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ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

BACTERIOLOGICAL ISOLATION AND ANTIMICROBIAL RESISTANCE PROFILES OF *ESCHERICHIA COLI* AND *SALMONELLA* ISOLATES, AND SURVEY ON ANTIMICROBIAL USE AND VACCINE MANAGEMENT IN POULTRY, CENTRAL ETHIOPIA

MSc THESIS

BY:

BELAYNEH SEIFU

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

MSc PROGRAM IN POULTRY HEALTH AND MANAGEMENT

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BISHOFTU, ETHIOPIA

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A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of

Science in Poultry Health and Management

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Bishoftu, Ethiopia

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by Belayneh Seifu, entitled: "**Bacteriological isolation and antimicrobial resistance profiles of *Escherichia coli* and *Salmonella* isolates, and survey on antimicrobial use and vaccine management in poultry, Central Ethiopia***"* and recommended that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Poultry Health and Management.

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STATEMENT OF THE AUTHOR

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

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Belayneh Seifu _____ _____

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LIST OF ABBREVIATIONS AND ACRONYMS

AM	Ampicillin
AMR	Antimicrobial resistance
AMX	Amoxacillin
APEC	Avian pathogenic *E. coli*
ASL	Above Sea Level
BPW	Buffered peptone water
C	Chloramphenicol
CDC	Centre for Disease Control and prevention
CLSI	Clinical and Laboratory Standards Institute
CN	Gentamicin
CSA	Central Statistical Agency
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EMB	Eosine Methylin Blue
EPEC	Enteropathogenic *E.coli*
ETEC	Enterotoxigenic *E.coli*
ExPEC	Extraintestinal Pathogenic *E.coli*
FAO	Food and Agriculture Organization
IMViC	Indole Methyl red Voges–Proskauer Citrate
ISO	International Organization for Standardization
KF	Cephalothin
LPS	Lipopolysaccharides
MDR	Multi-Drug Resistant
NA	Naldixic acid
OIE	Office International des Epizooties
OT	Oxy tetracycline
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid

RVS	Rappaport Vassiliadis
S	Streptomycin
SXT	Sulphamethazole-Trimethoprim
TE	Tetracycline
TSB	Typtone Soya Broth
TSI	Triple sugar iron
USD	United States Dollar
VP	Voges–Proskauer
VTEC	Vero-Toxigenic *E.coli*
WHO	World Health Organization
XLD	Xylose lysine deoxycholate

ABSTRACT

Escherichia coli and *Salmonella* are prominent bacterial pathogens responsible for avian Colibacillosis and Fowl typhoid in poultry productions, respectively. However, there is a paucity of comprehensive data regarding their prevalence and resistance to antimicrobials. This cross-sectional study was carried out between November 2023 and May 2024 in Central Ethiopia, aimed to determine the prevalence, patterns of antimicrobial resistance, and possible risk factors contributing to the development of resistance. A total of 313 swab samples were collected from randomly selected poultry farms, alongside with a structured questionnaire survey. The bacteriological examination revealed an overall prevalence of 30.35% *Escherichia coli* and 3.83% *Salmonella*. The highest rate of isolation for *Salmonella* was observed in Bishoftu (7.40%) and was absent in Addis Ababa and Mojo, while the prevalence of *Escherichia coli* varied significantly across the study sites, which found highest in Adama (41.26%) and lowest in Mojo (13.79%). Tissue swabs emerged as the primary carriers of both *Salmonella* (7.14%) and *Escherichia coli* (34.14%). Antimicrobial susceptibility testing through Kirby-Bauer disk diffusion method indicated complete resistance (100%) to cephalothin in both *Salmonella* and *Escherichia coli* isolates. *Escherichia coli* demonstrated the highest susceptibility to chloramphenicol (95.75%), whereas *Salmonella* exhibited full susceptibility to sulfamethoxazole-trimethoprim (100%). Multidrug resistance was prevalent, with 100% of *Escherichia coli* and 83.33% of *Salmonella* isolates displaying resistance to multiple drugs. The survey study indicated the widespread misuse of antibiotics and inadequate vaccine management. The administration of vaccines and medications by non-veterinarians were 41.38% and 55.17% in the farms, respectively. Furthermore, the survey showed disease as major challenge and the heightened non-therapeutic use of antibiotics in poultry farms. In conclusion, the study underscores the substantial prevalence and resistance exhibited by *Escherichia coli* and *Salmonella* in poultry establishments. To tackle these challenges, the study recommends for the enhancement of antibiotic stewardship, the improvement of vaccine management practices, and the execution of further comprehensive investigations.

Key words: *Antimicrobial resistance, Central Ethiopia, Escherichia coli, Multidrug resistance, Poultry, Prevalence, Salmonella,*

1. INTRODUCTION

Livestock and poultry production constitute significant components of agricultural development initiatives in Ethiopia. Among, the poultry farming emerges as a preferred avenue for advancing the nation, particularly benefiting women, youth, and individuals with lower to moderate levels of income (CSA, 2017). The sector also contributes significantly to the socio-economic growth by delivering proteins that help to ensure food security (Sambo *et al*., 2015; FAO, 2020). However, diseases pose a significant threat in developing countries because of their wide range of mortality, morbidities, the need for expensive treatments, and the impact on production performances (Abd El-Fatah *et al*., 2017; Salem and Attia, 2021). *Escherichia coli* and *Salmonella* are the causative agents of avian Colibacillosis and Fowl typhoid respectively, which are the major important and widespread infectious diseases caused by bacteria in poultry industry (Getinet *et al.,* 2017; Ronco *et al*., 2017).

Antibiotics have been utilized in the sphere of animal production for a duration exceeding half a century, serving as therapeutic agents, as well as prophylactic agents, or even in the capacity of growth promoters. The effectiveness and economic viability of the vast majority of these substances resulted in their widespread and unselective application (Al Sattar *et al*., 2023). Accordingly, the incorrect utilization and excessive application of these antimicrobial agents have fostered the emergence of microbial reservoirs that carry determinants of antimicrobial resistance in livestock, particularly in poultry (Hudson *et al.,* 2017; Ketema *et al*., 2018). This, in turn, leads to escalated expenses for drug choices, prolonged durations of production decline, and heightened mortality rates (Amir *et al*., 2019; Rodrigues *et al.*, 2022). One of the most crucial and economical methods for preventing disease, minimizing losses, and lowering the requirement for antibiotics in poultry productions is vaccination. However, due to problems in vaccine handling, storage, transportation, and administration, all of which call for novel approaches to improve its efficacy, the protection level of the vaccines are disputable (Heise *et al*., 2015; Wubet *et al.,* 2019).

Despite the remarkable impact of bacterial diseases specifically *E. coli* and *Salmonella* within poultry through impeding economic growth and food security in Ethiopia, there exists a critical gap in the scientific understanding of their prevalence, antibiotic resistance profiles and vaccine management in the sector (Habte *et al,*. 2017;

Ebsa *et al.,* 2019). The existing literatures highlight the value of poultry farming as an affordable form of protein, a contributor to socio-economic growth, and a means of employment for low-income individuals, particularly women and youth. In the study area, available studies focus on a single town and infection rather than a comprehensive examination of nearby areas which could be epidemiologically important in disease transmission and, still there is a shortage of reports on antibiotic usages, vaccination processes, antimicrobial resistance (AMR) and the intervention level of bacterial diseases (Telfer *et al*., 2008; Eguale, 2018).

The detailed information about prevalence rate and the AMR status of the *Salmonella* and *E. coli* as well as an overall antibiotic consumption scenario and vaccine managements are notably very few. Hence, there is a critical need for an in-depth investigation into the state of major bacterial diseases and antibiotic usages against bacteria affecting the poultry sector in Central Ethiopia, along with an assessment of the status and effectiveness of vaccines. Bridging this knowledge gap is essential to develop an effective disease control and prevention approaches, treatment bases, promoting the overall health and performances of poultry and safeguarding the economic interests of poultry producers. Therefore, this study was conducted with the following general and specific objectives:

General objective of the study:

- To evaluate the prevalence of *Salmonella* and *E. coli* in poultry farms, determine the antimicrobial susceptibility profiles of the isolates and assessing the antimicrobial usages and vaccine managements in Central Ethiopia.

Specific objectives:

- To determine the prevalence of *Salmonella* and *E. coli* in selected poultry farms of Central Ethiopia;
- To evaluate the antimicrobial resistance profiles of the isolates and
- To assess the antibiotic usages and vaccine management in the study areas

2. LITERATURE REVIEW

2.1. Avian Salmonellosis

Salmonella Gallinarum and *Salmonella Pullorum* specifically cause clinical diseases and result in substantial economic losses for poultry farmers, particularly in developing nations. Typically, this genus predominantly inhabits the digestive tracts of animal hosts, with its presence outside of these sites, such as the environment, water and food, indicating fecal contamination (Eriksson *et al.,* 2018). Recent findings from various regions of the world highlight *Salmonella* as a prevalent cause of bacterial food borne diseases globally, underscoring the ineffectiveness of control strategies targeting *Salmonella* contamination throughout the food chain (O'Bryan *et al.,* 2022). Consequently, a rise in the prevalence and persistence of *Salmonella enterica* within the gastro intestinal tracts of animals can lead to the creation of chronic carriers who asymptomatically excrete the organism in their feces, acting as sources for ongoing contamination and transmission of *Salmonella* through their contaminated products and faeces (Xiong *et al*., 2018).

2.1.1. Taxonomy and Characteristics of Salmonella

The *Salmonella* genus is the member of *Enterobacteriaceae* family, with two species, *Salmonella enterica (S. enteric)* and *Salmonella bongori (S.bongori)* depending on variations in their 16S rRNA sequence analysis (Eng *et al*., 2015; Ryan *et al*., 2017). Based on biochemical traits, *Salmonella enterica* is divided into six subspecies: *(I) Salmnella enterica subsp. Houtenae; (II) Salmonella enterica subsp. salamae; (IIIa) Salmonella enterica subsp. Arizonae; (IIIb) Salmonella enterica subsp. Diarizonae; and (VI) Salmonella enterica subsp. Indica* (Figure 1). In contrast, *S. bongori* contains less than 10 extremely rare serovars. Of these *Salmonella* subspecies, *S. enterica subsp. enterica* is the most prevalent and responsible for around 99% of infections in humans and animals. On the other hand, cold-blooded animals and the environment frequently contain *S. bongory* and *subspecies* of *S. enterica* other than *S. enterica subsp. enteric* (Mezal *et al*., 2014; Jajere, 2019).

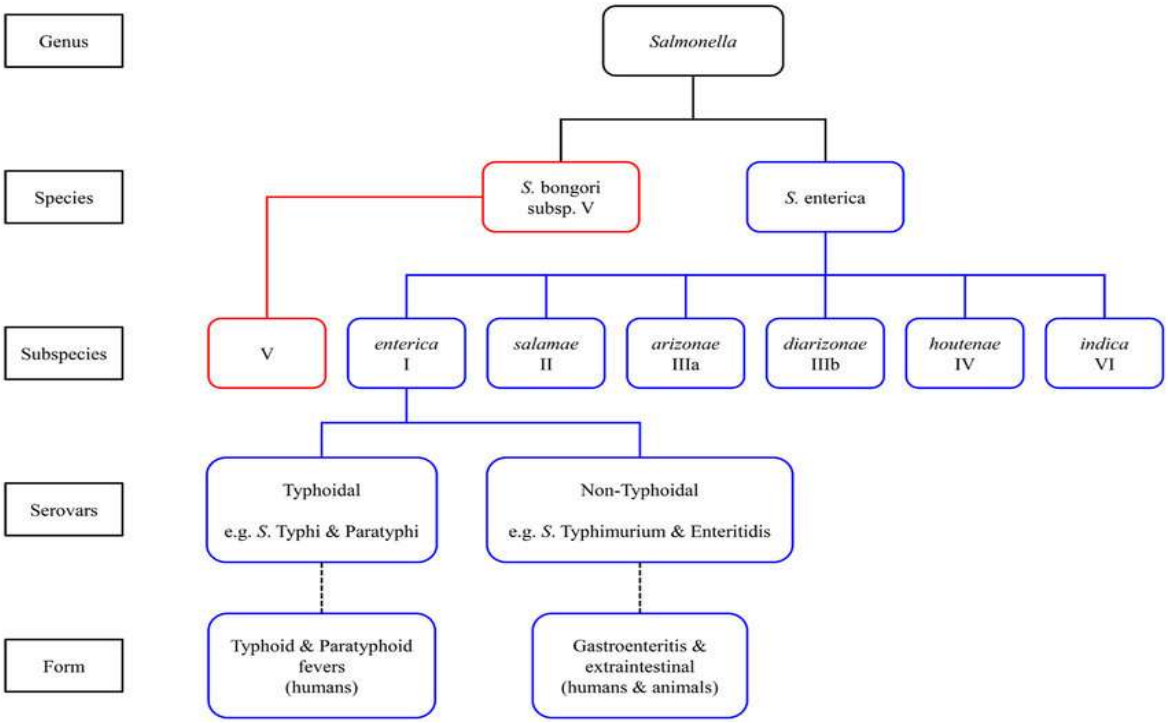

Figure 1: *Salmonella* species and subspecies classification

Source: (Hurley *et al*., 2014)

2.1.2. Biochemical characteristics of Salmonella

Most *Salmonella* serotypes thrive best at temperatures between 5°C to 47°C, with a peak occurring between 32 to 35°C (Tariq *et al*., 2022). Nevertheless, a small number of serotypes can grow at significantly broader temperature ranges, reaching 2-4°C as lower limit to 54°C as higher. *Salmonella* demonstrate susceptibility to higher temperature and is typically killed at surpassing 70°C. *Salmonella* can grow at pH values ranging from 4 to 9, with 6.5 to 7.5 being the optimal range (Wei *et al*., 2023). Despite the ability of *Salmonella* to endure conditions with water activity of less than 0.2, such as in dehydrated food products, their survival require higher water levels extending from 0.99 to 0.94 (Velasquez *et al*., 2018). While some monosaccharides, such as glucose, can be fermented by *Salmonellae* and produce gas, they are typically not able to ferment lactose, sucrose, or salicin. Typically, they are oxidase negative, catalase positive, and convert nitrates to nitrites. The organisms create hydrogen sulfide, decarboxylate lysine, arginine, and ornithine, and utilise citrate as their only

carbon source. They are Vogus-Proskauer test negative, methyl red test positive, and the indole test negative (Mikoleit, 2015; Geetha and Palanivel, 2018)

2.1.2. Epidemiology of Avian Salmonellosis

Salmonella are common environmental pathogens, predominantly intestinal bacteria that are typically found in human sewage, farm effluents, and any substance that has been contaminated by feces. Despite being found all over the world, Salmonellosis is believed to be more prevalent in regions with extensive animal husbandry, particularly in the production of chicken (Sato *et al*., 2007; OIE, 2008). There have been thousands of distinct *Salmonella* serovars described, and the number rises yearly with the discovery of new serovars (Mezal *et al*., 2014). The complexity of Salmonellosis epidemiology can be attributed in large part to the existence of over 2,500 unique serotypes, or serovars, with varying regional occurrences and separate reservoirs (Rounds *et al*., 2010; Chaka *et al*., 2012).

Geographical distribution

The epidemiology of *Salmonella* is complex due to its wide range of hosts and vectors, which are essential for effective transmission. Additionally, its ability to persist for extended periods in various climates, as well as its efficient fecal shedding from carrier animals and abundance of reservoir hosts, allows it to possess the necessary attributes for widespread distribution (Alzwghaibi *et al*., 2018; Ferrari *et al.,* 2019). Its epidemiology is intricate due to the presence of over 2,500 different serotypes, each with their own sources and local occurrences. Changes in dietary habits, cultivation methods, and distributing channels have resulted in a rising number of multistate outbreaks linked to both fresh produce and processed foods (Rounds *et al*., 2010).

The frequency of *Salmonella* in commercial poultry and their surroundings has been estimated to vary significantly, despite the fact that the bacteria has been isolated from flocks of chickens belonging to different species, including both broiler and layer types. They might indicate actual variations in *Salmonella* distribution between geographical areas and management regimes, but they may additionally simply reflect variations in the approaches employed for estimating the prevalence of the infections. According to sample types, handling and collection procedures, and detection techniques, the apparent incidence of *Salmonella* varies (Kabir, 2010). *Salmonella*

Gallinarum and *Salmonella Pullorum* are highly host adapted *biovars* that naturally occur in chickens; nevertheless, natural outbreaks have also been documented in turkeys, guinea fowl, quail, and pheasants (Medina *et al*., 2013).

Source of infection and mode of transmission

Poultry may acquire *Salmonella* in a variety of ways during the production cycle, including via interacting with carrier animals including cats, rodents, and insects. *Salmonella* is also spread by contaminated poultry feed, litter, water, and aerosol transmission (figure 2) (Raufu *et al.,* 2019; O'Bryan *et al*., 2022). On poultry farms, rodents and insects can serve as *Salmonella* carriers. In particular, because they can cross-contaminate and spread the infection to others in their group who are not sick, cockroaches can bring foodborne pathogens such as *Salmonella* into facilities that produce chickens (Liu *et al*., 2018; Ferrari *et al.,* 2019). Research has indicated that *S. Typhimurium*-infected cockroaches are capable of spreading the bacterium to the table egg. It has been shown that flies kept in poultry facilities can carry *Salmonella* (Leffer *et al*., 2010). Subsequently, the bacteria *S. enteritidis* is carried by the chicken mite (*Dermanyssus gallinae*) and the litter beetle (*Alphitobius diaperinus*) in poultry facilities. In addition to the mite blood meal, it is hypothesized that the chick oral consumption of crushed contaminated mites could be the main source of infection (Sparagano, 2009; Donoso *et al*., 2020).

There are two possible ways that *Salmonella* might contaminate eggs: horizontally and vertically. The serovar *S. enteritidis* is one such way. Before the egg is laid, the infection immediately develops in the yolk, vitelline membrane, and albumen in vertical or transovarial transmission. With *S. enteritidis*, the infection starts in the ovary and oviduct, two reproductive organs. As a result, germs begin to infect the egg in the oviduct even before the eggshell hardens. Eggs get contaminated through eggshell penetration from the colonized gastrointestinal tract (GIT) in horizontal or fecal-oral transmission (Ferrari *et al*., 2019; Morton *et al*., 2019).

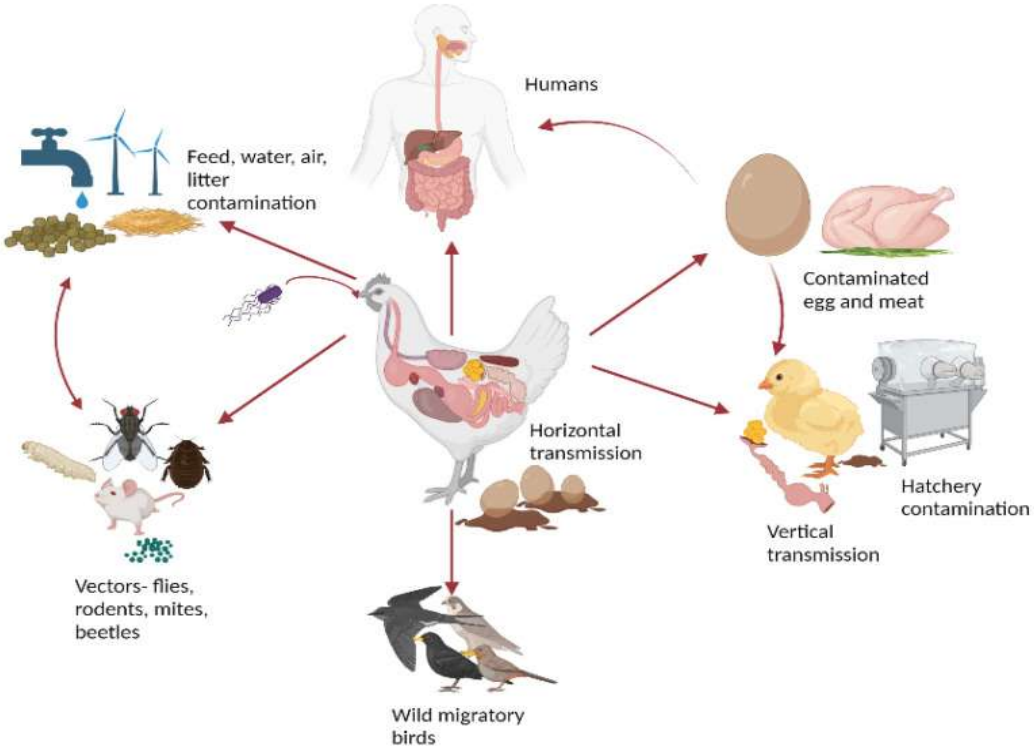

Figure 2: Various transmission routes of *Salmonella* in Poultry

Source: (Shaji *et al.,* 2023)

2.1.3. Pathogenesis and virulence factors

The main characteristic virulence aspect of *S. enterica*, which is its capacity to infiltrate or multiply intracellularly within the host's cells, has been studied using various techniques, including screening for weaker variants. As a result, many single genes involved in the virulence traits at the molecular and cellular levels have been found (Gerlach and Hensel, 2007; Pico-Rodríguez *et al*., 2024). Additionally, studies have shown that *S. enterica* produces a wide range of virulence factors, some of which are parts of the adhesion systems, including adhesins, invasins, fimbriae, hemagglutinins, exotoxins, and endotoxins. This is similar to many other enteropathogenic bacteria (kemal, 2014). Whether acting alone or in concert, these components allow *Salmonella* to attach itself to its host, adhere to, penetrate, and elude the host's defenses, including acidity in the stomach, gastrointestinal proteases, defensins, and the aggressins in the intestinal microbiota (Sabbagh *et al*., 2010; Yue and Schifferli, 2014)

2.1.4. Clinical signs and Postmortem findings

Clinical manifestations and pathological findings offer limited diagnostic purposes, as the presence of infection often occurs without apparent clinical manifestations or lesions. A precise diagnosis necessitates confirmation through the isolation, characterization, and/or identification of antibodies via serological analysis (Hafez, 2016). The frequency and kind of gross lesions are influenced by the progress of an infection, the pathogen's virulence, and the resistance level of the host. The pullorum disease lesions were the same as those observed in chicks suffering from fowl typhoid (Kumara *et al*., 2013). The characteristic look of the bronze-colored liver is a hallmark and easily identifiable lesion in most cases of fowl typhoid infections. Liver stained with bronze is typically observed in chicks between 7 and 15 days. Numerous grayish necrotic foci or necrotic patches, as well as evenly distributed reddish hemorrhagic foci throughout their surfaces, are evident in the afflicted chicken livers. In addition to exhibiting minor distension, the chicks which died in the early age group also showed mild liver discoloration, liver hypertrophy, and hepatic congestion. Diffuse regions of necrosis were also seen in a number of these victims (Rahman *et al.,* 2018).

Salmonellosis infections can have a variety of post-infection symptoms, depending on the infection's amount and opportunistic nature, which can lead to spontaneous outbreaks. However, clinical signs are the primary means of diagnosing the bacteria; general symptoms that may be shared by other diseases include dullness, severe depression, anorexia, appearing listless, and standing motionless with both eyes closed and head buried in the chest (Saha *et al*., 2012). The majority of the affected chicks experienced drooping wings with ruffled feathers, total inappetence, increasing thirst, and gradual weakening. In acute instances, the most common clinical symptom was diarrhea that ranged from watery to mucoid greenish yellow. In a few outbreaks, lameness in birds was also reported. Rarely, there may have been additional instances of minor respiratory distress. Birds that contracted acute paratyphoid disease typically died without exhibiting any early symptoms (Nazir *et al.*, 2012).

2.1.5. Diagnostic approaches of Salmonella

Bacterial culture method

The primary approach for precisely identifying the serotype and establishing a conclusive diagnosis of Salmonellosis is bacterial culture and detection. However, there are a number of variables that can affect the reliability of growing the organism, including the bacteriological techniques employed, the quantity of samples submitted, and the shielding mechanism of the organism. In addition Necropsy examination and typing can be used as base methods for the diagnosis. The conventional protocol for isolating *Salmonella* species entails a series of steps, including a pre-enrichment an enrichment phase, and plating on specific media (Ahmed *et al.*, 2014; Escamilla *et al.*, 2021).

The non-selective pre-enrichment medium helps the growth of *Salmonella* supplying nutrients for the recovery of sub-lethally wounded organisms while controlling the other competing bacteria. In a similar vein, adding inhibitors to selective media prevents non-*Salmonella* species from growing, which allows *Salmonella* bacteria to continue growing (Lee *et al*., 2015). As per ISO 6579:2002 criteria, Rappaport Vassiliadis (RVS) and Muller-Kauffmann Tetrathionate (MKTTn) are widely used for selective enrichment, whereas buffered peptone water is employed for the pre-enrichment stage (ISO, 2002). *Salmonella* colonies from selective plating media, like Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate (XLD), are usually confirmed by molecular detection, serological testing, and biochemical assays (Ahmed *et al.*, 2014).

Serological and Molecular methods

On farms, the whole blood test is utilized for a rapid diagnosis of *Salmonella Pullorum/Gallinarum*. Agglutination test and Enzyme-Linked Immunoabsorbent Assay (ELISA) are two techniques that can make the whole blood test a reasonably reliable diagnostic test in particular circumstances. Serum agglutination is used to separate somatic (O) and flagellar (H) antigens, which is necessary for serovar identification. Specific monoclonal or polyclonal antibodies that bind to somatic (O), flagella (H), and capsular (Vi) antigens are used in immunological procedures, primarily for the identification of *Salmonella* serovars (Geetha and Palanivel, 2018).

The determination of the serotype name is established based on the Kauffmann-White Scheme subsequent to a comprehensive serotyping of the *Salmonella* culture. However, due to changes and loss of surface antigens, the antigenicity of two or more identical *Salmonella* serotypes might vary, which reduces the effectiveness of serological approaches (Lee *et al*., 2015).

It is nowadays possible to diagnose avian Salmonellosis with *S. enteritidis* using a serological ELISA and molecular detections of the genetic materials of the bacteria. A real-time PCR technology and a recognized diagnostic are available for the quick and accurate detection of *Salmonella* genus- and serovar-specific (*S. enteritidis and S. typhimurium*) for the purpose of monitoring in the chicken food chain. *Salmonella* is detected using nucleic acid-based assays that focus on a particular nucleic acid sequence found in the bacterium (Ashwani *et al*., 2014; Escamilla *et al.*, 2021). PCR and DNA probe techniques are the primary methods employed in molecular analysis. These assays enable the detection of minimal organism quantities in samples and the efficient analysis of numerous samples for regular examination (Ahmed *et al.,* 2014). Because of its strong interspecies conservation, the *Salmonella invA* gene is a reliable and accurate target gene for molecular identification of their genus (Eng *et al*., 2015).

2.1.6. Public health and economic impacts of Avian Salmonellosis

Due to the possibility of human and animal pathogenicity in the majority of *Salmonella* strains, Salmonellosis poses a threat to public health. Exposure to avian salmonellosis can be harmful to a people health. The symptoms, which include severe gastroenteritis and diarrhea, are similar to those of food poisoning. On the other hand, it seems that birds mostly contract the disease from their surroundings and that affected birds are comparatively insignificant in the spread of the disease to humans and domestic animals. Due of concerns about food borne zoonotic transmission and public health, *Salmonella* is the focus of multiple global, domestic, and local surveillance initiatives (Kabir, 2018). The main cause of Salmonellosis, a frequent digestive disease in humans, is contaminated meat and poultry with *Salmonella*. Human infection frequently arises from contaminated poultry. With animal Salmonellosis serving as the primary reservoir, human cases of the disease have become significantly more common in recent years, contributing to the disease growing significance. *Salmonella* infections in humans are mostly caused by food

eaten with tainted raw eggs, egg products, or undercooked poultry meat (Priyantha *et al*., 2011; Gebremedhin *et al*., 2021).

Avian Salmonellosis results in significant economic losses due to decreased productivity and mortality. *Salmonella* in domesticated poultry can have complicated long-term implications that extend well beyond the immediate impact on the producers that are affected (Castro-Vargas *et al*., 2020). These diseases have several detrimental effects, including lower profits from operations involving chicken resources; human infection (morbidity, food safety, and quality); public expenditure for prevention or control; insufficient use of production potential; and decreased productivity for poultry farms causing production losses, treatment costs, market issues (Elina *et al*., 2011; Jajere, 2019).

2.1.7. Treatment, control and prevention

Clinical cases of *Salmonella* infection subside without the need for antibiotic treatment. However, those suffering from serious infections might need to receive efficient antibiotic therapy. Since many *Salmonella* species are prone to developing resistance to many medicines, it is important to examine the optimal antibiotic, dose, and duration of treatment (Eguale, 2018; Zhang *et al*., 2020).

Many antibiotics can mitigate morbidity and death, but not any of them are likely to eradicate the carrier from the flock of uninfected animals completely. It is not advised to treat infected flocks for pullorum and fowl typhoid sickness to lessen the persistence of the carrier status. Antibiotics are administered to day-old poults in the event of a paracolon infection in order to reduce mortality. However, even after treatment, birds may still carry and excrete the parasite. Several antibacterial medications reduce mortality in cases of paratyphoid infection, but they cannot completely eradicate the infection from the flock. The most sensitive medication for treating an infection with Salmonellosis was polymixin B. Furthermore, *Salmonella* infections are extremely susceptible to chloramphenicol (Kumara *et al*., 2013).

Salmonella can be prevented and controlled by putting the Hazard Analysis Critical Control Point (HACCP) concepts into practice. In order to prevent and control *Salmonella*, strict biosecurity protocols, suitable disinfection, avoiding stressful situations, and frequent vaccination are essential. Samples from various parts of the

poultry environment must be taken in order to assess the value of disinfection on poultry farms (Wibisono *et al*., 2020).

The main management protocols should involve the use of infection-free chicks and their placement in a thoroughly sanitized environment free of *S. gallinarum* and *S. pullorum*, along with rigorous biosecurity measures (Elina *et al.*, 2011). Kabir revealed proof concerning the possible use of probiotics in the mechanisms of competitive exclusion to suppress poultry strains of *Salmonella*. Pullorum and poultry typhoid can be treated with antibiotics, while the disease itself may be controlled with vaccinations (Kabir, 2010). Moreover, vaccinations against *Salmonella* help reduce the amount of the bacteria that animals harbor and stop specific serotypes from migrating from farms to facilities. However, using vaccination as the only method of control is not recommended. Ethiopians have received an attenuated live immunization made from the *S. Gallinarum 9R* strain. When the health of the breeding flock or the hatchery that produces the flock is uncertain, immunization against certain *Salmonella* serotypes at one day old may be recommended (Crouch *et al*., 2020).

2.2. Avian Colibacillosis

Avian Colibacillosis has been recognized as a significant global avian infectious disease affecting birds of all age groups, resulting in substantial economic implications for the poultry industry. The financial repercussions are primarily due to losses related to mortality and decreased productivity in infected birds, especially during the peak egg-laying phase and throughout the latter part of the laying cycle (Linden, 2015). The presence of Avian Pathogenic *Escherichia coli* (APEC) infections is linked to stress induced by various factors such as mycoplasma infections, infectious bursal disease (IBD) virus, infectious bronchitis (IB) virus, Newcastle disease (ND) virus, and environmental factors like temperature, humidity, ammonia, and farm dust. Additionally, *E. coli* has the capacity to penetrate the eggshell if contaminated by fecal matter, subsequently spreading to chicks during hatching, leading to increased early chick mortality rates and yolk sac infections (Kabir, 2010). *Escherichia coli* bacteria commonly reside in the avian digestive system, with most strains being non-pathogenic (Guabiraba & Schouler, 2015; Linden, 2015).

2.2.1. Taxonomy and characteristics of Escherichia coli

The bacterial species *E. coli*, a member of the *Enterobacteriaceae* family, was identified by the German-Austrian pediatrician Dr. Theodor Escherich (1857–1911) in 1885. The Gram-negative rod-shaped bacteria of the *Escherichia* genus can thrive in both anaerobic and aerobic conditions, with variations in flagella presence. They have the ability to ferment various carbohydrates, such as lactose and glucose, although specific strains exhibit characteristics like being oxidase negative and indole positive, indicating their inability to ferment lactose (Barnes *et al*., 2008; Quinn *et al.,* 2011).

Biochemical properties of the strain and the type of culture medium utilized influence the appearance of bacterial colonies. For example, lactose fermentation and acid production in MacConkey agar result in pink-colored strains, except for a few that remain orange-pale due to their inability to ferment lactose (Filho *et al*., 2013). The classification of different *E. coli* strains into distinct serotypes is based on three main antigens present in the bacterial cell: the O antigen, known as a somatic antigen derived from the Greek word "soma" meaning "body" and composed of polysaccharides (Stenutz *et al*., 2006), the H antigen located on the flagella (flagellar antigen), and the K antigen (capsular antigen) (Brugere-Picoux *et al*., 2015).

2.2.2. Epidemiology of Avian Colibacillosis

Geographical distribution

The organism is found all over the world and is frequently found in animals' and birds' digestive systems (Vegad, 2015). It is commonly found in a variety of environments, including soil, water, plants, decomposing organic materials, and the large intestines of people and animals. In Ethiopia reports of *E. coli* presence have also been documented in various locations involving poultry, other animals, and animal-derived products, including findings from cloacae samples collected from poultry farms. These findings collectively indicate the widespread distribution of the organism worldwide (Shecho *et al*., 2017).

Source of infection and mode of transmission

It spreads to other birds via the oral-fecal pathway and re-enters the bird environment through droppings. It suggests the primary reservoirs for pathogenic *E. coli* strains are the environment and avian intestines (Ewers *et al*., 2009). *Escherichia coli* can spread through vectors such as rodents, wild birds, flies, insects, mites, and darkling beetles (Barnes *et al*., 2008; Wales *et al*., 2010). Breeders who contain the bacterium in their reproductive system and pass it on to their progeny can transmit *Escherichia coli* either vertically or horizontally, directly or indirectly (Giovanardi *et al.*, 2005). The intestines of birds and their surrounding environment serve as the predominant carriers for strains of *E. coli* which possess the capability of inducing infection. Various vectors such as flies, insects, mites, rats, and wild birds are significant in the dissemination of *E. coli*, with transmission occurring either horizontally or vertically (Olakolu *et al*., 2020). The ovaries or other reproductive organs are the pathogen's means of entry into the offspring of the carrier breeder through the vertical transmission route (Panth, 2019).

2.2.3. Pathogenesis and virulence factors

Certain genes that code for the synthesis of particular bacterial cell components, or virulence factors, have been linked to the potential of APEC to cause disease. These factors have the ability to boost *E. coli* capacity to adhere to host cells, proliferate and attack avian cells, or hide *E. coli* from the host's immunological reaction. The most important virulence factors found in *E. coli* bacteria cells include adhesins like *type 1-F adhesin* and *P-adhesin,* iron-related factors like the *aerobactin* system made up of *iuc* and *iut* factors, *protectins* like *K1* and *iss* factor, toxins/bacteriocins like *stx1,2, hlyD* and *hlyF*, and other factors (Barnes *et al*., 2008; Landman *et al*., 2019).

It is unlikely that avian pathogenic *E. coli* (APEC) causes digestive illnesses. However, rarely, enterotoxigenic *E. coli* was identified from in poultry that have diarrhea, and diarrhea was deliberately generated by APEC intramuscular inoculation. On the other hand, from clinically healthy poultry, enteropathogenic *E. coli* (EPEC) were identified. Acute or chronic salpingitis can affect both layers and broilers (Dziva and Stevens, 2008). Both an infection of the left abdominal air sac and an ascending infection from the cloaca can cause salpingitis, however some researchers thought the latter was less frequent (Brugere-Picoux *et al*., 2015).

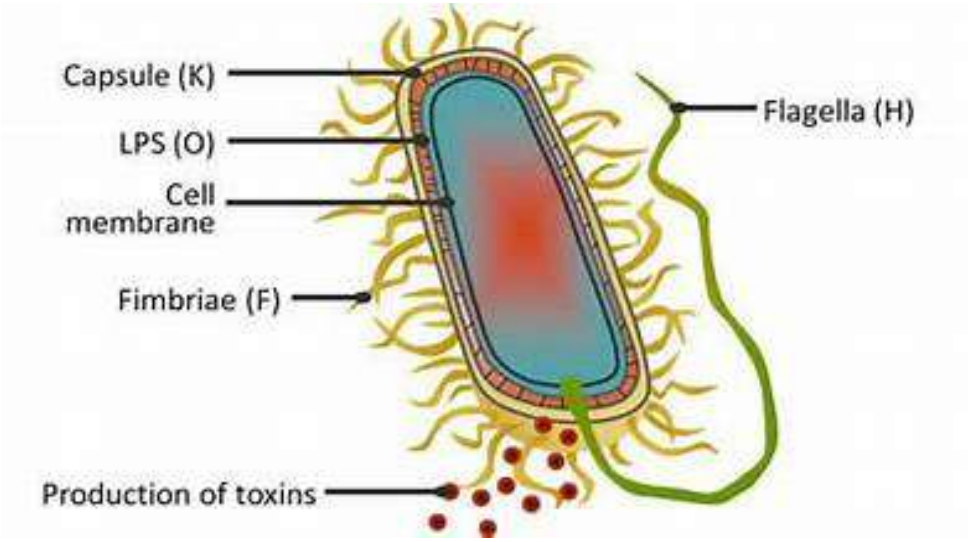

Figure 3: Important structures of pathogenic *E. coli*

Source: (EcL-lab.com Universite de Montreal)

Different avian systems can be affected by *E. coli*, leading to a variety of symptoms and either localized or systemic disease. One of the most prevalent types of *E. coli* infection in day-old chicks is the yolk sack infection (omphalitis), which is characterized by inflammation of the yolk sack and higher mortality (Dinev, 2007). *E. coli* can seriously infect the upper or lower respiratory tract in birds of various ages by entering the trachea or nasal route. Swollen head syndrome is a term used to describe face oedema and sinus enlargement in cases of localized upper respiratory tract illness (Landman and Van Eck, 2017).

2.2.4. Clinical signs and Postmortem Lesions

Birds of all ages can be impacted if their resistance is compromised by predisposing factors. Young chicks, less than 10 days old, are especially vulnerable when their resistance is diminished. In such cases, the infection's severity can range from mild to persistent, sometimes even presenting without any clinical signs. Poultry afflicted with colisepticemia may display signs of lethargy, reduced appetite, and cessation of drinking. The seriousness of the ailment can be inferred by the extent of decreased water intake (Nolan *et al.*, 2015). An infection caused by pathogenic *E. coli* can lead to various health issues in poultry, including acute septicemia, mushy chick sickness,

pericarditis, perihepatitis, and colisepticemia. Symptoms of colisepticaemia in birds typically encompass diarrhea, pasty vent, and loss of appetite, lethargy, difficulty breathing, and sneezing. Birds suffering from air sac illness often exhibit swollen, cloudy, and edematous air sacs with caseous deposits (Singh *et al*., 2011; Mellata, 2013).

Distinct pathological findings associated with colisepticaemia in birds involve salpingitis, mildly to moderately flaccid and wrinkled ovarian follicles with the rupture of theca wall leading to egg peritonitis, fibrinous perihepatitis, pericarditis, as well as congestion and regression of ovarian follicles (Oh *et al*., 2011). Common macroscopic lesions observed in cases of egg peritonitis linked to *E. coli* infection include the rupture of ovarian follicles, presence of amorphous yolk material in the peritoneal cavity, and strands adhering to the surface of the ova along with the serous lining of the intestines and oviduct (Srinivasan *et al*., 2013).

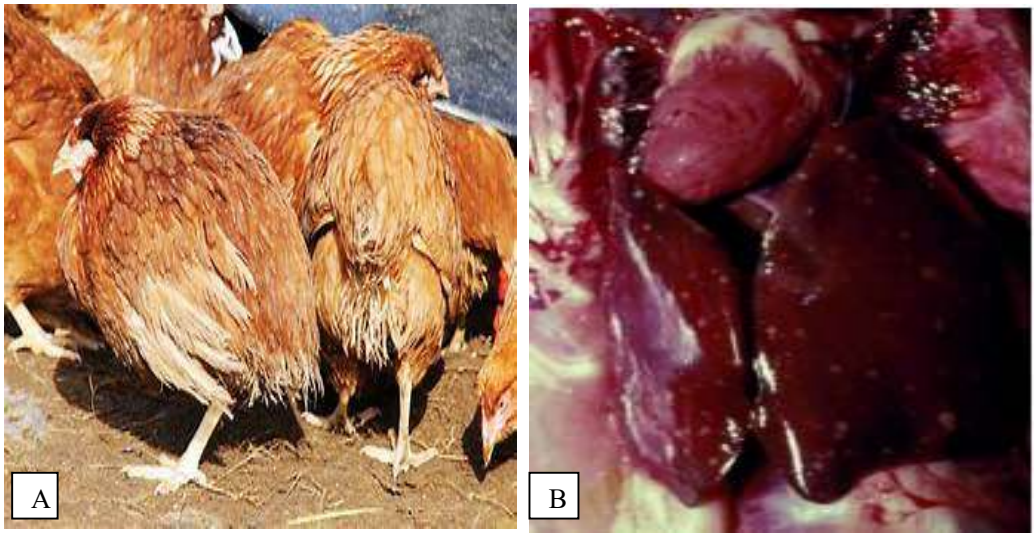

Figure 4: Pictures of clinical sign (A) and post-mortem finding (B) of APEC

Sources: A (chickencage.com), B (Nolan *et al*., 2015)

2.2.5. Diagnostic Approaches of Avian Colibacillosis

Colibacillosis diagnosis is commonly established through the scrutiny of macroscopic lesions and clinical signs. The identification of *E. coli* in cardiac blood and affected tissues like the liver, spleen, pericardium, or bone marrow is crucial for confirming the diagnosis. Previous studies have shown that isolation can be achieved within the first six hours to three days post infection in acute cases, but in subacute instances, it may take up to seven days. Contamination from the intestines is seldom a concern when fresh samples are utilized following standard bacteriological procedures. Utilization of selective media such as McConkey, eosinmethylene blue, or drigalski agar aids in the isolation process, with further confirmation through biochemical reactions on the isolated colonies (Nolan *et al*., 2015).

A common typing method employed is O-serotyping, which involves the detection of antibodies against key pathogenic serotypes like O78:K80 and O2:K1 using ELISA techniques based on sonicated *E. coli*. Another ELISA focuses on the fimbrial antigen but both have limited efficacy in identifying homologous APEC types. Virulence-associated factors found in strains isolated from Colibacillosis lesions are also present in fecal isolates from healthy chickens, rendering these traits unsuitable for APEC identification (Quinn *et al*., 2011).

2.2.6. Public Health significance of Avian Colibacillosis

The Public Health implications of Avian Colibacillosis are significant due to the common presence of *E. coli* in the lower intestines of warm-blooded organisms. While most *E. coli* strains are harmless, certain strains can lead to severe foodborne illnesses, with Shiga toxin-producing *E. coli* (STEC) such as O157:H7 being a major pathogen among over 700 identified serotypes. Non-O157:H7 serotypes like O26:H11, O103:H2, O111: NM and O113:H21 are also associated with disease transmission from animals to humans (Nataro and Kaper, 2021).

A strong phenotypic connection exists between *E. coli* of the O2:K1 serotype isolated from human urinary tract infections and septicemic poultry. Differentiation between these two groups was only possible by examining their plasmid contents. Comparison of *E. coli* strains isolated from sick humans in the same period and region as avian isolates revealed differences, particularly in avian isolates from healthy and diseased

birds suffering from airsacculitis and cellulitis (Anamaria *et al*., 2018). Colonization and persistence of avian *E. coli* are more limited in older birds, posing both animal welfare and public health risks as these zoonotic organisms have the ability to adapt to humans (Manges *et al*., 2007).

2.2.7. Treatment, Control and Prevention

Antibacterial agents have been employed in diverse manners for the management and treatment of Colibacillosis. The inadvertent misuse of antimicrobials has precipitated the emergence of antibiotic resistance, thereby diminishing the availability of efficacious antibiotics. Moreover, there exists a dearth in the development of innovative therapeutics (Quinn *et al.,* 2011). *Escherichia coli* exhibit varying degrees of resistance to commonly used antibiotics in the productions such as neomycin, oxytetracycline, amoxicillin, enrofloxacin, and ciprofloxacin. Gentamicin has been identified as an exceedingly effective treatment for Colibacillosis. A crucial aspect for successful treatment involves conducting an antibiotic susceptibility test to ascertain the vulnerability of the bacterial strain to antimicrobial intervention. Despite recommendations for alternative approaches such as prebiotics, probiotics, enzymes, digestive acidifiers, vitamins, immune enhancers, and anti-inflammatory medications, their utilization has not been met with enthusiasm. *Lactobacillus* and *Bacillus* species have been harnessed as probiotics to impede the colonization of *E. coli* in the gastrointestinal tract (Hossain *et al*., 2015; Linden, 2015).

The implementation of proper sanitation and hygiene measures plays a pivotal role in the prevention of Colibacillosis. It is imperative to effectively identify and address infection reservoirs and predisposing factors. Fumigating litter with methyl bromide and formaldehyde can prove to be efficacious in eradicating *E. coli*. Mitigating fecal contamination in eggs can be accomplished through regular collection, nest material sanitation, avoidance of ground eggs, disposal of cracked eggs, and prompt spraying or disinfection of eggs within two hours of laying (Davis & Morishita, 2005). Ensuring the quality of water by testing for coliforms and treating it with disinfectants is crucial. *E. coli* is susceptible to destruction through hot granulation techniques; however, precautions must be taken to prevent recontamination of food products. The adoption of closed irrigation systems (nipple) and chlorination of drinking water has demonstrated effectiveness in the management of Colibacillosis (Linden, 2015).

2.3. Antibiotic Usage in Poultry Production

In the forthcoming years, it is anticipated that the consumption of poultry meat will exceed that of pork and beef on a global scale. The rising demand for affordable protein sources worldwide, particularly in developing nations experiencing rapid growth in per capita income and population, serves as a significant contributing element (Belanger, 2015). Consequently, in order to meet the increasing need for chicken meat, a growing number of poultry are being raised in densely populated farms with limited outdoor areas. Within such production settings, the frequent application of sub-therapeutic doses of antibiotics is a common strategy to prevent bacterial infections due to overcrowding and unsanitary conditions (Aguirre, 2017). While the utilization of antibiotics in animal feed to enhance growth is prohibited in the United States and Europe, it remains permissible in numerous middle-income and low-income countries, and antimicrobials are easily accessible without a prescription from a veterinary professional or an animal health expert (Bhushan *et al*., 2017).

Antibiotics play a crucial role in the production of poultry and livestock, offering benefits to farmers and the economy alike by significantly enhancing poultry performance in a cost-effective manner. Nonetheless, there exists a potential risk of antibiotic-resistant strains of pathogenic and non-pathogenic organisms spreading into the environment and contaminating humans through the food chain, which could have adverse implications for public health (Apata, 2009; Van Boeckel *et al*., 2014). In commercial poultry farms, antimicrobials are predominantly used either as growth promoters or therapeutic agents for treating diseased flocks. A noteworthy aspect is that the majority of antibiotics employed in animal agriculture belong to the same class as those used in human medicine, even though some are exclusively reserved for animals (Landers *et al.,* 2012; Chattopadhyay, 2014). Additionally, a considerable proportion of these antibiotics are classified as "medically important" antimicrobials, which are the preferred choice for human treatment and encompass fluoroquinolones and cephalosporins. Studies have disclosed that numerous antibiotics, including colistin as a last resort option, are extensively utilized in commercial poultry systems to promote growth (Lee *et al*., 2012; Nhung *et al*., 2016).

In the poultry industry, farmers administer antibiotics for various purposes, such as utilizing them as therapeutic agents to treat infected animals, who typically receive a regimen involving high doses over a relatively short time frame (Murphy *et al*., 2017). Another common application of antimicrobials in poultry farming is their prophylactic role in preventing disease and serving as growth promoters. This practice involves administering sub-therapeutic doses of antibiotics to animals through feed or water when there are no visible signs of infection, but it is suspected. Periodically, antimicrobial drugs are administered over several days throughout the chicken's life cycle (Fung *et al,.* 2013; Agyare *et al.,* 2019).

2.3.1. Antimicrobial Resistance

Antimicrobial resistance is a condition in which microbial infections become resistant to the effects of antimicrobial drugs. The health of humans and animals remains seriously threatened by antimicrobial resistance, which increases the risk of infection and the difficulty of treating diseases brought on by resistant bacteria (Tang *et al*., 2017). Maintaining the efficacy of antibiotics in treating infections continues to pose a critical concern in veterinary and human health. Antimicrobial resistance is estimated to be the cause of 4.2 million deaths in Africa each year, and over the next 35 years, it is predicted to cause 300 million premature deaths globally (Chantziaras *et al*., 2014).

Common antimicrobials like tetracycline are often used in intensive chicken farming (Diarra and Malouin, 2014). The most commonly used antibiotics in Ethiopian areas that have the potential to produce poultry include tetracyclines, fluroquinolones, sulfadiazine and trimethoprim combined, Amoxycillin, colistin sulfate, and tylosin (Ejo *et al*., 2016),). In Ethiopia, microorganisms originating from food animals have been found to exhibit multidrug resistance to the listed antibiotics. Research has linked this to farmers' unrestrained use of antibiotics due to inadequate antibiotic control measures, particularly in poultry (Taddese *et al.,* 2020).

Antibiotics are unquestionably the mainstay of treatment for a large number of infectious diseases today; yet, the advent of antimicrobial resistance in veterinary and human medicine has sparked significant concerns for the health of the global population (Palma *et al*., 2020). Due to selection pressure, animal gut bacteria that are frequently exposed to various antibiotics at concentrations below therapeutic thresholds eventually become resistant to those medications. In highly cared-for food

animals such as chickens, which are regularly administered antibiotics as growth boosters to entire flocks, there is significant selection pressure for antibiotic-resistant bacteria in their gut flora (Marshall and Levy, 2011). *E. coli* is an essential part of the natural microbiota and is rapidly resistant to antibiotics that poultry consumes. Moreover, the potentiality of *E. coli* to transfer antibiotic resistance determinants to its other strains as well as different bacteria is well known (Rasheed *et al*., 2014).

2.3.2. Multidrug resistance

One key challenge to effectively treating infections is the evolution of concurrent resistance to various medications with distinct chemical structures and targets. Multidrug resistance (MDR) is a type of acquired resistance of microbes to treatment medicines characterized by distinct chemical structures and mechanisms of action (Wang *et al*., 2017). The over expression of many proteins that extrude the medication from the cell and lower its concentration below the effective level is the cause of multidrug resistance (MDR). Alternative antimicrobial drugs and ways to control bacterial infections are of vital importance, as the overuse and misuse of antibiotics have led to the fast development of MDR bacteria and their ineffectiveness against pathogens (Iacopetta *et al*., 2020).

There are numerous ways that bacteria can develop multidrug resistance. First, on resistance R-plasmids, bacteria typically generate many genes, each coding for resistance to a single medication, within a single cell. Moreover, increased expression of genes encoding multidrug efflux pumps, which eliminate a variety of medications, may lead to the emergence of multidrug resistance (Garcia *et al*., 2021). Last but not least, enzymatic inactivation of drugs through deterioration or the addition of a chemical group to them might result in MDR. Penicillin and tetracycline are two examples of drugs that can be rendered inactive by hydrolyzing them. For drug deactivation, transferring acetyl, phosphoryl, and adenyl groups is a common technique. Non-fermenting Gram-negative bacteria are increasingly known to be at major concern to human health due to their antimicrobial resistance (Reygaert, 2018).

2.4. Vaccines against Bacterial Diseases in Poultry

Bacterial vaccines present a promising approach to address antimicrobial resistance in the context of poultry farming. Unlike antibiotics, they pose a no risk of fostering AMR development, positioning them as a more sustainable substitute for antibiotics (Rabie *et al*., 2020). Mechanistically, bacterial vaccines induce an immune response in poultry against particular bacterial pathogens, thereby diminishing the necessity for antibiotic administration. Additionally, they provide long-lasting defense against bacterial infections, which lowers the need for antibiotic treatments (Redweik *et al*., 2020; Excler *et al*., 2021). Furthermore, debates persist regarding the effectiveness of bacterial vaccines, with research indicating variability in vaccine efficacy based on the targeted bacteria and the specific vaccine formulation. In order to reduce the need for antibiotics and control infections caused by bacteria in poultry, bacterial vaccinations have become an essential mechanism (Hoelzer *et al*., 2018; Islam and Rahman, 2023).

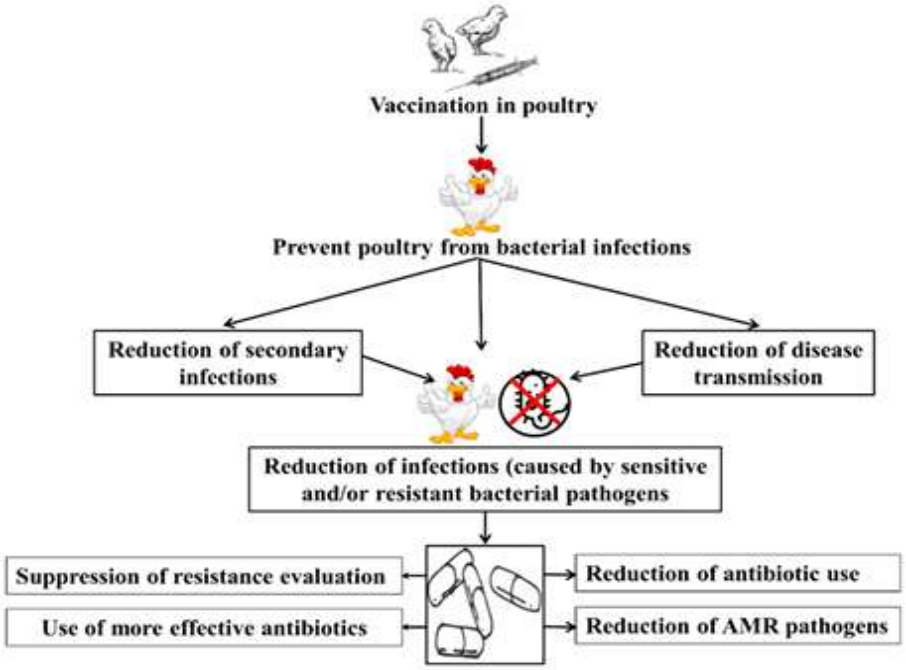

Figure 5: The role of bacterial vaccination in reducing antibiotic usage in poultry

Source: (Islam and Rahman, 2023)

2.4.1. Vaccines against Avian Salmonellosis

This vaccination is used to protect chickens from contracting *Salmonella*. It is made up of either live attenuated strains of bacteria that have been modified to lessen their virulence or inactivated bacteria. Avian species can be immunized against a particular host-specific serovar, such *Salmonella gallinarum*, to induce a targeted and potent immune response. Injections or drinking water can be used to administer the vaccination (Gast, 2007; Excler *et al.,* 2021). Most of the current vaccines are designed to specifically target serovar *Enteritidis* and *Typhimurium*. Usually, two doses of these vaccines are administered individually, four to six weeks apart, by subcutaneous injection after the birds are 10 to 14 weeks old (Islam and Rahman, 2023).

The aim of using a live attenuated vaccination is to decrease bacterial virulence without losing immunogenicity. Attenuation is frequently caused by mutations in genes encoding metabolic enzymes or by the loss of essential virulence components (Atterbury *et al*., 2010; Berghaus *et al*., 2011). The fact that the bacteria still release virulence factors which are crucial for inducing an immune system defense response is an advantage of the metabolic gene inactivation. The intestinal epithelial surface acts as a physical barrier that plays a major role in regulating the gut immune response to oral antigens (Papezova *et al*., 2008).

2.4.2. Vaccines against Avian Colibacillosis

Colibacillosis vaccination is an interesting technology that offers an alternate approach to disease management. The effectiveness of several vaccination forms, including live, subunit, and inactivated vaccines, has been investigated in relation to avoiding *E. coli* infection in chicken (Ghunaim *et al*., 2014). Live vaccinations offer greater protection against many serotypes and are frequently used in commercial poultry through mass application techniques like spraying. However, subunit vaccinations, which are given by injection and include certain proteins or *E. coli* virulence factors, have been shown to produce better heterologous immunity than inactivated vaccines (Islam and Rahman, 2023).

Inactivated vaccines, which contain several strains of *E. coli*, can be given intravenously and provide accurate homologous protection (Ghunaim *et al*., 2014).

The main protective effect of these vaccinations against an *E. coli* challenge is cell-mediated immunity, which is far more important for bird health than humoral immunity and antibodies (Filho *et al*., 2013; Yang *et al*., 2015). Since *Salmonella* and other intestinal commensals can be horizontally spread by these plasmids, reducing APEC in poultry is of utmost importance. Widely differing antigens have made vaccines with broad protection difficult to administer (Mohamed *et al.,* 2016). It is important to remember that APEC inhabits the gastrointestinal system as commensals and only move the lung epithelium once faeces aerosolize to cause Colibacillosis. Thus, it is possible to decrease the numbers of these bacteria in the gut and induce systemic immunity for extraintestinal resistance by administering live vaccinations orally (Sadeghi *et al*., 2015; Stromberg *et al*., 2017).

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

This study was conducted in randomly selected poultry farms of Central Ethiopia, specifically located in Oromia Regional State and Addis Ababa city administration, approximately in maximum radius of 100 km to Addis Ababa. Their topography is characterized by undulating mountains to flat land scapes, many lakes, rivers and hills. A total of twenty five poultry farms from six selected towns in Central Ethiopia including, Addis Ababa which is the capital city of the Federal Democratic Republic of Ethiopia, Located between 8.46° N Latitude and 38.35° E Longitude, covers approximately 519.482 km^2 area with an average elevation of 2300 meters above sea level (ASL), Adama located at 8.54°N 39.27°E at an elevation of 1712 meters ASL , 99 km Southeast of Addis Ababa, Bishoftu located at Latitude: 8.75°, Longitude: 38.98° at an elevation of 1,920 metres ASL, Dukem which is situated at 10 kilometers northwest of Bishoftu and 37 kilometers southeast of Addis Ababa. Its latitude and longitude are 8.48°N 38.54°E, and its elevation is of 1950 meters ASL, Mojo existing in a latitude and longitude of 8.39°N 39.5°E with an elevation between 1825 meters ASL and Gelan which is situated nearby to the peak Bushu Terara at a 8.82 ^{0}N and 38.85^0 E and at an elevation of 2,394 meters ASL are included in the study. The study areas are known for their large poultry productions and higher disease pressure for that farmers are prone to antibiotic dependence. There are several smallholder and commercial poultry farms in the areas (CSA, 2021).

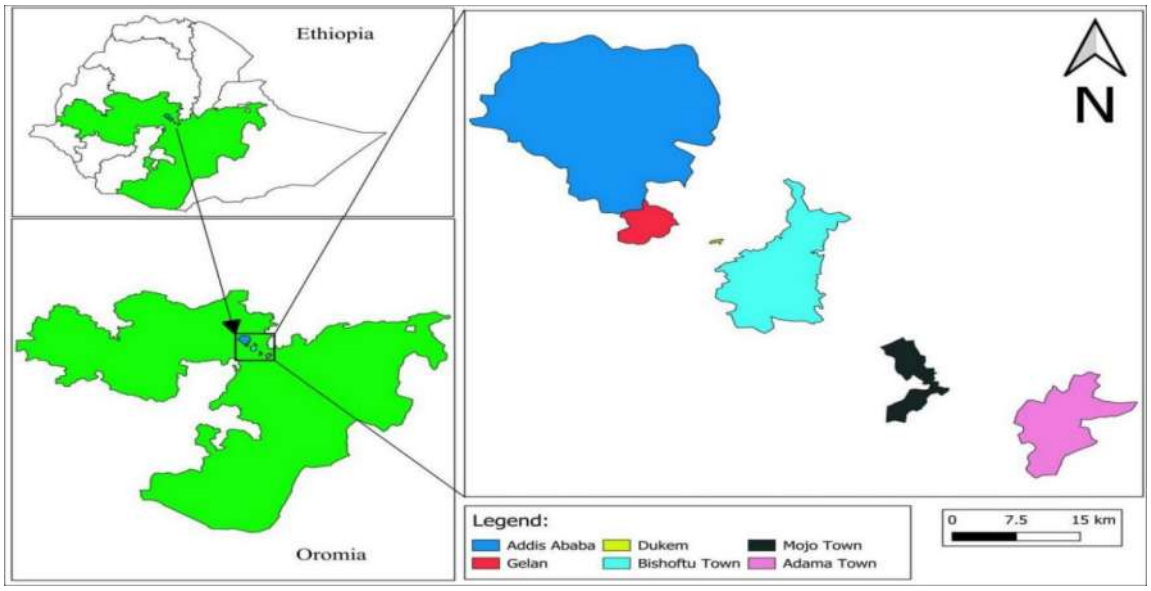

Figure 6: Map of the study areas, QGIS 3.36.0

3.2. Study Population

The study populations were chickens raised for varied purposes on poultry farms in the area. Different breed of chickens were included in the study including Bovans brown, Lohmanns, Sasso plus different strains of Broiler have been reared in the region and in the age limit of a month and above were included. According to how many chickens they had, the farms were divided into three categories: small scale (less than 1000 chickens), medium scale (1001–10,000 birds), and large scale (more than 10,001 chickens), according to (Vernooij *et al*., 2012). The farms which are owned by government, individuals as well as enterprises were assessed and on the way the farmers which are from the farms where samples taken were also used for survey. To protect the privacy of the data generated from farms, each farm was given a unique private code rather than having their identities publicized.

3.3. Study Design

A cross-sectional study was implemented from November 2023 to May 2024, at randomly selected poultry farms, with the aim of evaluating the prevalence and patterns of antibiotic resistance in *Salmonella* and *E. coli* species in poultry. The chickens raised on the selected farms underwent assessments for morbidity and mortality throughout the duration of the research, facilitating prompt post-mortem

examinations. In addition questionnaire data was collected from each farm from whom the bacteriological samples were taken to assess the antibiotic usages and vaccine managements on the poultry farms.

3.4. Sample Size Determination

The determination of the study sample size was based on the expected prevalence of the targeted bacteria in poultry and the desired level of precision, as outlined in the formula proposed by (Thrusfield 2018). The calculation of sample size from previous studies *E. coli* 17.3% (Shimelis, 2022) and *Salmonella* 24.3% (Belachew *et al*., 2021)

were calculated, and the larger value was taken as the appropriate sample size for both pathogens as the same type of samples were to be used. Accordingly, the sample size was established taking into account an expected prevalence of 24.3% for *Salmonella* (Belachew *et al*., 2021).

$$N = \frac{Z^2 * Pexp(1-Pexp)}{d^2}$$

Where:-

n = required sample size

Z = Z-score value at 95% confidence interval (1.96)

P_{exp} = Expected prevalence (24.3%)

d = desired absolute precision of 0.05

However, to increase the precision of the estimation, 10% of the calculated sample size was added, resulting in a total of 313 samples being examined in this study.

3.5. Sample Collection and Processing

3.5.1. Questionnaire Survey

Interviews with farmers and farm managers were used to gather objective-oriented data using a structured questionnaire. The questionnaire included details of contact information, biosecurity protocols, waste disposal procedures, flock handling practices, general farm history, disease management, conditions of housing, flock size, where to obtain drinking water, farm hygiene status, chicken breed, treatment and vaccination history, and others. Emphasis was given to vaccination practices and

antibiotic utilization bases that might have a role with disease pressure, vaccine failure and antimicrobial resistance development (Appendix 1).

3.5.2. Bacteriological sample collection

Cloacae, hand swabs, and tissue swabs were obtained from poultry farms chosen at random in accordance with the guidelines provided by the OIE (OIE, 2007). Representative chickens were selected from these randomly selected farms for sampling, and subsequently, their cloacae or tissues were swabbed to obtain samples for the isolation of the necessary bacteria. Moreover, hand swabs were taken from farm attendants or any farm member responsible for tending to the flock. Each sample was meticulously labelled with specific identification numbers, sampling dates, and the type of sample. A total of three hundred thirteen (313) cloacae, hand, and tissue swab samples were gathered from selected farms using sterile cotton-tipped swabs moistened with buffered peptone water. Every swab sample was placed in individual screw-capped sterile test tubes containing buffered peptone water and stored in an icebox with ice packs.

Suspected sick and deceased chickens underwent autopsies following established postmortem protocols (Morishita, 2019). In cases where visible lesions indicating potential diseases were observed, tissue swabs were obtained by aseptically incising vital organs using a scalpel blade and forceps. Prior to sampling, the surface of each organ was sterilized using isopropyl alcohol. Following the incision of each organ with a sterile scalpel blade, sterile cotton-tipped swabs were inserted and pooled swab samples of the organs were collected. These swabs were then placed in sterile test tubes containing buffered peptone water and transported to the CVMA Microbiology laboratory.

Isolation and Identification procedures of *Salmonella*

The methodologies employed for the isolation of *Salmonella* adhered to the techniques recommended by the International Organization for Standardization (ISO 6579, 2002). The isolation process comprised three main stages: pre-enrichment, enrichment, and plating on selective media, culminating in confirmation through biochemical tests. Consequently, all samples were pre-enriched individually in 10 ml of buffered peptone water (BPW) (Himedia GM614) and then incubated for 18–24

hours at 37 ± 1°C. Subsequently, Rappaport Vassiliadis *Salmonella* enrichment broth (Himedia MH1491) was utilized for the selective enrichment of all samples. A volume of 0.1 ml of the pre-enriched culture was aseptically transferred to test tubes containing 10 ml of Rappaport Vassiliadis *Salmonella* enrichment broth and incubated at 41.5 ± 0.5°C for 24 hours.

Finally, plating out and isolation were performed using Xylose Lysine Deoxycholate (XLD) agar (Blulux) plates. The surface of XLD agar the basis was suitably applied with a loop loaded with inoculums from RV broth. After that, the plates were turned over and incubated for 18 to 24 hours at 37^0C. Following incubation, typical *Salmonella* colonies were examined on the plates. On XLD agar, typical colonies were pink to red in color, with a black center or without, or they were colorless, very light, slightly shiny, and transparent (the color of the medium) with a dark tinted center surrounded by a light red area and yellow edge. Colonies that are positive for hydrogen sulfide (H_2S) are colorless or light pink with darker centers, while those that are positive for lactose are yellow or lack the distinctive blackening. In order to obtain additional validation through biochemical testing, presumed *Salmonella* colonies were selected from plating medium and subcultured on nutrient agar (Himedia M001) in a way that allowed isolated colonies to grow and incubate at 37^0C for 18–24 hours.

The following biochemical tests were performed on each suspected isolate of *Salmonella* to confirm it such as Triple Sugar Iron (TSI), Indole, Citrate Utilization, Methyl Red, and Vogues Proskauer (IMViC) tests. *Salmonella*-positive colonies were defined as those that produced red slant (alkaline), yellow butt (acid), blackening from hydrogen sulfide production and (gas production) on the butt, negative results for the Indole test, positive results for the Methyl red test, positive results for the citrate utilization (deep blue slant), and negative results for the VP test (ISO 6579, 2002; Quinn *et al*., 2004).

Isolation and Identification procedures of *E. coli*

The present study followed standard food microbiology procedures to identify *Escherichia coli*, as outlined in the WHO (2010) and ISO Standards catalog 07.100.30. Before being brought to the lab in a cold chain, the samples were first collected in sterile circumstances. After being swabbed, a sample was placed into BPW in a test tube, shipped with the cold chain unharmed, and incubated for 24 hours

at 37^0C. The culture suspension was subsequently transferred onto MacConkey (Himedia M008) agar and was cultured for 24 hours at 37^0C.

The pink-colored presumed *E. coli* colonies were subcultured onto Eosin Methylene Blue (EMB) (Accumix) agar the following day. Afterwards, colonies on EMB with a metallic green sheen were examined under a microscope with Gram's stain. After that, potential *E. coli* colonies were moved to nutrient agar for additional biochemical testing to confirm identification. Triple sugar iron agar and other biochemical tests (IMViC) tests were used for further characterization as per (Quinn *et al.*, 2004).

3.6. Antimicrobial Susceptibility Test

The testing of susceptibility in all isolates was carried out using the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar (Accumix), in adherence to the guidelines stipulated by the Clinical and Laboratory Standards Institute (CLSI, 2022). Evaluation of antimicrobial susceptibility in all isolates encompassed the application of 10 frequently utilized antimicrobials in both veterinary and human medical contexts within Ethiopia. The commencement of the procedure involved the retrieval of well-separated colonies, which were biochemically confirmed, from each isolate, and then grown on nutrient agar. These colonies were subsequently transferred into sterilized tubes containing 5 ml of tryptone soya (Himedia M001) broth. The broth culture was then incubated at a temperature of 37°C for a duration of 4 hours, achieving a turbidity standard of 0.5 McFarland.

A sterile cotton swab was immersed in the suspension, and the resultant bacteria were evenly spread across the entire surface of the Muller-Hinton agar plate. Following this step, the plates were left to air-dry at ambient conditions for 30 minutes. Subsequently, antibiotic discs with predetermined antimicrobial concentrations were meticulously placed onto the plate's surface in a specific layout using a sterile pair of forceps on Mueller-Hinton agar plates. The plates were then inverted and subjected to incubation at a temperature of 37°C for a period of 24 hours.

The antibiotics utilized in the discs consisted of ampicillin (AM, 10 µg), oxytetracycline (OT, 30 µg), chloramphenicol (C, 30 µg), tetracycline (TE, 30 µg), streptomycin (S, 10 µg), gentamycin (CN, 10 µg), nalidixic acid (NA, 30 g),

amoxicillin (AMX, 20 µg), cephalothin (KF, 30 µg), and sulphamethoxazole/trimethoprim (SXT, 25 µg). Following the incubation period, the diameter of the zone of inhibition for each antibiotic was measured in millimeters and compared with the corresponding internationally accepted standard zone diameter to interpret the test culture as resistant, intermediate, or sensitive, as per the guidelines outlined by CLSI (2022).

3.7. Data Management and Analysis

Epidemiological data derived from the surveys and laboratory examinations was initially encoded and entered into a database (Microsoft Excel Spreadsheet) for analysis using STATA version 14. Descriptive statistics were employed to evaluate the prevalence of each bacterium, and the Antimicrobial susceptibility patterns were also analysed and their respective statistical values were presented in frequency and percentages. By using Chi square analysis, the association between the prevalence rates across variables was examined. Logistic regression analysis was conducted to determine the Association between the prevalence of the bacteria and the predictor variables. Statistical significance of the results was reported by a P-value ≤ 0.05.

3.8. Ethical Clearance

The present investigation poses no significant risk to the study participants; however, it was carried out while adhering to all ethical guidelines established by Animal Ethics Review Committee of AAU, CVMA. Consequently, the findings derived from this research project maintained confidentiality regarding the farm that was examined. The team of researchers took care to strictly follow the ethical standards outlined by the university's review process with utmost respect. The Animal Ethics Review Committee of AAU, CVMA granted the ethical approval (Certificate Ref. No:VM/ERC/28/02/14/2023)- (Appendix 5).

4. RESULTS

4.1. Bacteriological isolation and Biochemical test results

Of 313 samples processed for this study (tissue swabs (n = 41), hand swabs (n = 28), and cloacal swabs (n = 244)), 30.35% and 3.83% samples found positive for *E. coli* and *Salmonella* respectively. Through bacteriological and biochemical analysis, the isolation rate of *E. coli* was determined to be relatively highest (41.26%) in Adama, followed by Bishoftu (35.80%), and was the lowest in Mojo (13.79%) with a statistical significance (P =0.040) and Pearson Chi^2 (χ^2) = 11.66. In addition, the findings also revealed that the prevalence of *Salmonella* is highest in Bishoftu (7.40%) with no detection from Addis Ababa and Mojo (Table 1).

Table 1: Prevalence of *E. coli* and *Salmonella* through bacteriological methods

Study area	**No of collected samples**	**No of positive samples for *E. coli* and %**	**No of positive samples for *Salmonella* and %**
Addis Ababa	39	8 (20.51)	0 (0.00)
Adama	63	26 (41.26)	3 (4.76)
Bishoftu	81	29 (35.80)	6 (7.40)
Dukem	49	16 (32.65)	2 (4.08)
Gelan	52	12 (23.07)	1 (1.92)
Mojo	29	4 (13.79)	0 (0.00)
Total	**313**	**95**	**12**

With regard to the type of samples, *E. coli* was found to be most prevalent in tissue swabs (34.14%), followed by cloacal swabs (31.96%) and lowest in hand swabs (10.71%) (P= 0.05). While no *Salmonella* was detected in samples obtained from hand swabs, it was exceedingly detected in tissue (7.14%) and relatively lower in cloacal swabs (3.73%). Based on breed characteristics, no *Salmonella* was isolated from Sasso whereas; Lohmanns were the highest (5.40%) harbors of *Salmonella* followed by Bovans (4.76%). On the other hand, Sasso (17.64%) and Bovans

(40.00%) were found to be the lowest and highest carriers of *E. coli*, respectively (P =0.03). One to two years old chickens had the highest rate of *E. coli* (45.76%), while the least amount (26.17%) was isolated from chickens under the age of one year (P =0.01). Conversely, it was determined that the age category of those less than one year old had the highest load of *Salmonella* (4.71%), whereas the age group of one to two years had the lowest (1.69%).

Among the isolates that are subjected to biochemical confirmations, small-sized farms had the highest prevalence rate of *E. coli* (38.59%), while large-scale farms had the lowest prevalence rate (23.8%). On the other hand, medium-sized farms had relatively the highest prevalence of *Salmonella* (5.29%) and lowest (1.75%) in small-scale farms. The prevalence of both *Salmonella* and *E. coli* were not statistically significant (P > 0.05) among different flock sizes (Table 2).

Table 2: Prevalence of *E. coli* and *Salmonella* cross various associated factors

Variables	Category	No of observations	% of *E. coli* positive samples	P-value	% of *Salmonella* positive samples	P-value
	< 1 year	191	26.17		4.71	
Age	1-2 years	59	45.76	0.01	1.69	0.98
	> 2 years	63	28.57		3.17	
Breed	Bovans brown	105	40.00		4.76	
	Broiler	46	28.26	0.03	2.17	0.38
	Lohmann brown	111	27.92		5.40	
	Sasso	51	17.64		0.00	
Flock size	Small scale	57	38.59		1.75	
	Medium scale	151	31.78	0.12	5.29	0.75
	Large scale	105	23.80		2.85	
Sample type	Cloacae swab	244	31.96		3.73	
	Hand swab	28	10.71	0.05	0.00	0.28
	Tissue swab	41	34.14		7.14	

4.2. Result of Antimicrobial Susceptibility Test

Antimicrobial susceptibility test (AST) was carried out on isolates of *Salmonella* and *E. coli* using ten (10) distinct antimicrobial disks (Table 3). Accordingly, AST was undertaken for 40 of the *E. coli* isolates, and the outcomes were classified into susceptible, intermediate, and resistant categories in relation to the tested antimicrobial agents at varying concentrations. Though the *E. coli* isolates exhibited a high degree of susceptibility to chlorampenicol (95.75%) followed by gentamicin (93.68%) and sulfametoxazole-trimetoprim (92.63%), however they have shown total (100%) resistance to cephalothin and 89.47% to Ampicillin (Table 3)

Table 3: Antibiotic susceptibility profile of *E. coli* isolates

Antimicrobial agents	Pattern of susceptibility (%)		
	Susceptible	**Intermediate**	**Resistant**
Ampicillin	4.21	6.32	89.47
Amoxacillin	88.42	6.32	5.26
Cephalothin	0.00	0.00	100.00
Chloramphenicol	95.79	3.16	1.05
Gentamicin	93.68	4.21	2.11
Oxytetracycline	9.47	6.32	84.21
Naldixic acid	75.79	16.84	7.37
Tetracycline	18.95	7.37	73.68
Streptomycin	77.89	14.74	7.37
Sulfametoxazole-trimetoprime	92.63	5.26	2.11

On the other hand, antimicrobial sensitivity test was performed on all (12) *Salmonella* isolates using ten commercially available antibiotic disks. The results showed varying degrees of sensitivity and resistance. Many isolates have different degrees of susceptibility and resistance, ranging from 0 to 100%. *Salmonella* isolates showed a high susceptibility to sulfametoxazole-trimethoprim (100%), followed by chloramphenicol and gentamicin (83.33%). Streptomycin and oxytetracycline were found to have a moderate rate of (33.33%), indicating an intermediate range of

susceptibility. Cephalothin exhibited the highest resistance, with resistance rates of 100% as shown below (Table 4).

Table 4: Antibiotic susceptibility profile of *Salmonella* isolates

Antimicrobial agents	**Pattern of susceptibility (%)**		
	Susceptible	**Intermediate**	**Resistant**
Ampicillin	0.00	25.00	75.00
Amoxacillin	66.67	25.00	8.33
Cephalothin	0.00	0.00	100.00
Chloramphenicol	83.33	16.67	0.00
Gentamicin	83.33	16.67	0.00
Oxytetracycline	41.67	33.33	25.00
Naldixic acid	75.00	16.67	8.33
Tetracycline	50.00	16.67	33.33
Streptomycin	58.33	33.33	8.33
Sulfametoxazole-trimetoprime	100.00	0.00	0.00

Escherichia coli demonstrated a higher level of resistance to a majority of medications in contrast to *Salmonella*, which displayed an elevated level of intermediate resistance profile. However, *E. coli* strains generally exhibited a higher degree of susceptibility towards the majority of antibacterial agents, with the exception of oxytetracycline, sulfamethoxazole-trimetoprime, and tetracycline as presented below (figure 7).

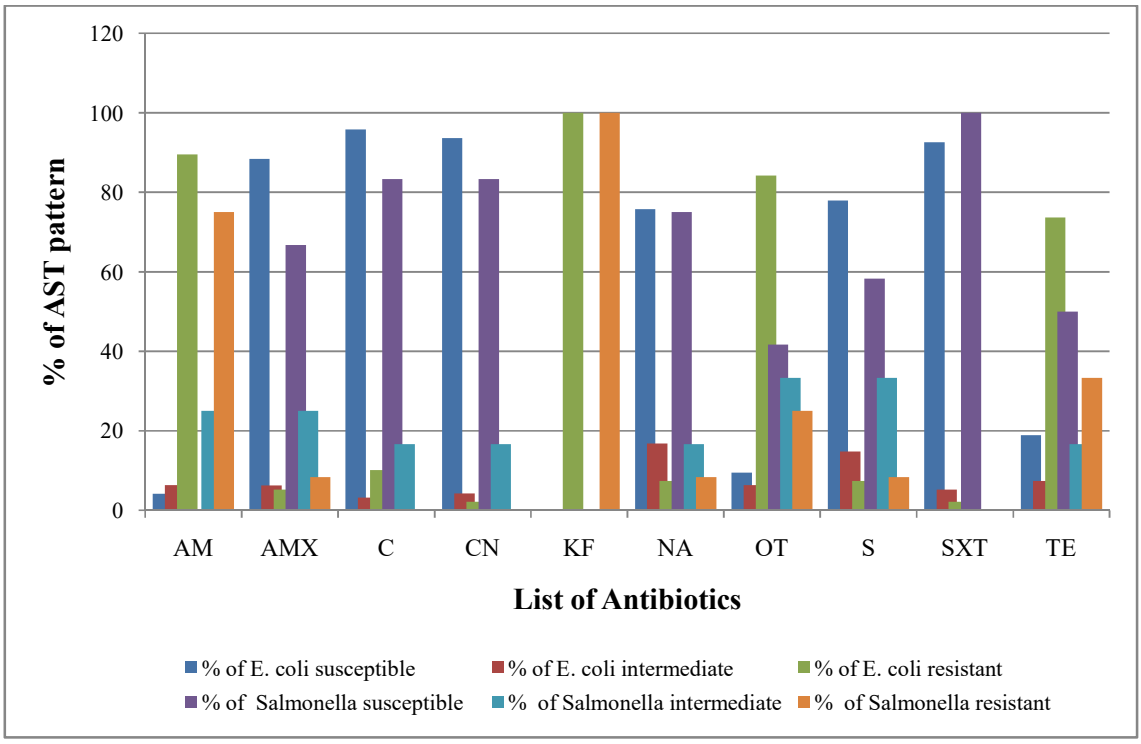

Figure 7: Graph on AST pattern of both *E. coli* and *Salmonella* for each antibiotic

Escherichia coli isolated from all cloacae, hand and tissue swab samples showed an average resistance (100%) to cephalothin followed by 95.66% to ampicillin, 88.13% to oxytetracyclin and 82.01% to tetracyclin, but intermediate to susceptible for naldixic acid (4.81%), streptomycin (4.81%) and amoxacillin (3.95%), however found highly susceptible to chloramphenicol, gentamycin and sulfamethoxazole-trimetoprim. Significant resistance variation was observed in *Salmonella* isolates across sample types. Accordingly, isolates of cloacal swabs showed highest resistance rates (100%) resistance to cephalothin, whereas tissue swabs isolates showed reduced resistance, with notable exceptions for cephalothin, ampicillin, gentamicin, and oxytetracyclines.

The overall resistance profile to antimicrobials was categorized as susceptible, intermediate or resistant based on the results of antimicrobial susceptibility testing for each antibiotic employed. This suggests that a significant proportion of the bacterial isolates exhibited susceptibility, alongside a noteworthy level of resistance. Specifically, 56% of the *E. coli* isolates displayed susceptibility, while 7% and 37% were determined as intermediate and resistant, respectively. Similarly, 26% of

Salmonella strains showed resistance to the tested antibiotics, while 18% and 56% presented intermediate and susceptible patterns, respectively as graphically presented below.

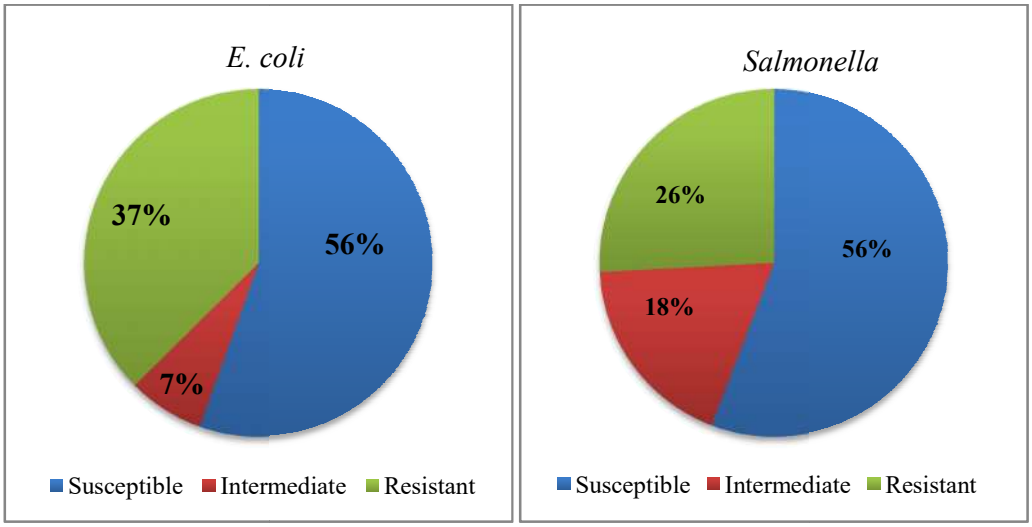

Figure 8: An overall AMR status of *E. coli* and *Salmonella*

4.3. Multi Drug Resistance

The present investigation demonstrated a 100% multidrug-resistance (MDR) of *E. coli* strains, with 91.58% of which exhibiting resistance to a minimum of three and a maximum of six antimicrobial agents. Predominantly, the antibiotic combinations leading to multidrug resistance in *E. coli* were cephalothin, ampicillin, oxytetracycline, and tetracycline. Among the *E. coli* strains, 46.31% displayed resistance to four antibiotics, while merely two strains demonstrated resistance to six antibiotics under consideration. Notably, none of the tested isolates exhibited susceptibility to the complete panel of antibiotics, as delineated below (Table 5).

Table 5: Results showing MDR profiles of *E. coli* isolates

Number of drugs	Name of drugs and their resistance pattern	Percentage of resistant isolates
Two	KF, OT AM, KF KF, TE	8.42
Three	AM, KF, TE AM, KF, OT KF, OT, TE	31.57
Four	AM, KF, TE, S AM, KF, OT, TE KF, OT, NA, TE AM, KF, OT, NA	46.31
Five	AM, C, KF, OT, TE AM, KF, OT, TE, S AM, KF, OT, NA, TE AM, AMX, KF, OT, TE	9.47
Six	AM, CN, KF, OT, TE, S AM, KF, OT, TE, S, STX	4.21

AM = ampicillin, AMX = amoxacillin, KF = cephalothin, C = chloramphenicol, CN = gentamicin, OT = oxy tetracycline, NA = naldixic acid, TE = tetracycline, S = streptomycin, SXT = sulphamethazole-trimethoprim

Regarding to *Salmonella*, a higher proportion of the isolates demonstrated resistance to either two or three different antibiotic combinations. The most dominant MDR combinations were cephalothin alongside ampicillin, and cephalothin, ampicillin and tetracycline each appeared three times among the twelve isolates. Only one isolate (8.33%) was discovered to be resistant to five antibiotics, the greatest combination in the aspect of *Salmonella*.

Table 6: Results showing MDR profiles of *Salmonella* isolates

Number of drugs	Name of drugs and their resistance pattern	Percentage of resistant isolates
Two	AM, KF KF, OT	33.33
Three	AM, KF, OT AM, KF, TE	33.33
Four	AM, KF, S, TE	8.33
Five	AM, AMX, KF, NA, TE	8.33

4.4. Results of survey on antibiotic and vaccine management

4.4.1. Overall Farm Characteristics

The questionnaire survey was done on 25 purposively selected poultry farms in the study areas, with farm attendants, owners, and managers to come up with accurate information about the general conditions on the farm. In this survey, owners made up the majority (62.07%) of the participants, followed by farm attendants (27.59%). In terms of poultry farming experience, 51.72% of respondents had one to five years of experience, while 44.83% had more than five years of experience in the sector. With regard to farm location, 9 farms were from Bishoftu, 4 from Adama, and equal number (3) from each Addis Ababa, Dukem, Gelan and Mojo were included. The majority of chicken breeds managed in the farms were Lohmann brown (37.93%) and Bovans brown (27.59%). 89.6% of farmers utilize commercial feed whereas only 10.34% use own-made feed for their flocks and the majority use municipal water (62.07%) as a primary source of drinking water (Table 6). As per the respondents' opinion, the main problem in their farming activity is feed price and availability, followed by disease interventions and shortage of farming lands.

Table 7: Overall Poultry farm characteristics in the study

Variables	Variables	Addis Ababa	Adama	Bishoftu	Dukem	Gelan	Mojo
Breed	Bovans brown	0	25	27.27	50	66.66	0
	Broilers strains	25	0	9.09	50	0	33.33
	Lohmann brown	25	75	54.54	0	0	33.33
	Sasso	50	0	9.09	0	33.33	33.33
Flock size	Small scale	0	25	9.09	50	0	0
	Medium scale	75	50	72.72	25	100	33.33
	Large scale	25	25	18.18	25	0	66.66
Role of respondent	Attendant	75	100	27.27	75	66.66	100
	Manager	25	0	54.54	0	33.33	0
	Owner	0	0	18.18	25	0	0
Source of feed	Commercial	100	75	81.81	100	100	100
	Own-produced	0	25	18.18	0	0	0
Source of water	Deep well water	25	0	36.36	50	66.66	66.66
	Municipal water	75	100	63.63	50	33.33	33.33
Main challenge in your farm	Disease	25	50	63.63	0	33.33	66.66
	Feed price	50	50	36.36	75	33.33	0
	Farm land	25	0	0	25	33.33	33.33

4.4.2. Bacterial Diseases and Antibiotic Usages

The survey data provided important insights into the practices and experiences of bacterial infections in poultry farms. The majority of respondents (68.97%) reported that they do not have their own veterinarian for consultation and antibiotics are the most commonly used treatment for bacterial diseases (75.86%), with herbal treatments and probiotics being less common. Remarkably, the primary reason for antibiotic use varies, according to respondents stating it is mainly for treatment (37.93%), prophylaxis (13.79%), growth promotion (24.14%), or a combination of the three (24.14%). The antibiotics that are most frequently used were amoxicillin, ampicillin, oxytetracycline, and tetracycline, with variation across location. Furthermore, the vast majority (82.76%) of survey respondents obtained antibiotics from veterinary pharmacies, with a smaller proportion from the open market and Veterinary clinics. On top of that, non-professionals were also involved in the non-prudent prescription of antibiotics and the means of antibiotic administration varies, with a strong preference (68.97%) for mixing medications with water, whereas mixing with feed was less preferred (Table 8).

Table 8: Bacterial diseases and antibiotic usages

Variables	Responses	Percentage
Do you have your own veterinarian?	Yes	31.03
	No	68.97
How do you treat bacterial diseases in your farm?	By antibiotics	75.86
	By herbal treatments	13.79
	By probiotics	10.34
Mainly for what purpose do you use antibiotics in your farm?	For treatment	37.93
	For prevention	13.79
	For growth promotion	24.14
	For all above mentioned	24.14
From where do you obtain antibiotics?	Vet.pharma.	82.76
	Other sources	13.79
	Vet. Clinics	3.45
Who is responsible to administer antibiotics to the chicken in your farm?	Self	55.17
	Veterinarian	44.83
How antibiotics administered in your farm?	Mixed with feed	31.03
	Mixed with water	68.97

4.4.3. Vaccine Sources, Utilization and Management

With regard to regular vaccination schedules for infections caused by bacteria, a minority (34.48%) of respondents follow the schedules, while the majority (65.52%) did not. The responsibility for on time vaccine administration varied among the respondents; 41.38% of them assigned the task to any personnel on the farm, while 31.03% and 27.59% rely on veterinarians and owners, respectively. Regarding vaccine source, majority (48.28%) was provided by the National Veterinary Institute (NVI) followed by private sellers (27.59%) and agricultural offices (24.14%). The

satisfaction rate with the vaccines used was mixed; some respondents (17.24%) reported dissatisfaction whereas large amount (48.28%) of the farmers expressed a partial level of satisfaction and neutral (24.14%), while others indicated that they were very satisfied (10.34%).

Table 9: Vaccine sources and management

Variables	Responses	Percentage
Do you have regular vaccination schedule of bacterial diseases?	Yes	34.48
	No	65.52
Who is responsible for vaccine administration in your farm?	Veterinarian	31.03
	Owner	27.59
	Any employee	41.38
From where do you access vaccines?	Agricultural offices	24.14
	Private suppliers	27.59
	NVI	48.28
How do you store vaccines in your farm case?	In cold places	37.93
	As recommended	37.93
	Not stored yet	24.14
Are you satisfied in the vaccines you used?	Very satisfied	10.34
	Partially satisfied	48.28
	Neutral	24.14
	Unsatisfied	17.24

5. DISCUSSION

5.1. Bacteriological isolation of *Salmonella* and *E. coli*

Salmonella and *Escherichia coli* are recognized as significant pathogens having the potential to induce severe health complications in poultry, resulting in morbidity, mortality, reduced productivity, and increased expenses associated with prevention and treatment emergence of antimicrobial resistance (Witkowska *et al.,* 2018). In the current study, which encompassed various study areas of Central Ethiopia, 30.35% of the samples examined from poultry exhibited positivity for *E. coli*. This is in agreement with the findings of previous studies by (Sarba *et al*., 2019) and (Gebeyehu *et al*., 2018) in Ethiopia and (Hariri *et al*., 2022) in Saudi Arabia who reported prevalence rates of 32.5%, 35.5%, and 29.2% respectively. However, this percentage is lower than the 92.9% report by (Mudenda *et al.* 2023) in Zambia. The rate of isolation in this study surpasses that of Nigeria at 10.6% as documented by (Matthew *et al.*, 2017) and Iraq at 16% according to (Al-Musawi *et al.*, 2016). The difference in prevalence rates of *E. coli* among these studies could be due to management factors, laboratory procedures and environmental factors.

The outcomes of the present study indicated that the presence of the *Salmonella* species was detected in 3.83% of the 313 samples obtained from various locations of central Ethiopia. This finding aligns relatively well with previous findings in Ethiopia, which indicated 2.9% (Dagnew *et al.,* 2020), 4.7% (Eguale, 2018), 2.9% (Bayu *et al*., 2013) and 2.5% in Zambia (Mpundu *et al*., 2019). Nevertheless, it falls below the prevalence rates which reported 25.4% (Karim *et al*., 2017) in Bangladesh, 16.7% (Abdi *et al*., 2017) and 14% (Mezene *et al*., 2020) in Ethiopia. Discrepancies in the prevalence and incidence frequencies across these studies may be linked to regional disparities, as well as variations in management practices and sanitary conditions. The transmission of *E. coli* and *Salmonella* infections predominantly arises from contaminated feed and water sources, in addition to factors such as the flock's age, vaccination records, and variances in diagnostic methodologies employed (Kuner *et al*., 2015).

The prevalence rate of *Salmonella* species in Lohmanns brown breeds was observed to be 5.4%, which was noted as the highest percentage among breed, followed by Bovans brown at 4.76%. Conversely, no *Salmonella* species were detected in Sasso breeds, a finding deemed not statistically significant. A similar scenario was documented in Ethiopia, where a study reported almost similar infection rate in Bovans brown chickens with the current study (Abunna *et al.*, 2017). However the recent report indicated higher prevalence in Bovans brown (32.83%) which is higher than our finding (Assefa and Teferi, 2022). The variation in infection rates among different breeds in this research may be attributed to genetic based disparities in disease resistance, length of time spent on the farm, and the intended purpose of production. Additionally, disparities in management practices, encompassing factors such as feed quality, water sanitation, flock density, and hygiene protocols, have the potential to impact the prevalence of *Salmonella*.

The present study unveiled that the prevalence of *E. coli* is most prominent in Bovans brown chickens, followed by broilers and Lohmanns browns, while the lowest rate of isolation was noted in Sasso breeds, with rates of 40%, 28.2%, 27.92%, and 17.64%, respectively, showing statistically significant difference ($P < 0.05$). Factors such as feeding habits, overcrowding, inadequate sanitation, and contaminated sources of water in might be responsible to elevate the probability of *E. coli* in farms rearing Bovans browns. Moreover, disparities in farming techniques, including biosecurity practices, vaccination regimens, and hygiene protocols, may have played a role in the heightened incidence of *E. coli* in Bovans brown breeds (Moraes *et al*., 2024).

This study achieved a relatively high rate of *E. coli* recovery from tissue swabs (34.14%) and cloacal swabs (31.96%); however, samples obtained from human hand swabs exhibited a lower *E. coli* load. This is possibly attributed to the implementation of proper hygienic practices and of personal protective equipment. The sample wise isolation of *E. coli* in chickens in this study closely resembled the findings (Robert *et al.*, 2006) in Thailand, who reported a 39% from cloacal and carcass swabs, but surpassed the 18% rate documented (Gokben and Adile, 2003) in Turkey. Comparatively, the *E. coli* recovery rate from cloacal swabs in this study was lower than that of a previous study in Qatar (52.3%) (Eltai *et al.,* 2018) and Malaysia (51.8%) (Ibrahim *et al*., 2021).

The overall incidence of *Salmonella* isolation from poultry tissue swabs in the present study was 7.31%, which holds great significance in terms of economic and public health implications within the industry. The status of *Salmonella* contamination in poultry tissue samples was found higher than the 2.7% observed by (Mengistu *et al*., 2011). Notably, cloacal swabs exhibited a 3.68% contamination rate, while no instances of positive *Salmonella* findings were detected in hand swab samples. The prevalence of *Salmonella* in cloacal swabs in this study was aligned with the finding of a study conducted in Kenya (3.6%) (Nyabundi *et al.,* 2017), but exceeded the rates reported by (Taddese *et al.*, 2019) in Southwestern Ethiopia (2.4%) and (Dagnew *et al.*, 2020) in Mojo town (0.3%). Conversely, the incidence was lower than the 23.2% prevalence documented in cloacal swabs from Asossa and Bambasi towns (Asmamaw *et al*., 2018).

The findings of this study revealed a higher proportion of *Salmonella*-positive isolates obtained from older chickens (4.71%) compared to younger chicks, implying an increased likelihood of acquiring *Salmonella* from the environment as age advances. This contradicts the finding of (Abdi *et al*., 2017), who reported a higher prevalence in younger chickens in southern Ethiopia, at 47.6%. Moreover, the study indicated that the rates of *E. coli* isolation within the age limits of 1 to 2 years and over 2 years were 28.57% and 45.76%, respectively, surpassing the rates in younger age groups (below one year) which yielded 26.17%. Consequently, the elevated occurrence of *E. coli* in adult chickens compared to young may be associated to the prolonged exposure period to infections experienced by adult birds. The isolation rate of *Salmonella* species from bacteriological cultures in this research was notably higher (23.25%) in farms with medium-sized flocks, aligning with previous findings in other regions of Ethiopia (Eguale, 2018; Dagnew *et al.,* 2020).

5.2. Antimicrobial Resistance Profiles of the Isolates

5.2.1. Antimicrobial Resistance Profiles of Salmonella Isolates

The current research delves into the investigation of susceptibility to both single and multiple drugs against two crucial bacterial strains. Both *Salmonella* and *E. coli* stand out as the primary causative agents of bacterial infections in poultry. In current study notable proportion (56%) of both *E. coli* and *Salmonella* isolates exhibited susceptibility to the antibiotics assessed, signifying that more than fifty percent of these bacterial variants remain responsive to conventional antimicrobial treatments. Nevertheless, 7% of *E. coli* demonstrated intermediate levels of resistance, with a worrisome 37% displaying full resistance. This significant degree of resistance observed in *E. coli* highlights the treatment challenges presented by these bacteria and emphasizes the need for ongoing surveillance and innovative therapeutic approaches. Within *Salmonella* isolates, 26% showed an average resistance to the antibiotics used which is particularly concerning due to involvement of *Salmonella* in foodborne diseases. Moreover, 18% of the isolates exhibited intermediate resistance, indicating the possibility of these variants developing complete resistance, thereby further complicating treatment regimens.

The findings of the antimicrobial susceptibility test indicated that all bacteriologically confirmed *Salmonella* isolates exhibited complete resistance to cephalothin. Apart from displaying the (100%) resistance to cephalothin, the *Salmonella* isolates also demonstrated varying and higher degrees of resistance to ampicillin (75%) among the antimicrobials tested. This outcome aligns with a study conducted in Debrezeyit (Bishoftu) and Mojo, which documented a 70% resistance rate of *Salmonella* isolates to ampicillin (Ali *et al*., 2020), surpassing the current resistance levels reported in Kafa, Southern Ethiopia, and Jimma by (Abda *et al*., 2021; Abdi *et al.,* 2017, and Taddese *et al.,* 2019) respectively. Conversely, another report from Ethiopia indicated a sensitivity rate of 69.5% to ampicillin (Mohammed and Dubie, 2022).

The most effective drug against *Salmonella* according to this study was found to be sulfamethoxazole-trimethoprim, with a susceptibility rate of (100%), consistent with the findings of (Moraes *et al*., 2024) reporting 97.8% susceptibility. However, this contrasts with reports from Ethiopia indicating resistance rates of 61.62% and 56% to sulfamethoxazole-trimethoprim (Belachew *et al.,* 2021; Ali *et al.*, 2020) respectively.

Furthermore, the isolates exhibited higher susceptibility to chloramphenicol and gentamicin, both with susceptibility rates of 83.33%, which is in line with the reported 83% susceptibility to chloramphenicol (Hamed *et al*., 2021) and 80% susceptibility to gentamicin by (Waktole *et al*., 2024). Studies in Ethiopia and Kenya have also shown higher susceptibility rates for both gentamicin and chloramphenicol compared to the present findings (Mohammed and Dubie, 2022; Taddese *et al*., 2019; Langata *et al*., 2019). Intermediate resistance to each of the tested drugs was observed in seven of the isolates tested; with the findings of the present investigation revealing intermediate resistance to widely employed antibacterial agents like oxytetracycline and streptomycin. This implies that *Salmonella* exhibits resistance to the usual attainable levels of antimicrobial agents, manifesting an intermediate resistance profile even with a standard dosing schedule as documented by (Abebe *et al*., 2013).

In current study, except for two isolates that displayed resistance to a single drug, the majority (83.33%), manifested resistance to multiple drugs and recognized as multi-drug resistant. This prevalence of MDR *Salmonella* aligns with previous research conducted in Ethiopia, with reported rates of 86.0% and 83.3% by Ali *et al.* (2020) and Zelalem *et al.* (2011) respectively. A significant portion of the isolates, (33.33%), exhibited resistance to two to three antimicrobials. Among the 12 isolates assessed for antimicrobial susceptibility, four, four, one, and one demonstrated resistance to 2, 3, 4, and 5 drugs, respectively. The combination of (AM, KF) and (AM, KF, TE) displayed the highest frequency of multi-drug resistances, being observed three times.

5.2.2. Antimicrobial Resistance Profiles of E. coli Isolates

The antimicrobial susceptibility test was carried out on a total of 40 *E. coli* isolates, with all isolates demonstrating resistance to cephalothin at a rate of 100%. Moreover, there were notable levels of resistance observed for ampicillin (89.47%), oxytetracycline (84.21%), and tetracycline (73.68%). This is in contrast to the reported resistance rate of 11% for cephalothin in Malaysia (Ibrahim *et al.*, 2021). Nevertheless, comparable results were identified in research conducted in Ethiopia (Shecho *et al*., 2017; Bushen *et al*., 2021) as well as in Zambia and Romania, where analogous resistance patterns for ampicillin, oxytetracycline, and tetracycline were documented (Mudenda *et al*., 2023; Sarker *et al.,* 2019). The tetracycline resistance pattern in this study was lower than the reported 100% (Shimelis, 2022) and 98.95%

(Rahman *et al*., 2020) rates but higher than the findings of (Sarba *et al*., 2019). The higher ampicillin resistance observed in this study aligned closely with the 89% reported (Yahiaoui *et al.*, 2015) and was higher than the rates reported (Shecho *et al*., 2017) at 34.6% and (Bedasa *et al*., 2018) at 76.9%; yet lower than the 100% reported by (Shimelis, 2022).

The antibiotic susceptibility test of *E. coli* revealed that the isolates displayed highest susceptibility to chloramphenicol (95.79%) and were notably susceptible to gentamycin (93.68%), sulfamethoxazole-trimethoprim (92.63%), and amoxicillin (88.42%). Furthermore, the isolates in this study exhibited moderate to higher susceptibility to streptomycin and nalidixic acid. These findings were in agreement with reports (Mudenda *et al*., 2023) and (Sarba *et al.*, 2019), which highlighted the high susceptibility of *E. coli* isolates to gentamycin, chloramphenicol, sulfamethoxazole-trimethoprim, and streptomycin, but differed from the report of (Ibrahim *et al.*, 2021) that indicated higher resistance to chloramphenicol, sulfamethoxazole-trimethoprim, streptomycin, and nalidixic acid. A higher intermediate resistance pattern was observed in nalidixic acid at 16.84%, followed by streptomycin at 14.74%.

This study revealed that all (100%) *E. coli* isolates were resistant to multiple drugs, with 91.5% showing resistance to three to six antibiotics. Among these combinations, cephalothin, ampicillin, oxytetracycline, and tetracycline were the most prevalent in driving multidrug resistance for *E. coli*. The MDR results of this study were consistent with findings in Malaysia, Bangladesh, and Ethiopia (Ibrahim *et al*., 2021; Al Azad *et al*., 2019; Abebe *et al*., 2014). The emergence of multidrug-resistant pathogens in poultry diseases can lead to treatment failures, prompting farmers to explore alternative antimicrobial options. This can exacerbate the selection pressure of antimicrobial resistance, making the treatment of infections increasingly challenging or even impossible (Uddin *et al*., 2021).

5.3. Survey on antibiotic usage and vaccine management

The pattern of antibiotic use found in this survey indicated that antimicrobial drugs were mostly used as a line of treatment by poultry farmers in Central Ethiopia. Antibiotics were utilized in most farms for different purposes; growth promotion (24.14%), therapeutic purposes (37.93%), and prophylactics (13.79%) in varying amounts. Some farms employed antibiotics for no particular reason at all. The results of this study support a previous analysis that found 36.2% of Nigerian farmers use antibiotics for therapeutic purpose regularly (Oluwasile *et al.,* 2014). This figure is considerably lower than the 100% of usage in Central Ethiopia (Mezene *et al*., 2020) and the 93.2% in Zambia (Chilawa *et al*., 2022). This is might be due to recent improvements and better accessibility of vaccines that protected their flocks from diseases. Furthermore, it was found that about 24.14% of farmers employed antibiotics to promote the growth of their flocks. This percentage is less than the 45% found in Pakistan (Habiba *et al.*, 2023) but not far from 32.2% (Mezene *et al*., 2020) in Ethiopia. Prophylactic antibiotic use was seen at a lower rate, which is less compared to those of other studies done in poultry farms of Ethiopia (56%), and Burkina Faso (50%). The reliance of poultry producers on antibiotics for therapeutic or prophylactic purposes could also be attributed to inadequate to environmental cleanliness, unhygienic practices, inadequate biosecurity, and other management flaws that heighten the risk of bacterial illnesses in Central Ethiopia.

In this survey, antibiotics were procured from veterinary pharmacies (82.76%) and the open market (13.79%) correspondingly. The study exhibited alignment compared to the previous findings reported by (Oluwasile *et al.*, 2014; Desta, 2015) and with a lower percentage of 26% procured from the open market according to (Mezene *et al*., 2020). The escalated acquisition of antibiotics from the open market by farm attendants could be attributed to their level of practice the prevailing medicine price inflation, and inadequate supervision of the veterinary drug supply chain. These erroneous responses from the participants signal an alarm regarding the inappropriate utilization of antibiotics within the area. The majority (82.76) of respondents source antibiotics from veterinary pharmacy which is lower than the 100% (Mezene *et al*., 2020), 92% (Oluwasile *et al*., 2014) and 87.8% (Chilawa *et al.*, 2023) in Ethiopia, Nigeria and Zambia respectively.

According to the survey, a larger percentage of participants (55.17%) used to prescribe and administer drugs on their own without help from a veterinarian. In the current study, amoxicillin, oxytetracycline, ampicillin and tetracycline were the most commonly utilized antibiotics which were also the main types of antibiotics for which bacteria developed resistance. These findings are in line with those of (Wongsuvan *et al*., 2018) from Thailand in poultry farms and (Fagbamila *et al*., 2017) from Nigeria in commercial poultry laying hens, who reported higher usages of amoxicillin and oxytetracycline. The unauthorized vending of antibiotics in unconventional and uncontrolled settings without legal authorization results in the inappropriate and unregulated utilization of these medications. This behavior significantly contributes to the formation and transmission of antimicrobial resistance (Spellberg *et al*., 2016).

The poultry farmers in this survey prioritize feed problem and disease as the main factors that challenging their production and productivity. This high (41.38%) disease pressure was in consistence with report of (Ovwigho *et al*., 2009). According to this survey, 48.28% of poultry farmers obtain vaccines from National Veterinary Institute, while 27.59% source them from private suppliers and the small proportion of respondents' access from agricultural offices. Regarding vaccine storage, an equal proportions (37.93%) of poultry producers store the vaccines in any cold place in their homes or as recommended by manufacturers. The remainder have not yet stored the vaccines. Storing vaccines in homes might cause reduced protection against pathogens because home storage conditions often lack the precise temperature control required to maintain vaccine efficacy. Vaccines typically need to be stored within specific temperature ranges to remain effective. Without proper refrigeration, the vaccines can degrade, lose potency, and ultimately fail to protect against diseases. Inconsistent or improper storage conditions in homes can thus lead to vaccine failure (Rabie and Amin, 2020).

Majority (65.5%) of the poultry farmers do not have vaccination schedules against bacterial diseases. Among the respondents, about 43% have vaccines administered by any personnel (non- veterinarian), 28.7% administer them themselves, and the rest by veterinarians. The survey also revealed mixed levels of satisfaction with the vaccine's protection, with 49% of farmers partially satisfied, 23% neutral, and the remainder unsatisfied. The low satisfaction level with vaccines among farmers in this survey is likely due to unwise vaccine management and improper vaccination protocols.

Majority of the farmers administer the vaccines themselves or use untrained personnel instead of professionals. This can lead to incorrect administration, improper storage, and handling errors, reducing the vaccine effectiveness and resulting in poor disease protection, which in turn causes dissatisfaction with the vaccine efficacy.

Despite the significant findings, there are some limitations in this study. One of the primary limitations of the study is only less than half of the *E. coli* isolates were subjected to antimicrobial susceptibility test since some of the most commonly used antibiotics in the sector were not evaluated due to the high cost and limited availability of antimicrobial discs on the market. Due to challenges with the PCR kit, the initial plan of this study to molecularly identify and characterize different *Salmonella* serotypes and *E. coli* species was not incorporated. Furthermore, some bacteriological cultures and biochemical experiments could not be performed as intended due to the lack of essential media and reagents. Moreover, the role of the unwise antibiotic and vaccine utilizations in AMR development was not associated in this study, which would require future researches incorporating the limitations of this study.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed significant proportions of samples originating from poultry establishments in Central Ethiopia were carriers of *E. coli* and *Salmonella*, on which their overall prevalence rates were 30.35% and 3.83%, respectively. The *E. coli* isolates exhibited higher resistance levels towards cephalothin, ampicillin, and oxytetracycline, displaying intermediate susceptibility to nalidixic acid and streptomycin, while elevated susceptibility to chloramphenicol, gentamicin, and sulfamethoxazole-trimethoprim. Similarly, the *Salmonella* isolates displayed substantial resistance to cephalothin, ampicillin, and tetracycline, moderate resistance to oxytetracycline and streptomycin, yet showcased high susceptibility to sulfamethoxazole-trimethoprim and gentamicin. Moreover, a notable proportion of multi-drug resistant isolates from both species were identified, underscoring the significance of chickens as potential sources of multi-drug resistant infections, posing a critical concern in treatment regimen. In Central Ethiopia, the indiscriminate and widespread application of antimicrobials in poultry farm settings and limited veterinary oversight in numerous establishments along with lower vaccine utilization against bacteria and poor vaccine managements were identified. The outcomes of this study provide substantiation for the emergence of antimicrobial resistance and multi-drug resistance in *Salmonella* and *E. coli* within poultry farms in Central Ethiopia. Based on the above conclusion, the following recommendations are forwarded:

- There should be implementation of effective biosecurity programs advocating a rigorous hygiene and sanitation protocols in poultry farms to mitigate bacterial contaminations.
- It is imperative to have strong antimicrobial stewardship and enforcing strict guidelines for antimicrobial use in poultry farms.
- Awareness creations and trainings to poultry farmers on antibiotic utilization and vaccine managements as well as risks associated with antimicrobial misuse are highly recommended.
- Further studies should be done in this area to get more information about antibiotic resistances profiles and associated risk factors in poultry.

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8. APPENDIX

Appendix 1: Informed Verbal consent form before conducting

Greeting: Hello, my name is Belayneh Seifu. I am working my MSc thesis in poultry health and management in College of Veterinary Medicine and Agriculture, Addis Ababa University. I would like to conduct questionnaire with you in few questions about antibiotic use, vaccine managements and handling on your poultry farm. The objective of this study is to collect baseline data on the role of antibiotics usages and vaccine managements in antimicrobial resistance of bacteria in poultry productions, which is required to improve the health status and production rates of the poultry and the human. Your contribution and consent for the questionnaire and observation is helpful in finding solutions to the existing problems related to the sector. Your name as well as address will not be indicated or specified in this form. All information that you give will be kept strictly confidential. Your participation is fully 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 cease 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!

This questionnaire aims to gather information about the prevalence, antimicrobial usages, vaccine management, and impact of common bacterial diseases in poultry farms. Your responses will contribute to understanding and improving poultry health.YOU HAVE FULL RIGHT TO CEASE OR REJECT THE SURVEY!

Thank you for your participation.

I. Farm and respondent details:

1. Farm Name: _____
2. Location (Town/District): _____ kebele_____
3. Position/Role of respondent on the Farm: _____
4. Years of Experience in Poultry Farming: _____

II. Management and flock history

1. Breed of Poultry in your Farm:

   A. Sasso B. Lohmann brown C.Bovans brown

   D. Deklab white E. mixture of___&____ F. Other

2. To which production system is the farm categorised under?

   A. Backyard scavenging (family owned) for subsistence

   B. Semi-intensive commercial farms

   C. Intensive commercial

3. Number of chicken found on your farm currently?

   A. Less than 1000 B. 1001-2500 C. greater than 2500

4. What is your primary purpose for poultry rearing?

   A. Food production (meat or eggs) for own consumption

   B. Trade of live birds or eggs

   C. Poultry rearing (sell live animals when they reach productive period)

   D. Other

5. What type of housing system is used on the farm?

   A. Cages

   B. Enriched cages

   C. Flooring system

   D. Aviary

   E. Free-range system

6. How do you keep your chickens? In houses;

   A. Built separate for birds

   B. They Share the same house with people

   C. They Share house with other animals D. Others

7. What type of feed do you use?

   A. Commercial feed

B. Own-produced feed

C. Kitchen waste/leftovers

D. A combination of the above

8. What is the source of water supply on your farm?

A. Municipal water supply

B. Deep well water

C. Rainwater

D. Surface water (pond, river, etc)

9. How often do you remove manure from the poultry houses?

A. Multiple times during every production round

B. After every production round

C. After several production rounds

10. How often are bacteriological analyses of the drinking water performed?

A. At least once a year

B. Every two years

C. Less frequent than every two years

D. Never

11. Are the poultry houses cleaned after each production cycle?

A. Yes B. No

12. Is a poultry health management programme, for which regular farm visits (e.g. by your veterinarian(s)) are performed, present on your farm?

A. Yes B. No

13. Which of the following aspects of your chicken farm are considered problems? (possible to choose more than one ….)

A. absence of sufficient feed

B. presence of predators

C. high disease intervention

D. inadequate services for animal health

E. insufficient budget

F. inadequate housing land

G. Other (describe)

14. Do you have your own veterinarian or other animal health professional?

A. Yes B. No

III. **History of Bacterial Diseases in the farm:**

1. Have you experienced outbreaks of bacterial diseases in your farm with in past 5 years?

   A. Yes B. No

2. If yes, please specify Awareness on the following Common Bacterial Diseases if any history (mark "X") infront!

   A. Fowl tyhoid ____ B. Avian colibacillosis____

   C. Fowl cholera_____ D. Avian Mycoplasma Infection___

   E. Infectious Coryza_____ F. Other (Specify) _____

3. How do you identify potential bacterial diseases in your poultry?

   A. Visual observation of clinical signs

   B. Behavioral changes

   C. Reduced egg production

   D. based on history

   E. laboratory testing

   F. Other

IV. Vaccination and Biosecurity Measures:

1. Which Biosecurity measures are implemented on your farm to prevent bacterial diseases?

   A. Vaccination C. regular cleaning and disinfections

   B. Fences D. Continuous supplementation of antibiotics

   E. avoiding the entrance of rodents and other carriers F. others

2. Did you vaccinated your flock against bacterial diseases?

   A. Yes B. No

3. If yes, please provide details of the vaccines used (date of vaccinating, route) for;

   A. fowl typhoid vaccine in the interval of____to____months through_____

   B. avian colibacillosis in the interval of____to____months through_____

   C. fowl cholera in the interval of____to____months through_____

4. Who administers vaccines on your farm?

   A. Farm owner B. Veterinarian

   C. Any employee in the farm D. Other

5. Where do you primarily source your vaccines?

   A. Veterinary Clinics B. Agricultural Bureaus

C. Private Suppliers D. NVI

6. How do you store vaccines on your farm?

A. In the way producers recommended B. in cold place in the house

C. In together with other drugs D. not stored yet

7. Do you think your vaccination protected your flock from bacterial disease?

A. Yes B. No

8. If no, what factors do you believe contribute to vaccine failure on your farm?

A. Production chain B. transportation ways C. storage

D. administration condition E. others

9. How satisfied are you with the efficacy of vaccines used on your farm?

A. Very Satisfied B. Satisfied C. Neutral

D. Unsatisfied E. Very Unsatisfied

10. How often do you clean the poultry house?

A. Daily B. Weekly

C. Monthly D. Other

11. Do you use disinfectants as part of your cleaning routine?

A. Yes B. No

12. How often do you clean and sanitize poultry equipment (feeders, waterers, etc.)?

A. Daily B. Weekly

C. Monthly D. Other

13. How do you manage poultry waste (bedding, manure, etc.) on your farm?

A. Regular Removal B. Composting

C. Burying D. Other

14. Do you have established hygiene practices like foot bathing for farm personnel and vehicles entering poultry houses?

A. Yes B. No

15. Do you maintain records related to hygiene and sanitation practices and diseases occurrence?

A. Yes B. No

V. Treatment Protocols and Antibiotic resistance:

1. How do you typically treat bacterial infections in your poultry? Using,

   A. Antibiotics B. Probiotics

   C. Herbal Remedies D. Other

2. For what purpose you use drugs in your farm case?

   A. For the treatment of sick chicken

   B. For prevention of diseases in healthy chicken

   C. For growth promotion D. other

3. How many times you use antibiotics in your flock as growth promoter/ disease prevention?

   A. Once a week B. twice a week C. every day

   D. every seventh day E. every fifteenth day

4. Among the followings which drug you use frequently? (choosing more than one is possible)

   A. Amoxycillin B. Chloramphenicol C. Tetracycline

   D. Oxy-tetracycline E. Streptomycin F. Naldixic acid G. Ampicillin H. Sulfamethoxazole-Trimethoprime I. other(specify)

5. Where do you obtain the drugs you use in your farm?

   A. Veterinary drug shop C. Human Pharmacy/drug shop

   B. Public Veterinary Clinic D. Open market E. Other sources

6. Who prescribe and administer drugs on your chicken?

   A. Self - administer B. veterinarians C. Other

7. How you determine the dosage before giving to birds?

   A. By reading the instructions on labels B. From prescription paper

   B. With assumption D. based on their bodyweight E. Other

8. How antibiotics are typically administered in your farm?

   A. Mixed with Feed B. Added to Drinking Water

   C. Injected D. Sprayed E. Other

9. Have you implemented or considered alternative strategies to reduce antibiotic usage on your farm? -

   A. Yes - B. No -

10. If yes, how do you implement the measures?

    A. By providing plant source antibiotics

B. by culling out those who get infected

C. By using vaccines during bacterial outbreaks in the farm

D. by strengthening biosecurity

11. What do you know about drug misuse in poultry production?

A. When given under-dose

B. When given over-dose

C. Use of drugs in the absence of disease

D. When stopped before the end of the recommended duration E. I don't know

12. How do you think that antibiotic resistance would be developed? It is when there is;

A. Use of drug over the recommended dose

B. Use of drug under the recommended dose

C. Continuous supply of drugs in animal feed

D. Use of human preparation drugs for animals

VI. Any Comments on the Survey

Is there anything else you would like to share regarding bacterial diseases in poultry farming and their vaccination strategies?

Thank you, once again!

Appendix 2: Media and Reagents used

1. Nutrient Agar M001

Nutrient Agar is used as a general purpose medium for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition

Ingredients	**Gms / Litre**
Peptone	5.000
Sodium chloride	5.000
HM peptone	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

Preparations

Suspend 28.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired ,the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour into sterile Petri plates.

2. Xylose-Lysine Deoxycholate Agar (XLD) Agar media (HIMEDIA, M031-500G, India)

Composition (gram/litre):

Ingredients	**Gms / Litre**
Yeast extract	3.0
L-Lysine	5.0
Lactose	7.5
Sucrose	7.5
Xylose	3.5
Sodium chloride	5.0
Sodium deoxycholate	2.5
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	15.0
Final pH (at 25°C)	7.4±0.2.

Preparation:

58.68 grams were suspended in 1000 ml of distilled water. Next, it was heated with frequent agitation until the medium boiled. Then it was transferred immediately to a water bath at 45-50 °C. Mixed well and poured in to sterile petri plates. DO NOT AUTOCLAVE!

3. Buffered Peptone Water (BPW)

Composition (gram/litre):

Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5
pH at 25 $\pm$ 0.2 ^{o}c	7.2

20 grams of dehydrated medium was added to 1 litre of distilled water. Mixed well and distributed into final containers. Then, it was sterilized by autoclaving at 121°C for 15 minutes.

Modified Rappaport Vassiliadis

Composition

Ingredients	Amount
Papaic digest of soyabean meal	4.5
sodium chloride	7.2
Mono-potassium phosphate	1.44
magnesium chloride	36.00
malachite green	0.036
Final PH at 25 ^{0}c	5.2 $\pm$ 0.2

Suspend 49.17 grams of the hydrated medium in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115 ℃ for 15 minutes.

5. MacConkey agar

Composition

Ingridients	Amount
Peptone (Pancreatic digest of gelatin)	17 gm
Proteose peptone (meat and casein)	3 gm
Lactose monohydrate	10 gm
Bile salts	1.5 gm
Sodium chloride	5 gm
Neutral red	0.03 gm
Crystal Violet	0.001 g
Agar	13.5 gm

Preparation:

Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water and Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Finally, Cool to 45-50°C and make sure to Mix well before pouring into sterile Petri plates.

6. Eosin Methylene blue Agar

Composition

Ingriedents	Amount
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.0065
Agar	15.0

Preparation:

Suspend 35.7g in 1 liter of distilled water and Bring to boil to dissolve completely. Sterilize by autoclaving at 121^0C for 15 minutes. Finally Cool to 45 to 50^0C and shake the medium in order to oxidize the methylene blue (restore its blue color and to suspend the precipitate which an essential part of the medium).

7. **Mueller Hinton Agar**

Composition:

Ingredients	Gms / Litre
HM infusion B from	300.000
Acicase	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

Preparations: Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Note: The performance of this batch has been tested and standardized as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

Gram's staining

Principle:

Bacteria also differ from one another chemically and physically and may react differently to a given staining procedure. This is the principle of differential staining. Differential staining can distinguish between types of bacteria based on the thickness of the cell wall. It divides bacteria into two groups: Gram negative and gram positive.

Procedure:

Fix the smear, Stain with the primary stain – crystal violet - for 30 seconds, Wash crystal violet off with water, Add iodine for 10 seconds (mordant) (the mordant can also be added without washing the crystal violet), Wash iodine off with water, Decolorize with ethyl alcohol for 10 – 20 seconds (decolorizing agent), Wash the alcohol off with water, Counter stain with safranin for 30 seconds, Wash the safranin off with water, Blot the smear dry, Observe under oil immersion.

Indole test

Principle:

Organisms those possess the enzyme tryptophanase can break down the amino acid tryptophan to indole. When indole reacts with para-dimethylaminobenzaldehye (Kovac's reagent) a pink -colored complex is produced. Tryptophan is plentiful in most media, but growth on blood agar or chocolate agar produces the best effects.

Procedure:

Take loopful of inoculum by touching the 3-5 representative colonies with inoculating loop from pure colonies and inoculate Tryptone soya broth tube. Incubate the tube at 37°C for 24 hours and cap left loosen to aerate the tube. After incubation, add 5-10 drops (0.5ml) of Kovac's reagent to the culture broth and agitate gently. Then observe the tube for color change within 5 minutes.

Citrate utilization test

Principle:

Citrate contains carbon. If an organism can use citrate as its only source of carbon the citrate in the media will be metabolized. Bromthymol blue is incorporated into the media as an indicator. Under alkaline conditions this indicator turns from green to blue. The utilization of citrate in the media releases alkaline bicarbonate ions that cause the media pH to increase above 7.4 causes the media blue.

Procedure:

Take loopful of inoculum by touching the center of 3-5 representative colonies with inoculating loop and streak it onto the surface of a Citrate slant. Incubate the tube aerobically at 35°C with cap left loosen for 22 hours. After 22 hrs incubation observe the tube for growth and color change.

Methyl red and Voges proskauer tests

Principle:

Some organisms produce acid from the metabolism of glucose in a sufficient quantity to produce a pH of 4.4 in the media. These acids are not further metabolized and are said to be stable acids. At a pH of 4.4 or less the pH indicator methyl red is a bright cherry red. While also some organisms initially produce acid from glucose metabolism but further metabolize the acid produced to neutral end products, such as acetoin, and 2, 3-butanediol. Initially the pH may drop to 4.4 but the neutral end products raise the pH so the methyl red test will be negative.

Procedure:

Take loopful of inoculum by touching the center of 3-5 representative colonies with inoculating loop from the pure isolated colonies and inoculate MR-VP broth with inoculum, incubated 37 ºC for 24 hours. Aseptically from incubated broth after 24 hrs transfer aliquot to two clean test tubes each with two ml of broth culture with sterile pipette. Add 5 drops of methyl red to for methyl red test and add 5 drops of 40% KOH and alpha-naphtanol for VP test.Then read the result immediately.

Triple sugar iron test

Principle:

Bacteria that ferment any of the three sugars in the medium will produce byproducts which will change the color of the red pH-sensitive dye (phenol red). A bacterium that is a nonlactose fermenter and ferments glucose, initially causes a yellow slant/yellow bottom (acid/acid reaction) after 8 hours, but then converts to a red slant/yellow

bottom after 24 hours (alkali/acid reaction). Where as if it ferments both lactose and glucose, it results in a yellow/yellow tube and remains that way due to the large amount of acid produced in the reaction.

Procedure:

By sterile inoculating loop touching the center of colony from isolated pure colony take loop full of inoculum. Streak the inoculum back and forth on Triple Sugar Iron agar in tube along the surface of the slant. Incubate the tube with the cap loosened at 35 ºC for 22 hours.

0.5 McFarland turbidity standards

Procedure:

Solution A (0. 48 M BaCl22H2O) 1,172g BaCl22H2O make up to 100ml with distilled water Solution B (0.18M H2SO4) 1ml H2SO4) (analar grade, sp.gr.1.84) make up to 100ml with distilled water.

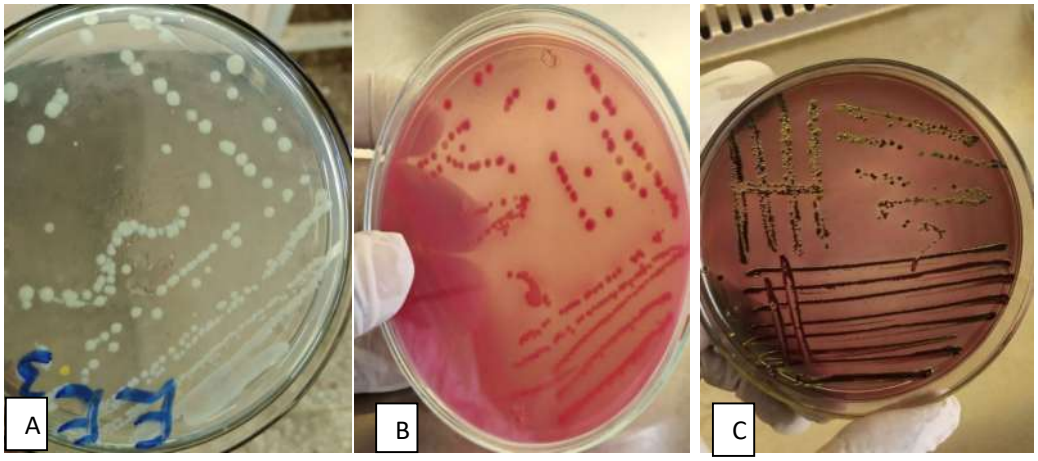
For standard check Add 0.5 ml solution A to 95.5 ml of solution B. shake vigorously and dispense in to 4-5ml sealed screw capped vials and store at room temperature in the dark place.

Appendix 3: Antibiotics used and their AST Breakpoints (CLSI, 2022)

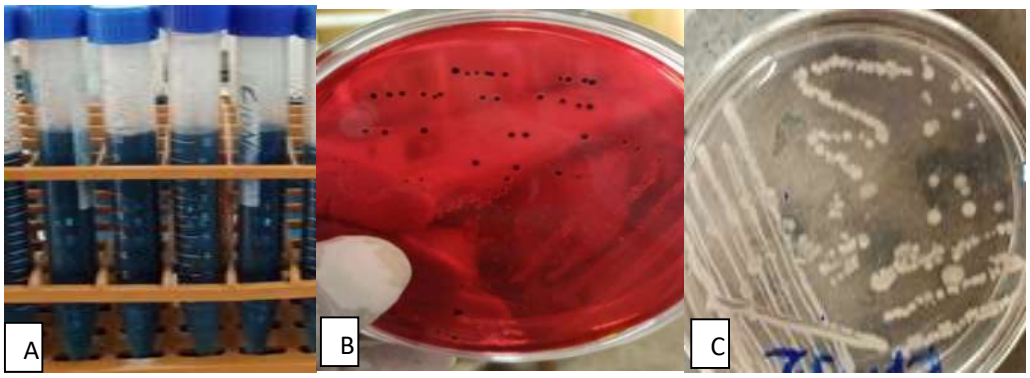
Name and symbol of the antibiotic	Concentration (µg/disc)	Zone diameter in millimeter		
		Susceptible	Intermediate	Resistant
Amoxacillin (AMC)	30	≥17	14-16	≤13
Ampicillin (AM)	10	≥17	14-16	≤13
Cephalothin (KF)	30	≥		≤
Chloramphenicol (C)	30			
Gentamycin (CN)	10	≥15	13-14	≤12
Nalidixic Acid (NA)	30			
Oxytetracycline (OT)	30			
Streptomycin (S)	25	≥15	12-14	≤11
Trimethoprime + Sulfa (SXT)	25	≥16	11-15	≤10
Tetracycline (TE)	30	≥15	12-14	≤11

Appendix 4: Different activities During the Study in Pictures

Pictures during sample collection A= tissue swab, B= cloacae swab, C= one of the farms from which samples taken

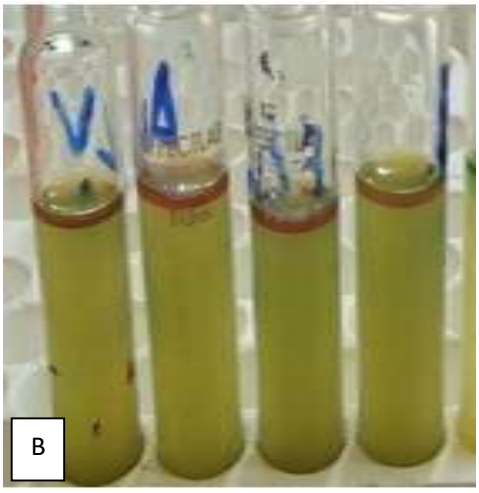


Pictures of *E. coli* colony in different media A=nutrient agar, B= Maconkey agar, C=EMB agar



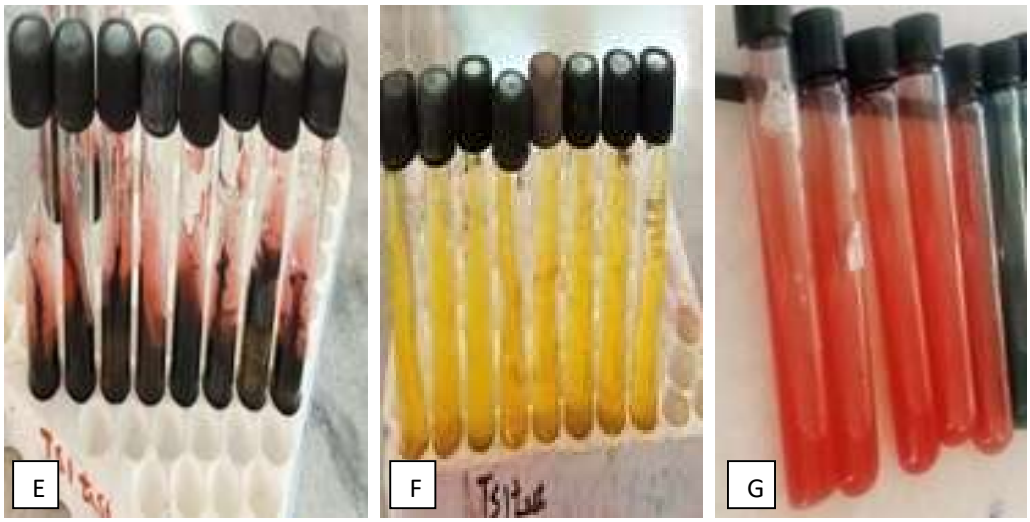
Pictures of *Salmonella* in broth and agar media
A=RV-broth, B=XLD agar, C=Nutrient agar

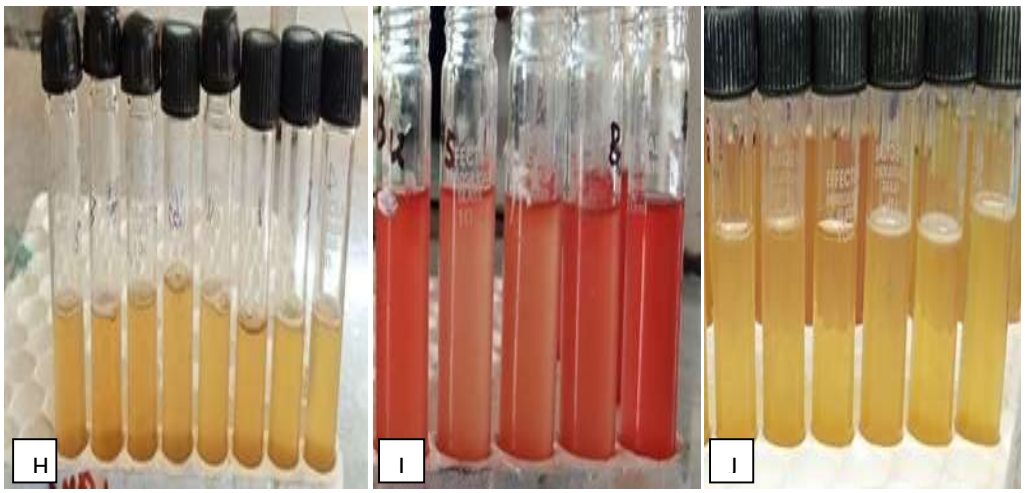

2 3 4 5 6 7 8 9 10
Indole test
A


B


C

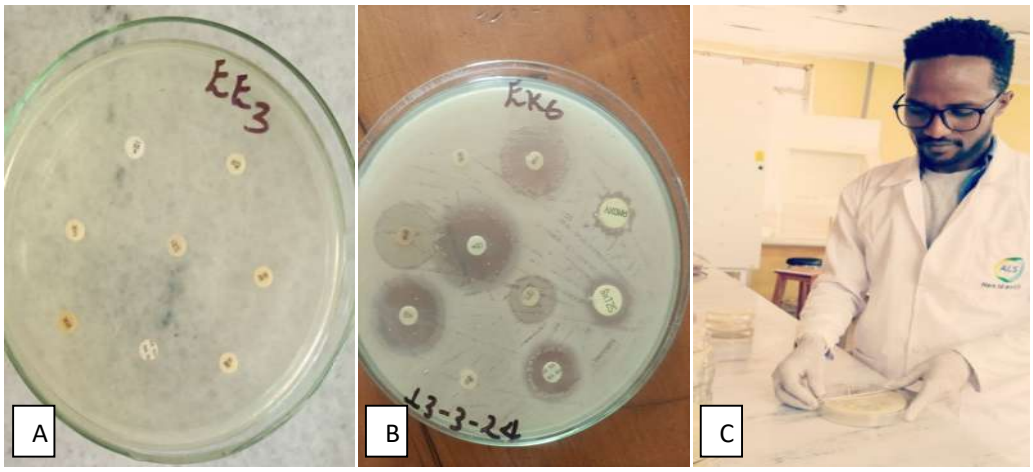

D





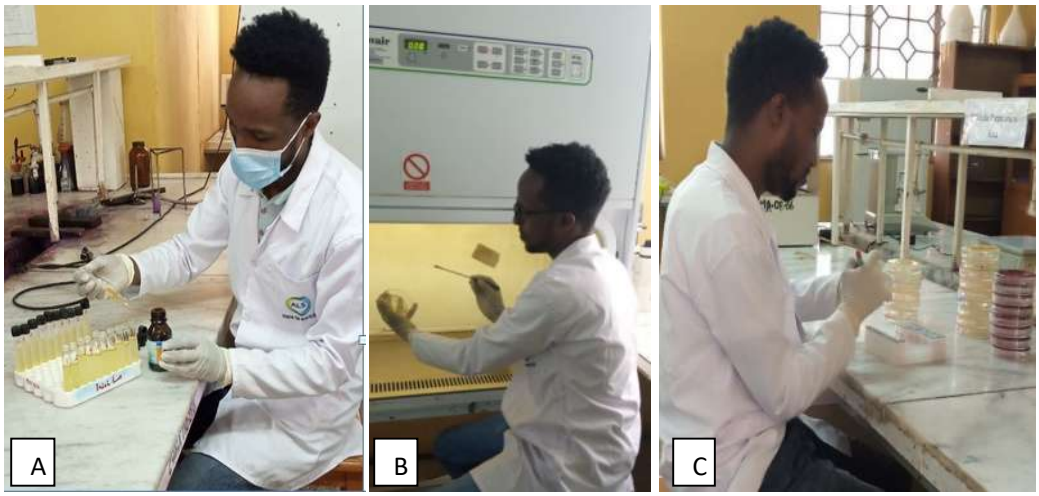
Pictures of media and results of biochemical tests

A= indole negative, B= indole positive, C=Simon's citrate negative, D= Simon's citrate positive, E= TSI (*Salmonella*), F= TSI (*E. coli*), G= TSI broth (negative control), H= VP negative, I= MR positive, J= MR-VP broth (negative control)



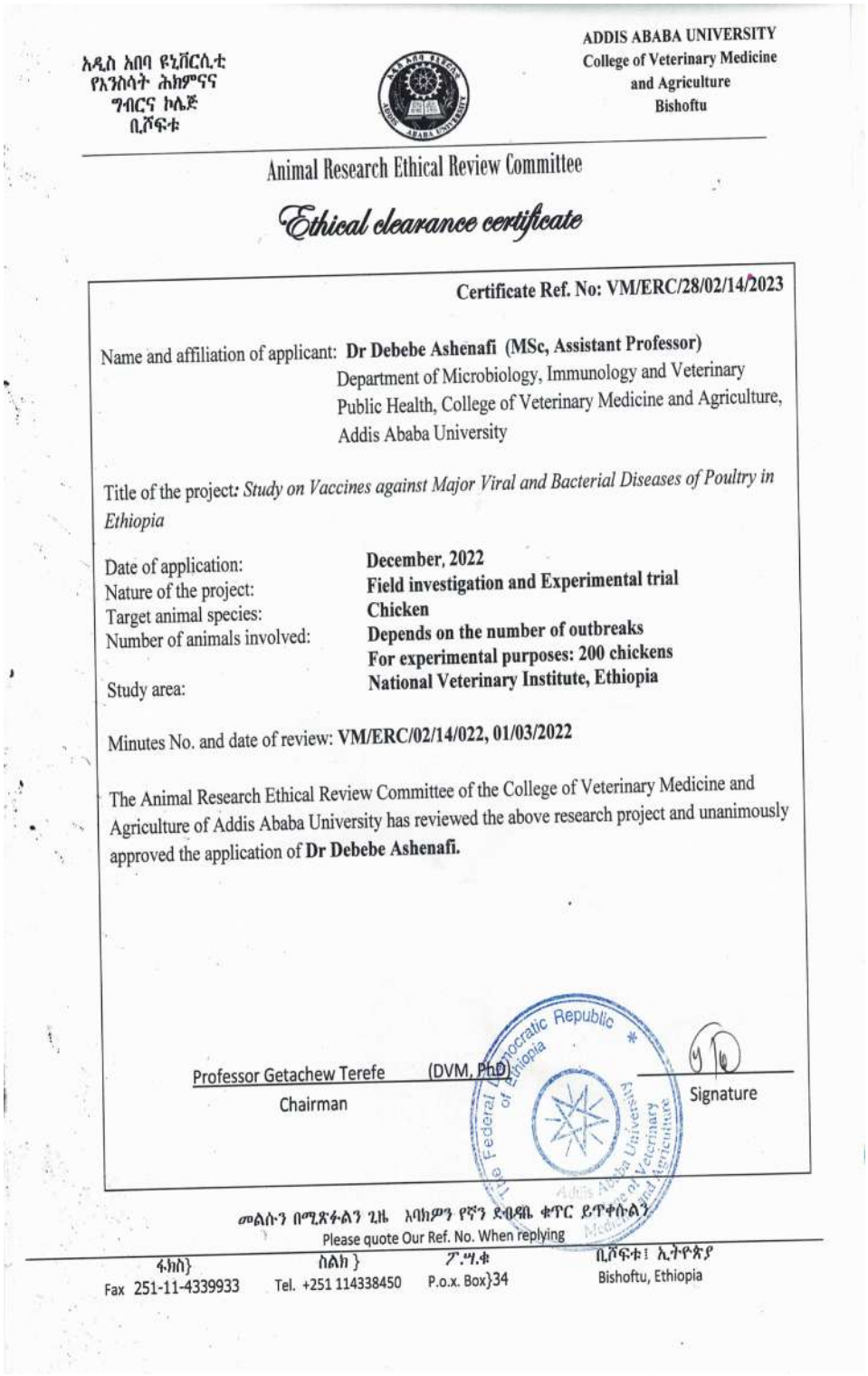
Pictures of antimicrobial susceptibility tests

A= plate containing antibiotic discs before incubation, B=antibiotic discs created zone of inhibition after incubation time, C= measuring zone of inhibition by calliper meter



Pictures of different activities in Microbiology laboratory A= while dropping Kovac's reagent in indole test B=sub-culturing C= labelling before preserving the isolates

Appendix 5: Ethical Clearance Certificate



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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
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Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/28/02/14/2023

Name and affiliation of applicant: **Dr Debebe Ashenafi (MSc, Assistant Professor)**
Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Study on Vaccines against Major Viral and Bacterial Diseases of Poultry in Ethiopia*

Date of application:	**December, 2022**
Nature of the project:	**Field investigation and Experimental trial**
Target animal species:	**Chicken**
Number of animals involved:	**Depends on the number of outbreaks** **For experimental purposes: 200 chickens**
Study area:	**National Veterinary Institute, Ethiopia**

Minutes No. and date of review: **VM/ERC/02/14/022, 01/03/2022**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of **Dr Debebe Ashenafi.**

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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

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Please quote Our Ref. No. When replying

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