Program Based Research Grant (PBRG)

Sub-project Completion Report

on

Determination of Antimicrobial Resistance and Residues in Livestock and Poultry Food Products and Feed in Bangladesh

Sub-project Duration

From July 01, 2018 to March 15, 2022

Coordinating Organization
Livestock Division
Bangladesh Agricultural Research Council (BARC)
Farmgate, Dhaka-1215

Project Implementation Unit
National Agricultural Technology Program-Phase II Project
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Implementing Organization

Bangladesh Agricultural University
Bangladesh Livestock Research Institute
Rajshshi University
Patuakhali Science and Technology University
Chattogram Veterinary and Animal Sciences University
Sylhet Agricultural University
Haji Mohammad Danesh Science and Technology University

Project Implementation Unit
National Agricultural Technology Program-Phase II Project
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215
January 2022

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Abbreviation and Acronyms

AST	Antimicrobial Susceptibility Testing
AMR	Antimicrobial Resistance
AMC	Amoxicillin
AMP	Ampicillin
BAU	Bangladesh Agricultural University
BLRI	Bangladesh Livestock Research Institute
CLSI	Clinical Laboratory Standard Institute
CFU	Colony Forming Unit
CVASU	Chattogram Veterinary and Animal Sciences University
CRO	Ceftriaxone
CN	Gentamicin
CIP	Ciprofloxacin
DOX	Doxycycline
EMB	Eosin Methylene Blue
HPLC	High Performance Liquid Chromatography
HSTU	Haji Mohammad Danesh Science and Technology University
MDR	Multidrug Resistance
MRL	Maximum Residue Level
TLC	Thin Layer Chromatography
TCBS	Thiosulfate-Citrate-Bile salts-Sucrose
TSI	Triple Sugar Iron
MHA	Mueller Hinton agar
OTC	Oxytetracycline
PCR	Polymerase Chain Reaction
PCR	Project Completion Report
P	Penicillin
PBRG	Program Based Research Grants
PSTU	Patuakhali Science and Technology University
RU	Rajshahi University
SAU	Sylhet Agricultural University
S	Streptomycin
SS	Salmonella Shigella Agar
SXT	Sulfamethoxazole and Trimethoprim
SPSS	Statistical Package for the Social Sciences
XLD	Xylose Lysine Deoxycholate
WHO	World Health Organization

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Executive Summary

Antimicrobial resistance (AMR) is one of the biggest threats to global health, food security, and development today. AMR can affect anyone, of any age, in any country. AMR occurs naturally, but misuse of antimicrobials in humans and animals is accelerating the process. AMR leads to longer hospital stays, higher medical costs and increased mortality. On the other hand, antimicrobial residues are metabolites found in trace amounts in any edible portion of the animal product after the administration. The antimicrobial residues in food animal in excess of the acceptable maximum residue limit may contribute to the development of AMR in animals or humans. Considering the paramount impact of AMR and residue in food chain, the present study was undertaken to screening the antimicrobial resistance and residues in livestock and poultry food products and feed in Bangladesh.

A pre-structured questionnaire survey was conducted among the 1520 farmers, veterinarians and informal prescribers (feed/ drug sellers). A number of 3280 samples consisting of 880 poultry products & by-products, 1440 livestock products & by products and 960 poultry feed were collected from thirty-three districts across the country. A number of 2320 samples except feed were tested for microbiological screening followed by phenotypic and genotypic resistance characterization. Similarly, 2,440 samples, excluding feces, were screened qualitatively and quantitatively for antibiotic residues.

The questionnaire survey revealed diversified knowledge, perception and practices on antimicrobial usage, resistance and residue among farmers, informal prescribers and veterinarians. There was a big knowledge and practice gaps exist in the rational use of antimicrobials in livestock and poultry farming practices. The knowledge, perception and practices of the farm owners revealed, 62.5% had knowledge on biosecurity; 95.7% heard about antibiotics; 68.2% heard about antimicrobial resistance, 52.2% know about antibiotic residue; 82% didn't have record on antibiotic use; 30.3% farm used antibiotic for preventive purposes; 80.2% didn't know about withdrawal period of antibiotics; 69.7% went to consult with veterinarian for treatment purposes; and 50% didn't know about shelf life of antibiotics. Microbiological analyses showed that the overall prevalence of *E. coli*, *Salmonella*, *Staphylococcus* and *Streptococcus* in livestock products and by-products were found to be 38.47%, 8.26%, 14.67% and 4.79%, respectively. Similarly, the overall prevalence of *E. coli*, *Salmonella*, *Staphylococcus* and *Campylobacter* in poultry products and by-products were found to be 51.59%, 17.61%, 21.93% and 4.43% respectively. The overall multidrug resistant (MDR) pattern of *E. coli*, *Salmonella*, *Staphylococcus* spp., *Streptococcus* spp. were found to be 88.33%, 75%%, 95% and 100% respectively regardless of origin. The phenotypic resistance revealed decreased susceptibility to penicillin, ampicillin, amoxicillin, levofloxacin, ciprofloxacin, tetracycline, neomycin, streptomycin and sulfamethoxazole-trimethoprim. While genotypic resistance showed consistent prevalence of plasmid-mediated beta lactamases (blaTEM, blaSHV and blaCMY) followed by sul1, tet (A), tet(B), aadA1, aac (3)-IV, ere(A) and mecA genes.

The prevalence of antimicrobial residue with different concentration in meat, milk, and egg, poultry feed samples were found to be 10.17%, 6.65%, 3.75% and 10.17% respectively by TLC method. Out of 75 meat samples, 62 were found positive by HPLC methods which includes amoxicillin (15), ampicillin (4), ceftriaxone (5), ciprofloxacin (6), oxytetracycline (23), doxycycline (4), streptomycin (3) and sulfonamides (2). Similarly, out of 35 milk samples, 32 were found to be positive by HPLC which includes amoxicillin (3), ampicillin (9), ceftriaxone (2), ciprofloxacin (7), oxytetracycline (3), gentamicin (5) and penicillin (2). Likewise, a

number of 65 feed samples were found to be positive in HPLC method which includes, ciprofloxacin (25), oxytetracycline (30), doxycycline (3), sulfonamides (6) and gentamicin (1). Furthermore, thirty-four egg samples were found to be positive by HPLC consisting of amoxicillin (2), ampicillin (1), ceftriaxone (11), and oxytetracycline (20). There seventeen meat samples had antibiotic residue above the MRL having amoxicillin (11), ampicillin (4), and oxytetracycline (2). Similarly, fourteen milk samples had antibiotic residue above the MRL consisting of amoxicillin (2), ampicillin (7), ciprofloxacin (1), gentamicin (2), oxytetracycline (1) and sulfonamide (1). Two egg samples were found above the MRL of oxytetracycline. Besides, the presence of residue in feed samples are alarming as antimicrobial use is prohibited in poultry feed as well as there is no standard MRL for feed samples in codex alimentarius standards. The mitigation study of antibiotic resistance and residue showed using probiotic, phytobiotic and their combination had significant influence on growth performance, carcass yield and dressing percentage after 28 days of treatment in broiler chicken. Supplementation of probiotic or combination of probiotic & phytobiotic in broiler feed could be the potential candidate alternative to antimicrobial as growth promoter for safe broiler production. Alongside, it was found in laboratory animal study (rat and mice) that whether probiotic, phytobiotic and spirulina may eliminate antibiotic drug residues from animal body. It may be assumed that supplementation of probiotic, phytobiotic and spirulina can eliminate drugs residue from the animal body.

Moreover, the presence of MDR pathogen in livestock and poultry derived food products indicated great public health hazard. In addition, antimicrobial residue in animal derived foodstuffs had catastrophic effect in public health. It may be concluded that rational use of antimicrobial as well as following the withdrawal period in the farming practices can reduce the emergence of resistance and transmission of residue in animal derived food products. In addition, awareness, motivation and knowledge among the producer, consumer, prescriber and policy maker would help in combating the resistance and residue in livestock and poultry food and feed in Bangladesh.

Keywords: Antimicrobials, poultry, livestock, feed, antimicrobial resistance, antimicrobial residue.

PBRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the PBRG sub-project:** Determination of Antimicrobial Resistance and Residues in Livestock and Poultry Food Products and Feed in Bangladesh
2. **Implementing organization (s):** Livestock Division, Bangladesh Agricultural Research Council, Farmgate, Dhaka-1215.
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4. **Sub-project budget (Tk.):** Total approved budget was Tk. 3,81,68,710.00. The component wise budget has given in **Table 1.**

Table 1: Component wise expenditure statement

Component	Total approved budget (Tk.)	Total fund released (Tk.)	Total fund spent (Tk.)	Unspent balance (Tk.)
Coordination Component (BARC)	6169021	3989954	3843782	146172
Component 1 (BAU)	6839362	6053112	6048748	790614
Component 2 (BLRI)	3940677	3173212	3088913	84299
Component 3 (RU)	3945500.00	3444794.00	3441640.00	3154.00
Component 4 (PSTU)	3999120	3546734	3077597	469137
Component 5 (CVASU)	4941960	4361934	4144503	217431
Component 6 (SAU)	4264750	3943606	3941351	2255
Component 7 (HSTU)	40,68,320	35,31,882	34,48,472	83,410
Total	38168710	32045228	31035006	1796472

5. Duration of the sub-project:

Start date (based on LoA signed): 01 July 2018
End date: March 15, 2022

6. Background of the sub-project:

Foodborne microorganisms are important pathogens that affect food safety and cause disease in humans worldwide through the consumption of foodstuffs, mainly animal products that are contaminated with vegetative pathogens or their toxins. Most of these microbes are zoonotic, which has important implications for both public health and the economic sector. Bacteria are pathogen responsible for two-thirds of human foodborne illnesses worldwide with high levels of contamination in developing countries. Edible animals are the main reservoir for many foodborne zoonotic bacterial pathogens, and foods of animal origin are the main routes of transmission. Meat, dairy products, and eggs are the main sources that people are exposed to zoonotic bacteria. *S*. aureus, *Salmonella* species, *Campylobacter* species, *L*. monocytogenes, and *E. coli* are the most important zoonotic bacterial pathogens that cause foodborne illness worldwide and deaths associated with the consumption of contaminated animal products. The production of toxins and virulent structural factors are responsible for the pathogenesis of these bacteria. These important zoonotic bacteria cause infections in humans, characterized primarily by gastrointestinal symptoms, including nausea, vomiting, diarrhea, abdominal cramps, and other pathogen-specific symptoms. Some bacteria can cause serious complications.

AMR is a global health and development threat. It requires urgent multi-sectoral action in order to achieve the Sustainable Development Goals (SDGs). WHO has declared that AMR is one of the top 10 global public health threats facing humanity. Misuse and overuse of antimicrobials are the main drivers in the development of drug-resistant pathogens. Lack of clean water and sanitation and inadequate infection prevention and control promotes the spread of microbes, some of which can be resistant to antimicrobial treatment. The cost of AMR to the economy is significant. In addition to death and disability, prolonged illness results in longer hospital stays, the need for more expensive medicines and financial challenges for those impacted. Without effective antimicrobials, the success of modern medicine in treating infections, including during major surgery and cancer chemotherapy, would be at increased risk.
Veterinary medicines, especially antibiotics, are among the most important components related to animal feed production. Generally, the main use of antibiotics in animals is for the treatment and prevention of diseases and growth promotion. Antibiotic usage in animals may result antibiotic residues in foodstuffs such as milk, egg and meat. These residues may cause various side effects

such as transfer of antibiotic resistant bacteria to humans, immunopathological effects, allergy, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol) and even carcinogenicity. The most important adverse effect of antibiotic residues is the transfer or antibiotic resistant bacteria to the humans due to the mobile properties of resistance. Because of these undesirable effects, it is important to regulate the use of antibiotics in food animals.

So far there was no comprehensive study undertaken on the determination of antimicrobial resistance and residues in livestock and poultry food products and feed in Bangladesh. Considering the paramount importance of food safety, the study was undertaken to isolate and identify foodborne pathogen along with characterization of their resistance pattern as well as detection of antibiotic residue in animal derived foods and feeds in Bangladesh. This study would generate national database on resistance and residue in animal derived foodstuffs and feed. The database would help to formulate policy and intervention in the food safety of livestock and poultry products and by-products.

7. **Sub-project general objective (s)**: Assessment of antimicrobial drug residues in livestock and poultry food products & feed and its mitigation program, and to determine the antimicrobial resistance & associated genes
8. **Sub-project specific objectives (component wise):**
   i. To assess the antimicrobial drug residues in livestock and poultry food products and feed, and its mitigation using alternate approach;
   ii. To isolate, identify and characterize antimicrobial resistant bacteria from different samples collected from sick or apparently healthy livestock and poultry;
   iii. To study of antimicrobial sensitivity/resistance pattern of each isolate with the detection of resistance genes;
   iv. To sequence and detect any changes in genomic level that alter their drugs sensitivity pattern;
   v. To provide recommendations/suggestions for the control of antimicrobial drugs residue and resistance;
9. **Implementing location (s):** The project was implemented by six components namely, Bangladesh Agricultural University (BAU), Bangladesh Livestock Research Institute (BLRI), Rajshahi University (RU), Patuakhali Science and Technology University (PSTU), Chattogram Veterinary and Animal Sciences University (CVASU), Sylhet Agricultural University (SAU) and Haji Mohammad Danesh Science and Technology University (HSTU).
10. **Methodology in brief** (with appropriate pictures):

Study Area: The study was conducted in the thirty-three districts under the eight administrative divisions of Bangladesh. The seven components of the project covered each one division with at least four districts while the component PSTU covered two divisions with nine districts. The details of the study area have given in **Table 2**.

Table 2: Study areas of the seven components

Component	Division	Districts
BAU	Mymensingh	Mymensingh, Sherpur, Netrokona, Jamalpur
BLRI	Dhaka	Gazipur, Rajbari, Manikgonj, Tangail
RU	Rajshahi	Rajsahi, Bogra, Sirajgonj, Naogaon
PSTU	Barisal	Barishal, Bhola, Patuakhali, Pirojpur and Barguna
	Khulna	Jessore, Magura, Khulna, Bagerhat
CVASU	Chittagong	Chattogram, Rangamati, Banderban, Cox's Bazar
SAU	Sylhet	Sylhet, Sunamgonj, Moulvi Bazar and Habiganj
HSTU	Rangpur	Rangpur, Dinazpur, Nilphamari, Panchagar

Questionnaire survey regarding antibiotic usage pattern, its residual knowledge and antibiotic resistance

Three individual pretested structured questionnaire survey was conducted among the farmer, veterinarian and informal prescribers (drug seller/ feed seller) to evaluate the present scenario of antimicrobial drugs usage, emergence of resistance and residues in livestock & poultry foods and feed. A number of 150 farmers (layer 25, broiler 25, cattle 30, duck 25, sheep 15, goat 15 and buffalo 15), 20 veterinarians and 20 drug/feed sellers in each eight divisions were interviewed by seven components.

Sampling design

A total 410 samples which includes 110 from poultry products, 180 from large and small animals and 120 feed samples were collected from each eight divisions by the seven components. Poultry products included broiler meat 20, layer meat 20, egg 30, broiler feces 20 and layer feces 20. Large and small animal samples included cattle meat 20, sheep/goat meat 20, buffalo meat 10, cattle milk 25, sheep/goat milk 25, buffalo milk 15, cattle feces 25, sheep/goat feces 25 and buffalo feces 10. A number of 120 feed samples consisting of 60 brand and 60 non-brand were collected from each eight divisions. Regardless of brand, among the 60 feed samples 30 were from broiler (10 starters, 10 growers and 10 finisher) and 30 were from layer (10 starters, 10 growers and 10 finishers). A number of 3,280 samples were collected throughout the country by all components. All collected samples for each division (290) except feed were tested for microbiological screening followed by phenotypic and genotypic resistance pattern. Similarly, all collected samples for each division (305) except feces were subjected to qualitative and quantitative antibiotic residue screening. The details of sampling design in each division are shown in **Table 3**.

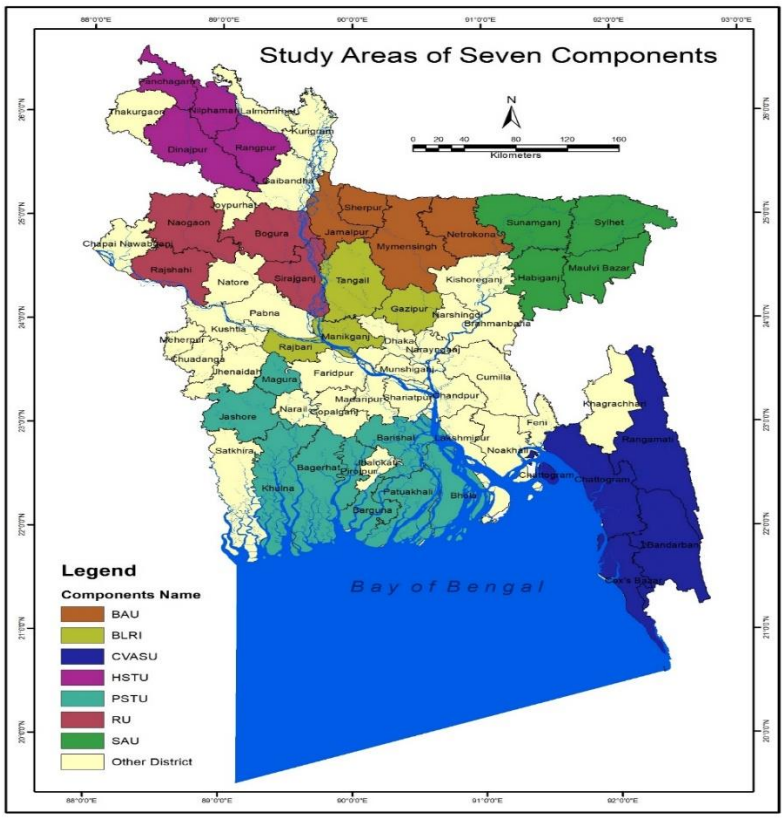

Fig 1: Study areas in the country

Table 3: Samples design for the surveillance of antimicrobial residues and resistance throughout the country

A. Poultry Products						
Meat		Broiler	20		40	110
		Layer	20			
Egg			20+10		30	
Feces		Broiler	20		40	
		Layer	20			
C. Large/ Small Animal						
Meat		Cattle	20		50	180
		Sheep/Goat	20			
		Buffalo	10			
Milk		Cattle	25		65	
		Sheep/Goat	25			
		Buffalo	15			
Faeces		Cattle	25		65	
		Sheep/Goat	25			
		Buffalo	15			
C. Feed						
Brand company	Broiler	Starter	10	30	60	120
		Grower	10			
		Finisher	10			
	Layer	Starter	10	30		
		Grower	10			
		Finisher	10			
Non Brand company	Broiler	Starter	10	30	60	
		Grower	10			
		Finisher	10			
	Layer	Starter	10	30		
		Grower	10			
		Finisher	10			

Qualitative and quantitative determination of antimicrobial drugs residue

A number of 305 samples except feces from each division were subjected to qualitative and quantitative determination of antimicrobial drugs residue by Thin Layer Chromatography (TLC) and Ultra High Performance Liquid Chromatography (UHPLC) method. Based on questionnaire survey result, commonly prescribed antimicrobials were selected for the determination of residues.

Preparation of standard: All standards; Oxytetracycline (OTC), Ciprofloxacin (CIP), Amoxicillin (AMC), Doxycycline (DOX), Gentamicin (GN), Ampicillin (AMP), Penicillin (P), Enrofloxacin (ENR), Erythromycin (E), Cloxacillin (C), Ceftriaxone (CRO), Levofloxacin (LVX), Neomycin (Ne), Tylosin (TYL), Streptomycin (S) and Sulfonamides (SXT) were collected from Merck-Germany. Each standard was prepared by dissolving 0.1g of antibiotic powder into four ml of methanol solution (Hussain et al., 2013).

Chemicals for extraction and mobile phase: Trichloroacetic acid, diethyl ether and phosphate buffer saline were used for extraction of antibiotics from sample. Methanol, acetonitrile, n-butanol, ammonia, chloroform, ammonium acetate, ethyl acetate, acetic acid and ethanol of analytical grade were used for mobile phase to detect different antibiotics. Water was filtered before using in a Milli-Q system, (Millipore, Bedford, MA, USA).

Sample preparation: Sample extraction procedure were performed according to Popelka et. al., 2005 and Ferdous et al., 2020 with light modification. Four gram (4 gm) of samples (meat, milk and feces) were taken in a falcon tube for TLC assay. Meat samples were cut into small pieces, grinded and blended. A 10 ml of Phosphate Buffer Saline (pH-6.5) was added to the samples and vortexed for one minute for homogenization. After homogenization 2 ml of 30% trichloroacetic acid was added to the sample and shake well immediately for protein precipitation and then centrifuged at 6000 rpm for 15 minutes. Supernatant was collected and filtered by Whatman 125 mm filter paper and funnel in another well cleaned falcon tube. Equal amount of diethyl ether was added to the filtrated fluid for de-fatation and left for 10 minutes at room temperature to become into separate layer. Upper layer was discarded and finally bottom layer was collected. This extraction procedure was repeated twice using diethyl ether. Then the extract was evaporated until dryness. By adding 2 ml of mobile phase, the dried sample was reconstituted and collected in a screw cap vial. Then, this extracted samples were kept into refrigerator for further analysis.

TLC apparatus: 0.25mm thickness TLC plate (MN-Germany), TLC tank (prepared locally) and UV detection box (prepared locally) were used to detect the antimicrobial residues from samples. F18W-Germany UV light of 254 nm and 365 nm wavelength were used as required in the UV detection box.

Thin Layer Chromatography: The procedure of TLC was done according to Tajick and Shohreh, 2006 with some modification. A 20×20 cm Silica plate were cut into 4×5 cm size pieces (MN-Germany). A straight line was drawn across these plates 2 cm (approx.) from the bottom edge by pencil. Below 1 cm from the plate's upper edge, another straight line was drawn across the plate. On the bottom line, desired spots were marked by pencil where analytes were dropped. 50 µl samples or standard were poured into the marked spot of the plate. Then, the plates were placed on TLC tank which contain mobile phase. Different mobile phases were used to detect different antibiotics. After placing the plates, TLC tank was covered by a lid and left until the mobile phase was reach the upper line. Then the plates were dried and 256 or 365 nm (as required) wave length UV light were used to visualize the residue spot in stationary phase in UV detection box. Spot was marked by pencil to calculate the retention factor (R_f). R_f value was measured by the formula: R_f = Distance travel by solvent/Distance travel by solute (Sample). If R_f value of standard spot and sample spot was same, it was considered as a similar compound (Ferdous et al., 2020; Anika et al., 2019).

Quantitative determination of antimicrobial drugs residue by Ultra High Performance Liquid Chromatography (UHPLC)

Quantification of antibiotic residues were done by Ultra High Performance Liquid Chromatography (UHPLC) method.

UHPLC apparatus: Thermo Scientific UltiMate 3000- Autosampler Column Compartment UHPLC from Waltham, Massachusetts, USA machine was used. Synchronys C18 reverse-phase stainless steel column (Carbon load 16%, 250 mm length, 4.6 mm diameter, 100 Å (10 nm) pore size, 5 µm partical size, surface area m2/g) from Thermo Scientific, Waltham, Massachusetts, USA were used as stationary phase. Refrigerator, Centrifuge machine (Table Top Centrifuge, DSC-200A-2, Taiwan), Ultrasonic bath (ISOLAB Laborgerate GmbH, Germany), Filter machine (Rocker 300, Taiwan), Rotary Evaporator (IKA-Werke GmbH and Co., Germany), homogenizer (Mini Vortex Mixer, VM-100-B, Taiwan) and 0.2 MFS syringe filter (Advantec MFD, Japan) were used for sample preparation.

Preparation of standard: Primary standard stock solution of each antibiotic was prepared by dissolving 10 mg of antibiotics into 10 mg of mobile phase (different for different antibiotic) to give

final concentration of 1 mg/ml. Stock solution was kept in amber glass vials to prevent photo-degradation and stored at -20ºC in refrigerator. Stock solution was used within 4 weeks of preparation (Cinquina et al., 2003). Secondary standard solution of each antibiotic was prepared by following the Maximum Residue Limits (MRL) values prescribed by Codex Alimentarius Commission (CAC) of FAO/WHO (Codex, 2015). Based on the MRL values, a linearity range (½, 1, 2, 4 and 6 times of MRL value) was selected to cover the lowest MRL values for each antibiotic. Then, primary standard solution was diluted with mobile phase to the required volume for the preparation of secondary standard solution (Chauhan et al., 2019).

Limits of Detection (LOD) and Limits of Quantification (LOQ): The base line noise approach was used to calculate LOD and LOQ. LOD was measured as the sample concentration that produces a peak three times higher than the base line noise level. LOQ was measured as concentration that is ten time higher than the base line noise level (ICH, 1997a).

Recovery: Recovery rate will be calculated based on the following equation (Harris, 2012):

$$\% \text{ recovery} = \frac{\text{Concentration of the spiked sample} - \text{Concentration of unspiked sample}}{\text{Concentration of added antibiotics in the spiked sample}}$$

Microbiological examination

(i) Sample collection: All collected samples (2320) except feed were tested for microbiological screening followed by detection of phenotypic and genotypic resistance pattern.

(ii)Transportation of sample: The samples for microbial analyses were brought to the microbiology laboratory of the different components by an insulated ice box with minimum delay (4-6 hrs.). For further investigation samples were processed as well as pre-enriched in selective medium.

(iii) Conventional culture method: Following pre-enrichment the samples were inoculated in different selective and non-selective bacteriological media (nutrient broth, nutrient agar, blood agar, EMB agar, XLD agar, MacConkey's agar, TCBS agar, MS agar, SS agar, chocolate agar, TSI agar etc.) for culture as well as to obtain pure isolate. The bacterial pathogen such as *E. coli*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Pasteurella*, *Corynebacterium* were isolated from livestock whilst *E. coli*, *Salmonella*, *Staphylococcus*, *Campylobacter*, *Pasteurella*, *Corynebacterium* were isolated from poultry sources sample.

(iv) Molecular detection: Chromosomal DNA was extracted from each pure culture by conventional boiling method. PCR was performed for confirmatory detection of each isolates. All PCR assays were adjusted in 25µL reaction mixture containing 2µL of DNA template, 12.5µL of 2x master mix (Go Taq Green Master Mix, Promega, USA), 0.5µL each of forward and reverse primers (10 pmol/µL), and remaining volume of nuclease free water. Amplicons were visualized after running at 100 V with 500 mA for 30 min in 1.5% agarose gel containing ethidium bromide. A 100-bp DNA ladder (Thermo Scientific, USA) was used as a size marker. The details of primers are given in **Table 4**.

Table 4: List of primers used for bacterial species identification

AMR Pathogens	Primers	Sequence (5′–3′)	Amplicon size (bp)	Reference
Salmonella spp.	F	TCATCGCACCGTCAAAGGAACC	284	Rahn et al., 1992
	R	GTGAAATTATCGCCACGTTCGGGCAA		
E. coli	F	CCCCCTGGACGAAGACTGAC	401	Ferasyi et al., 2020
	R	ACCGCTGGCAACAAAGGATA		
Staphylococcus spp.	F	CCTGAAACAAAGCATCCTAAAAA	155	Wang et al., 2019
	R	TAAATATACGCTAAGCCACGTCCAT		
Campylobacter jejuni	F	TGACGCTAGTGTTGTAGGAG	402	Wang et al., 1999
	R	CCATCATCGCTAAGTGCAAC		
Streptococcus spp.	F	AGCGGGGGAT AACTATTGGA	569	Shome et al., 2011
	R	TACGCATTTCACCGCTACAC		

Fig 2: Different steps for Polymerase Chain Reaction

Antimicrobial resistance pattern

(i) Determination of phenotypic resistance pattern: Antimicrobial susceptibility testing (AST) was made by the Kirby-Bauer disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2018). Briefly, two-three fresh colonies were suspended in 3 mL normal saline and the turbidity of the suspension was standardized to match with 0.5 McFarland standard (approximately 1.5×10^6CFU/mL). This bacterial inoculum was wiped over the surface of Mueller Hinton agar (MHA; Oxoid, UK) plate, onto which the antimicrobial disks were placed by using disk dispenser within 15 min. Plates were incubated for 24 hrs at 37°C prior to determination of results. The diameter of the zone of inhibition surrounding the disks was measured by conventional method and compared to the break points of CLSI. A number of 15 antimicrobials were used for AST such as penicillin (P, 10µg), ampicillin (AMP, 10µg), amoxicillin (AMC, 10µg), cloaxcillin (C, 10µg), ceftriaxone (CRO, 30µg), tetracycline (TE, 30µg), doxycycline (DO, 30µg) sulfamethoxazole-trimethoprim (SXT, 25µg), gentamicin (GN, 10µg), erythromycin (E, 10µg), ciprofloxacin (CIP, 5µg), streptomycin (S, 10µg), levofloxacin (LVX, 5µg), enrofloxacin (ENR, 5µg) and neomycin (NE, 30µg). The results of resistant, intermediate, or susceptible were interpreted according to the CLSI, 2018 and resistance pattern against at least three antibiotics was classified as multidrug resistant (MDR).

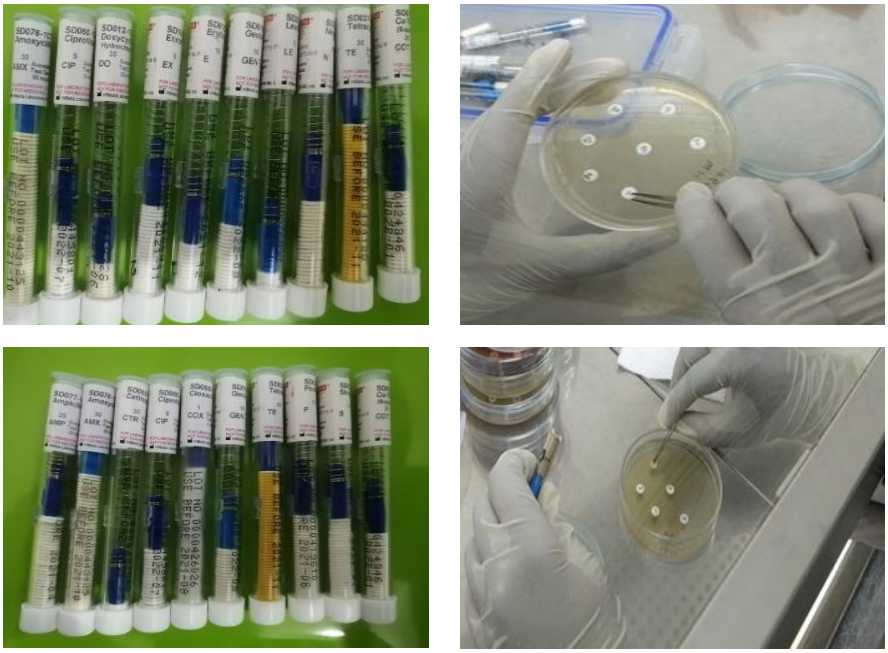

Fig 3: Antibiotic disk and placing of antibiotics on MH agar media

(ii) Determination of genotypic resistance pattern: *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Campylobacter* spp. isolates were screened by PCR for the detection of β-lactamase genes (blaTEM, blaSHV, and blaCMY), tetracycline resistant genes (tetA, tetB and tetC), sulfonamide resistant genes (sul1), streptomycin resistant gene (aadA1), gentamicin resistant gene (aac(3)-II), neomycin resistant gene (aph(3')-I), erythromycin resistant gene (ereA) and quinolone group resistant gene (gyrA). All PCR assays were adjusted in 25µL reaction mixture containing 2µL of DNA template, 12.5µL of 2x master mix (Go Taq Green Master Mix, Promega, USA), 0.5µL each of forward and reverse primers (10 pmol/µL), and remaining volume (µL) of nuclease free water. Uniplex PCR was performed in most of the resistance genes identification. Amplicons were visualized after running at 100 V with 500 mA for 30 min in 1.5% agarose gel containing ethidium bromide. A 100-bp DNA ladder (Thermo Scientific, USA) was used as a size marker. The list of primers to detect antibiotic resistance gene are given in **Table 5.**

Table 5: List of primers to detect antibiotic resistant genes

Class	Target Gene		Sequence (5′–3′)	Amplicon size (bp)	Reference
Gentamicin	*aac(3)-IV*	F	CTTCAGGATGGCAAGTTGGT	286	Van et al. 2008
		R	TCATCTCGTTCTCCGCTCAT		
Tetracycline	*tet*(A)	F	GGTTCACTCGAACGACGTCA	577	Randall et al. 2004;
		R	CTGTCCGACAAGTTGCATGA		
	tet(B)	F	CCTCAGCTTCTCAACGCGTG	634	
		R	GCACCTTGCTGATGACTCTT		
Beta lactams	bla_{TEM}	F	ATA AAA TTC TTG AAG AC	1076	Van et al., 2008
		R	TTA CCA ATG CTT AAT CA		
Beta lactams	bla_{SHV}	F	TCGCCTGTGTATTATCTCCC	768	Van et al., 2008
		R	CGCAGATAAATCACCACAATG		
Beta lactams	bla_{CMY}	F	TGGCCAGAACTGACAGGCAAA	462	Van et al., 2008
		R	TTTCTCCTGAACGTGGCTGGC		
Erythromycin	*ere(A)*	F	GCCGGTGCTCATGAACTTGAG	419	Van et al. 2008
		R	CGACTCTATTCGATCAGAGGC		
		R	GACCCCAAGTTTCCTGTAAGTG		
Sulfonamide	*sul1*	F	TTCGGCATTCTGAATCTCAC	822	Van et al., 2008
		R	ATGATCTAACCCTCGGTCTC		
Streptomycin	*aadA1*	F	TATCCAGCTAAGCGCGAACT	447	Van et al., 2008
		R	ATTTGCCGACTACCTTGGTC		
Methicillin-resistant	*mecA*	F	AAAATCGATGGTAAAGGTTGGC	533	Bühlmann et al., 2008
		R	AGTTCTGGAGTACCGGATTTGC		
		R	GTACTTTACGCCATGAACGT		

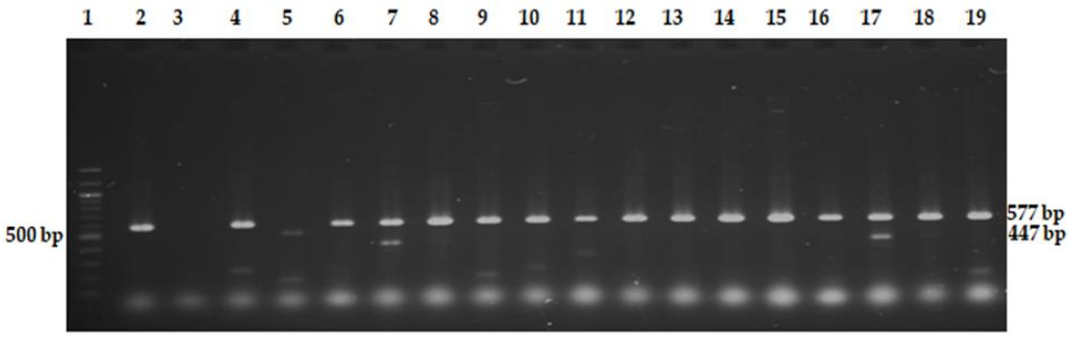

Fig 4: Results of PCR to determine the presence of tetA (tetracycline) and aadA1 (streptomycin) genes in phenotypically positive isolates. Lane 1: 100bp ladder, lane 2: positive control for tetA (577 bp), lane 3: negative control, lanes 4-19: different isolates of *E. coli*, *Salmonella* spp., *S. aureus* and *Streptococcus* spp.

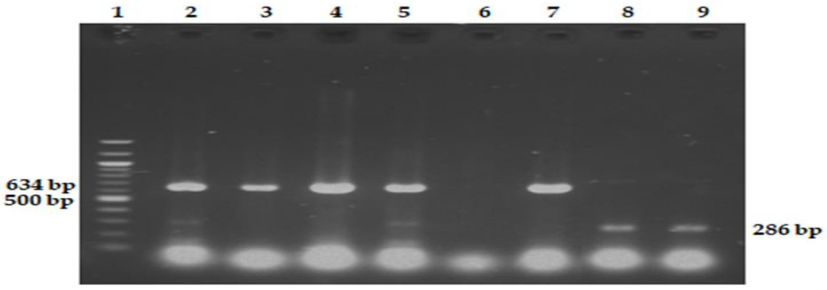

Fig 5: Results of PCR to determine tetB (tetracycline) and aac(3)-IV (gentamicin) genes in phenotypically positive isolates. Lane 1: 100bp ladder, lane 6: negative control, lane 7: positive control for tetB (634bp), lanes 1-5 and 8-9: different isolates of *E. coli*, *Salmonella* spp., *S. aureus* and *Streptococcus* spp.

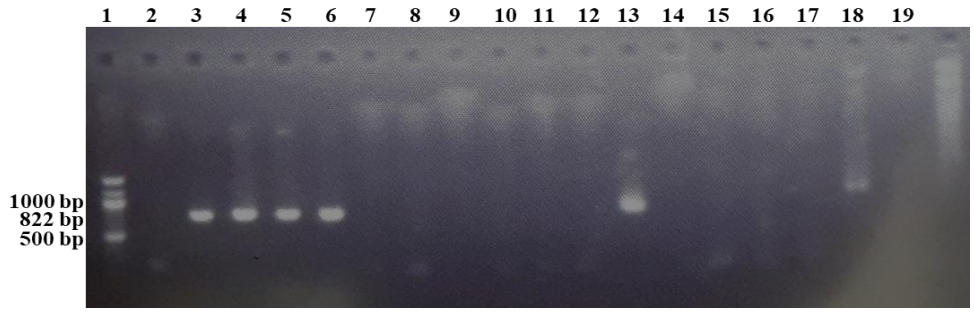

Fig 6: Amplification of sul1 (sulphonamide) gene in the phenotypically sulphonamide resistant isolates. Lane 1: 100bp DNA ladder; lanes 2-19: phenotypically resistant isolates.

Fig 7: PCR assay for the detection of the bla_{TEM} gene (964bp) in *E. coli*; Lane L: 1kb plus DNA ladder; Lane P: Positive control; Lane N: Negative control; Lane 1-7: bla_{TEM} gene

AMR Intervention Study

The experiment was carried out in the departments of Pharmacology and Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh-2202.All experimental procedures were performed according to the guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh [Approval number: AWEEC/BAU/2018(11)]. The comparative effects of antibiotic, phytobiotic, probiotic, and their combination on growth, production response, intestinal pH modification and gut bacterial population in broilers were studied. A total of 65-day old broiler chicks of either sex were collected from Kazi Farms and were used for this experiment. Five randomly selected day old chicks were sacrificed on first day for baseline bacterial analyses. The rest of the birds were then divided into five groups of having 12 birds in each group. The different groups are as follows- Group-A: standard diet, Group-B: standard diet with 0.1% antibiotic [Oxytetracycline hydrochloride, (Renamycin 100®), Renata Ltd. (Animal Health Division), Bangladesh], Group-C: standard diet with 0.1% phytobiotic [Galibiotic®, Square Pharmaceuticals Co. Ltd., Dhaka, Bangladesh], Group-D: standard diet with 0.15% probiotic [Bio-Top®, Pharma and Firm Co. Ltd., Dhaka, Bangladesh], and Group-E: standard diet with phytobiotic + probiotic 0.1 + 0.15%, respectively. Body weights were recorded and five birds from each group were sacrificed at 2nd and 4th weeks for pH measurements and bacteriological analysis. The total viable count (TVC), total coliform count (TCC), and total *Salmonella* count (TSC) of both feces and intestinal contents were recorded following the standard procedure (Ripon et al., 2019).

Alongside, another sets of animal experiment were done by using rat and mice whether they eliminate drug residues from their body with the following protocol. The animals were divided into five groups having 6 animals in each group for 7-day treatment period. The different groups are as follows; Group-A: control diet, Group-B: control diet with 0.1% antibiotic [Oxytetracycline hydrochloride, Oxy: (Renamycin 100®), Renata Ltd., Bangladesh], Group-C: control diet + antibiotic(Oxy) with 0.1% phytobiotic [Galibiotic®, Square Pharmaceuticals Co. Ltd., Dhaka,

Bangladesh], Group-D: control diet + antibiotic (Oxy) with 0.15% probiotic [Bio-Top®, Pharma and Firm Co. Ltd., Dhaka, Bangladesh], and Group-E: control diet + antibiotic (Oxy) with spirulina orally, 400 mg/kg body weight.

Statistical Analyses

MS Excel program was used for data entry and editing. The statistical analyses of the data were performed by using SPSS (Statistical Package for the Social Sciences) software version 20. Descriptive statistics such as frequency count and percentages, mean and standard deviation (SD) were used to sum up the data. Chi square ($\chi 2$) test was used to find the relationship between different categorical characteristics, such as the sociodemographic characteristics, knowledge, attitude and practice scores. P-values less than 0.05 ($p<0.05$) was considered statistically significant. Data analysis was performed using the R programming language (R-4.1.1 for Windows).

11. Results (with appropriate pictures)

Objective 1: To assess the antimicrobial drug residues in livestock and poultry food products and feed, and its mitigation using alternate approach

Baseline survey: A baseline survey was conducted to assess the knowledge, attitude and practices of professional veterinarian, informal prescribers, and farmers regarding use of antimicrobials as well as emergence of resistance in livestock and poultry value chain. A total number of 190 (100%) questionnaires for each component were interviewed by farmers, veterinarians and informal prescribers (drug/feed sellers) on knowledge, attitude and practices regarding antimicrobial usage and residue in food products and feeds. The salient findings of the questionnaire survey are presented below

Large animal farmer's knowledge, perception and practice: Most of the farm owners/caretaker were illiterate (27.2%) followed by higher secondary/diploma certificate holder (19.3%) and few of them were master's degree holder (2.7%). Regarding large animal farmers/ owners, 97.3% known about antibiotics; 65.3% heard about antibiotic resistance; 58% had biosecurity knowledge,

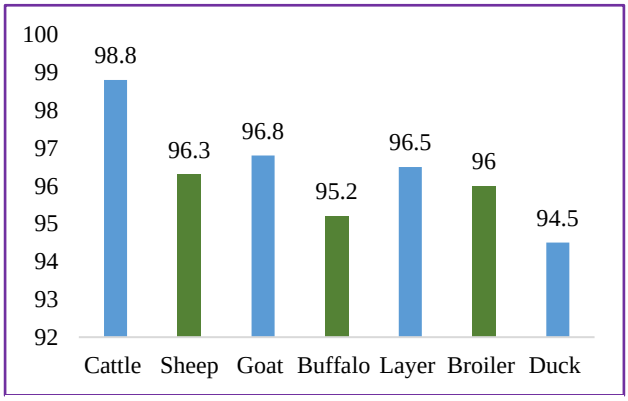

Fig 8: Knowledge of farmers about antibiotics

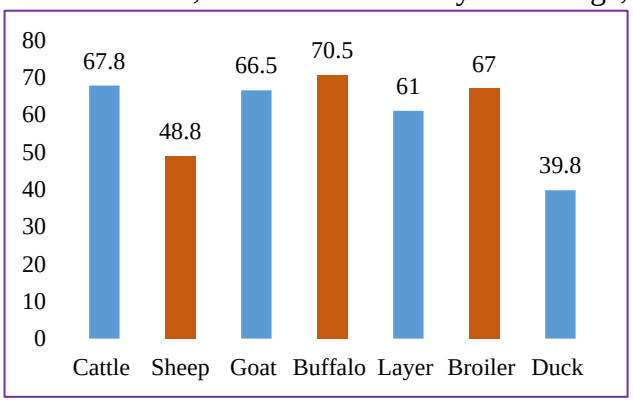

Fig 9: Knowledge of farmers about antibiotic residue

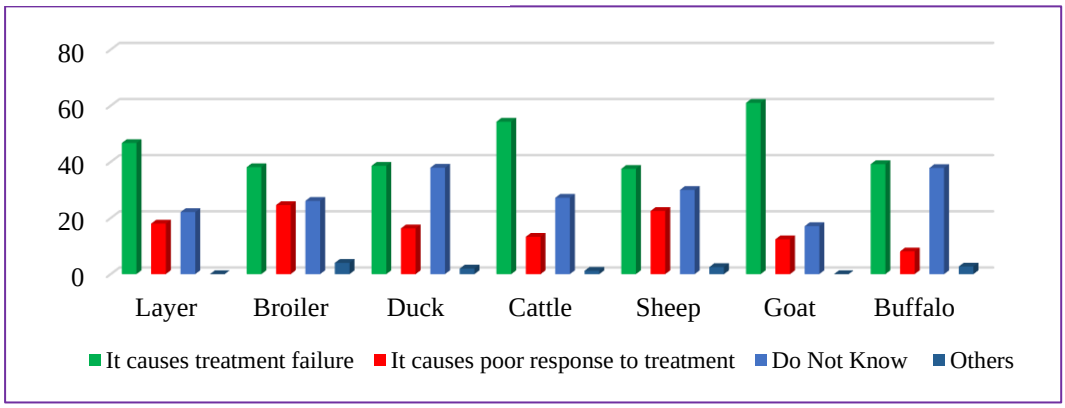

Fig 10: Perception of farmers about antibiotic resistance

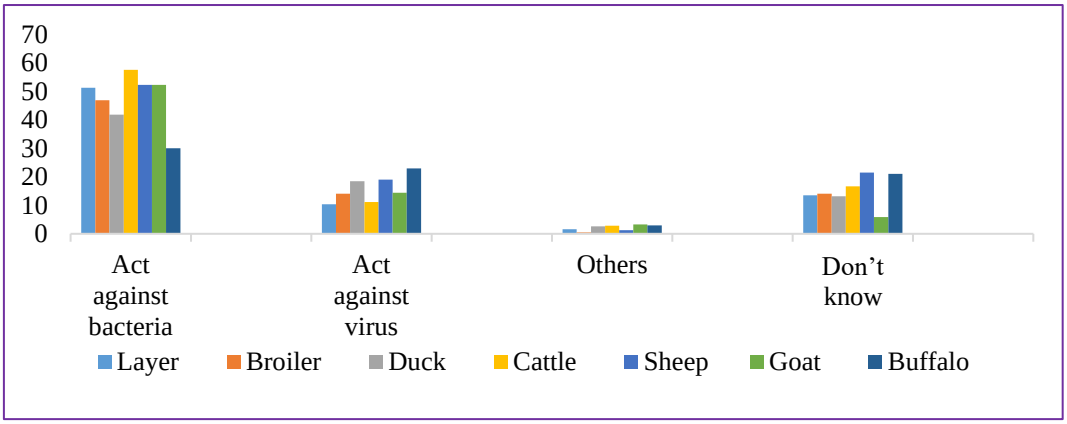

Fig 11: Perception of farmers about function of the antibiotics

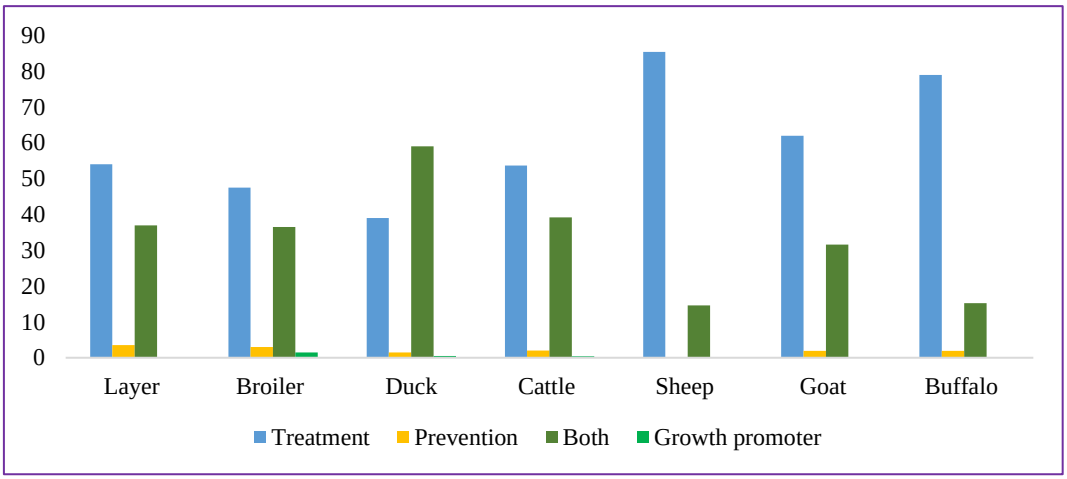

Fig 12: Purposes of uses of antibiotics in farm practices

77.2% consulted with veterinary practitioner for treatment of sick animals; 51.5% believed antibiotic resistance triggered treatment failure; 44% heard about antibiotic residue; 81.2% didn't have record the use of antibiotics; 64.7% used antibiotics for therapeutic purposes; 68.7% didn't have knowledge on antibiotic withdrawal period; 52.2% had knowledge on shelf life of antibiotics; most of the farmers used penicillin, oxytetracycline, streptomycin, gentamicin, ciprofloxacin, ceftriaxone and sulphur drugs in the treatment of animals. A significant association was found among the education of the large animal farmers and some knowledge parameters along with practices of the large animal farmers, i.e. cattle, sheep, goat and buffalo farmers (p >0.001).

***Poultry farmer's knowledge, perception and practice*:** Most of the poultry farm owners/caretaker were illiterate (19%) followed by secondary certificate holder (18.5%) and few of them were master's degree holder (5%). Regarding knowledge, perception and practices, highest number (27.5%) of poultry farms had population size within 1001-2000; 62.5% had knowledge on biosecurity; 95.7% heard about antibiotics; 68.2% heard about antimicrobial resistance, 52.2% known about antibiotic residue; 82% didn't have record on antibiotic use; 30.3% farm used antibiotic for preventive purposes; 19.8% known about withdrawal period of antibiotics; 69.7% went to consult with veterinarian for treatment purposes; 50% known about shelf life of antibiotics; most commonly used antibiotics were ciprofloxacin, enrofloxacin, levofloxacin, oxytetracycline, amoxicillin, sulphur drugs and tylosin. A significant association was found among the many parameters of knowledge and practices of poultry farmers, i.e. layer, broiler and duck farmer's knowledge and practices (p >0.001). A higher association was found among the biosecurity knowledge of the livestock and poultry farmers (p >0.001). Similarly, higher association was also observed about the cleaning of feeders, waterers, feces, floor and litter among the livestock and poultry farmers (p >0.001). The knowledge and practices of farmers are presented in different figures as shown below

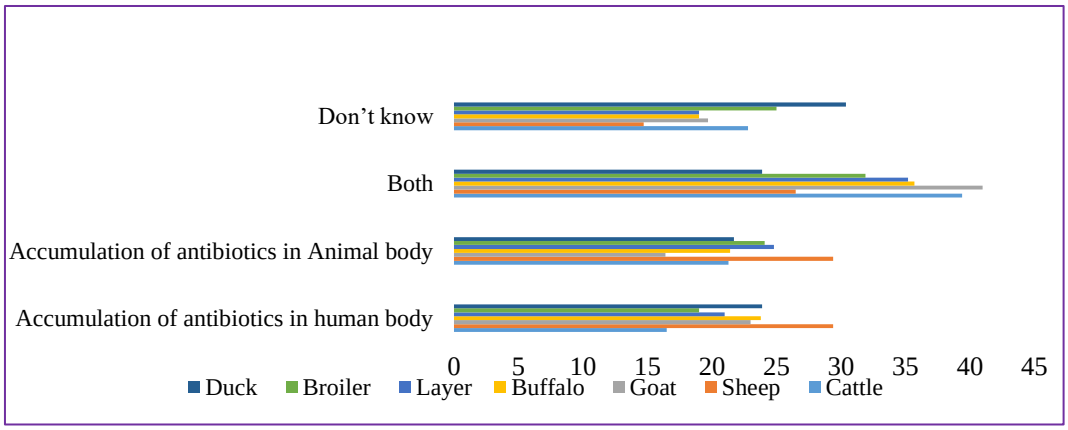

Fig 13: Perception of farmers about antibiotic residue

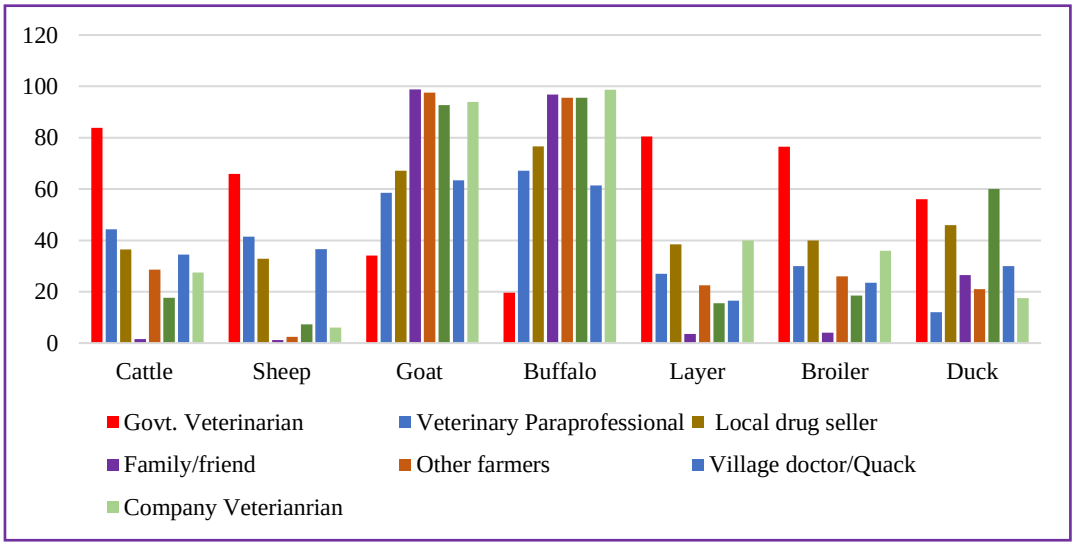

Fig 14: From whom usually you get suggestions or treatments for your sick animals

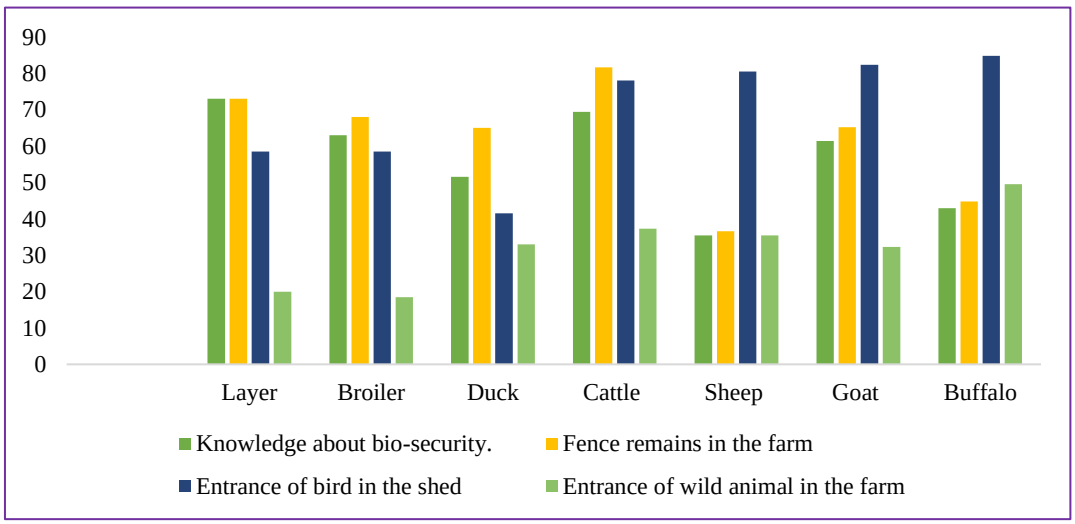

Fig 15: Biosecurity ststus of the livestock and poultry farms

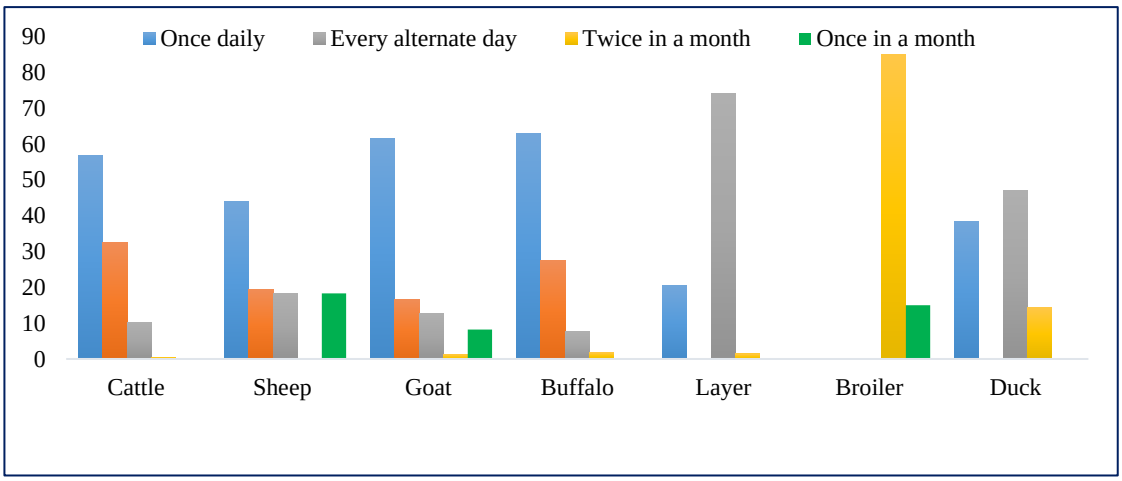

Fig 16: Farm practices (cleaning of feces, floor and litters)

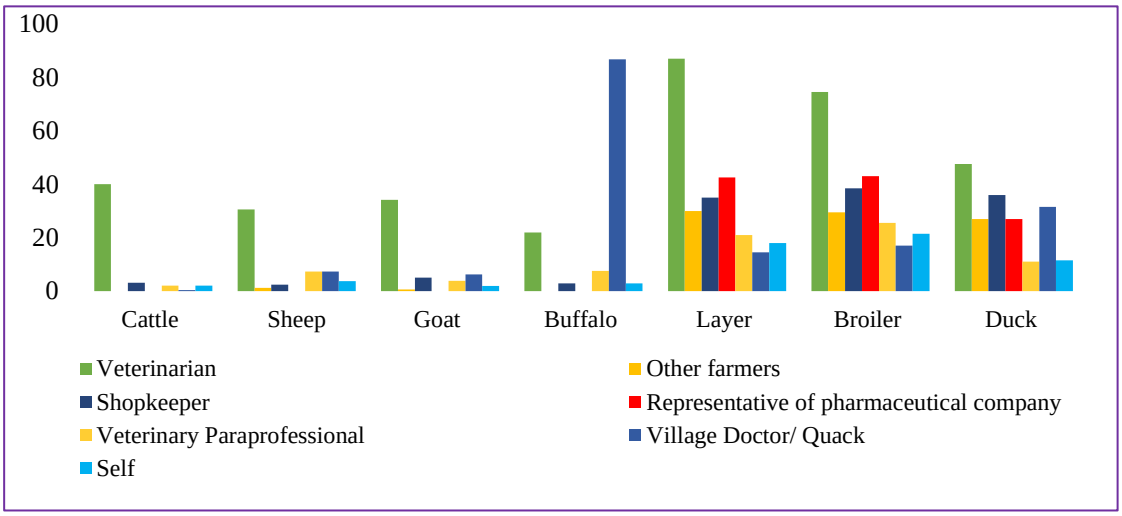

Fig 17: Use of antibiotics recommended by

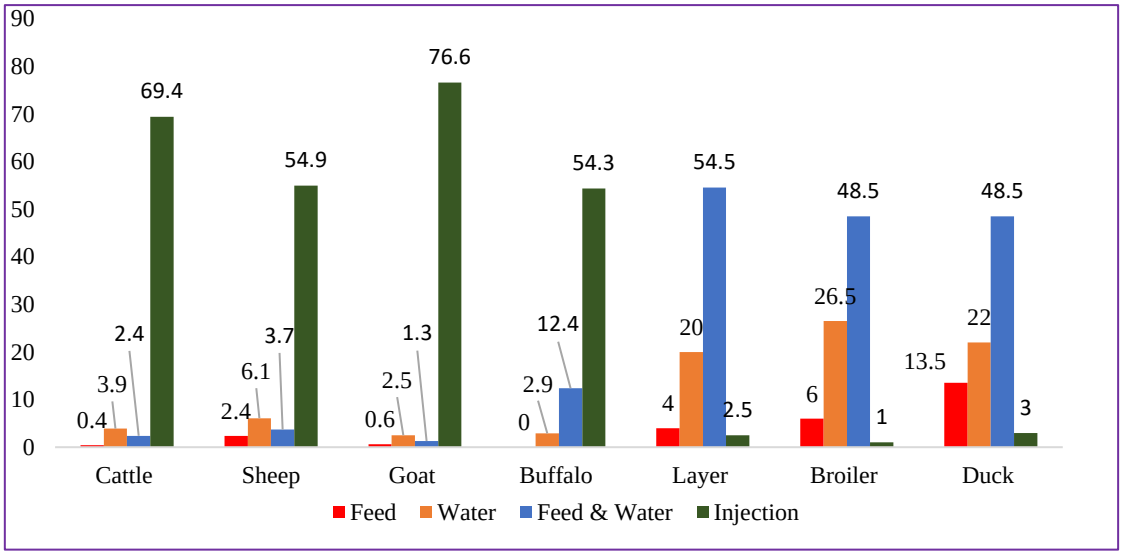

Fig 18: Routes of the uses of antibiotics

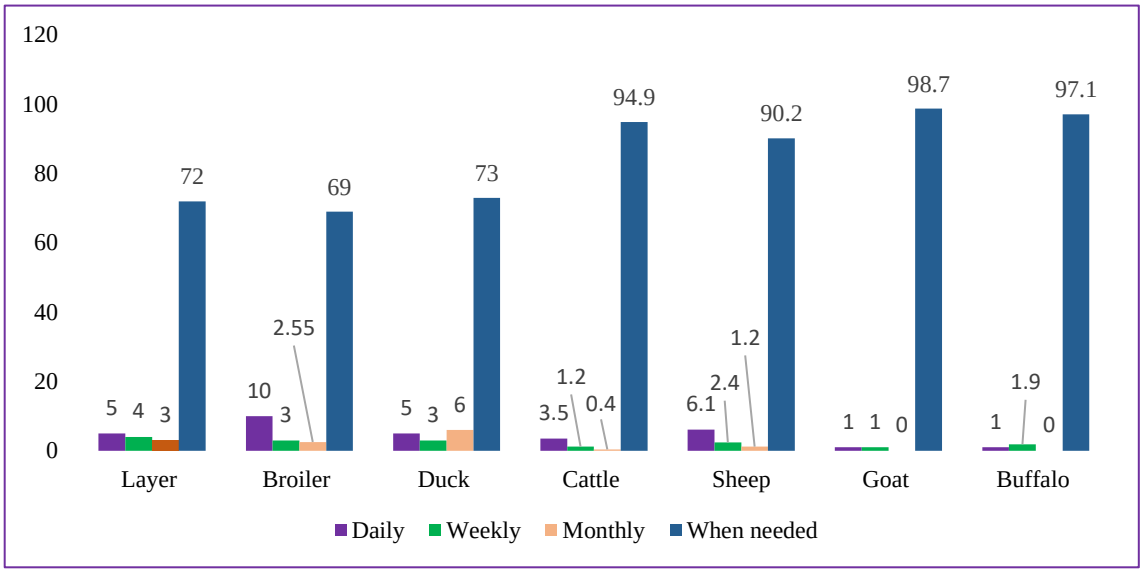

Fig 19: Frequency of the uses of antibiotics

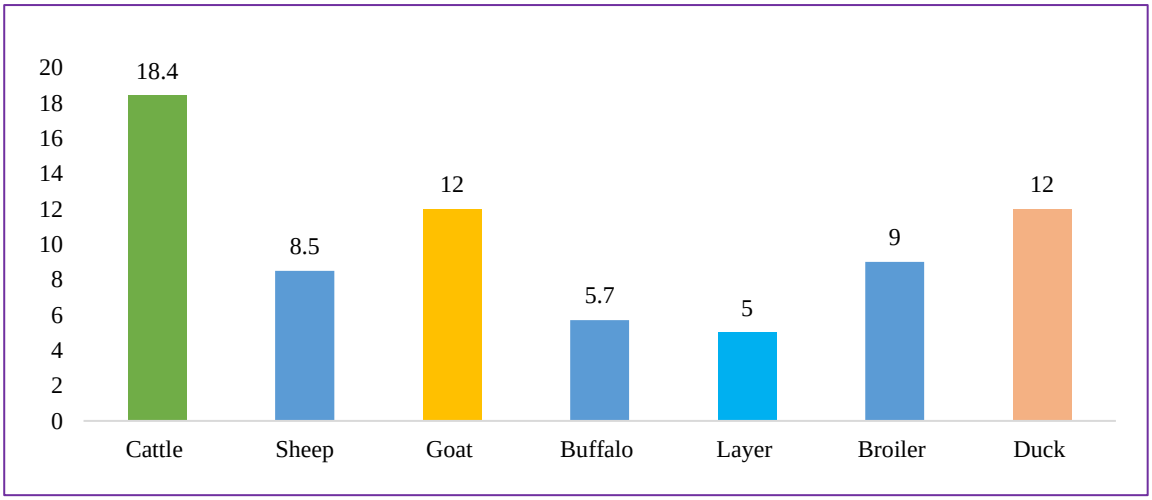

Fig 20: Withdrawal period followed by the farmers

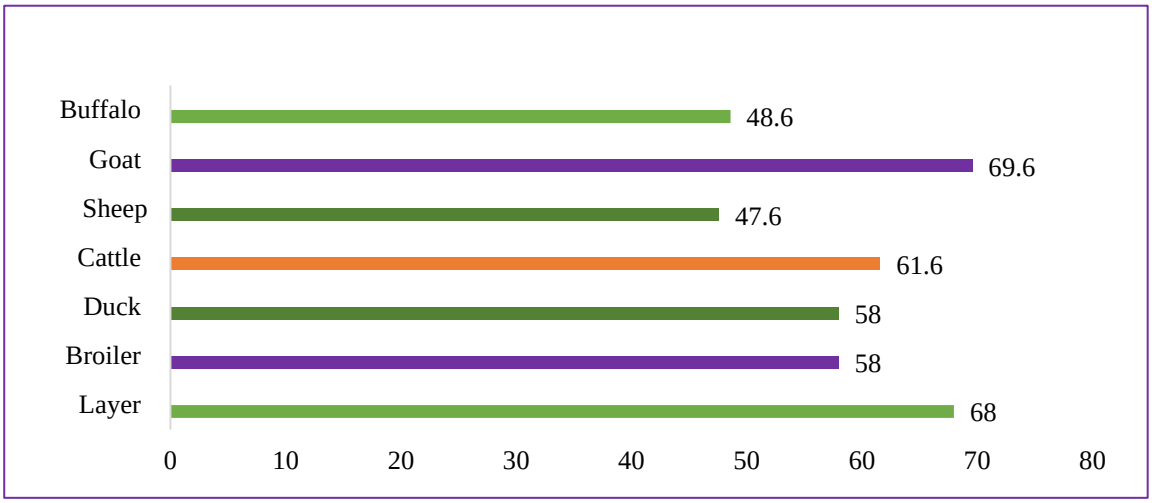

Fig 21: Completion of antibiotic doses

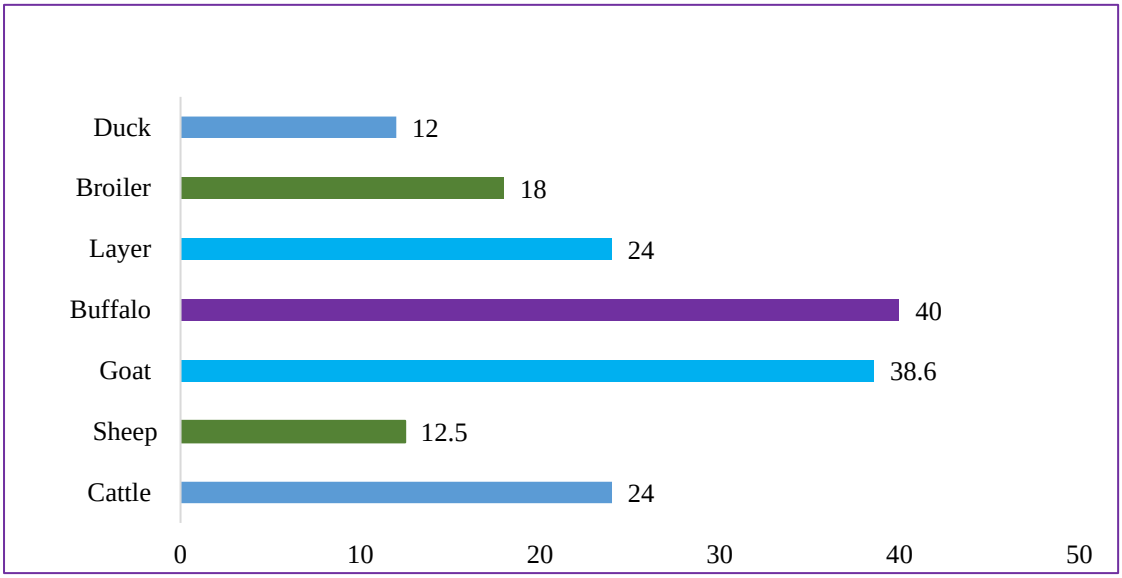

Fig 22: Record keeping of the uses of antibiotics

Informal prescriber's knowledge, perception and practice: Most of the (48.8%) informal prescriber aged between 30-40 years old; most of them (37.5%) were higher secondary/ diploma certificate holder followed by secondary holders (25.6%) and few of them were master's degree holders (8.1%). Regarding knowledge, perception and practice, 83.1% informal prescriber known about antimicrobial resistance; 73.1% believed antimicrobial resistance occurred due to incomplete course of antibiotics; 55.6% had no knowledge on antibiotic residue; 58.1% had no knowledge on withdrawal period of antibiotics; 72.5% believed antibiotics can work against viral diseases, 51.2% chosen antibiotics with dose on its own experience; they prescribed mostly penicillin, streptomycin, gentamicin, oxytetracycline, ciprofloxacin and ceftriaxone in the treatment of large animal diseases; in poultry treatment they prescribed mostly doxycycline, oxytetracycline, ciprofloxacin, enrofloxacin and levofloxacin.

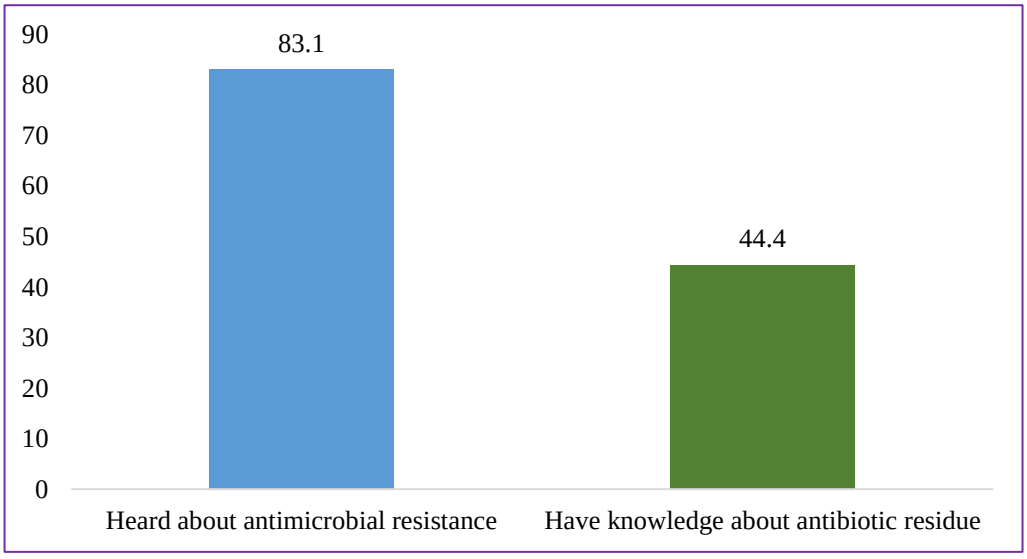

Fig 23: Knowledge about antibiotic resistance and residue

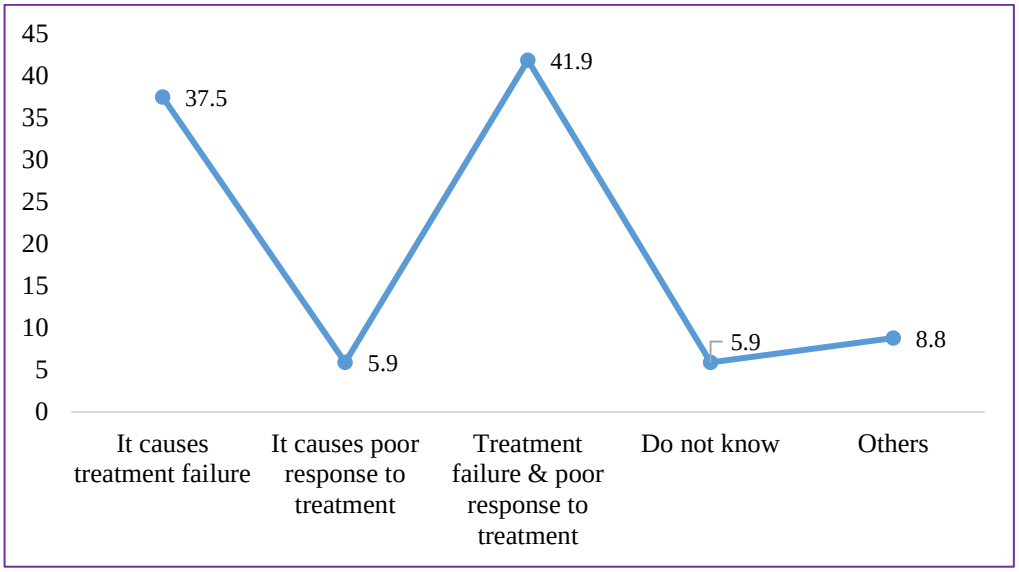

Fig 24: Knowledge about antibiotic resistance

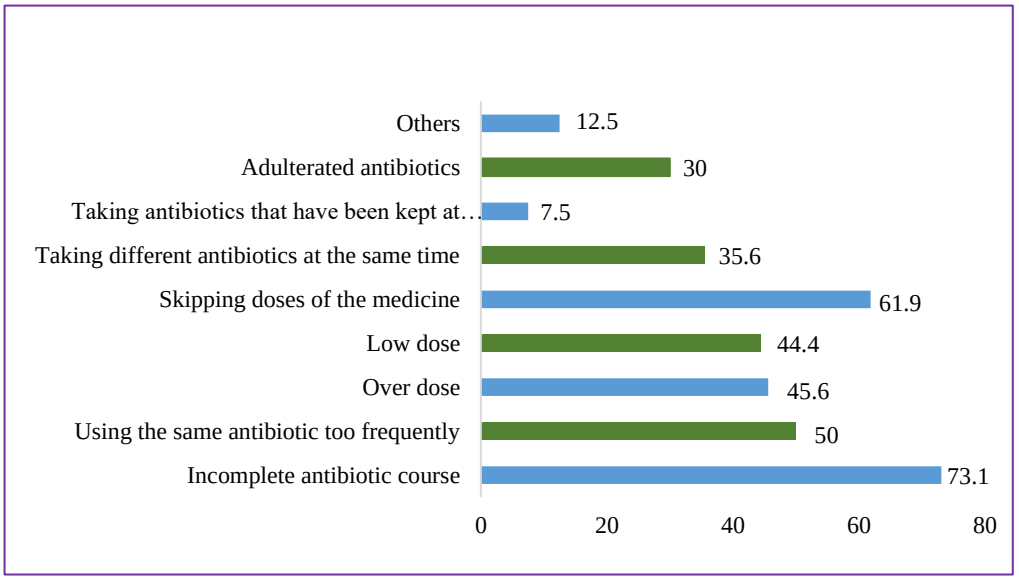

Fig 25: Knowledge about causes of antibiotic resistance

Veterinarian knowledge, perception and practice: Most of the veterinarian (42.5%) were aged between 30-40 years old; most of them awarded MS degree (66.9%) with basic DVM and few of them (2.5%) had PhD degree. All veterinarian had knowledge on antimicrobial resistance (100%) and antimicrobial residue (100%). Veterinarian believed incomplete dose (70.6%); frequent use of same antibiotics (64.4%); skipping doses of antibiotics (60%) are the main drivers of the emergence of antimicrobial resistance. Similarly, most of the veterinarian (44.4%) believed antibiotic residue is the metabolized antibiotic; 73.8% perceived about WHO classification of antibiotics "Aware"; few of them had knowledge on the uses of reserve type of antibiotics in livestock and poultry production; 63.8% used single antibiotic at a time; 97.5% asked to farmers prior prescribing antibiotics; 90% instructed to farmers about the course and doses of the antibiotics. In addition, they prescribed mostly penicillin (64.4%), oxy-tetracycline (70%), streptomycin (57.5%), gentamicin (75%), ciprofloxacin (61.3%) and ceftriaxone (54.4%) in livestock; most common prescribed antibiotics were doxycycline (72.5%), oxy-tetracycline (68.8%), ciprofloxacin (63.1%), enrofloxacin (63.8%) and levofloxacin (48.8%) in poultry production.

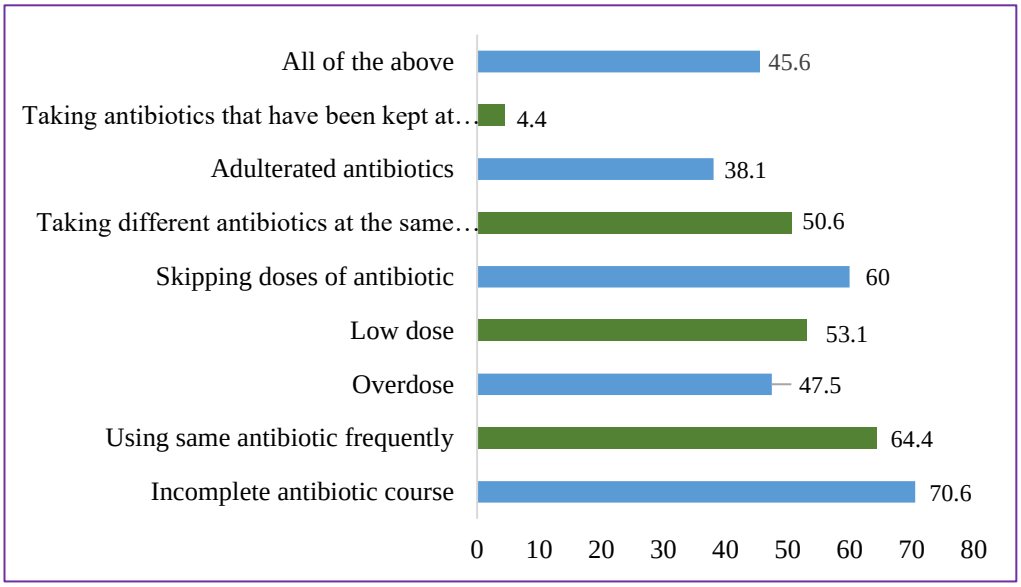

Fig 26: Knowledge about causes of antibiotic resistance

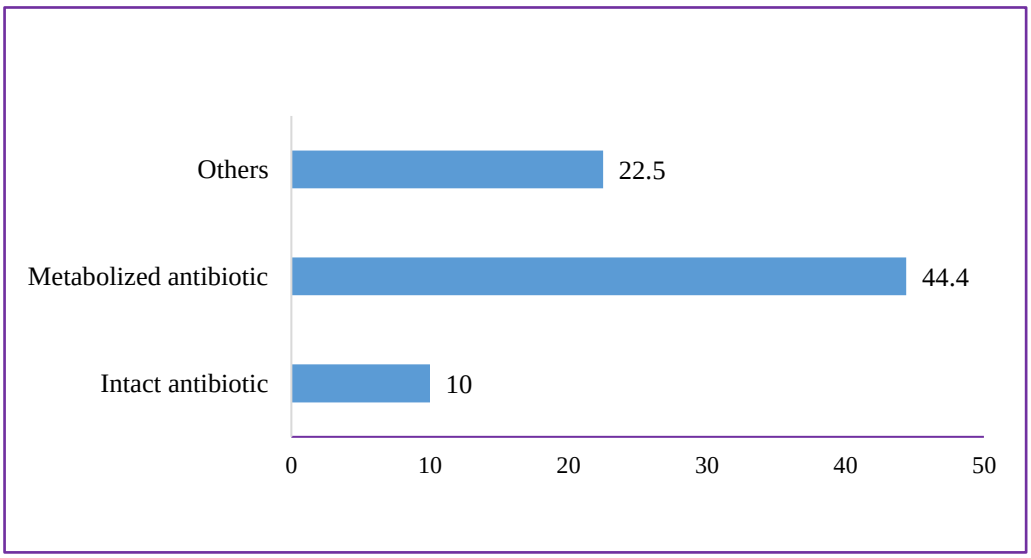

Fig 27: Knowledge about the deposition of antibiotic residue in animal body

Determination of antibiotic residue in animal food and feed samples

Qualitative assay for antimicrobial residue screening by TLC

A total number of 2335 samples were analyzed by Thin Layer Chromatography (TLC) method for the qualitative screening of nine antibiotics those are commonly used in livestock and poultry production system in the country. The samples were consisting of broiler meat (n=160), layer meat (n=160), cattle meat (n=160), goat meat (n=160), buffalo meat (n=70), cattle milk (n=200), buffalo milk (n=110), goat milk (n=200), eggs (n=240) and poultry feed (n=875) samples. The overall prevalence of antibiotic residue positive sample was found to be 8.9% (208/2335) through qualitative assay. **Table 6** summarizes the detailed prevalence of antibiotic residues in animal-derived food samples screened by TLC method.

Table 6: Prevalence of antimicrobial residue in animal-derived food and feed samples

Type of samples		No. of samples	No. of positive samples	Prevalence (%)	Average Prevalence (%)	Confidence interval (95% C.L)
Meat	Broiler	160	25	15.62	10.17	10.81-22.05
	Layer	160	20	12.5		8.24-18.52
	Cattle	160	16	10		6.25-15.63
	Goat	160	9	5.62		2.99-10.34
	Buffalo	70	5	7.14		3.09-15.66
Milk	Cattle	200	19	9.5	6.65	6.17-14.36
	Goat	200	10	5		2.74-8.96
	Buffalo	110	6	5.45		2.52-11.39
Egg	Poultry	240	9	3.75	3.75	1.99-6.97
Feed	Poultry	875	89	10.17	10.17	8.34-12.35
Total		2335	208	8.9		

Quantitative assay for antimicrobial residue screening by UHPLC

The quantitative screening was done by ultra high performance liquid chromatography (UHPLC) method for the determination of antibiotic residue concentration in animal-derived food products and poultry feeds. There nine antibiotics were screened such as P, AMC, OTC, SXT, CRO, CIP, CN, AMP and S. The meat samples contained P, AMC, OTC, SXT, CRO, CIP, AMP DOX but no P and CN residues were found in the meat samples. Similarly, the milk samples contained eight types of residues such as P, AMC, OTC, SXT, CRO, CIP, CN and AMP. Likewise, the egg samples had five types of antibiotic residues such as AMC, OTC, CRO, AMP and DOX. Further, feed samples contained five types of the antibiotics such as OTC, CIP, SXT, CN and DOX.

Average concentration of the antimicrobial residues in animal foods and feeds

Among animal-derived food commodities along with feed samples, the average concentration of P was found in cattle milk (32.19 µg/l) while average highest concentration of AMC was found in cattle meat (87.894 µg/kg). The average highest concentration of OTC was found in cattle milk (149.08 µg/l) whilst lowest average concentration was found in goat meat (3.48 µg/kg). The highest average concentration of SXT was found in cattle milk (121.63 whereas lowest average concentration was found in feed (0.50 µg/kg). The highest concentration of CRO was found in cattle meat (12.49 µg/kg) although lowest average concentration was found in egg (0.70 µg/kg). The highest average concentration of CIP was found in cattle milk (51.69 µg/l) though lowest average concentration was found in goat milk (35.16 µg/l). The highest average concentration of CN was found in cattle milk (93.68 µg/l) whilst lowest average concentration was found in buffalo milk (10.65 µg/l). The highest average concentration of AMP was found in goat meat (58.97 µg/kg) through lowest average concentration was found in goat milk (4.66 µg/l). The highest concentration of DOX was found in broiler meat (172.195 µg/kg) although lowest average concentration was found in egg (6.2 µg/kg). The average concentration of antibiotic residues in animal food and feeds are presented in **Table 7**.

Table 7: Average concentration of antibiotics in animal food and feeds (in µg/kg, µg/l)

Type of samples		Average concentration of antimicrobial residue (in µg/kg, µg/l)								
		P	AMC	OTC	SXT	CRO	CIP	CN	AMP	DOX
Meat	Broiler	-	9.30	62.00	-	0.42	45.14	-	-	172.195
	Layer	-	51.41	45.01	-	-	-	-	51.86	-
	Cattle	-	87.894	68.70	5.77	12.49	-	-	55.44	-
	Goat	-	56.56	3.48	90.64	0.45	-	-	58.97	-
	Buffalo	-	-	-	-	-	-	-	-	-
Milk	Cattle	32.19	3.59	149.08	121.63	11.805	51.69	93.68	32.44	-
	Goat	-	-	5.26	-	0.84	35.16	-	4.66	-
	Buffalo	-	26.8	-	-	-	-	10.65	42.87	-
Egg	Poultry	-	0.79	83.283	-	0.70	-	-	38.84	6.2
Feed	Poultry	-	-	26.84	0.50	-	37.14	31.05	-	45.75

Maximum residue limit (MRL) of antibiotics in meat samples

The highest concentration of AMC, OTC, SXT, CRO, AMP, CIP, DOX and S was found to be 125.77, 278.96, 5.772, 24.33, 59.24, 89.54, 521.12 and 151.33 µg/kg in meat samples. Out of 710 meat samples, 11 samples were found to be above the MRL level of AMC residue (1.55%). Similarly, the MRL of OTC exceeded in two meat samples out of 71 samples (0.28%). Similarly, the MRL of AMP was found in four meat samples (0.56%). The MRL value of SXT, CRO, CIP and DOX was not calculated due to lack standard reference value in CAC. The MRL value of different antibiotics in meat samples are presented in **Table 8.**

Table 8: Maximum residue level (MRL) value of different antibiotics in meat samples

Antibiotics	No. of positive samples	Maximum concentration (µg/kg)	Minimum concentration (µg/kg)	Mean ±SD (µg/kg)	MRL value[1] (µg/kg)	No. of samples above MRL value
AMC	15	125.77	1.09	53.71±35.52	50	11
OTC	23	278.96	0.147	57.04±96.89	200	2
SXT	2	5.772	90.64	48.20±60.01	-	-
CRO	5	24.33	0.425	5.26±10.65	-	-
AMP	4	59.24	51.64	55.43±4.24	50	4
CIP	6	89.54	0.29	45.14±35.84	-	-
DOX	4	521.12	12.73	172.19±236.11	-	-
S	3	151.33	42.9	85.19±58.01	600	-

[1]codex alimentarius commission

Maximum residue level of different antibiotics in milk samples

There eight antibiotics were found to be positive in milk samples with different concentrations. The maximum concentration of AMC, OTC, SXT, CRO, CIP, CN, AMP and P was found to be 26.8, 149.08, 121.63, 20.61, 174.04, 250.65, 86.53 and 64.33 µg/l in milk samples. Out of 510, two milk samples were found to be above the MRL of AMC (0.4%). The antibiotic residues of OTC, SXT, CIP, CN and AMP were found to be above the MRL value respectively in one, one, one, two and seven samples. The MRL value of P and CRO was not measured due to lack of standard reference value in CAC. The MRL value of different antibiotic residues are presented in **Table 9**.

Table 9: Maximum residue level (MRL) value of different antibiotics in milk samples

Antibiotics	No. of positive samples	Maximum concentration (µg/l)	Minimum concentration (µg/l)	Mean ± SD (µg/l)	MRL value[1] (µg/l)	No. of samples above MRL value
AMC	3	26.8	3.59	13.46±11.98	4	2
OTC	3	149.08	0.173	53.20±83.18	100	1
SXT	1	121.63	-	121.63±0	25	1
CRO	2	20.61	0.735	6.32±9.57	-	-
CIP	7	174.04	2.89	44.61±65.40	100	1
CN	5	250.65	4.25	81.82±109.64	200	2
AMP	9	86.53	3	30.51±28.29	4	7
P	2	64.33	0.59	32.195±28.47	-	-

[1]codex alimentarius commission

Maximum residue level of antibiotics in egg samples

Four antibiotic residues (CRO, AMP, AMC and OTC) were found in the egg samples. The maximum concentration of CRO, AMP, AMC and OTC in egg samples were found to be 1.69, 38.84, 1.02 and 462.06 µg/kg respectively. The MRL of OTC was found in a single egg sample while the MRL value of other antibiotic residues were not determined due to lack of standard reference value in CAC. The MRL value of different antibiotics in egg samples are presented in **Table 10**.

Table 10: Maximum residue level (MRL) value of different antibiotics in egg samples

Antibiotics	No. of positive samples	Maximum concentration (µg/kg)	Minimum concentration (µg/kg)	Mean ± SD (µg/kg)	MRL value[1] (µg/kg)	No. of samples above MRL value
CRO	11	1.69	0.39	0.70±0.34	-	-
AMP	1	38.84	-	38.8471005	-	-
AMC	2	1.02	0.56	0.79±0	-	-
OTC	20	462.06	0.78	83.28±142.71	400	1

[1]codex alimentarius commission

Maximum residue level of antimicrobial residue in feed samples

Five antibiotic residues were found in poultry feed samples (OTC, DOX, SXT, CIP and CN). The maximum concentration of OTC, DOX, SXT, CIP and CN were found to be 214.52, 126.91, 0.862, 184.74 and 31.051 µg/kg respectively. The MRL value of the antibiotic residue in feed samples were not determined as there was so far no standard available for the animal feed samples in CAC. The concentration of different antibiotic residues in feed samples are presented in **Table 11**.

Table 11: Maximum residue level (MRL) value of different antimicrobials in feed samples

Antibiotics	No. of positive samples	Maximum concentration (µg/kg)	Minimum concentration (µg/kg)	Mean ± SD (µg/kg)	MRL value (µg/kg)	No. of samples above MRL value
OTC	30	214.52	0.028	26.84±58.22	Unknown	-
DOX	3	126.91	0.5	45.75±70.439	Unknown	
SXT	6	0.862	0.142	0.50±0.281	Unknown	-
CIP	25	184.74	5.8	37.14±36.77	Unknown	-
CN	1	31.051	-	-	Unknown	-

Linearity index of antibiotic residues among the meat, milk and egg samples

Among the overall samples, the residue of CRO has increased linearly and steady in meat and milk samples. Similarly, the residue of CIP has increased linearly with greater concentration in feed and milk samples. Continually, the AMP residue increased linearly and steady in milk samples. Correspondingly, the AMC residue increased linearly and consistently in meat samples. Likewise, the OTC residue increased linearly and steady in egg samples. Otherwise, none of the residues were increased linearly, steady and consistently. The linearly index projected the intensity and severity of the problem. The linear regression analysis of different concentration of antibiotic residues are presented in **Figure 28.**

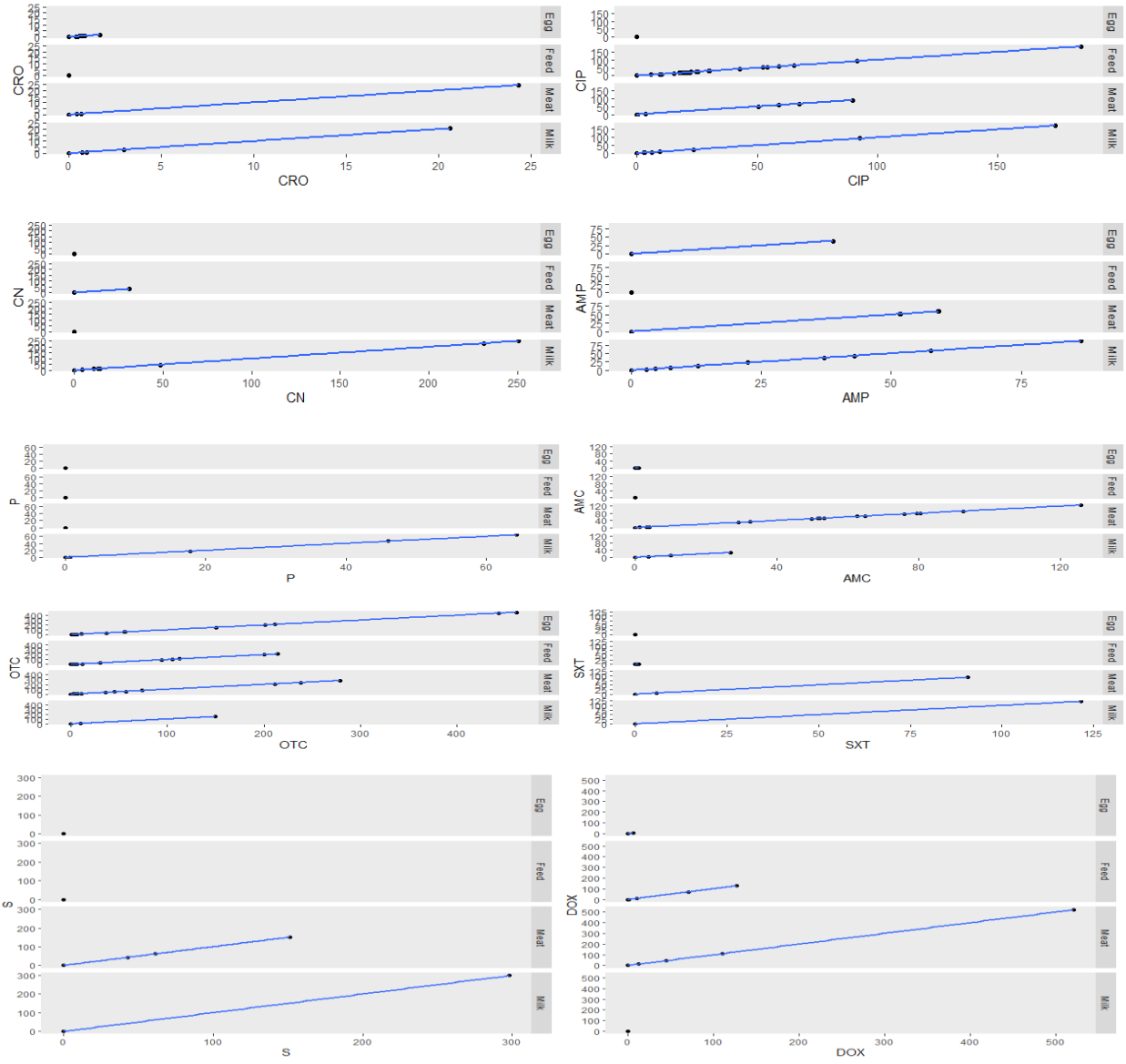

Fig 28: Linear regression analysis of different concentration of antibiotic residues

Alternate approach study to mitigate antimicrobial drugs residue and resistance

On day 7, the average body weights in different groups of birds were more or less similar. On day 14, the highest body weight was observed in Group E (Phytobiotic + Probiotic) followed by Group D (Probiotic), but on day 28 group D (probiotic) was highest. Body weight increased significantly ($P<0.05$) in all treatment groups (C, D and E) compared to control (A) group by the end of the experiment (**Table 12, Figure 29**). Regarding initial body weight, there was no significant

difference among the dietary groups. At the end of 28 days of age, the highest live weight was (1095±6.03 g/bird) found in broilers of group D (probiotic). This was followed by broilers belonging to group E (1065.0±18.93 g/bird) and group C (780±27.79 g/bird), respectively (**Table 12, Figure 29**). Another disparity was also seen with group D having the lowest feed conversion ratio at the end of the experiment.

Table 12: Production performance of the broilers supplemented with phytobiotic, probiotic and their combination (0-28 days)

Parameters	Treatment groups				
	A (Control)	B (Antibiotic)	C (Phytobiotic)	D (Probiotic)	E (Phyto+Probi)
ILW (g)	41.25±1.25	41.75±1.79	41±1.87	42±1.58	40.50±2.21
FLW (g)	665 ± 4.16	686 ± 2.08	780 ± 27.79	1095± 6.03	1065.0±18.93
LWG (g)	623.75±2.91	644.25±1.39	739±25.79	1053±4.90	1024.50±21.25
FI(g)	1310	1263	1352	1812	1793
FCR	2.1	1.96	1.83	1.72	1.75

Data are express as mean±SE. ILW; Initial live weight. FLW; Final live weight. LWG; Live weight gain. FI; Feed intake. FCR; Feed conversion ratio.

Surprisingly the combination group (E) had the highest weight gain regarding visceral organs (Table-33). This is clearly different from the final live weight gain where group D had the highest with the lowest feed conversion ratio. The probiotic group however came second to the combination group regarding visceral organ weight.

Table 13: Relative organ weight of broiler supplemented with phytobiotic, probiotic and their combination (0-28 days)

Groups	Liver (g)	Gizzard (g)	Heart (g)	Proventriculus (g)
A (Control)	27 ±1.48	21.20±0.75	4.40±0.60	6.40±0.50
B (Antibiotic)	25.80±0.86	20.27±1.39	4.60±0.50	5.60±0.50
C (Phytobiotic)	25 ±1.01	20.20±0.87	5±0.70	6.80±0.37
D (Probiotic)	40.20 ±0.37	23.20±0.86	6.20±0.58	7.20±0.58
E (Phyto+Probi)	41±0.71	25±0.95	7.10±0.54	7.80±0.58

Data are express as mean±SE, (n=10) *p< 0.001 of treatment groups vs control

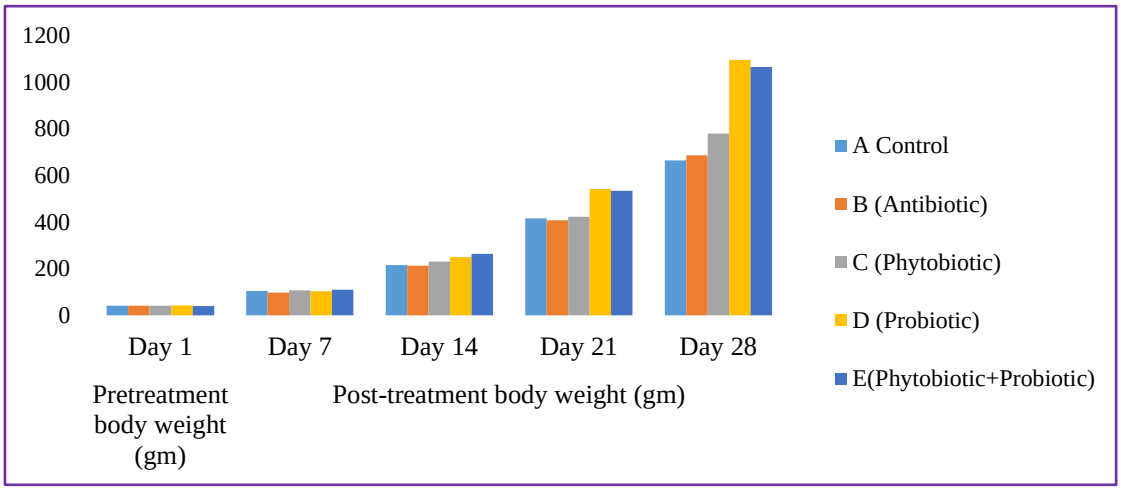

Fig 29: Body weight at different age

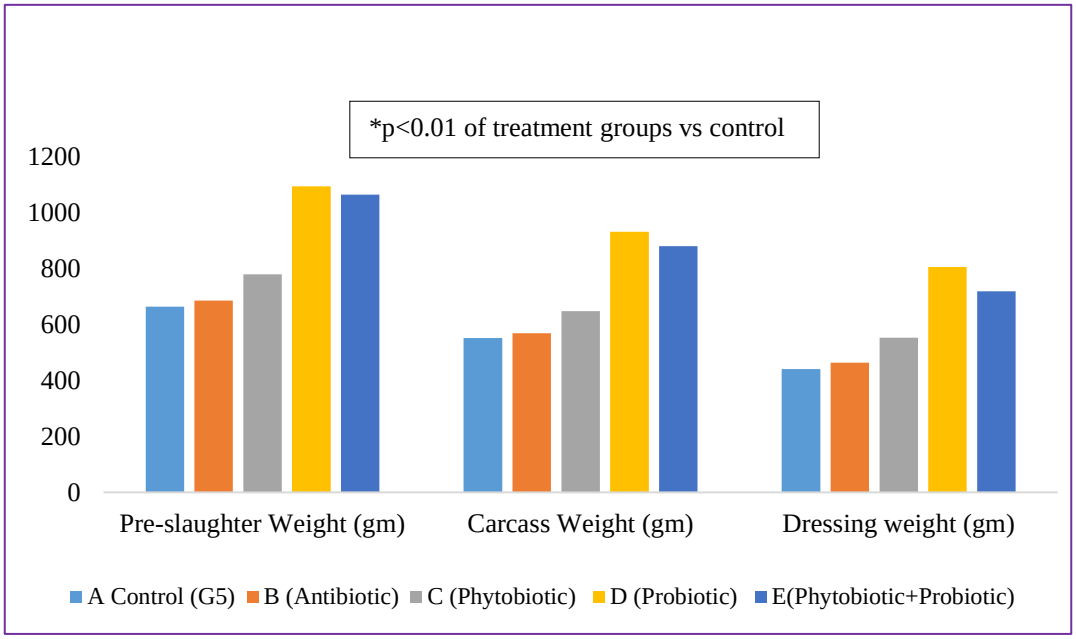

Fig 30: Production performance

As

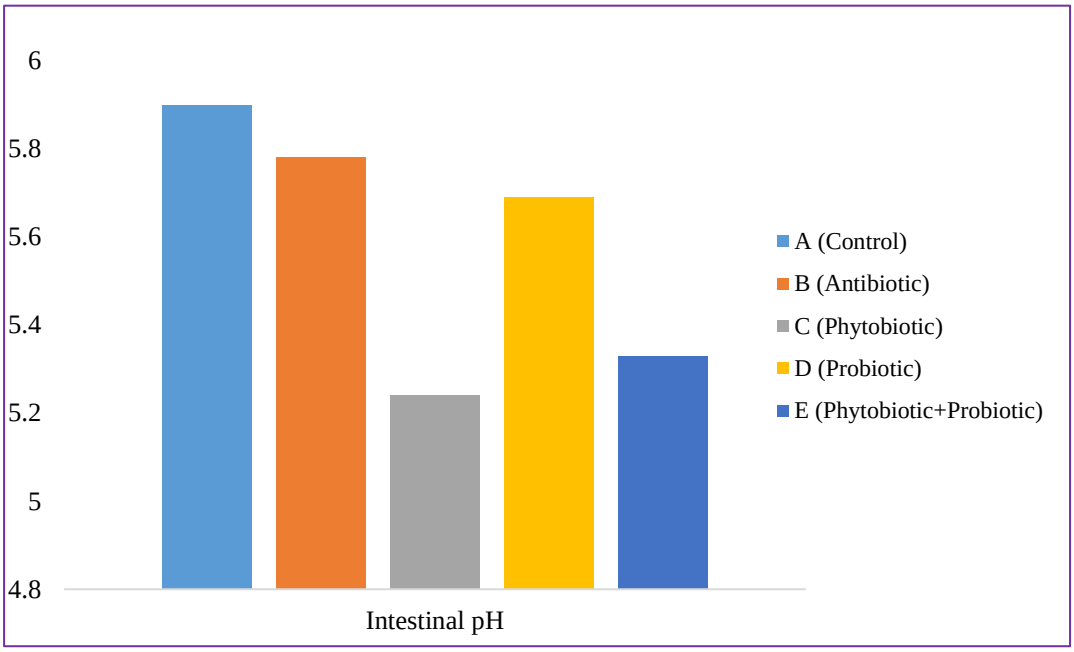

Fig 31: Intestinal pH at 28th day

shown in **Figure-10**, the pH of all the treatment groups was significantly decreased compared to control group ($p<0.05$). However, on day 28, we observed that intestinal pH in group C was significantly ($p<0.05$) lower than all other groups. Group E (combination) was second highest followed by group D (probiotic). TVC count increased in group E on day 28. However, second highest TVC was observed in control group (group A). No growth of *Salmonella* sp. was observed on day 28 in all the treatment groups. Total coliform count (TCC) in phytobiotic (Group C) was significantly ($p<0.05$) lower than all other groups (A, B, D and E) on day 28 (**Table 13**).

Table 14: Average CFU from intestinal content in different groups (n=12) of broilers

Groups	Groups		28th day			
	TVC (CFU/g sample)	TSC (CFU/g sample)	TCC (CFU/g sample)	TVC (CFU/g sample)	TSC (CFU/g sample)	TCC (CFU/g sample)
Group A	$1.10x\ 10^9$	2.50×10^9	1.06×10^9	1.70×10^9	0	$68.00\ x10^3$
Group B	0.11×10^9	1.60×10^9	$0.12\ x10^9$	$0.6\ x10^9$	0	7.00×10^3
Group C	0.12×10^9	8.00×10^9	1.32×10^9	1.26×10^9	0	0.024×10^3
Group D	$2.00\ x10^9$	$6.60\ x10^9$	1.28×10^9	0.54×10^9	0	48.00×10^3
Group E	0.06×10^9	$12.60\ x10^9$	1.38×10^9	1.80×10^9	0	0.38×10^3

Total viable cell count (TVC), total coliform count (TCC), and total salmonella count (TSC)

The results of haematological parameters; total erythrocyte count, packed cell volume, haemoglobin concentration, and erythrocyte sedimentation rate (TEC, PCV, Hb, ESR) respectively are presented in **Table-35**. Significant difference ($p<0.05$) among the mean values of TEC, Hb, PCV and ESR were observed among the different treatment groups at different age. TEC significantly *(*$p<0.05$) increased in the probiotics treated group (group D) compared to control group by day 28. The highest packed cell volume (22.00±1.03%) was recorded in combination group (E) and the lowest (18.00±1.02%) in group C by day 28. No Significant ($p<0.05$) differences were observed among the probiotics treated group compared to control groups. Highest erythrocyte sedimentation rate (7.50±1.7 mm) recorded in group (B) and the lowest was found (5.55±1.35 mm) in group A by the end of the experiment. No significant ($p<0.05$) differences were observed among the probiotics treated group and control groups.

Table 15: Haematology values of broilers at 2 weeks and 4 weeks respectively

	TEC		HB		PCV		ESR	
Group	14th day	28thday	14th day	28thday	14th day	28thday	14th day	28thday
Negative control(A)	$2.7\pm\ 0.02^{ab}$	2.5±0.02	7.3± 0.10	7.3±0.13	20.0±2.0	20.5±1.50	5.5± 1.50	5.6±1.35
Positive control(B)	$2.1\pm\ 0.02^{b}$	2.2 ± 0.02^{ab}	5.0 ± 0.20^{abc}	6.1 ± 0.10^{a}	20.1±1.01	21.5±1.55	7.0± 1.05	7.5 ± 1.7
Phytobiotic (C)	2.43±0.02a	2.8 ± 0.02^{b}	$6.5\pm\ 0.05^{c}$	5.7 ± 0.12^{ac}	20.0±1.0	18.0±1.02	5.5± 0.55	6.5±1.57
Probiotic (D)	2.4 ± 0.01^{b}	2.9 ± 0.01^{a}	$6.5\pm\ 0.15^{b}$	7.2 ± 0.05^{c}	21.5±0.50	21.5±0.57	6.0± 1.03	6.0±1.08
Combined (E)	$2.2\pm\ 0.01$	2.5±0.03	$6.7\ \pm0.10^{a}$	7.5 ± 0.07^{a}	18.5±1.50	22.0±1.03	6.5± 1.53	6.2±1.6

Data are express as mean±SE, (n=10)

From the present study, it has been observed that phytobiotic and its combination with probiotic supplementation showed significant influence on growth performance, carcass yield and dressing percentage after 28 days of treatment. However, phytobiotic (0.1%) alone checked the growth of pathogenic *Salmonella* spp. and significantly lower intestinal pH and the Total Coliform Count. This result indicates the improvement of gut health of broiler. Therefore, it might be concluded that phytobiotic may be an effective growth promoter instead of antibiotic in broiler industry to maintain food safety. Further dose dependent study of phytobiotic with larger number of birds and molecular study is strongly recommended.

Supplementation of probiotic or combination of probiotic & phytobiotic in broiler feed could be the potential candidates alternative to antibiotic as growth promoter for safe broiler production.

The animal experiment was conducted in rat and mice by using probiotic, phytobiotic and spirulina to eliminate drug residues from animal body. In this experiment oxytetracycline treated rats and mice were further treated with probiotic, phytobiotic and spirulina for 7 days. At the end of experiment animals were starved overnight. Anesthesia was done by lidocaine and soleus muscles were collected for analysis of oxytetracycline residues using HPLC. In this experiment probiotic, phytobiotic and spirulina showed lower oxytetracycline reside in muscles non-significantly (**Table-**

18). The limitation of this study was to use a single dose of oxytetracycline, probiotic, phytobiotic and spirulina. Therefore, further details dose dependent pharmacokinetic and pharmacodynamics studies are recommended to explore the possibilities.

Table 16: Oxytetracycline concentration in animal muscles supplemented with phytobiotic, probiotic and spirulina (on day 7 days)

Groups	OTC conc. in muscle (ppm)	MRL values of OTC in meat (ppm)
A (Control)	BDL	
B (Oxy)	123.61±0.95	200
C (Oxy+Phytobiotic)	101.93 ±1.81	
D (Oxy+Probiotic)	109.57 ±1.03	
E (Oxy+Spirulina)	95.00±1.05	

Data are express as mean±SE, (n=6)

Objective (ii): To isolate, identify and characterize antimicrobial resistant bacteria from different samples collected from sick or apparently healthy livestock and poultry

Prevalence of AMR pathogen in animal derived food products: A number of 953 four types of bacterial pathogen were isolated from livestock products and by-products (meat, milk and feces) which consisting of 554 *E. coli*, 119 *Salmonella*, 211 *Staphylococcus* and 69 *Streptococcus* respectively. On the contrary, a number of 851 bacterial pathogens were isolated from poultry products and by-products (meat, egg and feces) which consisting of 454 *E. coli*, 155 *Salmonella*, 193 *Staphylococcus* and 39 *Campylobacter* species.

Table 17: Isolation and identification of AMR bacterial pathogen from livestock products and by-products

Component	*E. coli*	*Salmonella* spp.	*Staphylococcus* spp.	*Streptococcus* spp.
BAU	112	12	72	22
CVASU	81	5	8	0
BLRI	70	10	15	29
SAU	60	51	36	0
HSTU	70	32	38	0
RU	37		39	18
PSTU	124	9	3	0
Total	554	119	211	69

Table 18: Isolation and identification of AMR pathogen from poultry products and by products

Component	*E. coli*	*Salmonella* spp.	*Staphylococcus* spp.	Campylobacter
BAU	90	16	53	0
CVASU	38	0	4	39
BLRI	50	6	5	0
SAU	107	77	61	0
HSTU	75	29	54	0
RU	35	22	10	0
PSTU	59	5	6	0
Total	454	155	193	39

The overall prevalence of *E. coli*, *Salmonella*, *Staphylococcus* and *Streptococcus* in livestock products and by-products were found to be 38.47%, 8.26%, 14.67% and 4.79% respectively. Similarly, the overall prevalence of *E. coli*, *Salmonella*, *Staphylococcus* and *Campylobacter* in poultry food products and by-products were found to be 51.59%, 17.61%, 21.93% and 4.43% respectively. The details of prevalence pattern are presented in **Table 19 and Table 20.** The overall

multidrug resistant pattern of *E. coli*, *Salmonella*, *Staphylococcus* spp., *Streptococcus* spp. were found to be 88.33%, 75%%, 95% and 100% respectively.

Table 19: Prevalence of AMR pathogen in livestock products and by products

Organisms	Total samples	Positive samples	Prevalence (%)	Confidence Interval (95% CL)
E. coli	1440	554	38.47	35.99-41.01
Salmonella	1440	119	8.26	6.95-9.80
Staphylococcus spp.	1440	211	14.67	12.92-16.57
Streptococcus spp.	1440	69	4.79	3.80-6.02

Table 20: Prevalence of AMR pathogen in poultry products and by products

Organisms	Total samples	Positive samples	Prevalence (%)	Confidence Interval (95% CL)
E. coli	880	454	51.59	48.29-54.88
Salmonella	880	155	17.61	15.24-20.27
Staphylococcus spp.	880	193	21.93	19.32-24.78
Campylobacter spp.	880	39	4.43	3.26-6

Objective 3: Study of antimicrobial sensitivity/resistance pattern of each isolate with the detection of resistance genes

Phenotypic resistance pattern

The phenotypic resistance revealed decreased susceptibility to penicillin, ampicillin, amoxicillin, levofloxacin, ciprofloxacin, tetracycline, neomycin, streptomycin and sulfamethoxazole-trimethoprim.

Antimicrobial Susceptibility Testing (AST) pattern of the AMR pathogen recovered from livestock products and by products

AST pattern of *E. coli*: The AST result shown highest resistance (96.19%) to penicillin followed by ampicillin (90.71%), amoxicillin (86.87%), oxytetracycline (78.32%), cloxacillin (70.37%) and sulfamethoxazole-trimethoprim (70.01%). Among the antibiotics, gentamicin (66.46%) was found highest susceptible. The detail AST pattern of *E. coli* is presented in **Figure 32**.

AST pattern of *Salmonella* spp.: The AST result predicted highest resistance to penicillin (95.15%) followed by ampicillin (91.48%), oxytetracylcine (82.2%), amoxicillin (73.1%), cloxacillin (67.85%) whilst highest susceptible was recorded in gentamicin (82.91%) followed by ceftriaxone (58.88%). The detail AST pattern of *Salmonella* is presented in **Figure 33**.

AST pattern of *Staphylococcus* spp.: The AST result projected highest resistance to cloxacillin (87.345) followed by amoxicillin (85.39%), penicillin (85.29%), ampicillin (76.28%), streptomycin (79.47%), oxytetracycline (67.74%) whereas highest susceptible was found in gentamicin (66.32%) followed by ciprofloxacin (49.46%) and ceftriaxone (49.36%). The detail AST pattern of *Staphylococcus* is presented in **Figure 34**.

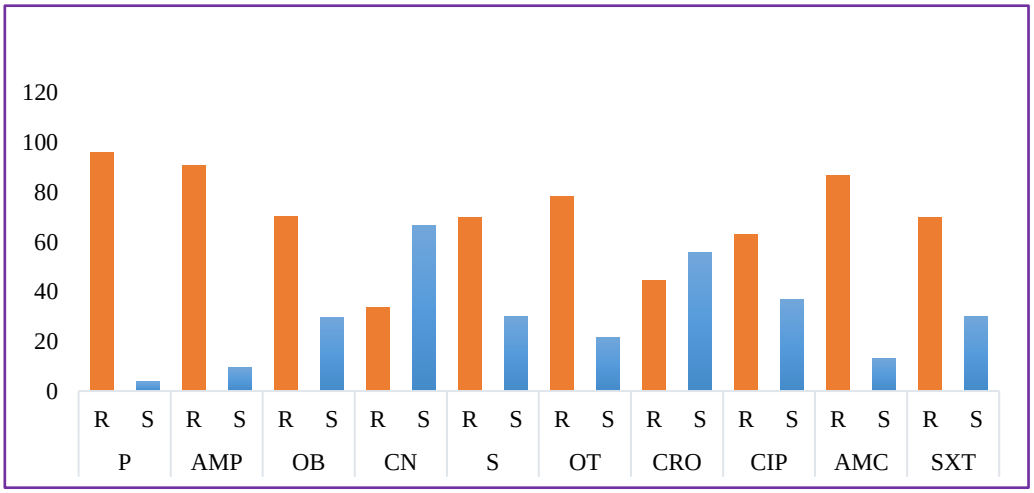

Fig 32: AST pattern on E. coli isolated from livestock

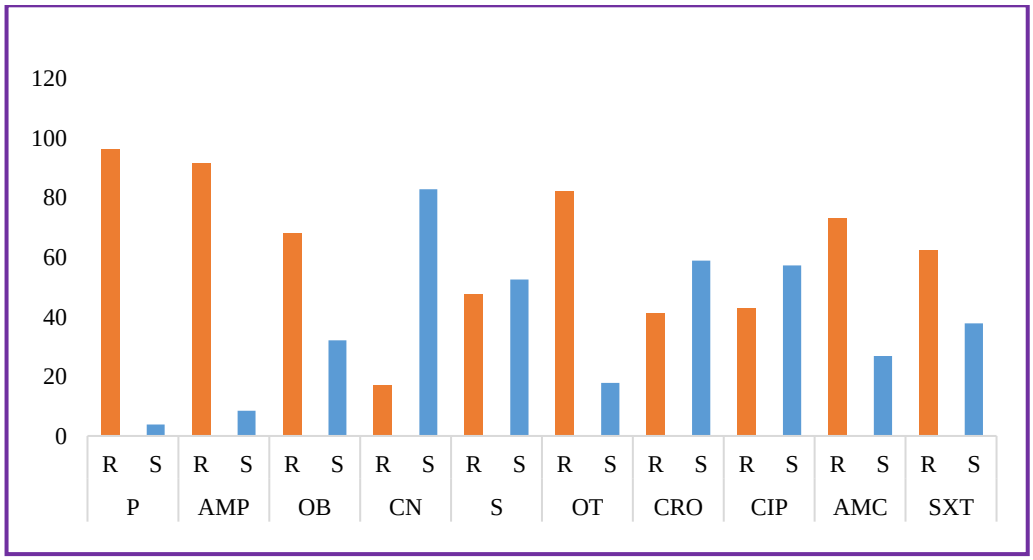

Fig 33: AST pattern of *Salmonella* recovered from livestock

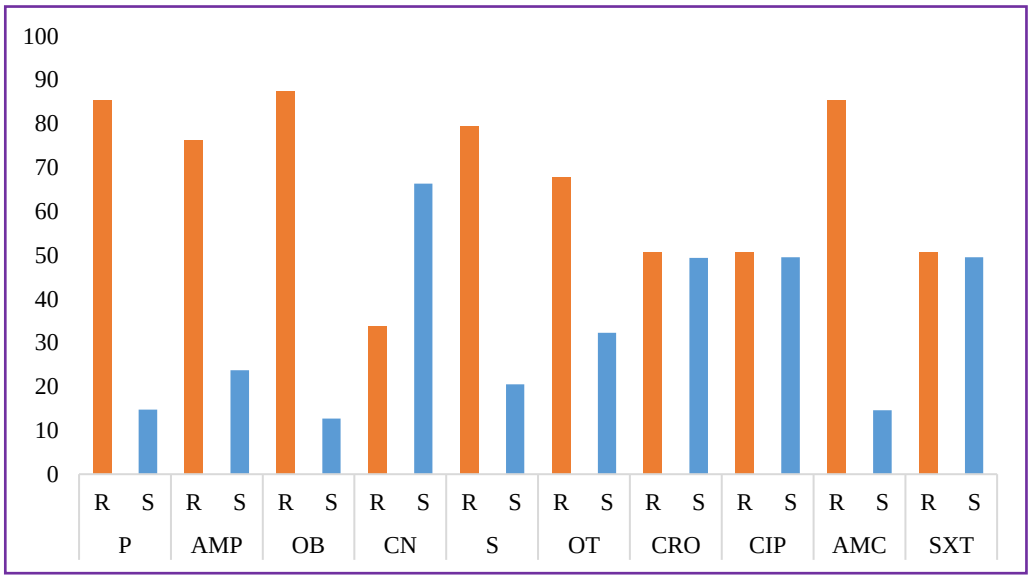

Fig 34: AST pattern of *Staphylococcus* spp. recovered from livestock

AST pattern of *Streptococcus* spp.: The AST result indicated greater resistance to penicillin, followed by cloxacillin (86.27%), oxytetracycline (84.5%), streptomycin (78.87%), amoxicillin (73.23%), sulfamethoxazole-trimethoprim (69.01%) whilst greater susceptible was observed to ciprofloxacin (7043%) and gentamicin (42.26%). The detail AST pattern of *Streptococcus* is shown in **Figure 35.**

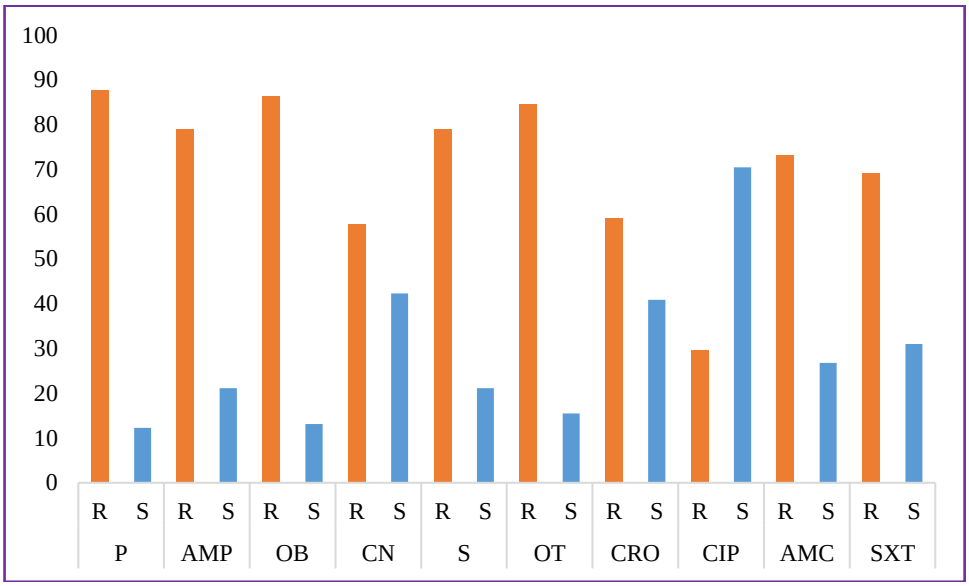

Fig 35: AST pattern of *Streptococcus* recovered from livestock

Antimicrobial susceptibility pattern of the AMR pathogen recovered from poultry products and by products

AST pattern of *E. coli* recovered from poultry: The AST result of *E. coli* showed higher resistance to erythromycin (97.74%) followed by enrofloxacin (91.63%), oxytetracycline (87.42%), ciprofloxacin (86.46%), sulfamethoxazole-trimethoprim (84.54%), tetracycline (75.82%) whereas highest sensitive was found to gentamicin (49.21%). The detail AST pattern of *E. coli* is showed in **Figure 36**.

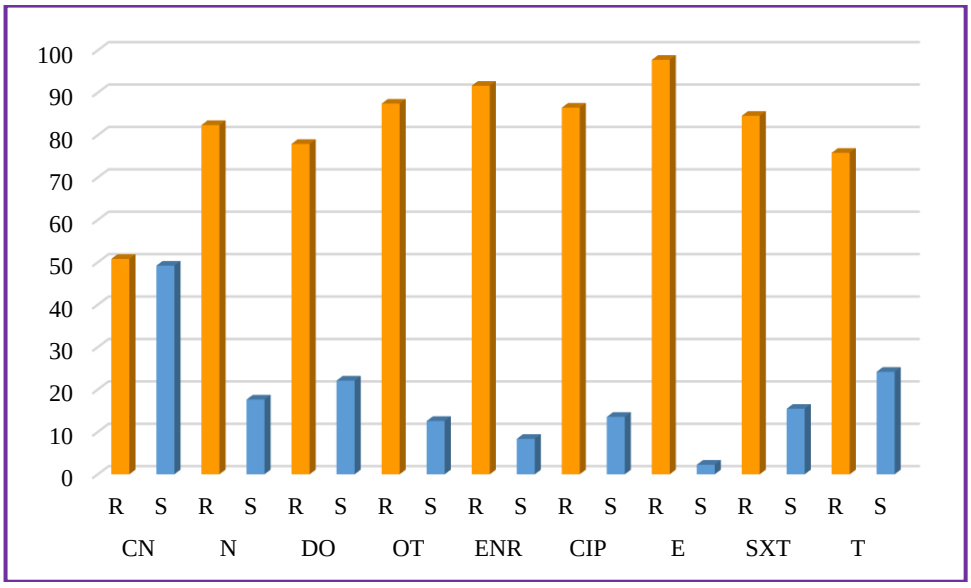

Fig 36: AST pattern of *E. coli* recovered from poultry

AST pattern of *Salmonella* recovered from poultry: The AST result of *Salmonella* shown higher resistance to ampicillin (93.97%) followed by oxytetracycline (89.04%), tetracycline (87.5%), doxycycline (85.71%), enrofloxacin (75.86%), erythromycin (72.41%), ciprofloxacin (70%) and

sulfamethoxazole-trimethoprim (63.63%), however highest sensitive was found to gentamicin (71.43%) and levofloxacin (52.81%). The detail AST pattern of *Salmonella* is outlined in **Figure 37**.

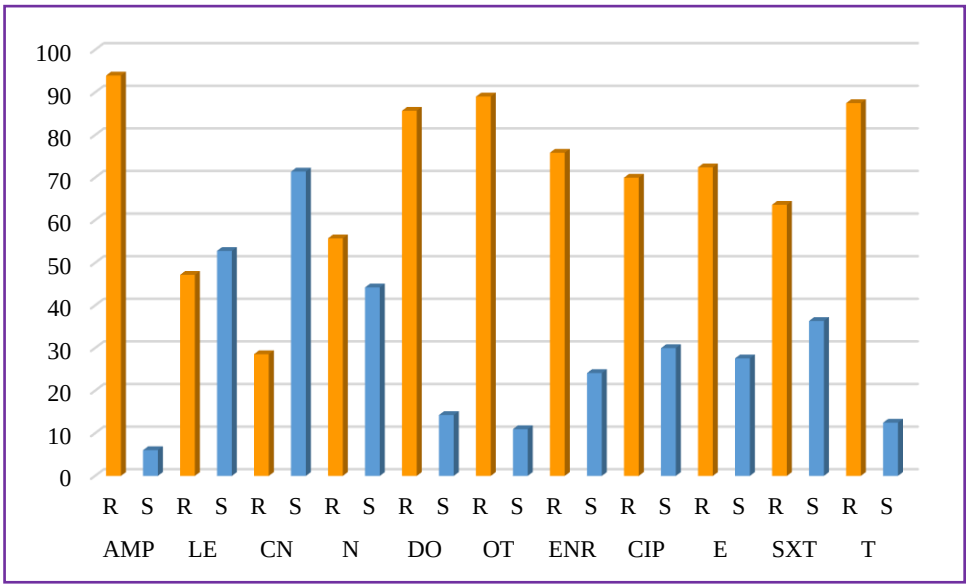

Fig 37: AST pattern of *Salmonella* recovered from poultry

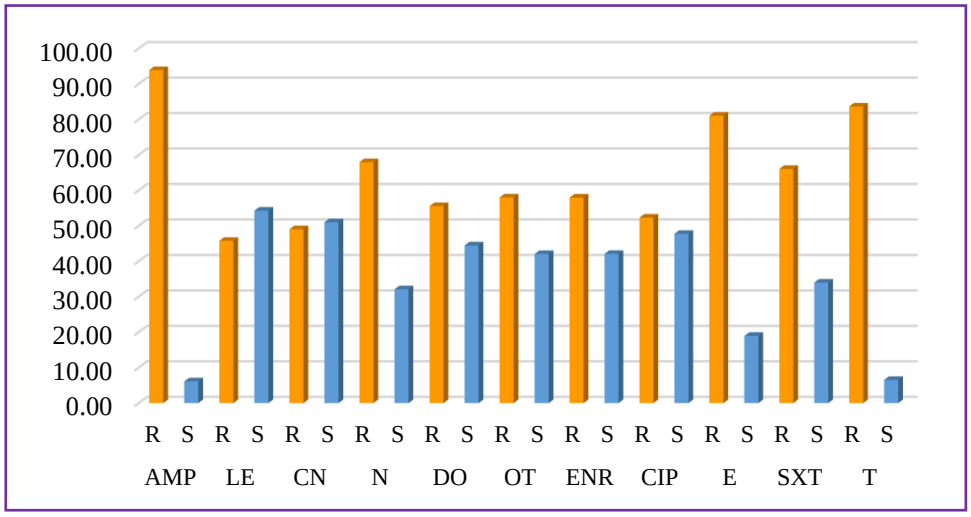

Fig 38: ASTpattern of *Staphylococcus* recovered from poultry

AST pattern of *Staphylococcus* recovered from poultry: The AST pattern predicted higher resistance to ampicillin (93.88%) followed by tetracycline (83.61%), erythromycin (81.05%), neomycin (67.91%), sulfamethoxazole-trimethoprim (63.63%), oxytetracycline (57.97%), enrofloxacin (57.97%), doxycycline (55.56%), however highest sensitive was found to gentamicin (49.02%) and levofloxacin (45.75%). The detail AST pattern of *Staphylococcus* is presented in **Figure 38**.

AST pattern of Campylobacter recovered from poultry: The AST pattern of *Campylobacter* species anticipated higher resistance to erythromycin (100%) followed by ciprofloxacin (85%), sulfamethoxazole-trimethoprim (85%), ampicillin (62.5%) and levofloxacin (60%) whilst higher sensitive was found to doxycycline (72.5%), oxytetracycline (50%), gentamicin (50%) and neomycin (50%). The AST pattern of Campylobacter species is presented in **Figure 39**.

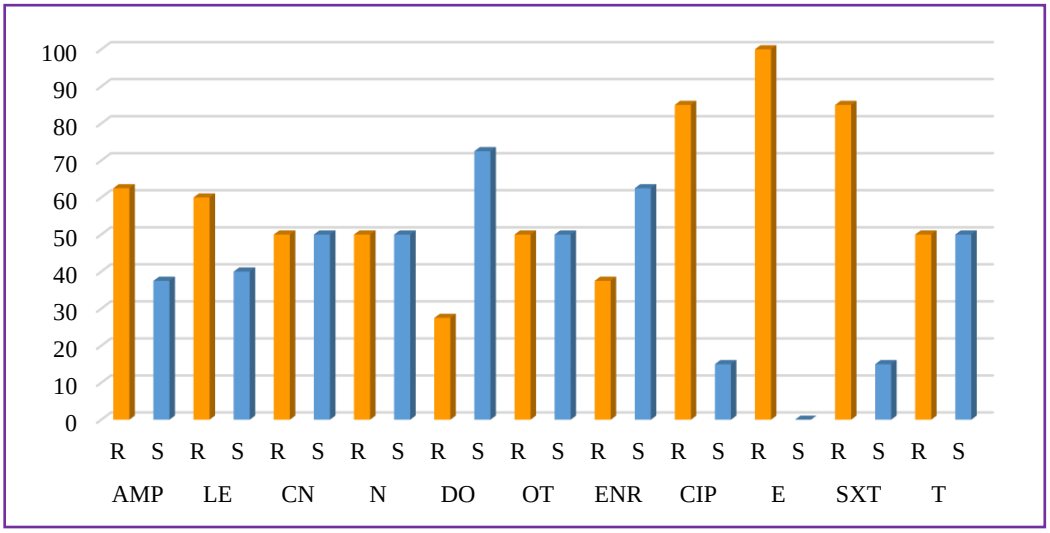

Fig 39: AST pattern of *Campylobacter* recovered from poultry

Genotypic Resistance Pattern of the Isolates

The genotypic resistance pattern showed resistance to ESBL gene (blaTEM, blaSHV, blaCMY), tetracycline gene (tetA, tetB), sulfonamide gene (sul1), streptomycin gene (aadA1), gentamicin gene (aac(3)-IV) and erythromycin gene (ere(A). Almost similar genotypic trends with some extent of deviations were found among the isolated foodborne pathogens. A shorter range of diversity and variability were found among the genotypic resistance pattern of different isolates against the resistance genes. Regardless of origin, all foodborne pathogens possessed antibiotic resistance gene (blaTEM, blaSHV, blaCMY, tetA, tetB, aadA1, aac(3)-IV, ere(A) with different percentage.

Genotypic resistance pattern of *E. coli*: The prevalence of antibiotic resistance gene, sul1, tet (A), tet (B), aac(3)-IV, ere(A), blaSHV, blaCMY, aadA1 and blaTEM was found to be 19.7%, 18.1%, 12.1%, 6.3%, 4.9%, 4.1%, 3.3%, 1.7% and 0.5% respectively in the *E. coil* isolates.

Genotypic resistance pattern of *Streptococcus* spp.: The prevalence of antibiotic resistance gene, sul1, tet (A), tet (B), aadA1, aac(3)-IV, ere(A), blaTEM, blaCMY, and blaSHV was found to be 21.5%, 17.5%, 7%, 3.5%, 3.5%, 3%, 3%, 1.5% and 0.5% respectively in the *Streptococcus* isolates .

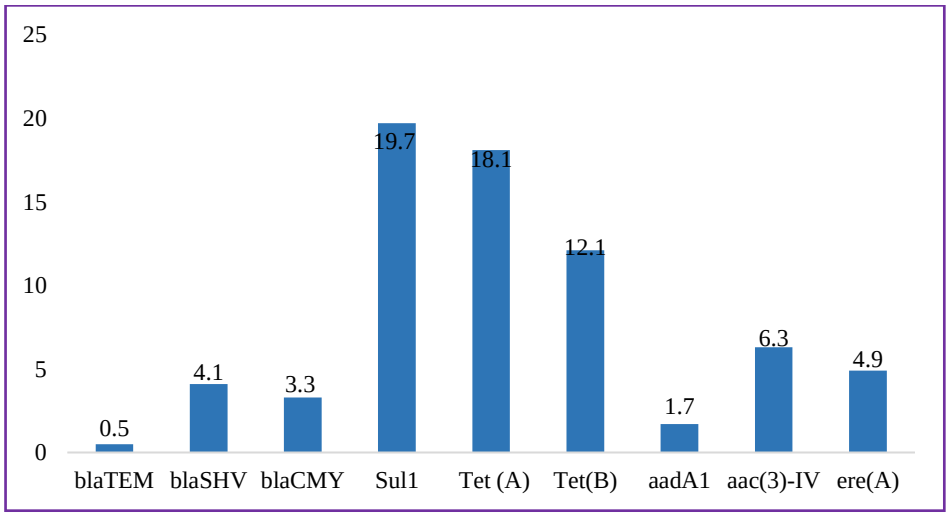

Fig 40: Genotypic resistance pattern of *E. coli* recovered from livestock and poultry

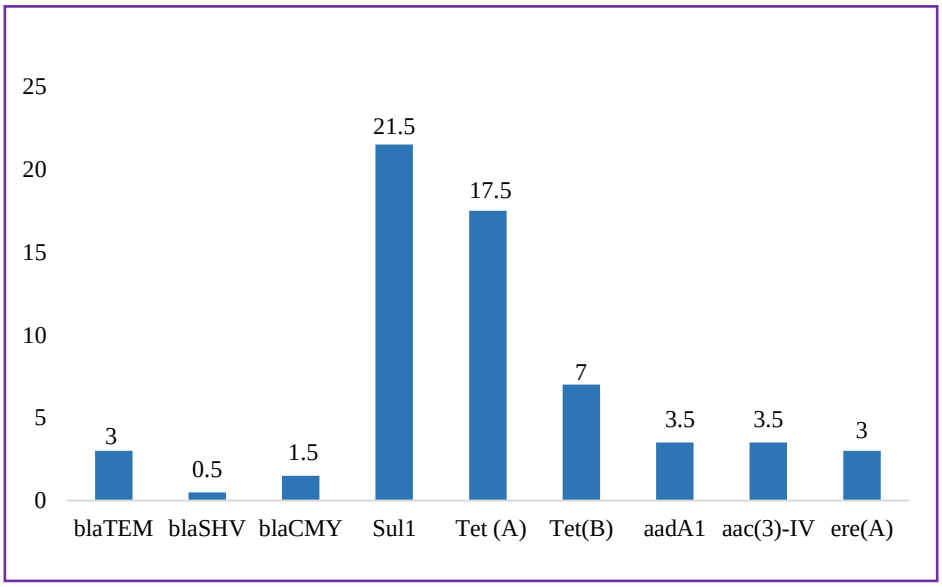

Fig 41: Genotypic resistance pattern of *Streptococcus* recovered from livestock and poultry

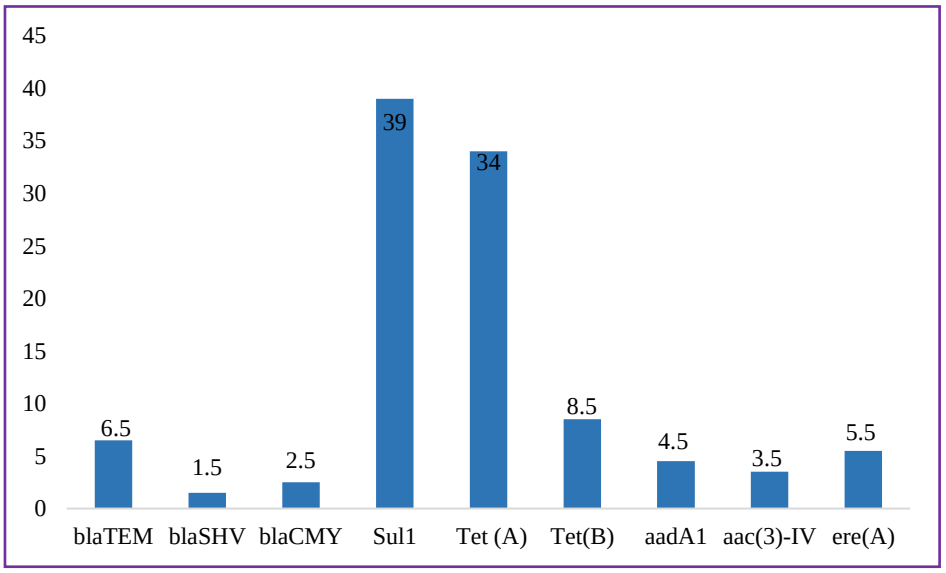

Fig 42: Genotypic resistance pattern of *Salmonella* recovered from livestock and poultry

Genotypic resistance pattern of *Salmonella* spp.: The prevalence of antibiotic resistance gene, sul1, tet (A), tet (B), blaTEM, ere(A), aadA1, aac(3)-IV, blaCMY, and blaSHV was found to be 39%, 34%, 8.5%, 6.5%, 5.5%, 4.5%, 3.5%, 2.5% and 1.5% respectively in the *Salmonella* isolates.

Genotypic resistance pattern of *Staphylococcus* spp.: The prevalence of antibiotic resistance gene, sul1, Tet (A), mecA, Tet (B), blaSHV, blaTEM, ere(A), aadA1, aac(3)-IV and blaCMY was found to be 30%, 29.5%, 20%, 5.5%, 4.5%, 3.5%, 3%, 2.5%, 2.5%, and 1.5% respectively in the *Staphylococcus* isolates.

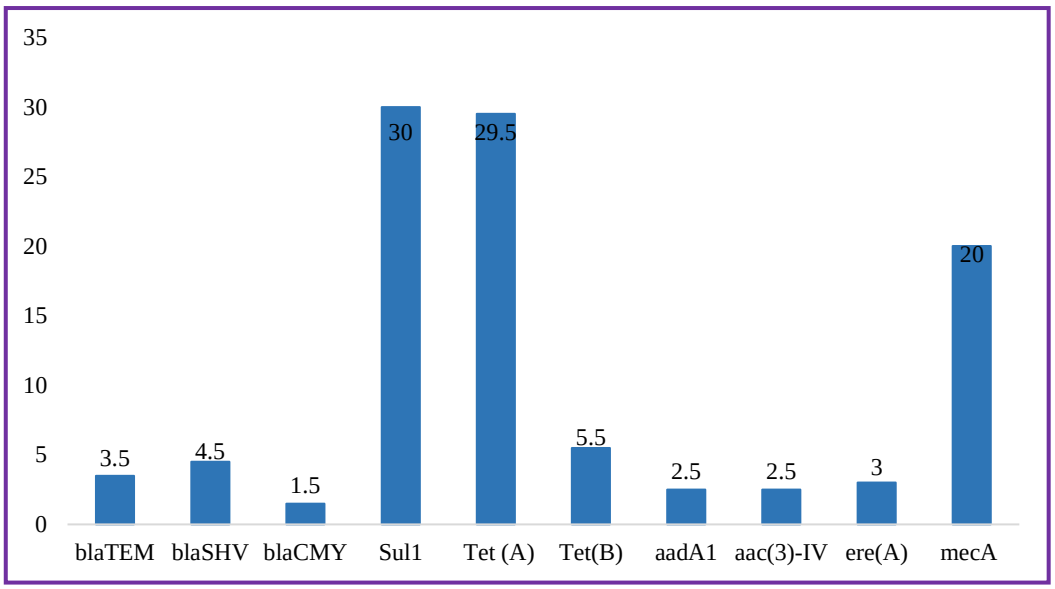

Fig 43: Genotypic resistance pattern of *Staphylococcus* recovered from livestock and poultry

Objective 4: To provide recommendations/suggestions for the control of antimicrobial drugs residue and resistance;

Zoonotic foodborne bacterial pathogens are the leading cause of human disease worldwide, with a significant burden in developing countries, in addition to public health problems, leads to enormous economic losses. *S. aureus*, *Salmonella* species, *Campylobacter* species, *L*. monocytogenes, and *E. coli* are the most common bacterial pathogens associated with foods of animal origin. Currently, these bacterial pathogens are of great concern to public health due to the emergence of multi-resistant strains. Based on the research findings, the following recommendations has been proposed for the control of antimicrobial drug residue and resistance in livestock and poultry foods and feed.

I. Key messages to the nations

- ✓ Animal derived food products (milk, meat and eggs) are mostly freed from antibiotic residues with allowable limits;
- ✓ Higher prevalence of multidrug resistant foodborne pathogen in animal derived food products carries serious public health concern;

II. Policy recommendations

- ✓ To strengthen continuous monitoring and surveillance of antimicrobial drug residue in milk, meat and eggs at local markets and farms;
- ✓ Strictly monitoring local feed producer and factories to prohibit the use of antimicrobial drugs in feed as a growth promoter;
- ✓ Probiotic, phytobiotic or spirulina could be use in feed as a growth promoter in broiler industries instead of antibiotics;
- ✓ Increase awareness among the livestock and poultry farmers to reduce the abuse and misuse of antimicrobials;
- ✓ To promote the food safety concern among the all stakeholders through mass media;
- ✓ The compulsory registration of all commercial livestock and poultry farms and feed business operators by the livestock department;
- ✓ To prohibit the use of antibiotics in farming practices without prescription of registered veterinarian;
- ✓ Adopting good husbandry practices including of proper biosecurity measures, cleaning, sanitation, hygiene, disinfection, quarantine, therapeutic regimens and appropriate vaccination programme;

- ✓ Building awareness and motivation to the livestock and poultry farmers, drug sellers, feed sellers and feed companies about antibiotic resistance and residues;

II. Research recommendations

- ✓ To disclose the root of the horizontal and vertical transmission of antimicrobial resistance and residue in animal derived food commodities;
- ✓ To develop alternatives to antibiotics (probiotics and prebiotics) in animal production system;
- ✓ To develop new antimicrobials for effective treatment against AMR pathogens;
- ✓ To improve biosecurity and vaccination model in the animal farming systems;
- ✓ Development of good agricultural practices in animal farm production system;

III. Recommendations for hygienic production of animal foods

- ✓ Selection of healthy and disease free animals for human consumption;
- ✓ HACCP principles should be followed in production and processing of animal products;
- ✓ Proper cleaning and disinfection of food processing places;
- ✓ Personal hygiene should be followed during processing of animal foods;
- ✓ Strictly maintain withdrawal period of the antimicrobials before harvesting and marketing of animal products;
- ✓ Precautionary measures to check for pathogen spread by people, vehicles and equipment

IV. Housekeeping recommendations

- ✓ Good kitchen hygiene practices and adequate cooking of the product;
- ✓ Proper cooking of the food product is very important measure. Moist heat treatment reduces the chance of spread of bacterial toxins;
- ✓ Proper cooling and refrigeration of food items along with maintenance of keeping quality;
- ✓ Avoiding cross contamination of cooked food;
- ✓ Chlorination of drinking water;
- ✓ Avoiding cross contaminations of cooked with other raw food materials;
- ✓ Avoid consumption of raw/ uncooked products especially milk;
- ✓ Last but the least is the promotion of health education;

Discussion

Food-producing animals are the major reservoirs for many foodborne pathogens such as *E. coli*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Campylobacter* species, and *Listeria monocytogenes*. The zoonotic potential of foodborne pathogens and their ability to produce toxins causing diseases or even death can recognize the severity of the situation (Norma and García, 2018). Foodborne pathogens cause millions of cases of sporadic illness and chronic complications, as well as large and challenging outbreaks in many countries and between countries. The magnitude of this problem is demonstrated by the significant proportion of the 1.5 billion annual diarrheal episodes in children less than 3 years of age that are caused by enteropathogenic microorganisms, which results in more than 3 million deaths per year (EFSA-ECDC, 2016). A surveys estimated that in the United States alone, bacterial enteric pathogens cause 9.4 million episodes of foodborne illness in humans, 55,961 hospitalizations, and 1,351 deaths each year (Scallan et al., 2011). However, it is estimated that the reported incidence of foodborne disease represents less than 1% to 10% of the real incidence (Scallan et al., 2011). The importance of food-producing animals as carriers of pathogenic bacteria is real; for example, beef is reported to be the vector of 7% of the 1.7 million cases of foodborne disease that was recorded from 1996 to 2000 in England and Wales (Anderson et al., 2009).

The term foodborne diseases or foodborne illnesses or more commonly food poisoning are used to denote gastrointestinal complications that occur following recent consumption of a particular food or drink. There is enormous social and economic strain on societies

due to food contamination. Each year around one-third of the world population is affected by foodborne pathogens especially in developing countries. Even in developed nation like US, billions are spent in treatment of foodborne diseases caused by major pathogens. Each year 48 million people are affected in US with foodborne illness (Scallan et al., 2011; CDC, 2013). International trade of animal products has aggravated the condition. The foodborne illness can be of three types i.e. food infection, food intoxication and toxico-infection. The rapid increase in human population and urbanization along with changing food habits has resulted increase in the consumption of animal product like meat, milk and egg as the animal protein are of higher value and rich in micronutrients like iron and zinc and vitamins (Broglia and Kapel, 2011). The foodborne pathogens can enter the food chain anytime between farm to fork. The pathogens cause disease by consumption of undercooked food and produce illnesses either by their presence or by production of toxins or both. Pregnant women, elderly people, immunocompromised people and children are at higher risk of infection with these pathogens (McCabe-Sellers and Beattie, 2004). The important foodborne pathogens of animal origin includes *Salmonella*, *Campylobacter, Escherechia coli, Staphylococcus, Clostridium, Yersisnia, Listeria, Arcobacter* and (Dhama et al., 2011a; Dhama et al., 2013a).

Escherichia coli*: E. coli* is among many pathogenic microorganisms which can get access to food of animal origin and is considered as a reliable indicator of contamination by manure, soil, and contaminated water (Disassa et al., 2017). Most of *E. coli* are normal inhabitants of the gastrointestinal tract of animals and humans ((Disassa et al., 2017; Abreham et al., 2019; Taye et al., 2013), while others are pathogenic to humans (Bekele et al., 2014). *E. coli* are of zoonotic in nature and constitute a public health hazard (Messele et al., 2019). Shiga toxin-producing *E. coli* are associated with several life-threatening foodborne outbreaks worldwide (Elmonir et al. 2018).

***Salmonella* spp**.: *Salmonella* spp. are the most important foodborne zoonotic bacterial pathogens in humans. *Salmonella* is widespread in nature (Kemal et al., 2015) and is the most important pathogenic bacterium in both humans and animals (Addis et al., 2011). They are the most commonly isolated bacterial pathogens in foodborne disease outbreaks (Balakrishnan et al., 2018) and account for about 93.8 million foodborne illnesses and 155,000 deaths per year worldwide (Heredia and Garcia, 2018). *Salmonella* has become the leading cause of foodborne illness and a serious public health problem in the world, with growing concern about the emergence and spread of antimicrobial resistant strains (Ejo et al., 2016), including in developed countries (Tegegne, 2019). Antibiotic-resistant *Salmonella* infections in humans and animals are a universal problem, especially in developing countries (Ejo et al., 2016).

***Staphylococcus* spp**.: It is a microorganism that occurs as commensals on the skin, nose, and mucous membranes of healthy people and animals (Tessema and S. Tsegaye, 2017; Lozano et al., 2016). However, it is a known opportunistic foodborne pathogen (Wang et al., 2016) that can cause several infectious diseases of varying severity (Lozano et al., 2016). It causes a wide range of diseases in humans and animals (Abraha et al., 2018). The presence of *S.* aureus in products for human consumption is important for the food industry, since some strains cause food poisoning (Tsepo et al., 2016). They are responsible for food spoilage, reduce food safety and shelf life, and cause food poisoning (Beyene et al., 2017). *S.* aureus is one of the main causes of food poisoning from the consumption of food contaminated with staphylococcal enterotoxins (EI-Jakee et al., 2013). The widespread use of antibiotics and the ability of bacteria to grow rapidly and acquire antimicrobial resistance have facilitated the emergence of resistant strains such as methicillin-resistant *S.* aureus (MRSA) (Adame-Gomez et al., 2106; Mardziah et al., 2019). The presence of MRSA in farm animals and the potential for cross-contamination in humans have been of great concern (Dehkordi et al., 2019).

***Campylobacter* species**: It is the leading cause of foodborne diarrhea in humans worldwide (Wieczorek et al., 2018), which is mainly due to contamination of food of animal origin (Kassa et al., 2007). *Campylobacter* can colonize most warm-blooded animals and poultry (Mughal, 2018). The zoonotic nature of *Campylobacter* species makes it clinically and economically important worldwide (Mdegela et al., 2006). They account for 15% of food-related illness-related hospital admissions and 6% of food-related illness-related deaths, and about 400 million cases are reported due to foodborne infection each year (Hagos et al., 2019; Nigatu et al., 2015). The economic losses due to *Campylobacter* infections are mainly related to the costs of treatment, the loss of productivity of infected people, and the costs of fighting the pathogen (Mdegela et al., 2006).

Antibiotic residue in animal derived foodstuffs

Antibiotics are used to treat diseases and improve animal production. These antibiotics can cause residues to build up in meat, milk, and eggs that are not allowed in food for human consumption. The most important side effect of antibiotic residues is the transfer of antibiotic resistant bacteria to humans due to mobile resistance properties. Due to these side effects, it is important to regulate the use of antibiotics in the animal production system.

Uses of antimicrobial in animal production

Antimicrobials have been used in agricultural production systems, particularly in animals, since the mid-1940s, shortly after they were used to treat human disease (Gustafson & Bowen, 1997; McEwen, 2006). Antimicrobial agents are widely used today in animal production systems for the treatment, prevention, and promotion of growth (Masud et al., 2020). A large number of antibiotics used in human medicine belong to the same category or have the same mode of action as antibiotics used in animal production. Several antibiotics that are used to treat bacterial infections in humans also have veterinary uses (Landers et al., 2012). In fact, the use of animal antibiotics to treat or prevent disease is closely adhered to their use in humans. Every year, a large number of drugs, including antibiotics, are used in farm animals to produce more animal foods (meat, eggs and milk) for human consumption around the world (Vazquez Moreno´ et al., 1990; Roura et al.,1992; Rassow and Schaper, 1996). In 2010, China, the United States, Brazil, India and Germany were the five countries with the highest levels of antibiotics consumed by food-producing animals (van Bijnen et al., 2011). The estimated global quantity of antibiotics used in food-producing animals was 63,151 (± 1560) tons (Boeckel et al., 2015). The abuse and overuse of antibiotics can lead to bacterial resistance, which is a major public health problem worldwide (Ferech et al., 2006; Liu et al., 1999; van Bijnen et al., 2011; Ahiabu et al., 2016; Al Tawfiq et al., 2010; Costelloe et al., 2010; Vaananen et al., 2006; You et al., 2008; Mather et al. 2012). The use of antibiotics in animal production systems is an important factor in the development of resistance to bacteria that can cause infections in humans (Molbak, 2004; Wassenaar, 2005). In Bangladesh, antimicrobial agents are often used to treat and control diseases in the agricultural system, especially of food producing animals, and are often used as feed additives to promote growth (Masud et al., 2020). In the case of poultry, the use of antibiotics promotes their efficient production and also improves their health and well-being by reducing the incidence of infection (Tollefson & Miller, 2000). In Bangladesh, antimicrobial agents are often used to treat and control diseases in agriculture, especially in food-producing animals, and are often used as food additives to promote growth (Hassan et al., 2021). The presence of antibiotic residues in foods of animal origin can be the point of entry into the human food chain. In recent years, the presence of antibiotic residues in milk, meat, egg, fish and aquaculture products has had a negative impact on their international trade (Mathews et al., 2003). Antibiotics are commonly used to treat, prevent, and promote the growth of farm animals, including doxycycline, colistin sulfate, neomycin, tetracycline, enrofloxacin, ciprofloxacin, and amikacin (Apata, 2009; Roess et al., 2013). Antibiotic residues are always likely to enter to the consumer products and to human food chains.

The antibiotic residues in animal derived food commodities (milk, meat and eggs) can lead to the progression of AMR pathogens

The possible effects of the extensive use of antibiotics in poultry and livestock have been reported elsewhere (Marshall & Levy, 2011). The use of antibiotics means that this type of drug can enter the human food chain in the form of drug residues in foods of animal origin, including meat, milk and eggs. This can occur if the withdrawal period is not allowed before marketing animal products for human consumption. This approach reduces the effectiveness of antibiotics for treatment of human diseases. In addition, the widespread use of antibiotics may also foster to the development of antibiotic-resistant strains and the resistance gene that can be transferred further to other pathogenic and non-pathogenic bacteria (Peterson & Kaur, 2018) or the human food value chain (Smith, 1969). Therefore, humans can get exposure antibiotic-resistant bacteria following ingestion of poultry, livestock, and fish products. Antibiotic resistance to human pathogens is a major global public health problem today (Martínez Martínez & Calvo, 2010). It is well known that there is a clear synergy between the use of antibiotics in animals and resistance to human pathogens (Hoelzer et al., 2017; Landers et al., 2012; Wegener, 2012). Obviously, the use of antibiotics in livestock is related to the development of resistance to antibiotics in humans. The World Health Organization (WHO) identified sub therapeutic application of antibiotics are the major risk factors for the emergence of antibiotic resistance to various microorganisms in human and animals (Landers et al., 2012; Prajwal et al., 2017). It has been reported that the residual effects of tetracycline may persist in food for a longer period of time, although withdrawal period has been properly maintained (Grossman, 2016). Antibiotic-contaminated waste and effluent from veterinary and human health settings can lead to deposition of antibiotic residues in soil and water, which then transfer resistant bacteria to various components of livestock production systems (Manyi-Loh et al., 2018). Food animals can receive various types of antibiotic agents directly for treatment or as growth promoters. If the withdrawal period for a particular antibiotic agent is not maintained, some antibiotic residues may remain in the animal derived food commodities.

The antibiotic residues in animal derived food products

In our study, the maximum concentration of amoxicillin residues in a single raw milk sample (4.59 µg/l) was found to be slightly above the MRL (4 µg/l) according to the Codex Alimentarius Commission (CAC). This finding is supported by the observation of previous researchers who found an MRL for amoxicillin in raw milk (Movassagh et al., 2010; Rahman et al., 2021). Likewise, the maximum concentration of ampicillin was detected in two milk samples (22.47 µg/l), which is four times higher than the MRL (4 µg/l), which is consistent with the results of previous studies (Anderson et al., 1996). The highest amoxicillin concentration (125.78 µg/kg) was measured in six meat samples above the MRL (50 µg/kg), which is consistent with previous observations (Jammoul and Darra, 2019).

The animal products that exceed the maximum allowed residue limit can cause serious problems (Treiber and Knauer, 2021). Heat treatment during cooking can reduce the risk of ingestion of residues of sulfonamides, tetracycline, and fluoroquinolones, but there is no guarantee that these antibiotic residues will completely break down or break down in animal products, especially chicken meat (Kühne et al., 2001; Gratacós-Cubarsí et al., 2007). The high stability of quinolones and beta-lactams represents a considerable risk to human health, since the residues of these antibiotics remain in the milk after heat treatment and thus can reach the consumer (Roca et al., 2010; Roca et al., 2011).

The antimicrobial residues in feed samples

In our study, five antimicrobial residues (OTC, SXT, CIP, CN and DOX) were found positive in feed samples with different concentrations. In a study, the mean concentration of 4-epichlortetracycline and chlortetracycline antibiotic residue were found 2722.12 and 3720.87µg/kg

respectively in India (Waghamare et al., 2020). The average concentration of chlortetracycline residue was found 77.17ppm in 2.81% poultry feed samples (Waghamare et al., 2006). In Europe, since 1998, the animal husbandry and broiler industries voluntarily stopped using all growth-promoting antibiotics, while the European Union has banned the use of all antibiotics as growth promoters since 2006. Most recently, in 2017, the United States [USFDA. part 558] also prohibited the use of tetracycline as a growth promoter (Jensen and Hayes, 2014). The "Fish Feed and Animal Feed Act, 2010" in Bangladesh has imposed ban the use of antimicrobial in animal and poultry feed samples (MoFL, 2010). Though, the use of antimicrobial in feed is not allowed but the result predicted some antibiotics were randomly used with feed either intentionally or unintentionally. The competent authority should enhance their monitoring and surveillance to the feed industry to prohibit the use of antibiotics in feed formulations for any reason whether preventive or growth promoter. The presence of antibiotic residues in feed may be due to the illegal use during production in factories or the application of additional growth promoter labels on farms (Waghamare et al., 2020).

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12. Research highlights (title, background, objectives, methodology, key findings, and key words):

Title: Determination of Antimicrobial Resistance and Residues in Livestock and Poultry Food Products and Feed in Bangladesh

Background & objectives: Antimicrobials are used in humans and animal sectors to treat the diseases and improve livestock production. The increasing use of antimicrobials might result in deposition of residues in livestock food products and to develop resistance to these drugs by the microorganisms, therefore many diseases are becoming difficult to treat both in humans and animal. Use of low dose antibiotics as growth promoter resulting antibiotic residues in food products having detrimental effects in human health. There is no reliable and nationwide data on antibiotic resistance and residue in livestock and poultry food products and feed in Bangladesh. Therefore, the objectives of the sub-project were to assess the status of antimicrobial residues in livestock and poultry food products along with identification of foodborne pathogen with their antibiogram and detection of resistance genes, and its mitigation by using alternate approach as growth promoter for safe food production in Bangladesh.

Methodology: The research work was conducted in 33 districts under eight administrative divisions of the country. A number of 3,280 samples were collected throughout the country by all seven components. Of all samples, 2320 samples were tested for microbiological screening followed by phenotypic and genotypic characterizations of the antibiotic resistance patterns of the foodborne pathogens. The 2440 samples were also screened qualitatively and quantitatively for antibiotic residues. Microbiological tests were performed according to conventional culture methods, followed by a polymerase chain reaction. The antibiotic residue was assessed by the TLC and HPLC method. Intervention studies were conducted in the poultry and laboratory animals to find out the alternatives to antibiotics as the growth promoter. In addition, a number of 1520 questionnaire were interviewed by the veterinarian, farmers and informal prescribers (drug seller and feed seller).

Key findings:

- ✓ Higher prevalence of foodborne pathogen in livestock and poultry food products indicated serious public health concern;
- ✓ Multidrug resistant pathogens in food chain has catastrophic effect in human and animal health;
- ✓ Antibiotic residue was found in animal derived food commodities with different extent.
- ✓ Maximum food commodities possessed allowable limit of antibiotic residue level.
- ✓ Farmers had inadequate knowledge and poor practices on the impact of antibiotic resistance and residue in animal derived foodstuffs;
- ✓ Higher prevalence of foodborne pathogen along with MDR indicated irrational use of antimicrobials in farming practices;

Keywords: Antibiotic residue; antibiotic resistance, livestock; food; feed; maximum residue level;

B. **Implementation Status**

1. **Procurement (Component wise):**

Components	Description of equipment and capital items	PP Target		Achievement		Remarks
		Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
BARC	(a) Office equipment	1	349,800	1	349,800	completed
	(b) Lab &field equipment	1	450,200	1	450,200	completed
BLRI	(a) Office equipment	2	44,000	2	43,830	completed
	(b) Lab &field equipment	2	100,000	2	99,850	completed
BAU	(a) Office equipment	7	183000	7	181600	Completed
	(b) Lab & field equipment	9	1,630,000	9	1,624,800	Completed
	(c) Other capital items	1	20,000	1	20,000	Completed
RU	(a) Lab & field equipment	2	6,50,0000	2	6,48,200	Completed
PSTU	(a) Office equipment	5	100000	5	99200	Completed
	(b) Lab & field equipment	10	1248500	10	1246500	Completed
CVASU	(a) Office equipment	6	2,05,000	6	2,05,000	Completed
	(b) Lab & field equipment	1	3,00,000	1	3,00,000	Completed
	(c) Other capital items	7	61,000	7	60,800	Completed
SAU	(b) Lab & field equipment		1,934,618		1,934,618	Completed
	(c) Other capital items		745,000		742,000	Completed
HSTU	(a) Office equipment	5	81,000	5	80,025	Completed
	(b) Lab &field equipment	1	4,90,000	1	4,87,000	Completed

2. **Establishment/renovation facilities: N/A**

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. **Training/study tour/ seminar/workshop/conference organized:**

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training			202	Three AMR trainings were organized at SAU, RU and BLRI	
(b) Workshop			150	One inception and two annual review workshop was organized at BLRI	
(c) Others (if any)					

C. **Combined financial and physical progress (component wise)**

Combined financial and physical progress						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	12889031	37490575	12868815	806	99.84%	
b. Field research/lab expenses and supplies	13570459		13262565	200000	97.73%	
c. Operating expenses	2535644		2440667	71506	96.25%	
d. Vehicle hire and fuel, oil & maintenance	631543		625776	0	99.086%	
e. Training/workshop/seminar etc.	512621		512621	0	100%	
f. Publications and printing	270000		229945	0	85.16%	
g. Miscellaneous	1050569		848026	199999	80.72%	
h. Capital expenses	6708861		6699361	0	99.85%	
Total	**38168728**	**37490575**	**37487776**	**472311**	**98.21%**	

1. BARC component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	3802362	5697686	3800521	0	100%	
b. Field research/lab expenses and supplies	200000		0	200000	0%	
c. Operating expenses	229995		160500	69495	93.33%	
d. Vehicle hire and fuel, oil & maintenance	26313		26313	0	100%	
e. Training/workshop/seminar etc.	512621		512621	0	100%	
f. Publications and printing	200000		200000	0	100%	
g. Miscellaneous	399774		199775	199999	98.24%	
h. Capital expenses	797956		797956	0	100%	
Total	**6169021**	**5697686**	**5697686**	**469494**	**99.93%**	

2. BLRI component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1466400	1468900	1468900	-	100%	
b. Field research/lab expenses and supplies	1760932	1746932	1746932	-	100%	
c. Operating expenses	299770	293271	291260	2011	99.32%	
d. Vehicle hire and fuel, oil & maintenance	170000	169945	169945	-	100%	
e. Training/ workshop/seminar etc.	-	0	0	-	-	
f. Publications and printing	-	0	0	-	-	
g. Miscellaneous	99895	99844	99844	-	100%	
h. Capital expenses	143680	143680	143680	-	100%	
Total	**3940677**	**3922572**	**3920561**	**2011**	**99.95%**	

3. BAU component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1886875	1871216	1871216	0	99.17%	Due to delayed PhD fellow and other staffs recurrent
b. Field research/lab expenses and supplies	2440710	2426970	2426970	0	99.43%	
c. Operating expenses	353515	345054	345054	0	97.60%	
d. Vehicle hire and fuel, oil & maintenance	212362	207000	207000	0	97.47%	
e. Training/workshop/ seminar etc.	0	0	0	0		
f. Publications and printing	40000	0	0	0	0	Thesis of PhD fellow not yet prepared
g. Miscellaneous	75000	75000	75000	0	100%	
h. Capital expenses	1830900	1826400	1826400	0	99.75%	
Total	**6839362**	**6751640**	**6751640**	**0**	**98.72%**	

4. RU Component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1355181.00	1355181.00	1355181.00	0.00	79.11%	
b. Field research/lab expenses and supplies	1413000.00	1411701.00	1411701.00	0.00	96.55%	
c. Operating expenses	292286.00	290916.00	290916.00	0.00	93.8%	
d. Vehicle hire and fuel, oil & maintenance	147868.00	147518.00	147518.00	0.00	99.76%	
e. Training/workshop/ seminar etc.	0.00	0.00	0.00	0.00	0	
f. Publications and printing	0.00	0.00	0.00	0.00	0	
g. Miscellaneous	87165.00	84800.00	84800.00	0.00	97.29%	
h. Capital expenses	650000.00	648200.00	648200.00	0.00	76.72%	
Total	3945500.00	3938316.00	3938316.00	0.00	**87.23%**	

5. PSTU Component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1077541	1077541	1077541	0	100%	
b. Field research/lab expenses and supplies	1070775	1070775	1070775	0	100%	
c. Operating expenses	364574	364574	364574	0	100%	
d. Vehicle hire and fuel, oil & maintenance	0	0	0	0	-	
e. Training/workshop/seminar etc.	0	0	0	0	-	
f. Publications and printing	14945	14945	14945	0	100%	
g. Miscellaneous	58005	58005	58005	0	100%	
h. Capital expenses	1405300	1405300	1405300	0	100%	
Total	**3991140**	**3991140**	**3991140**	**0**	100%	

6. CVASU component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	986948	986948	986948	0	100%	
b. Field research/lab expenses and supplies	2817733	2740133	2740133	0	100%	
c. Operating expenses	341479	337680	337680	0	100%	
d. Vehicle hire and fuel, oil & maintenance	75000	75000	75000	0	-	
e. Training/workshop/ seminar etc.	0	0	0	0	-	
f. Publications and printing	15000	15000	15000	0	100%	
g. Miscellaneous	140000	140000	140000	0	100%	
h. Capital expenses	565800	565800	565800	0	100%	
Total	**4941960**	**4860561**	**4860561**	0	100%	

7. SAU component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1092960	1088550	1088550	0	100%	
b. Field research/lab expenses and supplies	1934618	1934618	1934618	0	100%	
c. Operating expenses	375172	375172	375172	0	100%	
d. Vehicle hire and fuel, oil & maintenance	0	0	0	0	-	
e. Training/workshop /seminar etc.	0	0	0	0	-	
f. Publications and printing	0	0	0	0	100%	
g. Miscellaneous	120,000	120000	120000	0	100%	
h. Capital expenses	742,000	742000	742000	0	100%	
Total	**4,264,750**	**4260340**	**4260340**	**0**		

8. HSTU component						
Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	12,20,764	12,20,764	1219958	806	100%	Due to variation in Basic Salary of Co-PI
b. Field research/lab expenses and supplies	19,31,436	19,31,436	1931436	0.00	100%	-
c. Operating expenses	2,75,511	2,75,511	275511	0.00	100%	-
d. Vehicle hire and fuel, oil & maintenance	-	-	-	-	-	-
e. Training/workshop/seminar etc.	-	-	-	-	-	-
f. Publications and printing	-	-	-	-	-	-
g. Miscellaneous	70,602	70,602	70602	0.00	100%	-
h. Capital expenses	5,70,025	5,70,025	570025	0.00	100%	-
Total	**40,68,320**	**4068320**	**4067514**	**806**		

D. **Achievement of Sub-project by objectives (Tangible form): Technology generated/developed**

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
Information/ Knowledge/ policy has been generated rather than technology			

E: **Information/knowledge/policy generated**

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
To assess the antimicrobial drug residues in livestock and poultry food products and feed;	1. Pretested questioner survey was conducted among the livestock stakeholder for KAP analysis; 2. Qualitative antimicrobial residue detected by TLC; Quantitative antimicrobial residue detected by HPLC 3. Alternates to antibiotic were used to mitigate antimicrobial drug residue and resistance in poultry and laboratory animals;	1. Knowledge and practice of the stakeholders regarding antibiotic resistance and residue has disclosed; 2.Antimicrobial residue in livestock and poultry foods and feeds has detected 3. Probiotic and phytobiotic has used as a growth promoter in feed	1. Policy will be generated to trained up livestock stakeholders regarding reduction of AMU and AMR 2. Food safety of animal derived food products will be ensured; 3. Public concern on food safety will be resolved;

To isolate, identify and characterize antimicrobial resistant bacteria from different samples collected from sick or apparently healthy livestock and poultry;	Sample collection and processing is conducted; culture and biochemical test is conducted; Polymerase chain reaction is conducted; Sequencing of the isolates was done;	Common foodborne pathogen (*E. coli*, *Salmonella*, *Staphylococcus*, *Streptococcus* and *Campylobacter*) has detected in livestock and poultry foods	Evidence based scientific information on prevalence of common foodborne pathogen has been generated which will promote vendor and consumer awareness
Study of antimicrobial sensitivity/resistance pattern of each isolate with the detection of resistance genes;	Antimicrobial susceptibility testing is done by disk diffusion method; Resistance gene was detected by polymerase chain reaction;	Antimicrobial resistance profile of common foodborne pathogen has been disclosed	The research findings will provide best treatment options in AMR pathogen infections
Coordinate, monitor and evaluate the project activities throughout the project duration, and organization of seminar and workshop;	Organized a inception workshop, two annual review workshop, five trainings, three virtual meetings and several field monitoring visits	The project activities have coordinated and monitored successfully through inception meeting, annual review workshop, virtual meetings and field monitoring visit; About 200 veterinary professionals has trained up;	The project activities has been carried out smoothly and effectively
Organize a workshop for the collection/ preparation/ shipment of samples from the field to the laboratory for PI, Co-PI, and project relevant personals;	Organized resource sharing meetings and consultation with PI & Co-PI of the project;	Working protocol and Standard Operating Procedures (SOPs) has developed	Microbiological and pharmacological analysis of the samples has been carried out smoothly
The anticipated recommendations/ suggestions for the control of antimicrobial drug residues and resistance will be disseminated through seminar, symposium and mass media circulation.	The project final workshop is organized for finding sharing/ dissemination with scientific community; Leaflet /Brochure is developed for awareness of the people	The research findings has shared through final workshop and brochure.	The scientific community and public will be adhered with project findings.

F. **Materials Development/Publication made under the Sub-project:**

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	Leaflet/ bulletin	N/A	1. Determination of Antibiotic Residues in Milk and Assessment of Human Health Risk in Bangladesh. *Heliyon*, *7*(8), 2021. e07739. 2. Time dependent screening of antibiotic residues in milk of antibiotics treated cows. *J Adv Vet Anim Res* 2019; 6(4):516–520. 3. Dose-dependent response to phytobiotic supplementation in feed on growth, hematology, intestinal pH, and gut bacterial load in broiler chicken. *J Adv Vet Anim Res*, 6(2), 253-259. 4. Beneficial effects of probioticand phytobiotic as growth promoter alternative to antibiotic for safe broiler production. J Adv Vet Anim Res 2019; 6(3):409–415. 5. Serum biochemical changes and growth response study following probiotic and phytobiotic supplementation in broiler chickens. Asian Australas. J. Biosci. Biotechnol. 2019, 4 (1), 1-6
Journal publication	Published (5) Submitted (3)	Yes	
Video clip/TV program	N/A	N/A	
News Paper/ Popular Article	N/A	N/A	
Other publications, if any	N/A	N/A	
Popular articles			1. Antibiotic residues in milk: a public health concern. *'Safe Food Digest'* Vol 1 Issue 1, pp 31-33, October,2020 2. Antibiotic residues and resistance in Livestock food products and public health hazard. *WPSA-BB Newsletter*, September,2019 3. Antibiotic residues and resistance and our duties. Dainik Amadershomoy, 24th June,2019 4. Antibiotic resistance a global threat. *Dainik Amadershomoy*, 25th June,2019 5. Social approach regarding antibiotic residues and resistance in Livestock. *Dainik Amadershomoy*, 26th June,2019 6. Antibiotic resistance a global threat. *Daily kaler kantho*, 28th June,2019

G. **Description of generated Technology/knowledge/policy:**

i. Technology Fact Sheet (title, introduction, description, suitable location/ecosystem, benefits, name and contact address of author)

Title: Use of probiotic and phytobiotic for safe broiler production in Bangladesh

Introduction

The poultry industry has grown so fast alongside an irrational use of antibiotics to maximize profit and made it cost effective production during last few decades. The rising and indiscriminate use of antibiotics might result in deposition of residues in poultry food products having detrimental effects on public health; and due to development of resistance to these drugs by the microorganisms, many diseases are becoming difficult to treat both in humans and animal. These excreted antibiotics eventually accumulated in the environment and enter into human food chain as well as development of multidrug resistance bacteria. In this regard, to find out the alternatives is of paramount importance for the production of safe meat and egg.

Therefore, recently much research attention has been diverted towards the search for alternatives to antibiotic as in-feed growth promoter after banding of the antibiotic growth promoters by the EU, which in turn has resulted in the enhanced use of different types of feed additives in poultry feed. Along with our recently published data and evidences published elsewhere the use of probiotics,

prebiotics, synbiotic, phytobiotics, their combinations and spirulina as growth promoters has many potential benefits.

Description

Application of probiotics as additives for broiler chickens

The provision of animal feed containing probiotics as a feed additive to stimulate growth and prevent disease is not a novel dietary means. Probiotics are live microorganisms that are deliberately given to livestock with the aim of improving the balance of microbes in the digestive tract and reducing bad or pathogenic microbes such as Escherichia coli, Salmonella, and Clostridium and has a nutritional effect (Cheng et al 2014; Simon et al 2017). In broiler chickens, the most commonly used probiotics include Lactobacillus (L. acidophilus, L. casei, L. farciminis, L. plantarum, L. rhamnosus), Bacillus (B. cereus var. Toyoi, B. licheniformis, B. subtilis), Enterococcus (E. faecium), Pediococcus (P. acidilactici), Streptococcus (S. infantarius), several fungi including Saccharomyces cerevisiae and Kluyveromyces (Pandey et al 2015).

A number of researchers have reported the effect of probiotics on the productivity of broiler chickens. Pourakbari et al. (Pourakbari et al 2016) reported that probiotics can provide better body weight gain with a lower feed conversion ratio. The increase in body weight in broiler chickens caused by giving probiotics was also reported by Upadhaya et al (2015) and Islam et al (2014). Further, Hussein et al (2020) reported that the use of probiotics can prevent infection with necrotic enteritis, which can improve the harmful effects of broilers infected with C. perfringens in terms of performance, feed efficiency, meat quality and carcass characteristics. Peng et al (2016) stated that the use of L. plantarum had a positive effect on the growth of broiler chickens, reduces the content of E. coli in the cecum, and increases the number of lactic acid bacteria in the cecum and ileum. Probiotics have also been reported to ameliorate the negative effects of heat stress in broilers (Sugiharto et al 2017).

Phytobiotic applications as additives for broilers

Phytobiotics are natural bioactive compounds derived from plants that can stimulate appetite, help endogenous secretions such as enzymes, and have antimicrobial activity so that they can improve the performance and health of farm animals (Wati et al 2015; Jarriyawattanachaikul et al 2016). Phytobiotics can be used to replace the role of AGP as a growth promoter for broiler. This is due to the large number of active ingredients found in plant extracts, such as antioxidants, immunostimulants, and antimicrobials (Li et al 2015; Wati et al 2015; Jarriyawattanachaikul et al 2016). In previous studies, several bioactive compounds from plants have been identified as potential candidates that can stimulate the growth of beneficial bacteria such as lactobacilli and bifidobacteria and inhibit the growth of pathogenic bacteria (Fasina et al 2015; Hussein et al 2020).

The results of previous studies showed that phytobiotics are very beneficial when given to broiler chickens because they can inhibit pathogenic bacteria, improve intestinal health, increase antioxidant status and can improve digestive function, as well as improve chickens immune functions (Murugesan et al 2015; Jarriyawattanachaikul et al 2016; Nobakht et al 2016). Research conducted by Fallah et al (2013) to evaluate the effect of artichoke leaf meal (Cynara Scolymus L.) and menthe extract (Mentha piperita) as a phytobiotic in broiler chickens showed that there was a decrease in high-density lipoprotein (HDL) and low-density lipoprotein (LDL) concentrations. The use of nutmeg meat flour (Myristica frangrans Houtt) and clove leaves (Syzygium aromaticum L) as phytobiotics can reduce feed costs and reduce the use of antibiotics during the rearing of broiler chickens (Sapsuha et al 2019). However, the use of phytobiotics as feed additives on the growth performance of broiler chickens can vary depending on the biological factors of the plant itself, such as plant species, location for cultivation and conditions during harvesting, as well as storage conditions such as light, temperature, and storage time (Sugiharto 2016).

Suitable location/ecosystem: Anywhere in Bangladesh especially for broiler farms

Name and contact address of author: Dr. Kazi Rafiqul Islam**,** Professor**,** Department of Pharmacology**,** Bangladesh Agricultural University, Mymensingh-2202**,** Mobile: 01711-285766, E-mail: krafiq73@yahoo.com

ii. Effectiveness in Policy Support (if applicable)

H. **Technology/Knowledge generation/Policy Support (as applied):**

i. Immediate impact on generated technology (commodity & non-commodity)

1. Policy generated to minimize the food safety hazard of animal derived foodstuffs;
2. Use of probiotic, phytobiotic in feed as a growth promoter in broiler feed instead of antibiotics;
3. To reduce AMR burden through increasing awareness among the stakeholders about the prudent use of antimicrobial in livestock and poultry;

ii. Generation of new knowledge that help in developing more technology in future

1. Use of probiotic, phytobiotic in feed as a growth promoter alternative to antibiotics;
2. Technology transferred that help increase agricultural productivity and farmers' income;

iii. Policy Support

1. Continuous monitoring and surveillance of antimicrobial drug residue in milk, meat and eggs at local markets and farms;
2. Strictly monitoring local feed producer and factories to prohibit the use of antimicrobial drugs in feed as a growth promoter;
3. Probiotic, phytobiotic or spirulina could be used in feed as a growth promoter in broiler industries instead of antibiotics;
4. Increase awareness among the livestock and poultry farmers to reduce the abuse and misuse of antimicrobials;
5. To promote the food safety concern among the all stakeholders through mass media;
6. The compulsory registration of all commercial livestock and poultry farms and feed business operators by the livestock department;
7. To prohibit the use of antibiotics in farming practices without prescription of registered veterinarian;
8. Adopting good husbandry practices including proper biosecurity measures, cleaning, sanitation, hygiene, disinfection, quarantine, therapeutic regimens and appropriate vaccination programme;
9. Building awareness and motivation to the livestock and poultry farmers, drug sellers, feed sellers and feed companies about antibiotic resistance and residues;

I. **Information regarding Desk and Field Monitoring**

i. Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):

ii. Field Monitoring (date& no. of visit, name and addresses of team visit and output):

Monitoring team	Organization	Date (s) of visit	Outcome and suggestion
1. Dr. Mian Sayeed Hassan, NATP 2. Dr. Md. Abdul jalil Bhuyan, NATP 3. Dr. G.P. DAS, Research NATP 4. Dr. Md. Nowsher Ali Sarder, PIU-BARC, NATP-2.	BAU	17.02.2019	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project.

1. Dr. Md Rafiqul Islam, BARC 2. Dr. Md. Ismail Hossen, BARC	BLRI	14.05.2019	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project.
1. Dr. Md. Rafiqul Islam, BARC 2. Dr. Md. Ismail Hossen, BARC 3.Dr. Mohammad Showkat Mahmud, BARC	BAU	13.02.2020	Meeting with PI and CoPI about the progress of the project. Monitored the physical progress of the project.
1. Dr. Nazmun Nahar Karim, BARC 2. Dr. Md Rafiqul Islam, BARC 3. Dr. Md. Ismail Hossen, BARC	SAU	03.03.2020	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project.
1. Dr. Md Rafiqul Islam, BARC 2. Dr. Md. Ismail Hossen, BARC	RU	16.03.2020	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project.
1. Dr. Md. Rafiqul Islam, BARC 2. Dr. Kazi M.Kamaruddin, BARC 3. Dr. Md. Nure Alam Siddiky, BARC 4. Dr. Mohammad Showkat Mahmud, BARC	BLRI	28.7.2020	Meeting with PI and CoPI about the progress of the project. Monitored the physical progress of the project.
1. Dr. Md. Rafiqul Islam, BARC 2. Dr. Kazi M.Kamaruddin, BARC 3. Dr. Md. Nure Alam Siddiky, BARC	BAU	11.1.2021	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project
1. Dr. Md Rafiqul Islam, BARC 2. Dr. Md. Ismail Hossen, BARC	HSTU	25.02.2021	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project.
1. Md. Abdur Rahman, NATP 2. Dipak Kumar, NATP 3. Md. Hasan Mahmud, NATP	PSTU	06.10.2020	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project.
1. Dr. Md. Nure Alam Siddiky, BARC	PSTU	24.01.2021	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project.
1. Dr. Md Rafiqul Islam, BARC	HSTU	25.02.2021	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project
1. Dr. Md. Harunur Rashid, NATP 2. Mr. Mohammad Shahidul Islam, NATP 3. Dr. Mohammad Abdullah Al Faroque, NATP 4. Mr. Md. Ashequr Rahman, NATP	HSTU	18.03.2021	Meeting with PI and Co-PI about the progress of the project. Monitored the physical progress of the project

iii. Weather data, flood/salinity/drought level (if applicable) and natural calamities: N/A

J. Sub-project auditing (covers all types of audit performed)

Components	Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
BARC	Internal	No	3,843,782	Satisfactory	
BLRI	Internal	No	1,288,026	Satisfactory	
	Internal	No	1,115,718	Satisfactory	
BAU	FAPAD	No	1,697,026	Satisfactory	
	FAPAD	No	1,997,350	Satisfactory	
RU	FAPAD	No	731,238	Satisfactory	
	FAPAD	No	1,210,582.5	Satisfactory	
PSTU	PAPAD	No	792,017	Satisfactory	
	PAPAD	No	1,781,218	Satisfactory	
CVASU	PAPAD	No	19,86,352	Satisfactory	
	PAPAD	No	5,82,059	Satisfactory	
SAU	PAPPD	No		Satisfactory	
	PAPPD	No		Satisfactory	
HSTU	FAPAD	No	9,92,753	Satisfactory	
	FAPAD	No	9,22,421	Satisfactory	

K. **Lessons Learned**:

1. The findings of the research have given an insight about the presence of antimicrobial residue in livestock and poultry foods and feed in accordance with public health hazard;
2. The prevalence of common foodborne AMR pathogens with multidrug properties in livestock and poultry food chain has given public health attention;
3. The research findings would provide knowledge base scientific evidence in the rational or judicious use of antimicrobials for the treatment of human foodborne illness and therapeutic & preventive use in livestock & poultry farming practices;
4. Use of probiotic, phytobiotic in feed as a growth promoter in broiler industries instead of antibiotic growth promoter;
5. Farmers and informal prescribers has inadequate knowledge and poor practices about antibiotic resistance and residue;

L. Challenges (if any): The activities of the project were solely technical, sophisticated, demand driven and closely adhere with laboratory competency. The state of art laboratory for microbial analysis and chemical analysis including skilled human resources were prerequisite to accomplish the project activities very successfully. Some laboratory analysis could not perform well due to inadequate capacity and resources of the different project components. Besides, the project operation has faced challenges due to pandemic and aftermath.

M. Suggestions for future planning (if any):

1. The surveillance and monitoring of antibiotic drug residue and resistance in livestock and poultry food and feed chain should be undertaken in a regular and phase manner;
2. The capacity and competency of the laboratory and human resources should be improved with the demand of time to encounter residue and resistance;
3. Strictly monitoring local feed producer and factories to stop the use of antimicrobial drugs in feed;
4. Popularize the use of probiotic, phytobiotic or spirulina in feed as a growth promoter in broiler industries instead of antibiotic growth promoter;
5. To promote research to develop alternatives to antibiotics (probiotics and prebiotics) in animal production system;
6. To develop new antimicrobials for effective treatment against AMR pathogens;

7. To improve biosecurity and vaccination practices in the animal farming systems;
8. Development of good agricultural practices in animal farm production system;
9. Building awareness among the relevant stakeholders about the food safety concern;

Conclusions

The indiscriminate and irrational use of antibiotic in livestock production cycle as well as without following the withdrawal period of antibiotic can cause unexpected residue in animal derived food products. Higher antimicrobial residual concentration in animal derived food may have the catastrophic impact on public health. The antimicrobial residue may spillover in the environment and lead to resistant bacteria through the selective pressure. Humans can be exposed to antibiotic residues or directly to antibiotic-resistant bacteria, including pathogens, through foods or environments, and potentially become infected. Rather, antibiotic residue in the animal feed is unexpected since by law antibiotic use is prohibited in animal feed. It is suggested to ensure the prudent use of antibiotics is animal farm practices and strictly follow the withdrawal period before harvesting and marketing of animal products for human consumption.

Signature:……
Name of the Coordinator :
Dr. Nazmun Nahar Karim
Designation: Member Director
Livestock Division, (C. C.)
BARC, Farmgate, Dhaka-1215
Date:
Seal:

Signature……..
Name: Dr. Shaikh Mohammad Bokhtiar
(Head of the organization or authorized representative)
Designation: Executive Chairman (in charge)
BARC, Farmgate, Dhaka-1215
Date:
Seal:

Annexure 1: Photo Album

a. Questionnaire survey








b. Field Monitoring Visit








Annexure 2: Statistical Analyses

Table 1: Education status of large animal farmers of Bangladesh

Educational status of farm owner/caretaker	Division								Total	Chi-square value (p-value)
	Mymensingh	Dhaka	Rajshahi	Barisal	Khulna	Chittagong	Sylhet	Rangpur		
Illiterate	12	65	30	0	2	24	6	24	163	352.30*** (0.0001)
Primary (PSC)	13	10	0	8	4	10	13	21	79	
Junior Secondary (JSC)	8	0	0	12	9	7	10	11	57	
Secondary (SSC)	7	0	21	19	14	11	8	11	91	
HSC/Diploma	25	0	12	23	20	12	18	6	116	
Graduate	10	0	10	13	26	6	11	2	78	
Masters	0	0	2	0	0	5	9	0	16	
	75	75	75	75	75	75	75	75	600	

Table 2: Educational status and type of farm cross table of large animal farmers of Bangladesh

Educational status of farm owner/caretaker	Type of farm				Total	Chi-square value (p-value)
	Cattle	Sheep	Goat	Buffalo		
Illiterate	53	31	53	26	163	65.383*** (0.0001)
Primary (PSC)	17	13	21	28	79	
Junior Secondary (JSC)	24	10	17	6	57	
Secondary (SSC)	38	9	23	21	91	
HSC/Diploma	66	11	22	17	116	
Graduate	47	5	20	6	78	
Masters	10	3	2	1	16	
Total	255	82	158	105	600	

Table 3: Distribution of educational status of farm owner/caretaker according to their poultry farm types of Bangladesh

Educational status of farm owner/caretaker	Type of farm			Total	Chi-square value (p-value)
	Layer	Broiler	Duck		
Illiterate	28	39	47	114	36.38*** (0.0001)
Primary (PSC)	21	32	47	100	
Junior Secondary (JSC)	29	34	36	99	
Secondary (SSC)	42	34	35	111	
HSC/Diploma	46	38	25	109	
Graduate	32	22	10	64	
Masters	2	1	0	3	
Total	200	200	200	600	

**** Significant at 1% level of significance.*

Table 4: Information about management practices in large animal farms of Bangladesh

SL No.	Item/Statements		Responses numbers (%)										Chi-square value (p-value)
			Cattle		Sheep		Goat		Buffalo		Total		
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
1.	Have Knowledge about bio-security.		177 (69.4)	78 (30.6)	29 (35.4)	53 (64.6)	97 (61.4)	61 (38.6)	45 (42.9)	60 (57.1)	348 (58)	252 (42)	41.51^{***} (0.0001)
2.	Do you have any fence remains in the farm		208 (81.6)	47 (18.4)	30 (36.6)	52 (63.4)	103 (65.2)	55 (34.8)	47 (44.8)	58 (55.2)	388 (64.7)	212 (35.3)	78.41^{***} (0.0001)
3.	Have any entrance of bird in the shed		199 (78)	56 (22)	66 (80.5)	16 (19.5)	130 (82.3)	28 (17.7)	89 (84.8)	16 (15.2)	484 (80.7)	116 (19.3)	2.523^{NS} (0.471)
4.	Have any entrance of wild animal in the farm		95 (37.3)	160 (62.7)	29 (35.4)	53 (64.6)	51 (32.3)	107 (67.7)	52 (49.5)	53 (50.5)	227 (37.8)	373 (62.2)	8.423^{**} (0.038)
5.	Water supply	Direct	211 (82.7)		57 (69.5)		125 (79.1)		69 (65.7)		462 (77)		15.30^{**} (0.002)
		Reserved	44 (17.3)		25 (30.5)		33 (20.9)		36 (34.3)		138 (23)		
6.	Cleaning of waterer	Once daily	191 (74.9)		57 (69.5)		131 (82.9)		73 (69.5)		452 (75.3)		29.454^{***} (0.003)
		Once in a week	32 (12.5)		12 (14.6)		14 (8.9)		14 (13.3)		72 (12)		
		Twice in a week	20 (7.8)		9 (11)		13 (8.2)		11 (10.5)		53 (8.8)		
		Once in a month	12 (4.7)		1 (1.2)		00		3 (2.9)		16 (2.7)		
		Twice in a month	00		3 (3.7)		00		4 (3.8)		7 (1.2)		
7.	Supply of feed	a. Self-processed	156 (61.2)	99 (38.8)	47 (57.3)	35 (42.7)	106 (67.1)	52 (32.9)	66 (62.9)	39 (37.1)	375 (62.5)	225 (37.5)	---
		b. Purchased/Commercial	62 (24.3)	193 (62)	18 (22)	64 (78)	28 (17.7)	130 (82.3)	26 (24.8)	79 (75.2)	134 (22.3)	466 (77.7)	---
		ab.	37 (14.5)	218 (85.5)	17 (20.7)	65 (79.3)	24 (15.2)	134 (84.8)	13 (12.4)	92 (87.6)	91 (15.2)	509 (84.8)	---
8.	Cleaning of feeder	Once daily	205 (80.4)		57 (69.5)		135 (85.4)		81 (77.1)		478 (79.7)		47.231^{***} (0.0001)
		Once in a week	25 (9.8)		12 (14.6)		16 (10.1)		9 (8.6)		62 (10.3)		
		Twice in a week	12 (4.7)		10 (12.2)		7 (4.4)		9 (8.6)		38 (6.3)		
		Once in a month	13 (5.1)		1 (1.2)		00		00		14 (2.3)		
		Twice in a month	00		2 (2.4)		00		6 (5.7)		8 (1.3)		
9.	Cleaning of feces/floor/litter	Once daily	145 (56.9)		36 (43.9)		97 (61.4)		66 (62.9)		344 (57.3)		76.922^{***} (0.0001)
		Twice daily	83 (32.5)		16 (19.5)		26 (16.5)		29 (27.6)		154 (25.7)		
		Every alternate day	26 (10.2)		15 (18.3)		20 (12.7)		8 (7.6)		69 (11.5)		
		Twice in a month	1 (0.4)		00		2 (1.3)		2 (1.9)		5 (0.8)		
		Once in a month	00		15 (18.3)		13 (8.2)		00		28 (4.7)		

Table 5: Information about management practices in poultry farms of Bangladesh.

SL No.	Item/Statements		Responses numbers (%)								Chi-square Value (p-value)
			Layer		Broiler		Duck		Total		
			Yes	No	Yes	No	Yes	No	Yes	No	
1.	Have Knowledge about bio-security.		146 (73)	54 (27)	126 (63)	74 (37)	103 (51.5)	97 (48.5)	375 (62.5)	225 (37.5)	19.755*** (0.0001)
2.	Do you have any fence remains in the farm		146 (73)	54 (27)	136 (68)	64 (32)	130 (65)	70 (35)	412 (68.7)	188 (31.3)	3.037NS (0.219)
3.	Have any entrance of bird in the shed		117 (58.5)	83 (41.5)	117 (58.5)	83 (41.5)	83 (41.5)	117 (58.5)	317 (52.8)	283 (47.2)	15.463*** (0.0001)
4.	Have any entrance of wild animal in the farm		40 (20)	160 (80)	37 (18.5)	163 (81.5)	66 (33)	134 (67)	143 (23.8)	457 (76.2)	14.01*** (0.001)
5.	Water supply	Direct	122 (61)		115 (57.5)		125 (62.5)		362 (60.3)		1.10NS (0.577)
		Reserved	78 (39)		85 (42.5)		75 (37.5)		238 (39.7)		
6.	Cleaning of waterer	Once daily	118 (59)		117 (58.5)		89 (44.5)		324 (54)		65.227*** (0.0001)
		Once in a week	51 (25.5)		54 (27)		40 (20)		145 (24.2)		
		Twice in a week	19 (9.5)		23 (11.5)		71 (35.5)		113 (18.8)		
		Once in a month	7 (3.5)		5 (2.5)		00		12 (2)		
		Twice in a month	5 (2.5)		1 (0.5)		00		6 (1)		
7.	Supply of feed	a. Self-processed	90 (45)	110 (55)	43 (21.5)	157 (78.5)	58 (29)	142 (71)	191 (31.8)	409 (68.20	26.559*** (0.0001)
		b. Purchased/Commercial	110 (55)	90 (45)	157 (78.5)	43 (21.5)	109 (54.5)	91 (45.5)	376 (62.7)	224 (37.3)	32.157*** (0.0001)
		ab. Self-processed & Purchased/Commercial	00	200 (100)	00	200 (100)	33 (16.5)	167 (83.5)	33 (5.5)	567 (94.5)	69.841*** (0.0001)
8.	Cleaning of feeder	Once daily	3 (1.5)		46 (23)		101 (50.5)		150 (25)		215.817*** (0.0001)
		Once in a week	70 (35)		67 (33.5)		43 (21.5)		180 (30)		
		Twice in a week	26 (13)		32 (16)		56 (28)		114 (19)		
		Once in a month	22 (11)		10 (5)		00		32 (5.3)		
		Twice in a month	79 (39.5)		45 (22.50		00		124 (20.7)		
9.	Cleaning of feces/floor/litter	Once daily	41 (20.5)		00		77 (38.5)		118 (19.7)		530.381*** (0.0001)
		Twice daily	8 (4)		00		00		8 (1.3)		
		Every alternate day	148 (74)		00		94 (47)		242 (40.3)		
		Twice in a month	3 (1.5)		170 (85)		29 (14.5)		202 (33.7)		
		Once after sell	00		30 (15)		00		30 (50		

Table 6: Information about use of antibiotics in large animal farming of Bangladesh

SL No.	Statements	Positive Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
1. From whom you usually get suggestions or treatment for your sick animals												
a.	Veterinary practitioner	214 (83.9)	41 (16.1)	54 (65.9)	28 (34.1)	127 (80.4)	31 (19.6)	68 (64.8)	37 (35.2)	463 (77.2)	137 (22.8)	22.656^{***} (0.0001)
b.	Veterinary paraprofessional	113 (44.3)	142 (55.7)	34 (41.5)	48 (58.5)	52 (32.9)	106 (67.1)	45 (42.9)	60 (57.1)	244 (40.7)	356 (59.3)	5.574^{NS} (0.134)
c.	Local drug seller	93 (36.5)	162 (63.5)	27 (32.9)	55 (67.1)	37 (23.4)	121 (76.6)	37 (35.2)	68 (64.8)	194 (32.3)	406 (67.7)	8.153^{**} (0.043)
d.	Family/friend	4 (1.6)	251 (98.4)	1 (1.2)	81 (98.8)	5 (3.2)	153 (96.8)	00	105 (100)	10 (1.7)	590 (98.3)	4.058^{NS} (0.255)
e.	Other farmers	73 (28.6)	182 (71.4)	2 (2.4)	80 (97.6)	7 (4.4)	151 (95.6)	2 (1.9)	103 (98.1)	84 (14)	516 (86)	79.195^{***} (0.0001)
f.	Self	45 (17.6)	210 (82.4)	6 (7.3)	76 (92.7)	7 (4.4)	151 (95.6)	9 (8.6)	96 (91.4)	67 (11.2)	533 (88.8)	19.961^{***} (0.0001)
g.	Village doctor/Quack	88 (34.5)	167 (65.5)	30 (36.6)	52 (63.4)	61 (38.6)	97 (61.4)	55 (52.4)	50 (47.6)	234 (39)	366 (61)	10.275^{**} (0.016)
h.	Company professional	70 (27.5)	185 (72.5)	5 (6.1)	77 (93.9)	2 (1.3)	156 (98.7)	4 (3.8)	101 (96.2)	81 (13.5)	519 (86.5)	75.044^{***} (0.0001)
2. Have you ever heard about antibiotic?		252 (98.8)	3 (1.2)	79 (96.3)	3 (3.7)	153 (96.8)	5 (3.2)	100 (95.2)	5 (4.8)	584 (97.3)	16 (2.7)	---

Table 6: Information about use of antibiotics in large animal farming of Bangladesh continued.……

SL No.	Statements	Positive Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
3. What is antibiotic? (Respondent: 584; Cattle-252, Sheep-79, Goat-153, Buffalo-100)												
a.	Act against bacteria	145 (57.5)	107 (42.5)	32 (52.3)	47 (59.5)	80 (52.3)	73 (47.7)	30 (30)	70 (70)	287 (49.1)	297 (50.9)	---
b.	Act against virus	28 (11.1)	224 (88.9)	15 (19)	64 (81)	22 (14.44)	131 (85.6)	23 (23)	77 (77)	88 (15.1)	496 (84.9)	---
c.	Others	7 (2.8)	245 (97.2)	1 (1.3)	78 (98.7)	5 (3.3)	148 (96.7)	3 (3)	97 (97)	16 (2.7)	568 (97.3)	---
d.	Don’t know	42 (16.7)	210 (83.3)	17 (21.5)	62 (78.5)	9 (5.9)	144 (94.1)	21 (21)	79 (79)	89 (15.2)	495 (84.8)	---
ab.		21 (8.3)	231 (91.7)	11 (13.9)	68 (86.1)	28 (18.3)	125 (81.7)	14 (14)	86 (86)	74 (12.7)	510 (87.3)	---
bc.		6 (2.4)	246 (97.6)	2 (2.5)	77 (97.5)	3 (2)	150 (98)	5 (5)	95 (95)	16 (2.7)	568 (97.3)	---
ac.		2 (0.8)	250 (99.2)	00	79 (100)	2 (1.3)	151 (98.7)	1 (1)	99 (99)	5 (0.9)	579 (99.1)	---
abc.		1 (0.4)	251 (99.6)	1 (1.3)	78 (98.7)	4 (2.6)	149 (97.4)	3 (3)	97 (97)	9 (1.5)	575 (98.5)	---
4. Have you ever heard about antimicrobial resistance?		173 (67.8)	82 (32.2)	40 (48.8)	42 (51.2)	105 (66.5)	53 (33.5)	74 (70.5)	31 (29.5)	392 (65.3)	208 (34.7)	11.943*** (0.008)

Table 6: Information about use of antibiotics in large animal farming of Bangladesh continued..……

SL No.	Statements	Positive Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
5. What do you know about antibiotic resistance? (Respondent: 392; Cattle-173, Sheep-40, Goat-105, Buffalo-74)												
a.	It causes treatment failure	94 (54.3)	79 (45.7)	15 (37.5)	25 (62.5)	64 (61)	41 (39)	29 (39.2)	45 (60.8)	202 (51.5)	190 (85.5)	---
b.	It causes poor response to treatment	23 (13.3)	150 (86.7)	9 (22.5)	31 (77.5)	13 (12.4)	92 (87.6)	6 (8.1)	68 (91.9)	51 (13)	341 (87)	---
c.	Do not know	47 (27.2)	126 (72.8)	12 (30)	28 (70)	18 (17.1)	87 (82.9)	28 (37.8)	46 (62.2)	105 (26.8)	287 (73.2)	---
d.	Others	2 (1.2)	171 (98.8)	1 (2.5)	39 (97.5)	00	105 (100)	2 (2.7)	72 (97.3)	5 (1.3)	387 (98.7)	---
ab.		7 (4)	166 (96)	2 (5)	38 (95)	8 (7.6)	97 (92.4)	9 (12.2)	65 (87.8)	26 (6.6)	366 (93.4)	---
ad.		00	173 (100)	1 (2.5)	39 (97.5)	2 (1.9)	103 (98.1)	00	74 (100)	3 (0.8)	389 (99.2)	---
6. Have you ever heard about antibiotic residue?		127 (49.8)	128 (50.2)	34 (41.5)	48 (58.5)	61 (38.6)	97 (61.4)	42 (40)	63 (60)	264 (44)	336 (56)	---
7. What is an antibiotic residue? (Respondent: 264; Cattle-127, Sheep-34, Goat-61, Buffalo-42)												
a.	Accumulation of antibiotics in human body	21 (16.5)	106 (83.5)	10 (29.4)	24 (70.6)	14 (23)	47 (77)	10 (23.8)	32 (76.2)	55 (20.8)	209 (79.2)	---
b.	Accumulation of antibiotics in Animal body	27 (21.3)	100 (78.7)	10 (29.4)	24 (70.6)	10 (16.4)	51 (83.6)	9 (21.4)	33 (78.6)	56 (21.2)	208 (78.8)	---
c.	Both	50 (39.4)	77 (60.6)	9 (26.5)	25 (73.5)	25 (41)	36 (59)	15 (35.7)	27 (64.3)	99 (37.5)	165 (62.5)	---
d.	Don't know	29 (22.8)	98 (77.2)	5 (14.7)	29 (85.3)	12 (19.7)	49 (80.3)	8 (19)	34 (81)	54 (20.5)	210 (79.5)	---
8. Do you keep record of using any drug?												
a.	Always	44 (17.3)		5 (6.1)		18 (11.4)		4 (3.8)		71 (11.8)		79.46^{***}

b.	Most Frequently	25 (9.8)		10 (12.2)		26 (16.5)		10 (9.5)		71 (11.8)		(0.0001)
c.	Sometimes	84 (32.9)		15 (18.3)		22 (13.9)		19 (18.1)		140 (23.3)		
d.	Rarely	48 (18.8)		12 (14.6)		31 (19.6)		14 (13.3)		105 (17.5)		
e.	Never	43 (16.9)		19 (23.2)		36 (22.8)		34 (32.4)		132 (22)		
f.	Do not know	11 (4.3)		21 (25.6)		25 (15.8)		24 (22.9)		81 (13.5)		
9. Do you have any record of antibiotics used within past one year?		63 (24.7)	192 (75.3)	10 (12.2)	72 (87.8)	31 (19.6)	127 (80.4)	9 (8.6)	96 (91.4)	113 (18.8)	487 (81.2)	15.414^{***} (0.001)
SL No.	Statements	Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
10. Do you add antibiotics during self-feed processing?		20 (7.8)	235 (92.2)	3 (3.7)	79 (96.3)	3 (1.9)	155 (98.1)	2 (1.9)	103 (98.1)	28 (4.7)	572 (95.3)	10.492^{**} (0.015)
11. Purpose of antibiotic use-												
a.	Treatment	137 (53.7)	118 (46.3)	70 (85.4)	12 (14.6)	98 (62)	60 (38)	83 (79)	22 (21)	388 (64.7)	212 (35.3)	---
b.	Prevention	5 (2)	250 (98)	00	82 (100)	3 (1.9)	155 (98.1)	2 (1.9)	103 (98.1)	10 (1.8)	590 (98.3)	---
c.	Both (a+b)	100 (39.2)	155 (60.8)	12 (14.6)	70 (85.4)	50 (31.6)	108 (68.4)	16 (15.2)	89 (84.8)	178 (29.7)	422 (70.3)	---
d.	Growth promoter	1 (0.4)	254 (99.6)	00	82 (100)	00	158 (100)	00	105 (100)	1 (0.2)	599 (99.8)	---
ad.		10 (3.9)	245 (96.1)	00	82 (100)	7 (4.4)	151 (95.6)	4 (3.8)	101 (96.2)	21 (3.5)	579 (96.5)	---
cd.		2 (0.8)	253 (99.2)	00	82 (100)	00	158 (100)	00	105 (100)	2 (0.3)	598 (99.7)	---
12. Regular use of antibiotics to prevent any specific disease.		18 (7.1)	237 (92.9)	4 (4.9)	78 (95.1)	11 (7)	147 (93)	6 (5.7)	99 (94.3)	39 (6.5)	561 (93.5)	---
13. Which are the specific diseases? (Respondent-39; Cattle-18, Sheep-4, Goat-11, Buffalo-6)												
a.	Mastitis	(4.3)	244 (95.7)	1 (1.2)	81 (98.8)	4 (2.5)	154 (97.5)	1 (1)	104 (99)	17 (2.8)	583 (97.2)	4.207^{NS} (0.24)
b.	Fever	7 (2.7)	248 (97.3)	3 (3.7)	79 (96.3)	4 (2.5)	154 (97.5)	00	105 (100)	14 (2.3)	586 (97.7)	3.357^{NS} (0.34)
c.	Diarrhoea	13 (5.1)	242 (94.9)	3 (3.7)	79 (96.3)	8 (5.1)	150 (94.9)	1 (1)	104 (99)	25 (4.2)	575 (95.8)	3.642^{NS} (0.303)
d.	Foot and mouth disease	13 (5.1)	242 (94.9)	00	82 (100)	3 (1.9)	155 (98.1)	1 (1)	104 (99)	17 (2.8)	583 (97.2)	8.992^{**} (0.029)

e.	PPR	00	255 (100)	3 (3.7)	79 (96.3)	6 (3.8)	152 (96.2)	00	105 (100)	9 (1.5)	591 (98.5)	13.713*** (0.003)
f.	Anthrax	2 (0.8)	253 (99.2)	1 (1.2)	81 (98.8)	00	158 (100)	00	105 (100)	3 (0.5)	597 (99.5)	2.589NS (0.459)
g.	Black Quarter	4 (1.6)	251 (98.4)	00	82 (100)	00	158 (100)	00	105 (100)	4 (0.7)	596 (99.3)	5.448NS (0.142)
h.	Others	2 (0.8)	253 (99.2)	2 (2.4)	80 (97.6)	1 (0.6)	157 (99.4)	00	105 (100)	5 (0.8)	595 (99.2)	3.525NS (0.318)

SL No.	Statements	Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
14.	Does any of your prescriber mentioned about withdrawal period?	99 (38.8)	156 (61.2)	17 (20.7)	65 (79.3)	50 (31.6)	108 (68.4)	22 (21)	83 (79)	188 (31.3)	412 (68.7)	16.199*** (0.001)
15.	Use of antibiotics recommended by-											
a.	Veterinarian	102 (40)	153 (60)	25 (30.5)	57 (69.5)	54 (34.2)	104 (65.8)	23 (21.9)	82 (78.1)	204 (34)	396 (66)	---
b.	Other farmers	00	255 (100)	1 (1.2)	81 (98.8)	1 (0.6)	157 (99.4)	00	105 (100)	2 (0.3)	598 (99.7)	---
c.	Shopkeeper	8 (3.1)	247 (96.9)	2 (2.4)	80 (97.6)	8 (5.1)	150 (94.9)	3 (2.9)	102 (97.1)	21 (3.5)	579 (96.5)	---
d.	Representative of pharmaceutical company	00	255 (100)	00	82 (100)	00	158 (100)	00	105 (91.4)	00	600 (100)	---
e.	Veterinary Paraprofessional	5 (2)	250 (98)	6 (7.3)	76 (92.7)	6 (3.8)	152 (96.2)	8 (7.6)	97 (92.4)	25 (4.2)	575 (95.8)	---
f.	Village Doctor/ Quack	1 (0.4)	254 (99.6)	6 (7.3)	76 (92.7)	10 (6.3)	148 (93.7)	91 (86.7)	51 (48.6)	31 (5.2)	569 (94.8)	---
g.	Self	5 (2)	250 (98)	3 (3.7)	79 (96.3)	3 (1.9)	155 (98.1)	3 (2.9)	102 (97.1)	14 (2.3)	586 (97.7)	---
16.	Please mention the name of five common antibiotics you frequently use in livestock.											
a.	Penicillin	140 (54.9)	115 (45.1)	47 (57.3)	35 (42.7)	75 (47.5)	83 (52.5)	59 (56.2)	46 (43.8)	321 (53.5)	279 (46.5)	3.298NS (0.348)

b.	Tetracycline	61 (23.9)	194 (76.1)	21 (25.6)	61 (74.4)	53 (33.5)	105 (66.5)	28 (26.7)	77 (73.3)	163 (27.2)	437 (72.8)	4.719^{NS} (0.194)
c.	Doxycycline	23 (9)	232 (91)	12 (14.6)	70 (85.4)	16 (10.1)	142 (89.9)	9 (8.6)	96 (91.4)	60 (10)	540 (90)	2.47^{NS} (0.481)
d.	Oxytetracycline	180 (70.6)	75 (29.4)	71 (86.6)	11 (13.4)	99 (62.7)	59 (37.3)	64 (61)	41 (39)	414 (69)	186 (31)	18.306^{***} (0.0001)
e.	Streptomycin	86 (33.7)	169 (66.3)	47 (57.3)	35 (42.7)	65 (41.1)	93 (58.9)	46 (43.8)	59 (56.2)	244 (40.7)	356 (59.3)	14.958^{***} (0.002)

SL No. / Statements		Positive Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
f.	Gentamycin	142 (55.7)	113 (44.3)	34 (41.5)	48 (58.5)	67 (42.4)	91 (57.6)	43 (41)	62 (59)	286 (47.7)	314 (52.3)	11.49^{***} (0.009)
g.	Cephalexin	1 (0.4)	254 (99.6)	2 (2.4)	80 (97.6)	3 (1.9)	155 (98.1)	4 (3.8)	101 (96.2)	10 (1.7)	590 (98.3)	5.82^{NS} (0.121)
h.	Ceftriaxone	160 (62.7)	95 (37.3)	25 (30.5)	57 (69.5)	65 (41.1)	93 (58.9)	41 (39)	64 (61)	291 (48.5)	309 (51.5)	38.551^{***} (0.0001)
i.	Ciprofloxacin	111 (43.5)	144 (56.5)	53 (64.6)	29 (35.4)	80 (50.6)	78 (49.4)	49 (46.7)	56 (53.3)	293 (48.8)	307 (51.2)	11.467^{***} (0.009)
j.	Cloxacillin	8 (3.1)	247 (96.9)	2 (2.4)	80 (97.6)	3 (1.9)	155 (98.1)	5 (4.8)	100 (95.2)	18 (3)	582 (97)	1.884^{NS} (0.597)
k.	Cefixime	3 (1.2)	252 (98.8)	1 (1.2)	81 (98.8)	00	158 (100)	00	105 (100)	4 (0.7)	596 (99.3)	3.144^{NS} (0.37)
l.	Sulphar drug (Combined)	164 (64.3)	91 (35.7)	52 (63.4)	30 (36.6)	99 (62.7)	59 (37.3)	63 (60)	42 (40)	378 (63)	222 (37)	0.608^{NS} (0.895)
m.	Sulfadimidine	19 (7.5)	236 (92.5)	5 (6.1)	77 (93.9)	11 (7)	147 (93)	12 (11.4)	93 (88.6)	47 (7.8)	553 (92.2)	2.44^{NS} (0.486)
n.	Sulfadiazine	26 (10.2)	229 (89.8)	3 (3.7)	79 (96.3)	9 (5.7)	149 (94.3)	3 (2.9)	102 (97.1)	41 (6.8)	559 (93.2)	8.756^{**} (0.033)
o.	Sulfamethoxazole	3 (1.2)	252 (98.8)	00	82 (100)	00	158 (100)	00	105 (100)	3 (0.5)	597 (99.5)	4.079^{NS} (0.253)
p.	Ampicillin	22 (8.6)	233 (91.4)	6 (7.3)	76 (92.7)	13 (8.2)	145 (91.8)	8 (7.6)	97 (92.4)	49 (8.2)	551 (91.8)	0.194^{NS} (0.979)
q.	Amoxicillin	81 (31.8)	174 (68.2)	44 (53.7)	38 (46.3)	73 (46.2)	85 (53.8)	51 (48.6)	54 (51.4)	249 (41.5)	351 (58.5)	18.55^{***} (0.0001)
r.	Ceftiofur	40 (15.7)	215 (84.3)	3 (3.7)	79 (96.3)	3 (1.9)	155 (98.1)	00	105 (100)	46 (7.7)	554 (92.3)	41.173^{***} (0.0001)

s.	Lincomycin	3 (1.2)	252 (98.8)	1 (1.2)	81 (98.8)	00	158 (100)	1 (1)	104 (99)	5 (0.8)	595 (99.2)	1.857^{NS} (0.603)
t.	Azithromycin	14 (5.5)	241 (94.5)	4 (4.9)	78 (95.1)	10 (6.3)	148 (93.7)	1 (1)	104 (99)	29 (4.8)	571 (95.2)	4.446^{NS} (0.217)
u.	Amikacin	00	255 (100)	1 (1.2)	81 (98.8)	00	158 (100)	00	105 (100)	1 (0.2)	599 (99.8)	6.328^{NS} (0.110)
v.	Others	1 (0.4)	254 (99.6)	2 (2.4)	80 (97.6)	4 (2.5)	154 (97.5)	00	105 (100)	7 (1.2)	593 (98.8)	6.27^{*} (0.099)

Table 6: Information about use of antibiotics in large animal farming of Bangladesh continued.……

SL No./ Statements		Positive Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
18. Number of antibiotics use at a time.												
a.	Single	142 (55.7)	113 (44.3)	48 (58.5)	34 (41.5)	95 (60.1)	63 (39.9)	69 (65.7)	36 (34.3)	354 (59)	246 (41)	---
b.	Combined/Multiple	44 (17.3)	211 (82.7)	11 (13.4)	71 (86.6)	13 (8.2)	145 (91.8)	9 (8.6)	96 (91.4)	77 (12.8)	523 (87.2)	---
c.	Do not know	27 (10.6)	228 (89.4)	5 (6.1)	77 (93.9)	33 (20.9)	125 (79.1)	20 (19)	85 (81)	85 (14.2)	515 (85.8)	---
ab.	ab.	42 (16.5)	213 (83.5)	18 (22)	64 (78)	17 (10.8)	141 (89.2)	7 (6.7)	98 (93.3)	84 (14)	516 (86)	---
19. Frequency of use of antibiotics in livestock.												
a.	Daily	9 (3.5)	246 (96.5)	5 (6.1)	77 (93.9)	1 (0.6)	157 (99.4)	1 (1)	104 (99)	16 (2.7)	584 (97.3)	8.157^{**} (0.043)
b.	Weekly	3 (1.2)	252 (98.8)	2 (2.4)	80 (97.6)	1 (0.6)	157 (99.4)	2 (1.9)	103 (98.1)	8 (1.3)	592 (98.7)	1.66^{NS} (0.646)
c.	Monthly	1 (0.4)	254 (99.6)	1 (1.2)	81 (98.8)	00	158 (100)	00	105 (100)	2 (0.3)	598 (99.7)	2.844^{NS} (0.416)
d.	When needed	242 (94.9)	13 (5.1)	74 (90.2)	8 (9.8)	156 (98.7)	2 (1.3)	102 (97.1)	3 (2.9)	574 (95.7)	26 (4.3)	10.315^{**} (0.016)

Table 6: Information about use of antibiotics in large animal farming of Bangladesh continued.……

SL No.	Statements	Positive Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
20. Route of administration of antibiotics for livestock.												
a.	Feed	1 (0.4)	254 (99.6)	2 (2.4)	80 (97.6)	1 (0.6)	157 (99.4)	00		105 (100)	4 (0.7)	596 (99.3)	---
b.	Water	10 (3.9)	245 (96.1)	5 (6.1)	77 (93.9)	4 (2.5)	154 (97.5)	3 (2.9)		105 (97.1)	22 (3.7)	578 (96.3)	---
c.	Feed & Water	6 (2.4)	249 (97.6)	3 (3.7)	79 (96.3)	2 (1.3)	156 (98.7)	13 (12.4)		92 (87.6)	24 (4)	576 (96)	---
d.	Injection	177 (69.4)	78 (30.6)	45 (54.9)	37 (45.1)	121 (76.6)	37 (23.4)	57 (54.3)		48 (45.7)	400 (66.7)	200 (33.3)	---
e.	Others	00		00		00		00			00		
cd		3 (1.2)	252 (98.8)	3 (3.7)	79 (96.3)	1 (0.6)	157 (99.4)	5 (4.8)		100 (95.2)	12 (2)	588 (98)	---
bd		11 (4.3)	244 (95.7)	7 (8.5)	75 (91.5)	8 (5.1)	150 (94.9)	6 (5.7)		99 (94.3)	32 (5.3)	568 (94.7)	---
de		6 (2.4)	249 (97.6)	00	82 (100)	1 (0.6)	157 (99.4)	00		105 (100)	7 (1.2)	593 (98.8)	---
ad		11 (4.3)	244 (95.7)	2 (2.4)	80 (97.6)	5 (3.2)	153 (96.8)	6 (5.7)		99 (94.3)	24 (4)	576 (96)	---
abcd		30 (11.8)	225 (88.2)	15 (18.3)	67 (81.7)	15 (9.5)	143 (90.5)	15 (14.3)		90 (85.7)	75 (12.5)	525 (87.5)	---

Table 6: Information about use of antibiotics in large animal farming of Bangladesh continued……

SL No./ Statements		Responses numbers (%)										Chi-square Value (p-value)
		Cattle		Sheep		Goat		Buffalo		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	
21. Antibiotic course completed last time in livestock.		157 (61.6)	98 (38.4)	39 (47.6)	43 (52.4)	110 (69.6)	48 (30.4)	51 (48.6)	54 (51.4)	357 (59.5)	243 (40.5)	17.223^{***} (0.001)
22. Withdrawal period follows.		47 (18.4)	208 (81.6)	7 (8.5)	75 (91.5)	19 (12)	139 (88)	6 (5.7)	99 (94.3)	79 (13.2)	521 (86.8)	13^{***} (0.005)
23. Storage of drug in-												
a.	Store room	131 (51.4)	124 (48.6)	35 (42.7)	47 (57.3)	71 (44.9)	87 (55.1)	37 (35.2)	68 (64.8)	274 (45.7)	326 (54.3)	---
b.	Refrigerator	22 (8.6)	233 (91.4)	3 (3.7)	79 (96.3)	13 (8.2)	145 (91.8)	3 (2.9)	102 (97.1)	41 (6.8)	559 (93.2)	---
c.	Shed	61 (23.9)	194 (76.1)	19 (23.2)	63 (76.8)	43 (27.2)	115 (72.8)	30 (28.6)	75 (71.4)	153 (25.5)	447 (74.5)	---
d.	Others	20 (7.8)	235 (92.2)	24 (29.3)	58 (70.7)	30 (19)	128 (81)	34 (32.4)	71 (67.6)	108 (18)	492 (82)	---
ab		2 (0.8)	253 (99.2)	1 (1.2)	81 (98.8)	1 (0.6)	157 (99.4)	00	105 (100)	4 (0.7)	596 (99.3)	---
cd		19 (7.5)	236 (92.5)	00	82 (100)	00	158 (100)	1 (1)	104 (99)	20 (3.3)	580 (96.7)	---
24. Do you have any idea about self-life/expiry date of antibiotics?		162 (63.5)	93 (36.5)	41 (50)	41 (50)	62 (39.2)	96 (60.8)	48 (45.7)	57 (54.3)	313 (52.2)	287 (47.8)	25.68^{***} (0.0001)
25. Do you use antibiotics by yourself or without taking prescription from any		126 (49.4)	129 (50.6)	42 (51.2)	40 (48.8)	80 (50.6)	78 (49.4)	53 (50.5)	52 (49.5)	301 (50.2)	299 (49.8)	0.112^{NS} (0.99)
26. Do you follow the prescription of Veterinarian during purchasing of exact prescribed?		167 (65.5)	88 (34.5)	35 (42.7)	47 (57.3)	90 (57)	68 (43)	38 (36.2)	67 (63.8)	330 (55)	270 (45)	31.62^{***} (0.0001)

Table 6: Information about use of antibiotics in poultry farming of Bangladesh

SL No.	Statements	Responses numbers (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
1. From whom you usually get suggestions or treatment for your sick animals.										
a.	Veterinary practitioner	161 (80.5)	39 (19.5)	153 (76.5)	47 (23.5)	112 (56)	88 (44)	426 (71)	174 (29)	33.56 (0.0001)
b.	Veterinary paraprofessional	54 (27)	146 (73)	60 (30)	140 (70)	24 (12)	176 (88)	138 (23)	462 (77)	21.005^{***} (0.0001)
c.	Local drug seller	77 (38.5)	123 (61.5)	80 (40)	120 (60)	92 (46)	108 (54)	249 (41.5)	351 (58.5)	2.595^{NS} (0.273)
d.	Family/friend	7 (3.5)	193 (96.5)	8 (4)	192 (96)	00	200	15 (2.5)	585 (97.5)	7.795^{**} (0.02)
e.	Other farmers	45 (22.5)	155 (77.5)	52 (26)	148 (74)	53 (26.5)	147 (73.5)	150 (25)	450 (75)	1.013^{NS} (0.603)
f.	Self	31 (15.5)	169 (84.5)	37 (18.5)	163 (81.5)	21 (10.5)	179 (89.5)	89 (14.8)	511 (85.2)	5.172^{*} (0.075)
g.	Village doctor/Quack	33 (16.5)	167 (83.5)	47 (23.5)	153 (76.5)	60 (30)	140 (70)	140 (23.3)	460 (76.7)	10.193^{***} (0.006)
h.	Company professional	80 (40)	120 (60)	72 (36)	128 964)	35 (17.5)	165 (82.5)	187 (31.2)	413 (68.8)	26.865^{***} (0.0001)
2. Have you ever heard about antibiotic?		193 (96.5)	7 (3.5)	192 (96)	8 (4)	189 (94.5)	11 (5.5)	574 (95.7)	26 (4.3)	1.045^{NS} (0.593)
3. What is antibiotic? (Respondent 574: L-193, B-192, D-189)										
a.	Act against bacteria	99 (51.3)	94 (50.5)	90 (46.9)	102 (53.1)	79 (41.8)	110 (58.2)	268 (46.7)	306 (53.3)	---
b.	Act against virus	20 (10.4)	173 (89.6)	27 (14.1)	165 (85.9)	35 (18.5)	154 (81.5)	82 (14.3)	492 (85.7)	5.199^{*} (0.074)
c.	Others	3 (1.6)	190 (98.4)	1 (0.5)	191 (99.5)	5 (2.6)	184 (97.4)	9 (1.6)	565 (98.4)	---
d.	Don’t know	26 (13.5)	167 (86.5)	27 (14.1)	165 (85.9)	25 (13.2)	164 (86.8)	78 (13.6)	496 (86.4)	---

Table 6: Information about use of antibiotics in poultry farming of Bangladesh continued……

SL No.	Statements	Responses numbers (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
ab.	Act against bacteria + virus	32 (16.6)	161 (83.4)	34 (17.7)	158 (82.3)	32 (16.9)	157 (83.1)	98 (17.1)	476 (82.9)	---
bc.	Virus + Others	5 (2.6)	188 (97.4)	6 (3.1)	186 (96.9)	7 (3.7)	182 (96.3)	18 (3.1)	556 (96.9)	---
ac.	Bacteria + Others	1 (0.5)	192 (99.5)	2 (1)	190 (99)	1 (0.5)	188 (99.5)	4 (0.7)	570 (99.3)	---
abc.	Bacteria + virus + others	7 (3.6)	186 (96.4)	5 (2.6)	187 (97.4)	5 (2.6)	184 (97.4)	17 (3)	557 (97)	---
4. Have you ever heard about antimicrobial resistance?		122 (61)	78 (39)	134 (67)	66 (33)	153 (76.5)	47 (23.5)	409 (68.2)	191 (31.8)	---
5. What do you know about antibiotic resistance? (Respondent-409; Layer-122, Broiler-134 & Duck-153)										
a.	It causes treatment failure	57 (46.7)	65 (53.3)	51 (38.1)	83 (61.9)	59 (38.6)	94 (61.4)	167 (40.8)	242 (59.2)	---
b.	It causes poor response to treatment	22 (18)	100 (82)	33 (24.6)	101 (75.4)	25 (16.3)	128 (83.7)	80 (19.6)	329 (80.4)	---
c.	Do not know	27 (22.1)	95 (77.9)	35 (26.1)	99 (73.9)	58 (37.9)	95 (62.1)	120 (29.3)	289 (70.7)	---
d.	Others	00	122 (100)	4 (3)	130 (97)	2 (1.3)	151 (98.7)	6 (1.5)	403 (98.5)	---
ab		16 (13.1)	106 (86.9)	9 (6.7)	125 (93.3)	9 (5.9)	144 (94.1)	34 (8.3)	375 (91.7)	---
ac		00	122 (100)	2 (1.5)	132 (98.5)	00	153 (100)	2 (0.5)	407 (99.5)	---

Table 6: Information about use of antibiotics in poultry farming of Bangladesh continued……

SL No.	Statements	Responses numbers (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No			Yes	No	Yes	No	
6. Have you ever heard about antibiotic residue		105 (52.5)	95 (47.5)	116 (58)	84 (42)	92 (46)	108 (54)	313 (52.2)	287 (47.8)	---
7. What is an antibiotic residue? (Responded-313; Layer-105, Broiler-116 and Duck-92)										
a.	Accumulation of antibiotics in human body	22 (21)	83 (79)	22 (19)	94 (81)	22 (23.9)	70 (76.1)	66 (21.1)	247 (78.9)	---
b.	Accumulation of antibiotics in Animal body	26 (24.8)	79 (75.2)	28 (24.1)	88 (75.9)	20 (21.7)	72 (78.3)	74 (23.6)	239 (76.4)	---
c.	Both	37 (35.2)	68 (64.8)	37 (31.9)	79 (68.1)	22 (23.9)	70 (76.1)	96 (30.7)	217 (69.3)	---
d.	Don’t know	20 (19)	85 (81)	29 (25)	87 (75)	28 (30.4)	64 (69.6)	77 (24.6)	236 (75.4)	---
8. Do you keep record of using any drug?	Always	18 (9)		11 (5.5)		12 (6)		41 (6.8)		97.178^{***} (0.0001)
	Most Frequently	66 (33)		54 (27)		18 (9)		138 (23)		
	Sometimes	55 (27.5)		50 (25)		37 (18.5)		142 (23.7)		
	Rarely	41 (20.5)		50 (25)		41 (20.5)		132 (22)		
	Never	12 (6)		27 (13.5)		77 (38.5)		116 (19.3)		
	Do not know	8 (4)		8 (4)		15 (7.5)		31 (5.2)		
9. Do you have any record of antibiotics used within past one year?		48 (24)	152 (76)	36 (18)	164 (82)	24 (12)	176 (88)	108 (18)	492 (820	9.756^{***} (0.008)
10. Do you add antibiotics during self-feed processing?		13 (6.5)	187 (93.5)	10 (5)	190 (95)	12 (6)	188 (94)	35 (5.8)	565 (94.2)	0.425^{NS} (0.809)

SL No.	Statements	Responses numbers (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
11. Purpose of antibiotic use										
a.	Treatment	108 (54)	92 (46)	95 (47.5)	105 (52.5)	78 (39)	122 (61)	281 (46.8)	319 (53.2)	9.09^{***} (0.011)
b.	Prevention	7 (3.5)	193 (96.5)	6 (3)	194 (97)	3 (1.5)	197 (98.5)	16 (2.7)	584 (97.3)	---
c.	Both (a+b)	74 (37)	126 (63)	73 (36.5)	127 (63.5)	118 (59)	82 (41)	265 (44.2)	335 (55.8)	26.778^{***} (0.0001)
d.	Growth promoter	00	200 (100)	3 (1.5)	197 (98.5)	1 (0.5)	199 (99.5)	4 (7)	596 (99.3)	---
ad.		7 (3.5)	193 (96.5)	15 (7.5)	185 (92.5)	00	200	22 (3.7)	578 (96.3)	15.948^{***} (0.0001)
cd.		4 (2)	196 (98)	8 (4)	192 (96)	00	200 (100)	12 (2)	588 (98)	8.163^{**} (0.017)
12. Regular use of antibiotics to prevent any specific disease		59 (29.5)	141 (70.5)	84 (42)	116 (58)	39 (19.5)	161 (80.5)	182 (30.3)	418 (69.7)	24.055^{***} (0.0001)
13. Which are the specific diseases?										
a.	Salmonellosis	31 (15.5)	169 (84.5)	27 (13.50	173 (86.5)	17 (8.5)	183 (91.5)	75 (12.5)	525 (87.5)	4.745^{*} (0.093)
b.	Mycoplasmosis	27 (13.50	173 (86.5)	38 (19)	162 (86.5)	7 (3.5)	193 (96.5)	72 (12)	528 (88)	23.39^{***} (0.0001)
c.	Collibaccilosis	12 (6)	188 (94)	21 (10.5)	179 (89.5)	9 (4.5)	191 (95.5)	42 (7)	558 (93)	5.991^{**} (0.05)
d.	New Castle Disease	42 (21)	158 (79)	43 (21.5)	157 (78.5)	16 (8)	184 (92)	101 (16.8)	499 (83.2)	16.738^{***} (0.0001)
e.	Gumboro Disease	29 (14.5)	171 (85.5)	35 (17.5)	165 (82.5)	7 (3.5)	193 (96.5)	71 (11.8)	529 (88.2)	20.831^{***} (0.0001)
f.	Marek’s Disease	14 (7)	186 (93)	5 (2.5)	195 (97.5)	1 (0.5)	199 (99.5)	20 (3.3)	580 (96.7)	13.759^{***} (0.001)
g.	Infectious Bronchitis	8 (4)	192 (96)	4 (2)	196 (98)	00	200 (100)	12 (2)	588 (98)	8.163^{**} (0.017)
h.	Others	8 (4)	192 (96)	6 (3)	194 (97)	2 (1)	198 (99)	16 (2.7)	584 (97.3)	3.596^{NS} (0.166)

SL No.	Statements	Responses n (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
14. Does any of your prescriber mentioned about withdrawal period.		37 (18.5)	163 (81.5)	45 (22.5)	155 (77.5)	37 (18.5)	163 (81.5)	119 (19.8)	481 (80.2)	1.342^{NS} (0.511)
15. Use of antibiotics recommended by										
a.	Veterinarian	174 (87)	26 (13)	149 (74.5)	51 (25.5)	95 (47.5)	105 (52.5)	418 (69.7)	182 (30.3)	77.149^{***} (0.0001)
b.	Other farmers	60 (30)	140 (70)	59 929.5)	141 (70.5)	54 (27)	146 (73)	173 (28.8)	427 (71.2)	0.504^{NS} (0.777)
c.	Shopkeeper	70 (35)	130 (65)	77 (38.5)	123 (61.5)	72 (36)	128 (64)	219 (36.5)	381 (63.5)	0.561^{NS} (0.755)
d.	Representative of pharmaceutical company	85 (42.5)	115 (57.5)	86 (43)	114 (57)	54 (27)	146 (73)	225 (37.5)	375 (62.5)	14.123^{***} (0.001)
e.	Veterinary Paraprofessional	42 (21)	158 (79)	51 (25.5)	149 (74.5)	22 (11)	178 (89)	115 (19.2)	485 (80.8)	14.221^{***} (0.001)
f.	Village Doctor/ Quack	29 (14.5)	171 (85.5)	34 (17)	166 (83)	63 (31.5)	137 (68.5)	126 (21)	474 (79)	20.313^{***} (0.0001)
g.	Self	36 (18)	164 (82)	43 (21.5)	157 (78.5)	23 (11.5)	177 (88.5)	102 (17)	498 (83)	7.30^{**} (0.026)
17. Please mention the name of five common antibiotics you frequently use in poultry.										
a.	Penicillin	13 (6.5)	187 (93.5)	12 (6)	188 (94)	25 (12.5)	175 (87.5)	50 (8.3)	550 (91.7)	6.851^{**} (0.033)
b.	Tetracycline	25 (12.5)	175 (87.5)	28 (14)	172 (86)	34 (17)	166 (83)	87 (14.5)	513 (85.5)	1.694^{NS} (0.429)
c.	Doxycycline	101 (50.5)	99 (49.5)	113 (56.5)	87 (43.5)	125 (62.5)	75 (37.5)	339 (56.5)	262 (43.5)	5.859^{*} (0.053)
d.	Oxytetracycline	67 (33.5)	133 (66.5)	69 (34.5)	131 (65.5)	105 (52.5)	95 (47.5)	241 (40.2)	359 (59.8)	19.029^{***} (0.0001)
e.	Streptomycin	8 (4)	192 (96)	10 (5)	190 (95)	5 (2.5)	195 (97.5)	23 (3.8)	577 (96.2)	1.718^{NS} (0.424)
f.	Gentamycin	41 (20.5)	159 (79.5)	36 (18)	164 (82)	40 (20)	160 (80)	117 (19.5)	483 (80.5)	0.446^{NS} (0.800)
g.	Cephalexin	3	197	2	198	3	197	8	592	0.253^{NS}

		(1.5)	(98.5)	(1)	(99)	(1.5)	(98.5)	(1.3)	(98.7)	(0.881)
SL No.	Statements	Responses n (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
h.	Ceftriaxone	1 (0.5)	199 (99.5)	2 (1)	198 (99)	11 (5.5)	189 (94.50	14 (2.3)	586 (97.7)	13.311^{***} (0.001)
i.	Ciprofloxacin	149 (74.5)	51 (25.5)	152 (76)	48 (24)	157 (78.5)	43 (21.5)	458 (76.3)	142 (23.7)	0.904^{NS} (0.636)
j.	Enrofloxacin	112 (56)	88 (44)	104 (52)	96 (48)	74 (37)	126 (63)	290 (48.3)	310 (51.7)	16.071^{***} (0.0001)
k.	Cefixime/Levofloxacin	111 (55.5)	89 (44.5)	99 (49.5)	101 (50.5)	50 (25)	150 (75)	260 (43.3)	340 (56.7)	42.529^{***} (0.0001)
l.	Sulphar drug (Combined)	65 (32.5)	135 (67.50	60 (30)	140 (70)	100 (50)	100 (50)	225 (37.5)	375 (62.5)	20.267^{***} (0.0001)
m.	Sulfadimidine	17 (8.5)	183 (91.5)	14 (7)	186 (93)	15 (7.5)	185 (92.5)	46 (7.7)	554 (92.3)	0.330^{NS} (0.848)
n.	Sulfadiazine	10 (5)	190 (95)	11 (5.5)	189 (94.5)	10 (5)	190 (95)	31 (5.2)	569 (94.8)	0.068^{NS} (0.967)
o.	Sulfamethoxazole	8 (4)	192 (96)	8 (4)	192 (96)	8 (4)	192 (96)	24 (4)	576 (96)	---
p.	Amoxicillin	85 (42.5)	115 (57.5)	79 (39.5)	121 (60.5)	95 (47.5)	105 (52.5)	259 (43.20	341 (56.8)	2.663^{NS} (0.264)
q.	Cefixime	6 (3)	194 (97)	5 (2.5)	195 (97.5)	4 (2)	196 (98)	15 (2.5)	585 (97.5)	0.41^{NS} (0.815)
r.	Lincomycin	3 (1.5)	197 (98.5)	2 (1)	198 (99)	1 (0.5)	199 (99.5)	6 (1)	594 (99)	1.01^{NS} (0.603)
s.	Azithromycin	10 (5)	190 (95)	12 (6)	188 (94)	32 (16)	168 (84)	54 (9)	546 (91)	18.071^{***} (0.0001)
t.	Amikacin	2 (1)	198 (99)	2 (1)	198 (99)	4 (2)	196 (98)	8 (1.3)	592 (98.7)	1.014^{NS} (0.602)
u.	Vancomycin	00	200 (100)	00	200 (100)	00	200 (100)	00	600 (100)	---
v.	Erythromycin	18 (9)	182 (91)	27 (13.5)	173 (86.5)	30 (15)	170 (85)	75 (12.5)	525 (87.5)	3.566^{NS} (0.168)

Table 6: Information about use of antibiotics in poultry farming of Bangladesh continued……

SL No.	Statements	Responses n (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
w.	Ncomycin	16 (8)	184 (92)	20 (10)	180 (90)	28 (14)	172 (86)	64 (10.7)	536 (89.3)	3.918^{NS} (0.141)
x.	Kanamycin	00	200 (100)	00	200 (100)	00	200 (100)	00	600 (100)	---
y.	Chloramphenical	6 (3)	194 (97)	9 (4.5)	191 (95.5)	11 (5.5)	189 (94.5)	26 (4.3)	574 (95.7)	1.528^{NS} (0.466)
z.	Moxifloxacin	4 (2)	196 (98)	6 (3)	194 (97)	3 (1.5)	197 (98.5)	13 (2.2)	587 (97.8)	1.101^{NS} (0.577)
z1.	Flovphenicol	00	200 (100)	1 (0.5)	199 (99.5)	1 (0.5)	199 (99.5)	2 (0.3)	598 (99.7)	1.003^{NS} (0.606)
z2.	Colistin	68 (34)	132 (66)	53 (26.5)	147 (73.5)	13 (6.5)	187 (93.5)	134 (22.3)	466 (77.7)	46.602^{***} (0.0001)
z3.	Tiamulin	9 (4.5)	191 (95.5)	10 (5)	190 (95)	8 (4)	192 (96)	27 (4.5)	573 (95.5)	0.233^{NS} (0.89)
z4.	Tylosin	59 (29.5)	141 (70.5)	54 (27)	146 (73)	20 (10)	180 (90)	133 (22.2)	467 (77.8)	26.102^{***} (0.0001)
z5.	Others	11 (5.5)	189 (94.5)	12 (6)	188 (94)	14 (7)	186 (93)	37 (6.2)	563 (93.8)	0.403^{NS} (0.817)

Table 6: Information about use of antibiotics in poultry farming of Bangladesh continued……

SL No.	Statements	Responses n (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
18. Number of antibiotics use at a time.										
a.	Single	94 (47)	106 (53)	95 (47.5)	105 (52.5)	85 (42.5)	115 (57.5)	274 (45.7)	326 (54.3)	---
b.	Combined/Multiple	55 (27.5)	145 (72.5)	39 (19.5)	161 (80.5)	42 (21)	158 (79)	136 (22.7)	464 (77.3)	---

c.	Do not know	14 (7)	186 (93)	19 (9.5)	181 (90.5)	50 (25)	150 (75)	83 (13.8)	517 (86.2)	31.908*** (0.0001)
ab.	Single + Combined	37 (18.5)	163 (81.5)	47 (23.5)	153 (76.5)	23 (11.5)	177 (88.5)	107 (17.8)	493 (82.2)	---
19. Frequency of use of antibiotics in poultry.										
a.	Daily	10 (5)	190 (95)	20 (10)	180 (90)	10 (5)	190 (95)	40 (6.7)	560 (93.3)	---
b.	Weekly	8 (4)	192 (96)	6 (3)	194 (97)	6 (3)	194 (97)	20 (3.3)	580 (96.7)	---
c.	Monthly	6 (3)	194 (97)	5 (2.55)	195 (97.5)	12 (6)	188 (94)	23 (3.8)	577 (96.2)	---
d.	When needed	144 (72)	56 (28)	138 (69)	62 (31)	146 (73)	54 (27)	428 (71.3)	172 (28.7)	---
cd.		7 (3.5)	193 (96.5)	6 (3)	194 (97)	26 (13)	174 (87)	39 (6.5)	561 (93.5)	---
abcd.		25 (12.5)	175 (87.5)	25 (12.5)	175 (87.5)	00	200 (100)	50 (8.3)	550 (91.7)	

Table 6: Information about use of antibiotics in poultry farming of Bangladesh continued……

SL No.	Statements	Responses n (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
20. Route of administration of antibiotics for poultry.										
a.	Feed	8 (4)	192 (96)	12 (6)	188 (94)	27 (13.5)	173 (86.5)	47 (7.8)	553 (92.2)	---
b.	Water	40 (20)	160 (80)	53 (26.5)	147 (73.5)	44 (22)	156 (78)	137 (22.8)	463 (77.2)	---
c.	Feed & Water	109 (54.5)	91 (45.5)	97 (48.5)	103 (51.5)	97 (48.5)	103 (51.5)	303 (50.5)	297 (49.5)	---
d.	Injection	5 (2.5)	195 (97.5)	2 (1)	198 (99)	6 (3)	194 (97)	13 (2.2)	587 (97.8)	---
e.	Others	00	200	00	200	00	200	00	600	---

			(100)		(100)		(100)		(100)	
ab		13 (6.5)	187 (93.5)	11 (5.5)	189 (94.5)	1 (0.5)	199 (99.5)	25 (4.2)	575 (95.8)	---
bc		25 (12.5)	175 (87.5)	25 (12.5)	175 (87.5)	00	200 (100)	50 (8.3)	550 (91.7)	---
abc		00	200 (100)	00	200 (100)	25 (12.5)	175 (87.5)	25 (4.2)	575 (95.8)	---

Table 6: Information about use of antibiotics in poultry farming of Bangladesh continued……

SL No.	Statements	Responses n (%)								χ^2 value (p-value)
		Layer		Broiler		Duck		Total		
		Yes	No	Yes	No	Yes	No	Yes	No	
21. Antibiotic course completed last time in Poultry.		136 (68)	64 (32)	116 (58)	84 (42)	116 (58)	84 (42)	368 (61.3)	232 (38.7)	5.622* (0.06)
22. Withdrawal period follows.		10 (5)	190 (95)	18 (9)	182 (91)	24 (12)	176 (88)	52 (8.7)	548 (91.3)	6.232** (0.44)
23. Storage of drug in-										
a.	Store room	68 (34)	132 (66)	62 (31)	138 (69)	94 (47)	106 (53)	224 (37.3)	376 (62.7)	---
b.	Refrigerator	5 (2.5)	195 (97.5)	8 (4)	192 (96)	3 (1.5)	197 (98.5)	16 (2.7)	584 (97.3)	---
c.	Shed	106 (53)	94 (47)	126 (63)	74 (37)	95 (47.5)	105 (52.5)	327 (54.5)	273 (45.5)	---
d.	Others	5 (2.5)	195 (97.5)	3 (1.5)	197 (98.5)	7 (3.5)	193 (96.5)	15 (2.5)	585 (97.5)	---
ab		6 (3)	194 (97)	00	200 (100)	1 (0.5)	199 (99.5)	7 (1.2)	593 (98.8)	---
ac		10 (5)	190 (95)	1 (0.5)	199 (99.5)	00	200 (100)	11 (1.8)	589 (98.2)	---
24. Do you have any idea about self-life/expiry date of antibiotics?		113 (56.5)	87 (43.5)	104 (52)	96 (48)	83 (41.5)	117 (58.5)	300 (50)	300 (50)	9.48*** (0.009)

25. Do you use antibiotics by yourself or without taking prescription from any	88 (44)	112 (56)	98 (49)	102 (51)	121 (60.5)	79 (39.5)	307 (51.2)	293 (48.8)	11.46*** (0.003)
26. Do you follow the prescription of Veterinarian during purchasing of exact prescribed?	146 (73)	54 (27)	129 (64.5)	71 (35.5)	115 (57.5)	85 (42.5)	390 (65)	210 (35)	10.593*** (0.005)

NS=Not significant, ***= significant at 1%, ** at 5% and * at 10% level of significance.

Table 7: Information about KAP of informal prescribers of Bangladesh.

SL No.	Item/Statements	Response n (%)	
		Yes	No
7. Do you have Knowledge about antibiotics?			
a.	Act against bacteria	139 (86.9)	21 (13.1)
b.	Act against virus	50 (31.3)	110 (68.7)
c.	Others	20 (12.5)	140 (87.5)
d.	Don't know	6 (3.8)	154 (96.2)
A	Only a	101 (63.1)	59 (36.9)
B	Only b	12 (7.5)	148 (92.5)
C	Only c	3 (1.9)	157 (98.1)
D	Only d	6 (3.8)	154 (96.3)
ab		21 (13.1)	139 (86.9)
bc		00	00
ac		00	00
abc		17 (10.6)	143 (89.4)
8. Used antibiotics in livestock.			
a.	Penicillin	77 (48.1)	83 (51.9)
b.	Tetracycline	43 (26.9)	117 (73.1)
c.	Doxycycline	32 (20)	128 (80)
d.	Oxytetracycline	132 (82.5)	28 (17.5)
e.	Streptomycin	84 (52.5)	76 (47.5)
f.	Gentamycin	103 (64.4)	57 (35.6)
g.	Cephalexin	8 (5)	152 (95)
h.	Ceftriaxone	109 (68.1)	51 (31.9)
i.	Ciprofloxacin	97 (60.6)	63 (39.4)
j.	Cloxacillin/Enrofloxacin	23 (14.4)	137 (85.6)
k.	Cefixime/Levofloxacin	6 (3.8)	154 (96.2)
l.	Sulphar drug (combined)	70 (43.8)	90 (56.2)
m.	Sulphadimidine	35 (21.9)	125 (78.1)
n.	Sulphadiazine	20 (12.5)	140 (87.5)
o.	Sulphamethoxazole	9 (5.6)	151 (94.4)
p.	Ampicillin	30 (18.8)	130 (81.2)
q.	Amoxicillin	62 (38.8)	98 (61.2)
r.	Ceftiofur	4 (2.5)	156 (97.5)
s.	Lincomycin	3 (1.9)	157 (98.1)
t.	Azithromycin	14 (8.8)	146 (91.2)
u.	Amikacin	2 (1.3)	158 (98.7)
v.	Others	140 (87.5)	20 (12.5)

Table 7: Information about KAP of informal prescribers of Bangladesh (continued)

SL No.	Item/Statements	Response n (%)	
		Yes	No
9. Used antibiotics in poultry.			
a.	Penicillin	12 (7.5)	148 (92.5)
b.	Chlortetracycline	11 (6.9)	149 (93.1)
c.	Doxycycline	126 (78.8)	34 (21.2)
d.	Oxytetracycline	114 (71.2)	46 (28.8)

e.	Streptomycin	4 (2.5)	156 (97.5)
f.	Gentamycin	43 (26.9)	117 (73.1)
g.	Cephalexin	6 (3.8)	154 (96.2)
h.	Ceftriaxone	17 (10.6)	143 (89.4)
i.	Ciprofloxacin	112 (70)	48 (30)
j.	Enrofloxacin	84 (52.5)	76 (47.5)
k.	Levofloxacin	82 (51.3)	78 (48.8)
l.	Sulphar drug (Combined)	31 (19.4)	129 (80.6)
m.	Sulphadimidine	7 (4.4)	153 (95.6)
n.	Sulphadiazine	00	160 (100)
o.	Sulphamethoxazole	2 (1.2)	158 (98.8)
p.	Amoxicillin	74 (46.2)	86 (53.8)
q.	Cefixime	00	160 (100)
r.	Lincomycin	16 (10)	144 (90)
s.	Azithromycin	36 (22.5)	124 (77.5)
t.	Amikacin	20 (12.5)	140 (87.5)
u.	Vancomycin	00	160 (100)
v.	Erythromycin	20 (12.5)	140 (87.5)
w.	Neomycin	34 (21.2)	126 (78.8)
x.	Kanamycin	00	160 (100)
y.	Chloramphenicol	5 (3.1)	155 (96.9)
z.	Moxifloxacin	30 (18.8)	130 (81.2)
z1.	Flovphenicol	23 (14.4)	137 (85.6)
z2.	Colistine	33 (20.6)	127 (79.4)
z3.	Tiamuline	24 (15)	136 (85)
z4.	Tylosin	35 (21.9)	125 (78.1)
z5.	Others	20 (12.5)	140 (87.5)

Table 7: Information about KAP of informal prescribers of Bangladesh (continued)

SL No.	Item/Statements	Response n (%)	
		Yes	No
10. Conditions for using antibiotics			
a.	Bacterial diseases	151 (94.4)	9 (5.6)
b.	Viral disease	116 (72.5)	44 (27.5)
c.	Mycoplasma diseases	78 (48.8)	82 (51.2)
d.	Protozoal diseases	77 (48.1)	83 (51.9)
e.	Parasitic infection	2 (1.2)	158 (98.2)
f.	Unknown cause	51 (31.9)	109 (68.1)
g.	Other	27 (16.9)	133 (83.1)
11. How do you select antimicrobials and determine its dose?			
a.	Personal experience	82 (51.2)	78 (48.8)
b.	Consult with Veterinarian	110 (68.8)	50(31.2)
c.	Consult with drug seller	37 (23.1)	123 (76.9)
d.	Consult with representative of pharmaceutical company	93 (58.1)	67 (41.9)
e.	Consult with other prescribers	38 (23.8)	122 (76.2)
f.	Others	22 (13.7)	138 (86.3)
12. Heard about antimicrobial resistance?		133 (83.1)	27 (16.9)

13. Causes of antibiotic resistance.			
a.	Incomplete antibiotic course	117 (73.1)	43 (26.9)
b.	Using the same antibiotic too frequently	80 (50.0)	80 (50.0)
c.	Over dose	73 (45.6)	87 (54.4)
d.	Low dose	71 (44.4)	89 (55.6)
e.	Skipping doses of the medicine	99 (61.9)	61 (38.1)
f.	Taking different antibiotics at the same time	57 (35.6)	103 (64.4)
g.	Taking antibiotics that have been kept at home for a long time	12 (7.5)	148 (92.5)
h.	Adulterated antibiotics	48 (30.0)	112 (70.0)
i.	Others	20 (12.5)	140 (87.5)
14. Have knowledge about antibiotic residue.		71 (44.4)	89 (55.6)
15a. Have knowledge about antibiotic resistance?		136 (85)	24 (15)
15. What do you know about antibiotic resistance? (Respondent-136)			
a.	It causes treatment failure	51 (37.5)	85 (62.5)
b.	It causes poor response to treatment	8 (5.9)	128 (94.1)
a+b.	Treatment failure & poor response to treatment	57 (41.9)	79 (58.1)
c.	Do not know	8 (5.9)	128 (94.1)
d.	Others	12 (8.8)	124 (91.2)
16. Have any record of antibiotics sell? (Respondent only drug seller-58)		00	00
17. Purpose of antibiotic use			
a.	Therapeutic	91 (56.9)	69 (43.1)
b.	Prophylactic	1(0.6)	159 (99.4)
c.	Both (a+b)	60 (37.5)	100 (62.5)
d.	Growth promotor	8 (5)	152 (95)

Table 7: Information about KAP of informal prescribers of Bangladesh (continued)

SL No.	Item/Statements	Response n (%)	
		Yes	No
18. Sell of antibiotics recommended by Respondent only drug seller (58)			
a.	Veterinarian	9 (15.5)	49 (84.5)
b.	Other farmers	00	00
c.	Representative of pharmaceutical company	00	00
d.	Village doctor	00	00
e.	Quack	00	00
f.	All of the above	00	00
abcde		49 (84.5)	9 (15.5)
19. Number of antibiotics use at a time			
a.	Single	68 (42.5)	92 (57.5)
b.	Combined/Multiple	52 (32.5)	108 (67.5)
a+b.	Single & Combined/Multiple	40 (25)	120 (75)
20. Frequency of use of antibiotics in livestock/poultry			
a.	Daily	8 (5)	
b.	Weekly	1 (0.6)	
c.	Monthly	5 (3.1)	

d.	When needed	146 (91.3)	
21. Route of administration of antibiotics for livestock/poultry			
a.	Injection	147 (91.9)	13 (8.1)
b.	Oral	121 (75.6)	39 (24.4)
c.	Others	41 (25.6)	119 (74.4)
22. Do you suggest for the completion of antibiotic course?		151 (94.4)	9 (5.6)
23. Do you know about withdrawal period of antibiotics?		67 (41.9)	93 (58.1)
24. Do you suggest the farmer to follow the withdrawal period of antibiotics?		67 (41.9)	93 (58.1)
25. How do you store drugs? (Respondent only drug seller-58)			
a.	Store room	12 (20.7)	46 (79.3)
b.	Refrigerator	2 (3.4)	56 (96.6)
c.	Others	5 (8.6)	53 (91.4)
ab.	Store room & Refrigerator	20 (34.5)	38 (65.5)
ac.	Store room & Others	18 (31)	40 (690
abc.	Store room, Refrigerator & Others	1 (1.7)	57 (98.3)
26. Do you sell antibiotic yourself or without taking prescription from any veterinarian		36 (62.1)	22 (37.9)
27. Do you follow the prescription of veterinarian during selling of exact prescribed antibiotic		21 (36.2)	37 (63.8)

Table 8: Information about KAP of Veterinarian of Bangladesh.

SL No.	Item/Statements	Response n (%)	
		Yes	No
1. Causes of antibiotic resistance.			
a.	Incomplete antibiotic course	113 (70.6)	47 (29.4)
b.	Using same antibiotic frequently	103 (64.4)	57 (35.6)
c.	Overdose	76 (47.5)	84 (52.5)
d.	Low dose	85 (53.1)	75 (46.9)
e.	Skipping doses of antibiotic	96 (60)	64 (40)
f.	Taking different antibiotics at the same time	81 (50.6)	79 (49.4)
g.	Adulterated antibiotics	61 (38.1)	99 (61.9)
h.	Taking antibiotics that have been kept at home for long time	7 (4.4)	153 (95.6)
i.	All of the above	73 (45.6)	87 (54.4)
2. Do you have any knowledge about antibiotic residue?		160 (100)	0 (00)
3. What types of residues deposited in the meat/ milk/eggs?			
a.	Intact antibiotic	16 (10)	144 (90)
b.	Metabolized antibiotic	71 (44.4)	89 (55.6)
c.	Others	36 (22.5)	124 (77.5)
d.	Don’t know	00	160 (100)
ab		37 (23.1)	123 (76.9)
4. What are the adverse effects of antibiotic residue in food of animal origin?			
a.	Producing severe human health hazards	28 (17.5)	132 (82.5)
b.	Producing antibiotic resistant bacteria.	24 (15)	136 (85)

c.	Producing adverse effect in environment.	00	00
d.	All of the above.	65 (40.6)	95 (59.4)
ab		35 (21.9)	125 (78.1)
bc		5 (3.1)	155 (96.9)
ac		3 (1.9)	157 (98.1)
5. Do you have any knowledge about FAO categorized antibiotic aware?		118 (73.8)	42 (26.3)
6. Do you have any knowledge about reserve type of antibiotic?		119 (74.4)	41 (25.6)
7. What are the reserve antibiotics uses in case of livestock?			
a.	4th generation Cephalosporin	68 (42.5)	92 (57.5)
b.	5th generation Cephalosporin	24 (15.0)	136 (85.0)
c.	Monobactum	19 (11.9)	141 (88.1)
d.	Oxazolidinones	7 (4.4)	153 (95.6)
e.	Deptomycin	7 (4.4)	153 (95.6)
f.	Phosphomycin	18 (11.3)	142 (88.8)
g.	Tegacycline	10 (6.3)	150 (93.8)
h.	Polymyxin	20 (12.5)	140 (87.5)
i.	Others	53 (33.1)	107 (66.9)
8. What are the reserve antibiotics uses in case of poultry?			
a.	4th generation Cephalosporin	61 (38.1)	99 (61.9)
b.	5th generation Cephalosporin	22 (13.8)	138 (86.3)
c.	Monobactum	7 (4.4)	153 (95.6)
d.	Oxazolidinones	6 (3.8)	154 (96.2)
e.	Deptomycin	2 (1.3)	158 (98.8)
f.	Phosphomycin	7 (4.4)	153 (95.6)
g.	Tegacycline	4 (2.5)	156 (97.5)
h.	Polymyxin	39 (24.4)	121 (75.6)
i.	Others	60 (37.5)	100 (62.5)
9. Do you give advice to the owner about the withdrawal period of antibiotics?		148 (92.5)	12 (7.5)
10. Do you give advice to the farmers to keep record of antibiotics?		145 (90.6)	15 9.4)
11. Number of antibiotic used at a time?			
a.	Single	102 (63.8)	58 (36.3)
b.	Combined	37 (23.1)	123 (76.9)
a+b.	Single & Combined	21 (13.1)	139 (86.9)
12 Frequency of use of antibiotics in livestock/poultry?			
a.	Daily	6 (3.8)	154 (96.2)
b.	Weekly	4 (2.5)	156 (97.5)
c.	Monthly	6 (3.8)	154 (96.2)
d.	When needed	144 (90)	16 (10)
c+d.	Monthly & When needed	3 (1.9)	157 (98.1)
13 Do you mention the duration of use of antibiotics?		144 (90)	16 (10)
14 Do you ask the farmers about prior use of antibiotics?		156 (97.5)	4 (2.5)

15	Do you ask farmers whether the prior antibiotic course was completed or not?	138 (86.3)	22 (13.8)
16	Used antibiotics in livestock.		
a.	Penicillin	103 (64.4)	57 (35.6)
b.	Tetracycline	42 (26.3)	118 (73.8)
c.	Doxycycline	19 (11.9)	141 (88.1)
d.	Oxytetracycline	112 (70)	48 (30)
e.	Streptomycin	92 (57.5)	68 (42.5)
f.	Gentamycin	120 (75)	40 925)
g.	Cephalexin	1 (0.6)	159 (99.4)
h.	Ceftriaxone	87 (54.4)	73 (45.6)
i.	Ciprofloxacin	98 (61.3)	62 (38.8)
j.	Enrofloxacin/ Cloxacillin	28 (17.5)	132 (82.5)
k.	Levofloxacin/Cefixime	3 (1.9)	157 (98.1)
l.	Sulphar drug (combined)	80 (50)	80 (50)
m.	Sulphadimidine	45 (28.1)	115 (71.9)
n.	Sulphadiazine	12 (7.5)	148 (92.5)
o.	Sulphamethoxazole	21 (13.1)	139 (86.9)
p.	Ampicillin	41 (25.6)	119 (74.4)
q.	Amoxicillin	60 (37.5)	100 (62.5)
r.	Cefixime/Ceftiofur	2 (1.3)	158 (98.8)
s.	Lincomycin	1 (0.6)	159 (99.4)
t.	Azithromycin	24 (15)	136 (85)
u.	Amikacin	00	160 (100)
v.	Others	22 (13.7)	138 (86.3)
17.	Used antibiotics in poultry.		
a.	Penicillin	6 (3.8)	154 (96.2)
b.	Chlortetracycline	39 (24.4)	121 (75.6)
c.	Doxycycline	116 (72.5)	44 (27.5)
d.	Oxytetracycline	110 (68.8)	50 (31.3)
e.	Streptomycin	1 (0.6)	159 (99.4)
f.	Gentamycin	61 (38.1)	99 (61.9)
g.	Cephalexine	14 (8.8)	146 (91.2)
h.	Ceftriaxone	00	160 (100)
i.	Ciprofloxacin	101 (63.1)	59 (36.9)
j.	Enrofloxacin	102 (63.8)	58 (36.3)
k.	Levofloxacin	78 (48.8)	82 (51.3)
l.	Sulphar drug (Combined)	56 (35)	104 (65)
m.	Sulphadimidine	10 (6.3)	150 (93.8)
n.	Sulphadiazine	8 (5)	152 (95)
o.	Sulphamethoxazole	10 (6.3)	150 (93.8)
p.	Amoxicillin	74 (46.3)	86 (53.8)
q.	Cefixime	2 (1.3)	158 (98.8)
r.	Lincomycin	9 (5.6)	151 (94.4)
s.	Azithromycin	23 (14.4)	137 (85.6)
t.	Amikacin	14 (8.8)	146 (91.3)
u.	Vancomycin	00	160 (100)
v.	Erythromycin	26 (16.3)	134 (83.8)

w.	Neomycin	42 (26.3)	118 (73.8)
x.	Kanamycin	00	160 (100)
y.	Chloramphenicol	2 (1.3)	158 (98.8)
z.	Moxifloxacin	00	160 (100)
z1.	Flovphenicol	33 (20.6)	127 (79.4)
z2.	Colistin	40 (25)	120 (75)
z3.	Tiamulin	25 (15.6)	135 (84.4)
z4.	Tylosin	34 (21.3)	126 (78.8)
z5.	Others	20 (12.5)	140 (87.5)
18. Factors for prescribing antibiotics.			
a.	Type of organism/disease	156 (97.5)	4 (2.5)
b.	Severity of the disease	143 (89.4)	17 (10.6)
c.	Positive culture sensitivity test report	45 (28.1)	115 (71.9)
d.	Antibiotic resistance	74 (46.3)	86 (53.8)
e.	Drug instructions	91 (56.9)	69 (43.1)
f.	Owner's demand	8 (5.0)	152 (95)
g.	Economic status of the owner	94 (58.8)	66 (41.3)
h.	Availability at the local market	111 (69.4)	49 (30.6)
i.	Symptom	110 (68.8)	50 (31.3)
j.	Others	40 (25)	120 (75)
19. Do you think animal owners follow the prescription for antibiotic properly?			
a.	Always	17 (10.6)	
b.	Maximum times	104 (65)	
c.	Sometimes	34 (21.3)	
d.	Rarely	4 (2.5)	
e.	Do not follow	1 (0.6)	
20. Most preferred spectrum of antibiotics.			
a.	Narrow spectrum	37 (23.1)	
b.	Broad spectrum	123 (76.9)	
21. Prescribed more than one antibiotic in a single prescription?		55 (34.4)	105 (65.6)

Annexure-3

Questionnaire survey form regarding antibiotic usage pattern, its residual knowledge and antibiotic resistance (For Farmer's)

A. General information (সাধারণ জ্ঞাতব্য)

1. Name of the farm (খামারের নাম): .....

2. Name of the owner/caretaker (মালিক/তত্ত্বাবধায়কের নাম): .....

3. Address of the farm (খামারের ঠিকানা): .....

Phone (ফোন): .....E-mail (ই-মেইল): .....

4. Type of farm (খামারের ধরন): a) Layer [1] b) Broiler [2]

c) Duck [3] d) Cattle [4] e) Sheep [5]

f) Goat [6] g) Buffalo [7] h) Dairy [8]

i) Beef Fattening [9]

5. Total population (মোট সংখ্যা): .....

6. Age (birds/animals) (বয়স):

a) Single age group (একক) [1] b) Multiple age group (বহু) [0]

7. How many sheds in the farm (খামারে সেডের সংখ্যা): .....

8. Educational status of farm owner/caretaker (খামারমালিক/তত্ত্বাবধায়কের শিক্ষাগত যোগ্যতা):

a) Illiterate (অশিক্ষিত) [1] b) Primary (PSC) (প্রাথমিক/পিএসসি) [2] c) Junior Secondary (JSC) (জুনিয়র মাধ্যমিক/জেএসসি) [3]

d) Secondary (SSC) (মাধ্যমিক/এসএসসি) [4] e) HSC/Diploma (উচ্চ মাধ্যমিক/ডিপ্লোমা) [5] f) Graduate (স্নাতক) [6]

g) Masters/M (স্নাতোকত্তার) [7]

B. Information about management practices in poultry farms (মুরগীর খামারের ব্যবস্থাপনার চর্চা সম্পর্কে তথ্য)

1. Do you have any knowledge about bio-security (আপনার কি জৈব নিরাপত্তা সম্পর্কে কোন জ্ঞান আছে)?

a) Have (আছে) [1] b) Don't have (নাই) [0]

2. Do you have any fence remains in the farm (আপনার খামারে বেড়া/প্রাচীর আছে)?

a) Have (আছে) [1] b) Don't have (নাই) [0]

3. Have any entrance of bird in the shed (সেডে পাখী প্রবেশ করে)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

4. Have any entrance of wild animal in the farm (খামারে বন্য প্রাণি প্রবেশ করে)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

5. Water supply (পানি সরবরাহ)

a) Direct (সরাসরি) [1] b) Reserved (মজুদ রেখে) [0]

6. Cleaning of waterer (পানির পাত্র পরিস্কার করা)

a) Once daily (দিনে এক বার) [1] b) Once in a week (সপ্তাহে এক বার) [2]

c) Twice in a week (সপ্তাহে দুইবার) [3] d) Once in a month (মাসে এক বার) [4]

e) Twice in a month (মাসে দুইবার) [5]

7. Supply of feed (খাদ্য সরবরাহ)

a) Self processed (নিজ হাতে প্রস্তুত কৃত) [1] b) Purchased/Commercial (বাজার থেকে কেনা) [0]

8. Cleaning of feeder (খাদ্যের পাত্র পরিস্কার করা)

a) Once daily (দিনে এক বার) [1] b) Once in a week (সপ্তাহে এক বার) [2]

c) Twice in a week (সপ্তাহে দুইবার) [3] d) Once in a month (মাসে এক বার) [4]

e) Twice in a month (মাসে দুইবার) [5]

9. Cleaning of feces/floor/litter (মল-মুত্র/ফ্লোর/লিটার পরিস্কার করা)

a) Once daily (দিনে এক বার) [1] b) Twice daily (দিনে দুই বার) [2]

c) Every alternate day (এক দিন পর পর) [3] d) Twice in a month (মাসে দুই বার) [4]

e) Once in a month (মাসে এক বার) [5] f) Once after sell [6]

C. Use of Antibiotics in livestock and poultry (প্রাণী/ মুরগীতে অ্যান্টিবায়োটিকের ব্যবহার)

1. From whom you usually get suggestions or treatment for your sick animals/birds (কার কাছ থেকে অসুস্থ্য প্রাণীর/মুরগীর চিকিৎসা সমন্ধে মতামত পাওয়া যায়)?

a) Veterinary practitioner	1	
b) Veterinary paraprofessional	1	Put 1 if answer is Yes, otherwise put 0
c) Local drug seller	1	
d) Family/friend	1	
e) Other farmers	1	
f) Self	1	
g) Village doctor/Quack	1	
h) Company professional	1	

2. Have you ever heard about antibiotic (আপনিকি এন্টিবায়োটিক সম্পর্কে শুনেছেন)?

a) Yes (হ্যাঁ) 1 b) No (না) 0

3. What is antibiotic (এন্টিবায়োটিক কি)?

a) Act against bacteria (ব্যাকটেরিয়ার বিরুদ্ধে কাজ করে) 1

b) Act against virus (ভাইরাসের বিরুদ্ধে কাজ করে) 1

c) Others (অন্যান্য) 98

d) Don't know (আমি জানি না) 99

4. Have you ever heard about antimicrobial resistance (আপনিকি এন্টিবায়োটিক প্রতিরোধী সম্পর্কে শুনেছেন)?

a) Yes (হ্যাঁ) 1 b) No (না) 0

5.What do you know about antibiotic resistance (আপনি এন্টিবায়োটিক প্রতিরোধী সম্পর্কে কি জানেন)?

a) It causes treatment failure 1

b) It causes poor response to treatment 1

c) Do not know 99

d) Others (অন্যান্য) 98

6. Have you ever heard about antibiotic residue (আপনি কি এন্টিবায়োটিক অবশিষ্ট সম্পর্কে শুনেছেন)?

a) Yes (হ্যাঁ) 1 b) No (না) 0

7. What is antibiotic residues ? (এন্টিবায়োটিক অবশিষ্টকি?)

a) Accumulation of antibiotics in human body (মানুষের দেহে এন্টিবায়োটিক জমা হওয়া) 1

b) Accumulation of antibiotics in Animal body (প্রাণির দেহে এন্টিবায়োটিক জমা হওয়া) 1

c) Both (উভয়ই) 1

d) Don't know (আমি জানিনা) 99

8. Do you keep record of using any drug (আপনি কি ঔষধ ব্যবহারের রেকর্ড রাখেন)?

a) Always (সর্বদা) 1

b) Most frequently (প্রায়সময়ই) 2

c) Sometimes (কোন কোনসময়) 3

d) Rarely (কদাচিত) 4

e) Never (কখনোনা) 5

f) Do not know (জানিনা) 99

9. Do you have any record of antibiotics used within past one year (১বছরের অ্যান্টিবায়োটিক ব্যবহারের কোন রেকর্ড আছে)?

a) Have (আছে) 1 b) Don't have (নাই) 0

10. Do you add antibiotics during self-feed processing (আপনি প্রস্তুতকৃত খাবারে এন্টিবায়োটিক মেশান কিনা)?

a) Yes (হ্যাঁ) 1 b) No (না) 0

11. Purpose of antibiotic use (এন্টিবায়োটিক ব্যবহারের উদ্দেশ্য)

a) Treatment (প্রতিরোধক) 1 b) Prevention (প্রতিষেধক) 1

c) Both (a+b) (উভয়) 1 d) Growthpromoter (বৃদ্ধি তরান্বিত) 1

12. Regular use of antibiotics to prevent any specific disease (কোন রোগ প্রতিরোধের জন্য নিয়মিত অ্যান্টিবায়োটিক ব্যবহার করেন কিনা)?

a) Yes (হ্যাঁ) 1 b) No (না) 0

13. Which are the specific diseases (কোন নির্দিষ্ট রোগের ক্ষেত্রে)?

i) For poultry (পাখীর জন্য)

a) Salmonellosis (সালমোনেলোসিস) [1]

b) Mycoplasmosis (মাইকোপ্লাজমোসিস) [1]

c) Collibaccilosis (কলিব্যাসিলোসিস) [1]

d) New Castle Disease (রানীক্ষেত রোগ)) [1]

e) Gumboro Disease (গামবোরো রোগ) [1]

f) Marek's Disease (মারেক্স রোগ) [1]

g) Infectious Bronchitis (ব্রঙ্কাইটিস) [1]

h) Others (অন্যান্য)..... [98]

Put 1 if answer is Yes, otherwise put 0

ii) For livestock (প্রাণীর জন্য)

a) Mastitis (দুধজ্বর) [1]

b) Fever (জ্বর) [1]

c) Diarrhoea (উদারময়) [1]

d) Foot and mouth disease (ক্ষুরারোগ) [1]

e) PPR (ছাগলের প্লেগ) [1]

f) Anthrax [1]

g) Black Quarter [1]

h) Others (অন্যান্য)..... [98]

14. Does any of your prescriber mentioned about withdrawal period

(প্রেসক্রিপশনকারী এন্টিবোয়টিকের উইথড্রয়াল পিরিয়ড সমন্ধে লিখে কিনা)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

15. Use of antibiotics recommended by (কার পরামর্শে অ্যান্টিবায়োটিক ব্যবহার করেন)?

a) Veterinarian (ভেটেরিনারিয়ান) [1]

b) Other farmers (অন্য খামারি) [1]

c) Shopkeeper (দোকানদার) [1]

d) Representative of pharmaceutical company (ঔষধ কোম্পানির প্রতিনিধি) [1]

Put 1 if answer is Yes, otherwise put 0

e) Veterinary Paraprofessional [1]

f) Village Doctor/ Quack [1]

g) Self [1]

16. Please mention the name of five common antibiotics you frequently use in livestock

(৫টি কমন অ্যান্টিবায়োটিক এর নাম লিখুন যেটা প্রায় প্রাণীতে ব্যবহার করেন)

a) Penicillin [1]

b) Tetracycline [1]

c) Doxycycline [1]

d) Oxytetrecycline [1]

e) Streptomycin [1]

f) Gentamycin [1]

g) Cephalexine [1]

h) Ceftriaxone [1]

i) Ciprofloxacin [1]

j) Cloxacillin [1]

k) Cefixime [1]

l) Sulphar drug (Combined) [1]

m) Sulfadimidine [1]

n) Sulfadiazine [1]

o) SulphaMethoxazole [1]

p) Ampicillin [1]

q) Amoxycillin [1]

r) Ceftiofur [1]

s) Lincomycin [1]

t) Azithromycin [1]

u) Amikacin [1]

v) Others (অন্যান্য)..... [98]

17. Please mention the name of five common antibiotics you frequently use in poultry

(৫টি কমন অ্যান্টিবায়োটিক এর নাম লিখুন যেটা প্রায় পাখীতে ব্যবহার করেন)

a) Penicillin [1]

b) Chlortetracycline [1]

c) Doxycycline [1]

d) Oxytetrecycline [1]

e) Streptomycin [1]

f) Gentamycin [1]

g) Cephalexine [1]

h) Ceftriaxone [1]

i) Ciprofloxacin [1]

j) Enrofloxacin [1]

k) Levofloxacin [1]

l) Sulphar drug [1]

m) Sulphadimidine [1]

n) Sulphadiazine [1]

o) Sulphamethoxazole [1]

p) Amoxycillin [1]

q) Cefixime [1]

r) Lincomycin [1]

s) Azithromycin [1]

t) Amikacin [1]

u) Vancomycin [1]

Put 1 if answer is Yes, otherwise put 0

v) Erythromycin [1]

w) Ncomycin [1]

x) Kanamycin [1]

y) Chloramphenical [1]

z) Moxifloxacin [1]

z1) Flovphenicol [1]

z2) Colistin [1]

z3) Tiamulin [1]

z4) Tylosin [1]

z5) Others (অন্যান্য)..... [98]

18. Number of antibiotics use at a time (একই সময় অ্যান্টিবায়োটিক ব্যবহারের সংখ্যা)

a) Single (একক ভাবে) [1] b) Combined/Multiple (একাধিক) [1]

c) Do not know (জানিনা) [99]

19. Frequency of use of antibiotics in livestock/poultry (অ্যান্টিবায়োটিক ব্যবহারের ফ্রিকোয়েন্সি)

a) Daily (দৈনিক) [1] b) Weekly (সাপ্তাহিক) [1]

c) Monthly (মাসিক) [1] d) When needed (যখন প্রযোজন) [1]

20. Route of administration of antibiotics for livestock/poultry (অ্যান্টিবায়োটিক খাওয়ানোর পদ্ধতি)

a) Feed (খাদ্য) [1] b) Water (পানি) [1] c) Feed & Water (খাদ্য ও পানি) [1]

d) Injection (ইনজেকশন) [1] e) Others (অন্যান্য)..... [98]

21. Antibiotic course completed last time in livestock/poultry (শেষ এন্টিবায়োটিক কোর্স সম্পন্ন হয়েছে কিনা)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

22. Withdrawal period follows (উইথড্রয়াল সময়ের অনুসরণ)

a) Yes (হ্যাঁ) [1] b) No (না) [0]

23. Storage of drug (ঔষধ সংরক্ষণ)

a) Store room (স্টোর রুম) [1] b) Refrigerator (ফ্রিজ) [1]

c) shed (সেড) [1] d)Others (অন্যান্য)..... [98]

24. Do you have any idea about self-life/expiry date of antibiotics (আপনার অ্যান্টিবায়োটিকের মেয়াদ উত্তীর্ণ সম্পর্কে ধারণা আছে কিনা)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

25. Do you use antibiotics by yourself or without taking prescription from any Veterinarian (অ্যান্টিবায়োটিক ব্যবহার করেন নিজে বা ভেটেরিনারিয়ান-এর প্রেসক্রিপশন না নিয়ে)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

26. Do you follow the prescription of Veterinarian during purchasing of exact prescribed antibiotic (সঠিক এন্টিবায়োটিক কেনার ক্ষেত্রে ভেটেরিনারিয়ান এর প্রেসক্রিপশন অনুসরন করেন)?

a) Always (সব সময়) [1]

b) Sometimes influenced by dealer of shopkeeper (ডিলার বা দোকানদার কর্তৃক পরিবর্তন) [0]

Questionnaire survey form regarding antibiotic usage pattern, its residual knowledge and antibiotic resistance (For Veterinarian)

A. General information

1. Name of the Veterinarian: .....

2. Address of the Veterinarian: .....

Phone: .....E-mail: .....

3. Age of Veterinarian: .....

4. Educational status of Veterinarian:

Graduate [1] MS [2] PhD [3]

1. Cause of antibiotic resistance [1]

   a) Incomplete antibiotic course [1] Put 1 if answer is Yes, otherwise put 0

   b) Using same antibiotic frequently [1]

   c) Overdose [1]

   d) Low dose [1]

   e) Skipping doses of antibiotic [1]

   f) Taking different antibiotics at the same time [1]

   g) Adulterated antibiotics [1]

   h) Taking antibiotics that have been kept at home for long time [1]

   i) All of the above [1]

2. Do you have any knowledge about antibiotic residue?

   a) Yes [1] b) No [0]

3. What type of residues deposited in the meat/ milk/eggs

a) Intact antibiotic [1]

b) Metabolized antibiotic [1]

c) Others …… [98]

d) Don't know [99]

4. What are the adverse effects of antibiotic residue in food of animal origin?

a) Producing severe human health hazards eg. Nausea, vomiting, headache, diarrhea, cancer, liver & kidney Failure etc. [1]

b) Producing antibiotic resistant bacteria. [1]

c) Producing adverse effect in environment. [1]

d) All of the above. [1]

5. Do you have any knowledge about FAO categorized antibiotic 'AWaRe'?

a) Yes [1] b) No [0]

6. Doyou have any knowledge about reserve type of antibiotic?

a) Yes [1] b) No [0]

7. What are the reserve antibiotics uses in case of livestock?

a) 4th generation Cephalosporin [1]

b)5th generation Cephalosporin [1]

c) Monobactum [1]

d)Oxazolidinones [1]

e) Deptomycin [1]

f) Phosphomycin [1]

g) Tegacycline [1]

[1]

h) Polymyxin

i) Others…… 98

8. What are the reserve antibiotics uses in case of poultry?

a) 4th generation Cephalosporin 1

b) 5th generation Cephalosporin 1

c) Monobactum 1

d) Oxazolidinones 1

e) Deptomycin 1

f) Phosphomycin 1

g) Tegacycline 1

h) Polymyxin 1

i) Others…… 98

9. Do you give advice to theowner about the withdrawal period of antibiotics?

a) Yes 1 b) No 0

10. Do you give advice to the farmers to keep record of antibiotics?

a) Yes 1 b) No 0

Put 1 if answer is Yes, otherwise put 0

11. Number of antibiotics used at a time

a) Single 1 b) Combined 0

12. Frequency of use of antibiotics in livestock/poultry:

a) Daily 1 b) Weeks 1 c) Monthly 1 d) When needed 1

13. Do you mention the duration of use of antibiotics?

a) Yes 1 b) No 0

14. Do you ask the farmers about prior use of antibiotics?

1 0

a) Yes b) No

15. Do you ask farmers whether the prior antibiotic course was completed or not?

a) Yes [1] b) No [0]

16. Please mention five common antibiotics you frequently use in live stock.

a) Penicillin [1]
b) Tetracycline [1]
c) Doxycycline [1]
d) Oxytetrecycline [1]
e) Streptomycin [1]
f) Gentamycin [1]
g) Cephalexine [1]
h) Ceftriaxone [1]
i) Ciprofloxacin [1]
j) Cloxacillin [1]
k) Cefixime [1]
l) Sulphar drug (combined) [1]
m) Sulphadimidine [1]
n) Sulphadiazine [1]
o) Sulphamethoxazole [1]
p) Ampicillin [1]
q) Amoxycillin [98]
r) Cefixime [1]
s) Lincomycin [1]
t) Azithromycin [1]
u) Amikacin [1]

Put 1 if answer is Yes, otherwise put 0

v) Others.....

17. Please mention five common antibiotics you frequently use in poultry

a) Penicillin	1
b) Chlortetracycline	1
c) Doxycycline	1
d) Oxytetracycline	1
e) Streptomycin	1
f) Gentamycin	1
g) Cephalexine	1
h) Ceftriaxone	1
i) Ciprofloxacin	1
j) Enrofloxacin	1
k) Levofloxacin	1
l) Sulphar drug (Combined)	1
m) Sulphadimidine	1
n) Sulphadiazine	1
o) Sulphamethoxazole	1
p) Amoxycillin	1
q) Cefixime	1
r) Lincomycin	1
s) Azithromycin	1
t) Amikacin	1
u) Vancomycin	1
v) Erythromycin	1
w) Ncomycin	1
	1

Put 1 if answer is Yes, otherwise put 0

x) Kanamycin

y) Chloramphenical [1]

z) Moxifloxacin [1]

z1) Flovphenicol [1]

z2) Colistin [1]

z3) Tiamulin [1]

z4) Tylosin [1]

z5) Others (অন্যান্য)..... [98]

18. What are the factors you consider for prescribing antibiotics?

a) Type of organism/disease [1]

b) Severity of the disease [1]

c) Positive culture sensitivity test report [1]

d) Antibiotic resistance [1]

e) Drug instructions [1]

f) Owner's demand [1]

g) Economical status of the owner [1]

h) Availability at the local market [1]

i) Symptom [1]

j) Others..... [98]

19. Do you think animal owners follow the prescription for antibiotic properly?

a) Always [1]

b) Maximum times [2]

c) Sometimes [3]

d) Rarely [4]

[99]

e) Do not follow

20. Which spectrum of antibiotics do you prefer most?

a) Narrow spectrum [1] b) Broad spectrum [0]

21. Do you prescribe more than one antibiotic in a single prescription?

a) Yes [1] b) No [0]

Put 1 if answer is Yes, otherwise put 0

Questionnaire survey form regarding antibiotic usage pattern, its residual knowledge and antibiotic resistance (For Informal prescribers)

A. General information (সাধারণ জ্ঞাতব্য)

1. Name of the medicine seller or shop keeper (ঔষধ বিক্রেতা অথবা দোকানদারের নাম)

.....

2. Address of the medicine seller or shop keeper (ঔষধ বিক্রেতা অথবা দোকানদারের ঠিকানা)

.....

Phone (ফোন): ..... E-mail (ই-মেইল): .....

3. Age of the medicine seller or shop keeper (ঔষধ বিক্রেতাঅথবা দোকানদারের বয়স): .....

4. Educational status of medicine seller or shop keeper or informal prescribers

(ঔষধ বিক্রেতা অথবা দোকানদারের শিক্ষাগত যোগ্যতা):

a) Illiterate (অশিক্ষিত)	1	b) Primary (PSC) (প্রাথমিক/পিএসসি)	2	c) Junior Secondary (JSC) (জুনিয়রমাধ্যমিক/জেএসসি)	3
d) Secondary (SSC) (মাধ্যমিক/এসএসসি)	4	e) HSC/Diploma (উচ্চমাধ্যমিক/ডিপ্লোমা)	5	f) Graduate (স্নাতক)	6
g) Masters (স্নাতোকত্তর)	7				

Put code of respective answer (any one)

5. What is your position (in case of informal prescribers)?

a) Veterinary field officer (ভেটেরিনারি মাঠ কর্মকর্তা)	1
b) Compounder (কম্পাউন্ডার)	2
c) Dresser (ড্রেসার)	3
d) Village animal doctor/quack (কোয়াক)	4
e) Drug seller (ঔষধ বিক্রেতা)	5
f) Medicine Company professional (ঔষধ কোম্পানীরচাকুরীজীবি)	6
g) Others (অন্যান্য)	98

6. How long you are engaged in practice (কত দিন ধরে প্রাকটিস্ করেন)?

a) 1 Year (এক বছর) [1] b) 2 Years (দুইবছর) [2] c) 3 Years (তিনবছর) [3]

d) 4 Years (চারবছর) [4] e) 5 Years (পাঁচবছর) [5] f) Others (অন্যান্য)..... [98]

7. Do you have any knowledge about antibiotics (আপনার কি এন্টিবায়োটিক সম্পর্কে কোন জ্ঞান আছে)?

a) Act against bacteria (ব্যাকটেরি য়ার বিরুদ্ধে কাজ করে) [1]

b) Act against virus (ভাইরাসের বিরুদ্ধে কাজ করে) [1]

c) Others (অন্যান্য) ..... [98]

d) Don't know (জানিনা) [99]

8. Please mentionthe name of five common antibiotics that you use frequently in livestock (৫টি কমন অ্যান্টিবায়োটিক এর নাম লিখুন যেটা প্রায় প্রাণীতে ব্যবহার করেন)

Put 1 if answer is Yes, otherwise put 0

a) Penicillin [1]

b) Tetracycline [1]

c) Doxycycline [1]

d) Oxytetrecycline [1]

e) Streptomycin [1]

f) Gentamycin [1]

g) Cephalexine [1]

h) Ceftriaxone [1]

i) Ciprofloxacin [1]

j) Cloxacillin [1]

k) Cefixime [1]

l) Sulphar drug (Combined) [1]

m) Sulfadimidine [1]

n) Sulfadiazine [1]

o) SulphaMethoxazole [1]

p) Ampicillin [1]

q) Amoxycillin [1]

r) Cefixime [1]

s) Lincomycin [1]

t) Azithromycin [1]

u) Amikacin [1]

v) Others (অন্যান্য) ..... [98]

09. Please mention the name of five common antibiotics you frequently use in poultry

(৫টি কমন অ্যান্টিবায়োটিক এর নাম লিখুন যেটা প্রায় মুরগীতে ব্যবহার করেন)

a) Penicillin [1]

b) Chlortetracycline [1]

c) Doxycycline [1]

d) Oxytetrecycline [1]

e) Streptomycin [1]

f) Gentamycin [1]

g) Cephalexine [1]

h) Ceftriaxone [1]

i) Ciprofloxacin [1]

j) Enrofloxacin [1]

k) Levofloxacin [1]

l) Sulphar drug [1]

m) Sulphadimidine [1]

n) Sulphadiazine [1]

o) Sulphamethoxazole [1]

Put 1 if answer is Yes, otherwise put 0

p) Amoxycillin [1]

q) Cefixime [1]

r) Lincomycin [1]

s) Azithromycin [1]

t) Amikacin [1]

u) Vancomycin [1]

v) Erythromycin [1]

w) Ncomycin [1]

x) Kanamycin [1]

y) Chloramphenical [1]

z) Moxifloxacin [1]

z1) Flovphenicol [1]

z2) Colistin [1]

z3) Tiamulin [1]

z4) Tylosin [1]

z5) Others (অন্যান্য) ..... [98]

10. For which of the following illnesses or conditions you use antibiotics?

a) Bacterial diseases (ব্যাকটেরিয়াল ডিজিজ) [1]

b) Viral disease (ভাইরাল ডিজিজ) [1]

c) Mycoplasmal diseases (ছত্রাক ডিজিজ) [1]

d) Protozoal diseases (প্রোটোজোয়াল ডিজিজ) [1]

e) Parasitic infection (কৃমি) [1]

f) Unknown cause (অজানা কারণ) [1]

g) Other (অন্যান্য)..... [98]

11. How do you select antimicrobials and determine its dose?

a) Personal experience (ব্যক্তিগত অভিজ্ঞতা) [1]

b) Consult with Veterinarian (ভেরেরিনারিয়ান এর পরার্মশ) [1]

c) Consult with drug seller (ঔষধ বিক্রেতার পরামর্শ) [1]

d) Consult with representative of pharmaceutical company [1]

(ঔষধ কোম্পানীর প্রতিনিধির পরামর্শ)

e) Consult with other prescribers (অন্যান্য প্রেসক্রিপশন কারীর পরামর্শ) [1]

f) Others (অন্যান্য) ..... [98]

12. Have you ever heard about antimicrobial resistance (আপনি এন্টিবায়োটিক রেজিসটেন্স সমন্ধে কিছু জানেন কি)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

13. Please mention cause of antibiotic resistance (এন্টিবায়োটিক রেজিসটেন্স কারণ)

a) Incomplete antibiotic course (অসমাপ্ত কোর্স) [1]

b) Using the same antibiotic too frequently [1]

(একই ধরনের এন্টিবায়োটিক ব্যবহার)

c) Over dose (অতিরিক্ত মাত্রা) [1]

d) Low dose (কম মাত্রা) [1]

e) Skipping doses of the medicine (মাত্রা বাদ দেওয়া) [1]

f) Taking different antibiotics at the same time [1]

(একই সময়ে একাধিক এন্টিবায়োটিক গ্রহণ)

g) Taking antibiotics that have been kept at home for a long time [1]

(দীর্ঘ সময়ে ঘরে সংরক্ষিত এন্টিবায়োটিক গ্রহণ)

h) Adulterated antibiotics (ভেজাল এন্টিবায়োটিক গ্রহণ) [1]

i) Others (অন্যান্য) ..... [98]

14. Do you have any knowledge about antibiotic residue (আপনার কি এন্টিবায়োটিক অবশিষ্ট সম্পর্কে কোন জ্ঞান আছে)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

15. What do you know about antibiotic resistance (আপনি এন্টিবায়োটিক প্রতিরোধী সম্পর্কে কি জানেন)?

a) It causes treatment failure [1]

b) It causes poor response to treatment [1]

c) Do not know [1]

d) Others (অন্যান্য) ..... [98]

16. Do you have any record of antibiotics sell (Only for medicine seller or shop keeper) (আপনার অ্যান্টিবায়োটিক বিক্রয়ের কোন রেকর্ড আছে কিনা)?

a) Have (আছে) [1] b) Don't have (নাই)

17. Purpose of antibiotic use (এন্টিবায়োটিক ব্যবহারের উদ্দেশ্য)

a) Therapeutic (প্রতিরোধক) [1] b) Prophylactic (প্রতিষেধক) [0]

c) Both (a+b) (উভয়) [1] d) Growth promotor (বৃদ্ধি তরান্বিত) [1]

18. Sell of antibiotics recommended by (Only for medicine seller or shop keeper)

a) Veterinarian (ভেটেরিনারিয়ান) [1]

b) Other farmers (অন্য খামারি) [1]

c) Representative of pharmaceutical company (ঔষধ কোম্পানির প্রতিনিধি) [1]

d) Village doctor (পল্লী চিকিৎসক) [1]

e) Quack (কোয়াক) [1]

f) Others (অন্যান্য) ..... [98]

19. Number of antibiotics use at a time (একই সময় অ্যান্টিবায়োটিক ব্যবহারের সংখ্যা)

a) Single (একক ভাবে) [1] b) Combined/Multiple (একাধিক) [1]

20. Frequency of use of antibiotics in livestock/poultry

(প্রতিরোধের জন্য এন্টিবায়োটিক ব্যবহারের ক্ষেত্রে সময়কাল)

a) Daily (দৈনিক) [1] b) Weekly (সাপ্তাহিক) [2]

c) Monthly (মাসিক) [3] d) When needed (যখন প্রয়োজন) [4]

21. Route of administration of antibiotics for livestock/poultry (এন্টিবায়োটিক ব্যবহার করার পথ)

a) Injection (ইনজেকশন) [1] b) Oral (মুখে) [1] c) Others (অন্যান্য) ..... [98]

22. Do you suggest for the completion of antibiotic course (আপনিকি এন্টিবায়োটিক কোর্স সম্পন্ন করতে পরার্মশ দেন)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

23. Do you know about withdrawal period of antibiotics (আপনিকি এন্টিবায়োটিকের উইথড্রয়াল সময়ের সর্ম্পকে জানেন)?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

24. Do you suggest the farmer to follow the withdrawal period of antibiotics?

a) Yes (হ্যাঁ) [1] b) No (না) [0]

25. How do you storedrugs (আপনি কিভাবে ঔষধ সংরক্ষন করেন)? (Only for medicine seller or shop keeper)

a) Store room (স্টোর রুম) [1] b) Refrigerator (ফ্রিজ) [1] c) Others (অন্যান্য) [98]

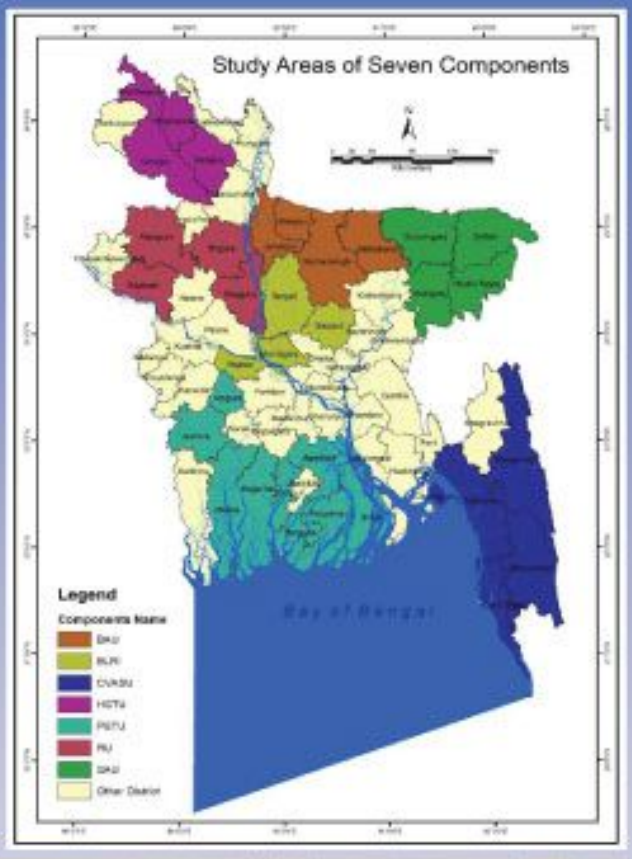
26. Do you sell antibiotic yourself or without taking prescription from any veterinarian (Only for medicine seller or shop keeper) (অ্যান্টিবায়োটিক বিক্রয় করেন নিজে বা ভেটেরিনারিয়ান-এর প্রেসক্রিপশন না নিয়ে)?

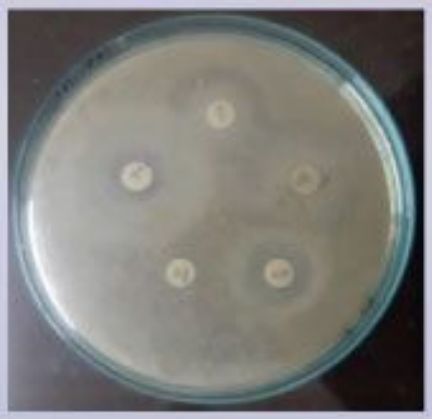
a) Yes (হ্যাঁ) [1] b) No (না) [0]

27. Do you follow the prescription of veterinarian during selling of exact prescribed antibiotic (Only for medicine seller or shop keeper)? (সঠিক এন্টিবায়োটিক বিক্রয়ের ক্ষেত্রে ভেটেরিনারিয়ান এর প্রেসক্রিপশন অনুসরন করেন কিনা)?

a) Always (সব সময়) [1]

b) Sometimes influenced by dealer of shopkeeper (ডিলার বা দোকানদার কর্তৃক পরিবর্তন) [0]





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