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POULTRY PRODUCERS' KNOWLEDGE, ATTITUDE AND PRACTICES REGARDING ANTIBIOTICS USE AND DETECTION OF MULTI-DRUG RESISTANCE PROFILE OF *ESCHERICHIA COLI* FROM SELECTED FARMS IN BISHOFTU TOWN, CENTRAL ETHIOPIA

BY

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Poultry Producers' Knowledge, Attitude and Practices Regarding Antibiotics Use and Detection of Multi-Drug Resistance Profile of *Escherichia Coli* from Selected Farms in Bishoftu Town, Central Ethiopia

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Title: Poultry Producers' Knowledge, Attitude and Practices Regarding Antibiotics Use and Detection of Multi-Drug Resistance Profile of *Escherichia Coli* from Selected Farms in Bishoftu Town, Central Ethiopia

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STATEMENT OF AUTHOR

First, I declare that this thesis my *bona fide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABR	Antibiotics Resistance
AHI	Animal Health Institute
AMC	Amoxicillin Clavulanic Acid
AML	Amoxicillin
AMP	Ampicillin
AMR	Antimicrobial Resistance
AMU	Antimicrobial Use
APEC	Avian Pathogenic *Escherichia coli*
AST	Antimicrobial Susceptibility Test
ATP	Adenosine Triphosphate
BHIA	Brain Heart Infusion Agar
BHIB	Brain Heart Infusion Broth
CIP	Ciprofloxacin
CN	Gentamycin
DNA	Deoxy Ribonucleic Acid
E. coli	*Escherichia coli*
EMB	Eosin Methylene Blue
HCCA	α-Cyano-4-Hydroxycinnamic Acid
KAP	Knowledge Attitude and Practice
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight
MDR	Multiple Drug Resistance
MHA	Muller Hilton Agar
MIC	Minimum Inhibitory Concentration
RNA	Ribonucleic Acid
S	Streptomycin
S3	Sulfonamide
SOP	Standard Operating Procedure
STM	Stuart Transport Media
SXT	Sulphamethoxazole-Trimethoprim
TE	Tetracycline
μL	Micro Litter

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ABSTRACT

Antibiotics are used at sub-therapeutic dose for growth promotion and prohylaxis in poultry are now on the rise which predisposes to the development and transfer of anti biotics resistance .A cross-sectional study was carried out to assess the knowledge, at titudes and practice of poultry producer regarding antibiotics use and resistance using semi-structured questionnaire to selected poultry farms in Bishoftu town, Ethiopia. Ei ghteen poultry farms were visited for sample collection to detect the resistance of *E. coli* from cloacal swab and litter samples using systematic random sampling, Identific ation of *E. coli* was carried out by MALDI-TOF. Kirby bauer disk antimicrobial susce ptibility test method was conducted to determine antibiotics resistance of *E. coli*. Desc riptive statistics, chis-quare, and logistic regression were used to determined the assoc iation of explanatory variable with knowledge, attitude and practice, antibiotic usage history, and resistance profile of *E.coli*. 61%, 67% and 56% of respondents in the stud y area had sufficient knowledge, good attitude and favorable practice on antibiotics us e and resistance, respectively. Knowledge of the usage of antibiotics and resistance was associated with respondents' age (> 45, OR = 3.6, p = 0.014), and farming experi ence (> 10, OR = 14.3, p = 0.00). The respondents' attitudes were also associated with their age (> 45, OR = 16, p = 0.001), education (Graduates, OR = 6.25, p = 0.007), and farming experience (> 10; OR = 5.4, p = 0.04). Practice of responders was associated with education level (Graduate, OR = 6, p = 0.01) and farming experience (>10, OR = 4.89, p = 0.004). According to correlation analysis, attitudes and the use of antibiotics as preventive medicine have a positive linear link (r = 0.81, p = 0.00), as well as attitudes and growth promotion (r = 0.47, p = 0.00). Additionally, there were s ignificant positive correlations between the usage of prophylactic antibiotics (r = 0.81 5, p = 0.00) and the stimulation of growth (r = 0.592,p = 0.00). Tetracycline, Sulpham ethoxazole + trimethoprim and Sulfonamide were commonly used in the study farms. *E. coli* was confirmed in 46% of cloacal sample, andin eleven farms by MALDI-TOF. 75% of the *E. coli* were showed multidrug antibiotisresistance. The study revealed co ncordance of farm antibiotic use purpose and resistance profile of the *E. coli,* warranti ng strict regulation to reduce antibiotics usage in poultry.

Keywords: *Antibiotic resistance, Attitudes, E. coli, Knowledge, Practice, Poultry farm, Bishoftu*

1. INTRODUCTION

Antibiotic kills or stops the spread of bacteria, produced by a micro-organisms. When an antibiotic is given to domestic animals at the right time and under the right conditions, vulnerable bacteria are killed, but resistant bacteria are left behind (Singh *et al*., 2018) due to resistance development to the given antibiotic (Madigan *et al*., 2014). By way of direct contact or environmental contamination, infected animals may release resistance bacteria into the environment, posing a risk to animals and people (Sri *et al*., 2018).

Bacteria counteract the actions of antibiotics by four well-known mechanisms, namely; drug inactivation, alteration in target binding sites, efflux activity and decreased permeability of bacterial membrane (Cox and Wright, 2013). Disk diffusion methods of antibiotic suceptibility is affordable, ease of use, and applicability to a wide range of bacterial species and medicines (Balouris *et al*., 2016).

Around 57 million poultry are thought to be present nationwide (CSA, 2021). Poultry production systems in Ethiopia are backyard, small-scale, medium and large-scale commercial production systems (FAO, 2019). All systems play a significant role in raising the socioeconomic status of the community and employment (Mohammed 2018).

Avian pathogenic colibacillosis (APEC) major bacterial diseases threatening the poultry industry all over the world (Sarba *et al*., 2019). The disease is caused by *Escherchia* coli (*E. coli)*; a gram negative and most *E. coli* species are and exist com mensally (Poirel, 2018). Tetracycline, Beta lactams, Aminoglycosides, Sulphonamide, and the Fluoroquinolones are used to treat poultry colibacillosis (Landoni *et al*., 2015). The chicken commercial used a lot of feed supplements containing tetracycline, chloramphenicol, and procaine penicillin in sub therapeutic levels to encourage growth and egg output (Marshall and Levy, 2011).

Numerous issues, including the possible transmission of infections resistant to antibiotics from animals to humans, have been brought up by the use of antibiotics in agricultural animals. Serious health consequences from this transfer could include treatment failures that could result in mortality and higher costs for both animal and human medicines (Friedman *et al*., 2016). Healthy chickens' gastrointestinal tracts may contain antibiotic-resistant bacteria, with faces and litter serving as potential transmission points (Laube, 2014) that could be spread from animals to humans either directly or indirectly through the food production chain (WHO, 2011). Investigations showed that human and poultry *E. coli* had a significant degree of genetic similarity (Vounba *et al*., 2019).

The incidence of antibiotic resistance has increased significantly in recent years (Marshall *et al*., 2011). Use of antibiotics for growth promotion, prophylaxis, and abuse of drugs without medical supervision or following improper diagnostic procedures are factors that contribute to antibiotic resistance development (Morar *et al*., 2012). Treatment of *E. coli* infections is challenging and complex due to the existence of strains that are resistant to antibiotics (Eltai *et al*., 2020). Previous studies in Ethiopia have reported antimicrobial resistance prevalence as high as 95% (Messele *et al*., 2017). The veterinary drug regulation authority of Ethiopia is not strong enough to enforce the drug use regulation in the veterinary sector (VDFACA, 2009). Antimicrobials are bought without prescriptions and administered by farm owners themselves (62%) are administered by either veterinarians (37.9%) or owners themselves (Agga *et al*., 2020).

According to studies conducted in central Ethiopia, almost 80% of those who grow food animals were unable to explain what antimicrobials were or how they were employed. Only 14.1% of the respondents were aware of antimicrobial resistance (AMR) and its effects, and 35.5% and 9.7% of them concurred that unwise antimicrobial use in animals could result in AMR in both animals and humans (Tufa *et al*., 2018).

The majority of the eggs and dressed poultry meat sold in Addis Abeba are produced in Bishoftu Town, which is home to the majority of Ethiopia's industrial-scale chicken farms (Paolo and Abebe, 2008). For any intervention to be successful and for the changes to be sustained, it should change the knowledge, attitudes and practices (KAP) of the target group (Brown, 2002). Despite this, studies on the knowledge, attitude, and practices on antibiotic usage and resistance in poultry farm; and antibiotics resistance profile of *E. coli* in poultry are deficient in Biahoftu town.

2. OBJECTIVES

General objective

- To gather information on the knowledge, attitude and practice of poultry producers regarding the use and resistance of antibiotics and to determine the multi-drug resistance profile of *E. coli* in poultry farms.

Specific objectives

- To identify *E. coli* from chicken cloaca and litter samples in selected poultry farm.
- To assess the type of antibiotic use in selected poultry farms.
- To ascertain the relationship between the knowledge, attitude and practice of chicken producers and antibiotic usage and resistance.
- To determine the antibiotic susceptibility profile of commercially available antibiotics used in selected poultry farm in the study area.

3. LITERATURE REVIEW

3. 1. Antibiotics

Antibiotics either kill bacteria by being cytotoxic or cytostatic to them, which enables the body's natural defenses, such as the immune system, to get rid of them. They frequently work by preventing the development of a bacterial cell, protein synthesis, DNA, and RNA, or by using a substance that disorganizes membranes (Levy and Marshall, 2004).

Since the invention of antibiotics 60 years ago, millions of metric tons of newer classes have been generated. However, due to the excessive and careless use of antibiotics, a considerable amount of the development of resistance strains has been attributed to this (Chopra *et al*., 2002).

3. 2. Antibiotics Resistance

Antibiotic development takes at least ten years to be approved for general use; in contrast, bacteria have a short time to develop resistance (Ahmad *et al*., 2017). Overuse, inappropriate prescribing, extensive agricultural use, the lack of novel antibiotics, regulatory barriers to producing new antibiotics, and an increase in worldwide trade and travel are some of the factors contributing to the antibiotic resistance dilemma (Ayukekbong *et al*., 2017). Bacteria might be develop multidrug-resistance to antibiotics which is lack of susceptibility in three or more antimicrobial categories (Food, 2011).

Drug resistance to antibiotics can arise naturally or be acquired from exogenous source. Intrinsic resistance is a trait that is shared universally within a bacterial species, is independent of previous antibiotic exposure (Cox and Wright, 2013). Acquired resistance occurs when a particular microorganism obtains the ability to resist the activity of an antibiotic agent to which it was previously susceptible. Target change, drug inactivation, reduced permeability, and efflux pumps are the causes of acquired bacterial resistance (Banin *et al*., 2017).

3. 3. Poultry Production System in Ethiopia

Around 57 million poultry are thought to be in the country (CSA, 2021). The poultry production system in Ethiopia can be divided into village or backyard commercial, small, medium and large-scale commercial production system based on the goal, breeds, flock size, housing, feeding, health, level of bio-security, and technology employed (FAO, 2019).

3. 4. Antibiotics Uses in Poultry

Antibiotic are used for a variety of purposes, including the treatment, prevention, and stimulation of growth (Economou *et al*., 2015). It is well known that more than 60% of all antibiotics are utilized, both therapeutically and non-therapeutically, in animal husbandry (Van Boeckel *et al*., 2015).

Resistance may develop as a result of the use of antimicrobial drugs as feed additives, which are often given at low doses over extended periods (Diarra and Malouin, 2014). The beneficial use of antibiotics in chickens, the potential spread of antibiotic resistant pathogenic and non-pathogenic strains of microorganisms into the environment (Apata, 2009). The chicken business used a lot of feed supplements containing tetracycline, chloramphenicol, and procaine penicillin at sub therapeutic levels to encourage growth and egg output (Marshall and Levy, 2011).

3. 5. *Escherichia coli*

The Enterobacteriaceae, which also comprises gram-negative bacteria, includes *Escherichia coli* appear as lone straight rods (Batt, 2014a). *E. col* functions as the typical intestinal flora seen in humans, animals, and birds (Quinn *et al*., 2015).

3. 5. 1. E. coli-related diseases in poultry

A variety of pathogenic isolates of the adaptable bacterium *E. coli* are capable of causing intestinal and extra intestinal infections, but the majority are safe for their hosts and are known as commensally isolates (Poirel, 2018). Avian pathogenic *E. coli* (APEC) leads to systemic infections in chickens either as a primary pathogen or secondary to Newcastle disease, avian influenza, mycoplasma infections, Infectious bursal disease, or environmental stresses by entering through oral and respiratory routes (Ghunaim, 2014).

It causes death (up to 20%) and morbidity in chicken, avian colibacillosis also causes a reduction in meat output (2% decrease in live weight, 2.7% decline in feed conversion ratio). APEC causes economic losses worth hundreds of millions of dollars for the global poultry industry (Guabiraba and Schouler, 2015). Treatment of Avian colibacillosis involves the use of a variety of antibiotics, such as, Aminoglyco sides, Tetracycline, Sulphonamide, & Fluoroquinolones (Landoni *et al*., 2015).

3. 5. 2. Escherichia coli antibiotic resistance mechanism

Antibiotic resistance mechanism in *E. coli* can be classified as follows

Drug inactivation: bacteria can inactivate medications in one of two ways: by actually degrading the drug, or by adding a chemical group to the drug. The most prevalent defense employed by gram-negative bacteria against Beta-lactam medications are Beta–lactamases (Kumar *et al*., 2013). The Enterobacteriaceae are the most often encountered family of plasmid-carrying Beta-lactamase genes (Schultsz and Geerlings, 2012).

Modification of drug targets: Antimicrobial targets such peptidoglycan, ribosome, nucleic acid enzymes and lipo-polysaccharides are beneficial for preventing infection by facilitating the binding of antibiotics, and any modification blocking drug binding and resulting in resistance (Varela *et al*., 2021). Quinolone resistance on *E. coli* through nucleotide substitutions (Rahman *et al*., 2017).

Reduced permeability: The structure and functions of the lipo-polysaccharides layer in gram-negative bacteria work as a barrier and confer on those bacteria an intrinsic resistance to specific families of powerful antibiotic drugs. The quinolone and Fluoroquinolones resistance in Enterobacteriaceae is caused by the plasmid-mediated, quinolone resistance gene (Blair *et al*., 2014).

Drug efflux pumps: All bacteria have efflux pumps, which are essential to their physiology and play a variety of roles including the removal of harmful metabolic byproducts and homeostasis maintenance (Varela *et al*., 2021).

E. coli uses a number of different drug efflux systems, including the ATP binding Cassette super-family (macrolides), major facilitator super-family (Norfloxacin, and Tetracycline), resistance-nodulation-cell division super-family (β-lactams and Tetracycline); small multidrug resistance family efflux pumps (Erythromycin); multidrug and toxic compound extrusion family (norfloxacin and Enrofloxacin) (Li and Nikaido, 2016).

3. 5. 2. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight

A new method for identifying microorganisms is matrix assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF) (Neelja *et al*., 2015). It is an ionization method that produces ions from big molecules with the least amount of fragmentation possible using a laser energy absorption matrix (Hillenkamp *et al*., 1991). In clinical microbiological laboratories, it has gained popularity as a tool for species identification since it has advantages over other immunological or biochemical techniques (Seng *et al*., 2009).

Most commonly there are three methods in current clinical microbiology laboratories sample preparation those are; Direct colony transfer method, extended extraction method (on target extraction method) as simple pretreatment approaches often used for identifications of common bacteria, such as gram-negative bacteria, Gram-positive bacteria, and mucinous bacteria, while Extraction (in-tube extraction method) as a more advanced but time-consuming preparation methods is used for difficult to identify microorganisms (Alex *et al*., 2017).

3. 6. Antibiotic Susceptibility Test Methods

Antibiotic susceptibility testing methods can be broadly divided into dilution method and diffusion method as follows:

3. 6. 1. Dilution method

It establishes the minimum inhibitory concentration (MIC) of an antibiotic. The MIC endpoint is defined as the lowest concentration of antimicrobial agent that, under appropriate incubation conditions, totally stops growth (Balouiri *et al*., 2016). The lengthy, manual process, potential for mistakes when preparing antimicrobial solutions for each test, and the comparatively high amount of reagents and space needed are the main drawbacks of the dilution approach (Jorgensen and Ferraro, 2009).

3. 6. 2. Diffusion method

Anti-bio gram provides qualitative results by categorizing bacteria as susceptible, intermediate or resistant (Jorgensen and Ferraro, 2009). Unable to differentiate between bactericidal and bacteriostatic effects since bacterial growth inhibition does not necessarily indicate bacterial death (Caron, 2012).

Agar disk-diffusion method cannot be utilized to estimate the MIC. Compared to other approaches, the disk-diffusion assay has numerous benefits, including simplicity, cheap cost, the capacity to test a large variety of bacteria and antimicrobial drugs, and the ease with which the data can be interpreted (Balouiri *et al*., 2016).

3. 7. Role of Antibiotic Uses for Antibiotic Resistant *E. coli* Occurrence

The prevalence of resistant bacteria in animals used for food production is closely correlated with the usage of antimicrobial drugs in husbandry (Baron *et al*., 2014).

The selection pressure for microorganisms resistant to antibiotics is heightened by the use of antibiotics in chicken production (Diarra and Malouin, 2014). They are frequently regarded as indicator bacteria for antibiotic resistance in populations of Gram-negative bacteria as a result of their prevalence and act as a model for research into the emergence of antibiotic resistance (Jubeh *et al*., 2020).

Animals' antimicrobial resistance to antibiotics, which contributes to the total burden of antimicrobial resistance, has been linked with excessive use of antibiotics in recent years, according to sufficient evidence (Marshall and Levy, 2011).

3. 8. Antibiotic Resistance's Impact on Public Health

Antibiotic-resistant bacteria can transfer from animals to humans' indirectly through food or less frequently, through direct contact (Spellbery, 2008). These bacteria, which asymptomatically colonize animal guts, may contribute to the epidemiological spread of resistance between humans and the food-producing animals (Chantziaras *et al*., 2014). *E. coli* strains are known to be cause colibacillosis in poultry, and some of them can cause serious human illnesses such hemolytic uremic syndrome and hemorrhagic colitis (Ferens and Hovde, 2011).

It has been suggested that the intestines of birds could be a source of *E. coli* with zoonotic potential that could spread from birds to people directly (Shecho *et al*., 2017). High genetic similarity between human and poultry *E. coli* suggests that the chicken may act as a reservoir for the human pathogen *E. coli* (Vounba *et al.,* 2019).

3. 9. Antibiotic Resistance Profile Reports of *E.coli* in Chickens in Ethiopia

The misuse and abuse of veterinary antibiotics have contributed to the development of bacterial resistance in *E. coli* (Table 1), which are present in animal intestines and transmit drug resistance genes between bacteria, contributing to the spread of drug resistance in Ethiopia has been summarized.

Table 1: Study on antibiotics resistance profiles of *E. coli* isolated from chickens in some parts of Ethiopia

Site	AST	Antibiotic disc	Resistance (%)	Reference
Eastern Ethiopia	Disc Diffusion	Ampicillin	92.30	Shecho *et al.*, 2017
		Amoxicillin	34.6	
		Streptomycin	34.61	
		Gentamycin	7.69	
		Tetracycline	76.92	
		Ciprofloxacin	0.0	
South west Ethiopia	Disc Diffusion	Ampicillin	91.	Bushen *et al.*, 2021
		Amoxicillin-clavulanic acid	66.7	
		Gentamicin	20.8	
		Tetracycline	75.0	
		Sulfamethoxazole-trimethoprim	70.8	
		Ceftriaxone	41.7	
		Ceftazidime	37.5	
Addis Ababa & Bishoftu	Disc Diffusion	Ampicillin	70.4	Messele *et al.*, 2017
		Erythromycin	40.7	
		Streptomycin	63.0	
		Gentamycin	0.0	
		Sulfamethoxazole–	0.7	
		Trimethoprim	48.1	
		Tetracycline	77.8	
Ambo	Disc Diffusion	Streptomycin	0.0	Sarba *et al.*, 2019
		Gentamicin	0.0	
		Tetracycline	46.3	
		Sulfamethoxazole-trimethoprim	0 .0	
		Ciprofloxacin	0.0	
		Nalidxic acid	7.3	

Key: AST-Antibiotics SusceptibilityTest

4. MATERIALS AND METHODS

4. 1. Description of the Study Area

The study was conducted in Bishoftu town, in the central highlands of Ethiopia Bishoftu is home to the majority of Ethiopia's industrial-scale chicken farms. Small and large commercial farms provide the majority of the eggs and dressed poultry meat to retailers in Addis Ababa (Paolo and Abebe, 2008).

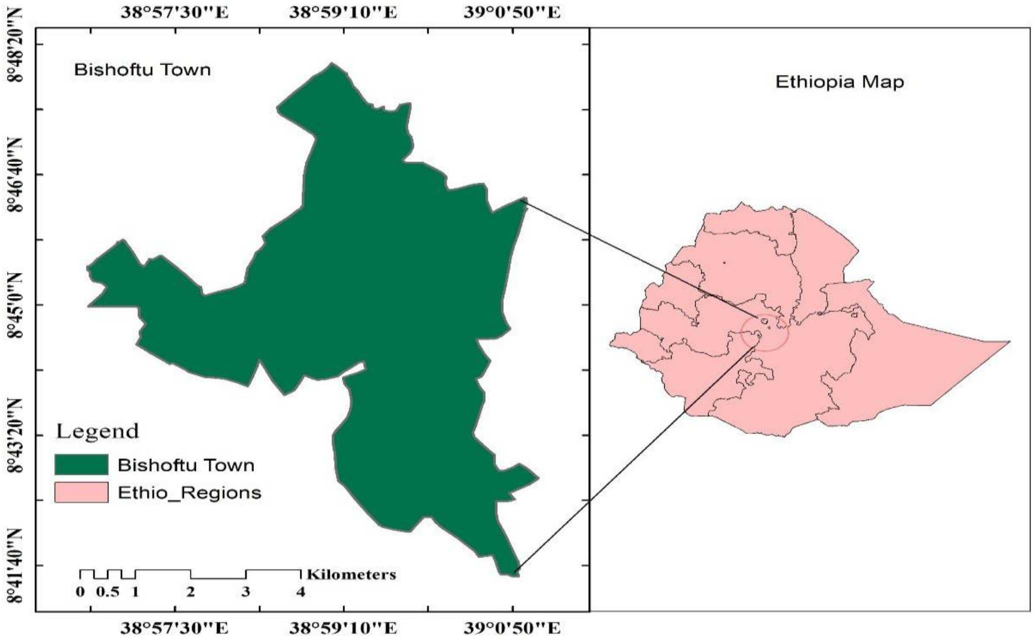


Figure 1: Map of the study site.

4. 2. Study Animals

A total of 244 poultry farms were registered in Bishoftu town with small (n = 122: 100 layers and 22 broilers), medium (n = 108: 78 layers and 30 broilers), and large (n = 14: 9 layers and 5 broiler) sacle production system (ADARDO, 2022). From 244 poultry farm producers, 110 (Small = 55: 45 layers and 9 broilers , Medium = 47: 34 layers and 13 broilers, large = 6: 4 layers and 2 broilers) were choosen for the pourpose of KAP assessements on antibiotics usage and resistance. The target population for antimicrobial susceptibility testing study comprised 18 (small = 9, medium = 7, large = 2) chicken farms selected by systematic random sampling method from 110 poultry farm used for the porpose of KAP assessments.

4. 3. Sample Size Determination

The sample size was estimated using the formula (Thrusfield, 2007), taking into account the expected prevalence of 37.0% (Messele *et al.*, 2017), 95% confidence interval, and the 5% type I error.

$$n = Z^2 * \frac{Pexp\,(1-Pexp)n}{d^2} \qquad \frac{1.96^2 * 0.37(1-0.37)}{0.05^2} = 358$$

Where, n = required sample size, Pexp = 37%, Z = z statistic for the level of confidence = 1.960, and d =desired absolute precision of 0.05. However, to improve precision, the sample was adjusted using (Lavrakas, 2008). 10% of 358 where 36 chickens were added, making the final number of birds used in the study 394.

The formula $0.25/SE^2$ (Hossein, 2005) was used to determine the sample size for the questionnaire for the KAP assessment, and it was discovered that 100 participants were required. However, 10% more was included to improve the precision, bringing the total number of participants to 110 poultry farms found in Bishoftu town. Where SE= 0.05 $SE^2 = 0.0025$ $0.25/0.0025 = 100$. However, of the total interviewed poultry producers, 108 participants (98. 2%) gave complete information whereas 2 (1.8%) were incomplete for most of the questions. Accordingly, two respondents were excluded from the analysis (Burns and Grove, 2011) and information from 108 respondents was used.

4. 4. Study Design and Sampling Strategy

To evaluate the knowledge, attitudes, and practices (KAP) of poultry producers and to identify the multidrug resistance profile of *E. coli* from cloaca swabs and litter samples from chicken farms, a cross-sectional study was conducted from November 2022 to June 2023. For this study, a total of 412 (10 grams of litter from each farm in total 18, and 394 cloaca swabs) samples were collected. Systematic random sampling was used to collect cloaca swab samples, which were allocated according to the number of hens on each farm.

4. 5. Sample Collection, Transportation and Storage

Using sterile cotton swabs provided by the microbiology department of the Animal health institute, cloaca swab samples were collected. Following suitable restraint, sterile cotton swabs were inserted and rotated into the poultry's cloaca before being transferred to a test tube containing five ml of broth Stuart transport media (CONDA). Total number of cloacal swab sample were allocated according to the number of poultry in the farm and finally 394 cloaca samples were collected from 18 poultry farms.

For each chicken farm, a bottle containing 10 grams of litter sample was taken from the center, middle, and corner of each poultry house using a sterile spoon that was inserted 2-5 centimeter below the surface (US Geological Survey, 2022). Following collection, the test tube's containing swabs and litter sample in universal bottle were immediately placed in an ice box contains ice packs.

Labeling was performed by using the initial letter of the animal's sex, the farm number, and the number of the sample retrieved from the farm. Litter sample represented by letter “L” with respective to the farm number. As soon as possible, samples were shipped in an icebox with an ice pack to the Animal Health Institute (AHI) in Sebeta. With the use of personal protective equipment and in accordance with SOP (Standard Operating Procuders), sampling procedures were carried out (WHO, 2003).

4. 6. Questionnaire

Knowledge, attitudes, and practices of poultry producers about the use of antibiotics and resistance in chicken farms were evaluated using semi-structured questionnaires (Appendix 1).

The question was created with the aid of a literature research on the KAP survey and international studies on antibiotics. During proposal defenses, the questionnaire's questions were internally examined for content. Based on evaluation's input and suggestions, questionnaire items were modified to better fit the local population. One responsible employee contributed from each farm.

The questionnaire had five sections and a total of 31 questions regarding to socio-demographic characteristics of the poultry producer, the producer's knowledge, attitude, and practices with regard to antibiotic use and resistance, and list of antibiotics for different purpose. These farms were visited, and their owners were contacted, to gauge their interest in taking part in the study. A semi-structured questionnaire was used to conduct in-person interviews with specialists in animal health.

4. 7. Isolation and Identification of *Escherichia coli*

All samples that were brought to the AHI reception desk were registered, given a lab code, and then transferred to the bacteriology laboratory where they were maintained at 4°C until processing. In this laboratory different procuders were done to isolate and identify the bacteria *E. coli*.

4. 7. 1. Isolation of E. coli

Cloacae sample in Stuart transport media (STM) were incubated at 37 °C for an overnight. After overnight incubation, Samples were then placed in to class II microbiology safety cabinet.

The day before, 394test tubes containing 5 ml of brain heart infusion broth (BHIB) (HIMEDIA) were prepared then after labeling each swab containing sample was transferred to respective test tube containing brain heart infusion broth media in to class II microbiology safety cabinet, then placed in incubator for 24 hours at 37 ^{0}C.

A total of 394 petridis containing Eosin Methylene Blue (EMB) (HIMEDIA) media prepared the day before, This prepared EMB media were labeled after checking the strality. Then sample in the BHIB were placed at room temperature and homogenized using a vortex. Then, from a test tube containing BHIB media, a loop full of culture suspension was extracted, streaked to an EMB containing Petridis in a sterile location, using sterile inoculating loop under a Bunsen burner. Following a 24 hour incubation period, EMB petridis were seen under a Bunsen burner. Colony that had a metallic shine was chosen and subculture on Brain Heart Infusion Agar (BHIA) media (CRITERION) for overnight at 37 ^{0}C for purpose of obtaining pure colony.

Using a cry vial tube (Thermo scientific) with 1.5 milliliter of a 20% glycerol and 80% BHIB media combination preservative, a pure colony was taken and added in the preservative under a Class II microbiological safety cabinet. Following that, samples in the cry vial were kept at -20 ^{0}C for further conformation testing.

For a sample of litter 50 ml of BHIB media and 10 gram of sample were combined, homogenized with a vortex mixer, and then incubated at 37 ^{0}C for an overnight period. The identical steps as with cloaca sample isolation were carried out after a 24-hour incubation period. Isolation and preservation of *E. coli* suspected culture was conducted following SOP of microbiology departments of AHI, Sebeta (Appendix 2).

4. 7. 2. Identification with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Biotyper

Sample preserved at -20 ^{0}C ware placed at +4 ^{0}C refrigerator for 24 hours. After 24 hours incubation it was placed at room temperature with minimum of 2 hours and sub cultured in to BHIA media and incubated at 37 ^{0}C for 24 hours. After observing the pure colony all sub cultured Petridis was transferred to MALDI-TOF machine class room.

Extended direct transfer sample preparation procedure method of sample preparation was used. A single colony was directly smeared onto the target plate, applied with 1 µL 70% formic acid, dried at room temperature, and then overlaid with 1 µL HCCA (α-Cyano-4-Hydroxycinnamic Acid) matrix solution. After air drying at room temperature, the samples were sequentially analyzed by using the Bruker MALDI-TOF Biotyper (Daltonics, 2012) **(**Appendix 3).

4. 8. Antimicrobial Susceptibility Test

Kirby Bauer disk method was used to determine whether or not a given species of bacteria is susceptible to a particular antibiotic by using antibiotic-impregnated disk (OXOID). Antibiotic were selected based on both the ones being used by the poultry farmers as well as the commonly available antibiotics in the study area which includes Ampicillin, Amoxicillin, Ciprofloxacin, Tetracycline, Gentamycin, Streptomycin, Sul fonamides, Sulphamethoxazole + trimethoprim, and Amoxicillin-clavulanic acid.

After obtaining pure culture by BHIB a McFarland 0.5 standardized suspensions of the bacteria were made (CLIS, 2022). Surface of the over-dried Mueller Hinton Agar (CRITERION) were swabbed with the dispersion. Antibiotic discs were placed on the plates with the aid antibiotic disc dispenser (OXIOD) in Class II safety cabinet by using sterile cotton. For each disc to make full contact with the agar surface, downward pressure was applied. The plates were placed incubator upside down position and left there for 18 hours at 37 ^{0}C (Appendix 4).

After 18 hours the zone of inhibition was determined by using measuring caliper, the clear zones of bacterial growth inhibition were quantified in millimeters. Based on the zone of inhibition and class of antibiotics used for the assays, the isolates were classified as "resistant (R), intermediately resistant (I), and sensitive (S)" based on the results of the test. These classifications are based on the performance standards of the Clinical and Laboratory Standard Institute (CLSI, 2022) (Appendix 5).

4. 9. Data Analysis

Data were classified, filtered, and coded using Microsoft Excel (Appendix 6). The dat a were analyzed using descriptive statistics, Chi square, a univariant and multivariate l ogistic regression. All statistically significant factors from chi square were included to the univariant analysis then subjected to multivariate analysis using backward stepwi se selection by removing least significant variable at each step based on p-value. The variance inflation factor was calculated to identify the presence of multicollinearity between the independent variables. Interaction between the independent variables ware also checked by command “testparm”.

Spearman's rank order correlation coefficient was used to define the relationship and direction of the association between KAP with antibiotics usage purpose were studied in this study to estimate the nature relationships.

The prevalence of *E. coli* in cloaca swab and litter samples was calculated by dividing the number of positive samples by the total number of samples analyzed. The percentages of antimicrobial resistance of each pattern were calculated including the multidrug resistance profile.

Analysis was performed using STATA software version 16 (Texas, USA). In all the analyses P-value of less than 0.05 was considered significant.

4. 10. Ethical Consideration

Ethical clearance for the study was taken from Addis Ababa University College of veterinary medicine and agriculture ethics and review committee (Appendix 8).

4. 11. Limitation of the study

Due to a lack of an antibiotic resistance gene kit, it was impossible to undertake molecular antibiotics resistance gene detection to determine which antibiotic resistant genes were present in the study area.

5. RESULTS

5. 1. Respondents' Socio-Demographic Characteristics

The socio-demographic characteristic of respondents were assessed and presented (Table 2). Of the total respondents, 78 (72.2%) were males, while 30 (27.8%) were females.

Table 2: Socio-demographic characteristics of poultry producers in Bishoftu (n = 108)

Characteristics	Category	N	100%
Sex	Female	30	27.8
	Male	78	72.2
Age (Year)	18-30	42	38.9
	31-45	36	33.3
	> 45	30	27.8
Level of education	Non-formal education	18	16.7
	Primary/ secondary school	36	33.33
	Graduate	54	50.00
Farming experience (Year)	< 5	42	38.9
	5-10	36	33.3
	> 10	30	27.8

Key: N-number

5. 2. Knowledge, Attitudes and Practices on Antibiotic Uses and Resistance

It is well known that the knowledge, attitude and practice of poultry producers can affect awareness regarding antibiotic usage in poultry farm and antibiotics resistance developments.

5. 2. 1. Knowledge

In this study poultry producers, who participated in the study 36 (33.3%) had "high knowledge" and 30 (27.8%) of them had "moderate knowledge" while 42 (38.9%) respondents had "low knowledge" regarding antibiotic use and resistance. Despite the fact that antibiotics were used in therapy, 38 (35.2%) of the respondents were unable to define what they meant. Only 40 (37%) of those who said they were familiar with antibiotics were able to classify them as drugs that either kill or suppress germs.

A total of 56 (51.9%) respondents did not know anything about antibiotic resistance. In 66 (61%) of the farms that raise poultry, antibiotics are given for prophylaxis, yet not in 42 (39%) of them. Antibiotics were not used to boost growth at 60 (55.6%) chicken farms, and 48 (44.4%) were in support of the practice.

In response to the question of whether there is a link between antibiotic usage in chicken and the emergence of antibiotic resistance, 36 (33.3%) respondents knew the relationship between the use of antibiotics use and the development of resistance, whereas 72 (66.7%) did not know about it. 36 (33.3%) of respondents did not know the use of antibiotics in animals can reduce the effect of antibiotics on humans.

5. 2. 2. Attitude

It's common knowledge that a certain medicinal regimen might be affected by the employees' attitude at a chicken farm. The vast majority of respondents to the study claimed to have a positive opinion of both antibiotic use and resistance. A "positive attitude" was found in 30 (27.8%) respondents, whereas 36 (33.3%) of them had a "negative attitude" towards antibiotic use and resistance in chicken farms, and 42 (38.9%) of them had a "moderate attitude."

The majority of respondents in the research area 81 (75.0%) accept that only individuals who are legally licensed to do so may sell and distribute antibiotics. When it came to the statement that using the same antibiotics over an extended period of time can cause antibiotic resistance, 78 (72.2%) respondents agreed, while 30 (27.8%) disagreed.

When poultry was not treated, increasing the dose of antibiotics was preferred, according to 38 respondents (35.2%). In addition, the use of antibiotics for non-therapeutic conditions contributed to the development of antibiotic resistance. Of the respondents, 30 (27.8%) were aware of this fact, while 78 (72.2%) were unaware of it.

A survey of poultry producers revealed that 56 (51.9%) were unaware that limiting the use of antibiotics for non-therapeutic purposes can prevent the emergence of antibiotic resistance. 48 (44.4%) of the responders in the study area were kept on antibiotics at the farm.

5. 2. 3. Practice

48 (44.4%) of the study's participants had a positive outlook on antibiotic use and resistance. While 36 (33.3%) of the farm workers were of the opinion that bad practice was being engaged in, 24 (22.2%) of the participants were practicing moderately.

90 (83.3%) of the respondents in the research area said they had not received any training on antibiotic use and resistance. When chickens become ill, 72 (66.7%) poultry breeders visit a veterinarian before giving them antibiotics. 42 respondents, or 38.9%, said they never check the antibiotics' expiration dates before using them.

60 (55.6%) of chicken breeders choose their medicine administration routes based on leaflets or prescriptions. In 78 (72.2%) of the chicken farm attendants polled for this topic, antibiotics were prescribed by veterinarians; among the remaining respondents, friends provided antibiotics in 12 (11.1%) of instances and self-prescribed in 18 (16.7%) of cases.

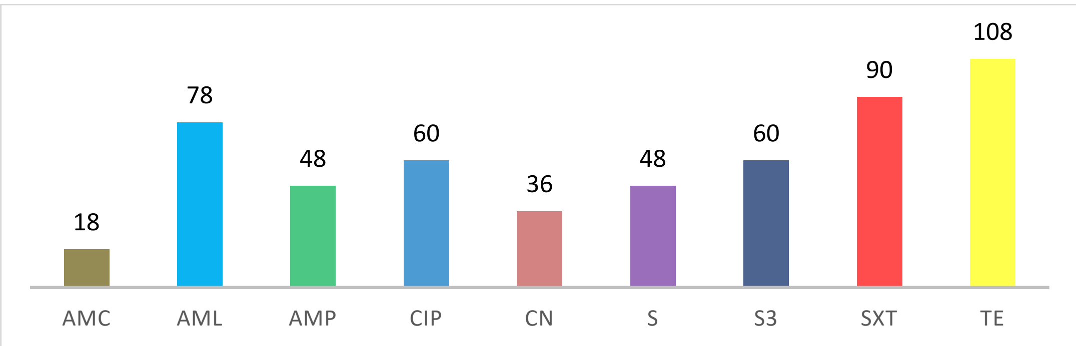
In terms of the dosage of antibiotics that should be used prior to using them on hens on a farm, 60 (55.6%) were determined by reading the leaflet or prescription, whereas 12 (11.11%) and 36 (33.33%) were determined, respectively, by advice from friends and prior experience. 48 (44.4%) of the attendees gave their poultry antibiotics themselves, while the other 50% had a veterinarian provide them.

Producers of poultry obtained antibiotics from veterinary clinics and pharmacies in proportions of 90 (83.3%) and 72 (66.7%), respectively. The sources of antibiotics for 12 (11.1%) and 24 (22.2%) of the population were human pharmacies and open marketplaces.

5. 3. Antibiotic Usage Purpose in Poultry

Antibiotics usage purpose assessed in the study area indicates that poultry producers were used for treatment purpose in all poultry farms, as growth promotion in 48 (44.4 %), and for prevention purpose 66 (61.1%) of poultry producer used in poultry farm.

As shown in figure 2, Tetracycline (100%), Sulphamethoxazole + trimethoprim (83.3%), and Sulfonamide (55.6%) were the three antibiotics that were most frequently prescribed in this study area.



Keys: AMP-Ampicillin, AML-Amoxicillin, AMC-Amoxicillin Clavulanic Acid, CIP-Ciprofloxacin, CN-Gentamycin, S-Streptomycin, S3- Sulfonamide, TE-Tetracycline SXT-Sulphamethoxazole + trimethoprim

Figure 2: Antibiotics used for the treatment of poultry diseases in selected study farms.

Tetracycline (83.3%), Sulphamethoxazole + trimethoprim (55.6%), Sulfonamides (55.6%), Ciprofloxacin (44.4%), Ampicillin (38.9%), & Amoxil (46.3%) were the antibiotics most frequently used for prophylaxis at the chicken farm under study. Tetracycline, Sulphamethoxazole + trimethoprim , and Sulfonamides were utilized in the research region to promote growth.

5. 4. Socio-demographic Characteristics with Knowledge, Attitude and Practice Regarding Antibiotic Usage and Resistance

Socio-demographic characteristics of respondents were examined to determine whether there was any association with the respondents' knowledge, attitudes, and practice regarding the use of antibiotics and antibiotic resistance.

Chi-square analysis indicates that a statistically significant association were found between Knowelage, attitude and practice with age, level of education and farming experience ($p < 0.05$) of poultry producers (Table 3).

Poultry producers over 45 years were 5 times more likely knowledgeable ($p = 0.014$) than those of respondents having age range of 18-30. Regarding the farming experience of the poultry producers, those of having greater than ten years farming experiance, and five to ten years farming experience were 14, and 3.7 more times more likely knowledgeable when compared to those of having less then five years experience, respectively (Table 3).

Analysis using multiple logistic regression respondents having age of over 45 years being 16.4 times more likely to show favorable attitudes ($p = 0.001$) than respondents in the category of 18 to 30 years. Respondents with more than 10 years of farming experience were 5.4 times more likely to show a positive attitude ($p = 0.04$) to those of respondents having less than five years experiance (Table 3)..

Respondent who were graduates had 6 times more likely have good practice as compared with those following no formal education ($p = 0.011$) (Table 3). Regarding farming experience and practice, it was found that those with more than ten years of experience were seven times more likely to have good practice than those with fewer than five years of experience ($p = 0.004$).

Table 3: Analysis Socio-demographic and Knowledge, Attitude and Practice of the poultry producers in Bishoftu town

Chi-square analysis output

SD	Knowledge		Attitudes		Practice	
	$\chi2$	P-value	$\chi2$	P-value	$\chi2$	P-value
Sex	0.022	0.88	0.831	0.362	1.662	0.197
Age (year)	13.385	0.001	14.204	0.001	8.229	0.016
Level of education	16.558	0.000	25.500	0.000	18.518	0.000
Farming experience (year)	31.358	0.000	17.614	0.000	18.5143	0.000

Univariant logistic regression analysis output

SD	Category	Knowledge		Attitudes		Practice	
		OR	P-value	OR	P-value	OR	P-value
Age (Year)	31-45	2	0.154	3.2	0.018	1.8	0.205
	> 45	6.7	0.001	10.9	0.000	4.2	0.005
	_cons	.75	0.356	.83	0.538	.56	0.068
Level of education	1^0 & 2^0 school	1.8	0.333	2	0.249	1.8	0.402
	Graduate	7.8	0.001	16	0.000	8.3	0.001
	_cons	.5	0.166	.5	0.166	.29	0.027
Farming experience	5-10	7.5	0.001	4.7	0.002	2.5	0.055
(Year)	> 10	15	0.000	8.7	0.001	10	0.000
	_cons	.4	0.007	.75	0.356	.4	0.007

Multiple logistic regression analysis output

SD	Category	Knowledge		Attitudes		Practice	
		OR	P-value	OR	P-value	OR	P-value
Age (Year)	31-45	2.64	0.132	4.2	0.022	2.2	0.159
	> 45	5.32	0.014	16.4	0.001	3.3	0.064
Farming experience	5-10	3.74	0.043	2.8	0.125	1.9	0.246
(Year)	> 10	14.3	0.000	5.4	0.040	7.2	0.004
Education level	1^0 & 2^0 school			1.4	0.636	.97	0.975
	Graduate			8.1	0.007	6.1	0.011
	_cons	.220	0.002	.13	0.003	.10	0.005

Keys: $\chi2$-Chi-square; 1^0-Primary; 2^0-Secondary; P-Probability; OR- Odd ratio; SD- Socio-demographic

5. 5. Socio-demographics Characteristics with Pattern of Antibiotic Use

All farm workers used antibiotics as treatment, regardless of their sex, age, level of education, or farming experience (Table 4). Level of education ($p = 0.000$), and farming experience ($p = 0.000$) were linked with the prophylactic and growth promotion use of antibiotics.

Table 4: Analysis of Socio-demographic with Antibiotic Usage Purpose in poultry farms in Bishoftu town

SD	Category	Rx	Prophylaxis				Growth promotions			
		Yes	Yes	No	χ2	p-value	Yes	No	χ2	p-value
Sex	Male	78	48	30	0.03	0.883	34	44	0.08	0.77
	Female	30	18	12			14	16		
Age (Year)	18-30	42	24	18	0.46	0.796	17	25	0.92	0.63
	31-45	36	23	13			18	18		
	> 45	30	19	11			13	17		
Education level	Non formal	18	3	15	25.44	0.000	3	15	13.94	0.001
	1^o&2^o school	36	19	17			13	23		
	Graduated	54	44	10			32	22		
Farming experience (Year)	< 5	40	17	23	15.32	0.000	10	30	22.69	0.000
	5-10	36	21	15			12	24		
	> 10	32	28	4			24	8		

Keys: χ2 – Chi-squre; P-Probability; Rx- Treatment; SD-Socio-demographic

According to multiple logistic regression analysis, education level and farming experience were significantly associated with the intention to use antibiotics as prophylaxis and growth promotion (Table 5). As the farming experience and level of education increase using of antibiotics as growth promotion and prohylaxis also increased.

Table 5: Analysis of Socio-demographic Character with Antibiotics Usage Purpose in Poultry Farms in Bishoftu Town is

Univariant logistic regression analysis output					
SD	Category	Prophylaxis		Growth promotion	
		OR	P-value	OR	P-value
Level of education	1^0 & 2^0 school	5.59	0.016	4.52	0.068
	Graduate	22	0.000	11.6	0.002
	_cons	.2	0.011	.125	0.006
Farming experience (Year)	5-10	1.894	0.170	1.5	0.425
	> 10	9.47	0.000	10.71	0.000
	_cons	.739	0.345	.3333	0.003
Multiple logistic regression analysis output					
SD	Category	Prophylaxis		Growth promotion	
		OR	P-value	OR	P-value
Farming experience (Year)	5-10	.853	0.776	.857	0.787
	> 10	4.25	0.034	6.31	0.002
Education level	1^0 & 2^0 school	3.74	0.077	2.42	0.311
	Graduate	15.4	0.000	6.58	0.028
	_cons	.206	0.014	.129	0.007

Keys: OR-Odd Ratio; P-Probability; SD-Socio-demographic

5. 6. Antibiotic Usage Purpose with Knowledge, Attitude and Practice of Poultry Producers

Attitude and practice of poultry producers regarding antibiotics usage and resistance were significantly associated with using of antibiotics for prophylaxis ($p < 0.05$) (Table 6). There was also an association of poultry producers regarding antibiotics usage and resistance with the use of antibiotics to encourage growth of poultry ($p = 0.000$).

Table 6: Analysis of antibiotic usage purpose with knowledge, attitude and practice of poultry producers in Bishoftu town

		Rx	**Prophylaxis**				**Growth promotions**			
KAP	Response	Yes	Yes	No	$\chi 2$	p-value	Yes	No	$\chi 2$	p-value
Knowledge	Yes	66	41	25	0.073	0.787	29	37	0.0	0.89
	No	44	25	17			23	19	18	
Attitudes	Yes	72	64	8	70.13	0.000	44	38	24.	0.000
	No	36	8	48			4	32	30	
Practice	Yes	60	44	16	45.63	0.000	44	28	24.	0.000
	No	48	4	44			4	32	3	

Keys: $\chi 2$-Chi-squre; KAP- Knowledge, Attitudes and Practice, P- probability

Correlation anylasis indicates that poultry producer are using antibiotics as growth promotion and prophylaxis without sufficient knowledge (Table 7), but using antibiotics as growth promotion and prophylaxis were correlated with the the attitude and practice of poultry producers.

Table 7: Correlation analysis of antibiotic usage pattern with the KAP of respondents

Antibiotics usage purpose	**KAP**	**r**	**P-value**
Prophylaxis	Knowledge	0.0260	0.7896
	Attitude	0.8058	0.0000
	Practice	0.8154	0.0000
Growth promotion	Knowledge	-0.0127	0.8959
	Attitude	0.4743	0.0000
	Practice	0.6500	0.0000

Key: KAP- ; Knowledge, Attitude and Practice; r -Correlation coefficient

5. 7. Antimicrobial Susceptibility Profiles

E. coli was confirmend in 181 (45.9%) coacal swabs by MALDI-TOF. *E. coli* was also confirmed in eleven farms from a total of 18 litter's sample. *E. coli* demonstrated resistance against Tetracycline (n = 152, 84)%), Amoxicillin (n =151, 83.4%), Sulfonamides (n = 82, 45.3%), Ciprofloxacin (n = 82, 45.3%), Ampicillin (n = 68, 37.6%), Streptomycin (n = 67, 37%), Sulphamethoxazole + trimethoprim (n = 65, 35.9%), Amoxicillin-clavulanic acid (n = 18, 9.9%), and Gentamycin (n = 2, 1.1%) for cloaca swab samples (Figure 3). 170 (93.92%) *E. coli* isolates from the cloaca showed resistance at least for one drugs and in 11 (6.08%) of the isolate resistance were not observed.

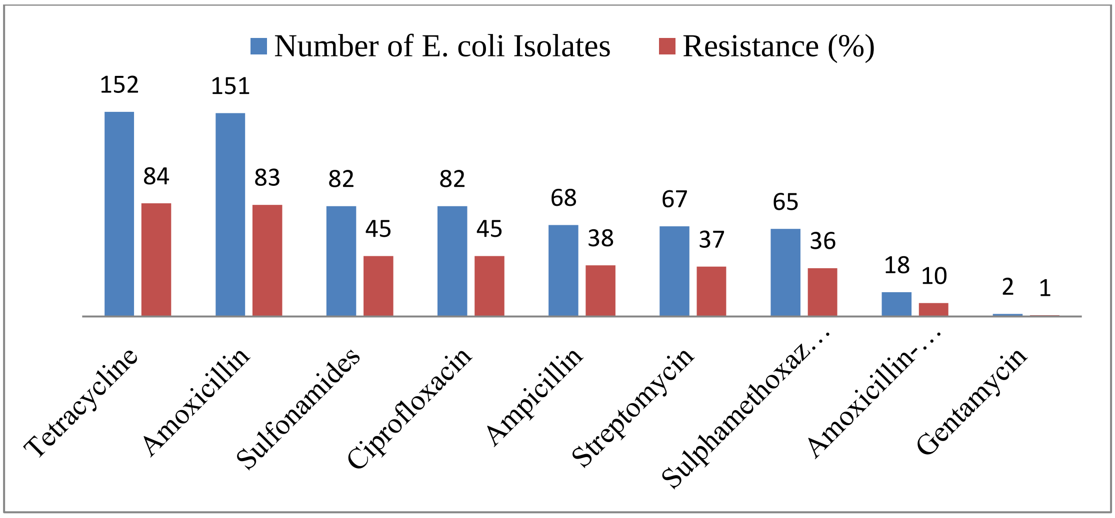


Figure 3: Rresistance profile of antbiotics for *E. coli* isolated from Cloaca swab sample

E. coli was found to have a high rate of resistance to the following drugs in the eleven litter sample (Figure 4): Amoxicillin (n = 9, 90%), Tetracycline (n = 7, 70%), Ampicil lin (n =5, 50%),Streptomycin (n = 3, 30%), and Sulfonamide (n = 2, 20%).

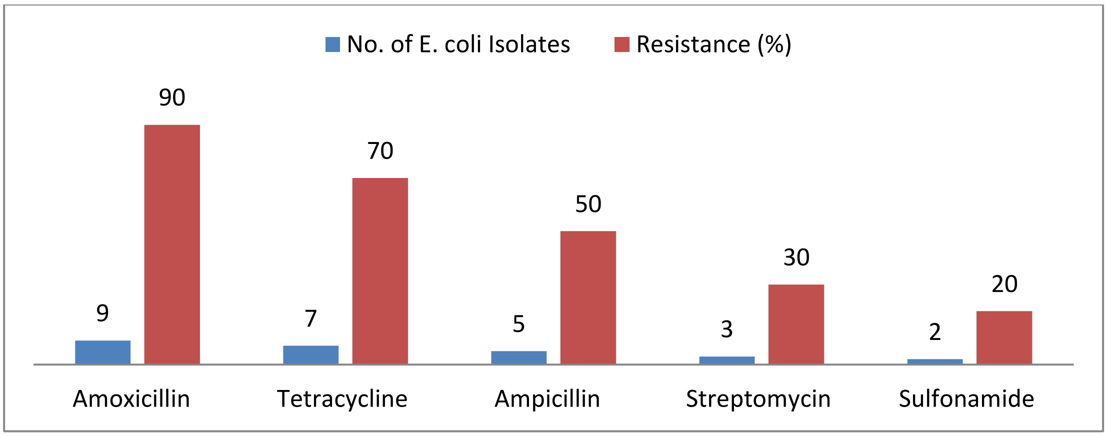


Figure 4: Resistance profile of antbiotics for *E. coli* isolated from litter sample

5. 8. Multi-drug resisance profile of *E. coli*

Multi drug resistance (MDR) profile of *E. coli* isolates from cloaca swabs used in this study showed resistance to 2 (1.1%), 7 (3.86%), 13 (7.18%), 21 (11.60%), 27 (14.91%), 21 (11.60%), and 45 (24.86%) isolates showed MDR to 9, 8, 7, 6, 5, 4, and 3 class of antibiotic, respectively.

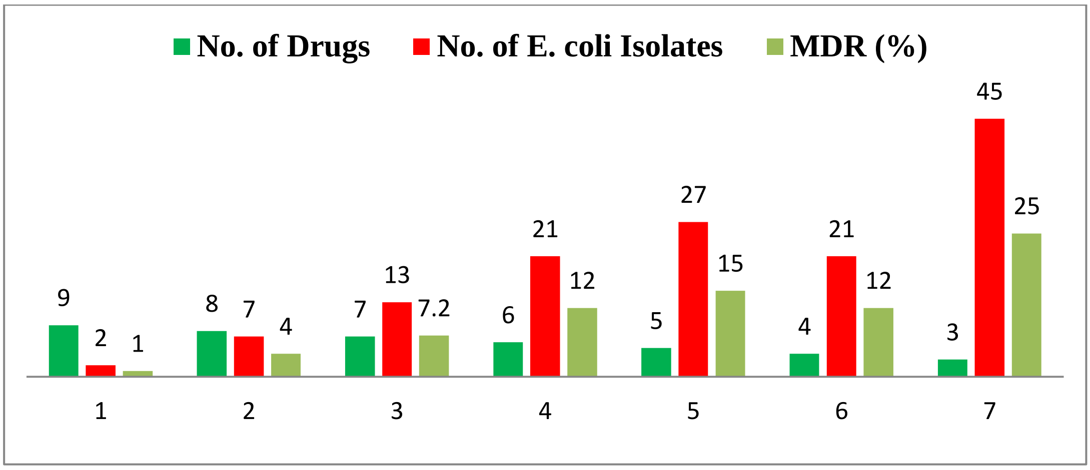


Figure 5: Multiple drug resistanceof *E. coli* cloaca swab sample

Seven isolate of *E. coli* from litter sample showed multiple drug resistance at leas for three class of antibiotics. Multi-drug resistance profile of *E. coli* identified from litters sample was recorded (Table 8). In one farm *E. coli* isolate showed multie drug resistance for five antibiotics in different class where as five *E. coli* isolate showed MDR for three class of antibiotics.

Table 8: Multi-drug resistance profile of *E. coli* isolated from litter sample

Antibiotics	Frequency	Resistance *E. coli*	No. Class of antibiotics	%
AML, CIP, TE	1	3	3	30
AMP, AML, TE, S3	1	1	3	30
AMP, AML, TE, S	1	1	3	
AML, CIP, TE, S	1	1	4	
AMP, AML, TE, S, S3, AMC	1	1	5	10

Keys: AMC-Amoxicillin-clavulanic Acid; AML-Amoxicillin; AMP-Ampicillin; CIP-Ciprofloxacin; S-Streptomycin; S3-Sulfonamides; TE-Tetracycline

6. DISCUSSIONS

Knowledge, attitude and practice on antibiotic usage and resistance among the poultry farm attendants of many countries including, Ethiopia has only been studied to a limited extent. This study done to assessed the knowledge, attitude and practice of the poultry producer regarding antibiotic use and resistance, and to determine multidrug resistance profile of *E. coli* in poultry farm.

In the current study 61% of respondents have knowledge regarding antibiotic use and resistance closure to reports of 70% of satisfactory knowledge about antibiotics use and resistance (Oluwasile *et al*., 2014). This study was higher than reports of 50.5% on livestock farm owners/workers (Geta and Kibret, 2021). This study dissimilar to reports of 80% (Gebeyehu *et al*., 2021), 94% livestock producer were not knowledgeable about AMR and AMR (Tufa *et al*., 2023) in Ethiopia. this difference might be assciated with the species difference and management of the animals.

The use of antibiotics for diseases that are not actually treatable with these medications is another factor contributing to abuse, and as a result, to the development of resistance. It is therefore crucial to understand which conditions can be treated with antibiotics (Widayati *et al.,* 2011). The percentage of respondents who knew what an antibiotic was 64.8%, lower than the reported of 90.6% in Zambia (Chilawa *et al*., 2023) and 92% in Bangladesh (Hassan *et al*., 2021). Only 37% of respondents in the current study correctly identified the purpose of antibiotics. This data suggests that the participants' knowledge of antibiotics is only undeveloped.

Respondents' awareness in this study about antibiotic resistance was 48.2%, which was lower than reports of 56.7% (Hassan *et al*., 2021), and better than 14.1% (Tufa *et al*., 2018) in Ethiopia, and 29.2% (Chilawa *et al*., 2023) in Zambia. This might be assciated with lack of trainig related to the antibiotics use and resistance.

Poultry producer were assessed the relationship between antibiotic use and antibiotic resistance and 33.3% of the respondents to this study were not aware of the connection between antibiotic use and antibiotic resistance. This finding was higher than previous studies of 22.4% of people incorrectly demonstrated their understanding of how using certain antibiotics can lead to resistance (Tesfaye, 2017). This outcome was lower than 36.3% reports in Ethiopia (Geta and Kibret, 2021), and 62.1% findings from Bangladesh (Hassan *et al*., 2021). This might be associated with the use of antibiotics other than treatment is now increasing. This might be associated with the sciodemographic characterstics of the respondentsl; for example educational level where half of the respondents were followed non formal education, and primary and or secondary education; as well as associated with the farming experience; most of the respondents in the study area had experience of less than then years experiences.

Due to AMR, which is becoming more prevalent, the use of antibiotics in animals is coming under more examination (Anderson *et al*., 2003). In this study, 66.7% of respondents did not understand that using antibiotics on animals can lessen their impact on humans. This result was higher than the 43.2% that (Tesfaye, 2017) stated. This might be assciated with lack of traning to the poultry producer.

In this study respondents above the age of 45 were 5 tims more likely to be knowledgeable compared to than those between the ages of 18-30 age. This finding was supported by reports of older farmers had 1 times more likely knowledge (Oluwasile *et al.*, 2014). This might be associated with most of the respondents above the age of 45 were having greater thn ten years experience and were in the category of education level who were graduated.

Poultry producer with more than ten years of experience were fourteen times more likely to be knowledgeable compared to responders with less than five years of experience. This result supported data from Ethiopia (Tufa *et al.,* 2023) and Bangladesh (Hassan *et al.,* 2021) that farmers' knowledge rose considerably with farming experience. It indicates that most of experienced respondents in the study area were in the category of having experience greater than ten years.

In the current, 66.7% of farm workers had desirable attitudes toward antibiotic use and resistance, which was higher than reports of 52.8% Geta. and Kibretn (2021) in Ethiopia, and closure to 68% reports of Oluwasile *et al*., (2014). This study were on contrary to previous reports 97% unfavorable attitudes toward contributing factors to AMR among livestock producers (Tufa *et al*., 2023), 71.7% negative attitudes (Chilawa *et al.*, 2023) from Zambia. This might be as a result of this study was at poultry farm level while the other studies were includes the extensive farming system and species of animal reared were poultry and ruminates.

The primary causes of antibiotics resistances include antimicrobial use fragmented governance in the livestock production industry (Ekakoro *et al.,* 2018). 75% of respondents agreed that only individuals who are legally licensed to do so should sell and distribute antibiotics. This result was higher to reports of 53.8% of livestock producers (Geta and Kibret, 2021) but comparable to reports of 78.6% of commercial laying hens in Nigeria (Adebowale *et al.,* 2016). This might be related with the current inflation cost of drugs, lack of understanding of irrational use of antibiotics, and lack of strict control measure of antibiotics in the study area.

According to this study, 35.18% of respondents increased their antibiotic dose or frequency when their poultry was not cured, compared to 64.8% of respondents who did not. The findings of this study did not support statements that 71.1% of Bangladeshis did not increase their antibiotic dosage or frequency (Hassan *et al.*, 2021). This also related with lack of traninig about antibiotics use and lack of understanding related with irrational use of antibiotics in the study area.

Respondents, 72.2% were aware of using the same antibiotics over an extended period of time can result in antibiotic resistance. This outcome was comparable of a research in Thailand, where 66.67% of participants agreed that prolonged use of identical antimicrobial medications can result in the development of antimicrobial resistance (Nuangmek *et al.,* 2018*).* This might be related with lack of understanding on irrational use of antibiotics effect in the study area.

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In this study 72.2% respondents of thought that using antimicrobial drugs for non-therapeutic purposes might not lead to the development of antibiotic resistance. This finding was higher than that of 40% (Nuangmek *et al*. 2018) and 53.8% (Geta and Kibret, 2021) reported. This difference might be associated with lack of understandin g on how antibiotic are used, for what purpose are used, and under what circumstancesantibiotics are used.

According to 48.2% of respondents, antibiotic use in chickens results in the growth of bacteria that are resistant to treatment and cause illnesses in humans lower than 64.8%

reports (Geta and Kibret, 2021) in Ethiopia. Nearly half of (44.4%) of respondents store antibiotics in the poultry farm this finding was higher to reports that one third of respondents agreed to keep unused antibiotics at home (Tesfaye, 2017), which might be another indicator of antibiotic misuse.

Misconceptions regarding antibiotics may be significantly impacted by variations in how drug restrictions are implemented, which affect how readily available antibiotics are in various nations (Belkina *et al*., 2014).

In the current study, 83.33% of respondents were obtained antibiotics from veterinary pharmacies, which was lower than the finding of 100% (Mezene *et al*., 2020) in Ethiopia, 92% of antibiotics were purchased from licensed drug stores (Oluwasile *et al.*, 2014), and 87.8% (Chilawa *et al*., 2023) for government shops shuttered in Zambia, but higher than 70.3% were obtained from drug companies or cooperatives with a legal permit (Geta and Kibret, 2021) in nort west Ethiopia.

In this study, antibiotics were purchased from human pharmacies (11.11%) and open market (22.2%) respectively. This study was greater than the previous finding's 16.2% reports (Mezene *et al.,* 2020) and higher than reports of 8.6% sourced antibiotics from local merchants (Oluwasile *et al*., 2014), which was lower than 26% from the open market (Mezene *et al*., 2020). Increased farm attendant purchases of antibiotics from the open market may be related to the farm attendants' practice level (55.6%), current medicine price inflation, and low level supervision of the veterinary drug supply chain. These incorrect responses from respondents serve as a warning sign for the continent's inappropriate use of antibiotics.

A knowledgeable animal health practitioner must be consulted in order to provide a proper diagnosis and provide guidance on the best course of treatment in order to prevent the unnecessary use of antibiotics (Pinault, 2003). In this study, more than half of the respondents (66.7%) said they consulted a veterinarians, and 33.3% said they did not consult veterinarians. This result was comparable to 88% of respondents who agreed that consulting Animal health community practitioner before using antibiotics on animals is important (Tufa *et al*., 2023), and 64.2% of respondents who said they visit veterinarians when sick poultry need to be treated (Chilawa *et al*.,

2023) from Zambia, in contrast to claims that 22.34% of respondents sought advice from a local animal health worker (Sawadogo *et al*., 2023).

Antibiotic resistance, which is a problem for Bishoftu as well, is commonly regarded to have been facilitated by insufficient dosing, incomplete courses, and indiscriminate drug usage (Awad and Al-saffar, 2010). In this study, 55.6% of farm workers used prescription papers or leaflets to calculate antibiotic dosage. Compared to statements that 46.3% of participants in a study conducted in Zambia followed the manufacturer's instructions (Chilawa *et al*., 2023).

In this study, 66.7% of the respondents stated that veterinarians had advised or prescribed antibiotics for chickens. This conclusion was superior to that of 61.9% of prescriptions written by veterinarians and veterinary assistants (Mezene *et al*., 2020) and 50% written by veterinary doctors (Oluwasile *et al*., 2014).

According to prior experience, 11.1% and 16.7% of participants in the current study received prescriptions from their friends, which was lower than the 38.1% of participants who did not receive prescriptions based on their own or their friends' prior experiences (Mezene *et al*., 2020). In Burkina Faso, more than 31.9% of farmers utilized veterinary medications without a prescription (Sawadogo *et al*., 2023).

Regarding dosage determination, 55.6% of farm attendants relied on reading prescription paper or late leaf information that did not match 76.6% of reports (Mezene *et al*., 2020). In the current study, antibiotics were administered by a veterinarian to chickens at half of the respondents' poultry farms, which was an improvement over reports of 37.9% by veterinary professionals (Mezene *et al*., 2020) and 76.9% without a prescription (Chilawa *et al*., 2023) in Zambia.

In this study, 44.4% of the respondents gave antibiotics to their chickens on their own, which contrasts with reports that 14% of farmers gave medicines to their animals on their own (Tufa *et al.,* 2023), but is lower than reports that 76.5% of respondents gave antibiotics on their own (Mezene *et al*., 2020), According to reports, 43.1% of people self-medicate (Oluwasile *et al*., 2014), which is in line with the current findings. Due in large part to farmers' statements of positive experience, the lack of veterinary services, and the higher expense of veterinary services, self-medication is widespread among chicken farmers. It is likely that the dosage schedule has a sizable impact on the chance of resistance strains developing in a certain host (Wilson, 2010).

In contrast to studies from Bangladesh, where 94.1% of respondents always check the expiration date of antibiotics before using them, just 47.2% of respondents in the present study indicated they always do so. It is almost identical to the reported 17.1% of farmers in Burkina Faso who attended training on chicken production (Sawadogo *et al*., 2023) that 17.6% of respondents in this survey received on antibiotic usage and resistance.

Practice level of the study participant was significantly associated with age, level of education and farming experience. Farming experience and level of education were strongly associated with respondents' practices regarding antibiotic usage and resistance which was similar to education level was a factor that favorably influenced some practices (Sawadogo *et al*., 2023).

All of the farms used antibiotics for treatment. This current finding is consistent with 100% (Mezene *et al*., 2020) and 93.2% (Chilawa *et al*., 2023) in Zambia. This study did not support the conclusions of studies in Nigeria that found 36.2% (Oluwasile *et al*., 2014), 63.3% (Joshua *et al*., 2018), and 78% (Bamidele *et al*., 2022), and this shows how frequently people seize antibiotics, and the high rates of use may be a contributing factor to the AMR issue.

The use of antibiotics for prophylaxis was 61.11%, which is close to earlier estimates of 56% (Mezene *et al*., 2020) in Ethiopia, and 50% (Sawadogo *et al*., 2023) in Burkina Faso, but lower than78% (Bamidele *et al*., 2022) in Nigeria, and 89.8% (Chilawa *et al*., 2023) in Zambia. This might be associated with the level of attitudes and practice in the study area somewhat better, so the repondents mor tend to use antibiotics as a prophylaxis,

In this study, 44.44% of the respondents were used to promote growth. It was greater than estimates for Ethiopia of 32.2% (Mezene *et al*., 2020), 6.9% (Oluwasile *et al.,* 2014), Nigeria of 3.3% (Joshua *et al*., 2018), and Zambia of 19.3% (Chilawa *et al.,* 2023). On the other hand, this outcome was in opposition to reports from chicken farms which claimed of the farms utilized antibiotics to promote growth 86%

(Adelowo *et al*., 2014). Using of antibiotics as a growth promotion more related to because of modt of farms are business oriented.

Tetracycline (100%), Sulphamethoxazole + trimethoprim (83.33%), Sulfonamides (55 .56%), Streptomycin (44.4%), Ciprofloxacin (55.6%), Ampicillin (44.4%), and Amoxicillin (72.2%) were the antibiotics that were used the most frequently in the current study. This is in line with the findings of (Mezene *et al*., 2020), who found that Fluoroquinolones (41.5%), Sulphamethoxazole + trimethoprim (94.1%), and Tetracycline (100%) were the most commonly utilized medications in Ethiopia. This results was supported by the findings that Oxytetracycline (86.4%), Gentamicin (35.2%), and Sulfonamide (18.2%) were used (Chilawa *et al*., 2023). Multidrug resistance profiles was noted in this finding for the most commonly used antibiotics, and MDR pattern has been reported in bacteria from food animals in Ethiopia and other countries (Mebrat *et al*., 2016). This might be linked to the unrestricted use of antibiotics by poultry farm workers due to lack of antibiotic control policies, particularly in developing countries.

Associations between education level and the use of antibiotics for growth promotion and prevention were found in this study. It shows that respondents used antibiotics more frequently for growth promotion or prevention, which is entirely inappropriate, as education level increased. This was confirmed by research findings by (Sawadogo *et al*., 2023).

Using of antibiotics for prophylaxis and growth promotion was associated with the attitude and practice of antibiotics usage and resistance. Those having good practice and positive attitudes were tending to use antibiotics for non-therapeutic purpose.
Antimicrobials may be used carelessly in human health care, veterinary care, and agriculture, which encourages the creation and spread of microbes that are resistant to them (Simonsen *et al*. 2004).

83.4% of *E. coli* isolate from cloaca swabs showed resitance to Amoxicillin in the current study. The results of lower than 100% reported in Jordan by Abu-Basha *et al*., (2012), and 100% in Nigeria reported by Joshua *et al.*, (2018). The current finding was in contrast to Shecho *et al*., (2017), who report 34.6% in eastern Ethiopia.This difference might be related with using of Amoxicillin as a treatment for poultry diseases and as prophylaxis in the current study.

Ampicillin resistance in this study was 37.6% closure to reported 89% by (Messai *et al*., 2015), but lower from 76.9% (Bedasa *et al*., 2018), 74.1% (Cheikh, 2010) reported in Senegal, and 76.05% (Ranasinghe *et al*., 2022) in Sri Lanka. The current finding was in closure to Shecho *et al*., (2017), who report 34.6% in eastern Ethiopia.This difference might be related with using of Ampicillin as a treatment for poultry diseases and as prophylaxis in the current study.

According to the current study, 98.9% of *E. coli* isolates were found to be susceptible to Gentamycin, compared to 93% (Sarba *et al*. 2019), and 100% (Amare *et al*. 2013) and in Ethiopia. Studies in southwest Ethiopia reported a higher rate of resistance 20.8% than this study (Bushen *et al*., 2021). The resistance profile of Gentamycin in this study is lower than rportes of different scholar such as 15.0% in Vietnam (Nguyen *et al*., 2015), 70% (Joshua *et al*., 2018) in Nigeria, 57.2% in Jordan (Ibrahim *et al*., 2019), and 35% in India (Singh *et al.,* 2019). This might be related to gentamycine in study are were used in lower manner as indicate in the patterns of antibiotics usage in this study.

In current study, *E. coli* showed 37% of Streptomycin resistance which is lower than reports of 63% Messele *et al*., (2017) and 85% Sarba *et al*., (2019) in Ethiopia, 69.2% Zahraei and Farashi, (2006), and 67% Zakeri and Kashefi, (2012), and 92.2% reported by Ibrahim *et al*., (2019). The current finding, however, was greater than reports from eastern Ethiopia, where the rate was 34.6% (Shecho *et al*., 2017). This might be linked with the current study focused on farms, and AST determination based on CLIS, 2022.

Sulfamethoxazole-trimethoprim resistance was reported in the current study at 35.9%, which was lower than 48.1% in backyard production systems (Messele *et al*., 2017), 92.3% (Shecho *et al*., 2017), 70.8% (Bushen *et al*., 2021) in Ethiopia, 90% (Joshua *et al*., 2018) in Nigeria, and 90.9% (Singh *et al*., 2019) in India. The widespread use of this association in avian disease, particularly in the non-specific treatment of coccidiosis and to colibacillosis, may account for the high rates of resistance. This might be Antibiotics are applied sub-therapeutically with out sufficient knowledge; Study focused on farms, and AST determination based on CLIS 2022.

The current studies on Ciprofloxacin resistance profiles were 45.3%, which is greater than reports of susceptibility 100% (Shecho *et al*. 2017) in Ethiopia and reports in Vietnam which were 24.2% (Nguyen *et al*., 2015) on home and small-scale chicken farms respectively. This study brings to a close data from seven European nations that point to a higher incidence of Ciprofloxacin resistance 57.6% (Nulty *et al*, 2016). However, variations in sampling techniques and breakpoints for interpreting susceptibility test findings across research from various geographical areas, such comparisons should be evaluated with caution.

This study found a higher rate of Tetracycline resistance (84%), which is higher than previous studies that found resistance rates of 77.8% (Messele *et al*., 2017) in backyard production systems in Addis Ababa and Bishoftu, 76.9% (Shecho *et al*., 2017) in eastern Ethiopia, 75% (Bushen *et al*., 2021) in southern Ethiopia, and 46.3% (Sarba *et al*., 2019). The Tetracycline resistance pattern in these studies was lower than where it was reported to be 87% (Aggad *et al*. 2010) in Algeria, 98.2% (Cheikh N., 2010) in Senegal, 85.1%, (Rahimi, 2013) in Iran, 90.4% (Benameur *et al*. 2014) in Algeria, and 94% (Halfaoui *et al*., 2017) in Algeria, but higher than that in India, where it was reported to be 57% (Singh *et al.,* 2019). This might be because this molecule is being used excessively as a growth factor, a preventive, a cure, or both.

A limited range of studies have reported antibiotic resistance to Amoxicillin-clavulanic acid. The resistance to the Amoxicillin-clavulanic acid drug reported in the current study 9.9% was lower compared with previous report in in southern Ethiopia 66.7% (Bushen *et al*., 2021). This finding was lower than data reported in Algeria which was 43.1% (Halfaoui *et al*., 2017). This might be associated with Amoxicillin-clavulanic acid in the current study area usage pattern somewhat in limited range and due to nature of drug it is beta lactaminase inhibitor.

In this study, 85.1% of the *E. coli* isolates from the cloaca swabs had phenotypic resistance to various families of antibiotics, indicating that multidrug resistance is a real issue. The high percentage of drug resistance suggests that antibiotics are being misused for a variety of purposes, particularly prophylaxis (61.1%) and growth promotion (44.4), according to this study's findings on the pattern of antibiotic use in the study area. When several antibiotics are used concurrently during a prophylaxis or therapy, the chance of developing multiple resistances rises.

The most widely used antibiotics in poultry had the highest levels of resistance in our study, which provided proof of this phenomenon. This might be the cause of the therapeutic failures in colibacillosis, and the resistance issue raised serious health issues. It's crucial to be concerned about antibiotic resistance and how it affects human infections (Joshi S *et al*., 2012).

This study found that MDR to three to seven antibiotic classes looked to be the most prevalent among MDR *E. coli,* which is closely in line with the findings of (Sarba *et al*., 2019), who reported that two to four antimicrobials appeared to be the most common among MDR *E. coil*. This may be due to the fact that comparable drugs have been used carelessly and extensively in chicken farms and sub therapeutically application of antibiotics in the study area.

46.4% of the isolates exhibited resistance involving 2-4 antibiotics, which is less than the reports of 92.5% isolates exhibiting multidrug resistance involving 2 to 4 antimicr obial (Bedasa *et al*., 2018) & 92.3% of isolates exhibited multidrug resistance involvi ng 2 to 4 antimicrobial (Mude *et al.,* 2017). The isolate in this study had the highest possible rate of resistance to three drugs, at 23.2%. Seven drug resistances were present in 37.7% of the isolate. The highest drug resistance to 11.05% isolates in this study was caused by the combination of Amoxicillin, Ciprofloxacin, and Streptomycin.

7. CONCLUSION AND RECOMMENDATIONS

According to the current study, 38%, 33%, and 44% of respondents lacked the necessary attitudes, actions, or knowledge regarding the use of antibiotics and resistance, respectively. The knowledge, attitudes, and methods of a chicken producer are influenced by age and framing experience. According to respondents' educational level and farming experience, antibiotics were used by 44.4% and 61.1% of respondents in this study for growth promotion and prophylaxis, respectively, while all poultry farmers utilized antibiotics as treatments. Producer attitude and behavior were linked to antibiotic usage among chicken farmers as a growth enhancer and prophylactic, but knowledge was irrelevant. In the research area, antibiotics were generally utilized unreasonably. For instance, 44.4% failed to check the expiration date, 72.2% did not investigate the effect of non-therapeutic use on the emergence of antibiotic resistance, 35% increased the amount and frequency of antibiotics when poultry was not cured, and 50% of respondents administered antibiotics to poultry alone or with friends' assistance. The majority of survey respondents (83%) claimed to have had no training on antibiotic use or resistance. Sulfonamide, Sulphamethoxazole + trimethoprim, and Tetracycline were used in this experiment as treatments, preventative measures, and growth promoters to illustrate the resistance profiles of *E. coli*. In this study, 86.7% of the E. coli isolates had at least two antibiotic resistances. The findings of this study will be useful in developing further in-depth research plans. As a result, the following suggestions are made:

- The owner of chicken farms needs to be made aware of how the use of sub-therapeutic doses of antibiotics results in the development of antibiotic resistance.
- To improve the knowledge, attitude, and practices of poultry producers about the use of antibiotics and antibiotic resistance, awareness campaigns should be developed.
- There should be effective controls measures in place to reduce the unwarranted use of antibiotics in poultry farm as sub-therapeutically purposes.

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9. APPENDICES

Appendix 1: Assessments of knowledge, attitude and practice of poultry producer regarding antibiotics use and resistance

Part I: version of data collection materials (questioners)

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Biomedical Science

Knowledge, Attitude and Practices Poultry Producer Regarding Antibiotic Usage and Resistance in Bishoftu, Ethiopia

<u>Verbal consent form before conducting interview</u>

Greeting:

Hello, my name is ______________. I am working my MSc thesis in veterinary pharmacology in collage of veterinary medicine in Addis Ababa University. I would like to interview you a few questions about antibiotic use on your poultry farm. The objective of this study is to collect baseline data on antibiotics use in poultry production, which is important to improve the health status of the poultry so as to safeguard the safety of poultry meat, egg reaching consumer from poultry farm Your cooperation and willingness for the interview and observation is helpful in identifying problems related to the subject matter. Your name will not be written in this form. All information that you give will be kept strictly confidential. Your participation is voluntary and you are not obliged to answer any question you do not wish to answer. If you are not still comfort with the interview please feel free to drop it any time you want. Do I have your permission to continue?

If yes, shall I continue to the next page?

If no, thank you!!!!

General instruction

Almost all questions have pre-coded response. So it is important to follow the following instructions while you are interviewing respondents and recording their answer.

Region___________________Zone________________District/Wereda__________

Towen____________________Kebele__________________________________

Location: (Latitude______________ Longitude _________________ (With GPS)).

Date of Interview______________ /___________________/_________________

Time Started:__________________________ Time Ended:___________________

Participant ID Number ____

Section I: Socio-Demographic Characteristics of the Participants

1. Sex:

Male [ ] Female [ ]

2. Age (year):

18-30 [ ] 31-45 [ ] Greater than 45 [ ]

3. Education level:

No formal education [ ] Primary or secondary school [ ]

Graduate [ ]

4. Farming experience (year):

Less than five [ ] Five to ten [ ] Greater than ten [ ]

Section II: Questions about Knowledge of poultry producer regarding antibiotic use and resistance

1. Do you know antibiotics?

Yes [ ] No [ ]

2. Define what does mean antibiotics?

____

____

____

3. Do you have awareness about antibiotic resistance?

Yes [ ] No [ ]

4. Do you use for treatment of disease?

Yes [ ] No [ ]

5. Do you use for prophylaxis of disease?

Yes [ ] No [ ]

6. Do you use for growth promotion for poultry?

Yes [ ] No [ ]

7. Is there a relationship between antibiotic use in poultry and development of antibiotic resistance?

Yes [ ] No [ ]

8. The use of antibiotic in animals can reduce the effect of antibiotics in humans?

Yes [ ] No [ ]

Section III: Questions about Attitudes of poultry producer regarding antibiotic use and resistance

1. Do you believe usage of the same antibiotic drugs for long period of time can lead to antibiotic resistance?

Agree [ ] Disagree [ ]

2. Do you believe usage of Antibiotic drugs for non-therapeutic (prophylaxis and growth promotion) purpose lead to antibiotic resistance?

Agree [ ] Disagree [ ]

3. Do you believe sale and distribution of antibiotics shall only be done by persons permitted to do so by law?

Agree [ ] Disagree [ ]

4. Do you believe that use of antibiotics in poultry causes the emergence of resistant bacteria which cause diseases in humans?

Agree [ ] Disagree [ ]

5. Do you believe restriction of antibiotic usage for non-therapeutics purpose can reduce antibiotic resistance development?

Agree [ ] Disagree [ ]

6. Do you think increasing the dose of antibiotic and frequency is better when poultry was not cure?

Agree [ ] Disagree [ ]

7. Do you prefer to keep unused antimicrobials at farm in case there may be a need for them?

Agree [ ] Disagree [ ]

Section IV: Questions about practice of poultry producer regarding antibiotic use and resistance

1. Do you receive any training related to antibiotic use?

Yes [ ] No [ ]

2. Do you consult a veterinarian before using antibiotic when animals get sick?

Yes [ ] No [ ]

3. Do you get prescription before use antibiotics?

Yes [ ] No [ ]

4. Do you check the expiry date of the antibiotics before using it?

Yes [ ] No [ ]

5. Routs of drug administration determined by based on leaflets or prescription?

Yes [ ] No [ ]

6. Who prescribe antibiotics in your farm for intended use?

Veterinarian [ ] Recommended by friends [ ]

Self-prescription [ ]

7. How an antibiotics dosage determined before usage in chicken in your farm?

Reading leaflet [ ] Recommended by friends [ ] Self-based [ ]

8. Who provides treatment to your poultry in case of sickness?

Veterinarian [ ] Friends [ ] Self-administration [ ]

9. Where do you get antibiotic?

Veterinary pharmacy [ ] Veterinary clinic [ ]

Human pharmacy [ ] Open market [ ]

Section V: Questions regarding antibiotic used in the poultry farm

1. Mentions antibiotics you used in your poultry farm for treatment purpose?

____

2. Mentions antibiotics you used in your poultry farm for prophylaxis purpose?

____

3. Mentions antibiotics you used in your poultry farm for growth promotion purpose?

____

Part II: Oromiffa version of data collection materials (questioners)

Madaallii beekumsa, ilaalchaa fi shaakala oomishtoota qamadii itti fayyadama antibaayootikii fi dandeettii ittisuu ilaalchisee

Yuunivarsiitii Addis Ababa
Kolleejjii Fayyaa Beeyladaa fi Qonnaa
Kutaa Saayinsii Baayoomedikaalaa
Beekumsa, Ilaalchaa fi Hojimaata Oomishaa Shimbirroo Itti Fayyadama
Antibaayootikii Ilaalchisee fi Dandeettii Bishooftuu, Ethiopia

Af-gaaffii gaggeessuu dura unka hayyama afaanii

Nagaa gaafachuu:

Akkam jirtu, maqaan koo ______________ jedhama. Yuunivarsiitii Addis Ababaatti barruu qorannoo koo MSc farmaakooloojii beeyladaa koolaajii qoricha beeyladaa keessatti hojjechaa jira. Waa'ee itti fayyadama antibaayootikii qonna qamadii keessan irratti gaaffii muraasa isin gaafachuu barbaada. Kaayyoon qorannoo kanaa oomisha qamadii keessatti fayyadama antibaayootikii irratti ragaa bu'uuraa walitti qabuudha, kunis nageenya foon qamadii, hanqaaquu fayyadamaa qonna qamadii irraa ga'u eeguuf haala fayyaa qamadii fooyyessuuf barbaachisaa dha. Tumsi fi fedhiin keessan af-gaaffii fi ilaalchaaf rakkoolee dhimmichaan walqabatan adda baasuuf gargaara. Maqaan kee bifa kanaan hin barreeffamu. Odeeffannoon ati kennitu hundi iccitii cimaa ta'ee ni eegama. Hirmaannaan keessan fedhii keessanii waan ta'eef gaaffii deebii kennuu hin barbaanne kamiyyuu deebisuuf dirqama hin qabdan. Yoo ammallee interview sanaan jajjabina hin arganne ta'e yeroo barbaaddanitti bilisaan gadi dhiisaa. Itti fufuuf hayyama keessan qabaa?

Yoo eeyyee ta'e gara fuula itti aanutti itti fufaa?

Lakki yoo ta'e galatoomaa!!!!

Qajeelfama waliigalaa

Gaaffiiwwan hundi jechuun ni danda’ama deebii dursanii koodii ta’e qabu. Kanaafuu yeroo deebii kennitoota af-gaaffii gootuu fi deebii isaanii galmeessitu qajeelfama armaan gadii hordofuun barbaachisaa dha

Naannoo__________________Godinna _____________Aanaa/Weredaa__________

Toween____________________Kebele____________________________________ .

Bakka: (Laatituudii_________ Loongituudii __________________ (GPS waliin)).

Guyyaa Af-gaaffii_________ /____________________/__________________

Yeroo Jalqabame:___________________ Yeroo Xumurame:____________________

Lakkoofsa eenyummaa hirmaattotaa ______________________________________

Kutaa I: Amaloota Hawaas-Dimoogiraafii Hirmaattotaa

1. Sala:

Dhi [ ] Dhala [ ] .

2. Umurii (waggaa):

18-30 [ ] 31-45 [ ] 45 ol [ ]

3. Sadarkaa barnootaa:

Barnoota idilee hin qabu [ ]

Mana barumsaa sadarkaa tokkoffaa ykn sadarkaa lammaffaa [ ]

Eebbifamuu [ ]

4. Muuxannoo qonnaa (waggaa):

Shan gadi [ ] Shan hanga kudhanitti [ ] Kudhan ol [ ]

Kutaa II: Gaaffiiwwan waa’ee Beekumsa oomishaa qamadii itti fayyadama antibaayo otikii fi dandeettii ittisa isaa ilaalchisee

1. Antibaayootikii beektaa?

Eeyyee [ ] Lakki [ ]

2. Antibaayootikii jechuun maal jechuudha?

____

3. Waa’ee dandeettii farra baakteeriyaa ofirraa ittisuu irratti hubannoo qabduu?

Eeyyee [ ] Lakki [ ]

4. Wal'aansa dhukkubaaf ni fayyadamtaa?

Eeyyee [ ] Lakki [ ]

5. Dhukkuba ittisuuf ni fayyadamtaa?

Eeyyee [ ] Lakki [ ] .

6. Guddina qamadii guddisuuf ni fayyadamtuu?

Eeyyee [ ] Lakki [ ] .

7. Fayyadama antibaayootikii qamadii keessatti fi guddina dandeettii farra baakteeriyaa ofirraa ittisuu gidduu hariiroon jiraa?

Eeyyee [ ] Lakki [ ] .

8. Fayyadamni antibaayootikii bineensota irratti dhiibbaa antibaayootikii nama irratti qabu hir'isuu danda'aa?

Eeyyee [ ] Lakki [ ] .

Kutaa III: Gaaffiiwwan waa'ee Ilaalcha oomishtoota qamadii itti fayyadama antibaayootikii fi dandeettii ittisuu ilaalchisee

1. Qorichoota farra baakteeriyaa walfakkaataa yeroo dheeraaf fayyadamuun qoricha farra baakteeriyaa ofirraa ittisuu danda'a jettanii amantaa?

Walii galuu [ ] Walii hin galu [ ]

2. Qorichoota Antibaayootikii kaayyoo wal'aansa hin taaneef (ittisa fi guddina guddisuu) fayyadamuun qoricha farra baakteeriyaa ofirraa ittisuu fida jettanii amantaa?

Walii galuu [ ] Walii hin galu [ ]

3. Gurgurtaa fi raabsi antibaayootikii namoota seeraan hayyamamaniin qofa ta'a jettanii amantaa?

Walii galuu [ ] Walii hin galu [ ]

4. Fayyadamni qoricha farra baakteeriyaa qamadii keessatti baakteeriyaa dandeettii dhukkuba kana dandamatu kan nama irratti dhukkuba fidu akka uumamu taasisa jettanii amantaa?

Walii galuu [ ] Walii hin galu [ ]

5. Kaayyoo qoricha hin taaneef itti fayyadama antibaayootikii daangessuun guddina dandeettii farra baakteeriyaa hir'isuu danda'a jettanii amantaa?

Walii galuu [ ] Walii hin galu [ ]

6. Yeroo qamadii hin fayyinetti doosiin qoricha farra baakteeriyaa fi irra deddeebiin dabaluu wayya jettanii yaaddu?

Walii galuu [ ] Walii hin galu [ ]

7. Farra maaykiroobiyaanii hin fayyadamne yoo barbaachisummaan jiraachuu danda'e qonna keessatti kaa'uu ni filattaa?

Walii galuu [ ] Walii hin galu [ ]

Kutaa IV: Gaaffiiwwan waa'ee shaakala oomishaa qamadii itti fayyadama antibaayootikii fi dandeettii ittisuu ilaalchisee

1. Leenjii itti fayyadama antibaayootikii wajjin walqabatu ni argattu?

Eeyyee [ ] Lakki [ ]

2. Yeroo bineensonni dhukkubsatan qoricha farra baakteeriyaa fayyadamuu dura ogeessa beeyladaa mariisisuu?

Eeyyee [ ] Lakki [ ]

3. Antibaayootikii fayyadamuu dura ajaja ogeessa fayyaa ni argatta?

Eeyyee [ ] Lakki [ ]

4. Qoricha farra baakteeriyaa fayyadamuu dura guyyaa dhumaa isaa ni ilaaltuu?

Eeyyee [ ] Lakki [ ]

5. Daandiiwwan bulchiinsa qoricha kan murtaa'an barruulee ykn ajaja ogeessa fayyaa irratti hundaa'uun?

Eeyyee [ ] Lakki [ ]

6. Qonna keessan keessatti qoricha farra baakteeriyaawwan akka itti fayyadama yaadamaniif eenyutu ajaja?

Ogeessa beeyladaa [ ] Hiriyyootaan kan gorfamu [ ] .

Ofiin ajaja ogeessa fayyaa [ ]

7. Qonna keessan keessatti hanqaaquu keessatti fayyadamuu dura doosiin antibaayootikii akkamitti murtaa'a?

Barruu dubbisuu [ ] Hiriyyootaan kan gorfamu [ ]

Ofitti bu'uura [ ]

8. Yoo dhukkubni qamadii keessaniif yaala eenyutu kenna?

Ogeessa beeyladaa [ ] Hiriyyoota [ ] Ofiin of bulchuu [ ]

9. Antibaayootikii eessaa argatta?

Faarmaasii beeyladaa [ ] Kilinika beeyladaa [ ] .

Faarmaasii namaa [ ] Gabaa banaa [ ]

Kutaa V: Gaaffiiwwan antibaayootikii qonna qamadii keessatti fayyadaman ilaalchisee

1. Antibaayootikii qonna qamadii keessan keessatti yaalaaf itti fayyadamtan ni kaasa?

____

2. Antibaayootikii qonna qamadii keessan keessatti kaayyoo ittisaaf itti fayyadamtan ni eereera?

____

3. Antibaayootikii qonna qamadii keessan keessatti guddina guddisuuf itti fayyadamtan ni kaasa?

____

Appendix 2: Media preparation procedures

Stuart Transport Media

Procedures

1. Suspend 14.1 grams in 1000 ml double distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense into tubes with screw caps to give a depth of approximately 7 cm.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes and after sterilization tighten the caps.
5. Cool the tubes immediately in an upright position using
6. Incubate for 24 hours to check sterility

Brain Heart Infusion Broth

Procedures

1. Suspend 37.0 grams in 1000 ml distilled water.
2. Dispense into tubes and sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.
3. Cool the tubes immediately in an upright position using.
4. Incubate for 24 hours to check sterility

Eosin Methylene Blue Agar

Procedures

1. Suspend 35.96 grams in 1000 ml distilled water.
2. Mix until suspension is uniform.

3. Heat to boiling to dissolve the medium completely.

4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

5. Cool to 45-50°C and shake the medium in order to oxidize the methylene blue

6. Immediately dispense in to Petridis in laminar flow hood (keep it for 20 minutes)

7. Label and Incubate for 24 hours to check sterility

Brain Heart Infusion Agar

Procedures

1. Suspend 52.0 grams in 1000 ml distilled water.

2. Heat to boiling to dissolve the medium completely.

3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

4. Cool at 45-50°C

5. Mix and add into sterile Petridis, keep it until solidify

6. Label and Incubate for 24 hours to check sterility

Preservative Media Preparation

Procedures

1. Suspend 37.0 grams of Brain Heart Infusion Broth media in to 1000 ml distilled water.

2. Boil until completely dissolved

3. Mix Glycerol in combination of 80% BHIB with 20%of glycerol in laminar flow hood.

4. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

5. Placed in to water bath with temperature of 45-50 ^{0}C

6. Incubate at 37 ^{0}C over night to check sterility

Muller Hilton Agar

Preparation

1. Weigh appropriate amount of dehydrated Mueller-Hinton agar powder (follow manufacturer's instructions on bottle) and place in flask.

2. Add 1 liter of distilled water and swirl to disperse powder.

3. Place over a hot plate with a magnetic stirrer and heat until powder is dissolved

4. Carefully remove agar from heat and dispense in desired aliquots into containers of choice

5. Autoclave at 121°C for 15 min.

6. Allow to cool in a 48°C water bath

8. Arrange sterile Petri plates on a level surface to give uniform depth in laminar flow hood.

9. For disk diffusion tests, pour accurately measured volumes of molten agar into plates.

The agar when poured on Petri dishes should be 4mm

11. Allow plates to solidify at room temperature with plate lids slightly ajar.

12. Label and incubate for overnight at 37 ^{0}C.

Appendix 3: MALDI-TOF biotyper bacterial identification procedures

Preliminary preparation For MALDI – TOF Biotyper

1. Remove matrix and BTS from storage and warm to room temperature (up to 30 minutes)

2. Turn on the class II microbiological safety cabinet and clean inside with appropriate disinfectant (70% ethanol).

3. Mix the matrix using vortex mixer.

Extended direct transfer sample preparation procedure

1. For each sample smear an isolated colony as a thin film directly onto a sample position using a sample applicator.

2. Overlay the sample position with 1 µL 70% formic acid for all samples

3. Air-dry sample positions at room temperature

4. Deposit 1 µL of BTS onto each of the assigned BTS QC positions.

5. Dry the spots at room temperature.

6. Overlay each sample position and BTS QC position with 1 µL HCCA matrix solution. Use a new pipette tip for each sample position to avoid cross-contamination.

7. Air-dry the spots at room temperature.

8. Load the MALDI target plate into the MALDI-TOF.

Appendix 4: Antimicrobial susceptibility test

Procedure

1. 0.5 McFarland turbidity standard preparation step

a. A, 8.5 gram of sodium chloride was measured and mixed with 1000 ml of distilled water and Shake vigorously

b. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes and after sterilization

c. Placed in dark place until preparation of MHA media.

2. Preparation of Inoculums

a. Contact 4 or 5 isolated colonies to be tested using sterile inoculums rings or needles.

b. Suspend the organism in 5 mL of sterile saline.

c. Vortex the saline tube to create a smooth suspension.

d. Adjust the turbidity of this suspension to a 0.5 McFarland standard.

e. Use this suspension within 15 minutes of preparation.

3. Inoculation of the MHA Media

a. Dip a sterile swab into the inoculums tube

b. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid.

c. The dried surface of an MH agar plate is inoculated with a swab and the entire agar surface is streaked three times. Rotate the plate about 60 degrees each time to ensure that the inoculums is evenly distributed.

d. Leave the lid slightly open and let the plate sit at room temperature for 3-5 min, but no more than 15 minutes, to dry the surface.

4. Placement of the Antibiotic Disks

a. Place the appropriate antimicrobial-impregnated disks on the surface of the agar.

b. Once all disks are in place, replace the cap, flip the plate, and place it in an air incubator at 35°C for 16 to 18 hours.

5. Incubation of the Plates

A temperature range of 35°C ±2°C is required.

6. Measuring Zone Sizes

After incubation, use a ruler or caliper to measure the zone sizes to the nearest millimeter, disk diameter is included in the measurement.

Interpretation and Reporting of the Results

After 24 hours of incubation, use a metric ruler to measure the zone of inhibition and include the diameter of the disc in the measurement.

Compare the result with CLSI guidelines to report the result. The results are reported as Susceptible (S), Intermediate (I), or Resistant (R).

Appendix 5: Questionary data anaylasis procuders

The answers to knowledge questions were classed as "right" when the response was "yes" and "wrong when the response was "no" or "I don't know." When answering the attitude questions, "agree" with a positive statement was regarded as a "positive" attitude. The opposite was regarded as a "negative" attitude. Responses to questions about chicken producer methods were coded as "good" or "poor. Data were classified by assigning a 1 to "correct, positive, and good" and a 0 to "incorrect, negative, and poor" to a particular knowledge, attitude, and practice question, respectively.

Answers to open questions were classified into categorical factors (correct vs. wrong information; positive vs. negative attitude; good vs. poor practice). For each KAP item, the percentages of "appropriate" answers (i.e., right answers in the knowledge question, positive attitudes in the attitude question, and good practices in the in practice part) were calculated. A modified Bloom's cutoff point was used to categorize the participants' overall KAP score.

Participants' overall knowledge score was classified as high, moderate, or low if it was 7-8, 4-6, or 0-4 points. The attitude score was likewise assessed as good (6-7), moderate (4-5), or poor if it was less than 4 points. Similarly, the overall score for practice ranged from 0 to 9 and was classified as good, fair, or poor if the score was 8-9, 5-7, or less than 4 points, respectively.

The final KAP scores were dichotomized for further analysis, and those answers 50% and above recorded as good (score of both good and moderate) in a knowledge, attitude (score of both good and moderate), and practice (score of both good and moderate) section of the questionnaire were considered to have sufficient good knowledge, good attitudes, and good practice, while those answers 50% score recorded as low knowledge, poor attitudes, and poor practice.

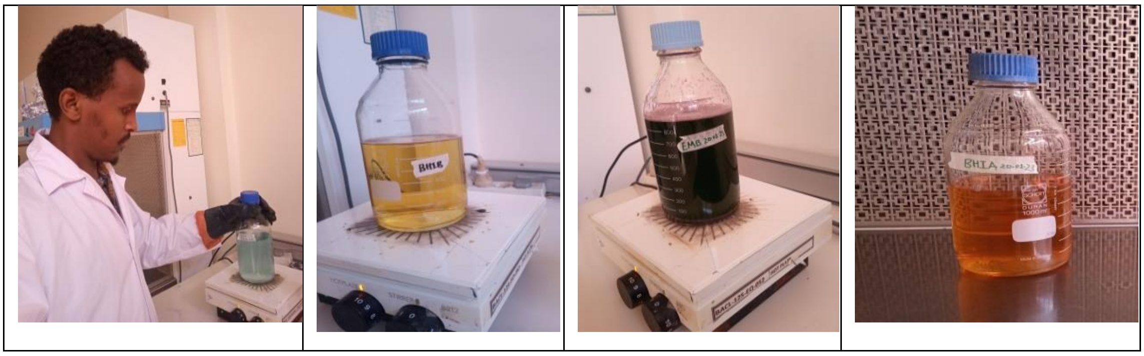
Appendix 6: Interpretative category and zone diameter breakpoints

Antibiotic agent	Disc potency (μ g)	Zone diameter in mmillimeter		
		Susceptible	Intermediate	Resistance
Ampicillin	10	≥17	14-16	≤13
Amoxicillin	2	≥17	14-16	≤13
Ciprofloxacin	10	≥26	22-25	≤21
Tetracycline	30	≥15	12-14	≤11
Gentamycin	10	≥15	13-14	≤12
Streptomycin	10	≥15	12-14	≤11
Sulfonamides	300	≥17	13-16	≤12
Sulphamethoxazole +trimethoprim	25	≥16	11-15	≤10
Amoxicillin-clavulanic acid	30	≥18	14-17	≤13

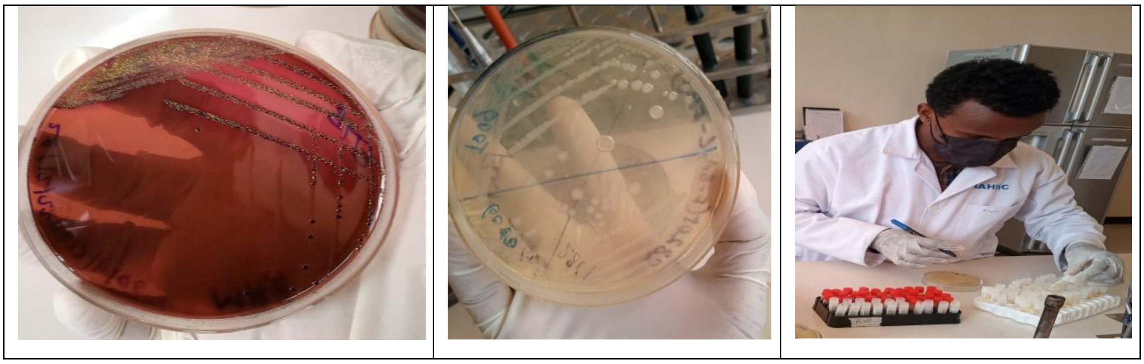
Appendix 7: Pictures for sample collection and laboratory work

Picture 1: Sample collection

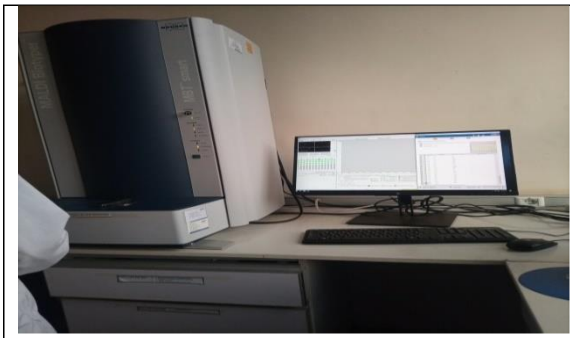
Picture 2: Deep liter bedding

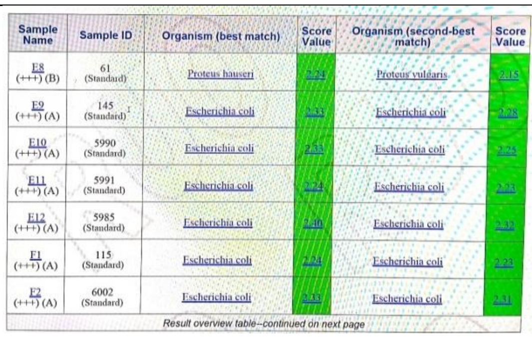


Picture 3: STM, BHIB, EMB BHIA,



Picture 4: *E. coli* colony in EMB BHIA and Preservation of suspected colony



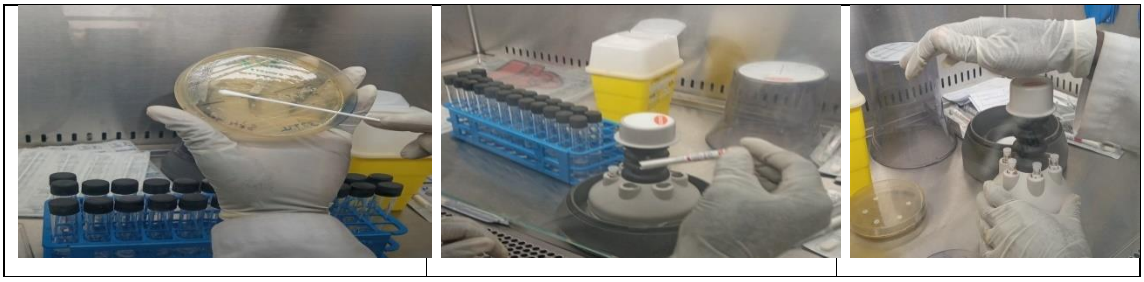


Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
E8 (+++) (B)	61 (Standard)	Proteus hauseri	2.24	Proteus vulgaris	2.15
E9 (+++) (A)	145 (Standard)	Escherichia coli	2.33	Escherichia coli	2.28
E10 (+++) (A)	5990 (Standard)	Escherichia coli	2.33	Escherichia coli	2.25
E11 (+++) (A)	5991 (Standard)	Escherichia coli	2.24	Escherichia coli	2.23
E12 (+++) (A)	5985 (Standard)	Escherichia coli	2.40	Escherichia coli	2.32
F1 (+++) (A)	115 (Standard)	Escherichia coli	2.24	Escherichia coli	2.23
F2 (+++) (A)	6002 (Standard)	Escherichia coli	2.33	Escherichia coli	2.31

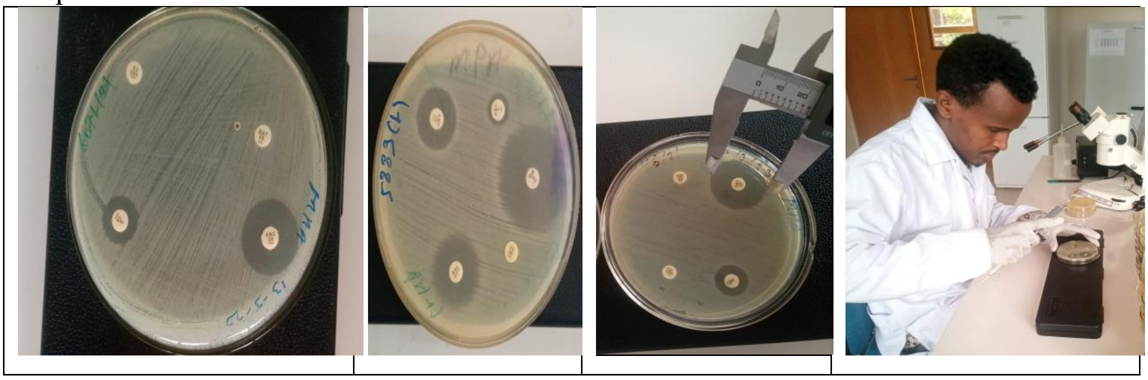
Result overview table--continued on next page

Picture 5: MALDI-TOF and result format of confirmed *E. coli*

Picture 6: Preparation of Antibiotics disc and McFarland measurement



Picture 7: Culturing *E. coli* on MHA and placing of antibiotics disc on Oxide dispenser



Picture 8: Measurements zone of inhibition of antibiotics disc in *E. coli* cultured MHA.

Appendix 8: Ethical clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/05/01/15/2023

Name and affiliation of applicant: **Dehinenet Terefe (DVM, MSc Student)**
Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *knowledge, attitude and practice assessment of farm attendants, antibiotic resistance screening and resistance gene detection of E. coli isolated from Chickens in Bishoftu, Ethiopia*

Date of application:	**December, 2022**
Nature of the project:	**Farm investigation**
Target animal species:	**Domestic chicken**
Number of animals involved:	**347**
Study area:	**Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/01/15/022, 16/12/2022**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Dehinenet Terefe provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when deemed necessary

The Federal Democratic Republic of Ethiopia
Addis Ababa University College of Veterinary Medicine and Agriculture

Professor Getachew Terefe (DVM, PhD) Signature
Chairman

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Please quote Our Ref. No. When replying

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