Thesis ref.no____________

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

ASSESSMENT OF FARM MANAGEMENT PRACTICES, ANTIMICROBIAL USAGE, POULTRY HEALTH AND WELFARE: ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *E. COLI* IN POULTRY FARMS AT THE STARTER PHASE IN BISHOFTU, ETHIOPIA

BY

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MSc PROGRAM IN POULTRY HEALTH AND MANAGEMENT

JUNE, 2024

BISHOFTU, ETHIOPIA

Assessment of Farm Management Practices, Antimicrobial Usage, Poultry Health and Welfare: Isolation and Antimicrobial Susceptibility Profile of *E. Coli* in Poultry Farms at the Starter Phase In Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture,

Department of Parasitology and Pathology

MSc Thesis

A Thesis Submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in the Partial Fulfillment of the Requirements for the Degree of Masters of Science in Poultry Health and Management

By

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Advisor: Hika Waktole (Associate Professor)

June, 2024

Bishoftu, Ethiopia

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As members of the examining board of the final MVSc thesis open defense, we certify that we have read and evaluated the thesis prepared by Kebene Temesgen "**Assessment of Farm Management Practices, Antimicrobial Usage, Poultry Health and Welfare: Isolation and Antimicrobial Susceptibility Profile of *E. Coli* in Poultry Farms at the Starter Phase In Bishoftu, Ethiopia"** and recommend it to be accepted as fulfilling the thesis requirement for the degree of **Masters of Science in Poultry Health and Management**.

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I hereby certify that I have read the revised version of this thesis prepared under my direction

and recommend that it be accepted as fulfilling the thesis/dissertation requirement.

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DECLARATION

This is to certify that the thesis, entitled: "**Assessment of Farm Management Practices, Antimicrobial Usage, Poultry Health and Welfare: Isolation and Antimicrobial Susceptibility Profile of *E. Coli* in poultry farms at the starter phase in Bishoftu, Ethiopia''** was accepted in partial fulfillment of the requirements for the award of the degree of Master of Veterinary Science in Poultry Health and Management by the college of veterinary medicine and agriculture, Addis Ababa University conducted by **Kebene Temesgen** was a genuine work carried out by her under my guidance. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the research thesis requirements.

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STATEMENT OF THE AUTHOR

I **Kebene Temesgen** hereby declare and affirm that the thesis entitled "**Assessment of Farm Management Practices, Antimicrobial Usage, Poultry Health and Welfare: Isolation and Antimicrobial Susceptibility Profile of *E. Coli* in Poultry Farms at the Starter Phase in Bishoftu, Ethiopia''** is my own work conducted under the supervision of **Associate Professor Hika Waktole.** I have followed all the ethical principles of research in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I have adequately cited and referenced all the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and I have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. This thesis is submitted in partial fulfillment of the requirement for a degree from the Post Graduate Studies at Addis Ababa University, College of Veterinary Medicine and Agriculture. I further declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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Name: Kebene Temesgen Signature: _______________

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TABLE OF CONTENTS **PAGES**

ACKNOWLEDGEMENTS

First and foremost, I would like to praises and thanks God, the Almighty, for his incredible help, and give me the capacity to do this research and in all activities throughout my life. My Lord! I have experienced your guidance day by day. You are the one who let me finish my master's degree. I will keep on trusting you for my future.

I would like to express my deep and sincere gratitude to my research advisor Hika Waktole (Associate Professor) for his overall intellectual guidance, encouraging and supporting me at each step until the final writing of this paper. Also, I would like to give special thanks to Addis Ababa University for giving me a scholarship opportunity and supporting me financially to attend the program.

I am extremely grateful to my family for their love, prayers, caring and sacrifices for educating and preparing me for my future. My family your prayer for me was what sustained me this far.

Last but not least; I would like to express my utmost gratitude to the poultry producer in Bishoftu for their willingness and support during the questionnaire survey and sample collection. The work in this study would not have been possible without the help and support of them.

LIST OF TABLES

TABLES **PAGES**

LIST OF FIGURES

FIGURES **PAGES**

LIST OF APPENDICES

APPENDIX **PAGES**

LISTS OF ABBREVIATIONS AND ACRONYMS

ADARDO	Ada’a District Agricultural and Rural Development Office
AMR	Antimicrobial Resistance
AMU	Antimicrobials Use
APEC	Avian Pathogenic *Escherichia Coli*
AST	Antibiotic Susceptibility Testing
CLSI	Clinical and Laboratory Standard Institute
DOC	Day Old Chick
E. coli	*Escherichia Coli*
EMB	Eosin Methylene Blue
MDR	Multidrug Resistance
MHA	Muller Hinton Agar
WOAH	World Organization for Animal Health
χ2	Chi-Square

ABSTRACT

In view of the expanding trend of poultry sector, the starter phase of poultry production requires effective management, health, welfare and judicious antimicrobial usage. Suboptimal practices during the starter phase can result in reduced productivity and increased spread of disease. A cross-sectional study was conducted from October 2023 to May 2024 with the targeted objectives of assessing farm management practices, poultry health and welfare, antimicrobial usage and susceptibility profile of *E. coli* in poultry farms during the starter phase in Bishoftu town. Methodologically, the study employed questionnaire surveys and bacteriological isolation of *E. coli*. The questionnaire survey revealed that 69%, 50%, 70%, 42% and 65% of respondents had good management practices in terms of housing and brooding, chick quality and procurement, health and biosecurity, welfare and antimicrobial usage, respectively. The study's findings indicated several managemental practices were not properly addressed. The study found that farmers' educational level, experience and role significantly ($p<0.05$) impact on the management of housing and brooding, as well as maintenance of health and biosecurity and rational use of antimicrobials. Education also significantly influenced ($\chi2=28.6$; $p=0.000$) the promotion of chicken welfare within the farms. Risk factors like breed also impact chick quality and procurement management and maintenance of health and biosecurity ($p<0.05$). Differences in farm size also played a significant ($p<0.05$) influence in maintaining health and biosecurity, promoting animal welfare and ensuring the rational use of antimicrobials. The present study disclosed that 52.7% (116/220) isolation of *E. coli* from cloacal swabs. Further, Antimicrobial susceptibility testing revealed all isolates showed 100% resistance to oxacillin and penicillin, 88% to vancomycin, 64% to oxytetracycline, 48% to amoxicillin, 32% to sulphamethoxazole-trimethoprim. To promote effective management of poultry farms, particularly during the starter phase targeted interventions are recommended for effective poultry farm management, focusing on promoting poultry health, and welfare and judicious use of antimicrobials for sustainable farming practices.

Keywords: *Antimicrobial usage, Biosecurity, Chick quality, E. coli, Health, Resistance, Starter Phase, Welfare*

1. INTRODUCTION

In Ethiopia, there are about 56.5 million chickens which are rendering essential proteins, and employment opportunities for youth and women and contribute to self-sufficiency and household needs (CSA, 2021; Gedeno *et al.*, 2022). The sector secures the livelihoods and food security of millions of people worldwide. The increasing demand for poultry meat and eggs is due to population growth, rising incomes and urbanization (Mottet and Tempio, 2017; Wong *et al.*, 2017). In Ethiopia, poultry production mainly involves raising chickens for egg or meat production or both (Wilson, 2010).

Poultry production is a popular choice due to its efficiency and high feed conversion rates, making it an attractive choice for meeting the growing demand for animal protein. However, the industry also faces challenges (Nkukwana, 2018). Among the many threats faced by poultry farmers, especially during the starter phase, suboptimal practices can lead to reduced productivity, increased disease spread and potential health impacts (Van Limbergen *et al.*, 2020). The occurrence of the diseases is often attributed to inadequate biosecurity measures. These measures include the implementation of hygiene regulations in poultry farms, especially during the starter phase and preventive measures such as vaccinations (Goualie *et al.*, 2020).

Poultry production management is a complex process involving bird rearing, from caring for day-old chicks to achieving production goals (Habte *et al.*, 2017). Optimal management practices are crucial for efficient and sustainable production. These practices aim to create optimal conditions for optimal performance from birds. Effective management includes maintaining appropriate brooding, rearing, growth and egg-laying conditions in the poultry house, monitoring health, administering recommended vaccinations and implementing a feeding program (Glatz and Pym, 2013; Attamah *et al.*, 2023). This can be achieved by controlling diseases, maintaining feed efficiency, handling waste and ensuring proper hygiene in poultry houses (Hamra, 2010).

The World Organization for Animal Health (WOAH) defines good animal welfare as a state where animals are healthy, comfortable, well-nourished and can exhibit their natural behaviors. Animal welfare means a balance between the animal itself and its environment. In practice, this can be understood as providing them with ensuring health, comfort and stress avoidance (Appleby *et al.*, 2018). To achieve this, measures like disease prevention, veterinary care, proper housing, nutritional management, humane handling and humane slaughter are required (Senevirathnaa *et al.*, 2023). Additionally, various management practices, such as stocking density, feeding, litter quality management, ventilation, temperature control, handling, pre-slaughter catching, transportation and slaughter, can affect the welfare of broiler chickens (De jong and Guemen, 2011).

Animal welfare practices and commitment to animal husbandry can reduce chicken suffering and premature mortality and increase productivity (Hartcher and Lum, 2020). This leads to reduced disease and antimicrobial resistance, a global health concern (Wilcox *et al.*, 2024). Implementing health management measures is crucial to protect poultry from disease. Maintaining a clean environment and high standards of cleanliness and containment minimizes the likelihood of disease introduction (Serbessa *et al.*, 2023). Prioritizing health management measures can reduce outbreaks and the spread of disease, increase consumer confidence in poultry products and reduce the need for antimicrobials (Olutumise *et al.*, 2023).

Antimicrobials are crucial in modern medicine for treating infectious diseases in humans and animals (Gray *et al.*, 2021). They improve health, productivity and economic returns by reducing disease incidence, morbidity and mortality. However, non-therapeutic antimicrobials used for growth promotion and feed efficiency enhancement are often considered non-therapeutic (Hassan *et al.*, 2021). Poultry farming is a significant use of antimicrobials, often used to treat and prevent diseases. Chickens have the highest antimicrobial use (AMU) values among all feed animal species. The use of antibiotics can contribute to the emergence of antimicrobial resistant *E. coli* strains, posing a serious threat to human health (Panth, 2019).

Colibacillosis is a common poultry disease caused by avian pathogenic *Escherichia coli* (APEC) infection. Local APEC infection can cause respiratory tract lesions and systemic infection can cause severe cardiac and liver damage, compromising poultry health and welfare. *E. coli* is a natural inhabitant of chickens' intestinal tract but can cause disease under stressful conditions like overcrowding, poor ventilation, malnutrition, extreme temperatures and immunosuppression (Lutful, 2010). This disease leads to economic losses in the poultry industry, including morbidity, lack of flock growth uniformity, reduced production, increased condemnation in slaughterhouses and mortality (Panth, 2019).

In poultry production, the starter phase is of utmost importance in achieving high performance outcomes at the end of the production cycle and it requires effective management, judicious antimicrobial use, health and welfare. Despite this, suboptimal practices during the starter phase can lead to reduced productivity, increased disease spread and potential health impacts (George and George, 2023). Therefore, assessing farm management practices, antimicrobial usage, poultry health and welfare in poultry farms during the starter phase is essential for ensuring chicks' health, growth and welfare, promoting responsible practices and formulating policy recommendations.

General Objective:

The general objective of the present study was to assess farm management practices, antimicrobial usage, poultry health and welfare: isolation and antimicrobial susceptibility profile of *E.coli* in poultry farms at the starter phase in Bishoftu town.

Specific Objectives:

- To assess the farm management practices of poultry farms during the starter phase
- To assess poultry health and welfare practices in poultry farms at the starter phase
- To assess antimicrobial usage during the starter phase and
- To isolate *E. coli* and detect their antimicrobial susceptibility test

2. LITERATURE REVIEW

2.1. Starter Phase of Poultry Production

The starter phase of poultry refers to the initial stage of raising poultry, specifically chicks, from hatch to 3 to 6 weeks of age depending on the chicken breed before they transit to the next phase, known as the grower phase. During the starter phase, the chicks require special care and attention to ensure their health, growth and development. Brooding is the period after hatching when chicks require special care for their health and survival. The first two weeks of life can be affected by damage, such as inefficient temperature regulation and stress exposure, leading to loss of uniformity in the flock (Idiaye *et al.,* 2022). To ensure chicks' comfort, it is essential to create a comfort zone with access to heat, feed and clean water. Poor conditions during brooding can significantly harm flock performance, including reduced growth, poor feed conversion, increased disease susceptibility and high mortality rates (Fairchild *et al.,* 2009).

2.2. Getting ready for a new production cycle

After completing the previous production cycle, it is crucial to prepare the facilities for the next cycle to ensure optimal conditions. All housing facilities and equipment must be thoroughly cleaned and disinfected to avoid problems in the subsequent cycle. This includes the removal of all litter and organic matter that disease causing agents over an extended period (Collett *et al.,* 2020). Preparing a brooder for the arrival of chicks is critical to the success of poultry operations. The health and environment of the chicks must be carefully considered during the preparation of the brooding facilities (Duguies *et al.,* 2016). The house should be fumigated and left unstocked for 1-2 weeks after fumigation. Two weeks before the arrival of the chicks, the brooder, waterer, feeder and litter should be cleaned and disinfected and the brooder should be properly set up two days before the arrival of the chicks (Shyfullah, 2018).

2.3. Reception of the Chicks

Chicks are delivered to farms on the day they are hatched, coming from breeders of similar age and genetic background. In an all-in, all-out system, all chicks enter a house on the same day and leave the same day to be transported to a processing plant (Houghton, 2011). Upon arrival, it is crucial to count chicks near the heat source, provide sufficient feeding and drinking areas and maintain room temperature. Monitoring chicks' comfort is crucial, as they may become discomfort if the temperature is too hot or too cold (Duguies *et al.*, 2016). When collecting chicks, they should be uniform, alert, free of deformities and show no signs of infection. They should also have access to feed and water,and the house temperature and ventilation should be adequate (Manual, 2011).

2.3.1 Distribution of the birds in the shed

To prevent overcrowding and competition, it's crucial to maintain a uniform distribution of birds in the shed. This involves consistent lighting, temperature, and humidity conditions across all farm areas (Corkery *et al.*, 2013). Strategic placement of feeders and water troughs also contributes to uniformity. Therefore, these elements should be evenly distributed (De SG Barros *et al.*, 2020). It is essential to proportion the number of feeders and water troughs to the density of birds to prevent competition and crowding around these resources (Sirovnik *et al.*, 2018).

2.3.2 The beginning of the feeding

As the chicks can eat from it, fill the plastic feeder or feeder to the appropriate level (Ussery, 2022). Being sure that chicks have easy and immediate access to both feed and water is essential because a disruption in the water supply would affect the feed intake and vice versa. Therefore, continuity in supplies must always be guaranteed and feeders and water troughs must contain feed and water a few hours before the reception (Ussery, 2011).

In the first week of life, chicks should multiply their weight by four and a half times due to quick eating and early feed intake. A high-quality feed with adequate physicochemical characteristics is preferred and physical activity during the first days is beneficial (Neves *et al.,* 2014). Farm staff can forcibly stimulate chicks to move, but always carefully. To ensure correct feeding at the reception, it must be checked that after 24 hours, more than 90% of the chicks have their crop full of feed, which is a crucial point in broiler management (Gajana *et al.,* 2011).

2.4. Feeding in the Starter Phase

Typically, the first thing the chicks have access to when they arrive at the farm is feed and water (Mottet and Tempio, 2017). When they arrive, the chicks are 36 to 48 hours old or even older, as hatching and breeding times, processing and transport times vary. Withholding feed and water from chicks during this period can lead to suboptimal development such as loss of body weight and impaired immune competence compared to early feeding (Panth, 2019).

The starter phase is a crucial learning phase for birds, requiring early and appropriate feed and water intake to develop their digestive system and immunity (Jha *et al.,* 2019). Early water intake counteracts dehydration effects and increases feed digestibility (Von Keyserlingk *et al.,* 2016). During this phase, the protein content is highest and the metabolizable energy content is lowest, as birds need a higher protein content to support muscle structure development and a higher energy content for rapid fattening at the end of the cycle (Babiker *et al.,* 2011).

During the brooding period, chicks are provided with pre-starter and starter feed in crumbles form (Mabusela, 2004). These feeds should be easily digestible, as young chicks lack some of the digestive enzymes that digest carbohydrates and amino acids found in older birds (Ravindran and Abdollahi, 2021). They stimulate appetite and maximize nutrient absorption, supporting high growth rates. Careful management and extra feed bowls are necessary for easy access to feed. The feed should be clean, free of mold and have a uniform particle size with minimal fines (Manual, 2011).

2.5. Environmental Factors in the Starter Phase

2.5.1 Temperature

The brooding phase is critical in the life of chickens. In this early phase, the ability to self-regulate body temperature is not yet fully functional (Tracey, 2014). Maintaining the correct environmental temperature for chicks during the first two weeks is essential. Chicks are poikilothermic at hatching and the first five days, meaning their body temperature is determined by the temperature of their environment. Ambient temperature affects a chick that cannot properly control its metabolism and maintain its body temperature (Adegbenro *et al.*, 2023). Brooding aims to keep chicks in their comfort zone, preventing energy loss and heat gain (Brake and Yahav, 2012). Achieving the best brooding temperature can improve final body weight, feed conversion and flock uniformity (Tracey, 2014).

Thermoregulation is fully developed between 7 and 14 days. Chicks that are too hot or too cold have suboptimal feed intake, growth rate and feed conversion (Manual, 2011). When there are problems with heat management in a house, the birds exhibit different behaviors. If the temperature is below the required level, chicks crowd around the heat source, while if it's too high, they move away from the heat source, drink more water, breathe through an open mouth, open their wings and reduce feed intake, potentially leading to cannibalism (Habte *et al.*, 2017). A chick's ability to maintain a healthy body temperature depends on the ambient temperature, as chicks' body temperature decreases as the room temperature decreases and the chick's body temperature increases as the room temperature increases (Yahav and Giloh *et al.*, 2012). Poor growth, poor feed conversion and greater disease susceptibility could arise from cooling or overheating during this critical time (Sesay, 2022).

2.5.2 Ventilation

Ventilation is important for birds' environment, providing fresh air, removing moisture, limiting harmful gases, controlling humidity and maintaining good litter conditions (Bist and Chai, 2022). Good ventilation is especially important when brooding chicks, but drafts should be avoided to prevent respiratory diseases. In addition to the stress of illness, birds kept in a hot, poorly ventilated house will not eat or drink normally. This results in stunted, poorly developed pullets (Miles *et al.*, 2004). Proper ventilation is essential for poultry houses to maintain oxygen supply, remove exhaust gases and dust (Bist *et al.*, 2023) and also important for preventing coccidiosis, a disease that can lead to severe mortality and permanent stunting of surviving birds (McDougald *et al.*, 2020).

2.5.3 Lighting

Lighting is a crucial environmental factor in poultry housing systems, influencing growth, development, productivity and welfare. Good light programming enhances birds' behavior, health, feed intake, productivity and welfare, leading to more profitable production. Light is critical throughout a chick's life, from incubation to sales (El-Sabrout *et al.*, 2022). Adequate light is needed during brooding and throughout the flock life, with 24-hour lighting (60-watt LED lamps) provided during the first 7 days to stimulate feed consumption and digestive system development. This helps chicks find feed and water and adapt to their new environment (Ashabranner, 2023).

2.5.4 Litter materials

Litter material in poultry houses is essential for absorbing fecal waste from birds, making the floor easy to manage, and serving as a bedding material for chickens (Shepherd *et al.*, 2017). Good management practices are crucial for these materials. Common materials in Ethiopia include wood shavings and chopped teff straws. If these are not available, other lightweight, non-compressible, absorbent materials like wheat hulls can be used, provided they don't harm chickens or put them under stress (Habte *et al.*, 2017).

Litter depth should be between 10 and 20 cm, ensuring it is clean and free from dust particles from feces, feathers, skin and litter. Wet litter has a greater negative impact on performance, carcass quality, health, animal welfare and profitability (Habte *et al.,* 2017). It is recommended to manage litter with approximately 25% moisture. Proper litter conditions are maintained by managing drinkers and ventilation systems. Proper disposal of used litter, including original litter material, poultry manure, feathers and spilled feed, is crucial to minimize disease spread. Proper management of these systems is essential for bird health and profitability (Malone, 2006).

2.6. Health and Biosecurity

Poultry health and biosecurity are imperative for ethical and sustainable production. Disease outbreaks can lead to mortality, decreased growth rates and costly losses. Commercial poultry facilities face risks of viral and bacterial contagions (Collett *et al.,* 2020). Rigorous protocols are implemented across the production chain to prevent and contain illness. Biosecurity starts with designing poultry houses to exclude pathogens, using smooth materials and restricted access. Hygienic procedures, regular disinfection of equipment and vehicles, and perimeter buffer zones separate farms are essential. Mortality disposal follows biosecurity protocols to avoid disease spread (George and George, 2023).

Employing an all-in, all-out strategy for flock management based on age groups effectively eliminates the spread of infection between flocks (Collineau *et al.,* 2020). Day-old chicks can be vaccinated for common poultry viruses and bacteria based on region. Vaccines protect against newcastle disease, marek’s disease, infectious bronchitis, avian influenza, and others. Additional vaccines may be applied during grow-out (George and George, 2023). This is very important to maintain the health and productivity of the birds, strengthen immunity and prevent the spread of disease within the flock (Dhama *et al.,* 2011).

2.7. Assessment of Chicken Welfare

Poultry welfare can be assessed using various indicators, including health, management, physiological responses to stress and behavioral responses. Changes in animal management or husbandry practices can improve animal welfare (Bhadauria and Bhanja, 2017; Bensassi *et al.,* 2019; Rowe *et al.,* 2019). Welfare depends more on what the animal feels than on its reaction. Animal welfare is currently an important prerequisite for intensive poultry production (Moura *et al.*, 2006). However, the lack of effective animal welfare assessments poses a challenge for establishing regulations and developing knowledge about animal welfare. Despite these challenges, animal welfare remains a crucial prerequisite for intensive poultry production (Alonso *et al.*, 2020).

Poultry welfare can be considered in the context of the housing environment and management practices to which it is exposed (Voogt *et al.,* 2023). A key factor affecting broiler chicken welfare is management, which includes stocking density. High stocking densities can negatively affect chicken production, health and welfare (Sugiharto, 2022). Beak trimming, stocking density, free access to feed, heat stress and air pollutants have become important issues regulated in several countries (Moura *et al.,* 2006). Poor environmental management can compromise air and litter quality, leading to a higher prevalence of contact dermatitis, particularly hock burns and footpad dermatitis. Therefore, it is crucial to address these issues to ensure the welfare of chickens in indoor systems (Ly, 2018).

2.8. Antimicrobial Usage in Poultry Farms

Antimicrobials used in animal production for disease prevention and growth promotion have led to a decline in mortality and morbidity rates in animals (Imam *et al.,* 2020; Magnusson, 2021). AMU in poultry production systems increases the risk of antimicrobial resistance (AMR) (Al Sattar *et al.,* 2023; Manimaran *et al.,* 2023). Inappropriate or unrestricted use of antimicrobials in humans and animals can lead to the development of resistance traits (Gray *et al.,* 2021). AMR is the ability of microorganisms to tolerate antimicrobial drugs used to treat diseases, causing treatment failure and downstream impacts on animal welfare, food safety, food security and public health. AMR can be transmitted between humans, animals and the environment through direct contact and indirectly through the environment and food supply (Kiambi *et al.*, 2021).

Antimicrobials are commonly administered orally to chickens to reduce morbidity and mortality (Nolan *et al.,* 2013). Also used for prophylaxis and growth promotion in developing countries. However, in recent years increased resistance to first-line antimicrobials, such as third-generation cephalosporins and colistin, has been reported for *E. coli* isolates. The emergence of bacterial resistance to critically important antibiotics is a major problem in veterinary and human medicine (Gao *et al.,* 2018).

2.9. *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 commensal isolates (Pokharel *et al.,* 2023). The development of avian colibacillosis is influenced by inadequate environmental conditions, respiratory diseases, immunosuppressive factors and animal metabolism. Control can be achieved through environmental control, antibiotics, preventive vaccinations, controlling moisture, ventilation and drinking water chlorination (Da Rosa *et al.*, 2019).

Avian pathogenic *E. coli* (APEC) enters chickens through the mouth and respiratory systems and causes systemic infections either as a primary pathogen or as a secondary infection to other pathogens such as infectious bronchitis, newcastle disease, avian influenza, mycoplasma infections, infectious bursal disease and environmental stresses like high dust, overcrowding and ammonia levels (Kathayat *et al.*, 2021). These strains cause severe respiratory and systemic diseases and threaten food security and the welfare of birds worldwide (Guabiraba and Schouler, 2015).

APEC can cause various diseases in poultry, including perihepatitis, airsacculitis, pericarditis, egg peritonitis, salphingitis, coligranuloma, omphalitis, cellulitis, osteomyelitis/arthritis, respiratory tract infection, swollen head syndrome, septicemia, and polyserositis (Kathayat *et al.,* 2021). Additionally, *E. coli* can invade the eggshell and spread to chickens upon hatching, causing high early chick mortality and yolk sac infection (Panth, 2019). It can cause up to 20% mortality in chickens and significantly impact the global poultry industry. It leads to a decrease in meat output, live weight, feed conversion ratio, hatching rates, egg production, mortality and costs associated with treatment and prophylaxis (Joseph *et al.,* 2023).

The incidence of antibiotic resistance has increased significantly in recent years (Kassem *et al.,* 2016). 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 (Mdegela *et al.,* 2021). Antimicrobial treatment plays a crucial role in decreasing the prevalence and mortality rate related to avian colibacillosis. *E. coli* has susceptibility to numerous antibacterial agents. However, isolates of *E. coli* from poultry are often resistant to one or more antibiotics, particularly if they have been used widely in the poultry industry for a long period like tetracyclines (Casalino *et al.*, 2023).

Treatment options for *E. coli* infections usually entail the use of antibiotics, including aminoglycosides, tetracycline, sulphonamide, and fluoroquinolones (Landoni *et al.*, 2015). However, due to the bacterium's acquired resistance to these compounds, antibiotics that were once effective are now ineffective in combating the infection. Unfortunately, resistance to two or more classes of antibiotics has become widespread in veterinary medicine (Stachelek *et al.*, 2021).

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

The inappropriate use of veterinary antimicrobials have played a role in the emergence of bacterial resistance in *E. coli*, in Ethiopia, as summarized in (Table 1).

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

Site	AST	Antibiotic disc	Resistance	Reference
Addis Ababa and Bishoftu	Disc Diffusion	Ampicillin	70.4%	Messele *et al.*, 2017
		Erythromycin	40.7%	
		Streptomycin	63.0%	
		Gentamycin	0.0%	
		Sulfamethoxazoletrimethoprim	0.7%	
		Trimethoprim	48.1%	
		Tetracycline	77.8%	
Eastern Ethiopia	Disc Diffusion	Ampicillin	92.3%	Shecho *et al.*, 2017
		Amoxicillin	34.6%	
		Streptomycin	34.61%	
		Gentamycin	7.69%	
		Tetracycline	76.92%	
		Ciprofloxacin	0.0%	
Ambo	Disc Diffusion	Streptomycin	0.0%	Sarba *et al.*, 2019
		Gentamicin	0.0%	
		Tetracycline	46.3%	
		Sulfamethoxazoletrimethoprim	0.0%	
		Ciprofloxacin	0.0%	
		Nalidxic acid	7.3%	
Southwest Ethiopia	Disc Diffusion	Ampicillin	91.0%	Bushen *et al.*, 2021
		Amoxicillin-clavulanic acid	66.7%	
		Gentamicin	20.8%	
		Tetracycline	75.0%	
		Sulfamethoxazoletrimethoprim	70.8%	
		Ceftriaxone	41.7%	
		Ceftazidime	37.55	

Key: AST-Antibiotics Susceptibility Test

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Bishoftu town, central Ethiopia, which is 47 km south-east of Addis Ababa at an elevation of 1850 meters above sea level in the central highlands of Ethiopia. It is located at 9°N latitude and 40°E longitude. A bimodal rainfall pattern exists in the area, with a short rainy season from March to May and a longer wet season from June to September. It has an annual rainfall of 866 mm of which 84% is in the long rainy season. The dry season extends from October to February. The area's average annual maximum and lowest temperature are 26°C and 14°C, respectively, with 61.3% relative humidity. A total of 244 registered poultry farms were recognized, with 122 small-scale (100 layers and 22 broilers), 108 medium-scale (78 layers and 30 broilers), and 14 large-scale (9 layers and 5 broilers) farms (ADARDO, 2022). Bishoftu is home to a large number of poultry farms and has a long history of poultry farming, with numerous experienced farmers.

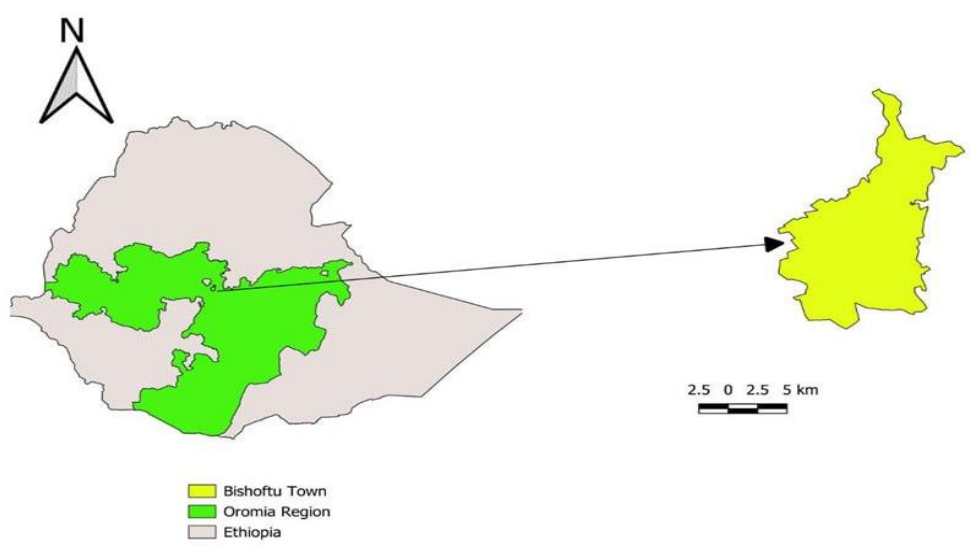


Figure 1: Map of Ethiopia showing location of study area, Bishoftu town (QGIS Version 3.3).

3.2. Study Population

The study encompasses the starter phase of poultry production in Bishoftu town, including layer, broiler and dual-purpose commercial poultry farms. The first weeks of a chick's life is known as the starter phase, which typically lasts from day-old to 3-6 weeks, depending on the chicken breed. The study population therefore consists of small-scale, medium and large-scale poultry farms at the starter phase found in Bishoftu town. Flock sizes were divided into small-scale (100-1000 birds), medium-scale (1001–10,000 birds) and large-scale (more than 10,000 birds) operations (Wondmeneh *et al.*, 2017).

3.3. Study Design and Sampling Technique

A cross-sectional study was conducted between October 2023 and May 2024 in Bishoftu town to assess farm management practices, antimicrobial usage, poultry health and welfare during the starter phase. The study also aimed to determine the antimicrobial susceptibility profile of *E. coli* isolated from cloacal swabs. The selection of farms was a purposive sampling method based on the age of the chicks from day-old to 3-6 weeks depending on the chicken breed, while cloacal swab collection followed a simple random sampling technique. Data collection involved face-to-face questionnaire surveys with farm owners, managers and workers using structured closed-ended questions.

3.4. Sample Size Determination

The sample size of the respondents was determined using the formula proposed by (Arsham, 2007) at a standard error of 0.05 with a confidence level of 95% was used for farm management practices, antimicrobial usage, poultry health and welfare of poultry at the starter phase in poultry farms in Bishoftu town. Then using the formula below the sample size was determined.

$$N=0.25/SE^2$$

where,

N= sample size

SE= standard error.

SE = 0.05 SE^2 = 0.0025 0.25/0.0025 = 100. Therefore, 100 respondents were required for questionnaire survey.

To determine the sample size required for the *E. coli* isolation for the antimicrobial susceptibility test the standard formula for simple random sampling for an infinite population was utilized, considering the expected prevalence of *E. coli* in Bishoftu town 17.3% which was reported by (Shimelis, 2022). The required sample size was calculated using the formula described by (Thrusfield, 2007) with a 95% confidence interval and a desired precision of 5% (d^2).

$$n=\frac{1.96^2 pexp(1-pexp)}{d^2}$$

Accordingly, the sample size used were 220 chickens for cloacal swab samples.

3.5. Data Collection Methods and Study Procedures

3.5.1. Questionnaire interviews

A study was conducted to assess farm management practices, antimicrobial usage, poultry health and welfare during the starter phase. A structured closed-ended questions were designed to gather respondents' information on these practices. Before the interview, the objectives of the study were explained to each respondent and their willingness and verbal consent of the participants was obtained. Before evaluating the real study, the structured questions were tested on ten farms that did not participate in order to enhance question clarity, determine relevance and assess the efficient use of time.

The questions had seven sections and 48 questions, covering sociodemographic characteristics, farm management practices of housing and brooding management category including housing, space, ventilation, lighting, heat, temperature and bedding materials; chick quality and procurement category including chick sourcing**,** health inspection, vaccination, and feeding practice; health and biosecurity categories include health monitoring, abnormalities checking, vaccination and veterinary advise, footbaths, timely bird treatment, visitor allowance, cleaning and disinfection of farm equipment, advisory services on poultry health care services and production and healthcare provider; the welfare category for the chick includes awareness level, space allocation, lighting conditions and structural enrichment and antimicrobial usage practices categories include antimicrobials usage, common antimicrobials types, dosage calculations and veterinary consultation of poultry farmers during the starter phase. Each interview was completed within 20-25 minutes. Additionally, the date and time of the farm visit, the location of the farm and general characteristics of the farm were all recorded. Each farm's completed form was submitted to the Kobo Toolbox server project.

3.5.2 Sample collection and transportation

Of 100 poultry farms selected for survey data clocal swabs were collected based on willingness of the poultry producers. After finishing the brooding time of the chick cloaca swab samples were collected using sterile cotton swabs. Following suitable restraint, sterile cotton swabs were inserted and rotated into the poultry's cloaca to take a cloacal sample. A total number of cloacal swab samples (220) were allocated according to the number of chickens in the farm.

The collected swab samples were placed in a sterile test tube containing 10ml buffered peptone water as transport media and then all samples were labeled and transported by icebox containing ices to the Veterinary Public Health Laboratory of Addis Ababa University, College of Veterinary Medicine and Agriculture for bacteriological analysis. To ensure the integrity of the samples, the samples were maintained at a temperature of

4°C in a refrigerator until analysis was take place when immediate bacteriological culturing is difficult to culture all samples at a time.

3. 6. Isolation and Identification of *Escherichia coli*

3.6.1 Culturing on agar media

Primary isolation was done by using solid mediums: MacConkey agar (Oxford, England). Samples were cultured on MacConkey agar for the first step of primary isolation and incubated at 37°C for 24 hours. Following 24 hours of incubation, the plate was observed for typical colony characteristics of *Escherichia coli*, which are likely to appear as pink colonies and lactose fermenters. Based on the characteristics of colony appearance, a single suspected colony was streaked on EMB agar for a second step of isolation and incubated at 37°C for 24 hours. After incubation, the plate was observed for green metallic sheen colonies, and the metallic sheen-forming colonies were transferred to nutrient agar for biochemical tests (Snyder and Atlas, 2006).

3.6.2 Biochemical tests

The most appropriate biochemical tests conducted include the Indole test, Methyl Red (MR) test, Voges Proskauer (VP) test and Citrate Utilization Test. The tests were conducted by taking a pure colony from nutrient agar. The indole test indicates a positive reaction for *Escherichia coli*, which reacts positively to the methyl blue test but negatively to the Voges Proskauer test. A citrate utilization test for *Escherichia coli* showed a negative reaction for this test (Warpala *et al.*, 2020).

3.7 Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was performed according to the Clinical and Laboratory Standards Institute's agar disc diffusion technique. The antimicrobial susceptibility testing of the isolates was performed with the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2022) and Kirby-Bauer Disk Diffusion Susceptibility Test Protocol on Muller Hinton agar medium. From the identified isolate by the biochemical test, a loopful of well-grown colonies on nutrient agar were transferred with the sterile loop into sterile tubes containing 2ml of normal saline solution (0.85% NaCl). The inoculated colonies are mixed well with saline solution by vortex until a smooth suspension is formed. Saline solution (if the suspension is more turbid) or colonies (if the suspension is less turgid) were added to the suspension until it achieved the 0.5 McFarland turbidity standards. Then sterile cotton swabs were dipped into the suspension and the bacteria was swabbed uniformly over the entire surface of the Muller Hinton Agar plate (Hudzicki, 2009).

The plates were held at room temperature for 3 minutes in a biosafety cabinet to allow drying. Eight antimicrobial disks with the known concentration of antimicrobial were placed on the Muller Hinton Agar plate; eight of them in a circular pattern and one at the center and the plates were placed in the incubator at 37^0c for 22 hours. All these eight antimicrobial discs were OXOID company's products and include Oxytetracycline (OT30µg), Trimethoprim-Sulfamethoxazole (SXT 23.75µg), Amoxicillin (AX 10µg), Streptomycin (S 10µg), Trimethoprim (TRIMS 5µg), Vancomycin (VANS 30µg), Oxacillin (OXA-1 30µg) and Penicillin G (P1 10µg). The diameters of the clear zone of inhibition produced by diffused antimicrobial on lawn-inoculated bacterial colonies were measured to the nearest millimeters using a ruler. All eight zones of inhibition against eight antimicrobial agents for each isolate were recorded and compared with standards and interpreted as resistant, intermediate and susceptible according to a published interpretive chart (CLSI, 2022).

3.8. Data Management and Analysis

The raw data from the questionnaire survey gathered through Kobo Toolbox server was retrieved as Excel files, carefully reviewed for errors, coded and subsequently imported into the data analysis software. Then analyzed using Stata/IC 14.2. Additionally, the raw data generated from the laboratory work from the study area was arranged, organized, coded and entered into an Excel spreadsheet 2010. The laboratory results of *E. coli* isolation and their AMR profile were mostly described in proportion.

For the questionnaire survey, descriptive statistics were used to characterize sociodemographics, farm features, housing and brooding management practice, chick quality and procurement, health and biosecurity, welfare and antimicrobial usage-related factors were reported as counts and percentages using descriptive statistics. The demographic variables of the visited farms including sex, age category, education level, experience, role in the farm and breed of the chick, purpose of the production, age of the chick, and farm size were considered as predictor/ independent variables.

Housing and brooding management practices, chick quality and procurement, health and biosecurity, welfare and antimicrobial usage practices were considered outcome/ dependent variables. Outcome variables were assessed by one point if correct and zero if incorrect. The total grades per participant were used to determine the mean scores, which served as an average for good and poor practice.

Responses to questions about farmers' practices were either “yes vs no”or multiple choice, with a latter being dichotomized as correct and incorrect. Data were coded by giving one to correct answers and zero to incorrect responses to a specific question or item. The percentages of appropriate responses and acceptable management methods were determined for each dependent variable item. The relationship between demographics and farm features and dependent variable was investigated using a chi-square and fishers exact test of association between housing and brooding management practices, chick quality and procurement, health and biosecurity, welfare and antimicrobial usage practices and possible explanatory independent variables. The 5% significance level was used to interpret the association results.

3.9. Ethical Clearance

Before the start of this work research ethical clearance, ref. no: VM/ERC/03/15/16/2024 was obtained from the Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University. So the research was conducted by keeping the welfare of animals and with complete research ethics of Addis Ababa University (Appendix 9).

4. RESULTS

4.1. Socio-demographic Characteristics of the Respondents

The socio-demographic characteristics of respondents were assessed and presented in Table 2. Among the total respondents, most of them (70%) were males, while (30%) were females. The majority of the respondents were found in the 26-35(40%) age group and most of them have less than 5 years of poultry farming experience (44%), most of them (39%) were graduates, and most of them (62%) were owners of the farm.

Table 2: Socio-demographic characteristics of poultry producers in Bishoftu (n = 100).

Variables	Category	Frequency	Percentage
Sex	Male	70	70%
	Female	30	30%
Age	18-25	3	3%
	26-35	40	40%
	36-45	26	26%
	>45	31	31%
Level of education	Informal	19	19%
	Primary	16	16%
	Secondary	26	26%
	Graduate	39	39%
Experience	0-5 years	44	44%
	5-10 years	35	35%
	>10 years	21	21%
Role in the farm	Owner	62	62%
	Manager	9	9%
	Worker	29	29%

4.2. Association of Risk factors with House and Brooding Management, Chick Quality and Procurement, Health and Biosecurity, Chicken Welfare and Antimicrobial Usage Practice

The statistical analysis using Pearson's chi-square and Fisher's exact test indicated that the sex of respondents did not have a significant ($p>0.05$) influence on the management of housing and brooding, chick quality and procurement management, as well as the maintenance of health and biosecurity, keeping welfare and rational use of antimicrobials. However, the age of the respondents showed a significant ($p<0.05$) influence on maintaining chicken welfare and rational use of antimicrobials.

The study found that farmers' educational level was found to have a significant ($p<0.05$) influence on the management of housing and brooding, as well as the maintenance of health and biosecurity, welfare and rational use of antimicrobials. While it did not influence chick quality and procurement management. The experience of the respondents also had a significant ($P<0.05$) influence on the management of housing and brooding, as well as the maintenance of health and biosecurity and rational use of antimicrobials. But not on chick quality and procurement management and keeping chicken welfare.

Additionally, the role of the respondents in the farm was found to have a significant ($P<0.05$) influence on the management of housing and brooding, as well as the maintenance of chicken welfare and rational use of antimicrobials. But not on chick quality and procurement management and keeping health and biosecurity (Table 3).

Table 3: Assessment of risk factors with house and brooding management practice, chick quality and procurement, health and biosecurity, chicken welfare and antimicrobial usage (n=100).

Variables			**House and brooding management**		**Chick quality and procurement**		**Health and biosecurity**		**Chicken welfare**		**AMU**	
	Category	Respondents	χ2	P-value	χ2	P-value	χ2	P-value	χ2	P-value	χ2	P-value
Sex	Male	70	0.10	0.75	2.12	0.14	2.03	0.2	3.73	0.053	0.1	0.754
	Female	30										
Age	18-25	3	7.77	0.051	2.67	0.45	6.90	0.08	15.47	0.001	12.05	0.007
	26-35	40										
	36-45	26										
	>45	31										
Level of educated	Informal	19	19.8	0.000	5.74	0.13	8.30	0.04	28.6	0.000	20.65	0.000
	Primary	16										
	Secondary	26										
	Graduate	39										
Experience (in years)	0-5	44	6.14	0.046	0.39	0.82	6.3	0.04	3.91	0.14	14.73	0.001
	5-10	35										
	>10	21										
Role in the farm	Owner	62	6.94	0.031	3.93	0.140	4.32	0.12	35.31	0.000	16.86	0.000
	Manager	9										
	Worker	29										

N.b: Respondents frequency stands for all outcome variables.

4.3. General Poultry Farm Information

The findings showed that the purpose of poultry farming was predominately for egg production 61% out of 100 poultry farms, the flock size consisted of 37 small-size, 56 medium and 7 large-scale farms. In the present study, Bovans Brown 43% were the dominant breed, followed by Cobb 500 23%, Lohmann Brown 18% and Sasso 16%, in the farms of Bishoftu and all of the farms use deep litter housing systems for poultry management (Table 4).

Table 4: General characteristics of studied poultry farm information (n=100).

Characteristics	Category	Frequency	Percentage
Breeds of chicks	Bovans brown	43	43%
	Lohmann brown	18	18%
	Cobb 500	23	23%
	Sasso	16	16%
Purpose of chicken production	Egg	61	61%
	Meat	23	23%
	Dual	16	16%
Age of the chicks	1week	13	13%
	2 weeks	10	10%
	3 weeks	16	16%
	4 weeks	24	24%
	5 weeks	17	17%
	6 weeks	20	20%
Farm size	Small	37	37%
	Medium	56	56%
	Large	7	7%
Housing system	Deep litter system	100	100%

4.4. Association of Risk Factors with Characteristics of Poultry Farms

The study assesses the relationship between risk factors, such as chick breeds, production purpose, chick age, and farm size, with different aspects of poultry farm management including house and brooding management, chick quality and procurement, health and biosecurity measures, chicken welfare and antimicrobial usage.

The study findings indicate that the breed of the chicks had a significant ($p<0.05$) impact on chick quality and procurement management, as well as the maintenance of health and biosecurity practices in poultry farming. However, it did not have a significant influence on the management of housing and brooding, promotion of chicken welfare and the rational use of antimicrobials. Additionally, the purpose of production was found to significantly ($P<0.05$) influence the maintenance of health and biosecurity practices but did not have significant effects on other aspects of poultry farming.

Furthermore, the age of the chicks was shown to have a significant ($p<0.05$) influence on the management of housing and brooding, while not significantly affecting chick quality and procurement management, health and biosecurity practices, the promotion of chicken welfare, and the rational use of antimicrobials.

Moreover, farm size was found to play a significant ($P<0.05$) role in maintaining health and biosecurity, promoting animal welfare and ensuring the rational use of antimicrobials, but did not significantly influence brooding house management and chick quality and procurement (Table 5).

Table 5: Association of risk factors with characteristics of poultry farms (n=100).

Variables			**House and brooding management**		**Chick quality and procurement**		**Health and biosecurity**		**Chicken welfare**		**AMU**	
	Category	Responses	χ2	P-value	χ2	P-value	χ2	P-value	χ2	P-value	χ2	P-value
Breed	Bovans	43	3.09	0.382	10.46	0.005	11.94	0.012	2.17	0.538	1.68	0.640
	Lohmann	18										
	Cobb 500	23										
	Sasso	16										
Purpose	Egg	61	1.03	0.596	3.68	0.159	10.52	0.007	2.16	0.343	1.20	0.549
	Meat	23										
	Dual	16										
Age of chicken	1week	13	14.93	0.011	3.53	0.574	6.26	0.197	10.11	0.072	2.69	0.762
	2weeks	10										
	3weeks	16										
	4weeks	24										
	5weeks	17										
	6weeks	20										
Farm size	Small	37	4.77	0.097	1.86	0.525	6.44	0.038	30.10	0.000	17.97	0.000
	Medium	56										
	Large	7										

N.b: Respondents frequency stands for all outcome variables.

4.5. Assessment of House and Brooding Management Practice in Poultry Farms

The study assessed poultry house and brooding management practices focusing on various activities conducted on the farm. The findings revealed that a large majority (88%) of respondents clean and disinfect the house and equipment before the arrival of chicks. Only 20% of respondents have separate brooding and finishing houses on their farms. In terms of brooding house conditions, a significant proportion of farmers (85% and 78%) provide adequate space and ventilation; lighting and heat for their chicks respectively.

Regarding the heat source during brooding, the majority of respondents (86%) use both electric and charcoal, while 9% use only charcoal and 5% use electric power. It was observed that 60% of respondents allowed their chicks to stay up to 3 weeks in the brooding house, with varying durations reported by other respondents. Furthermore, the study found that controlling the temperature of the brooder house and gradually reducing it as the chicks grow is a common practice among 67% of farmers.

About 69% of the farmers had a good house and brooding management that activities such as cleaning and disinfecting before chick arrival, separating brooding and finishing houses and providing adequate space and ventilation; lighting and heat in the brooding house have a significant impact ($p<0.05$) on poultry house and brooding management practices in the study area (Table 6).

Table 6: House and brooding management practices (n=100).

House and brooding management	Responses	Percentage	$\chi2$	P-Value
Did you clean and disinfect the house and equipment before the arrival of the chicks?	Yes	88%	15.90	0.000
	No	12%		
Do you have brooding and finishing houses separately?	Yes	20%	8.84	0.004
	No	80%		
Does the brooder house provide adequate space and ventilation for the chicks?	Yes	85%	5.68	0.017
	No	15%		
Is there adequate lighting and heat in the brooding house?	Yes	78%	21.74	0.000
	No	22%		
What is a source of heat used during brooding?	Electric	5%	6.11	0.051
	Charcoal	9%		
	Both electric and charcoal	86%		
What is the duration in the brooding house (weeks)?	1 week	3%	3.62	0.279
	2 weeks	30%		
	3 weeks	60%		
	4 weeks	7%		
Is the temperature in the brooder house properly controlled and gradually reduced as the chick grows?	Yes	67%	2.66	0.154
	No	33%		

Average mean score=69%

Good housing and brooding$\geq$69%

Poor housing and $\leq$69%

4.6. Assessment of Chick Quality and Procurement Practice

The study findings indicated that a majority of respondents (73%) obtained their day-old chicks from reputable hatcheries, while 27% sourced them through middlemen. It was observed that 64% of farmers conducted thorough inspections of the chicks upon arrival to detect any signs of illness, stress or deformities. Additionally, the majority (91%) of the respondents ensured that the chicks had received proper vaccinations. In terms of feeding practices, 85% of respondents provided chicks with feed Ad libitum. The study also revealed that 84% of farmers used tap water as the primary source of drinking water for their chickens, while 16% relied on well water.

Approximately 50% of the farmers demonstrated good chick quality and procurement practices, including the origin of day-old chicks, inspection for health and deformities upon arrival, vaccination status, and feeding schedule during the starter phase, which were found to be significant ($p<0.05$). These results emphasize the importance of adopting scientifically sound practices in chick management and highlight the crucial role of vaccination and feeding in ensuring the health and well-being of poultry chicks (Table 7).

Table 7: Chick quality and procurement practices (n=100).

Chick quality and procurement	Responses	Percentage	$\chi 2$	P-Value
Where is the source of your DOC?	Well-known supplier	73%	46.45	0.000
	Middleman	27%		
Do you inspect the chicks upon arrival for any signs of illness, stress, and deformities?	Yes	64%	10.70	0.001
	No	36%		
Do you ensure that the chicks have been properly vaccinated before they arrive at the farm?	Yes	91%	31.39	0.000
	No	9%		
What is the feeding schedule for the starter phase?	Ad libitum	85%	8.78	0.003
	Adjusted based on growth	15%		

Average mean score=50%

Good chick quality and procurement ≥50%

Poor chick quality and procurement≤50%

4.7. Assessments of Poultry Health And Biosecurity

The study findings revealed that a significant proportion of farmers (89%) implemented regular health monitoring schemes for their chicks. Among these farmers, 42% conducted comprehensive checks on various aspects such as behavior, droppings, feathers, eyes, legs, smell and sound, while 47% focused primarily on behavior and droppings. Notably, 67% of respondents administered vaccines in the morning, with smaller percentages using the afternoon (15%), evening (9%) or random (9%) timings.

Moreover, a substantial majority (80%) of respondents sought veterinary guidance to develop vaccination programs. It was observed that 95% of poultry farms had footbaths at the entry of their poultry houses and 79% ensured quick treatment of sick birds and removal of dead birds from poultry houses. Additionally, most respondents (87%) always clean and disinfect their farm equipment. The study also revealed that 52% of respondents restricted visitors from entering their farms, while 37% did so infrequently, and 11% allowed visitors regularly. The majority 74% of the respondents have advisory services on poultry health care services and production. 31% of the respondents call the health care provider at the first sick. However, 40% of the respondents call health care providers at death one or more than one chick.

About 70% of the respondents had good poultry health and biosecurity practices on the farm through implementing regular health monitoring schemes, conducting thorough health checks, consulting veterinarians for vaccination programs, ensuring timely treatment and removal of sick or dead birds, maintaining cleanliness and biosecurity practices, availing advisory services for poultry health care, and promptly contacting healthcare providers in case of chick mortality events which were found to be significant ($p<0.05$). These practices collectively play a vital role in safeguarding animal health and biosecurity on poultry farms (Table 8).

Table 8: Assessment of poultry health and biosecurity (n=100).

Health and farm biosecurity	Responses	Percentage	$\chi 2$	P-value
Do you have regular health monitoring schemes for your chicks?	Yes	89%	21.28	0.000
	No	11%		
If your response is 'yes', in the above question do you check for the abnormalities of:	Behavior, droppings, feathers, eyes, legs, smell, sound	42%	10.58	0.001
	Behavior, droppings	47%		
What is the time of the day vaccines are administered?	Morning	67%	1.17	0.670
	Afternoon	15%		
	Evening	9%		
	Random	9%		
Do you consult with a veterinarian to develop a vaccination program?	Yes	80%	48.00	0.000
	No	20%		
Are there footbaths available at the entry of poultry houses?	Yes	95%	0.63	0.596
	No	5%		
Did you ensure timely treatment of sick birds and removal of dead birds?	Yes	79%	37.15	0.000
	No	21%		
How often farm equipment are cleaned and disinfected?	Always	87%	10.64	0.001
	Sometimes	13%		
How often visitors are allowed inside a farm?	Often	11%	3.49	0.175
	Rarely	37%		
	Never	52%		
Do you have advisory services on your poultry health care services and production?	Yes	74%	43.31	0.000
	No	26%		
If the answer is yes, in the above question in what case/condition do you call the health care provider?	At first sick	31%	49.90	0.000
	At death one or more than one chick	40%		
	No call	29%		
Average mean score =70% Good health and biosecurity ≥70% poor health and biosecurity≤ 70%				

4.8. Assessments of Chicken Welfare in Poultry Farms

A welfare assessment was conducted to identify essential practices for ensuring the welfare of chicks on poultry farms. The findings revealed that only 19% of respondents had an awareness of animal welfare, primarily acquired through educational means. Notably, 92% of respondents allocated sufficient space for chicks to engage in natural behaviors and exercise, while 79% provided appropriate lighting conditions to enhance bird welfare. However, it is concerning that none of the respondents offered structural enrichment, such as perches or elevated tiers, for chicks to rest and support optimal skeletal development.

About 42% of the farmers had good chicken welfare practices such as awareness of animal welfare, obtaining this knowledge through education, and providing adequate lighting conditions in the starter phase housing which were found to be significant ($p<0.05$) influenced chick welfare (Table 9).

Table 9: Assessments of chicken welfare (n=100).

Welfare assessment	Responses	Percentage	χ2	P-value
Do you have animal welfare awareness?	Yes	19%	76.88	0.000
	No	81%		
If your response to the above question is yes, how did you get it?	Education	19%	76.88	0.000
	No	81%		
Is the housing space sufficient to allow the chicks to move, exercise, and exhibit natural behaviors?	Yes	92%	2.31	0.198
	No	8%		
Are proper lighting conditions provided in the starter phase housing to promote bird welfare?	Yes	79%	7.07	0.005
	No	21%		
Do you provide structural enrichment such as perches or elevated tiers for the chicks to rest and for optimal skeletal development?	No	100%		
Average mean score= 42% Good welfare ≥42% Poor welfare ≤ 42%				

4.9. Assessments of Antimicrobial Usage in Poultry Farms

An assessment was conducted to assess antimicrobial usage during the starter phase in poultry farms. The results showed that all poultry farms utilized antimicrobials for preventive measures during the starter phase, with 80% using them for growth promotion and 60% for treatment purposes. The most commonly used antimicrobials were oxytetracycline (46%), enrofloxacin (31%), sulphamethoxazole (15%), and amprolium (8%). Notably, 43% of respondents use prescription papers or leaflets to calculate antibiotic dosage and it knows about antimicrobial resistance or failed to cure diseases.

Furthermore, it was observed that 53% of respondents consistently sought advice from veterinarians before administering antimicrobials during the starter phase, while 47% did not consult veterinarians. Interestingly, a significant number of respondents (93%) used multivitamin supplements as an alternative for disease prevention in chicks.

About 65% of the respondents had good practices of antimicrobial usage during the starter phase. Factors such as utilizing prescription papers or leaflets for calculating antibiotic dosages, understanding antimicrobial resistance implications, seeking veterinary advice before antimicrobial use, and incorporating multivitamin supplements for disease prevention which were found to be significant ($p<0.05$) influenced antimicrobial usage practices in the study area (Table 10).

Table 10: Assessment of antimicrobial usage in the poultry farms (n=100).

Antimicrobial usage	Response	Percentage	χ2	P-value
Do you use antimicrobials during the starter phase?	Yes	100%		
Which antimicrobial drugs are frequently used at the starter phase?	Oxytetracycline	46%	1.90	0.594
	Enrofloxacin	31%		
	Sulphamethoxazole	15%		
	Amprolium	8%		
Do you use prescription papers or leaflets to calculate antibiotic dosage?	Yes	43%	84.75	0.000
	No	57%		
What are the reasons for administering antimicrobials during the starter phase? (Select all that apply)	Prophylaxis	100%	1.32	0.516
	Growth promotor	80%		
	Disease treatment	60%		
Have you heard about antimicrobial resistance (failed to cure diseases)?	Yes	43%	84.75	0.000
	No	57%		
Is the veterinarian's advice before antimicrobial usage during the starter phase?	Yes	53%	56.70	0.000
	No	47%		
Do you use multivitamins supplements as alternative disease prevention for your chicks?	Yes	93%	4.812	0.028
	No	7%		
Average mean score=65% Good AMU≥65% Poor AMU≤65%				

4.10. Antimicrobial Susceptibility Profiles of *E. coli*

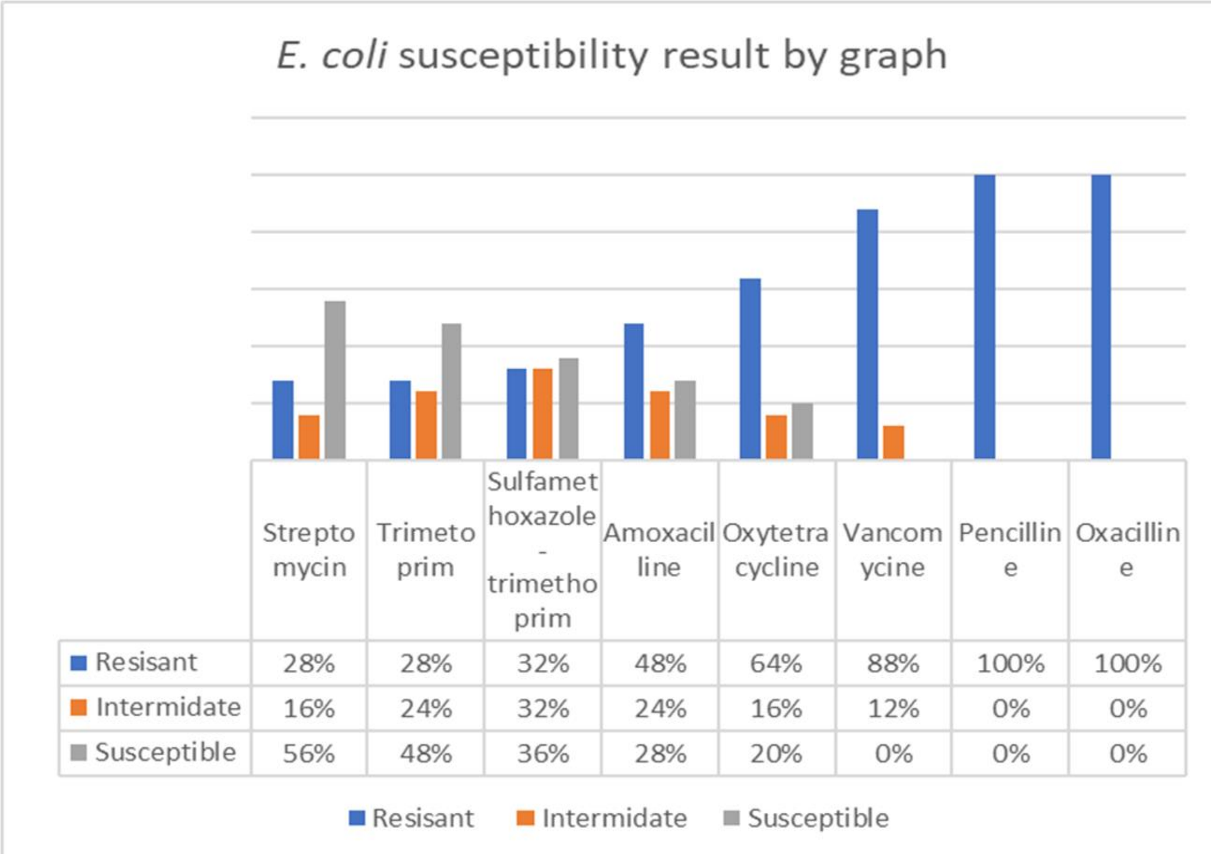
A total of 220 samples were collected from 16 commercial poultry farms found in Bishoftu town. Upon conducting cultural morphology on MacConkey, Eosin Methylene Blue agars, and biochemical identification, it was found that 52.7% (116/220) of the cloacal swabs examined were positive for *E. coli*.

Furthermore, susceptibility testing of 25 *E. coli* isolates to 8 antibiotics revealed that all isolates revealed 100% resistance to oxacillin and penicillin. Vancomycin showed the next highest resistance at 88%, followed by Oxytetracycline (64%), Amoxicillin (48%), Sulphamethoxazole-trimethoprim (32%), Trimethoprim (28%), and Streptomycin (28%).

On the other hand, 56% of the isolates were sensitive to Streptomycin, followed by Trimethoprim (48%), Sulphamethoxazole-trimethoprim (36%), Amoxicillin (28%), and Oxytetracycline (20%). Additionally, 32% of the isolates showed intermediate susceptibility to Sulphamethoxazole-trimethoprim, followed by Amoxicillin (24%), Trimethoprim (24%), Oxytetracycline (16%), Streptomycin (16%), and Vancomycin (12%) as it is illustrated in fig 2 below.

This result provides valuable insights into the prevalence of *E. coli* in poultry farms during the starter phase and the susceptibility patterns of the isolates to various antibiotics, highlighting potential concerns regarding antimicrobial resistance in poultry production during the starter phase.

Figure 2: *E. coli* susceptibility result by graph



5. DISCUSSION

The study highlights the necessity of effective farm management practices, judicious antimicrobial use and attention to poultry health and welfare are crucial for the starter phase to maintain sustainable poultry farming. No previous study has been conducted in Ethiopia concerning the assessment of farm management, antimicrobial usage, poultry health and welfare in poultry farms at the starter phase. Even though it is new, the result found from this study is crucial in contributing to an understanding of these factors in poultry farms of Bishoftu town.

According to this study, the thorough cleaning and disinfection of poultry houses before the introduction of new chicks is a common practice on poultry farms, with 88% of farms following this protocol. This result is supported by the practice that is essential for maintaining proper hygiene and preventing the transmission of diseases among poultry. It is crucial to prevent new chicks from being exposed to any debris or contaminants left behind by the previous flock (Hakeem and Lu, 2021).

The study revealed that majority of farmers, (80%), did not maintain separate brooding and finishing houses on their farms. This finding contrasts with research conducted in Aguata, Nigeria, where 92.5% of farmers had distinct brooding and finishing facilities (Attamah *et al.,* 2023). This lack of segregation poses a potential risk of disease transmission from the early chick stage to maturity, as chickens are housed together throughout both phases of growth. This difference might be due to financial constraints, especially among small-scale farmers and the limited availability of land for constructing dedicated brooding and finishing houses.

The study revealed that 60% of participants allowed their chicks to remain in the brooding house for up to 3 weeks, while 30% opted for 2 weeks, 7% extended it to 4 weeks, and 3% limited it to just 1 week. On average, the chicks spent approximately 2.5 weeks in the brooding houses, aligning closely with findings in Aguata, Nigeria, where chicks were reported to stay for 2.62 weeks (Attamah *et al.*, 2023). This duration contrasts with recommendations by (Hamilton *et al.,* 2016), suggesting an 11-day brooding period. Additionally, a study in Gampaha district, Sri Lanka, found that 74.4% of farmers kept chicks in the brooder for 7-10 days (Senevirathnaa *et al.,* 2023). These variations might be highlighting the diversity in brooding practices among poultry farmers worldwide.

The study found that 67% of farmers in the surveyed area regulate the temperature within the brooder house, gradually adjusting it as the chicks grow. This percentage is lower compared to the 98.75% reported in a study conducted in Kaduna State (Egwuma *et al.*, 2018). Proper regulation of temperature and humidity in poultry production facilities is crucial for maintaining bird health and optimizing productivity. Inadequate environmental control can weaken the immune system of chickens and lead to heat stress (Adriano and Djamessi, 2017).

A notable finding from the study is that 73% of respondents indicated procuring their day-old chicks from reputable hatcheries. This aligns with the results of a study conducted in Kaduna State (Egwuma *et al.*, 2018) which reported 69.5%. Acquiring well-hatched and healthy chicks from reliable sources helps farmers avoid potential production costs associated with addressing health issues in the chicks. Additionally, this practice can contribute to reducing mortality rates, as the quality of hatching can impact the survival rates of the birds (Yoho *et al.,* 2008).

Upon chick arrival, 64% of respondents inspected them for signs of illness, stress or deformities. This practice enables farmers to identify potential health issues early on when they are more manageable. By isolating sick or stressed chicks, farmers can prevent the spread of disease to the rest of the flock. Additionally, ensuring proper vaccination of chicks before arrival reported by 91% of respondents reduces the risk of flock loss due to preventable diseases. Vaccinated chicks are more likely to succeed, reducing mortality rates (Yoho *et al.,* 2008).

In the study, it was found that 89% of farmers have implemented monitoring systems to evaluate the health of their chicks. Notably, this percentage is lower compared to a recent study in the Gampaha district of Sri Lanka where 97.9% of respondents reported purposefully checking their flock for sick or injured birds (Senevirathnaa *et al.*, 2023). This regular monitoring practice allows farmers to promptly identify early signs of illness in their chicks, facilitating timely and effective treatment. This variance may be the importance of consistent monitoring practices in poultry farming for disease prevention and disease treatment.

In the current study majority of respondents (80%) consult veterinary professionals to develop vaccination programs for their poultry. However, this finding is higher than a study conducted in the Ibadan metropolis of Nigeria, where only 42.87% of respondents reported consulting veterinary personnel before vaccination (Isegbe *et al.,* 2024). This difference might be a potential gap among poultry farmers in Nigeria compared to those in Bishoftu, Ethiopia importance of engaging veterinary expertise in vaccination planning for optimal poultry health management.

In this study, a high percentage (95%) of poultry farms have footbaths, indicating a strong commitment to biosecurity practices that reduce the risk of disease introduction by workers and visitors. This proactive approach aligns with the findings of another study in Bishoftu (Waktole *et al.,* 2023), where all respondents reported using footbaths. In contrast, studies in Nigeria have reported much lower rates of footbath use, with only 1.25% of farmers in Aguata (Attamah *et al.,* 2023) and a small percentage in Ekiti State using foot dips at the entrance of their poultry houses (Ajewole and Akinwumi, 2014). This difference suggests a potential gap in biosecurity practices among poultry farmers in Nigeria compared to those in Bishoftu, Ethiopia, and the importance of promoting and implementing biosecurity measures to enhance poultry health and productivity.

The study revealed that the vast majority (79%) of farmers ensure the timely treatment of sick birds and the timely removal of dead birds from their poultry houses, which significantly increases the likelihood of successful recovery for sick birds and minimizes the risk of disease transmission. Moreover, the vast majority (87%) of the respondents consistently maintain high levels of cleanliness and disinfect their farm equipment. This aligns with research by (Attamah *et al.*, 2023), and (Egwuma *et al.*, 2018) reported that 63.5% of farmers in Kaduna State adhere to similar hygiene practices.

In this study majority (74%) of the respondents indicated that they have access to advisory services on poultry health care services and production. This result was higher than 58.1% of animal health clinics give poultry curative health services. These findings imply that advisory services on poultry health care provide farmers with expert guidance on disease prevention, diagnosis and treatment, helping them maintain the health and productivity of their flocks. Advisory services on poultry health care and production empower farmers with the knowledge and tools they need to optimize flock health, increase production and ensure the sustainability of their poultry operations (Asfaw *et al.*, 2021).

According to the study, just 19% of respondents know about animal welfare, which they acquired through education. This suggests that many farmers lack access to information, possess limited knowledge, and have not received training in best practices for animal welfare. This result contrasts with consumer awareness of avian welfare has grown in recent years, and as a result, consideration for higher-quality meat production has increased (El-Sabrout *et al.*, 2022). This suggests a notable gap in information accessibility, limited knowledge distribution, and insufficient training in optimal animal welfare practices among the farmers of poultry in Bishoftu town.

However, it is significant that most (92%) of respondents provide their chicks with sufficient space to move around, exercise, and engage in natural behaviors. This suggests that providing adequate space helps prevent overcrowding, which can lead to stress and negatively impact chick health, growth, immune function and welfare. Creating a stress-free environment through sufficient space allows chicks to feel comfortable and secure (El-Sabrout *et al.*, 2022). Furthermore, 79% of respondents reported providing appropriate lighting conditions for their chicks. Good light programming has been shown to enhance bird behavior, health, feed intake, productivity, body weight and overall well-being, aiming to improve poultry welfare and optimize production (Soliman and El-Sabrout, 2020). In Bishoftu, farmers may not have direct knowledge about welfare, but they indirectly promote chick welfare by providing sufficient space and appropriate lighting during the starter phase. This demonstrates a practical application of animal welfare principles, even in the absence of formal knowledge about welfare practices among farmers.

In this study, all respondents (100%) used antibiotics for prophylaxis purposes during the starter phase, which is higher than estimates of 56% (Mezene *et al.*, 2020) in Ethiopia and 89.8% (Chilawa *et al.*, 2023) in Zambia. This might be due to various factors such as differences in farming practices, accessibility of veterinary services, awareness and knowledge about antimicrobial resistance and socioeconomic factors influencing farmers' decision-making.

In this study, 80% of the respondents used antimicrobials for growth promoters. This finding aligns with 86% of chicken farms which claimed that the farms utilized antibiotics to promote growth (Adelowo *et al.*, 2014). However, disagreement results in Ethiopia of 32.2% (Mezene *et al.*, 2020), 6.9% in Ogun state (Oluwasile *et al.*, 2014), Nigeria of 3.3% (Joshua *et al.*, 2018), and Zambia of 19.3% (Chilawa *et al.*, 2023). This difference might be influenced by factors such as the perceived benefits of antibiotic use in promoting animal growth and productivity, economic incentives for farmers, limited access to alternative management practices and a lack of awareness about the potential risks associated with antibiotic misuse.

During the starter phase, 60% of the respondents used antimicrobials for treatment purposes. This current finding is lower than 100% (Mezene *et al.,* 2020) in Ethiopia, 93.2% (Chilawa *et al.,* 2023) in Zambia, 78% (Bamidele *et al.,* 2022), and 63.3% (Joshua *et al.,* 2018). However, this study is higher than in Nigeria at 36.2% (Oluwasile *et al.,* 2014). This could be influenced by factors such as high disease burden and potentially lower awareness about the risks of antimicrobial resistance and awareness campaigns on responsible antibiotic use.

In this study, 43% of the respondents used prescription papers or leaflets to calculate antibiotic dosage. This result is similar to statements that 46.3% of participants in a study conducted in Zambia followed the manufacturer's instructions (Chilawa *et al.,* 2023). However, this study was lower than 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). The variation might be due to education and training of farmers, awareness of antimicrobial resistance, availability of resources and the enforcement of regulations around antibiotic use in livestock farming, including requirements for veterinary oversight, which may impact the regularity of using prescription papers for dosing calculations.

Respondents' awareness in this study about antibiotic resistance was 43%, which was lower than reports of 56.7% (Hassan *et al.,* 2021), and higher than 14.1% (Tufa *et al.,* 2018) in Ethiopia, and 29.2% (Chilawa *et al.,* 2023) in Zambia. This might be due to exposure to information about antimicrobial resistance. The study population in this particular study may have had different characteristics compared to the populations in the other studies, which could explain the variation in awareness levels.

In this study, 53% of respondents consulted veterinarians before antimicrobial usage during the starter phase. This result is lower than 64.2% of respondents who said they visit veterinarians when sick poultry need to be treated (Chilawa *et al.,* 2023) from Zambia and higher than that 22.34% of respondents sought advice from a local animal health worker (Sawadogo *et al.,* 2023). This might be due to the availability and accessibility of veterinary services.

In this study oxytetracycline resistance was found to be (64%), which is lower 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, 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) and 94% (Halfaoui *et al.,* 2017) in Algeria. However higher than that in India, where it was reported to be 57% (Singh *et al.,* 2019) and 46.3% (Sarba *et al.,* 2019) in Ethiopia. This might be due to differences in antimicrobial use practices and the management practices employed on poultry farms, such as biosecurity measures, and hygiene practices, which can affect the spread of antimicrobial resistance. Farms with better biosecurity measures and disease prevention strategies may have lower resistance rates compared to those with poor management practices.

Amoxicillin resistance was reported in the current study at 48%, which was higher than 34.6% in eastern Ethiopia (Shecho *et al*., 2017). The current finding was in contrast with 100% reported in Jordan by (Abu-Basha *et al.,* 2012), and 100% in Nigeria reported by (Joshua *et al.*, 2018). This difference might be related to using Amoxicillin as a treatment for poultry diseases and as prophylaxis in the current study. These results suggest the possibility of vertical transmission of resistant strains to newly hatched chicks from parent flocks and seem to indicate that the treatment with amoxicillin increased the resistance of *E. coli* to other antibiotics.

Sulfamethoxazole-trimethoprim resistance was reported in the current study at 32%, which was lower than 92.3% (Shecho *et al.,* 2017), 70.8% (Bushen *et al.,* 2021), 48.1% in backyard production systems (Messele *et al.,* 2017) 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 colibacillosis, may account for the high rates of resistance. The lower reported sulfamethoxazole-trimethoprim resistance in the current study compared to other studies may be attributed to several factors. One possible reason could be differences in the patterns of antimicrobial usage in poultry production systems across different regions and countries. Additionally, variations in antimicrobial stewardship practices, regulations, and enforcement across different regions can also influence the prevalence of antimicrobial resistance.

In the current study, *E. coli* showed 28% resistance to Streptomycin 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), 67% (Zakeri and Kashefi, 2012), 92.2% reported by (Ibrahim *et al.,* 2019), and reports from eastern Ethiopia, where the rate was 34.6% (Shecho *et al.,* 2017). The lower reported streptomycin resistance in the current study compared to other studies may also be attributed to several factors. One possible reason could be differences in the patterns of streptomycin usage in poultry production systems across different regions and countries. Additionally, differences in poultry management practices, biosecurity measures, and hygiene standards among various production systems and geographical locations may also contribute to variations in streptomycin resistance patterns.

In the present study, *E. coli* revealed 100% resistance to pencilline and oxacilline, and 88% resistance to vancomycine. This is consistent with the findings of (Neville *et al.*, 2021; Levitus *et al.,* 2023), who reported that vancomycine shows very low activity against *E. coli*, indicating a high degree of resistance. The study conducted by (Pormohammad *et al*., 2019) also revealed the highest resistance of oxacilline against *E.coli*. This is because all pencilline, oxacilline and vancomycine act against gram-positive bacteria and have low action or resistance against gram-negative bacteria.

6. CONCLUSION AND RECOMMENDATIONS

The starter phase of poultry production is a crucial period that can significantly impact the overall performance of the flock at the end of the production cycle. It is essential to control all factors during this phase to ensure proper development. According to the current findings, significant percentages of respondents had suboptimal management practices in areas such as housing and brooding (31%), chick quality and procurement (50%), health and biosecurity (30%), chicken welfare (58%), and antimicrobial usage (35%). The educational level of the respondents was found to have a statistically significant effect ($P<0.05$) on their management practices in parts such as house and brooding, health and biosecurity, chick welfare, and antimicrobial usage. A substantial majority (81%) of the respondents in this study were unaware of poultry welfare practices. Furthermore, all poultry farmers employed antibiotics as preventive measures. In the research area, antibiotics were generally used irrationally. For instance, 47% of the respondents administered antimicrobials without consulting veterinarians and 57% used antimicrobials without utilizing prescription papers or leaflets to determine the appropriate dosage. Additionally, the prevalence of *E. coli* was discovered in the study area and the commonly used antibiotics for prevention and treatment were resistant. The resistance profile of *E. coli* showed the highest resistance to penicillin, oxacillin, vancomycin, oxytetracycline, amoxicillin, sulphamethazole-trimethoprim, trimethoprim, and streptomycin in order.

Based on the above conclusion, the following recommendations are forwarded:

- Development of targeted interventions to improve poultry farm management practices, particularly during the starter phase is highly recommended.
- Provide training and education to farmers on best practices for poultry farm management, poultry health and welfare and antimicrobial usage practices.
- Conduct regular monitoring of antimicrobial resistance patterns in *E. coli* isolates from poultry farms to track changes over time.
- Further research should be conducted to investigate the impact of management practices on poultry health, welfare, and antimicrobial resistance during the starter phase.

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8. APPENDICES

Appendix 1: Assessments of farm management practices, antimicrobial usage, poultry health, and welfare in poultry farms at the starter phase in Bishoftu, Ethiopia

Addis Ababa University College of Veterinary Medicine and Agriculture

Department of Pathology and Parasitology

Poultry Health and Management Program

Assessments of farm management practices, antimicrobial usage, poultry health, and welfare in poultry farms at the starter phase in Bishoftu, Ethiopia

Verbal consent form before conducting interview

Greeting: Hello, my name is Kebene Temesgen. I am working my MSc thesis in poultry health and management at the college of Veterinary Medicine at Addis Ababa University. I would like to interview you a few questions about farm management practices, antimicrobial usage, poultry health, and welfare during the starter phase. The objective of this study is to collect baseline data on farm management practices, antimicrobial usage, poultry health, and welfare during the starter phase, which is important to improve the health status of the poultry so your 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!!!!

Date __________________ Questionnaire no. _______

Socio-demographic characteristics of the respondents

1. Which of the following best describes your sex?

A. Male

B. Female

2. Which of the following best describes your age groups (in years)?

A. 18-25 years

B. 26-35 years

C. 36-45 years

D. >45 years

3. Role in the farm?

A. Owner B. Manager C. Attendant

4. Educational level:

A. No formal education B. Primary school

C. High school D. Diploma and above

5. How many years of experience in poultry farming?

A. 1-5 years B. 5-10years C. >10

General Farm Information

1. Name of the farm: ________________

2. Location: latitude________ longitude: _______

3. Breed of chickens

A. Bovans brown B. Lohmann brown D. Cobb500 F. Sasso

4. Purpose of chicken production A. Egg B. Meat C. Dual

5. Age of the chicks A. 1 week B. 2 weeks C. 3 weeks

D. 4 weeks E. 5 weeks F. 6 weeks

6. Type of housing system A. deep litter system B. cages system

7. Size of farm A. small B. medium C. large

Questionnaire to assess poultry farm management practices, poultry health, and welfare during the starter phase

Housing and brooding management practices

1. Did you clean and disinfect the house and equipment before the arrival of the chicks?

A. Yes

B. No

2. Do you have brooding and finishing house separately?

A. Yes

B. No

3. Does the brooder house provide adequate space and ventilation for the chicks?

A. Yes

B. No

4. Is there adequate lighting and heat in the brooding house?

A. Yes

B. No

5. What is a source of heat used during brooding?

A. Electric power supply

B. Charcoal

C. Both electric power and charcoal

6. What is the duration in the brooding house (weeks)?

A. 1 week

B. 2 weeks

C. 3 weeks

D. 4 weeks

E. 5 weeks

7. Is the temperature in the brooder house properly controlled and gradually reduced as the chick grows?

A. Yes

B. No

8. What are the bedding materials you use to absorb moisture to ensure comfort and hygiene for the chicks?

A. Straw

B. Wood shavings

Chick quality and procurement:

9. Where is the source of your DOC?

A. Well-known supplier

B. Local chicken supplier

C. Middleman

10. Do you inspect the chicks upon arrival for any signs of illness, stress, or deformities?

A. Yes

B. No

11. Do you ensure that the chicks have been properly vaccinated before they arrive at your farm?

A. Yes

B. No

Feeding and watering:

12. What is the feeding schedule for the starter phase?

A. Adlibitum

B. Adjusted based on growth

C. Fixed feeding program

13. What type or form of starter feed do you use for your DOC?

A. Crumble

B. Pellets

C. Mesh

14. What is the source of drinking water for chicks?

A. Wells

B. Tap water

Health, and biosecurity:

15. Do you have regular health monitoring schemes for your chicks?

A. Yes

B. No

16. If your response to above question is ‘Yes’, do you check for the abnormalities of: (More than 1 response is possible)

A. Behavior

B. Droppings

C. Feathers

D. Eyes

E. Legs

F. Sound and smell

17. What is the time of the day vaccines are administered?

A. Morning

B. Afternoon

C. Evening

D. Random

18. Do you consult with a veterinarian to develop a vaccination program?

A. Yes

B. No

19. Are there footbaths available at the entry of poultry houses?

A. Yes

B. No

20. Did you ensure timely treatment of sick birds and removal of dead birds?

A. Yes

B. No

21. How often visitors are allowed inside a farm?

A. Often

B. Rarely

C. Never

22. How often farm equipment are cleaned and disinfected?

A. Always

B. Sometimes

23. Do you have advisory services on your poultry health care services and production?

A. Yes

B. No

24. If the answer is ‘yes’ to above, in what case/condition do you call the health care provider?

A. At first sick

B. At death one or more than one chick

Animal Welfare:

25. Do you have animal welfare awareness?

A. Yes

B. No

26. If your response to the above question is yes, how did you get it?

A. Through training

B. I am professional

27. Do you provide sufficient space for the chicks to move, exercise, and exhibit natural behaviors?

A. Yes

B. No

28. Are proper lighting conditions provided in the starter phase housing to promote bird welfare?

A. Yes

B. No

29. Do you provide structural enrichment such as perches or elevated tiers for the chicks to rest and for optimal skeletal development?

A. Yes

B. No

Questionnaires to assess antimicrobial usage practices in poultry farms during the starter phase:

30. Do you use antimicrobials during the starter phase?

A. Yes

B. No

31. Which antimicrobial drugs are frequently used at the starter phase? More than one answer is possible

A. Oxytetracycline

B. Enrofloxacin

C. Amprolium

D. Sulphamethoxazole

E. Piperazine

F. Others

32. Do you used prescription papers or leaflets to calculate antibiotic dosage?

A. Yes

B. No

33. What are the reasons for administering antimicrobials during the starter phase? (Select all that apply)

A. Disease Prevention

B. Disease Treatment

C. Growth Promotion

34. Have you heard/know about antimicrobial resistance (failed to cure diseases)?

A. Yes

B. No

35. Is the veterinarian’s advice before antimicrobial usage during the starter phase?

A. Always

B. Sometimes

C. Rarely

36. Do you use multivitamins supplements as alternative disease prevention for your chicks?

A. Yes

B. No

Appendex 2: Picture of the chicks during brooding


23

Picture of the chicks too cold

Picture of the chicks too hot

Appendix 3: Picture for questionnaire survey, sample collection and laboratory work

Appendix 4: Media preparation procedures

Buffered Peptone Water

Preparation

- ✓ Suspend 20.0 g of the powder in 1 liter of distilled
- ✓ Mix well. Heat to boil shacking frequently until completely dissolved and sterilize in autoclave at 121°C for 15 min.

MacConkey Agar

Preparation

- ✓ Suspend 50 grams of the medium in one liter of distilled water.
- ✓ Mix well until a uniform suspension is obtained. - Heat with frequent gentle agitation and boil for one minute.
- ✓ Sterilize in autoclave at 121º C for 15 minutes.
- ✓ Cool to 45-50 °C, and pour into Petri dishes.
- ✓ Allow the plates to solidify and place them upside down to avoid excessive moisture in the surface of the medium.

Eosin Methylene blue Agar

Preparation:

- ✓ Suspend 36g of EMB agar in 1 liter of distilled water.
- ✓ Mix until suspension is uniform.
- ✓ Heat to boiling to dissolve the medium completely.
- ✓ Sterilize by autoclaving at 15 lbs pressure (121^0C for) 15 minutes.
- ✓ Cool to $45\text{-}50^0$C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue color and to suspend the flocculent precipitate.

Nutrient Agar

Preparation:

- ✓ Suspend 28 grams of nutrient agar powder in 1 liter of distilled water.
- ✓ Mix well and leave to stand until the mixture is uniform.
- ✓ Heat with gentle agitation and boil for one or two minutes, or until completely dissolved.
- ✓ Sterilize by autoclaving at 121°C for 15 minutes.

- ✓ Dispense in sterile Petri dishes and wait for the medium to solidify

Muller Hinton Agar (Criterion, C6421, USA)

Preparation:38.04 grams of the medium was combined with one liter of deionized water stirred to mix thoroughly. Then it was dissolved completely by boiling and autoclaved at 121 ^{0}C for 15 minutes. Final pH: 7.3 + 0.1 at 25 ^{0}C.

List of Biochemical Tests

Indole Test:

Principle: Indole test is performed to determine the ability of the organism to split tryptophan molecule into Indole. Indole is one of the metabolic degradation products of the amino acid tryptophan. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with the production of Indole, Pyruvic acid and ammonia.

Procedure: Inoculate Tryptone broth with the test organism and incubate for 24hrs at 37^{o}C. Then add 5 drops of Kovacs reagent down the inner wall of the tube.

Interpretation: Development of bright red color at the interface of the reagent and the broth within seconds after adding the reagent is indicative of the presence of Indole and is a positive test .

Methyl Red Test

Principle: To test the ability of the organism to produce and maintain stable acid end products from glucose fermentation and to overcome the buffering capacity of the system.

Procedure: Inoculate the MR/VP broth with a pure culture of the test organism and incubate at 35^{o}C for 48 to 72 hrs. Add 5 drops of MR reagent to the broth.

Result interpretation:

- Positive result is red (indicating pH below 6).
- Negative result is yellow (indicating no acid production).

Voges Proskauer Test

Principle: To determine the ability of the organisms to produce neutral end product acetyl methyl carbinol (acetoin) from glucose fermentation.

Procedure: Inoculate pure culture of the test organism into VP broth and incubate for 24 hrs at 37^{o}C. Aliquot 1 ml of the broth to a sterile test tube and add 0.6ml of VP reagent (5% Alpha-Naphthol and ethanol) followed by 0.2ml of VP reagent (40% Potassium Hydroxide). Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes.

Interpretation:

- Positive: Pinkish red color at the surface of the medium.
- Negative: Yellow color at the surface of the.

Reagents used in biochemical tests

- Methyl red reagent: used for methyl red test
- ∝- naphthol, strong alkali (40% KOH), and atmospheric oxygen
- Indole Kovac's reagent

Appendix 6: Antimicrobial susceptibility test

Procedure 1. 0.5 McFarland turbidity standard preparation step

- ✓ A, 8.5 gram of sodium chloride was measured and mixed with 1000 ml of distilled water and Shake vigorously
- ✓ Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes and after sterilization
- ✓ Placed in dark place until preparation of MHA media.

2. Preparation of Inoculums

- ✓ Contact 4 or 5 isolated colonies to be tested using sterile inoculums rings or needles.
- ✓ Suspend the organism in 5 mL of sterile saline.
- ✓ Vortex the saline tube to create a smooth suspension.
- ✓ Adjust the turbidity of this suspension to a 0.5 McFarland standard.
- ✓ Use this suspension within 15 minutes of preparation.

3. Inoculation of the MHA Media

- ✓ Dip a sterile swab into the inoculums tube
- ✓ Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid.

- ✓ 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.
- ✓ 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

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

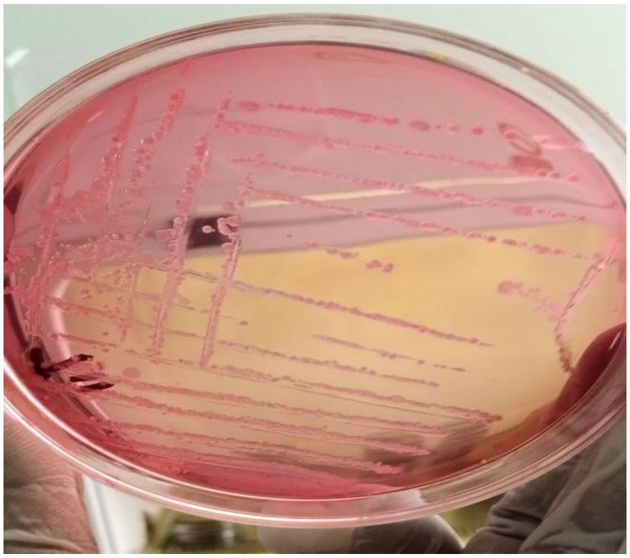
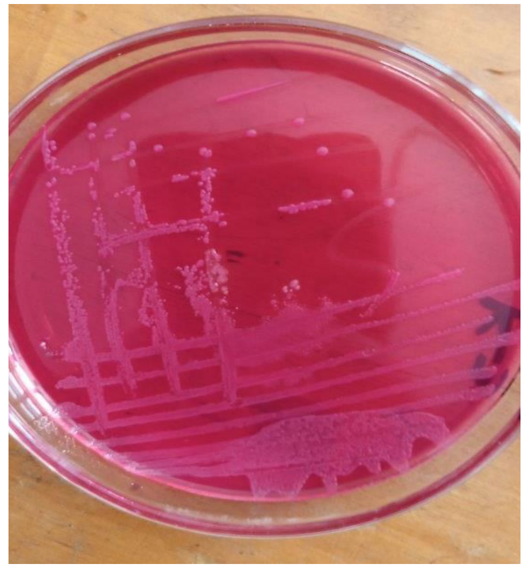
6. Measuring Zone Sizes After incubation, use a ruler or caliper to measure the zone sizes to the nearest millimeter, disk diameter is included in the measurement. Interpretation and Reporting of the Results After 24 hours of incubation, use a metric ruler to measure the zone of inhibition and include the diameter of the disc in the measurement.

Compare the result with CLSI 2022 guidelines to report the result. The results are reported as Susceptible (S), Intermediate (I), or Resistant (R).

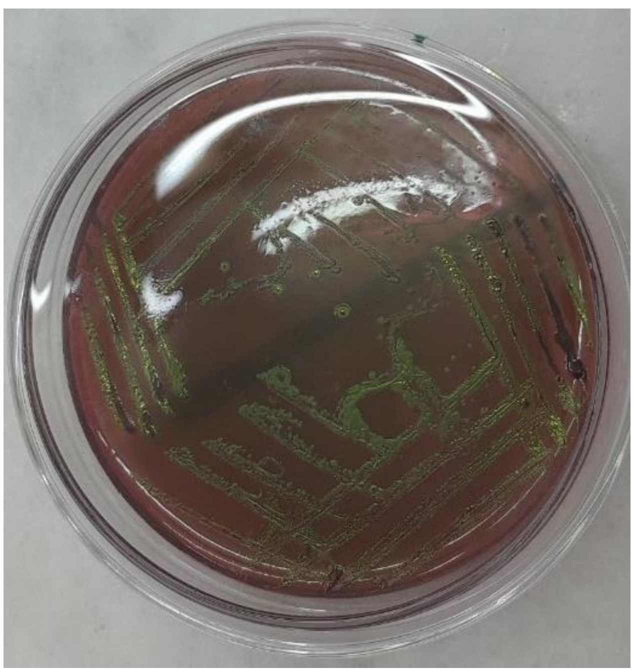
Appendix 7: Interpretative category and zone diameter breakpoints

Antimicrobial agent	Disk content (µg)	Zone diameter in millimeters		
		S	I	R
Amoxicillin	30	≥ 17	14–17	≤ 13
Oxytetracycline	30	≥ 15	12–14	≤ 11
Sulphamethoxazole +trimethoprim	1.25/23.75	≥ 16	11–15	≤ 10
Trimethoprim	5 µg	≥ 16	11–15	≤ 10
Streptomycin	25 µg	≥ 15	12–14	≤ 11
Penicillin	1 unit (10 µg)	≥ 17	_	≤ 16
Oxacillin	1 µg	≥ 25	_	≤ 24
Vancomycin	5 µg	≥ 17	15–16	≤14

Appendix 8: Supportive Pictures for *Escherichia coli* identification

E. coli colony in MaCconkey agar




E. coli colony in EMB

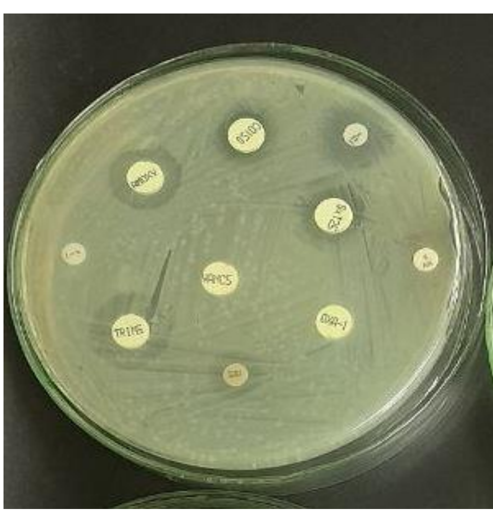
Indole test (+ve)

MRtest (+ve)

VP test (-ve)

Citrate utilization test (-ve)

Appendix 9: Antimicrobial sensitivity test plate and measurements zone of inhibition of antibiotics disc in *E. coli* cultured MHA.



Appendix 10: Ethical clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Ethical clearance certificate

Certificate Ref. No: VM/ERC/03/15/16/2024

Name of Applicant: **Kebene Temesgen Ayana (DVM, MSc student)**

Address: Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Assessment of farm management practices, antimicrobial usage, poultry health and welfare: detection and antimicrobial susceptibility profile of E. coli species in poultry farms at the starter phase in Bishoftu town, Central Ethiopia*

Date of application:	**December, 2023**
Nature of the project:	**Farm investigation**
Target animal species:	**Chicken**
Number of animals involved:	**220**
Study area:	**Bishoftu- Ethiopia**

Minutes No. and date of review: **VM/ERC/03/16/024, 16/05/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of **Kebene Temesgen Ayana.**

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Please quote Our Ref. No. When replying

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