EPIDEMIOLOGY OF FARM *ESCHERICHIA COLI* INFECTION AND ANTIBIOGRAM OF *ESCHERICHIA COLI* IN COMMERCIAL CHICKENS IN CHATTOGRAM, BANGLADESH

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Roll No: 0118/05

Registration No.: 529

Session: 2018-2019

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

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June 2020

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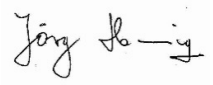

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List of abbreviations

Abbreviations	Elaborations
AM	Antimicrobials
AMR	Antimicrobial resistance
ANOVA	Analysis of variance
APEC	Avian pathogenic *E. coli*
AST	Antimicrobial susceptibility testing
BA	Blood agar
BALZAC	Behavioural adaptations in live poultry trading and farming systems and zoonosis control in Bangladesh
BBS	Bangladesh bureau of statistics
BER	Bangladesh economic review
BHI	Brain heart infusion
BPW	Buffered peptone water
CDC	Centers for disease control and prevention
CIAs	Critically important antimicrobials
CLSI	Clinical and laboratory standards institute
CRD	Chronic respiratory disease
DLO	District livestock officer
DLS	Department of livestock services
DOC	Day old chick
E. coli	*Escherichia coli*
EMB	Eosin methylene blue
ESBL	Extended-spectrum beta-lactamase
EUCAST	European committee on antimicrobial susceptibility testing
FAO	Food and agriculture organization
GDP	Gross domestic product
IB	Infectious bronchitis
IBD	Infectious bursal disease
LRT	Likelihood-ratio test
MAC	MacConkey
MALDI-TOF	Matrix assisted laser desorption/isonization time of flight
MHA	Mueller Hinton agar
MS	Master of science
ND	Newcastle disease
OECD	Organization for economic co-operation development

OHPH	One health poultry hub
OIE	Office international des epizooties (World organization for animal health)
OR	Odds ratio
OTC	Oxytetracycline
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PRTC	Poultry research and training centre
ROC	Receiver operating characteristic
USD	United States dollar
USDA	United States department of agriculture
WHO	World health organization
WPSA	World's poultry science association
%	Percentages
≥	Greater than or equal to
≤	Less than or equal to
95% CI	95% confidence interval

Abstract

Escherichia coli (*E. coli*), a Gram-negative bacterium of the family Enterobacteriaceae, is a commensal to the digestive tract of human and animals, can cause extra intestinal infections and serious food poisoning in human. Avian pathogenic ***E. coli***, strains can also cause systemic disease in poultry, which is called colibacillosis. Sign of colibacillosis include omphalitis, acute fatal septicemia or subacute pericarditis, airsacculitis, salpingitis, cellulitis and peritonitis. Poultry farm level *E. coli* prevalence and risk factors associated with biosecurity measures have not been reported yet in Bangladesh. In addition, indiscriminate use of antimicrobials in commercial chicken industry associated with poor biosecurity might play a role in the emergence and dissemination of multi-drug resistant *E. coli*. Hence, a cross-sectional study was conducted on 140 commercial broiler and layer farms in Chattogram in 2019. This study aimed to assess the farm level *E. coli* prevalence and describe antimicrobial susceptibility pattern of *E. coli* (farm and individual level) and determine associated potential risk factors related to biosecurity. Pool of cloacal (from 5 birds) and environmental swabs (5 sites) were collected from each per farm. Data on demographics, husbandry practices and antimicrobial application were collected with questionnaire, while a physical inspection of the farms was also conducted. *E. coli* was isolated by using selective culture media and antimicrobial susceptibility testing was performed by standard disc diffusion for 12 common antimicrobials of veterinary and/or human health importance. The total of 74.7% broiler and 84.2% of layer farm tested *E. coli* positive (at least one sample per farm tested positive). For environmental samples, 50.6% of broiler and 73.7% of layer, and for cloacal swabs, 54.2% of broiler and 63.2% of layer farms tested positive. Broiler farms having an isolation shed for sick birds had lower odds of farm level occurrence of *E. coli* (OR=0.4; 95% CI: 0.1-1.0). In case of layer farms that only partly conducted cleaning and disinfecting of farm surfaces and equipment had greater odds of *E. coli* (OR=3.4; 95% CI: 1.0-11.3) occurrence. Regardless of farm types, in average, *E. coli* isolates were resistant to 10 of the 12 antibiotics tested per farm. On broiler farms, 100% of isolates were resistant to amoxicillin, ampicillin, erythromycin and cephalexin followed by pefloxacin (98.8%), sulfamethoxazole and trimethoprim (96.5%), enrofloxacin (95.4%), doxycycline (94.2%); on layer farms, 100% of isolates were resistant to amoxicillin, ampicillin, erythromycin and cephalexin followed by pefloxacin (98.8%), sulfamethoxazole and

trimethoprim (96.3%), doxycycline (92.5%). On the other hand, Colistin sensitivity was high (~97%) for both production types. Interestingly, the antibiotic resistance to *E. coli* on broiler farms significantly increased with the level of education ($p<0.05$), but this was not the case for farms. This research highlighted a high prevalence of *E. coli* as well as high level of antimicrobial resistance on commercial poultry farms. Use of antimicrobial guidelines along with routine monitoring of antimicrobial susceptibility should be implemented to further reduce the antimicrobial resistance in Bangladesh.

Keywords: *E. coli* prevalence, Antimicrobial resistance, risk factors, chicken farms, Chattogram, Bangladesh

Chapter-I: Introduction

Commercial poultry production is a fast-growing industry in Bangladesh. It is a production type that requires less investment compared to other businesses (BER, 2021). The poultry population in Bangladesh is around 356.3 million with 296.6 million chicken in 2019-20 (DLS, 2020). There are 65000-70000 commercial farms in Bangladesh which are supported by 8 big poultry companies, many feed companies (~200) and pharmaceutical companies (~30) (WPSA, 2017; BPICC, 2020). In Chattogram district there are 1685 (577) layer farms, 7819 (4910) broiler farms and 1258 (295) Sonali farms (Personal communication: Dr. Md. Reajul Huq, DLO, Chattogram, 2020). Despite its tremendous growth over the last two decades, the poultry sector in Bangladesh struggled due to the presence of diseases like Colibacillosis, Salmonellosis, Fowl Cholera, Fowl Typhoid, Infectious coryza, Newcastle Disease, Infectious Bursal Disease, Infectious Bronchitis, Aflatoxicosis, CRD, ascites, Fatty liver hemorrhagic syndrome, egg bound and some deficiency disorders etc. (Islam et al., 2009; Roy et al., 2012; Al Mamun et al., 2019). About 30% annual mortality of chickens has been reported to occur due to disease outbreaks (Badruzzaman et al., 2015; Hamid et al., 2017). Colibacillosis caused by *Escherichia coli* (*E. coli*) is an important diseases that large mortalities (up to 94%) and decreases in production (Kabir, 2010; Nolan et al., 2013; Lupindu, 2017). It affects poultry of all ages (9.5-36.7%) (Rahman et al., 2004; Kabir, 2010). Colibacillosis in chicken is endemic in Bangladesh (Biswas et al., 2006). There are approximately 100 *E. coli* serotypes of which strains O1, O2 and O78, and to some extent O15 and O55 serotypes are the most pathogenic causing severe infections in poultry and survive for longer time in poultry house (Gross and Gyles, 1994; Chart et al., 2000; Kahn and Line, 2010). Based on pathogenicity there are six pathotypes of *E. coli*: verotoxigenic, enterotoxigenic, enteroinvasive, enteropathogenic, enteroaggregating and diffusely adherent (Nataro and Kaper, 1998; Vogt and Dippold, 2005). In poultry, there are different forms of colibacillosis reported: omphalitis, acute fatal septicemia or subacute pericarditis, airsacculitis, salpingitis, cellulitis and peritonitis (Nolan et al., 2013). In addition, sign reported include watery diarrhea, anorexia, weakness, loss of appetite (Kim et al., 1996) and pericarditis, perihepatitis, air sacculitis, exudate in abdominal cavity and fibrin as covering on multiple organs as oviduct, alveoli, liver, heart and lungs (Linden, 2015) are reported to be common manifestations of colibacillosis in

poultry. *E. coli* prevalence on broiler farms range between 66% to 86% (Kmetova, 2009; Mandal et al., 2021) and 59.3% to 81.7%, while on layer farms 56.4% to 82.8% have been estimated to be infected (Hossain et al., 2008; Mamun et al., 2016; Das et al., 2020; Ievy et al., 2020) in Bangladesh. Factors associated with Colibacillosis in poultry are age, ground water, density of farms, health status of chicken, housing condition, cannibalism, hygiene, pest control and biosecurity measures (Van den Bogaard et al., 2001; Vandekerchove et al., 2004; Ibrahim et al., 2019). Strict farm biosecurity and farm hygiene practices are the effective to prevent and control Colibacillosis in poultry (Yuvraj, 2019), however these practices are poorly implemented on small to medium poultry farms in Bangladesh (Shah et al., 2004). Antibiotics are widely used in poultry in Bangladesh – they are used as prophylactics as well as growth promoters (Schwarz et al., 2016). Easy access of antibiotics without the prescription by registered veterinarians and incompleteness of antibiotic courses along with sub-therapeutic doses and violation of drug withdrawal period are common in Bangladesh (Imam et al., 2020; Mutua et al., 2020; Phares et al., 2020). The indiscriminate use of antibiotics might contribute to the development of multi-drug resistant pathogens including *E. coli*. Previous studies reported that the frequent use of antimicrobial drugs as feed additives and administration at low concentrations (sub-therapeutic dose), give rise to selective pressure that may lead to development of resistant strains among commensal and pathogenic *E. coli* (Zhao et al., 2005; Apata, 2009; Zakeri and Kashefi, 2012; Diarra and Malouin, 2014). There is also a public health risk that antibiotic resistant pathogens like *E. coli* strains can be transmitted to humans via food or direct contact with infected poultry (Van den Bogaard et al., 2001; Marshall and Levy, 2011; Agyare et al., 2018). Antibiograms of common bacterial pathogens including *E. coli* have previously been produced in Bnagladesh (Akter et al., 2007; Hashem et al., 2012; Ievy et al., 2020; Mandal et al., 2021). In a recent study, some *E. coli* isolates were resistant to streptomycin, ceftriaxone, cefotaxime, gentamycin, clotrimoxazole and ampicillin (Pacifici, 2018; Hassan, 2020). Matin et al. (2017) found *E. coli* isolates multidrug resistant against ampicillin and cephalexin. However, only a limited antibiotics have been tested and risk factors that drive acquire resistance for *E. coli* at farm level have not been identified.

The present study was therefore conducted with the following objectives

1) To estimate the prevalence of *E. coli* infection in commercial chickens at farm level in Chattogram, Bangladesh

3) To determine the potential risk factors associated with the occurrence *E. coli* infection in commercial chickens at farm level

3) To assess antimicrobial pattern of *E. coli* in commercial chickens at individual and farm level

4) To determine potential risk factors associated with the occurrence AMR of *E. coli* at farm level

1.1.Outcomes of the study

1) Farm level *E. coli* prevalence and associated factors determined in commercial broiler and layer chicken in Chattogram, Bangladesh
2) Individual and farm level antibiogram pattern of *E. coli* and associated farm level factors determined
3) Made specific suggestions to control AMR at the farms of studied areas

Chapter-II: Review of Literature

The goal of this chapter was to review the previous research findings associated with the Master's thesis "**Epidemiology of Farm *Escherichia coli* Infection and Antibiogram of *E. coli* in Commercial Chickens in Chattogram Bangladesh**" to identify the scientific gaps and accordingly justify the current research. Various 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, farming challenges, *E. coli* and *E. coli* prevalence, associated risk factors, antimicrobial usage and antimicrobial resistance, antimicrobial resistance of *E. coli* in poultry with associated risk factors and consequences of AMR on poultry and public health.

2.1. Poultry farming in Bangladesh and it's challenges

Bangladesh is an agriculture based developing country with a huge population of 160 m (Rezvi, 2018). The progressively growing poultry sub-sector has proved to be an attractive economic activity, next to the Garments sector (USDA, 2019), accounting for 14% of the total value of livestock outputs (Islam et al., 2016) considered it as more beneficial than any other agricultural sub-sector for quick profit, income generation, poverty reduction and cheaper and rich animal protein production (Islam et al., 2016; Rahman et al., 2017). Poultry meat contributes 37% of total meat production of livestock origin in Bangladesh (WPSA, 2017). Poultry meat and eggs are also well accepted by all religions, social, economic and demographic groups (Simon, 2009).

The demand for poultry meat and eggs are fulfilled by locally grown backyard poultry (chicken, duck, goose) as well as from commercial chicken in different scales: small (flock size: 500-2500), medium (2501-5000) and large-scale (>5000) poultry enterprises (Personal communication: BALZAC project, 2018).

Commercial poultry farms are growing at a rate of 15% a year, with investment in the sector expected to double in the next decade. The poultry sector is gearing up for exporting by 2024 (OHPH, 2020). In Bangladesh, there were a total of 356.3 million poultry (including 296.6 million chickens) in the 2019-2020 production years (DLS, 2020). There are 65-70 thousand commercial chicken farms in various scales which are

supported by 16 grandparent farms, 206 small and large-scale breeder farms and 198 registered feed mills producing 5.3-5.4 million metric tonnes industrial feeds (WPSA, 2017). Predominant poultry companies in Bangladesh are Aftab Poultry, Aman Poultry, Bangladesh Rural Advancement Committee (BRAC), CP Bangladesh, Kazi farms, Nourish Poultry, Paragon Poultry etc. (Hamid et al., 2017). Commonly available commercial chicken strains in Bangladesh are Cobb 500, Ross 308, Habbard, Indian River meat, Tiger Sasso and Arber acre (broiler) and Hyline Brown/White, ISA Brown, Novogen Brown, Novogen White, Shaver 579, Hi-Sex Brown/White, and Bovine White (layer) (Hamid et al., 2017; USDA, 2019).

Although the poultry sector has remarkably intensified over the last two decades in Bangladesh, per capita animal protein consumption from poultry is still low (6.3 kg in Bangladesh vs. 2.4 kg in India, 6.6 kg in Pakistan, 48.7 kg in Malaysia, 7.8 kg in Indonesia, 7.8 kg in Thailand, 14 kg in China, 16.2 kg in Viet Nam, 17.7 kg in Japan and 18.7 kg in Korea) (Kawsar et al., 2013; WPSA, 2017; OECD, 2020). However, due to high-income generation and population growth with urbanization, demand for poultry meat and eggs has been increased (Islam and Jabbar, 2010; Hamid et al., 2017).

There are multiple challenges in poultry farming in Bangladesh such as lack of sustainable development policies and their implementation, insufficient veterinary services at door step, lack of skilled manpower, many endemic and epidemic poultry diseases, poor disease surveillance and data management systems along with poor laboratory support, poor strategies of disease prevention and control measures, excessive reliance on feed dealer and unstable market price (Kawsar et al., 2013; Rahman et al., 2015; Msoffe et al., 2016; Masud et al., 2020). Monopoly by the large industries with high pricing of the products also make drawbacks. Government and private sectors should plan to overcome such imbalances (Rahman et al., 2017). Over or indiscriminate usage of antibiotics in poultry is not only causing economic loss, but also causing antibiotic resistance which is posing serious public health threat (Chowdhury et al., 2021).

Poultry disease is the top most challenge in poultry rearing in different countries including Bangladesh. Commonly reported poultry diseases in Bangladesh are colibacillosis, salmonellosis, infectious coryza, fowl cholera, necrotic enteritis, infectious bursal disease, Newcastle disease, avian influenza, infectious bronchitis,

avian leucosis and fowl pox (Roy et al., 2012; Al-Mamun et al., 2019). In addition, the public health hazards from consuming foods with high antibiotic residues will remain a critical issue (Hafez and Attia, 2020). As this MS research is focused on *Escherichia coli*, the following literatures are focused on this.

2.2. *Escherichia coli*

Escherichia coli (*E. coli*), a Gram-negative and rod-shaped bacterium under the family Enterobacteriaceae, is a commensal to the digestive tract of warm human and animals, can cause extra intestinal infections (Guerra et al., 2003; Brenner et al., 2005; Lupindu, 2017). The genera *Escherichia* diverged around 102 million years ago, which coincides with the divergence of their hosts (Battistuzzi et al., 2004). Most *E. coli* strains are harmless, but some strains such as enterotoxigenic, enteropathogenic, enteroinvasive, or enterohaemorrhagic according to the presence of specific virulence factors can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination (Nataro and Kaper, 1998; Vogt and Dippold, 2005).

Generally, *E. coli* was provoked by several influencing factors: environmental factors, viral infections, mycoplasma infections, and immune suppression (Ewers et al., 2004). Generally, young birds are more susceptible than adults to severe infections (Rodriguez-Siek et al., 2005; Dziva and Stevens, 2008; Kabir, 2010). *E. coli* causes local and systemic infections in poultry, including pericarditis, air sacculitis, peri hepatitis, egg peritonitis, yolk sac infection (omphalitis), respiratory tract infection, coligranuloma, swollen head syndrome, cellulitis and septicaemia (Jordan et al., 2005; Matter et al., 2011; Matin et al., 2017; Azza et al., 2018; Swelum et al., 2021). ***E coli***, strains having specific virulence factors and causing systemic disease in poultry are termed avian pathogenic *E. coli* (APEC) (Ewers et al., 2003).

Colibacillosis in chickens refers to any local or systemic infection caused entirely or partly by *E. coli* strains (Nolan et al., 2013). It is the principle cause of colibacillosis in poultry characterized by inappetence, diarrhoea, dehydration, weight-loss, increase mortality and reduced infected birds' productivity (Kabir, 2010; Ibrahim et al., 2019). It might be the most frequent and most devastating bacterial infections in young birds, including developing embryos (Goren, 1978). Only a few specific serotypes are associated with colibacillosis that results from shifting organisms from the lower

intestine to other digestive parts or respiratory organs of the poultry (Stromberg et al., 2017). Colisepticaemia is a severe systemic form of infection (Dho-Moulin and Fairbrother, 1999; Saif et al., 2003). Omphalitis is a major factor responsible for early chick mortality during the first few days after hatching (Fasenko and O'Dea, 2008). It is accounting for large economic losses in poultry by causing mortality rates up to 25% during the first week of life (Cortés et al., 2010).

The horizontal infections with *E. coli* occur by the contact with other birds, in addition to fecal and oral routes. Contrarily, the *E. coli* vertical transmission was reported from breeders through eggshell contamination (Nolan et al., 2013; Swelum et al., 2021). Vertical transmission from healthy parents resulting in high first-week mortality in the offspring illustrates the potential of the emergence and spreading of bacteria in animal husbandry (Petersen et al., 2006).

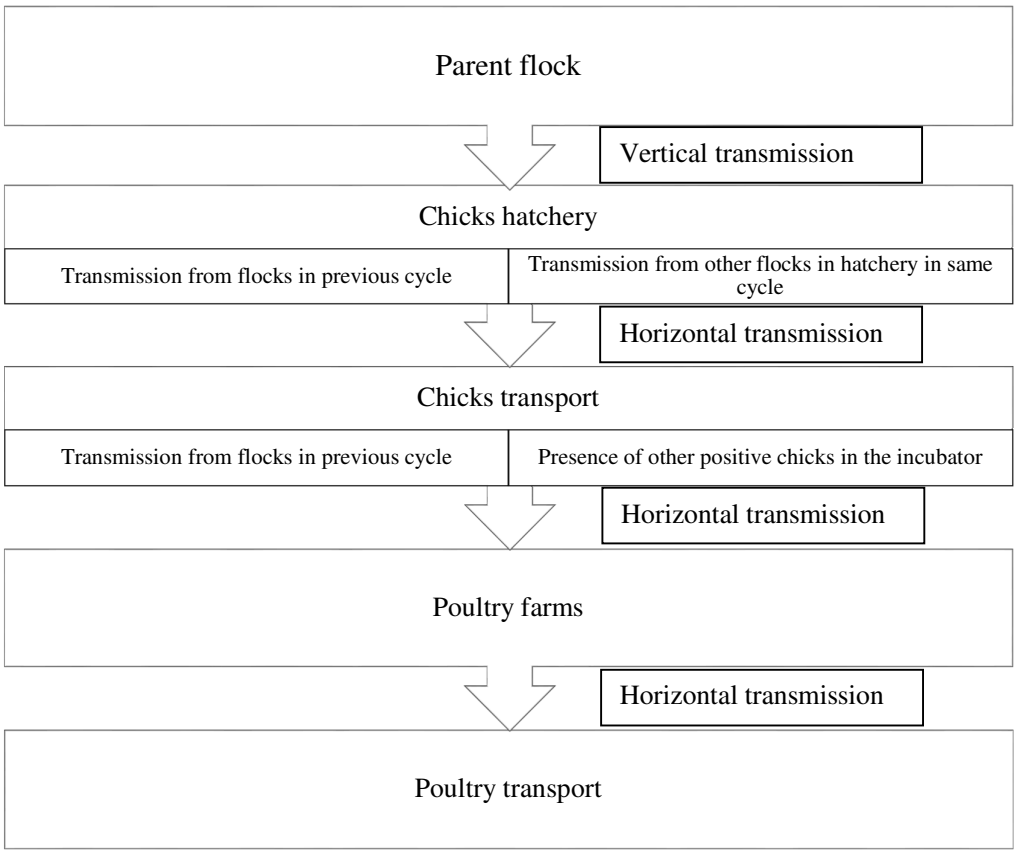

Figure 2.1. Transmission cycle of *E. coli* in poultry (Plaza Rodríguez et al., 2018)

2.2.1. Prevalence of *E. coli* in poultry

The prevalence of *E. coli* is varied by level (individual and farm), production system and geographical areas. Previously documented *E. coli* prevalence at different levels are presented in table below.

Table 2.1: Reported prevalence of *E. coli* in broiler poultry in different countries

Country	**Prevalence in broiler**		**Reference**
	Farm level, %	**Individual level, %**	
India		73	Bhardwaj et al. (2021)
India		85.3	Chowdhury et al. (2022)
Pakistan		59	Afridi et al. (2020)
Pakistan		89.2	Azam et al. (2019)
Nepal		36	Khanal et al. (2019)
Thailand		39	Hanson et al. (2003)
Thailand	60.4		Rodroo et al. (2021)
Malaysia		51.8	Ibrahim et al. (2021)
Malaysia		60.8	Elmi et al. (2021)
Indonesia		100	Wibisono et al. (2020)
Egypt		59	
Bangladesh		59.3	Das et al. (2020)
Bangladesh		61.7	Sarker et al. (2019)
Bangladesh		63.6	Hossain et al. (2008)
Bangladesh		81.7	Mamun et al. (2016)
Bangladesh	66		Kmetova, (2009)
Bangladesh	86		Mandal et al. (2021)

Table 2.2: Reported prevalence of *E. coli* in layer poultry in different countries

Country	Prevalence in layer		Reference
	Farm level, %	Individual level, %	
India	75.5		Samanta et al. (2014)
Malaysia		66	Elmi et al. (2021)
Indonesia		90	Wibisono et al. (2020)
Bangladesh		56.4	Hossain et al. (2008)
Bangladesh		78.7	Jakaria et al. (2012)
Bangladesh		82.8	Ievy et al. (2020)

The above literature suggests much published studies on estimating *E. coli* infection are at individual level. The cited Bangladesh studies were either small scale or not-properly followed epidemiological study design. Therefore, a study to estimate farm level *E. coli* is justified.

2.2.2. Factors in association with *E. coli*

Risk factors associated with the occurrence of *E. coli* in commercial poultry farm at individual or farm level have previously been reported in many published studies across the world.

Disinfection of floor between production cycles significantly lowered the odds of having a positive *E. coli* isolate, among the tested samples (OR = 0.1, p = 0.01, 95% CI 0.03–0.6) (Mo et al., 2016). Wet cleaning significantly decreased the farm *E. coli* prevalence (OR = 0.4, p = 0.044, 95% CI 0.2-1.0) than dry cleaning (Course et al., 2021). An increase in space of the cages with reducing layer hen density decreased the risk of *E. coli* infection by 33% (OR=0.8, p = 0.001, 95% CI 0.6-0.9) (Vandekerchove et al., 2004; Landman and Cornelissen, 2006). Broiler farms used ground water as drinking water were reported to be more prone to *E.coli* infection than that of supply water (OR = 18.1, p = 0.005; 95% CI 2.5-133.4) (Ibrahim et al., 2019). The use of no separate shoes for farm staff and visitors was reported to increase farm *E. coli* prevalence (OR=8.6; p value = 0.033; 95% CI 1.2-62.6) than those farms having separate shoes for farm staff and visitors (Mandal et al., 2021). Nguyen et al. (2015) also reported that change shoes/boots practice on the farm (OR=3.4, 95% CI=1.0–11.8) were associated with the presence of ESBL-producing *E. coli* on the farm.

However, there are gaps in conducting systematic and large-scale epidemiological studies in Bangladesh with the proper statistical analysis to determine potential risk factor analysis associated with the occurrence at farm level.

2.3. Antimicrobial usage and antimicrobial resistance

Antimicrobials comprised the major components of veterinary drugs (Donoghue, 2003) which are widely and indiscriminately used in commercial poultry in Bangladesh and neighbouring countries as prophylactic, therapeutic as well as growth enhancer (Snary et al., 2004; Awogbemi et al., 2018). Antimicrobial agents that are used commonly in poultry in this country are amoxicillin, tetracycline, doxycycline, oxytetracycline, ciprofloxacin, enrofloxacin, norfloxacin, erythromycin, neomycin, colistin sulfate, tylosin tartrate, tiamulin, sulphadiazine and trimethoprim-sulfonamide (Asaduzzaman, 2000; Hasan et al., 2011; Islam et al., 2016; Ferdous et al., 2019; Imam et al., 2020; Rousham et al., 2021). Most frequently used antimicrobial agents according to the certain overseas studies were amoxicillin, oxytetracycline, enrofloxacin, furazolidone, erythromycin, streptomycin, neomycin, gentamicin, tylosin and trimethoprim-sulphonamide (Persoons et al., 2012; Oluwasile et al., 2014; Al-Mustapha et al., 2020; Kasabova et al., 2021).

Antibiotics are commonly used to control Avian Pathogenic *E. coli* (APEC) infections in poultry (Agunos et al., 2012). Antimicrobial groups considered for treating colibacillosis cases in poultry worldwide are tetracyclines, sulfonamides, penicillins, aminoglycosides, cephalosporins, fluoroquinolone/quinolones, chloramphenicols, polymyxins, macrolides and lincosamides (Agunos et al., 2012; Landoni and Albarellos, 2015; Kathayat et al., 2021).

In developing countries and also in Bangladesh, most of the time, these antimicrobials are administered without seeking veterinary prescription and can be purchased over counter (Imam et al., 2020; Mutua et al., 2020; Phares et al., 2020). Because of indiscriminate usage previously sensitive antimicrobial agents are becoming resistance (Michael et al., 2014). Antimicrobial resistance is the resistance of a microbe to an antimicrobial agent that was used effectively in treating or preventing an infection caused by that microbe (Sykes, 2010; Prestinaci et al., 2015; Reygaert, 2018). The frequent use of antimicrobial drugs as feed additives, extensive use and also

administered at low concentrations (sub-therapeutic dose), give rise to selective pressure that may lead to development of resistant strains among commensal and pathogenic *E. coli* (Zhao et al., 2005; Apata, 2009; Zakeri and Kashefi, 2012; Diarra and Malouin, 2014).

2.4. Antimicrobial resistance in *E. coli* in poultry and associated risk factors

Resistant *E. coli* is frequently isolated from live chickens and strains with multiple resistance to tetracycline, streptomycin, sulfonamides, gentamycin, fluoroquinolones (Agyare et al., 2018; Varga et al., 2019). The previously reported AMR prevalence status against *E coli* in poultry is presented in table below:

Table 2.3: Reports on AMR prevalence of *E. coli* in different countries

Country	Antimicrobial agent	Prevalence, %	Reference
Bangladesh	Ampicillin	58-100	Kmetova, (2009); Al Azad et al. (2019); Sarker et al. (2019); Ievy et al. (2020)
	Amoxicillin	84.6	Hassan et al. (2014)
	Erythromycin	64-100	Hossain et al. (2008); Kmetova, (2009); Al Azad et al. (2019); Ievy et al. (2020)
	Enrofloxacin	55.5-100	Hassan et al. (2014); Ievy et al. (2020)
	Doxycycline	53.8-79.1	Hassan et al. (2014); Saha et al. (2020); Mandal et al. (2021)
	Gentamicin	8.3-51	Al Azad et al. (2019); Ievy et al. (2020); Saha et al. (2020)
	Sulfamethoxazole-trimethoprim	94.6-100	Bashar et al. (2011); Al Azad et al. (2019); Sarker et al. (2019)
	Neomycin	20	Kmetova, (2009); Bashar et al. (2011)
	Azithromycin	31.6	Saha et al. (2020)
	Colistin	7.8-26.5	Al Azad et al. (2019); Ievy et al. (2020); Saha et al. (2020); Mandal et al. (2021)
India	Ampicillin	29.2-96.1	Sahoo et al. (2012); Balasubramaniam et al. (2014); Muglikar et al. (2019); Khasa and Singh, (2020)
	Amoxicillin	16.7-71.4	Sahoo et al. (2012); Balasubramaniam et al. (2014); Khasa and Singh, (2020)

	Erythromycin	18.5	Kumar and Kumar, (2020)
	Enrofloxacin	31.6-91	Joshi et al. (2012); Balasubramaniam et al. (2014); Muglikar et al. (2019); Khasa and Singh, (2020)
	Cephalexin	16.7-73.7	Joshi et al. (2012); Khasa and Singh, (2020)
	Gentamicin	25-76.6	Muglikar et al. (2019); Khasa and Singh, (2020); Chowdhury et al. (2022)
	Sulfamethoxazole-trimethoprim	65.8	Kumar and Kumar, (2020)
	Neomycin	31.6	Joshi et al. (2012)
	Pefloxacin	26.3-88	Joshi et al. (2012); Balasubramaniam et al. (2014)
Pakistan	Ampicillin	93.5-98.6	Kamboh et al. (2018); Azam et al. (2019)
	Amoxicillin	85-93.9	Kamboh et al. (2018); Tahir et al. (2021)
	Erythromycin	27	Tahir et al. (2021)
	Enrofloxacin	50-77.1	Kamboh et al. (2018); Tahir et al. (2021)
	Doxycycline	61.2-84.4	Kamboh et al. (2018); Latif Baloch and Magsi, (2019)
	Gentamicin	34.1-78.8	Kamboh et al. (2018); Latif Baloch and Magsi, (2019)
	Sulfamethoxazole-trimethoprim	68-77.6	Latif Baloch and Magsi, (2019)
	Neomycin	53	Azam et al. (2019); Latif Baloch and Magsi, (2019)
Thailand	Ampicillin	78.5-100	Mooljuntee et al. (2010); Chansiripornchai et al. (2011); Homjan et al. (2018); Lawwyne et al. (2019); Tansawai et al. (2019)

	Amoxicillin	70.2-100	Chansiripornchai et al. (2011); Homjan et al. (2018); Lawwyne et al. (2019); Thomrongsuwannakij et al. (2020)
	Erythromycin	80-100	Mooljuntee et al. (2010); Chansiripornchai et al. (2011); Nuangmek et al. (2018)
	Enrofloxacin	24-50	Chansiripornchai et al. (2011); Lawwyne et al. (2019)
	Doxycycline	30-61.2	Chansiripornchai et al. (2011); Tansawai et al. (2019)
	Cephalexin	10-72	Chansiripornchai et al. (2011); Lawwyne et al. (2019)
	Gentamicin	20-43	Chansiripornchai et al., (2011); Lawwyne et al. (2019); Tansawai et al. (2019)
	Sulfamethoxazole-trimethoprim	26.7-64.5	Mooljuntee et al. (2010); Nuangmek et al. (2018); Lawwyne et al. (2019); Tansawai et al. (2019); Thomrongsuwannakij et al. (2020)
	Neomycin	62	Chansiripornchai et al. (2011)
	Colistin	24	Chansiripornchai et al. (2011)
Malaysia	Ampicillin	51.9-87.5	Elmi et al. (2021); Ibrahim et al. (2021)
	Amoxicillin	21.2	Elmi et al. (2021)
	Erythromycin	100	Ibrahim et al. (2021)
	Doxycycline	66.4	Elmi et al. (2021)
	Gentamicin	20.2-23.3	Elmi et al. (2021); Ibrahim et al. (2021)
	Sulfamethoxazole-trimethoprim	74.2-83.3	Elmi et al. (2021); Ibrahim et al. (2021)

The AMR prevalence data in Table 2.3 reflects the alarming situation of antimicrobial resistance in poultry of Bangladesh and other countries. However, farm level exploration of AMR pattern are not common and has not systematically studied in Bangladesh.

Multiple past studies reported significant risk factors associated with the occurrence of AMR against *E coli* at different levels (Individual and farm). The results are given in Table 2.4.

Table 2.4: Reported risk factors associated with AMR in *E. coli* in different countries

Country	**Significant factor**	**Category or categories**	**Odds ratio or other ratio**	**Confidence interval,**	***p* value**	**Ref**
Malaysia	Water source	Pump water	2.0	1.2-3.4	0.01	Elmi et al. (2021)
		Surface water	1.6	0.9-2.7	0.08	
	Farm size	Small scale	2.5	1.3-4.8	0.004	
	Source of sample	Sewage samples	7.4	1.0-156.9	0.09	
Vietnam	Farm size	Small	6.4	2.7-15.0	<0.001	Nguyen et al. (2015)
	Use of lincosamide	Yes	4.7	1.2-19.0	0.028	
	Use of tetracycline	Yes	2.0	1.2-3.4	0.011	
	Day old chick from other sources	Yes	4.9	1.2-20.0	0.026	
	Use of commercial feed	Yes	2.5	1.1-4.1	0.001	
	Experience		1.0	0.9-1.0	0.004	

Bangladesh	Season	Winter	8.4	1.1-63.9	0.04	Mandal et al. (2021)
	Follow veterinarian prescription	No	18.5	2.0-173.9	0.011	
Nigeria	Absence of lavatory	Yes	4.3	1.6-11.9	0.01	Aworh et al. (2019)
	Diarrhea in last 3 months	Yes	3.3	1.3-8.5	0.02	
	Work exposure	>10 years	0.3	0.1-0.9	0.04	
Cameroon	Lack of training	Yes	0.13	0.03-0.6	0.01	Moffo et al. (2021)
	Frequency of digestive tract diseases	High	0.1	0.02-0.4	0.001	
	Experience in poultry farming	>5 years	11.7	1.1-121.1	0.04	

Risk factors determined for the occurrence of AMR in poultry in Table 2.4 reflects the overall picture of AMR in Asia and African countries. However, in Bangladesh there were a few studies which justify the present study to determine potential risk factors associated the occurrence of AMR at farm level.

2.5. Consequence of antimicrobial resistance on poultry and public health

Antimicrobial resistance has emerged as a global health security worldwide (Aworh et al., 2019). Poultry and poultry environment act as a potential source for resistant *E. coli* and source of human infection (Stromberg et al., 2017). People involved in livestock farming have been shown to have higher rates of carriage of antimicrobial resistant bacteria. In some studies, it has been shown that resistant *E. coli* can spread from chickens to humans directly or via food (Norizuki et al., 2017; Amir et al., 2019; Mandal et al., 2021).

The socioeconomic implications of AMR include increased cost and duration of treatment while the public health implications include decreased ability to treat common infections resulting in increased human suffering and ultimately death (Michael et al., 2014; Prestinaci et al., 2015; Li and Webster, 2018)

2.6. Summary of the review

This review indicates information gaps about assessing farm level *E.coli* prevalence in commercial chicken in Bangladesh and associated factors. The review points to inconsistent AMR prevalence study against *E. coli* for human important antibiotics as well as potential risk factors associated with the occurrence AMR of *E. coli* at farm level. Moreover, the aforementioned cited Bangladeshi studies were not epidemiologically well designed. Therefore, the study aimed to appraise farm *E. coli* prevalence, associated risk factors and antibiogram pattern of *E. coli* in Chattogram, Bangladesh.

Chapter-III: Materials and Methods

3.1. Study area

Chattogram, a sub-tropical region, is one of the oldest districts of Bangladesh, located in the south-eastern part of Bangladesh (21º54´ and 22º59´N and 91º17´ and 92º13´E), with an area of 5284.92 sq. km. It has featured with sea, different rivers, hills and mountains and low and high land along with diverse ethnic groups (Muslim, Hindu, Buddhists, Christians and a range of tribes). The population size of this district is 7616,352 with a population density of 1442 sq. km (BBS, 2013). This district constitutes of 15 upazilas (sub-districts) and 3 metro thanas. The literacy rate of this district is 58.9% (BBS, 2013). The main professions of population of this district are fishing, whole sale and retail trade, manufacturing, hotel/restaurant business, and education (BBS, 2013). Chattogram has around 3.5 million poultry population, regardless of production types, which contribute to 0.95% of total population (N=365 million) in Bangladesh (DLS, 2020). Poultry farm distribution of Chattogram is 4882 broiler farms, 295 Sonali farms, 559 layer farms, 20 breeder farms and household farms (Personal communication: Dr. Md. Reajul Huq, DLO, Chattogram, 2020). Antimicrobials are widely used for different purposes (therapeutics, prophylactic or both) in poultry sectors across the country including Chattogram (Lagha et al., 2017; Mund et al., 2017; Mehdi et al., 2018). Hence, Chattogram was chosen for investigating antimicrobial usage and antimicrobial resistance in commercial poultry farms.

3.2. Study type and duration

A cross-sectional study was carried out on commercial poultry farms (broiler and layer) in Chattogram for 6 months, from February to July 2019.

3.3. Population

3.3.1. Reference population

All commercial poultry (commercial broiler and layer) farms belonging to Chattogram district were considered as the reference population.

3.3.2. Source population

To cover maximum geographical area of Chattogram district, Gupta et al. (2021) selected eight upazilas according to some criteria such as presence of water bodies,

forests, hills and distance from Chattogram city. Poultry farms belonging to these upazilas of Chattogram district were chosen as the source population for the present study. These included Anowara, Chandanaish, Fatickchari, Lohagara, Patiya, Rangunia, Raozan and Sitakunda.

3.3.3. Epidemiological unit and sampling frame

Without considering the poultry production type a farm having at least 500 birds was defined as the smallest unit of the sampling farms. Accordingly, there were total of 1748 commercial poultry farms (1493 broiler and 255 layer farms) and distribution of the farms in the sampling frame by upazillas (See Table 3.1). The sampling frame was developed by Gupta et al. (2021) through consultation with the relevant stakeholders or offices: Chattogram Livestock Services, government and private poultry practitioners, feed and chick dealers and pharmaceuticals representatives. Then Gupta et al. (2021) selected farms by using simple random sampling.

Table 3.1: Total number of poultry farms in sampling frame in studied upazilas

Upazilla	**Broiler farm**		**Layer farm**	
	No of farms	Size: Min-Max	No of farms	Size: Min-Max
Anwara	234	500-4000	9	500-5000
Chandanaish	199	500-5500	18	1000-6500
Fatickchari	180	500-4800	36	500-5500
Lohagara	180	500-3500	40	1000-13000
Patiya	199	500-5000	40	500-5000
Rangunia	231	500-3000	40	500-7000
Raozan	144	500-3500	27	500-6000
Sitakunda	126	500-7000	45	500-8000
Total	1493	500-7000	255	500-13000

3.4. Sample size calculation

A total of 139 farms were required assuming expected prevalence of 90% (if a farm having 50% of commonly used antibiotics become resistant, then this farm was classified as an AMR farm), ±10 precision (as there was no published ARM prevalence estimate at farm level), 95% Confidence Interval and 1 design effect (Formula: N = Design effect * $p(1-p)/E^2$) (OpenEpi, 2013).

3.5. Sampling technique

A proportionate probability of random sampling technique was applied to recruit the required number of farms (N=83 broiler farms and N=57 layer farms). Some farms were excluded as they were not operating or had no birds during field visit and neighboring farms were included as replacement.

If a farm had one shed, data and sample were then collected from that shed. If a farm had more than 1 shed and same kind of antimicrobials used in all sheds, data and sample were taken from the shed with oldest chickens. If a farm had more than 1 shed and multiple antimicrobials used in different sheds, data and sample were taken from the shed with highest number of antimicrobials used.

Table 3.2: Farm distribution according to production type in studied upazilas

Upazilla	**Broiler farm**		**Layer farm**	
	No of farms	Size: Min-Max	No of farms	Size: Min-Max
Anwara	13	500-4000	2	500-5000
Chandanaish	11	500-5500	4	1000-6500
Fatickchari	10	500-4800	8	500-5500
Lohagara	10	500-3500	9	1000-13000
Patiya	11	500-5000	9	500-5000
Rangunia	13	500-3000	9	500-7000
Raozan	8	500-3500	6	500-6000
Sitakunda	7	500-7000	10	500-8000
Total	83	500-7000	57	500-13000

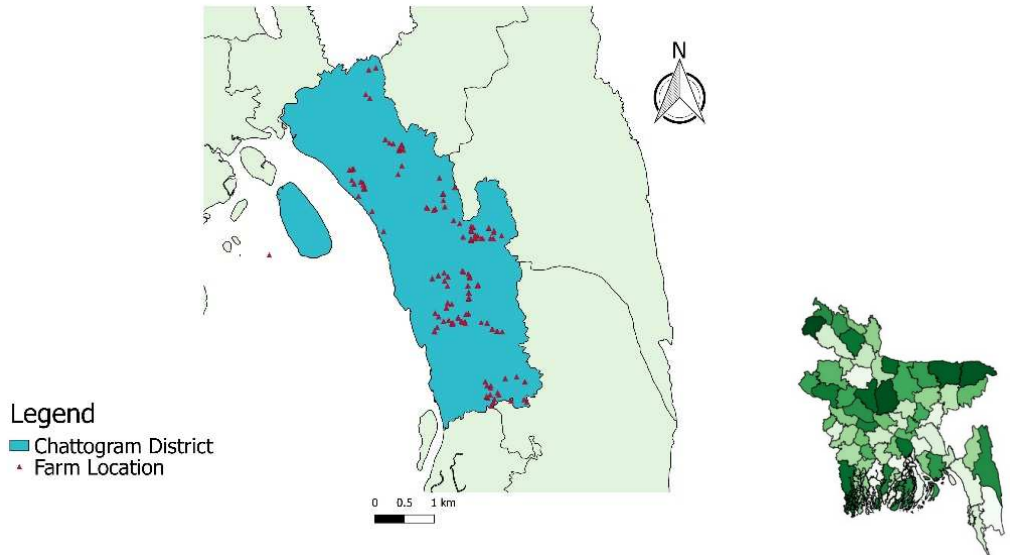

Figure 3.1. Location of selected poultry farms in Chattogram district

3.6. Data collection

3.6.1. Questionnaire development, validation and administration

According to the targeted objectives of the study a questionnaire was drafted. Before drafting a through literature review and consultation with experts was performed to identify areas to develop questionnaire. The drafted questionnaire was then peer-reviewed to identify any gaps and updated accordingly. Afterwards the updated version of the questionnaire was piloted on 3 broiler farms and 3 layer farms to check the consistency of the question and total time required to administer. The findings of the piloting were taken into account to finalize the questionnaire.

The questionnaire consisted of information related to i) farm characteristics including farm location, number of sheds, population of birds, age etc, ii) husbandry practices like farm hygiene, biosecurity, water bath facility, cleaning and disinfection, disposal of dead birds and wastage etc, iii) types of antimicrobials used, purposes, amount used, route of administration, duration etc. Closed ended, open ended and mixed types of questions were included in the questionnaire. The full questionnaire is given as **Appendix-I**. The farms used for questionnaire piloting was not included for the main study.

A 3-member team made all the field trips during the study period. Each day 4-5 farms were covered. Among the team members one member conducted the interview, one

collected the samples and one took the photographs and close inspection. Before visiting the field the team leader communicated with the local veterinarian to select farmers to set date of interview for data collection and biological sampling. A verbal consent was taken from each participant farmer before administering the questionnaire and sample collection. All the farmers had given a soap and a liquid hand-wash as token gift.

3.7. Sample collection, transportation and preservation

Cloacal swab and environment swab samples were obtained from each selected farm. Cloacal swabs were collected from randomly selected 5 birds per farm and then pooled in a 15-ml sterile falcon tube containing Stuart transport medium (Neogen, Lansing MI). Environmental swab samples were collected from middle and 4 corners of each selected farm and then pooled in a 15 ml sterile falcon tube containing buffered peptone water (BPW) (Neogen, Lansing MI). All tubes were then labeled with unique identity numbers and kept in an ice box. Within 4 to 6 hours, samples were transferred to the laboratory and kept in -20ºC for further analysis at PRTC lab of CVASU.

3.8. Lab evaluation

3.8.1. Sample preparation

E.coli was isolated from both sample types (Cloacal and environmental pools) by standard microbiological methods according to the procedure of Quinn et al. (2002). Before starting of laboratory work, each sample pooled swab was mixed with BPW (full) in a ratio of 1:10 and incubated at 37ºC for 18-24 hours as a purpose of enrichment.

3.8.2. Bacteriological test

10 µl pre-enriched cultured broth was streaked onto MacConkey (MAC) agar (Neogen, Lansing MI) surface and incubated overnight at 37°C aerobically. Any bright, pink colored transparent smooth raised colonies were suspected colonies on MC agar and then streaked on eosin methylene blue (EMB) agar (Neogen, Lansing MI). Plates were incubated at 37ºC for 18-24 hours. After incubation the plates were examined for the presence of typical colonies of *E. coli*. Yellow green characteristic metallic sheen on EMB agar were observed. Suspected colonies was verified and confirmed by the following

biochemical tests: triple sugar iron (TSI) agar (Neogen, Lansing MI) slant reaction (Yellow slant, yellowbutt, presence of gas bubbles and absence of black precipitate in the butt), indole reaction and citrate utilization test.

Suspected colonies were then transferred to 5% blood agar (BA) (Blood agar base, Oxoid®, Oxoid Ltd, Basingstoke, United Kingdom). After overnight incubation at 37°C these were grown in brain heart infusion (BHI) broth (Neogen, Lansing MI). All the positive isolates were stored at -80°C using 50% glycerol for further use. The detailed bacteriological test protocols are given in **Appendix-II**.

3.8.3. Cultural sensitivity test

Cultural sensitivity test of disk diffusion method was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018a) using Kirby-Bauer disc diffusion assay. *E. coli* colonies from BA were mixed with the phosphate buffer saline (PBS) by vortexing and the turbidity was adjusted to the MacFarland 0.5 turbidity standard. Then the broth was streaked on Mueller Hinton (MH) agar (Difco Laboratories, Sparks, MD, USA) plate. Antibiotic discs were applied aseptically on the surface of the inoculated plates with the help of a sterile pair of forceps. The antimicrobial agents (Hi-media) tested were amoxicillin (30 µg), ampicillin (25 µg), cephalexin (25 µg), doxycycline (30 µg), erythromycin (15 µg), enrofloxacin (5 µg), gentamicin (10 µg), neomycin (30 µg), azithromycin (30 µg), colistin (10 µg), pefloxacin (5 µg) and sulfonamide and trimethoprim (25 µg). The plates were then inverted and incubated at 37°C for 16 to 18 hours. After incubation the plates were examined and the diameters of the zones of complete inhibition were observed. The breakpoints for the interpretation of resistance and susceptibility were those recommended by the CLSI guideline (CLSI, 2018b) and EUCAST guideline (EUCAST, 2018). All breakpoints were not available in one guideline, thereby both guidelines were followed. Zones of inhibition were classified as susceptible, intermediate and resistant categories based on the CLSI guideline. The detailed cultural sensitivity test procedure is given in **Appendix-III**.

3.9. Statistical analysis

3.9.1. Data entry and cleaning

Field and laboratory data were entered into Microsoft Excel 2016. Data cleaning, coding and integrity were checked for validation and consistency, and then exported to STATA IC-13 (StataCrop, 4905, Lakeway Drive, College Station, Texas 77845, USA) for epidemiological analysis. Descriptive analysis and risk factor analysis were conducted on different data sets.

3.9.2. Descriptive analysis

The prevalence of *E. coli* at farm level was calculated by number of case farms divided by total number of studied farms tested. The farm prevalence of *E. coli* was then distributed by production types (broiler/layer) and sample types (cloacal swab/environmental swab). Summary statistics of antibiogram of *E. coli* at farm level by farm type (broiler/layer) was calculated. The results were expressed as frequency number, percentage, mean and 95% CI.

3.9.3. Risk factor analysis (*E. coli* infection)

Risk factor analysis was conducted on the data generated (outcome and exposure data) from the broiler and layer farms separately.

3.9.3.1. Univariate analysis for the occurrence of *E. coli* at farm level

Fisher's exact test was used to assess the difference of proportion of farm *E coli* cases (binary response variable) between different categories of each farm biosecurity practices. Of 13 factors at broiler farm only two factors were found statistically significant ($p \leq 0.2$). They were i.) "Have isolation shed for sick birds (Yes/partially or No)" and ii) "Disinfecting and cleaning the farm surfaces and equipment weekly (Yes/partially or No)" and therefore were forwarded to conducting the logistic regression model to assess their adjusted effect on the farm level occurrence of *E coli* in broiler farms.

Of 14 factors at layer farm only two factors were revealed as statistically significant (($p \leq 0.2$). They included i) "Washing egg tray being brought back from market (Yes or partially)" and ii) "Employee living within farm premises (Yes or No)". However, these factors were not forwarded to constructing the logistic regression model due to

insufficient frequency number against each category of the factor even after regrouping was made.

3.9.3.2. Multivariate analysis for the occurrence of *E. coli* at broiler farm level

A multivariable logistic regression model was constructed with the aforementioned factors ("Have isolation shed for sick birds" and ii) Disinfecting and cleaning the farm surfaces and equipment weekly. We used backward stepwise selection of variables with an inclusion threshold of 0.05 (Likelihood Ratio test: LRT p value). We assessed for interaction between factors by constructing two-interaction product terms for the significant main effect actors in the model, forcing them into the model and examining changes in the coefficients and P values of the main effects. The presence of confounding factors was investigated by removing one of the variables and assessing changes in the coefficient change of more than 15% was considered to indicate the presence of confounding variables. Independence of the factors was checked by Fisher's exact test. We used Hosmer-Lemeshow test to calculate model χ^2 statistic and McFadden's pseudo-R^2 (the coefficient of determination) to explain variance and measure goodness-of-fit for multivariate regression model. The results were presented as adjusted Odds Ratio (OR), 95% CI and p value.

3.9.4. Risk factor analysis (antibiogram)

Risk factor analysis was conducted on the data generated (AMR and exposure data) from the broiler and layer farms separately.

3.9.4.1. Univariate analysis for the status of antibiogram at farm level

The factors were assessed by using either t-test or 1-Anova.

In case of broiler farms mean number of antibiotic resistant types to *E. coli* isolates per farm significantly varied by farm size, farmer's experience, farmer's education, checking and decontamination of vehicles before entering into farm and decontamination of vehicle before leaving farm ($p \leq 0.2$).

In case of layer farms average number of antibiotic resistant types to *E. coli* isolates per farm significantly differed by having washing facility before entering to farm, do hand washing before entering into farm, changing clothes/shoes before entering into farm (visitors), disinfecting and cleaning the farm surfaces and equipment weekly,

employees having training on bio-security measures (at least once), employee living within farm premises and presence of other birds/animals in the farm ($p \leq 0.2$).

3.9.4.2. Multivariate analysis for the status of AMR at broiler farm level

As overall sample size of farms of each production type was small only three more relevant significant factors for each production type were forwarded to the multivariate linear regression analysis. They were farm size, farmer's experience and farmer's education (for broiler); having washing facility before entering to farm, do hand wash washing before entering into farm and employees having training on bio-security measures (at least once) (for layer).

A multivariable linear regression model was constructed with the aforementioned factors. We used backward stepwise selection of variables with an inclusion threshold of 0.05. We assessed for interaction and confounding between factors using the LRT in a similar procedure as discussed earlier for the logistic model analysis. Variance inflation factors (VIF) for the factors were examined to diagnose collinearity and to identify highly correlated factors to avoid a duplication of effects. A VIF value of more than 0 indicates serious collinearity. The Cook–Weisberg test was used to examine the homogeneity of variance and whether the overall data fitted the model. The results were presented for each adjusted factor as a coefficient, P value and 95% CI.

Chapter-IV: Results

4.1. Farm level prevalence of *E. coli* in commercial chicken farms in Chattogram, Bangladesh

Farm level prevalence estimate of *E. coli* was 74.7% in broiler and 84.2% in layer. In broiler farms, farm level prevalence estimate of *E. coli* was 54.2% and 50.6% for cloacal and environmental samples, respectively. In layer farms, farm level prevalence estimate of *E. coli* was 63.2% and 73.7% for cloacal and environmental samples, respectively (Table 4.1).

Table 4.1: Farm level prevalence estimate of *E. coli* in commercial chickens in Chattogram (N=140), Bangladesh

	No. of broiler farms (N=83)			**No. of layer farms (N=57)**		
Types of sample	**No of +ve**	**%**	**95% CI**	**No of +ve**	**%**	**95% CI**
Cloacal	45	54.2	42.9-65.2	36	63.2	49.3 – 75.6
Environment	42	50.6	39.4-61.7	42	73.7	60.3 – 84.5
Either one	62	74.7	63.9-83.6	48	84.2	72.1 – 92.5

4.2. Risk factor analysis for farm level occurrence of *E. coli* in commercial chickens in Chattogram, Bangladesh

4.2.1. Univariate association between farm level occurrence of *E. coli* and each of farm level factors in commercial chickens in Chattogram, Bangladesh

Two of the factors were found significantly associated with the farm level occurrence of *E. coli* in broiler chickens ($p \leq 0.2$). They were i) farms not having an isolation shed for sick birds (regrouped categories) and ii) weekly cleaning and disinfecting the farm surfaces and equipment (regrouped categories) **(Table 4.2)**. These two factors were forwarded to conducting the logistic regression model to assess their adjusted effect on the farm level occurrence of *E. coli* in commercial broiler chickens in Chattogram.

Two factors of i) washing egg tray being brought back and ii) employee living within farm premises were significantly associated with the farm level occurrence of *E. coli* in layer chickens in Chattogram ($p \leq 0.2$) **(Table-4.2)**. However, these factors were not forwarded to constructing the logistic regression model due to insufficient frequency number against each category of the factor.

Table 4.2: Univariate association between the farm level occurrence of *E. coli* and each of farm level factors in commercial chickens in Chattogram, Bangladesh

Variable	Categories	Broiler farm			Layer farm		
		Farm positive (either of the samples)			Farm positive (either of the samples)		
		Yes n (%)	No n	*P* (Fishers exact)	Yes n (%)	No n	*P* (Fishers exact)
Have isolation shed for sick birds	No	42 (82.4)	9	0.04	8 (88.9)	1	0.67
	Partial	17 (58.6)	12		36 (81.8)	8	
	Yes	3 (100.0)	0		4 (100)	0	
Have isolation shed for sick birds (Regrouped)	No	42 (82.4)	9	0.06	8 (88.9)	1	0.57
	Yes /Partial	20 (58.6)	12		40 (83.3)	8	
Have washing facility before entering to farm	No	22 (78.6)	6	0.28	18 (78.3)	5	0.31
	Yes	40 (74.1)	14		29 (87.9)	4	
	Partial	0 (0)	1		1 (100.0)	0	
Have washing facility before entering to farm (Regrouped)	No	22 (78.6)	6	0.61	18 (78.3)	5	0.45
	Yes/Partial	40 (72.7)	15		30 (88.2)	4	
Do hand washing before entering in to farm	No	23 (74.2)	8	1.00	22 (78.6)	6	
	Yes	28 (75.7)	9		23 (88.5)	3	
	Partial	11 (73.3)	4		3 (100.0)	0	

Do hand washing before entering in to farm (Regrouped)	No	23 (74.2)	8	1.00	22 (78.6)	6	0.25
	Yes/Partial	39 (75.0)	13		26 (89.7)	3	
Changing clothes/shoes before entering in to farm (Employees)	No	54 (74.0)	19	0.06	33 (80.5)	8	0.46
	Yes	7 (100.0)	0		14 (93.3)	1	
	Partial	0 (0.0)	1		1 (100.0)	0	
	NA	1 (50.0)	1		0 (0.00)	0	
Changing clothes/shoes before entering in to farm (Employees) [Regrouped]	No/NA	55 (73.3)	20	0.67	33 (80.5)	8	0.22
	Yes/Partial	7(87.5)	1		15 (93.8)	1	
Changing clothes/shoes before entering in to farm (Visitors)	No	59 (74.7)	20	0.48	38 (86.4)	6	0.46
	Yes	2 (100.0)	0		6 (85.7)	1	
	Partial	0 (0.0)	1		1 (100.0)	0	
	NA	1 (100.0)	0		3 (60.0)	2	
Changing clothes/shoes before entering in to farm (Visitors) [Regrouped]	No/NA	60 (75.0)	20	1.00	41 (83.7)	8	0.78
	Yes/Partial	2 (66.7)	1		7 (87.5)	1	
Checking and decontamination of vehicles before entering in to farm	No	29 (64.4)	16	0.11	15 (88.2)	2	0.78
	Yes	4 (80.0)	1		13 (86.7)	2	
	Partial	2 (100.0)	0		2 (66.7)	1	
	NA	27 (87.1)	4		18 (81.8)	4	

Checking and decontamination of vehicles before entering in to farm	No/NA	56 (73.7)	20	0.67	33 (84.6)	6	0.90
	Yes/Partial	6 (85.7)	1		15 (83.3)	3	
Decontamination of vehicles before leaving farm	No	30 (63.8)	17	0.11	17 (89.5)	2	0.89
	Yes	4 (100.0)	0		9 (81.8)	2	
	Partial	1 (100.0)	0		4 (80.0)	1	
	NA	27 (87.1)	4		18 (81.8)	4	
Decontamination of vehicles before leaving farm [Regrouped]	No/NA	57 (73.1)	21	0.32	35 (85.4)	6	0.70
	Yes/Partial	5 (100.0)	0		13 (81.3)	3	
Functioning foot bath facility	No	60 (74.1)	21	1.00	39 (86.7)	6	0.47
	Yes	2 (100.0)	0		8 (72.7)	3	
					1 (100.0)	0	
Source of drinking water	Deep well	29 (72.5)	11	0.78			
	Shallow well	32 (76.2)	10		39 (86.7)	6	0.32
	Pond	1 (100.0)	0		9 (75.0)	3	
Disinfecting and cleaning the farm surfaces and equipment weekly	No	8 (53.3)	7	0.06	28 (82.4)	6	0.64
	Yes	42 (76.4)	13		20 (87.0)	3	
	Partial	12 (92.3)	1				
	No	8 (53.3)	7	0.05	1 (50.0)	1	0.23
	Yes/Partial	54 (79.4)	14		41 (83.7)	8	

Disinfecting and cleaning the farm surfaces and equipment weekly [Regrouped]					6 (100.0)	0	
Washing egg tray being brought back from market	No	0	0	-			
	Yes	0	0		1 (50.0)	1	0.18
	Partial		0		47 (85.5)	8	
	NA	0	0				
					8 (80.0)	2	0.83
Employees having training on biosecurity measures (at least once)	No	45 (73.8)	16	1.00	36 (83.7)	7	
	Yes	2 (100.0)	0		2 (100.0)	0	
	NA/Other	15 (75.0)	5		2 (100.0)	0	
Employees having training on biosecurity measures (at least once) [Regrouped]	No/ NA/Other	60 (74.1)	21	1.00	10 (83.3)	2	0.92
	Yes	2 (100.0)	0		38 (84.4)	7	
Employee living within farm premises	No	11 (55.0)	9	0.74	45 (84.9)	8	0.34
	Yes	19 (46.3)	22		1 (50.0)	1	
	NA/Other	12 (54.6)	10		2 (100.0)	0	
Employee living within farm premises [Regrouped]	No/ NA/Other	32 (76.2)	10	0.8	47 (85.5)	8	0.18

	Yes	30 (73.2)	11		1 (50.0)	1	
Presence of other birds/animals in the farm	No	14 (66.7)	7	0.58	8 (72.7)	3	0.44
	Yes	16 (80.0)	4		38 (86.4)	6	
	NA	32 (76.2)	10		2 (100.0)	0	
Presence of other birds/animals in the farm [Regrouped]	No/NA	46 (73.0)	17	0.76	10 (76.9)	3	0.41
	Yes	16 (80.0)	4		38 (86.4)	6	
					32 (88.9)	4	0.44
					6 (75.0)	2	
					10 (76.9)	3	
					42 (85.7)	7	0.44
					6 (75.0)	2	

4.2.2. Multivariate logistic regression analysis between farm level occurrence of *E. coli* and significant factors determined in univariate analysis in broiler chickens in Chattogram, Bangladesh

Neither confounding (>15% difference) nor interaction (p>0.05) was detected in the multivariate logistic regression model. The factors in the model were independent (p>0.05, chi-square test). The model was also fitted well (p=0.147; Goodness of fit test; ROC: 0.70). After adjustment of the factors each other the farms having an isolation shed for sick birds had lower odds of farm level occurrence of *E. coli* in comparison to those having no isolation shed (OR=0.4; 95% CI: 0.1-1.0). Those farms adopting weekly cleaning and disinfecting the farm surfaces and equipment had greater odds of farm level occurrence of *E. coli* (OR=3.4; 95% CI: 1.0-11.3) than those not adopting weekly cleaning and disinfection of farm surface and equipment **(Table 4.3)**.

Table 4.3: Multivariate logistic regression analysis between farm level *E. coli* and significant factors identified in univariate analysis (p=0.2 or less) in commercial chickens in Chattogram, Bangladesh

		Broiler farm		
Factor	**Categories**	**OR**	**95% CI**	***P***
Have isolation shed for sick birds	No	1.0		
	Partially/Yes	0.4	0.1-1.0	0.04
Cleaning and disinfecting the farm surfaces and equipment weekly	No	1.0		
	Partially/Yes	3.4	1.0-11.3	0.04

OR: Odds ratio; CI: Confidence Interval

4.3. Descriptive results of antibiogram of *E. coli* isolates at commercial chicken farm in Chattogram, Bangladesh

Regardless of farm types average 10 antibiotics per farm become resistant to *E. coli* isolates. Only 1-2 antibiotics per farm remained sensitive (Table 4.4).

Table 4.4: Summary statistics of antibiogram of *E. coli* at commercial level in Chattogram, Bangladesh

Production types (No of farms)	**Intermediate resistance: Mean (95% CI)**	**Resistance: Mean (95% CI)**	**Sensitive: Mean (95% CI)**
Broiler (62)	0.2 (0.1 to 0.3)	10.5 (10.3 to 10.7)	1.4 (1.2 to 1.6)
Layer (48)	0.3 (0.1 to0.5)	9.6 (9.2 to 9.9)	2.1 (1.8 to 2.5)

CI: Confidence Interval

4.4. Risk factor analysis for the farm level occurrence of antimicrobial resistance against *E. coli* isolates

4.4.1. Univariate association between the farm level occurrence of antimicrobial resistance and each of farm level factors in commercial chickens in Chattogram, Bangladesh

For broiler farms mean antibiotic resistant types to *E. coli* isolates per farm significantly varied by farm size, farmer's experience, farmer's education (regroup), checking and decontamination of vehicles before entering into farm and decontamination of vehicle before leaving farm (p≥0.2) **(Table 4.5)**.

For layer farms average antibiotic resistant types to *E. coli* isolates per farm significantly differed by having washing facility before entering to farm (regroup), do hand washing before entering into farm (regroup), changing clothes/shoes before entering into farm (visitors), disinfecting and cleaning the farm surfaces and equipment weekly, employees having training on bio-security measures (at least once), employee living within farm premises (regroup) and presence of other birds/animals in the farm (p≥0.2) **(Table 4.5)**.

As overall sample size was small only three more relevant significant factors for each production type were forwarded to the multivariate linear regression analysis. They were farm size, farmer's experience and farmer's education (for broiler); having washing facility before entering to farm, do hand washing before entering into farm and employees having training on bio-security measures (at least once).

Table 4.5: Univariate association between farm level average antimicrobial resistant types (intermediate resistance and resistance together) and each of farm level factors in commercial chickens in Chattogram, Bangladesh

Factor	**Categories**	**Broiler farm**		**Layer farm**	
		Mean	***p*** **t –test or 1 way ANOVA**	**Mean**	***p*** **t –test or 1 way ANOVA**
Farm size	Small	2.3	0.05	9.7	0.93
	Medium	2.3		9.6	
				9.5 (medium)	
Farmer's experience (Years)	0-5	2.3	0.12	9.3	0.41
	6-10	2.3		9.9	
	>10	2.4		9.5	
Farmer's experience (Years) [Regrouped]	0-5	2.3	0.97	9.3	0.40
	6 or more	2.4		9.7	
Farmer's education	No education	2.4	0.11	7	0.25
	Up to primary	2.3		9.8	
	Up to secondary	2.4		9.5	
	Up to higher secondary	2.3		9.9	
	Graduate	2.3		9.6	

Farmer's education [Regrouped]	No education or up to primary	2.3	0.09	9.2	0.58
	Up to secondary	2.4		9.5	
	Above secondary to graduate	2.3		9.8	
Have isolation shed for sick birds	No	2.3	0.80	9.6	0.77
	Partial	2.4		9.5	
	Yes	2.4		10	
Have isolation shed for sick birds (Regrouped)	No	2.3	0.73	9.6	0.92
	Yes /Partial	2.4		9.6	
Have washing facility before entering to farm	No			9.3	0.40
	Yes			9.8	
	Partial			10	
Have washing facility before entering to farm (Regrouped)	No	2.4	0.41	9.3	0.18
	Yes/Partial	2.3		9.8	
Do hand washing before entering in to farm	No	2.4	0.29	9.2	0.13
	Yes	2.3		9.8	
	Partial	2.4		10.3	

Do hand washing before entering in to farm (Regrouped)	No	2.4	0.34	9.2	0.06
	Yes/Partial	2.3		9.9	
Changing clothes/shoes before entering in to farm (Employees)	No	2.4	0.84	9.7	0.54
	Yes	2.3		9.3	
	Partial	-		10	
	NA	2.3			
Changing clothes/shoes before entering in to farm (Employees) [Regrouped]	No/NA	2.4	0.45	9.7	0.34
	Yes/Partial	2.3		9.3	
Changing clothes/shoes before entering in to farm (Visitors)	No	2.3	0.47	9.8	0.01
	Yes	2.4		9.2	
	Partial	-		10	
	NA	2.4		7.7	
Changing clothes/shoes before entering in to farm (Visitors) [Regrouped]	No/NA	2.3	0.84	9.6	0.49
	Yes/Partial	2.4		9.3	
Checking and decontamination of vehicles before entering in to farm	No	2.4	0.18	9.5	0.98
	Yes	2.3		9.5	
	Partial	2.4		9.5	
	NA	2.3		9.7	

Checking and decontamination of vehicles before entering in to farm[Regrouped]	No/NA	2.4	0.33	9.6	0.85
	Yes/Partial	2.3		9.5	
Decontamination of vehicles before leaving farm	No	2.4	0.13	9.5	0.96
	Yes	2.3		9.4	
	Partial	2.3		9.8	
	NA	2.3		9.7	
Decontamination of vehicles before leaving farm [Regrouped]	No/NA	2.4	0.21	9.6	0.88
	Yes/Partial	2.3		9.5	
Functioning foot bath facility	No	2.3	0.84	9.7	0.32
	Yes	2.4		9	
Source of drinking water	Deep well	2.3	0.29		
	Shallow well	2.4		9.6	0.69
	Pond	2.4		9.5	
Disinfecting and cleaning the farm surfaces and equipment weekly	No	2.4	0.97	11	0.03
	Yes	2.3		9.7	
	Partial	2.4		8.5	
Disinfecting and cleaning the farm surfaces and equipment weekly [Regrouped]	No	2.4	0.46	11	0.24
	Yes/Partial	2.3		9.6	

Washing egg tray being brought back from market	No	-	-	9.4	0.34
	Yes	-		9.6	
	Partial	-		11	
	NA	2.3		9	
Employees having training on biosecurity measures (at least once)	No	2.3	0.59	9.6	0.16
	Yes	2.4		10	
	NA/Other	2.3		8	
Employees having training on biosecurity measures (at least once) [Regrouped]	No/ NA/Other	2.3	0.84	9.6	0.73
	Yes	2.4		10	
Employee living within farm premises	No	2.3	0.75	9.1	0.06
	Yes	2.3		9.8	
	NA/Other	2.4		8	
Employee living within farm premises [Regrouped]	No/ NA/Other	2.3	0.5	8.9	0.04
	Yes	2.3		9.8	
Presence of other birds/animals in the farm	No	2.3	0.5	9.9	0.03
	Yes	2.4		9	
	NA	2.3		8.9	
Presence of other birds/animals in the farm [Regrouped]	No/NA	2.3	0.82	9.7	0.21
	Yes	2.4		9	

4.4.2. Multivariate linear regression analysis between farm level occurrence of antimicrobial resistance (intermediate resistance and resistance together) and selected significant factors in univariate analysis in commercial chickens in Chattogram, Bangladesh

Neither confounding (>15% difference) nor interaction (p>0.05) was found in the multivariate linear regression models (one for broiler farms and one for layer farms). The factors in the model for each model were independent (Variance inflation factor=1.4-2.8). The model was also fitted well (p=0.17-0.90, heteroskedasticity test) (**Table 4.6**).

For broiler farms after accounting the factors each other average antibiotic resistant types to *E. coli* isolates increased with the increase level of education (p<0.05) (**Table 4.6**).

None of the factors was evident as significant with average antibiotic resistant types to *E. coli* isolates for layer farm (**Table 4.6**).

Table 4.6: Multivariate linear regression analysis between farm level average antimicrobial resistant types (intermediate resistance and resistance together) and significant factors identified in univariate analysis (p=0.2 or less) in commercial chickens in Chattogram, Bangladesh

Factor	Categories	Broiler farm			Layer farm		
		Coefficient	95% CI	*P*	Coefficient	95% CI	*p*
Farm size	Small	Referral					
	Medium	0.07	-0.6 to 0.07	0.83			
Farmer's experience (years)	0-5	Referral					
	6 or more	0.3	-0.2 to 0.9	0.249			
Farmer's education	No education or up to primary	Referral					
	Up to secondary	0.5	0.1 to 1.0	0.015			
	Above secondary to graduate	0.5	-0.02 to 1.0	0.059			
Have washing facility before entering to farm [Regrouped]	No				Referral		
	Yes/Partial				-0.1	-1.5 to 1.2	0.85
Do hand washing before entering in to farm [Regrouped]	No				Referral		
	Yes/Partial				0.62	-0.7 to 1.9	0.34
Employee living within farm premises [Regrouped]	No/ NA/Other				Referral		
	Yes				0.7	0.2 to 1.6	0.12

4.5. Distribution of antibiogram pattern of *E. coli* isolates obtained from commercial chicken farms in Chattogram, Bangladesh

Antimicrobial susceptibility testing revealed that, *E. coli* isolates obtained from broiler chicken farms were displayed 100% resistance to amoxicillin, ampicillin, erythromycin and cephalexin followed by pefloxacin (98.8%), sulfamethoxazole and trimethoprim (96.5%), enrofloxacin (95.4%), doxycycline (94.2%), azithromycin (82.6%), neomycin (80.2%) and gentamycin (58.1%), whereas 97.7% of those isolates were susceptible to colistin.

For layer chicken, *E. coli* isolates were displayed 100% resistance to amoxicillin, ampicillin, erythromycin and cephalexin followed by pefloxacin (98.8%), sulfamethoxazole and trimethoprim (96.3%), doxycycline (92.5%), enrofloxacin (73.8%) and azithromycin (71.3%). However, 95% of the isolates were displayed susceptible to colistin.

Table 4.7: Frequency distribution of antibiogram pattern of *E. coli* isolates obtained from commercial chickens in Chattogram, Bangladesh (86 isolates from 83 broiler farms and 80 isolates from 80 layer farms)

Antimicrobial type	**Broiler farm**			**Layer farm**		
	R **n (%)**	**I** **n (%)**	**S** **n (%)**	**R** **n (%)**	**I** **n (%)**	**S** **n (%)**
Amoxicillin	86 (100)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)
Ampicillin	86 (100)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)
Erythromycin	86 (100)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)
Enrofloxacin	82 (95.4)	0 (0)	4 (4.7)	57 (73.8)	5 (6.3)	18 (22.5)
Doxycycline	81 (94.2)	5 (5.8)	0 (0)	74 (92.5)	6 (7.5)	0 (0)
Gentamycin	50 (58.1)	5 (5.8)	31 (36.0)	23 (28.8)	2 (2.5)	55 (68.8)
Sulfamethoxazole and Trimethprim	83 (96.5)	0 (0)	3 (3.5)	77 (96.3)	0 (0)	3 (3.8)
Neomycin	69 (80.2)	9 (10.5)	8 (9.3)	27 (33.8)	10 (12.5)	43 (53.8)
Azithromycin	71 (82.6)	0 (0)	15 (17.4)	57 (71.3)	0 (0)	23 (28.8)
Cephalexin	86 (100)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)
Pefloxacin	85 (98.8)	0 (0)	1 (1.2)	79 (98.8)	0 (0)	1 (1.3)
Colistin	2 (2.3)	0 (0)	84 (97.7)	4 (5.0)	0 (0)	76 (95.0)

***R**=Resistant, **I**=Intermediate, **S**=Sensitive;*

Chapter-V: Discussion

The current study was conducted to estimate commercial poultry farm level *E. coli* prevalence, antibiogram of *E. coli* and associated risk factors. In this chapter, significant findings of the study, their implications, limitations, conclusions, recommendations and future directions have thoroughly been discussed under various headings as follows.

5.1. Farm level *E. coli* prevalence

The overall farm *E. coli* prevalence was high in both poultry production types, higher in layer farms (74.7% vs. 84.2%). Variable farm *E. coli* prevalence was reported by many preceding studies: 66% to 100% (broiler) and 78.7% to 82.8% (layer) in different parts of Bangladesh (Kmetova, 2009; Jakaria et al., 2012; Hadiujjaman et al., 2016; Al Azad et al., 2019; Ievy et al., 2020; Mandal et al., 2021), 73% (broiler) and 75.5% (layer) in India (Samanta et al., 2014; Bhardwaj et al., 2021), 36% (broiler) in Nepal (Khanal et al., 2019), 39% to 61.4% (broiler) in Thailand (Hanson et al., 2003; Rodroo et al., 2021), 60.8% (broiler) and 66% (layer) in Malaysia (Elmi et al., 2021) and 53.0% (broiler) in China (Liu et al., 2021). These discrepancies in the prevalence of *E. coli* in poultry farms might be linked with differences in isolation methods, geographic locations, hygienic practices, sanitation, and other management practices in farms (Ievy et al., 2020). The high prevalence of *E. coli* infections in poultry (broiler) could be associated with the accumulation of *E. coli* aerosols in the atmosphere of chicken barns that are inhaled by chickens into the respiratory tract (Ibrahim et al., 2019).

5.2. *E. coli* isolation rate

In this study *E. coli* isolation rate was as follows: environmental (50.6% in broiler and 73.7% in layer) and cloacal swabs (54.2% in broiler and 63.2% in layer). These results are consistent with the earlier studies (Hadiujjaman et al., 2016; Ibrahim et al., 2021). However, a past study reported lower *E. coli* isolation rate from environmental swabs (33.3%) than that of cloacal swab (82% in broiler and 78.7% in layer) in Mymensingh, Bangladesh (Jakaria et al., 2012; Saha et al., 2020).

5.3. Risk factors for the occurrence of farm level *E. coli*

In case of broiler farms those were having isolation sheds for sick birds had lower odds of farm level occurrence of *E. coli* in comparison to those having no isolation sheds (OR=0.4; 95% CI: 0.1-1.0). This is natural because mixing of sick birds (due to infectious disease) and healthy birds will increase the chance of spreading infection quickly among healthy birds (Morishita and Derksen, 2021).

Cleaning and disinfection of farm surface and equipments are important measure for lowering the spread of *E. coli* within the farm and from one flock to the next (Mo et al., 2016; Maertens et al., 2020). Unlike the cited studies the present study found that those farms adopting weekly cleaning and disinfecting the farm surfaces and equipment had greater odds of farm level occurrence of *E. coli* (OR=3.4; 95% CI: 1.0-11.3) than those not adopting weekly cleaning and disinfection of farm surface and equipment. Disinfectants may be found at lower concentration due to underdosing or residual organic debris, insufficient cleaning, dilution by remaining rinsing water and biofilm formation those cause exposure of bacteria to subinhibitory concentration of disinfectants that could lead to develop initially susceptible bacteria. Repeated exposure to subinhibitory disinfectant concentration may reduce susceptibility to disinfectants (Soumet et al., 2016; Maertens et al., 2019). This might be due to the dilution effect by susceptible bacteria due to soiled environment which is supported by findings of a Belgian study suggesting that a dirty environment may lead to a decrease in occurrence of resistant bacteria due to a more diverse microbiota and a dilution effect by susceptible bacteria (Persoons et al., 2011).

In case layer farms the present study found "washing egg tray being brought back" and "employee living within farm premises" reduce farm level occurrence of *E. coli*. These promising findings are in line with some earlier studies, for example Ferdous et al. (2019) reported cleaning and disinfection of egg tray before brought back inhibits the bacterial entrance to the farm. Employee living within the farm premises also may restrict entrance of bacterial pathogens from outside to the farm.

5.4. Antibiogram pattern of *E. coli* in commercial poultry farm

Regardless of farm types average 10 antibiotics per farm become resistance to *E. coli* isolates in this current study and these findings are aligned with other Bangladeshi and international studies (Kmetova, 2009; Hassan et al., 2014; Brower et al., 2017; Al Azad et al., 2019; Enany et al., 2019; Liu et al., 2021). Only very few antibiotics remained sensitive against *E. coli*. Continuous exposure to antimicrobials induces selection pressure to organisms such as commensal *E. coli* (Oz et al., 2014). It admits that *E. coli* isolates became resistant to commonly used poultry treating antimicrobials may lead to therapeutic impasses and become a major problem for human and animal health (Abbassi et al., 2017). Our results along with the results of the cited studies clearly indicate the indiscriminate use of antimicrobials which need to be addressed by introducing proper guidelines of antimicrobial use and a strong monitoring system along with introducing awareness campaign by inviting all relevant stakeholders.

5.5. Antibiogram pattern in broiler farm

The prevalence of resistance of common antimicrobials was quite high in this study, for example 100% each of amoxicillin, ampicillin, erythromycin and cephalexin. Similar resistance pattern was previously reveled by many Bangladeshi studies such as 100% ampicillin resistance (Al Azad et al., 2019; Sarker et al., 2019), 91.4% and 100% erythromycin (Hossain et al., 2008; Al Azad et al., 2019), 79.1% doxycycline (Mandal et al., 2021), 51% gentamicin (Al Azad et al., 2019), 94.6% and 100% Sulfamethoxazole and trimethoprim (Al Azad et al., 2019; Sarker et al., 2019).

The results of the current study and proceeding Bangladeshi studies closely correspond to many overseas studies such as amoxicillin resistance 87.5% in Malaysia (Ibrahim et al., 2021), 94% in Pakistan (Kamboh et al., 2018), and 70.2% in Thailand (Thomrongsuwannakij et al., 2020), erythromycin resistance 100% in Thailand and Malaysia (Mooljuntee et al., 2010; Ibrahim et al., 2021). Resistance to enrofloxacin was reported 77% and 84.4% to doxycycline and 78.8% gentamicin in Pakistan (Kamboh et al., 2018).

In this study colistin remained sensitive at very significant level (97%) with is corroborated with the studies conducted in Pakistan and Malaysia (100% susceptibility to colistin) (Ibrahim et al., 2021; Tahir et al., 2021). Random use of colistin is

apparently banned in livestock in Bangladesh (MoFL, 2010; Hassan et al., 2021) and there are ongoing awareness campaigns on judicious use of antimicrobials in poultry rearing and keeping this drug for human use among relevant stakeholders which may help colistin to be still sensitive against *E. coli*. It is also noteworthy to say that colistin is considered as last line agent as they are used to treat infections due to multi-drug resistance bacteria that are non-responsive to other classes of antibiotics. Several countries have also banned colistin in animal use (Maron et al., 2013; Walsh and Wu, 2016).

5.6. Antibiogram pattern in layer farm

Resistance pattern of different antimicrobials against *E. coli* was also very high in layer farm in the present study, for example 100% resistance to amoxicillin, ampicillin, erythromycin and cephalexin followed by pefloxacin (98.8%), sulfamethoxazole and trimethoprim (96.3%), doxycycline (92.5%), enrofloxacin (73.8%) and azithromycin (71.3%). However, 95% of the isolates remained to be susceptible to colistin. The similar resistance pattern of *E. coli* isolates was observed in different studies in Bangladesh that reported resistance of 100% to ampicillin (Ievy et al., 2020), 84.6% to amoxicillin (Hassan et al., 2014), 93.6% and 97.2% to erythromycin (Hossain et al., 2008; Ievy et al., 2020), 100% to enrofloxacin (Hassan et al., 2014), 100% to pefloxacin (Hassan et al., 2014). Resistance to doxycycline 53.8% (Hassan et al., 2014), 8.3% to gentamicin (Ievy et al., 2020), which are contrast to this study. Susceptibility to colistin was reported 88.9% (Ievy et al., 2020).

In neighboring countries, resistant to ampicillin was reported 100% in Thailand (Nuangmek et al., 2018) but 42% in India (Balasubramaniam et al., 2014) which is lower than our study. A moderate rate of resistance to amoxicillin and enrofloxacin 46% of each was reported in India (Balasubramaniam et al., 2014). However, a very high resistance to erythromycin (100%) was reported in Thailand (Nuangmek et al., 2018). Greater resistance levels were also reported for 46% and 100% to gentamicin in India and Thailand, respectively (Balasubramaniam et al., 2014; Nuangmek et al., 2018). Resistance to pefloxacin 88% (Balasubramaniam et al., 2014), 31.6% to neomycin and 73.7% to cephalexin (Joshi et al., 2012) were reported in India. 100% susceptibility to colistin was reported in Thailand (Nuangmek et al., 2018) which was also observed in the present study.

The high resistance could also be because of the lower prices for these antimicrobial agents and also the availability of the antimicrobial agents in Bangladesh particularly, which make the poultry farmers to easily afford them as suggested by Aworh et al. (2020). As discussed earlier a proper antimicrobial use guideline with strong monitoring system can only reduce the indiscriminate use of antimicrobials and thus reduce AMR. Colistin still remaining sensitive in this study is a good news for treating critical human cases.

Antimicrobial resistance in poultry pathogens results in treatment failure, leading to economic losses as well as burden of untreated poultry diseases but importantly act as a source of resistant bacteria to human (Nhung et al., 2017).

Resistance can be declined when antibiotic use is decreased and discontinued for sometimes, for example an earlier study found that resistant strains are replaced by susceptible strains when the selection pressure is removed (Phillips et al., 2004). Therefore, the antibiotics that become already resistant should stop applying in the field for a certain time, nationally or globally. Quality veterinary services are essential for mitigating misunderstanding about antimicrobial use in animal and bacterial resistance. One Health Approach is necessary to decrease the burden of AMR (Yang et al., 2019).

5.7. Risk factors for the occurrence of farm level antimicrobial resistance to *E. coli*

Risk factor analysis identified that number of antibiotic resistance to *E. coli* per broiler farm significantly increased with the increase level of education in this study which might be because educated farmers may use their own judgment, ignoring the consultation with registered veterinarians, to select and use antimicrobials for their farm birds. Educated farmers seek help for farm practices and poultry health care through Google, Youtube and other platforms instead of consulting veterinarians.

Sources of drinking water and sewage system were identified important risk factors for increasing antimicrobial resistance to *E. coli* (Ibrahim et al., 2019; Elmi et al., 2021). Beside these, farmer's experience and lack of training were also responsible for increasing AMR of *E. coli* in poultry (Nguyen et al., 2015; Aworh et al., 2019; Moffo et al., 2021).

None of the factors was evident as significant with the number of antibiotic resistance to *E. coli* per layer farm. However, available literature found the following potential factors associated with increased number of antibiotic resistance per farm: farm size, sources of day-old chicks and use of commercial feed (Nguyen et al., 2015; Moreno et al., 2019).

5.8. Limitations of the study

Information bias (particularly recall bias) might have happened because of interviewees' responses were mostly based on their memories. There were a few farms that had maintained registered books for farm database. However, before starting the main field study the questionnaire was properly piloted and field investigators (veterinarians) were properly trained to prevent from recording incorrect information.

Chapter-VI: Conclusion, Recommendations and Future direction

6.1. Conclusion

In this study, the overall farm *E. coli* prevalence was high. Farms having an isolation shed for sick birds reduce the *E. coli* prevalence in broiler farms. Farms adopting weekly cleaning and disinfecting the farm surfaces and equipment had greater odds of farm level occurrence of *E. coli*. In layer farms, washing egg tray being brought back and employee living within farm premises were significantly associated with the farm level occurrence of *E. coli* in Chattogram.

Irrespective of production types *E. coli* was confirmed resistant against amoxicillin, ampicillin, cephalexin, erythromycin, pefloxacin, enrofloxacin, doxycycline and trimethoprim-sulfonamide in broiler and layer farms. However, gentamicin, neomycin and colistin remain sensitive in both farm types. Number of antibiotic resistance to *E. coli* per broiler farm significantly increased with the increase level of education.

6.2. Recommendations

A proper, feasible and applied farm biosecurity protocol along with antibiotic use protocol should be prepared and implemented in poultry farms of the study areas with the help of veterinarian (public and private), producers and other stakeholders. Improve farm management and vaccination can help reduce *E. coli* infection. Some other intervention measures such as prolonged vacancy period, providing acidified litter, reducing bird stress, maintaining optimum temperature etc. should be introduced.
Resistant antimicrobials identified in the study should be stopped immediately and identified sensitive antimicrobials should be used judiciously. Selection of antibiotics for treatment should be justified based on antimicrobial susceptibility testing results of disc diffusion.

Routine monitoring of AMR at field level should be executed by the veterinarian. Diagnostic facilities, especially culture sensitivity testing facility should be enhanced at field level. National or local treatment protocol as antibiotic selection for infectious diseases should be established. National Action Plan (NAP) for AMR containment should be coordinated and implemented strictly.

Alternative to antibiotics like probiotics, prebiotics, synbiotics and postbiotics can have a beneficial effect on gut health in controlling infections.

These findings of *E. coli* and AMR prevalence with associated risk factors should be discussed with the farmers participated in the study along with local veterinarians and feed and drug dealers as well as policy makers to make aware about risk of *E. coli* and indiscriminant use of antimicrobials and AMR.

Veterinarian should aware the farmers to maintain withdrawal period of antibiotics. Regulating authorities and registered veterinarian should implement antimicrobial stewardship to seize the rising AMR threats.

6.3. Future directions

6.3.1. The current study was restricted to Chattogram district. So, any future study should be thought of wider geographical coverage.

6.3.2. Determination of minimum inhibitory concentration should be used for any future antibiogram study.

6.3.3. Molecular characterization of *E. coli* isolates and antimicrobial resistance genes should be explored in future.

References

Abbassi, M.S., Kilani, H., Zouari, M., Mansouri, R., El Fekih, O., Hammami, S. and Chehida, N.B. 2017. Antimicrobial resistance in *Escherichia coli* isolates from healthy poultry, bovine and ovine in Tunisia: a real animal and human health threat. Journal of Clinical Microbiology Biochemical Technology. 3(2): 19-23.

Afridi, O.K., Ali, J. and Chang, J.H. 2020. Next-generation sequencing based gut resistome profiling of broiler chickens infected with multidrug-resistant *Escherichia coli*. Animals. 10(12): 2350.

Agunos, A., Léger, D. and Carson, C. 2012. Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada. The Canadian Veterinary Journal. 53(12): 1289.

Agyare, C., Boamah, V.E., Zumbi, C.N. and Osei, F.B. 2018. Antibiotic use in poultry production and its effects on bacterial resistance. In: Kumar, Y., editor. Antimicrobial Resistance-A Global Threat. 1st ed. IntechOpen. pp. 33-50.

Akter, M., Choudhury, K., Rahman, M. and Islam, M. 2007. Seroprevalence of salmonellosis in layer chickens with isolation, identification and antibiogram study of their causal agents. Bangladesh Journal of Veterinary Medicine. 5(1 and 2): 39-42.

Al-Mamun, M., Islam, M. and Rahman, M.M. 2019. Occurrence of poultry diseases at Kishoregonj district of Bangladesh. MOJ Proteomics and Bioinformatics. 8(1): 7-12.

Al-Mustapha, A.I., Adetunji, V.O. and Heikinheimo, A. 2020. Risk perceptions of antibiotic usage and resistance: a cross-sectional survey of poultry farmers in Kwara State, Nigeria. Antibiotics. 9(7): 378.

Al Azad, M., Rahman, A., Rahman, M., Amin, R., Begum, M., Ara, I., Fries, R., Husna, A., Khairalla, A.S. and Badruzzaman, A. 2019. Susceptibility and multidrug resistance patterns of *Escherichia coli* isolated from cloacal swabs of live broiler chickens in Bangladesh. Pathogens. 8(3): 118.

Amir, M., Riaz, M., Chang, Y.F., Akhtar, S., Nadeem, H., Ahmad, Z. and Nadeem, M. 2019. Spread of antibiotic resistant *Escherichia coli* from broiler to human populations. Pakistan Journal of Agricultural Sciences. 56(4): 977-983.

Apata, D. 2009. Antibiotic resistance in poultry. International Journal of Poultry Science. 8(4): 404-408.

Asaduzzaman, M. 2000. Livestock sector, economic development and poverty alleviation in Bangladesh. In: Mandal, M.A.S. (ed). Changing rural economy of Bangladesh. Bangladesh Economic Association, Dhaka, Bangladesh. pp. 42-53.

Awogbemi, J., Adeyeye, M. and Akinkunmi, E. 2018. A survey of antimicrobial agents usage in poultry farms and antibiotic resistance in *Escherichia coli* and *Staphylococci* isolates from the poultry in Ile-Ife, Nigeria. Journal of Infectious Diseases Epidemiology. 4(1): 1-9.

Aworh, M.K., Kwaga, J., Okolocha, E., Harden, L., Hull, D., Hendriksen, R.S. and Thakur, S. 2020. Extended-spectrum ß-lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. One Health Outlook. 2(1): 1-11.

Aworh, M.K., Kwaga, J., Okolocha, E., Mba, N. and Thakur, S. 2019. Prevalence and risk factors for multi-drug resistant *Escherichia coli* among poultry workers in the Federal Capital Territory, Abuja, Nigeria. PloS One. 14(11): e0225379.

Azam, M., Mohsin, M. and Saleemi, M.K. 2019. Virulence-associated genes and antimicrobial resistance among avian pathogenic *Escherichia coli* from colibacillosis affected broilers in Pakistan. Tropical Animal Health Production. 51(5): 1259-1265.

Azza, A., Dahshan, A.H.M., El-Nahass, E.-S. and Abd El-Mawgoud, A.I. 2018. Pathogenicity of *Escherichia coli* O157 in commercial broiler chickens. Beni-Suef University Journal of Basic Applied Sciences. 7(4): 620-625.

Badruzzaman, A.T.M., Noor, M., Mamun, M.A., Husna, A., Islam, K.M., Alam, K.J. and Rahman, M.M. 2015. Prevalence of diseases in commercial chickens at Sylhet Division of Bangladesh. International Clinical Pathology Journal. 1(5): 23.

Balasubramaniam, A., Arthanari Eswaran, M., Suresh, P. and Sukumar, K. 2014. Detection of tetracycline resistance determinant tetA gene and antimicrobial resistance pattern in *Escherichia coli* isolates recovered from healthy layer chickens. Veterinary World. 7(9): 635-638.

Bashar, T., Rahman, M., Rabbi, F.A., Noor, R. and Rahman, M.M. 2011. Enterotoxin profiling and antibiogram of *Escherichia coli* isolated from poultry feces in Dhaka district of Bangladesh. Stamford Journal of Microbiology. 1(1): 51-57.

Battistuzzi, F.U., Feijao, A. and Hedges, S.B. 2004. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. BioMed Central Evolutionary Biology. 4(1): 1-14.

BBS. 2013. Bangladesh Bureau of Statistics. District Statistics 2011: Chittagong. Government of the People's Republic of Bangladesh, Dhaka-1207. [cited: 2020 Apr 14]. Available from: http://203.112.218.65:8008/WebTestApplication/userfiles/Image/District%20Statistics/Chittagong.pdf.

BER. 2021. Bangladesh Economic Review. EN. Ministry Of Finance. www.google.com. [cited: 2020 May 2]. Available from: https://mof.portal.gov.bd/sites/default/files/files/mof.portal.gov.bd/page/e8bc0eaa_463d_4cf9_b3be_26ab70a32a47/Banglades%20Economic%20Review%202019%20Eng.zip.

Bhardwaj, K., Shenoy, S., Baliga, S., Unnikrishnan, B., Baliga, B.S. and Shetty, V.K. 2021. Research Note: Characterization of antibiotic resistant phenotypes and linked genes of *Escherichia coli* and *Klebsiella pneumoniae* from healthy broiler chickens, Karnataka, India. Poultry Science. 100(6): 101094.

Biswas, P.k., Uddin, G.M., Barua, H., Roy, K., Biswas, D., Ahad, A. and Debnath, N.C. 2006. Causes of loss of Sonali chickens on smallholder households in Bangladesh. Preventive Veterinary Medicine. 76(3-4): 185-195.

BPICC. 2020. Bangladesh Poultry Industries Central Council. [cited: 2021 Feb 3]. Available from: http://www.bpicc-poultry.com/pages.php?pageID=basic-info&mID=2.

Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M., Boone, D.R., Vos, P.D., Goodfellow, M., Rainey, F.A. and Schleifer, K.H., 2005. Bergey's Manual of Systematic Bacteriology: The Proteobacteria; part B: the gammaproteobacteria. In: Bergey's Manual of Systematic Bacteriology: The Proteobacteria; part B: the gammaproteobacteria. pp. 1106-1106.

Brower, C.H., Mandal, S., Hayer, S., Sran, M., Zehra, A., Patel, S.J., Kaur, R., Chatterjee, L., Mishra, S. and Das, B. 2017. The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. Environmental Health Perspectives. 125(7): 77015.

Chansiripornchai, N., Mooljuntee, S. and Boonkhum, P. 2011. Antimicrobial sensitivity of avian pathogenic *Escherichia coli* (APEC) isolated from chickens during 2007-2010. The Thai Journal of Veterinary Medicine. 41(4): 519.

Chart, H., Smith, H., La Ragione, R. and Woodward, M.J. 2000. An investigation into the pathogenic properties of *Escherichia coli* strains BLR, BL21, DH5α and EQ1. Journal of Applied Microbiology. 89(6): 1048-1058.

Chowdhury, M., Bardhan, R., Pal, S., Banerjee, A., Batabyal, K., Joardar, S., Mandal, G., Bandyopadhyay, S., Dutta, T. and Sar, T. 2022. Comparative occurrence of ESBL/AmpC beta-lactamase-producing *Escherichia coli* and *Salmonella* in contract farm and backyard broilers. Letters in Applied Microbiology. 74(1): 53-62.

Chowdhury, S., Ghosh, S., Aleem, M.A., Parveen, S., Islam, M., Rashid, M., Akhtar, Z. and Chowdhury, F. 2021. Antibiotic usage and resistance in food animal production: what have we learned from Bangladesh?. Antibiotics. 10(9): 1032.

CLSI. 2018a. Performance standards for antimicrobial disk susceptibility tests. 13th ed. CLSI standard M02.Clinical and Laboratory Standards Institute.Wayne, PA. [cited: 2020 APR 28]. Available from: https://clsi.org/standards/products/microbiology/documents/m02/.

CLSI. 2018b. Performance standards for antimicrobial susceptibility testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018. [cited: 2020 APR 30]. Available from: http://file.qums.ac.ir/repository/mmrc/CLSI-2018-M100-S28.pdf.

Cortés, P., Blanc, V., Mora, A., Dahbi, G., Blanco, J.E., Blanco, M., López, C., Andreu, A., Navarro, F. and Alonso, M.P. 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Applied Environmental Microbiology. 76(9): 2799-2805.

Course, C.E., Boerlin, P., Slavic, D., Vaillancourt, J.-P. and Guerin, M.T. 2021. Factors associated with *Salmonella enterica* and *Escherichia coli* during downtime in commercial broiler chicken barns in Ontario. Poultry Science. 100(5): 101065.

Das, A., Dhar, P.K., Dutta, A., Jalal, M.S., Ghosh, P., Das, T., Barua, H. and Biswas, P.K. 2020. Circulation of oxytetracycline and ciprofloxacin-resistant commensal *Escherichia coli* strains in broiler chickens and farm environments, Bangladesh. Veterinary World. 13(11): 2395.

Dho-Moulin, M. and Fairbrother, J.M. 1999. Avian pathogenic *Escherichia coli* (APEC). Veterinary Research. 30(2-3): 299-316.

Diarra, M.S. and Malouin, F. 2014. Antibiotics in Canadian poultry productions and anticipated alternatives. Frontiers in Microbiology. 5: 282.

DLS. 2020. Department of Livestock Services. Livestock Economy at a Glance. Livestock Economics Section, DLS, Government of the People's Republic of Bangladesh, Dhaka-1207. [cited: 2020 Dec 26]. Available from: http://dls.portal.gov.bd/sites/default/files/files/dls.portal.gov.bd/page/ee5f4621_fa3a_40ac_8bd9_898fb8ee4700/2020-08-17-14-40-87b80574013fcc8738b7dd205b5619d1.doc.

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

Dziva, F. and Stevens, M.P. 2008. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian Pathology. 37(4): 355-366.

Elmi, S.A., Simons, D., Elton, L., Haider, N., Abdel Hamid, M.M., Shuaib, Y.A., Khan, M.A., Othman, I., Kock, R. and Osman, A.Y. 2021. Identification of risk factors associated with resistant *Escherichia coli* isolates from poultry farms in the east coast of peninsular Malaysia: A cross sectional study. Antibiotics. 10(2): 117.

Enany, M.E., Algammal, A.M., Nasef, S.A., Abo-Eillil, S.A., Bin-Jumah, M., Taha, A.E. and Allam, A.A. 2019. The occurrence of the multidrug resistance (MDR) and the prevalence of virulence genes and QACs resistance genes in *E. coli* isolated from environmental and avian sources. AMB Express. 9(1): 1-9.

EUCAST. 2018. European committee on antimicrobial susceptibility testing. Breakpoint tables for interpretation of MICs and zone diameters, version 9.0, 2019. [cited: 2020 APR 30]. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tab les/v_9.0_Breakpoint_Tables.pdf.

Ewers, C., Janßen, T., Kießling, S., Philipp, H.-C. and Wieler, L.H. 2004. Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. Veterinary Microbiology. 104(1-2): 91-101.

Ewers, C., Janßen, T. and Wieler, L.H. 2003. Avian pathogenic *Escherichia coli* (APEC). Berliner und Munchener tierarztliche Wochenschrift. 116(9-10): 381-395.

Fasenko, G. and O'Dea, E. 2008. Evaluating broiler growth and mortality in chicks with minor navel conditions at hatching. Poultry Science. 87(3): 594-597.

Ferdous, J., Sachi, S., Zakaria Al Noman, S., Hussani, Y.A.S. and Sikder, M.H. 2019. Assessing farmers' perspective on antibiotic usage and management practices in small-scale layer farms of Mymensingh district, Bangladesh. Veterinary World. 12(9): 1441.

Goren, E. 1978. Observations on experimental infection of chicks with *Escherichia coli*. Avian Pathology. 7(2): 213-224.

Gross, W.G. 1994. Diseases due to *Escherichia coli* in poultry. In: Gyles, C.L., editor. *Escherichia coli* in domestic animals and humans. Wallingford (United Kingdom): CAB International. pp. 237-259.

Guerra, B., Junker, E., Schroeter, A., Malorny, B., Lehmann, S. and Helmuth, R. 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. Journal of Antimicrobial Chemotherapy. 52(3): 489-492.

Gupta, S.D., Hoque, M.A., Fournié, G. and Henning, J. 2021. Patterns of avian influenza A (H5) and A (H9) virus infection in backyard, commercial broiler and layer chicken farms in Bangladesh. Transboundary Emerging Diseases. 68(1): 137-151.

Hadiujjaman, M., Rahman, M.M., Ahasan, M.D., Banu, M.A., Khatun, M.M. and Islam, M.A. 2016. Isolation and identification of *Escherichia coli* from apparently healthy chicken of selected areas of Bangladesh. International Journal of Natural and Social Sciences. 3(3): 15-23.

Hafez, H.M. and Attia, Y.A. 2020. Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. Frontiers in Veterinary Science. 7(516): 1-16.

Hamid, M., Rahman, M., Ahmed, S. and Hossain, K. 2017. Status of poultry industry in Bangladesh and the role of private sector for its development. Asian Journal of Poultry Science. 11(1): 1-13.

Hanson, R., Kaneene, J.B., Padungtod, P., Hirokawa, K. and Zeno, C. 2003. Prevalence of *Salmonella* and *E. coli*, and their resistance to antimicrobial agents, in farming communities in northern Thailand. Southeast Asian Journal of Tropical Medicine and Public Health. 33(Suppl 3): 120-126.

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

Hashem, M.A., Elahi, M.F., Mannan, M.A., Kabir, M.H.B., Kashem, M.A. and Pallab, M.S. 2012. Isolation, identification and antibiogram of *Escherichia coli* from broiler at Chittagong District in Bangladesh. Wayamba Journal of Animal Science. 6: 312-316.

Hassan, M.M. 2020. Scenario of antibiotic resistance in developing countries. In: Mareș, M., Erin, S.H., Lai, K., Cristina, R., editors. Antimicrobial Resistance-A One Health Perspective. London, UK: IntechOpen. [cited: 2021 Feb 06]. Available from: https://www.intechopen.com/chapters/74593

Hassan, M.M., Amin, K.B., Ahaduzzaman, M., Alam, M., Faruk, M.S. and Uddin, I. 2014. Antimicrobial resistance pattern against *E. coli* and *Salmonella* in layer poultry. Research Journal for Veterinary Practitioners. 2(2): 30-35.

Hassan, M.M., Kalam, M., Alim, M., Shano, S., Nayem, M., Khan, R., Badsha, M., Al Mamun, M., Hoque, A. and Tanzin, A.Z. 2021. Knowledge, attitude, and practices on antimicrobial use and antimicrobial resistance among commercial poultry farmers in Bangladesh. Antibiotics. 10(7): 784.

Homjan, P., Awaiwanont, N., Meeyam, T., Ngamsanga, P., Punyapornwithaya, V. and Pichpol, D. 2018. Antimicrobial resistance against avian pathogenic *Escherichia coli* (APEC) isolated from colibacillosis in chicken, Thailand. Conference proceedings of the 5th Food Safety and Zoonoses Symposium for Asia Pacific. Veterinary Public Health Centre for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Chiag Mai, Thailand, 6th-7th July 2018. Chiag Mai, Thailand. Abstract Book, p 69-72.

Hossain, M.T., Siddique, M.P., Hossain, F.M.A., Zinnah, M.A., Hossain, M.M., Alam, M.K., Rahman, M.T. and Choudury, K.A. 2008. Isolation, identification, toxin profile and antibiogram of *Escherichia coli* isolated from broilers and layers in Mymensingh district of Bangladesh. Bangladesh Journal of Veterinary Medicine. 6(1): 1-5.

Ibrahim, R.A., Cryer, T.L., Lafi, S.Q., Basha, E.-A., Good, L. and Tarazi, Y.H. 2019. Identification of *Escherichia coli* from broiler chickens in Jordan, their

antimicrobial resistance, gene characterization and the associated risk factors. BMC/BioMed Central Veterinary Research. 15(1): 1-16.

Ibrahim, S., Wei Hoong, L., Lai Siong, Y., Mustapha, Z., CW Zalati, C., Aklilu, E., Mohamad, M. and Kamaruzzaman, N.F. 2021. Prevalence of antimicrobial resistance (AMR) *Salmonella* spp. and *Escherichia coli* isolated from broilers in the east coast of peninsular Malaysia. Antibiotics. 10(5): 579.

Ievy, S., Islam, M., Sobur, M., Talukder, M., Rahman, M. and Khan, M.F.R. 2020. Molecular detection of avian pathogenic *Escherichia coli* (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. Microorganisms. 8(7): 1021.

Imam, T., Gibson, J.S., Foysal, M., Das, S.B., Gupta, S.D., Fournié, G., Hoque, M.A. and Henning, J. 2020. A cross-sectional study of antimicrobial usage on commercial broiler and layer chicken farms in Bangladesh. Frontiers in Veterinary Science. 7:576113. 7(576113): 1-11.

Islam, A., Trisha, A., Das, M. and Amin, M. 2009. Retrospective study of some poultry diseases at Gaibandha district in Bangladesh. Bangladesh Journal of Veterinary Medicine. 7(1): 239-247.

Islam, K.S., Shiraj-Um-Mahmuda, S., Hazzaz-Bin-Kabir, M. 2016. Antibiotic usage patterns in selected broiler farms of Bangladesh and their public health implications. Journal of Public Health in Developing Countries. 2(3): 276-284.

Islam, S.M.F. and Jabbar, M.A. S. 2010. Consumer preferences and demand for livestock products in urban Bangladesh. Research Report 23. Nairobi, Kenya, ILRI (International Livestock Research Institute). [cited: 2021 Jul 13]; Available from: https://www.ilri.org/publications/consumer-preferences-and-demand-livestock-products-urban-bangladesh

Jakaria, A.T.M., Islam, M.A. and Khatun, M.M. 2012. Prevalence, characteristics and antibiogram profiles of *Escherichia coli* isolated from apparently healthy chickens in Mymensingh, Bangladesh. Microbes and Health. 1(1): 27-29.

Jordan, F.T.W., Williams, N.J., Wattret, A. and Jones, T. 2005. Observations on salpingitis, peritonitis and salpingoperitonitis in a layer breeder flock. Veterinary Record. 157(19): 573-577.

Joshi, S., Singh, R. and Singh, S.P. 2012. Antibiotic resistance profile of *Escherichia coli* isolates from colibacillosis in and around Pantnagar, India. Veterinary World. 5(7): 405.

Kabir, S.M., 2010. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International Journal of Environmental Research and Public Health. 7(1): 89-114.

Kahn, C.M. and Line, S. 2010. The Merck veterinary manual. 1oth Ed. Merck & Co. Inc. Whitehouse station, NJ, USA. pp. 2824-2827.

Kamboh, A.A., Shoaib, M., Abro, S.H., Khan, M.A., Malhi, K.K. and Yu, S. 2018. Antimicrobial resistance in Enterobacteriaceae isolated from liver of commercial broilers and backyard chickens. Journal of Applied Poultry Research. 27(4): 627-634.

Kasabova, S., Hartmann, M., Freise, F., Hommerich, K., Fischer, S., Wilms-Schulze-Kump, A., Rohn, K., Käsbohrer, A. and Kreienbrock, L. 2021. Antibiotic usage pattern in broiler chicken flocks in Germany. Frontiers in Veterinary Science. 8:578. 8:673809

Kathayat, D., Lokesh, D., Ranjit, S. and Rajashekara, G. 2021. Avian pathogenic *Escherichia coli* (APEC): An overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens. 10(4): 467.

Kawsar, M.H., Chowdhury, S.D., Raha, S.K. and Hossain, M.M. 2013. Impact of management intervention on productive performance and profitability of small-scale broiler farming in Bangladesh. Livestock Research for Rural Development. 25(5): 2013.

Khanal, S., Kandel, M. and Shah, M.P. 2019. Antibiogram pattern of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. isolates from broiler chicken. Nepalese Veterinary Journal. 36: 105-110.

Khasa, V. and Singh, P. 2020. Isolation and antibacterial susceptibility of *Escherichia coli* from poultry. Journal of Pharmacognosy and Phytochemistry. 9(1): 1182-1184.

Kim, H.J., Kang, M.I. and Chung, U.I. 1996. Survey on enteric diseases in chicken. Korean Journal of Veterinary Research. 36(4): 1007-1012.

Kmetova, M. 2009. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Internet Journal of Food Safety. 11: 19-23.

Kumar, A. and Kumar, M. 2020. Antimicrobial spectrum of *Escherichia coli* isolated from poultry faecal samples in and around Ranchi. International Journal of Current Microbiology and Applied Sciences. 9(9): 2921-2925.

Lagha, A.B., Haas, B., Gottschalk, M. and Grenier, D. 2017. Antimicrobial potential of bacteriocins in poultry and swine production. Veterinary Research. 48(1): 1-12.

Landman, W.J. and Cornelissen, R.A. 2006. *Escherichia coli* salpingitis and peritonitis in layer chickens: an overview. Tijdschrift Voor Diergeneeskunde. 131(22): 814-822.

Landoni, M.F. and Albarellos, G. 2015. The use of antimicrobial agents in broiler chickens. The Veterinary Journal. 205(1): 21-27.

Latif Baloch, A. and Magsi, R.A. 2019. Prevalence of multiple drug resistance among avian pathogenic *E. coli* isolates from commercial poultry. Pak-Euro Journal of Medical and Life Sciences. 2(4): 83-88.

Lawwyne, L.D., Ruenghiran, C., Viriyarampa, S., Chumsing, S. and Tulayakul, P. 2019. Prevalence and antimicrobial resistance of *Escherichia coli* and *Salmonella* spp. isolated from poultry drinking water and cloacal swab compared of two regions in Thailand. International Journal of Infectious Diseases. 79(Suppl 1): 21.

Li, B. and Webster, T.J. 2018. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. Journal of Orthopaedic Research®. 36(1): 22-32.

Linden, J. 2015. Colibacillosis in layers: an overview. The Poultry Site. [cited: 2021 May 10]; Available from: https://www.thepoultrysite.com/articles/colibacillosis-in-layers-an-overview.

Liu, C., Wang, P., Dai, Y., Liu, Y., Song, Y., Yu, L., Feng, C., Liu, M., Xie, Z., Shang, Y. and Sun, S. 2021. Longitudinal monitoring of multidrug resistance in *Escherichia coli* on broiler chicken fattening farms in Shandong, China. Poultry Science. 100(3): 100887.

Lupindu, A.M. 2017. Isolation and characterization of *Escherichia coli* from animals, humans, and environment. In: Samie, A. editor. *Escherichia coli*-Recent Advances on Physiology, Pathogenesis and Biotechnological Applications. London, United Kingdom: IntechOpen Limited. pp. 187-206.

Maertens, H., De Reu, K., Meyer, E., Van Coillie, E. and Dewulf, J. 2019. Limited association between disinfectant use and either antibiotic or disinfectant

susceptibility of *Escherichia coli* in both poultry and pig husbandry. BMC Veterinary Research. 15(1): 1-12.

Maertens, H., Van Coillie, E., Millet, S., Van Weyenberg, S., Sleeckx, N., Meyer, E., Zoons, J., Dewulf, J. and De Reu, K. 2020. Repeated disinfectant use in broiler houses and pig nursery units does not affect disinfectant and antibiotic susceptibility in *Escherichia coli* field isolates. BMC Veterinary Research. 16(1): 1-11.

Mamun, M.M., Parvej, M.S. Ahamed, S., Hassan, J., Nazir, K.H.M.N.H., Nishikawa, Y. and Rahman, M.T. 2016. Prevalence and characterization of shigatoxigenic *Escherichia coli* in broiler birds in Mymensingh. Bangladesh Journal of Veterinary Medicine. 14(1): 5-8.

Mandal, A.K., Talukder, S., Hasan, M.M., Tasmim, S.T., Parvin, M.S., Ali, M.Y. and Islam, M.T. 2021. Epidemiology and antimicrobial resistance of *Escherichia coli* in broiler chickens, farmworkers, and farm sewage in Bangladesh. Veterinary Medicine and Science. 8(1): 187-199.

Maron, D.F., Smith, T.J. and Nachman, K.E. 2013. Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Globalization and Health. 9(1): 1-11.

Marshall, B.M. and Levy, S.B. 2011. Food animals and antimicrobials: impacts on human health. Clinical Microbiology Reviews. 24(4): 718-733.

Masud, A.A., Rousham, E.K., Islam, M.A., Alam, M.-U., Rahman, M., Mamun, A.A., Sarker, S., Asaduzzaman, M. and Unicomb, L. 2020. Drivers of antibiotic use in poultry production in Bangladesh: dependencies and dynamics of a patron-client relationship. Frontiers in Veterinary Science. 7:78.

Matin, M.A., Islam, M.A. and Khatun, M.M. 2017. Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. Veterinary World. 10(1): 29.

Matter, L.B., Barbieri, N.L., Nordhoff, M., Ewers, C. and Horn, F. 2011. Avian pathogenic *Escherichia coli* MT78 invades chicken fibroblasts. Veterinary Microbiology. 148(1): 51-59.

Mehdi, Y., Létourneau-Montminy, M.P., Gaucher, M.L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Côté, C., Ramirez, A.A. and Godbout, S. 2018. Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition. 4(2): 170-178.

Michael, C.A., Dominey-Howes, D. and Labbate, M. 2014. The antimicrobial resistance crisis: causes, consequences, and management. Frontiers in Public Health. 2: 145.

Mo, S.S., Kristoffersen, A.B., Sunde, M., Nødtvedt, A. and Norström, M. 2016. Risk factors for occurrence of cephalosporin-resistant *Escherichia coli* in Norwegian broiler flocks. Preventive Veterinary Medicine. 130: 112-118.

Moffo, F., Mouiche, M.M.M., Djomgang, H.K., Tombe, P., Wade, A., Kochivi, F.L., Dongmo, J.B., Mbah, C.K., Mapiefou, N.P., Ngogang, M.P. and Awah-Ndukum, J. 2021. Poultry litter contamination by *Escherichia coli* resistant to critically important antimicrobials for human and animal use and risk for public health in Cameroon. Antibiotics. 10(4): 402.

MoFL. 2010. Fish Feed and Animal Feed Act, 2010. Ministry of Fisheries and Livestock. Government of the Peoples Republic of Bangladesh Dhaka. file:///C:/Users/USER/AppData/Local/Temp/Fish%20feed%20and%20Animal%20feed%20act-2010.pdf.

Mooljuntee, S., Chansiripornchai, P. and Chansiripornchai, N. 2010. Prevalence of the cellular and molecular antimicrobial resistance against *E. coli* isolated from Thai broilers. The Thai Journal of Veterinary Medicine. 40(3): 311-315.

Moreno, M.A., García-Soto, S., Hernández, M., Bárcena, C., Rodríguez-Lázaro, D., Ugarte-Ruíz, M. and Domínguez, L. 2019. Day-old chicks are a source of antimicrobial resistant bacteria for laying hen farms. Veterinary Microbiology. 230: 221-227.

Morishita, T.Y. and Derksen, T. 2021. Biosecurity. In: Greenacre, C.B., Morishita, T.Y., editors. Backyard Poultry Medicine and Surgery: A Guide for Veterinary Practitioners. 1st ed. Ames, IA: John Wiley & Sons Inc. pp. 107-116.

Msoffe, G. and Ngulube, P. 2016. Farmers' access to poultry management information in selected rural areas of Tanzania. Library and Information Science Research. 38(3): 265-271.

Muglikar, D.M., Kalyani, I.H., Desai, D., Patel, J.M., Patel, D.R., Makwana, P. and Solanki, J.B. 2019. Serotyping and antimicrobial susceptibility pattern of avian pathogenic *Escherichia coli*. International Journal of Current Microbiology and Applied Sciences. 8(12): 505-511.

Mund, M.D., Khan, U.H., Tahir, U., Mustafa, B.E. and Fayyaz, A. 2017. Antimicrobial drug residues in poultry products and implications on public health: A review. International Journal of Food Properties. 20(7): 1433-1446.

Mutua, F., Sharma, G., Grace, D., Bandyopadhyay, S., Shome, B. and Lindahl, J. 2020. A review of animal health and drug use practices in India, and their possible link to antimicrobial resistance. Antimicrobial Resistance Infection Control. 9(1): 1-13.

Nataro, J.P. and Kaper, J.B. 1998. Diarrheagenic *Escherichia coli.* Clinical Microbiology Reviews. 11(1): 142-201.

Nguyen, V.T., Carrique-Mas, J.J., Ngo, T.H., Ho, H.M., Ha, T.T., Campbell, J.I., Nguyen, T.N., Hoang, N.N., Pham, V.M., Wagenaar, J.A., Hardon, A., Thai, Q.H. and Schultsz, C. 2015. Prevalence and risk factors for carriage of antimicrobial-resistant *Escherichia coli* on household and small-scale chicken farms in the Mekong Delta of Vietnam. Journal of Antimicrobial Chemotherapy. 70(1): 2144-2152.

Nhung, N.T., Chansiripornchai, N. and Carrique-Mas, J.J. 2017. Antimicrobial resistance in bacterial poultry pathogens: a review. Frontiers in Veterinary Science. 4(126): 1-17.

Nolan, L.K., Barnes, H.J., Vaillancourt, J.-P., Abdul-Aziz, T. and Logue, C.M. 2013. Colibacillosis. In: Saif, Y.M., editor. Diseases of Poultry. 12th ed. Blackwell Publishing. pp. 751-805.

Norizuki, C., Wachino, J.I., Suzuki, M., Kawamura, K., Nagano, N., Kimura, K. and Arakawa, Y. 2017. Specific bla CTX-M-8/IncI1 plasmid transfer among genetically diverse *Escherichia coli* isolates between humans and chickens. Antimicrobial Agents and Chemotherapy. 61(6): e00663-00617.

Nuangmek, A., Rojanasthien, S., Chotinun, S., Yamsakul, P., Tadee, P., Thamlikitkul, V., Tansakul, N. and Patchanee, P. 2018. Antimicrobial resistance in ESBL-producing *Escherichia coli* isolated from layer and pig farms in Thailand. Acta Scientiae Veterinariae. 46(1): 8.

OECD, 2020. Organisation for Economic Co-operation and Development. Meat consumption (indicator). doi: 10.1787/fa290fd0-en. [cited: 2020 May 8]. Available from: https://data.oecd.org/agroutput/meat-consumption.htm.

OHPH, B. 2020. One Health Poultry Hub, Bangladesh. [cited: 2021 Jan 11]. Available from: https://www.onehealthpoultry.org/where-we-work/bangladesh/.

Oluwasile, B.B., Agbaje, M., Ojo, O.E. and Dipeolu, M.A. 2014. Antibiotic usage pattern in selected poultry farms in Ogun state. Sokoto Journal of Veterinary Sciences. 12(1): 45-50.

OpenEpi. 2013. Open Source Epidemiologic Statistics for Public Health. [cited: 2020 Jan 15]. Available from: https://www.openepi.com/.

Oz, T., Guvenek, A., Yildiz, S., Karaboga, E., Tamer, Y.T., Mumcuyan, N., Ozan, V.B., Senturk, G.H., Cokol, M., Yeh, P. and Toprak, E. 2014. Strength of selection pressure is an important parameter contributing to the complexity of antibiotic resistance evolution. Molecular Biology Evolution. 31(9): 2387-2401.

Pacifici, G.M. 2018. Multidrug resistance in infants and children. International Journal of Pediatrics. 6(2): 7055-7080.

Persoons, D., Dewulf, J., Smet, A., Herman, L., Heyndrickx, M., Martel, A.N., Catry, B., Butaye, P. and Haesebrouck, F. 2012. Antimicrobial use in Belgian broiler production. Preventive Veterinary Medicine. 105(4): 320-325.

Persoons, D., Haesebrouck, F., Smet, A., Herman, L., Heyndrickx, M., Martel, A., Catry, B., Berge, A.C., Butaye, P. and Dewulf, J. 2011. Risk factors for ceftiofur resistance in *Escherichia coli* from Belgian broilers. Epidemiology and Infection. 139(5): 765-771.

Petersen, A., Christensen, J.P., Kuhnert, P., Bisgaard, M. and Olsen, J.E. 2006. Vertical transmission of a fluoroquinolone-resistant *Escherichia coli* within an integrated broiler operation. Veterinary Microbiology. 116(1-3): 120-128.

Phares, C.A., Danquah, A., Atiah, K., Agyei, F.K. and Michael, O.T. 2020. Antibiotics utilization and farmers' knowledge of its effects on soil ecosystem in the coastal drylands of Ghana. PloS One. 15(2): e0228777.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R. and Waddell, J. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy. 53(1): 28-52.

Plaza Rodríguez, C., Correia Carreira, G., Käsbohrer, A. A. 2018. Probabilistic transmission model for the spread of extended-spectrum-β-lactamase and AmpC-β-lactamase-producing *Escherichia coli* in the broiler production chain. Risk Analysis. 38(12): 2659-2682.

Prestinaci, F., Pezzotti, P. and Pantosti, A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathogens and Global Health. 109(7): 309-318.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J. and Leonard, F.C. 2002. Veterinary microbiology and microbial disease. Blackwell science. Iowa State University Press, Ames, Iowa, USA. pp. 536.

Rahman, M.A., Samad, M.A., Rahman, M.B. and Kabir, S.M. 2004. Bacterio-pathological studies on salmonellosis, colibacillosis and pasteurellosis in natural and experimental infections in chickens. Bangladesh Journal of Veterinary Medicine. 2(1): 1-8.

Rahman, M.S., Jang, D.H. and Yu, C.J. 2017. Poultry industry of Bangladesh: entering a new phase. Korean Journal of Agricultural Science. 44(2): 272-282.

Rahman, S.M., Roy, B.K., Shahriar, S.I. and Nipa, F.Y. 2015. Poultry Industry in Bangladesh: Issues and Challenges. International Journal of Business, Management and Social Research. 2(1): 71-79.

Reygaert, W.C. 2018. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiology. 4(3): 482.

Rezvi, M.R. 2018. The factors of declining agricultural growth in Bangladesh and its impact on food security. South Asian Journal of Social Studies and Economics. 1(1): 1-9.

Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J. and Nolan, L.K. 2005. Characterizing the APEC pathotype. Veterinary Research. 36(2): 241-256.

Rodroo, J., Intanon, M., Kreausukon, K., Kongkaew, A., Bender, J. and Awaiwanont, N. 2021. Occurrence of extended-spectrum beta-lactamase producing *E. coli* in broiler farm workers and the farm environment in Chiang Mai-Lamphun, Thailand. Veterinary Integrative Sciences. 19(1): 23-35.

Rousham, E.K., Asaduzzaman, M., Mozmader, T.A.U., Amin, M.B., Rahman, M., Hossain, M.I., Islam, M.R., Mahmud, Z.H., Unicomb, L. and Islam, M.A. 2021. Human colonization with extended-spectrum beta-lactamase-producing *E. coli* in relation to animal and environmental exposures in Bangladesh: An observational one health study. Environmental Health Perspectives. 129(3): 37001.

Roy, S.R., Rahman, M.B., Hassan, J. and Nazir, K.N.H. 2012. Isolation and identification of bacterial flora from internal organs of broiler and their antibiogram studies. Microbes and Health. 1(2): 72-75.

Saha, O., Hoque, M.N., Islam, O.K., Rahaman, M., Sultana, M. and Hossain, M.A. 2020. Multidrug-resistant avian pathogenic *Escherichia coli* strains and association of their virulence genes in Bangladesh. Microorganisms. 8(8): 1135.

Sahoo, T.K., Sahoo, L., Sarangi, L.N., Panda, S.K. and Panda, H.K. 2012. Prevalence, isolation, characterisation and antibiogram study of pathogenic *Escherichia coli* from different poultry farms of Odisha. Journal of Advanced Veterinary Research. 2(3): 169-172.

Saif, Y.M, Barnes, H.J., Glisson, J.R., FadlyAM, M. and Swayne, D.E. 2003. Diseases of poultry. Iowa State University Press. pp. 56.

Samanta, I., Joardar, S.N., Das, P.K., Das, P., Sar, T.K., Dutta, T.K., Bandyopadhyay, S., Batabyal, S. and Isore, D.P. 2014. Virulence repertoire, characterization, and antibiotic resistance pattern analysis of *Escherichia coli* isolated from backyard layers and their environment in India. Avian Diseases. 58(1): 39-45.

Sarker, M.S., Mannan, M.S., Ali, M.Y., Bayzid, M., Ahad, A. and Bupasha, Z.B. 2019. Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. Journal of Advanced Veterinary and Animal Research. 6(3): 272.

Schwarz, S., Shen, J., Kadlec, K., Wang, Y., Michael, G.B., Feßler, A.T. and Vester, B. 2016. Lincosamides, streptogramins, phenicols, and pleuromutilins: mode of action and mechanisms of resistance. Cold Spring Harbor Perspectives in Medicine. 6(11): a027037.

Shah, M.S.U.D., Khan, S.A., Aslam, A., Rabbani, M., Khan, K.A. and Rai, M.F. 2004. Effect of experimental yolk sac infection with *Escherichia coli* on immune status of broiler chicks. Pakistan Veterinary Journal. 24(3): 125-128.

Simon, P. 2009. Commercial egg and poultry meat production and consumption and poultry trade worldwide. Proceedings of the 6th International Poultry Show and Seminar, Dhaka, Bangladesh, 5th-7th March 2009. Dhaka, Bangladesh. Abstract Book, p 11.

Snary, E.L., Kelly, L.A., Davison, H.C., Teale, C.J. and Wooldridge, M. 2004. Antimicrobial resistance: a microbial risk assessment perspective. Journal of Antimicrobial Chemotherapy. 53(6): 906-917.

Soumet, C., Méheust, D., Pissavin, C., Le Grandois, P., Frémaux, B., Feurer, C., Le Roux, A., Denis, M. and Maris, P. 2016. Reduced susceptibilities to biocides and resistance to antibiotics in food-associated bacteria following exposure to

quaternary ammonium compounds. Journal of Applied Microbiology. 121(5): 1275-1281.

Stromberg, Z.R., Johnson, J.R., Fairbrother, J.M., Kilbourne, J., Van Goor, A., Curtiss 3rd, R. and Mellata, M. 2017. Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health. PloS One. 12(7): e0180599.

Swelum, A.A., Elbestawy, A.R., El-Saadony, M.T., Hussein, E.O., Alhotan, R., Suliman, G.M., Taha, A.E., Ba-Awad, H., El-Tarabily, K.A. and Abd El-Hack, M.E. 2021. Ways to minimize bacterial infections, with special reference to *Escherichia coli*, to cope with the first-week mortality in chicks: an updated overview. Poultry Science. 100(5): 101039.

Sykes, R. 2010. The 2009 Garrod lecture: the evolution of antimicrobial resistance: a Darwinian perspective. Journal of Antimicrobial Chemotherapy. 65(9): 1842-1852.

Tahir, A., Khan, M.A., Bibi, K., Bibi, S., Rauf, F. and Ayaz, F. 2021. Prevalence of colibacillosis in young broiler chicks and antibiogram of *Escherichia coli* in different areas of Hazara region. Advancements in Life Sciences. 8(3): 238-240.

Tansawai, U., Walsh, T.R. and Niumsup, P.R. 2019. Extended spectrum ß-lactamase-producing *Escherichia coli* among backyard poultry farms, farmers, and environments in Thailand. Poultry Science. 98(6): 2622-2631.

Thomrongsuwannakij, T., Blackall, P.J., Djordjevic, S.P., Cummins, M.L. and Chansiripornchai, N. 2020. A comparison of virulence genes, antimicrobial resistance profiles and genetic diversity of avian pathogenic *Escherichia coli* (APEC) isolates from broilers and broiler breeders in Thailand and Australia. Avian Pathology. 49(5): 457-466.

USDA, 2019. United States Department of Agriculture. Bangladesh Grain and Feed Annual, 2019. [cited: 2021 Jan 21]. Available from: https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Grain%20and%20Feed%20Annual_Dhaka_Bangladesh_4-2-2019.pdf.

Van den Bogaard, A.E., London, N., Driessen, C.A. and Stobberingh, E.E. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. Journal of Antimicrobial Chemotherapy. 47(6): 763-771.

Vandekerchove, D., De Herdt, P., Laevens, H. and Pasmans, F. 2004. Risk factors associated with colibacillosis outbreaks in caged layer flocks. Avian Pathology. 33(3): 337-342.

Varga, C., Guerin, M.T., Brash, M.L., Slavic, D., Boerlin, P. and Susta, L. 2019. Antimicrobial resistance in fecal *Escherichia coli* and *Salmonella enterica* isolates: a two-year prospective study of small poultry flocks in Ontario, Canada. BMC Veterinary Research. 15(1): 1-10.

Vogt, R.L. and Dippold, L. 2005. *Escherichia coli* O157: H7 outbreak associated with consumption of ground beef, June–July 2002. Public Health Reports. 120(2): 174-178.

Walsh, T.R. and Wu, Y. 2016. China bans colistin as a feed additive for animals. The Lancet Infectious Diseases. 16(10): 1102.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. 2020. Antimicrobial resistance on *Escherichia coli* from poultry production on Blitar, Indonesia. Indian Journal of Forensic Medicine and Toxicology. 14(4): 4131.

WPSA, 2017. World's Poultry Science Association. Poultry in Bangladesh: Key Facts & Figures. [cited: 2020 Oct 14]. Available from: http://wpsa-bb.com/poultry-at-a-glance/.

Yang, Y., Ashworth, A.J., Willett, C., Cook, K., Upadhyay, A., Owens, P.R., Ricke, S.C., DeBruyn, J.M. and Moore Jr, P.A. 2019. Review of antibiotic resistance, ecology, dissemination, and mitigation in US broiler poultry systems. Frontiers in Microbiology. 10(2639): 1-10.

Yuvraj, P., 2019. Colibacillosis in poultry: A review. Journal of Agriculture Natural Resources. 2(1): 301-311.

Zakeri, A. and Kashefi, P. 2012. Antimicrobial susceptibilities of avian *Escherichia coli* isolates in Tabriz, Iran. African Journal of Biotechnology. 11(19): 4467-4470.

Zhao, S., Maurer, J.J., Hubert, S., De Villena, J.F., McDermott, P.F., Meng, J., Ayers, S., English, L. and White, D.G. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Veterinary Microbiology. 107(3-4): 215-224.

Appendix-I

Assessment of antimicrobial usage on commercial poultry farms and, attitudes and behaviours of antimicrobial usage by commercial poultry farmers and attitudes and behaviours of antimicrobial sales and distribution by traders of antimicrobials in Bangladesh

<u>Demographic/Socioeconomic characteristics of the interviewee</u>

(Tick the boxes and fill in the blanks)

Date of interview:	_____ (day)	_____(month)	_____(Year)
Farm ID			
Name of the interviewee:			
What is your farm type?	0= Meat type (Broiler) 1= Egg type (Layer)		
Status of the interviewee on farm:	0=Owner 1=Manager 2=Worker 3=Owner's spouse 4=Owner's son 5=Owner's daughter 6=Other _____		

Poultry Information

How many chickens do you have in the farm today?			
What is your current production system?	0=All-in-All out	1=Continuous	2=Both
How many sheds you have in your farm?			
Do you use antimicrobial/antibiotics/ medicine/ vitamins/minerals in your farm?	0=No	1=Yes	
If yes, do you use different amount of antimicrobial/medicine/vitamins/antibiotics in different sheds?	0=No	1=Yes	
1. If yes, in which shed is the highest amount of antimicrobial/medicine/vitamins or antibiotics used? THIS IS THE SHED TO BE SAMPLED (If we get ans here then ques 21 will not appear)	0=Shed 1 3= Shed 4	1= Shed 2 4= Shed 5	2=Shed 3 5=Shed 6 6=Other shed (specify)____
If the same amount of antimicrobial/medicine/vitamins are used accross, do you have birds of different age on your farm?	0=No	1=Yes	
2. If yes, in which shed are the oldest birds? THIS IS THE SHED TO BE SAMPLED If no (All birds are of the same age), then THE SHED TO BE SAMPLED will be selected randomly.	0=Shed 1 3= Shed 4	1= Shed 2 4= Shed 5	2=Shed 3 5=Shed 6 6=Other shed (specify)____

How many chickens you have in the shed today from which faecal sample is taken?			
What is the age of the poultry in the shed from which faecal sample is collected?	_____ (day)	_____ (month)	_____ (Year)
What are the ages of the poultry from other sheds?			
If, all in all out, then collect the age for one batch (as all the chickens are of same age, so all sheds will be of same ages)			
If, continuous, then collect age for different batches			
1st Shed of same age	_____ (day)	_____ (month)	_____ (Year)
2nd Shed of same age	_____ (day)	_____ (month)	_____ (Year)
3rd Shed of same age	_____ (day)	_____ (month)	_____ (Year)
4th Shed of same age	_____ (day)	_____ (month)	_____ (Year)
5th Shed of same age	_____ (day)	_____ (month)	_____ (Year)
6th Shed of same age	_____ (day)	_____ (month)	_____ (Year)
Others_____			

Farm bio-security and hygiene related information

(Answers will be observed/asked by the interviewer)

Is the farm surrounded by a protective fence?	0=No	1=Yes	
3. In addition to the people involved in rearing poultry (listed in ques 23), who has access to your farm?	0=Feed suppliers	1=Other farm owners	2=Other farm workers
	3=Relatives	4=Egg traders	5=Poultry traders
	6=Poultry vaccinator	7=Government Veterinarians	8=Private Veterinarians
	9=Feed delivery person	10=Owner/worker from another farm	11=Others _____
Does anyone who is involved in poultry keeping go to other commercial poultry farms?	0=No	1=Yes	
If yes in question 23, then how frequently does he/they visit in the last month?	0=daily	1=consecutive days	2=once in a week
	3=once in a fortnight	4=once in a month	5=others _____

(Answers will be observed/asked by the interviewer)

(Tick appropriate answers)	**Yes**	**Partial**	**No**
1. Do you isolate the sick birds in a separate shed?			
2. What do you do with dead birds?			
3. What do you do with your manure?			
4. Does washing facility exist for the visitors/employees before entering farm/shed/premises?			
5. Do the visitors/employees use washing facility before entering farm/shed?			
6. Do the employees change clothes and shoes before entering the farm/shed?			
7. Do the visitors change clothes and shoes before entering the farm/shed?			
8. Are the vehicles checked and decontaminated before entering farm?			
9. Are the vehicles decontaminated when leaving the farm?			
10. Do you have footbaths available and used, and disinfectant water changed within 6 hours?			
11. What types of water you allow for drinking or cooling at the farm?			
12. Do you weekly disinfect and clean the farm surfaces and equipments?			
13. Are egg trays washed when bringing back from market?			
14. Are farm employees given training on biosecurity measures?			
15. How long do you keep the shed empty between two consecutive batches?			
16. Do farm workers live within the farm premises?			
16.1. If yes, do they rear their own poultry birds within the farm premises?			

Other demographic and Farm information

Mobile number of the interviewee:			
Address of the farm:			
Name of the poultry farm:			
Village:			
Ward:			
Union			
Upazilla/Thana:			
Latitude:			
Longitude:			
Experience of the interviewee in poultry farming:	0=< 6 months	1= 6-12 months 2= 1-5 years	3= 6-10 years 4=>10 years
Age (in years)			
Gender:	0=Male	1=Female	
Education:	0=No education	1=Up to Primary	2=Up to Secondary
	3=Up to higher secondary	4=Graduate	5=Post graduate
	6=Dakhil	7=Fazil	
Marital status:	0=Single	1=Married	2=Divorced

	3=Widow	4=Others _____	
Religion:	0=Muslim	1=Hindu	2=Christian
	3=Buddhist		
Which is the source provides the largest income to your household?	0=Poultry rearing	1=Livestock rearing	2=Fishing
	3=Daily worker	4=Grocery	5=Non-Government Organization
	6=Family business	7=Agriculture	8=Government organization
			9=Others_____
Monthly Net Income (in BDT)			
What type of breed/strain you have in the farm currently? (THIS QUES will come if interviewer ticks egg type)	0=Novogen Brown 1=White Hyline Brown 2=White Shaver 579	3=ISA Brown 4=Hi-Sex Brown	5=White Bovine White 6= Others_____
What type of breed/strain you have in the farm currently? (THIS QUES will come if interviewer ticks meat type)	1=Cobb 500 2=Ross 308	3=Indian River Meat 4=Tiger Sasso	5=Habbard and Arber acre

Appendix-II

Pre-enrichment in Buffered peptone water

The swab sample stored at -20 ℃ was thawed at room temperature and inoculated into Buffered peptone water (Neogen, Lansing MI) at a ratio of 1:10 and then incubated between 34℃ and 38°C for 18 h. After incubation, they were then separately incubated in MacConkey (MAC) Agar.

MacConkey (MAC) Agar inoculation

Samples properly grown in buffered peptone water were further inoculated into MAC (Neogen, Lansing MI) agar and incubated at 37 ℃ for 18-24 h. Pink, round medium-sized colonies were suspected as *E. coli* (Lupindu, 2017). Positive samples in MAC agar were then inoculated in Eosin Methylene Blue (EMB) agar for differentiating the organisms.

Eosin Methylene Blue (EMB) inoculation

Samples properly grown in MAC agar were inoculated into EMB (Neogen, Lansing MI) agar and incubated at 37 ℃ for 24 h. The positive growth in EMB indicates the presence of *E. coli*.

Biochemical tests

Further confirmation of *E. coli* is supported by some specific biochemical tests.

Triple sugar iron test

Same samples were also inoculated in triple sugar iron (Neogen, Lansing MI) agar. Development of yellow color in slant, yellow in butt, presence of gas bubbles and absence of black precipitate in the butt indicates positive for *E. coli.*

Indole test

The indole reacts with the aldehyde in the Kovac's reagent and give a red or a pink ring at the top of the tube. Peptone water in a tube, which contains tryptophan, was inoculated with *E. coli*. The mixture was incubated overnight at 37°C. Then, a few drops of Kovac's reagent were added to the mixture and formation of a red or a pink colored ring at the top is a positive reaction. *E. coli* is indole-positive bacteria.

Citrate utilization test

Suspected samples were inoculated in Simmons citrate (Neogen, Lansing MI) agar that ferment citrate and change of color from greenish to royal blue. No color change indicates negative result. *E. coli* is citrate negative.

Maintenance of pure culture and stock

For isolation of pure culture the bacteria were grown in selective media: EMB agar were again sub-cultured in blood agar (Oxoid®, Oxoid Ltd, Basingstoke, United Kingdom). After confirmation of pure culture by observation of colonies in BA, colonies were reinoculated in brain heart infusion (Neogen, Lansing MI) broth and incubated at 37 °C for 24 h for bacterial multiplication as per manufacture instruction. 50% glycerol solution was prepared by diluting 100% glycerol with phosphate buffered saline. Then 700 µl overnight cultures were transferred in sterilized cryovial with 300 µl of 50% glycerol and stored at -80°C as stock for longer time preservation. Entire procedure of bacteriological culture has been attached as sketch below.

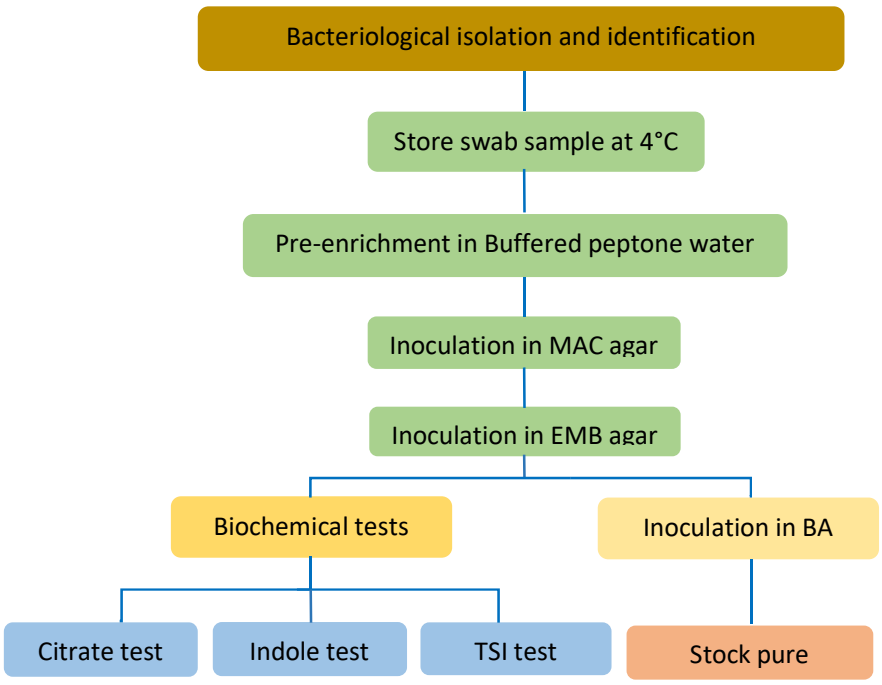

Figure 1. Flow chart of bacteriological isolation and identification

Appendix-III

Mueller-Hinton agar plate preparation

Mueller-Hinton (Oxoid®, Oxoid Ltd, Basingstoke, United Kingdom) agar plates were prepared according to manufacture instruction. MHA plates were stored at 4 ℃ in sealed packages. These plates were removed from refrigerator at least 15 minutes before use. If excess moisture on agar surface, the plates were then placed in a laminar flow hood at room temperature to remove access liquid till dry

Preparation of inoculum

Subculture of *E. coli* was prepared the previous day. Using a sterile inoculating loop, four or five isolated colonies from subculture were touched and suspended in 2 ml sterile saline. After vortexing the saline tube, turbidity of the suspension was adjusted with 0.5 McFarland standard to achieve an equivalent turbidity.

Mueller-Hinton agar plate inoculation

A sterile cotton swab was dipped into the 0.5 McFarland adjusted suspension and rotated against the side of the tube with firm pressure to remove excess fluid. MHA plate was inoculated by streaking the swab three times over the entire plate for an even distribution of inoculum and the rim of the agar. Leaving the lid ajar, allowed the plate to sit at room temperature at least 3 to 5 minutes.

Placement of discs to inoculated agar plates

Antimicrobial-impregnated disks were placed on the agar surface by using a multidisc dispenser. Each disk was pressed with sterilized forceps to ensure complete contact with agar surface. Then the plates were inverted and placed in an incubator to set 35°C ± 2°C.

Measuring zones and interpreting results

All plates were examined after 16 to 18 hours incubation. Plates were placed in front of dark background and recorded the zone diameter by using centimeter scale.

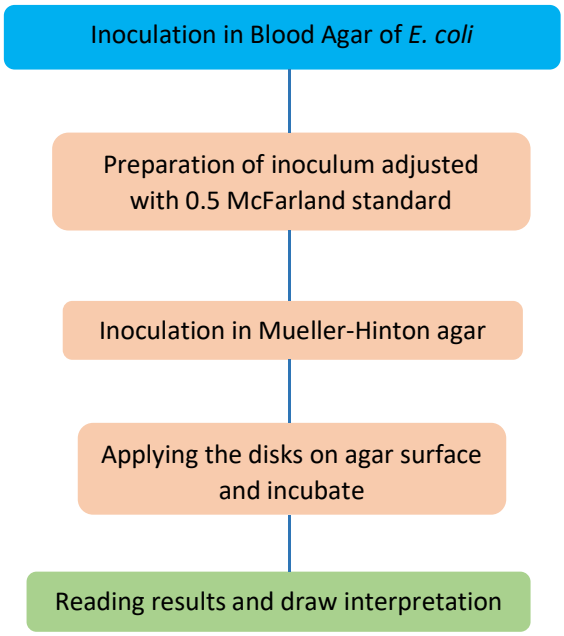

Figure 1. Disk diffusion AST process

Short Biography

Shetu Bhusan Das has been serving as an Upazila Livestock Officer (ULO) in the Department of Livestock Services (DLS), under the Government of Bangladesh since 2012. He passed the Secondary School Certificate Examination, SSC, in 2000 obtaining 1st Division and subsequently passed Higher Secondary Certificate Examination, HSC, in 2002 awarding with 1st Division. Then Mr. Das obtained his Doctor of Veterinary Medicine Degree in 2007 from Chattogram Veterinary and Animal Sciences University, CVASU, Bangladesh. Now, he is a candidate for the degree of MS in Epidemiology under the Department of Medicine and Surgery, Faculty of Veterinary Medicine, CVASU. He has immense interest to work in veterinary epidemiology and zoonoses research.