hear the words 'Antibiotic Resistance'?	1= Yes 0= No
5.1. If yes, from whom?	1= Vets 2= Dealers 3= MR's 4= Other farmers 5= School/college/University Curriculum 6= Mass Media 7= Others________________

Appendix II

Antimicrobial usage Data Collection Sheet

Farm Serial No.: Address:
Starting date of current batch No. of DOC:

Day	Name of the medicine used	Amount and time of drug used (dosage used)	Way of administer-ation	Amount of feed/water provided (kg or litter)	Amounts of drug bought and amounts of drug used (package number)	Cause of medicine use	Medicine prescriber	Who supplied the medicine?	Description of disease/ Condition	Number of Dead Bird(s)
						1) Therapeutic 2) Preventive 3) Growth Promotion 4) Others____	1) Govt. Vet 2) Private company Vet 3) Feed dealer 4) Self 5) Other farmers 6) Others__	1) Pharmacy 2) Feed dealer 3) MR 4) Vets---		
						1) Therapeutic 2) Preventive 3) Growth Promotion 4) Others___	1) Govt. Vet 2) Private company Vet 3) Feed dealer 4) Self 5) Other farmers 6) Others__	1) Pharmacy 2) Feed dealer 3) MR 4) Vets---		

Appendix III

Date tags for antimicrobial packages

১	২	৩	৪	৫
৬	৭	৮	৯	১০
১১	১২	১৩	১৪	১৫
১৬	১৭	১৮	১৯	২০
২১	২২	২৩	২৪	২৫
২৬	২৭	২৮	২৯	৩০
৩১	৩২	৩৩	৩৪	৩৫
৩৬	৩৭	৩৮	৩৯	৪০

Appendix IV

Formula used to calculate antimicrobial usage in broiler farms in Cumilla, Bangladesh

Metric	**Calculation**	**Requirements**	**Reference**
Total milligrams (mg)	Total mg= ∑(Total volume or amount of antimicrobials used × mg of active ingredients in the drug) Total mg per enterprise= total mg (through feed) + total mg (through water) + total mg (parenteral, injection) + total mg (intramammary) Note: Only total mg (through water) was available in the study	Volume or amount of active antimicrobial used per dose, duration of use, amount of water administered	Agunos et al. (2017); Mills et al. (2018)
mg per population correction unit (mg/PCU)	mg/PCU= $\frac{\text{Total mg}}{\text{PCU (kg)}}$ When PCU= (Total number of birds) × (Standard weight, 1 kg)	PCU (kg) provided by ESVAC. i.e. broiler chickens (1kg), total mg of antibiotic used	Agunos et al. (2017); Mills et al. (2018); EMA, (2014)
Number of Defined Daily Dose per PCU (nDDD/PCU)	nDDD/PCU= $\frac{\text{Total mg}}{\text{DDDesvac x PCU (kg)}}$	Total mg of antibiotic used, total PCU, ESVAC standards defined daily dose for antibiotics	Mills et al. (2018); Khan et al. (2021)
Number of Defined Course Dose per PCU (nDCD/PCU)	nDCD/PCU= $\frac{\text{Total mg}}{\text{DCDesvac x PCU (kg)}}$	Total mg of antibiotic used, total PCU, ESVAC standards defined course dose for antibiotics	Mills et al. (2018); Khan et al. (2021)
Treatment Frequency (TF) per day	TF=$\sum\frac{Animals\ treated\ x\ Treatment\ days}{Animals\ in\ the\ population}$ TF per day= $\frac{TF}{Average\ Production\ Cycle}$	Animal treated is defined as the average of birds present in treatment	Kasabova et al. (2021);

Metric	Calculation	Requirements	Reference
		duration, DOC number is considered as animals in the population	Khan et al. (2021)
Animal treatment days (ATD) per cycle	ATD= $\frac{(Flock\ population) * (Number\ of\ treatment\ days\ in\ specific\ flock) * (days\ at\ risk)}{(Flock\ population) * (\ Production\ length\ in\ specific\ flock)}$	Number of treatment days, days at risk which is defined as the mean production lengths	Bos et al. (2013); modified by Agunos et al. (2017)

Appendix V

Purpose of antimicrobial usage in total treatment courses according to antimicrobial preparation (N=40 farms, no of treatment course=154, range 1-5)

Active substance*	Prevention	Treatment	Growth promotion	Prevention and growth promotion	Prevention and treatment	Total
Amoxicillin	15 (20.2%)	12 (17.6%)	1 (100%)		1 (25%)	29 (18.8%)
Amprolium		1 (1.5%)				1 (0.7%)
Chlortetracycline		1 (1.5%)				1 (0.7%)
Cephalexin	3 (4.1%)	1 (1.5%)		2 (28.6%)		6 (3.9%)
Ciprofloxacin	1 (1.4%)	6 (8.8%)		1 (14.3%)		8 (5.2%)
Colistin sulphate		3 (4.4%)				3 (1.9%)
Doxycycline	3 (4.1%)	1 (1.5%)				4 (2.6%)
Enrofloxacin	20 (27%)	4 (5.9%)			2 (50%)	26 (16.9%)
Flumequine	4 (5.4%)					4 (2.6%)
Gentamycin		1 (1.5%)				1 (0.7%)
Levofloxacin		5 (7.4%)		1 (14.3%)		6 (3.9%)
Metronidazole		2 (2.9%)				2 (1.3%)
Neomycin Sulphate	1 (1.4%)	2 (2.9%)				3 (1.9%)
Norfloxacin	1 (1.4%)	1 (1.5%)				2 (1.3%)
Oxytetracycline	2 (2.7%)	3 (4.4%)		2 (28.6%)		7 (4.6%)
Pefloxacin	1 (1.4%)	1 (1.5%)				2 (1.3%)
Sulphaclozine	2 (2.7%)	2 (2.9%)				4 (2.6%)
Tilmicosin		2 (2.9%)				2 (1.3%)
Toltrazuril	4 (5.4%)	5 (7.4%)				9 (5.8%)
Tylosin	2 (2.7%)	1 (1.5%)				3 (1.9%)
Amoxicillin MP$^{\alpha}$					1 (25%)	1 (0.7%)
Amprolium MP1$^{\beta}$		2 (2.9%)				2 (1.3%)
Amprolium MP2$^{\gamma}$	3 (4.1%)	1 (1.5%)				4 (2.6%)
Colistin Sulphate MP$^{\delta}$	2 (2.7%)	1 (1.5%)				3 (1.9%)

Doxycycline MP1$^{\varepsilon}$	1 (1.4%)					1 (0.7%)
Doxycycline MP2$^{\zeta}$	2 (2.7%)					2 (1.3%)
Doxycycline MP3$^{\eta}$		3 (4.4%)				3 (1.9%)
Sulphachlorpyridazine MP$^{\theta}$	4 (5.4%)					4 (2.6%)
Sulphadiazine MP$^{\kappa}$		1 (1.5%)				1 (0.7%)
Sulphadimethoxine MP$^{\lambda}$	2 (2.7%)	3 (4.4%)		1 (14.3%)		6 (3.9%)
Tylosin MP1$^{\mu}$		1 (1.5%)				1 (0.7%)
Tylosin MP2$^{\rho}$	1 (1.4%)	2 (2.9%)				3 (1.9%)

* In case of mixed preparations, main preparation is written;

MP- Mixed preparation; **α-** Amoxicillin + colistin sulphate, **β-** Amprolium + Sulfaquinoxaline sodium; **γ-** Amprolium + sulfaquinoxaline sodium + vitamin K; **δ-** Colistin sulphate + trimethoprim; **ε-** Doxycycline + colistin sulphate; **ζ-** Doxycycline + neomycin; **η-** Doxycycline + oxytetracycline; **θ-** Sulphachlor-pyridazine + trimethoprim; **κ-** Sulphadiazine+ trimethoprim; **λ-** Sulphadimethoxine + sulphadimidine + diaverdine + nicotinamide + vitamin K3; **μ-** Tylosin + doxycycline; **ρ-** Tylosin + doxycycline + bromhexin HCL

Biography

Md. Abu Shoieb Mohsin passed the Secondary School Certificate Examination, SSC, in 2010 obtaining GPA 5.00 (A+) and then Higher Secondary Certificate Examination, HSC, in 2012 obtaining GPA 5.00 (A+). Mr. Shoieb obtained his Doctor of Veterinary Medicine Degree in 2017 from Chattogram Veterinary and Animal Sciences University, CVASU, Bangladesh. Now, he is a candidate for the degree of Master of Science in Epidemiology under the Department of Medicine and Surgery, Faculty of Veterinary Medicine, Chattogram Veterinary and Aniaml Sciences University. He has immense interest working on the surveillance of antimicrobial usage and antimicrobial resistance.