ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF BIOMEDICAL SCIENCES

ANTIBIOGRAM STUDY OF SALMONELLA AND ESCHERICHIA COLI ISOLATES AND ASSESSMENT OF KAP OF POULTRY PRODUCERS IN CONVENTIONAL POULTRY FARMS IN HOSSANA TOWN, CENTRAL ETHIOPIA

BY

HABTAMU ENDALE

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Antibiogram study of *Salmonella* and *Escherichia coli* isolates and assessment of KAP of poultry producers in conventional poultry farms in Hossana town, central Ethiopia

MSc Thesis

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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 Habtamu Endale entitled: **Antibiogram study of *Salmonella* and *Escherichia coli* isolates and assessment of the KAP of poultry producers in conventional poultry farms in Hossana town, central Ethiopia** and recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of science in Veterinary Pharmacology.

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I hereby affirm that this thesis is my original work and that all sources of materials utilized in its preparation have been properly acknowledged. This thesis is submitted as part of the requirements for the Master's degree in Veterinary Pharmacology at Addis Ababa University, College of Veterinary Medicine and Agriculture. It has been deposited in the college library for the purpose of being made available to borrowers in accordance with the library's regulations. I certify that this thesis has not been submitted to any other institution for the purpose of obtaining any academic degree, diploma, or certificate.

Habtamu Endale

Signature _____ Date _____

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LIST OF ABBREVIATIONS AND ACRONYMS

ARB	Antimicrobial resistant bacteria
ARG	Antimicrobial resistance gene
AMU	Antimicrobial use
AMS	Antimicrobial stewardship
BPW	Buffered peptone water
ESBL	Extended-spectrum β-lactamase
FMT	Fecal microbiota transfer
MAR	Multiple antimicrobial resistance
MGE	Mobile genetic elements
MOI	Multiplicity of infection
R score	Resistance score

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ABSTRACT

Antimicrobial resistance is a serious health threat to creatures that depend on antimicrobials for the prevention and relief of infections. Foodborne *Salmonella* and *Esherchia coli* O157:H7 are critical causes of antimicrobial resistance associated morbidity and mortality. Fast growing poultry production along with antimicrobial misuse is a critical hotspot for the emergence and spread of antimicrobial resistance. A cross-sectional study aimed at investigating the antimicrobial resistance pattern of *Salmonella* and *E. coli* O157:H7 by disc diffusion method and assessment of KAP of poultry producers was executed in conventional poultry farms in Hossana Town, Central Ethiopia from October 2023 to May 2024. Up on bacteriological, biochemical and serological tests, 27 *Salmonella* and 20 *E. coli* were isolated and identified from a total of 228 cloacal swabs and postmortem samples. In disc diffusion test, all *Salmonella* and *E. coli* isolates were resistant to tested antimicrobial agents. *Salmonella* was resistant to ampicillin (100%), sulfamethoxazole and cefoxitin (85.1%), tetracycline (77.8%), trimethoprim/sulfamethoxazole (74.1%), amoxicillin-clavulanic acid (66.6%), ceftriaxone and streptomycin (55.6%), ciprofloxacin (51.9%), ampicillin/sulbactam (48.1%), trimethoprim (44.4%) and cefotaxime (40.7%). It showed high susceptibility to meropenem (81.48 %), gentamicin (66.67%), chloramphenicol (81.48%) and azithromycin (81.48%). *E. coli* O157:H7 was resistant to ampicillin (100%), cefotaxime and tetracycline (90.00%), cefoxitin and trimethoprim (70.00%), sulfamethoxazole (65.00%), amoxicillin clavulanic acid and ceftriaxone (55.0%) and ampicillin/sulbactam (50.00%). It was highly susceptible to azithromycin 90.00%, gentamicin (75.00%), chloramphenicol (60.00%), ciprofloxacin (55.00%) and meropenem (50.00%). All *Salmonella* and 80.00% of *E. coli* exhibited MDR. A lower portion, 22.20%, 19.44% and 25.00% of the poultry producers have good knowledge, attitude and practice on AMU and AMR, respectively. Poultry farms in the study site were found crucial hotspots for AMR *Salmonella* and *E. coli* and the low KAP levels of the poultry producers further exacerbate antimicrobial misuse and AMR spread. Monitoring antimicrobial dispensing, awareness of stakeholders on AMR prevention, and improving poultry producers' KAP on antimicrobial use and resistance are paramount to tackling this global problem.

Keywords: *E. coli O157:H7, antimicrobial, antimicrobial resistance, poultry, Salmonella*

1 INTRODUCTION

1.1 Background

Antimicrobial resistance (AMR) poses a threat to contemporary medicine, public health, and the accomplishment of sustainable development goals. Antimicrobial resistant bacteria (ARB) infections contribute significantly to the worldwide disease burden. Antimicrobial-resistant bacteria were estimated to cause 1.27 million deaths in 2019 (WHO, 2024). Even if AMR occurs naturally, inappropriate use of antimicrobials significantly accelerates this process, making the misuse of antimicrobials in animals one of the main causes of AMR (Mikecz *et al.*, 2020; Pokharel *et al.*, 2020). Increasing AMR in human and veterinary medicine raises concerns about the irresponsible use of antimicrobials. Further use of antimicrobials in animal production raises concerns about the potential public health consequences of ARB from animals (Coyne *et al.*, 2019). Transmission of AMR within the population; to the other livestock and the humans is facilitated by a mobile genetic element (MGE), such as a plasmid (Shih *et al.*, 2023).

Utilization antimicrobials in animal production is widespread worldwide due to the unprecedented increase in the consumption of animal proteins, it is reported that about 8,164,662 kg of antimicrobials are used globally per year from which 70% is non-therapeutically (Bushen *et al.*, 2021). In Africa, about 3558 tons of the antimicrobials were used in animal production in 2020 (Mshana *et al.*, 2021). In many countries around the world, they are also used for non-therapeutic purposes, such as improving feed efficiency and promoting growth in livestock (Lekshmi *et al.*, 2017; Hosain *et al.*, 2021). Currently, a significant fraction (about 80%) of animals and birds involved in food production are given medication for either a part or the majority of their lives (Lees *et al.*, 2021; Wallinga *et al.*, 2022). In low-income countries, the demand for livestock produces has risen due to the modernization of veterinary services and the use of modern animal production methods. However, meeting this demand has led to excessive or improper use of antimicrobial agents (Geta and Kibret, 2021). In developing countries, this unregulated and irrational antimicrobial use highly hastens AMR in livestock.

According to (Gebeyehu *et al.*, 2021; Geta and Kibret, 2021; Dejene *et al.*, 2022; Tufa *et al.*, 2023) livestock producers in different parts of Ethiopia, use antimicrobials inappropriately and irresponsibly therapeutically and non-therapeutically. In different scales of poultry production

including conventional farms (Rashid *et al.*, 2019), antimicrobials are widely used for prevention (prophylaxis) and treatment of infections (Roth *et al.*, 2019; Aworh *et al.*, 2021); growth promotion (Aworh *et al.*, 2021; Rahman *et al.*, 2022; Umair *et al.*, 2022); in some area to improve the egg production in layers (Ferdous *et al.*, 2019a; Hosain *et al.*, 2021; Kiambi *et al.*, 2021); and to compensate the lack of adequate hygienic conditions (Kamboh *et al.*, 2018; Kumar *et al.*, 2019). This leads to the emergence of AMR in both innocuous and pathogenic bacteria (eg. *E. coli* and *Salmonella*) contributing to global AMR in humans and animals. Antimicrobial resistant *Salmonella* and *E. Coli* in poultry can make chemotherapy of associated diseases futile culminating in ensuing financial losses in the sector. Beyond this, they cause non-antimicrobial responding infection in humans followed by higher morbidity and mortality, as they are zoonotic (Nhung *et al.*, 2017; Varga *et al.*, 2019b).

Drug-and multi-drug resistant pathogens from poultry can reach and pose a health risk in humans through the food chain by consuming meat or eggs contaminated by the resistant pathogen or gene (Mouttotou *et al.*, 2017; Raut *et al.*, 2023); and direct contact with diseased or healthy poultry shedding the pathogen and their environment (Cheng *et al.*, 2019; de Mesquita Souza Saraiva *et al.*, 2022). β-lactamase (ESBL) genes of *Enterobacteriaceae* isolated from human blood culture are similar to those in human rectal swab samples and most of those isolated from retail raw meat of chicken suggesting that the AMR happened in animal farming has a vital impact on public health (Pormohammad *et al.*, 2019; Ramos *et al.*, 2020). In addition, resistant bacteria reach humans through untreated poultry excreta used as manure in the garden, mainly through the vegetables eaten fresh (Freitag *et al.*, 2018; Mansaray *et al.*, 2022).

E. coli (mainly Shiga toxin-producing, *E. coli* O157) and *Salmonella* (*S. Typhi, S. Typhimurium* and *S. Paratyphi*) are the most common foodborne zoonotic pathogens associated with animal products (Romero-Barrios *et al.*, 2020; Stearns *et al.*, 2022). AMR in bacteria of *Enterobacteriaceae* family like *E. coli* and *Salmonella* indicates the presence of drug-resistant strains in the community (Pavez-Munoz *et al.*, 2021). Studies in different parts of Ethiopia by (Bekele and Ashenafi, 2010; Shecho *et al.*, 2017; Eguale, 2018; Sarba *et al.*, 2020; Bushen *et al.*, 2021; Tigabie *et al.*, 2023) shown that poultry production is a vital hotspot of antimicrobial resistant *E. coli* and *Salmonella*. These studies reported that both *E. coli* and *Salmonella* were resistant to different antimicrobials commonly used in both human and animal health care.

1.2 Statement of Problem

Poultry production in Ethiopia has been steadily growing in recent years, with both large-scale and small-scale poultry farms contributing to the overall production. The emergence and spread of AMR in poultry production centers is a growing global concern that poses a significant threat to poultry health and production and public health owing to the failure of chemotherapy. In spite of this, there is no published research data showing the AMR profile of *Salmonella* and *E. coli* O157:H7 in conventional poultry farms in Hossana town and surroundings, central Ethiopia.

1.3 Research Objectives

1.3.1 General Objective

The major objective of the current work was to investigate the AMR profiles of *Salmonella* and *E. coli* O157:H7 isolates and assess the KAP of poultry producers in conventional poultry farms in Hossana Town, Central Ethiopia.

1.3.2 Specific objectives

- To determine the antimicrobial resistance pattern of *Salmonella* and *E. coli* O157:H7 from postmortem and cloacal swabs in conventional poultry farms.
- To assess poultry producers' knowledge, attitude and practice towards antimicrobial use and resistance in conventional poultry farms in the study area.
- To identify multiple antimicrobial resistance index, multidrug resistance and resistance score for *Salmonella* and *E. coli* O157:H7

2 LITERATURE REVIEW

2.1 Health and Economic Significance of *E. coli* O157:H7 and *Salmonella* in Poultry

The bacteria, *E. coli* O157:H7 and *Salmonella* belonging to the family *Enterobacteriaceae*, more recently named *Enterobacterales* are Gram-negative, rod-shaped, facultative anaerobic bacterium that affect poultry and other animals (Janda and Abbott, 2021). From a health perspective, both *E. coli* O157:H7 and *Salmonella* can cause infections in poultry, leading to various diseases. These infections can result in reduced growth rates, decreased egg production, and increased mortality rates among the birds (Gedeno *et al.*, 2022; Mak *et al.*, 2022). *E. coli* is a versatile bacterium that can cause a wide range of diseases mild gastroenteritis to severe infections in poultry, including yolk sac infection (omphalitis), respiratory tract infection, airsacculitis, pericarditis and septicemia (Nolan *et al.*, 2013; Swelum *et al.*, 2021). The two most virulent serovars in avian species, *Salmonella gallinarium* and *S. pullorum* (Fowl Typhoid), cause systemic infection and significant financial losses in the poultry sector. Nevertheless, infections with other low avian-specific serovars but with zoonotic significance such as *S. typhimurium, S. enteritidis, S. heidelberg, S. infantis, S. senftenberg,* and other serovars common in poultry (El-Saadony *et al.*, 2022).

Outbreaks of *E. coli* and *Salmonella* in poultry infections can result in significant financial losses for poultry farmers and the industry. Infected birds may require medical treatment, which adds to the production costs. Moreover, the presence of these pathogens in poultry products can lead to trade restrictions and bans, limiting market access and affecting export opportunities (Mak *et al.*, 2022). In addition, outbreaks of *E. coli* and *Salmonella* in poultry can have detrimental effects on the poultry industry. Contaminated poultry products can be recalled, leading to financial losses for poultry producers and suppliers. Moreover, consumer confidence in poultry products may decline, leading to decreased demand and reduced market prices. This can have a cascading effect on the entire poultry value chain, including farmers, processors, distributors, and retailers (Gedeno *et al.*, 2022; Abreu *et al.*, 2023). Beyond this, when these bacteria develop resistance against antimicrobials, they pose extremely high health and economic threat as they don’t respond to commonly used antimicrobials, necessitating repeated chemotherapy as well as the death of the chicken.

2.2 Multidrug Resistance, Multiple Antimicrobial Resistance Index and Resistance Score

Multidrug-resistant (MDR) pathogens are microbes possessing ability to resist the effect of three or more antimicrobial classes invitro. Across the world, MDR gram bacteria is increasing significantly higher rate making the pipeline of treatment narrower (Alkofide *et al.*, 2020). The overuse of antibiotics by farm owners in poultry farms, a common practice in developing countries, is a major reason for the development of MDR bacteria. This overuse typically occurs without consulting any veterinarians and without any previous testing of the animals. The dissemination of MDR bacteria to humans exposes the population to risk, especially the immunocompromised individuals, and exacerbates healthcare costs, and ultimately increases the usage of antibiotics (Tawyabur *et al.*, 2020). Several livestock-to-human MDR *E. coli* transmissions result from poultry product consumption, making poultry surveillance an essential public health entry point for preventing dissemination of MDR (Shawa *et al.*, 2021).

The multiple antimicrobial resistance index (MAR) index is calculated based on the number of antibiotics to which a bacterial isolate shows resistance. It is calculated by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested. The resulting value ranges from 0 to 1, with higher values indicating greater levels of MDR. MAR index combines data on antibiotic consumption and resistance patterns to provide a comprehensive assessment of AMR at both individual and population levels (Elshebrawy *et al.*, 2022). MAR index considers factors such as antibiotic use practices, surveillance data on resistant pathogens, and clinical outcomes associated with resistant infections. MAR index play a crucial role in monitoring and combating the spread of ARB. By providing insights into the prevalence and trends of AMR, MAR index role in public health policies and interventions to preserve the effectiveness of existing antibiotics (Karim *et al.*, 2023).

Antimicrobial resistance score (R score) is a quantitative measure of the resistance of microorganisms to antimicrobial agents. It is a numerical value that represents the degree of resistance of a particular microorganism to a specific antimicrobial agent. The R score is calculated based on the minimum inhibitory concentration (MIC) of the antimicrobial agent, which is the lowest concentration of the agent that inhibits the growth of the microorganism (Johnson *et al.*, 2017).

The R score provides a quantitative measure of antimicrobial resistance, allowing for more accurate comparisons between different microorganisms and antimicrobial agents. The R score is based on a standardized method of calculation, making it possible to compare results from different laboratories and studies. The R score has clinical relevance, as it can be used to predict the likelihood of treatment failure and guide antibiotic therapy (Bhattarai *et al.*, 2024).

2.3 Emergence of AMR in the Poultry and Its Contributing Factors

Antimicrobial resistance can develop in the natural environment where there are no scientifically synthesized antimicrobials since antimicrobials are extracted from natural things that have direct contact and antimicrobial activity on the microbes. To escape this malicious effect, microbes including pathogens develop preventive measures, which also work for currently used antimicrobials (Andersson *et al.*, 2020; Endale *et al.*, 2023). However, in the newer world emergence of AMR is hastened by factors consociating with human practices like drug over- and misuse, insufficient infection stoppage, and dearth of awareness); animal-associated practices like rife antimicrobial application in livestock and aquaculture, drug residue in food of animal origin); ecological features including release of active antimicrobials into water bodies, land, and waste systems) and wildlife-consociated aspects such as the wildlife consumption of active antimicrobials or antimicrobial treated carcasses or other substances (Al Amin *et al.*, 2020; Velazquez *et al.*, 2022; Nardulli *et al.*, 2023). Rife application and inapt use of antimicrobials in human and animal health care and agriculture have momentously contributed to the worldwide emergence and dissemination of AMR. The different study advocates that our hefty reliance on antimicrobials, along with the linkage among public health, animal farming and animal health pushed the emergence as well as the spread of drug resistant microbes. Consequently, this has culminated in a global epidemic where various frequently used antimicrobials become futile in fighting infections (Karakonstantis and Kalemaki, 2019).

As documented by (Adamowicz *et al.*, 2020) co-cultures of *E. coli* and *S. enterica* under model experimental systems developed distinct AMR pathways from monocultures of either species grown under the same experimental circumstances. Additionally, it was shown that *Salmonella* spp. and *E. Coli* can directly communicate with one another in the same environment through bacterial communication, which increases antimicrobial tolerance.

Transferability of plasmids containing antimicrobial resistance genes (ARGs) from *S. enterica* to *E. coli* and suggested that similarities between the plasmids isolated from the chicken gut of *S. enterica* and those found in pathogenic *E. coli*, another gut resident, may be indicative of interspecies transmission. Therefore, AMR for species living in the same habitats may be impacted by both bacterial signaling and genetic material transfer. Even when collected from the same sample, AMR phenotypes in *E. coli* do not correlate well with AMR phenotypes in *S. enterica*, indicating that additional genetic research on complicated AMR dynamics is necessary to comprehend this discrepancy (Baker *et al.*, 2024). Opportunistic pathogens like *E. coli* and *S. enterica* which are commonly found in livestock exhibit significant drug resistance and also possess the potential to infect humans (zoonotic potential). These bacteria have the capability to exchange genetic material not only within their own species but also potentially between different species. This mechanism of genetic transfer plays a critical role in the spread of antimicrobial resistance (AMR) among various organisms (Ikhimiukor *et al.*, 2022; Leonard *et al.*, 2022; Baker *et al.*, 2024).

2.3.1 Excessive Application of antimicrobials in poultry production

As the demand for animal-originated proteins like egg and poultry meat increases extremely, more intensified and commercial production of poultry is practiced widely. This in turn raises the application of antimicrobials both prophylactically and therapeutically since there is a higher risk of infection in intensified farming (Mdegela *et al.*, 2021). As the drugs are used excessively, the exposure of microbes to the antimicrobials increases this in turn facilitates the microbes to develop protocols help them to evade the effect of antimicrobials they have encountered or the related ones (Agyare *et al.*, 2018; Moffo *et al.*, 2022). As the antimicrobials are used widely, those microbes susceptible to those antimicrobials are removed by leaving that are resistant to antimicrobials in every ecological system where drugs where used. When the susceptible microbes are avoided, the surviving resistant ones propagate exponentially, leading to the wide spread of AMR (Kim *et al.*, 2021; Ngai *et al.*, 2021). This is because antimicrobials can abolish beneficial microorganisms like they destroy pathogenic microbes, which creates favorable conditions for the propagation of ARB (Panwar *et al.*, 2021). Consequently, the outstanding ARB subsist and reproduce, harboring resistance traits in their genome that can be transferred to other bacteria (Manohar *et al.*, 2020; Mancuso *et al.*, 2021).

Furthermore, indiscriminate use of antimicrobials also makes those non-pathogenic microbes like normal flora of *E. Coli* develop resistance and act as the reservoir of resistance genes (Mutua *et al.*, 2017; Peterson and Kaur, 2018; Wanja *et al.*, 2020; Koju *et al.*, 2022). As the microbes exchange their genetic material with the same or different species of bacteria, genes responsible for the drug resistance can also handover to the pathogenic bacteria through integrases, plasmids and transposases rendering the recipient resistant to the drug. Since the genes responsible for drug resistance are carried in mobile genetic substance plasmids and transposons which are prone to quick genetic exchange (Messele *et al.*, 2017; Ahmad *et al.*, 2021; Ma *et al.*, 2021).

As the extra antimicrobials are consumed, the prospect of AMR also rises, because individuals suffering from infection due to resistant bacteria frequently require extra antimicrobials for treatment. This further increases the chance of selection pressure favoring the resistant bacteria in both normal bacterial flora and pathogenic ones, culminating in the emergence and propagation of multidrug-resistant bacteria (Murray *et al.*, 2022). In general, AMR is an upshot of antimicrobial use, as bacterial genes mutate imprudently or up on contact with certain drugs enabling them to amend or circumvent drug targets (Naveed *et al.*, 2020). Nonetheless, the consociation between the two is not forthright. The variables of bacterial contact with antimicrobials and the development of AMR are intricate and involve multiple factors influencing the emergence and propagation of AMR from a bacterial standpoint (Bungau *et al.*, 2021; Uddin *et al.*, 2021). For instance, an investigation by (Laxminarayan *et al.*, 2016) demonstrated that indiscriminate utilization of drugs highly contributes to the emergence of *Staphylococcus aureus* resistant to methicillin and tuberculosis resistant to multiple drugs. Too, excessive usage of antiviral drugs like oseltamivir (Tamiflu) for prophylactic and therapeutic effects for influenza can end in the emergence of influenza strains (H1N1pdm09) resistant to the drug (Smyk *et al.*, 2022). Besides, inducible genomes encoding erythromycin resistance methylase carried in *mycobacteria* and *S. aureus* are lone synthesized on the induction by certain antimicrobials, where the bacteria swiftly evolve resistance to those specific drugs (Van and Paterson, 2016).

2.3.2 Inappropriate and extralabel use of antimicrobials in poultry

Improper utilization of antimicrobials, including the usage of broad-spectrum antimicrobials; under-prescription; use of antimicrobials to treat viral diseases; unnecessary antimicrobial prophylaxis; prescription without laboratory evidence; non-prescription sale; polypharmacy and administration of drug through the wrong route all contribute for the emergence of AMR (Dache *et al.*, 2021; Nkinda *et al.*, 2022; Salam *et al.*, 2023). Research indicates that when second-line antimicrobials, like macrolides and second-generation cephalosporins, are used to treat infections that are acute and self-limiting or that can be successfully treated with first-line antimicrobials like amoxicillin or penicillin alone, microbes are under selective pressure. This ultimately results in the microbes becoming resistant to the effects of these drugs, which is known as antimicrobial resistance (Karakonstantis and Kalemaki, 2019).

When poultry infected by a certain bacteria is a drug lower the dose needed to fully inhibit or destroy prevailing bacteria is prescribed (under-prescription), the surviving microbes develop resistance due to the ineffective challenge of the under-prescribed drug (Acharya and Wilson, 2019). This is because of one or two reasons that a lower amount of the drug triggers genetic mutation in the microbe resulting in amendment or circumvention of the drug site (Uddin *et al.*, 2021). Second, the microbe left unaffected by the drug gets a free and suitable environment as the drug removes a certain number of competing microbes resulting in quick multiplication of the resistant microbes (Endale *et al.*, 2023). Antimicrobials can also cause deviations in gene countenance which make the bacteria extra hurtful, augment mutation and more dissemination of AMR among bacterial populations (Iwu *et al.*, 2020). For example, low levels of drugs have been found to facilitate strain divergence in microbes like *Pseudomonas aeruginosa*. Also, subinhibitory concentrations of piperacillin and/or tazobactam persuade broad proteomic amendments in *Bacteroides fragilis* (Ventola, 2015).

The use of antimicrobials inappropriately in poultry production for the sake of animal health eminence and productivity improvement exceeds human antimicrobial usage globally. Analogous classes of antimicrobics critical for human medicine are also prescribed for animals (Jans *et al.*, 2018). Drug misuse in poultry and ensuing AMR is a rising problem globally in humans as well as livestock health (Velazquez *et al.*, 2022).

Extra-label drug use involves the utilization of an antimicrobial in an animal in a way that is not in harmony with the FDA-approved label. This comprises administration of the drug in a species or for an ailment or condition not explained on the label; administration at dosages, frequencies, or routes not indicated on the label; or aberration from the indicated withdrawal period (Da *et al.*, 2023). When drugs are given out of the label, the active ingredient of the antimicrobial is absorbed, distributed or excreted at a higher or lower rate than the normal pharmacokinetics of the drug. This is also corroborated by the fact as the size of the animal becomes higher, the body surface area to weight ratio declines, and the physiological processes become slower. These conditions in turn impact the pharmacokinetics of the antimicrobials in different animal species (Nair and Jacob, 2016). Thus, the use of a drug not recommended for poultry poses AMR associated with the wrong pharmacokinetics of the given drug. Administering antimicrobials at a dose that does not efficiently eliminate or inhibit the bacteria recorded on the label, or when the labeled dose is insufficient (due to AMR), ends in ineffective antimicrobial exposure and fire AMR (Papich, 2021). For example, as the multi-drug resistant *K. pneumoniae* strain SW1780 with resistant gene PSW1780-KPC in the plasmid is exposed to the subinhibitory levels of drugs like cefotaxime, ciprofloxacin, amikacin, and meropenem, transfer of resistance gene to the *E. coli* strain J53 was increased significantly (Ding *et al.*, 2022).

2.3.3 Use of antimicrobials as a feed additive in poultry production

Antimicrobials are extensively used in livestock farming as feed additives to boost nutrient utilization and improve animal health and overall productivity (Ferdous *et al.*, 2019b; Rahman *et al.*, 2022). However, the custom of using antimicrobials as feed additives is one of the leading causes of the emergence of AMR (Tian *et al.*, 2021). Since, the farmers apply antimicrobials to the feed at sub-therapeutic doses which an animal gains lower concentration, antimicrobials impose selection pressure which fuels the synthesis of resistant genes in the bacteria. This resistant gene synthesis stimulatory effect occurs in the bacteria found in feed as well as in the animal body (Pokharel *et al.*, 2020). As documented by (Suresh *et al.*, 2018) feed additive antimicrobials are administered to poultry with the aim of as growth promoters, at sub-therapeutic doses, antimicrobials augment growth rate, feed conversion and accordingly, the performance of broiler chicken.

The continual and non-cautious application of antimicrobials as feed additives poses the selection and dissemination of antimicrobial-resistant strains of fowl pathogens like *Salmonella* and *E. coli*. Another scholar (Ferdous *et al.*, 2019b) from Mymensingh district, Bangladesh reported that, poultry farmers use antimicrobials as egg lying promotors in layer chickens. The sub-lethal concentration of bactericidal drugs induces bacteria to develop reactive oxygen species, which can affect the genetic material of bacterium resulting in a buildup of mutations. This, in turn, interferes with error-correcting restoration systems, which finally make the bacterium multidrug resistant (Kohanski *et al.*, 2010; Qi *et al.*, 2023). For instance, (Gutierrez *et al.*, 2013) showed that exposure of *Salmonella* and *E. coli* to sub-inhibitory level beta-lactams like penicillin and ampicillin induce mutagenesis via the synthesis of reactive oxygen that raises the RpoS-regulon gene which induce PolIV gene this in turn downregulate MutS gene (responsible for DNA-replication fidelity). Furthermore, antimicrobials added in poultry feed can facilitate the handover of resistance genes by bacteriophages, thereby endorsing the dissemination of AMR. It is worth stating that sub-inhibitory levels of drugs also foster horizontal ARG exchange, which is the principal mechanism responsible for the dissemination of AMR (Iwu *et al.*, 2020; Yashi *et al.*, 2022; Castaneda-Barba *et al.*, 2023).

3 METHODS AND MATERIALS

3.1 Description of Study Area

The investigation was conducted in Hossana town, central Ethiopia from October 2023 to June 2024. Hossana town, the regional administrative city of central Ethiopia comprises three sub-cities and eight kebeles (the lowest governmental administrative units). It is located 232 km northwestern from Addis Ababa, the capital city of Ethiopia. Geographically the town is located at 70 22' N latitude and 370 28' E longitude while topographically, its altitude ranges from 1560m to 2688m above m.s.l. The mean annual temperature and precipitation are 21^{o}C and 750.9mm, respectively (Tesfay *et al.*, 2022) (Figure 3).

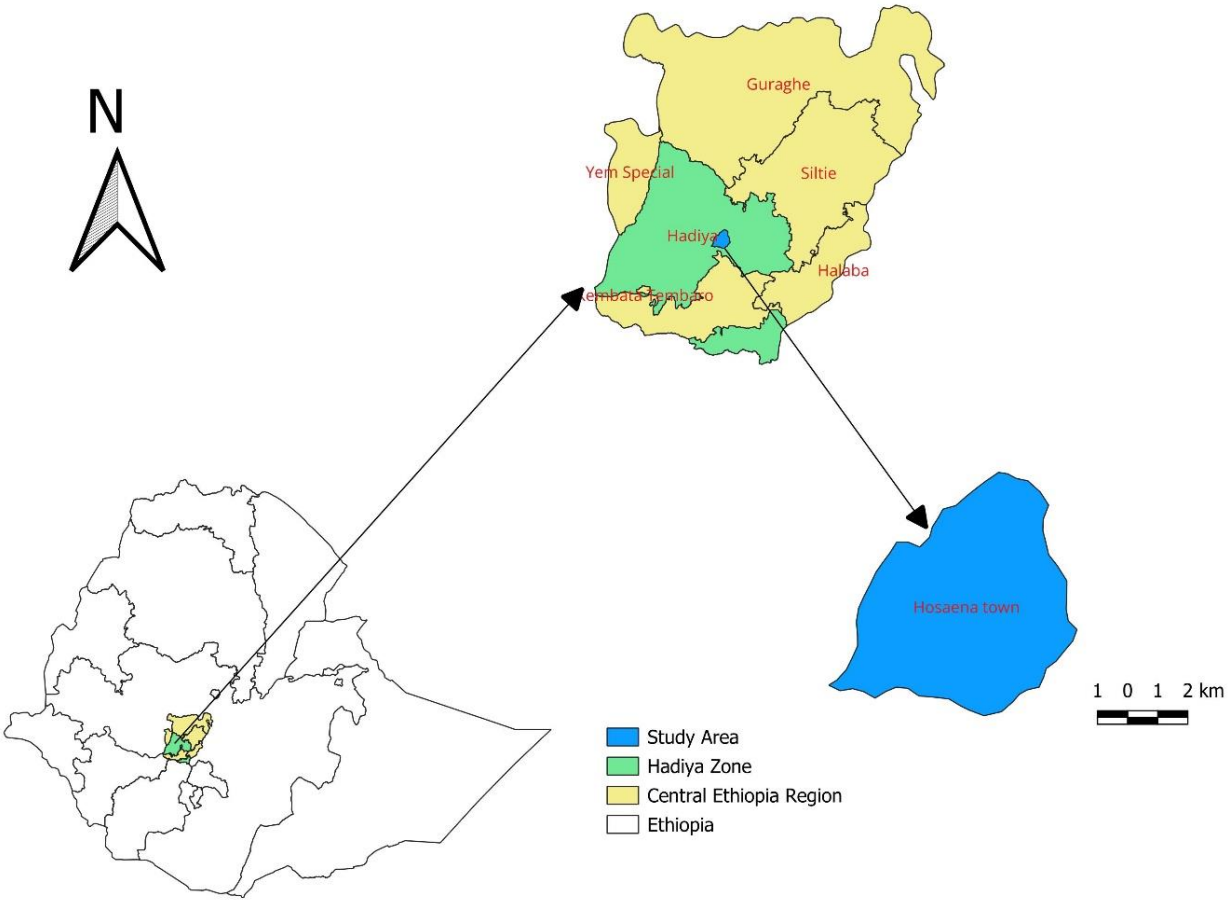


Figure 1. Map of the study area (Hossana town)

3.2 Study Design

A cross sectional study followed by simple random and purposive sampling was undertaken to isolate, identify and characterize phenotypic resistance patterns of *Salmonella* and *E. coli* from conventional poultry farms from October 2023 to June 2024. The number of samples was allocated to each farm proportionally according to the stocking size. The cloacal swabs were collected from clinically sick and apparently healthy chickens by visiting each farm at different days a single farm was revisited within a short period. A double marking on their legs and back plums by permanent marker was used to identify those sampled from non-sampled ones. A permanent marker was used to identify those sampled from non-sampled. Postmortem samples from freshly dead chickens were collected through continued contact with farm owners or managers and farm visits. In addition, the KAP of the producers on AMU and AMR was assessed and graded by scoring after a face-to-face interview.

3.3 Study Animals

For the current investigation chickens raised in conventional private and governmental poultry farms found in Hossana town, central Ethiopia were included. Layers and broilers of both sexes and different age groups including young (≤6 months) and adults (> 6 months) (Carol and Peter, 2005), apparently healthy, clinically sick, and freshly dead were included.

3.4 Sampling Technique and Sample Transportation

A total of 207 chickens, 200 live and 7 dead were sampled during the current investigation. About 200 cloacal swab samples (130 from apparently healthy and 70 from clinically sick chickens) and 28 organ samples (7 livers, 7 lungs, 7 ovaries and 7 kidneys) were collected during the research session. While sampling, apparently healthy chickens were selected randomly from each farm, while clinically sick chickens (those showing clinical signs like diarrhoea, pasted feathers around the vent, ruffled feathers, swollen navels, loose appetite depression and others) were and freshly dead chicken sampled purposively. For cloacal swabs, the wings of the chickens were held with one hand, keeping their heads down to expose their caudal parts. Then, a sterile cotton swab moistened with buffered peptone water was inserted into the cloacae and trundled inside several times.

Then the swabs were immediately transferred to test tubes containing 10ml of buffered peptone water (BPW) and kept in an icebox after appropriate labeling of all required information as adopted by (Meteab and Abed, 2018). For the postmortem sample, those freshly dead chickens with a history of the aforementioned symptoms were collected from the farms included and transported immediately to the Wachemo University veterinary laboratory for the necropsy. Then the necropsy was undertaken by following the procedures adopted by (Jagne and Buckles, 2021) and visceral organs including the kidney, liver and ovaries were sampled in sterile universal plastic bottles containing BPW aseptically. Then the samples were transported through an icebox to the Wachemo University Medical Microbiology Laboratory and bacteriological procedures were done on the same day (Figure 4). The number of respondents for the questionnaire was determined from 57 people engaged in poultry production at 19 conventional poultry farms by using a formula developed by Cochran (Mathewos *et al.*, 2023) considering a standard error of 5% at 95% confidence levels.

$$n = \frac{n0}{(1 + \mathrm{n0/population})}$$

where population size 57; n0= Cochran's required return sample size = 118; and n is the total required sample. The substitution of the values in the above formula gives 36, the total number of respondents required.

3.5 Inclusion and Exclusion Criteria for Farms for Antibiogram Study

Inclusion criteria

Those farms volunteered to provide informed consent and willing to cooperate fully with the study protocols, including providing access to farm records and allowing sample collection. Those who regularly raise poultry to avoid discontinuation of sampling; and whose management system was intensive were included in the current investigation.

Exclusion criteria

Farms in which the owners were not willing to cooperate with the full study protocol and unwilling to allow access to the farm at the required time. Those farms practicing extensive and semi-intensive management systems, small-scale and backyard farms were excluded from the study.

3.6 Bacteriological Methods

3.6.1 Bacterial isolation and identification and isolate preservation

Isolation and identification of *E. coli* o157:H7: In the current investigation, *E. coli* O157H7 was isolated and identified by adopting standard bacteriological and biochemical tests respectively. The BPW with cloacal swabs were incubated at 37°C for 24 hours. Then, through a cotton-tipped swab, bacterial suspension was streaked on the MacConkey Sorbitol Agar which is the selective and differential medium for *E. coli* O157H7 and incubated at 37°C aerobically for 24 hours. The next day, colorless colonies were sub-cultured on MacConkey agar at 37°C for 18-24 hours to get a pure colony and stored on a nutrient agar slant at 4°C. After that, the isolates were further characterized by biochemical tests including Triple sugar iron (TSI) agar, indole product and sulfide indole motility (SIM) test as adopted by (Sarba *et al.*, 2019). Each organ samples were crushed by a kind maceration through pestle and mortar, mixed distinctly with 10ml of BPW and incubated at 37°C overnight. A loopful of the culture suspension was streaked onto MacConkey Sorbitol Agar and incubated for 24 hours at 37°C aerobically. Afterward, the same steps as the cloacal swabs were followed as shown by (Quinn *et al.*, 2011).

Isolation and identification of *Salmonella*: The cloacal swabs in the BPW were incubated at 37°C for 18-24 hours to promote bacterial recovery. Then, 1ml of the pre-enriched sample was introduced to Rappaport-Vassiliadis (RV) broth and incubated at 42°C for 24 hours to afford *Salmonella* selective enrichment over other competitive microbes. A loopful of inoculant from RV was grown on the Xylose Lysine Deoxycholate (XLD) agar/ Salmonella-Shigella (SS) agar and incubated at 37°C for 24-48 hours and the plates were observed for red (XLD) and colorless (SS) colonies with blackened centers typical features of *Salmonella* (ISO, 2002). Suspicious *Salmonella* colonies from XLD/SS were sub-cultured on the MacConkey. Afterward, colorless colonies on the MacConkey were aseptically picked and stored on a nutrient agar slant for further biochemical characterization (Andrews, 2011). Then, these isolates were screened by biochemical tests including triple sugar iron, indole production test, Simon's Citrate test and SIM test. Organ samples were crushed by gentle maceration by pestle and mortar and one gram of minced tissues was mixed distinctly with 10ml BPW and incubated at 37°C for 18-24 hours to promote bacterial growth. After that, the same procedures adopted for cloacal swabs in this section were performed (ISO, 2002).

Once the bacteria were isolated, the isolates from confirmed colonies were inoculated in the 1.5ml volume nutrient broth (NB) and incubated for six hours at 37^0C. Then, 0.5 ml of NB containing colony suspension was transferred into the sterile serum screw vials. Next, 0.5 ml of 40% glycerin already prepared in distilled water by autoclaving was added by pasture pipette making the volume 1ml. After that, preserved at -70^0C temperature until serotyping and an antibiogram evaluation was performed (Bacteriological Analytical Manual, 1998).

3.6.2 Serological test

The isolated *Salmonella* and *E. coli* O157:H7 were serotyped at Wolaita Sodo Regional Veterinary Laboratory based on O (somatic) and H (flagellar) antigens according to the protocol documented by White-Kauffmann-LeMinor and the antigenic formula designated by (Grimont and Weill, 2007) as previously adopted by (Wang *et al.*, 2020; Elshebrawy *et al.*, 2022). Beyond this, the user guidelines of the kit manufacturers were followed accordingly. The antiserums used in the current study include *S. Typhimurium* (1,4,[5],12:i:1,2), *S. Gallinarum* and *S. Pullorum* (O1,9,12), S. *Enteritidis* (1,9,12:g,m), *S. Dublin* (1,9,12[Vi]:g,p) and *E. coli* (157:7). The *Salmonella* serovars were designated accordingly to their agglutination reaction with the antiserum used and those positive for the *Salmonella* polyvalent antiserum but not reactive with all available antiserums were interpreted as other serovars. Since *S. Gallinarum* and *S. Pullorum* have the same genetic structure, biochemical tests including dulcitol fermentation and ornithine decarboxylation were used to differentiate among them. Those fermenting dulcitol and but not decarboxylating ornithine were interpreted as *S. Gallinarum*. Those did not ferment dulcitol but decarboxylate ornithine were taken as *S. Pullorum* as adopted by (Ribeiro *et al.*, 2009; Haque *et al.*, 2021). For *E. coli*, the target isolate was the O157:H7 strain, thus it was confirmed serologically by slide agglutination as previously implemented by (Nada *et al.*, 2023) by using monovalent O157and H7 antisera. The isolate showing agglutination in O157 antiserum and H7 monovalent antiserum were confirmed as *E. coli* O157:H7, baes on kit manufacturers guideline.

3.7 Antibiogram of the Isolated Bacteria

Phenotypically confirmed isolates of both *E. coli* and *Salmonella* isolates were subjected to antimicrobial susceptibility testing using the disk diffusion (Kirby-Bauer disc diffusion) method.

A bacterial suspension was prepared by transferring 2-4 colonies grown overnight on nutrient agar to a glass tube containing 5 ml of sterile normal saline water with a sterile inoculating loop. The suspension was vortexed and visually matched with a 0.5 MacFarland standard for turbidity (Tendencia, 2004). Then, the isolates in the suspension were grown on the Mueller Hinton agar with a sterile cotton swab carefully covering the whole surface of the media to produce full growth across the media. After allowing to dry for 30 minutes at room temperature and checked for extra moisture, discs that had been impregnated with specific concentrations of antimicrobials were placed on the bacterial lawn and incubated at 37°C for 18 to 24 hours.

For the current investigation, 16 antimicrobial agents that are commonly used in both veterinary and human health care. These includes, penicillins (ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), sulbactam/Ampicillin (30 µg)); cephalosporins (cefotaxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg)); phenicols (chloramphenicol (30µg)); carbapenems (meropenem (10 µg)); fluoroquinolones (ciprofloxacin (10 µg)), folate pathway inhibitors (sulfamethoxazole (30 µg), trimethoprim/sulfamethoxazole (10 µg), trimethoprim (5 µg)); aminoglycosides (streptomycin (10 µg), gentamicin (10 µg)); tetracyclines (tetracycline (30 µg), oxytetracycline (30µg)) and macrolide (erythromycin (15 µg)). Afterward, the bacteria were interpreted as sensitive, intermediate, or resistant against applied antimicrobials by measuring the inhibition zones in millimeters using a ruler by adopting the Clinical and Laboratory Standards Institute's cut points (CLSI, 2020) (). This antibiogram study was conducted in the Wachemo University medical microbiology laboratory. Those resistant to at least one drug of three or more antimicrobial classes were classified as MDR (Cilloniz *et al.*, 2019).

3.8 Materials, Equipment, Reagents and Chemicals

Throughout the current study session from sampling to the result reading, the following materials, equipment, reagents and chemicals were used. The materials glove, laboratory coats, biohazard baskets, marker pen, data sheet, cotton, aluminum foil and cotton-tipped swab. The equipment used includes icebox, bunsen burner, laboratory incubator (DNP 9052, ARI medical china), safety cabinet (ABS-78IIB3, ARI medical china), autoclave, refrigerator (AMR168, ARI medical china), volumetric flasks, test tube rack, spatula, pasture pipette, microscopic slide,

measuring balance, inoculating loops, test tube, forceps, Petri dishes, vortex, pestle and mortar, scissors, scalpel blade, and universal bottles.

The reagents and chemical like distilled/ laboaratory grade water, ethanol (70%), sterile saline solution, buffered peptone water, 40% glycerin, (HiMedia™, M1494I Pvt. Ltd., India), MacConkey Sorbitol Agar (HiCrome™, M1340, Pvt. Ltd., India), MacConkey agar (HiMedia™, M081 Pvt. Ltd., India), MacConkey agar (HiMedia™, M081 Pvt. Ltd., India) Triple sugar iron agar (Huankai Microbial, HCM 014, Sci. & Tech. Co., Ltd., China), indole product (HiMedia™, R008, Pvt. Ltd., India), Rappaport-Vassiliadis broth (HiMedia™, M1467 Pvt. Ltd., India), Xylose Lysine Deoxycholate agar (Biomrk, B819, Pvt. Ltd., India), Salmonella Shigella agar (Biomark, B361, Pvt. Ltd., India), Nutrient agar (HiMedia™, M001, Pvt. Ltd., India), Mueller Hinton agar (Sisco Research Laboratory, MM019, Pvt. Ltd., India), antimicrobial discs (listed in section 3.7.3) (Thermo Scientific™ Oxoid™, India), *Salmonella* antiserum (SSI Diagnostica A/S, Herredsvejen 2, 3400 Hillerod, Denmark), O157 and monovalent H7 antiserum (BD Difco™, fisher scientific, BD 221545, Gothenburg-Sweden), Kovac's reagent (Merck Millipore, 109293, Sigma Aldrich, Germany), Simon citrate agar (Merck Millipore, S 8539, Sigma Aldrich, Germany) and SIM media (HiMedia™, M180 Pvt. Ltd., India F) were used.

3.9 Assessment of Poultry Producers' KAP Towards Antimicrobial Use and Resistance

The KAP of conventional poultry farm producers towards AMU and AMR were assessed by instilling questions ascertaining the issue. In the first round, a questionnaire was instilled to 10 respondents to test the questionnaire and the overall response of the target persons. Afterward, the questionnaire was restructured following the responses of the pretest respondents and was instilled through face-to-face interviews by explaining the idea of the questionnaire in plain language the respondents could understand. The questionnaire was targeted at the owners and the permanent employees of the farm. These individuals were interviewed on issues of knowledge including 12 questions, attitude including 7 questions and practice including 11 questions on drug use and AMR for about 30 minutes in their work station (Annex 5). In a dichotomous question, the correct answers were scored as one, while the incorrect were scored as zero. In questions with three options, correct, neutral and incorrect answers were scored as 2,

1, and 0, respectively. Scores ranging from 1 to 5 were assigned to responses on the five-point Likert scale (strongly agree = 5, agree = 4, neutral = 3, disagree = 2, and strongly disagree = 1) type questions. Finally, all scores were added up to change to discrete variables and rated on a 100-point scale (%), with the maximum probable score as 100% (Wang *et al.*, 2022). The knowledge of respondents was graded as good (76%-100), moderate (51%–75%), or poor (<50%); attitude as good (80%-100), fair (60%–75%), or poor (<50%); and practices as good (80%-100), fair (60%–79%), or poor (<51%) with moderate modification of Bloom's original cutoff points as adopted by (Baig *et al.*, 2020).

3.10 Ethical Approval and Consent of Participation

All investigations conducted on animals were conducted in stern accordance with the World Organization for Animal Health's guiding principles of animal welfare. Ethical approval for the current investigation was obtained from Addis Ababa University, College of Veterinary Medicine and Agriculture Institutional Animal Care and Use Committee (Reference number VM/ERC/02/10/162024). In addition to this, full permission and consent of the farm owners were obtained with full rights of the owners kept by explaining the purpose of the study and the level of effect of the investigation on their animals clearly with understandable language. While sampling live chickens, all the strict hygienic, safety, as well as welfare measures, were adopted.

3.11 Data Management and Statistical Analysis

Data collected from the questionnaire survey and laboratory study was entered into a Microsoft Excel Spreadsheet and analyzed using STATA version 14.0 for Windows (Stata Corp. College Station, TX, USA). The frequency of resistance, intermediate susceptibility and susceptibility of isolates to the tested antimicrobials was computed. Before data analysis, the normality test was done graphically using the Shapiro–Wilk test; thereafter, the rate of antimicrobial resistance of the isolates against each drug was determined by computing the frequency table. The multiple antimicrobial resistance (MAR) index was identified and interpreted by adopting the formula developed by Krumperman (1983) as a/b, in which ‘a’ the number of antimicrobials to which test isolates exhibited resistance, and ‘b’ denotes the entire number of antimicrobials tested. Isolates showing MAR index ≥0.2 were considered to be isolated from an origin where many antimicrobials are used most frequently with a high menace of contamination (Krumperman,

1983). The antimicrobial resistance (R) score was computed by adopting the model used by (De *et al.*, 2019). A score of 0, 0.5 and 1 were allocated for each isolate showing susceptibility, intermediate susceptibility, and resistance, respectively, to specific antimicrobial agents. Then, R score was defined by calculating the ratio of the sum of scores to the total number of antimicrobial agents subjected, those isolates showing R scores of 0 and 1 are designated as pandrug-susceptible and pandrug-resistant, respectively. The concurrent resistance to different antimicrobial agents was checked by computing spearman pairwise correlation test. Differences in MAR and R score with chicken type and genus of bacteria were computed by the Kruskal-Wallis test. In addition, spearman rank correlation was used to identify parallel resistance to the antimicrobials. The association of sociodemographic factors with the KAP was analyzed by linear regression at a 0.05 significance level and 95% confidence level. The correlation among the knowledge, attitude and practice of the respondents was evaluated by Pearson pairwise correlation at a 0.05 significance level.

4 RESULTS

4.1 Bacterial Isolation and Identification

In this investigation, from a total of 228; 200 cloacal swabs and 28 organ samples, 47 (20.61%) were positive for both *Salmonella* 27 (11.84%) and *E. coli* 20 (8.77%). Out of 20 *E. coli* isolates, 15 (75.00%) were from cloacal swabs and 5 (25.00%) were from postmortem samples. Of the 27 *Salmonella* isolates, 19 (70.37%) were from cloacal swabs and 8 (29.63%) were from postmortem samples. Out of 15 *E. coli* and 19 *Salmonella* cloacal swab isolates, 10 (66.7%) and 15 (78.95%) were isolated from healthy-looking chicken and 5 (33.3%) and 4 (21.05%) were from diseased chicken, respectively.

4.2 Serological test

A total of 29 bacterial isolates were identified as *Salmonella* through bacteriological and biochemical tests. From this, 27 isolates were confirmed as *Salmonella* and serotyped to different serovars by serological slide agglutination test. Up on serological test, *Salmonella* serogroup D serovars *S. Enteritidis*, *S. Gallinarum*, *S. Pullorum*, and *S. Dublin* and serogroup B *S. Typhimurium* and unknown serotype (other) with respective frequencies of 9(33.3%), 5(18.5%), 3(11.1%), 1(3.7%) and 7(25.9%) and 2(10.5%) were identified. Among the Salmonella serovars, *S. Enteritidis* and *S. Typhimurium* most frequent. Regarding *E. coli*, interestingly all the isolates (20) identified in bacterial culture and biochemical tests were serologically confirmed as *E. coli* O157:H7.

4.3 Antibiogram Profile of *Salmonella* Isolates

In the current investigation, all 27 *Salmonella* isolates exhibited resistance to ≥5 antimicrobial agents tested at different rates. The highest rate of resistance was recorded against ampicillin 27 (100%) followed by sulfamethoxazole and cefoxitin 23(85.1%), tetracycline 21(77.8%), trimethoprim/sulfamethoxazole 20(74.1%), amoxicillin-clavulanic acid 18(66.6%), ceftriaxone and streptomycin 15(55.6%), ciprofloxacin 14(51.9%), ampicillin/sulbactam 13(48.1%), trimethoprim 12(44.4%) and cefotaxime 11(40.7%). Higher susceptibility was recorded to the remaining antimicrobial agents meropenem 22(81.48%), gentamicin 18(66.67), chloramphenicol 25(92.59%), and azithromycin 22(81.48%). Regarding the class of

antimicrobials tested, higher resistance level was recorded in penicillin groups (100% in AMP) followed by folate synthesis inhibitors (85.19% to sulfamethoxazole) and cephalosporins (74.07% to cefoxitin). Conversely, good invitro antimicrobial activity was documented in antimicrobial classes including carbapenem (81.48% to meropenem), macrolides (81.48% to azitromycin), aminoglycosides (66.67% to gentamicin), phenicol (66.67% to chloramphenicol) and quinolones (48.15% to ciprofloxacin) (Table 1).

Table 1. Antibiogram profile of *Salmonella* (n=27)

Antimicrobial classes	**Antimicrobial agents**	**Number of susceptible isolates (%)**	**Number of intermediate isolates (%)**	**Number of resistant isolates (%)**
Penicillins	AMC	6(22.22)	3(11.11)	18(66.67)
	AMP	0(0.0)	0(0.0)	27(100)
	AMS	10(37.04)	4(14.81)	13(48.15)
Cephalosporins	CTX	8(29.63)	8(29.63)	11(40.74)
	CRO	11(40.74)	1(3.70)	15(55.56)
	FOX	2(7.41)	5(18.52)	20(74.07)
Aminoglycosides	S	12(44.44)	0(0.0)	15(55.56)
	CN	18(66.67)	6(22.22)	3(11.11)
Folate synthesis inhibitors	SMX	1(3.70)	3(11.11)	23(85.19)
	SXT	7(25.93)	0(0.0)	20(74.07)
	TRI	11(40.74)	4(14.81)	12(44.44)
Phenicols	C	18(66.67)	7(25.93)	2(7.41)
Carbapenem	MEM	22(81.48)	0(0.0)	5(18.52)
Macrolides	AZM	22(81.48)	4(14.81)	1(3.70)
Tetracyclines	TE	6(22.22)	0(0.0)	21(77.78)
Quinolone	CIP	13(48.15)	0(0.0)	14(51.85)

AMP-Ampicillin, AMC-Amoxicillin/clavulanic acid, AMS-Ampicillin/Sulbactam, CTX-Cefotaxime, CRO-Ceftriaxone, FOX-Cefoxitin, MEM-Meropenem, CIP-Ciprofloxacin, C-Chloramphenicol, SMX-Sulfamethoxazole, SXT-Trimethoprim/sulfamethoxazole, TRI-Trimethoprim, S-Streptomycin, CN-Gentamicin, TE-Tetracycline, AZM-Azithromycin

4.3.1 Species-level antimicrobial resistance of Salmonella isolates

Out of 9 *S. Enteritidis* isolates, 9 (100%) exhibited resistance against ampicillin, cefoxitin and sulfamethoxazole. From tested isolates, 7(77.8%) were resistant to amoxicillin/clavulanic acid, 3(44.4%) were resistant against ampicillin/sulbactam 6(66.7%) were resistant to trimethoprim/sulfamethoxazole and streptomycin, 5(55.6%) have shown non-susceptibility to ceftriaxone, tetracycline and ciprofloxacin. The majority 7(77.8%), 6(66.7%) and 5(55.6%) of the *S. Enteritidis* were susceptible to azithromycin, meropenem and chloramphenicol and gentamicin, respectively. Of the 5 *S. Gallinarum* 5(100%), 4(80%) and 3(60%) resistant against ampicillin and trimethoprim/sulfamethoxazole, streptomycin, amoxicillin/clavulanic acid, cefoxitin, sulfamethoxazole and trimethoprim and tetracycline, respectively. Conversely, all, 4 (80.0%) were susceptible to azithromycin and ciprofloxacin, meropenem, cefotaxime and gentamicin, respectively. All *S. Typhimurum* were non-susceptible to ampicillin, and tetracycline. About, 85.7% (6/7), 71.4% (5/7), 57.1% (4/7) and 42.9% (3/7) of the tested *S. Typhimurium* were resistant to sulfamethoxazole and trimethoprim/sulfamethoxazole, ampicillin, cefotaxime, ceftriaxone and trimethoprim, and streptomycin and ciprofloxacin, respectively. None of the *S. Typhimurium* were resistant to chloramphenicol, azithromycin and Gentamicin and the majority 85.7% (6/7) were susceptible to meropenem. From *S. pullorum* tested, all 100% (3/3), and 66.7% (2/3) exhibited non-susceptibility to ampicillin, cefoxitine and sulfamethoxazole, and amoxicillin/clavulanic, ampicillin/sulbactam, cefotaxime, streptomycin and tetracycline, respectively. The single isolate *S. Dublin* exhibited non-susceptibility to ampicillin, ceftriaxone, sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim and tetracycline. The two unknown serovars of *Salmonella* isolates were resistant to cefotaxime and meropenem (Table 2).

Table 2. Species-level antimicrobial resistance profile of *Salmonella* isolates

	***Salmonella* serotypes**																							
Antimicrobials	***S. Enteritidis (9)***				***S. Gallinarum (5)***				***S. Typhimurium (7)***				***S. Pullorum (3)***				***S. Dublin (1)***				***Other (2)***			
	R	**I**	**S**	**%R**	**R**	**I**	**S**	**%R**	**R**	**I**	**S**	**%R**	**R**	**I**	**S**	**%R**	**R**	**I**	**S**	**%R**	**R**	**I**	**S**	**%R**
AMC	7	1	1	77.8	3	0	2	60.0	5	0	2	71.4	2	1	0	66.7	0	1	0	0	1	0	1	50.0
AMP	9	0	0	100	5	0	0	100.0	7	0	0	100	3	0	0	100	1	0	0	100	0	0	2	100
AMS	3	1	4	33.3	2	3	0	40.0	1	2	4	14.3	2	1	0	66.7	0	0	1	0.0	1	0	1	50.0
CTX	3	4	2	33.3	1	3	1	20.0	3	0	4	42.9	2	0	1	66.7	0	1	0	0.0	2	0	0	100
CRO	5	3	1	55.6	4	0	1	80.0	3	0	4	42.9	1	2	0	33.3	1	0	0	100	1	1	0	50.0
FOX	9	0	0	100	3	1	2	60.0	4	3	0	57.1	3	0	0	100	0	0	1	0.0	1	1	0	50.0
S	6	0	3	66.7	4	0	1	80.0	3	0	4	42.9	2	0	1	66.7	0	0	1	0.0	0	0	2	0.0
CN	2	2	5	22.2	1	0	4	20.0	0	2	5	0.00	0	0	3	0.0	0	0	1	0.0	0	2	0	0.0
SMX	9	0	0	100	3	2	0	60.0	6	1	0	85.7	3	0	0	100	1	0	0	100	1	0	1	50.0
SXT	6	0	3	66.7	5	0	0	100	6	0	1	85.7	1	0	2	33.3	1	0	0	100	1	0	1	50.0
TRI	3	2	4	33.3	3	0	2	60.0	3	1	3	42.9	1	2	0	33.3	1	0	0	100	1	1	0	50.0
C	1	2	6	11.1	2	2	1	40.0	0	2	5	0.0	0	0	3	0.0	0	0	1	0.0	0	2	0	0.0
MEM	3	0	6	3.33	1	0	4	20.0	1	0	6	14.3	0	1	2	0.0	0	0	1	0.0	2	0	0	100
AZM	1	1	7	11.1	0	0	5	0.0	0	0	7	0.00	0	0	3	0.0	0	0	1	0.0	0	1	1	0.0
TE	5	1	3	55.6	3	0	2	60.0	7	0	0	100	2	1	0	66.7	1	0	0	100	1	0	1	50.0
CIP	5	0	4	55.6	1	0	4	20.0	4	1	2	57.1	1	0	2	33.3	0	0	1	0.0	1	0	1	50.0

R= resistant; I= intermediate; S= susceptible; %R= frequency of resistant; AMP-Ampicillin, AMC-Amoxicillin/clavulanic acid, AMS-Ampicillin/Sulbactam, CTX-Cefotaxime, CRO-Ceftriaxone, FOX-Cefoxitin, MEM-Meropenem, CIP-Ciprofloxacin, C-Chloramphenicol, SMX-Sulfamethoxazole, SXT-Trimethoprim/sulfamethoxazole, TRI-Trimethoprim, S-Streptomycin, CN-Gentamicin, TE-Tetracycline, AZM-Azithromycin

4.4 Antibiogram Profile of *E. coli* O157:H7 Isolates

All the 20 *E. coli* O157:H7 isolates were found resistant to at least three antimicrobial agents tested. The highest rate of resistance was recorded against ampicillin 20(100%) followed by cefotaxime and tetracycline 18(90.0%), cefoxitin and trimethoprim 14(70.0%), sulfamethoxazole 11(65.0%), amoxicillin-clavulanic acid and ceftriaxone 11(55.0%) and ampicillin/sulbactam 10(50.0%). Interestingly none of the *E. coli* O157:H7 isolates possessed nonsusceptibility to the azithromycin to which 18(90.0%) were susceptible with 2(10.0%) exhibiting intermediate susceptibility. Better invitro antimicrobial activity was documented for the antimicrobial agents including gentamicin 15(75.0%), chloramphenicol 12(60.0%), ciprofloxacin 11(55.0%), meropenem 10(50.0%) and sulfamethoxazole-trimethoprim 9(45.0%). In relation to the antimicrobial class under study, a higher resistance trend was recorded in penicillin groups, followed by cephalosporins and tetracyclines and folate synthesis inhibitors. In contrast, a higher susceptibility rate was documented for macrolides followed by phenicols, aminoglycosides, quinolones and carbapenems (Table 3).

Table 3. Overall antibiogram profile of *E. coli* O157:H7 isolates

Antimicrobial classes	**Antimicrobial agents**	**Susceptible isolates n(%)**	**Intermediate isolates n(%)**	**Resistant isolates n(%)**
Penicillins	AMC	8(40.0)	1(5.0)	11(55.0)
	AMP	0(0.0)	0(0.0)	20(100)
	AMS	3(15.0)	7(35)	10(50.0)
Cephalosporins	CTX	0(0.0)	2(10.0)	18(90.0)
	CRO	5(25.0)	3(15.0)	12(55.0)
	FOX	3(15.0)	3(15.0)	14(70.0)
Aminoglycosides	S	7(35.0)	6(30.0)	7(35.0)
	CN	15(75.0)	1(5.0)	4(20.0)
Folate synthesis	SMX	4(20.0)	3(15.0)	13(65.0)
inhibitors	SXT	9(45.0)	4(20.0)	7(35.0)
	TRI	6(30.0)	0(0.0)	14(70.0)
Phenicols	C	12(60.0)	5(25.0)	3(15.0)
Carbapenem	MEM	10(50.0)	3(15.0)	7(35.0)
Macrolides	AZM	18(90.0)	2(10.0)	0(0.0)
Tetracyclines	TE	0(0.0)	2(10.0)	18(90.0)
Quinolone	CIP	11(55.0)	5(25.0)	4(20.0)

AMP-Ampicillin, AMC-Amoxicillin/clavulanic acid, AMS-Ampicillin/Sulbactam, CTX-Cefotaxime, CRO-Ceftriaxone, FOX-Cefoxitin, MEM-Meropenem, CIP-Ciprofloxacin, C-Chloramphenicol, SMX-Sulfamethoxazole, SXT-Trimethoprim/sulfamethoxazole, TRI-Trimethoprim, S-Streptomycin, CN-Gentamicin, TE-Tetracycline, AZM-Azithromycin

4.5 MDR, Multiple Antimicrobial Resistance (MAR) Index and Resistance (R) score

All 27 *Salmonella* isolates exhibited MDR to a minimum of 3 and maximum of 8 antimicrobial classes out of 9 antimicrobial groups tested. From the *Salmonella* isolates, 1(3.7%), 1(3.7%), 4(14.8%), 12(44.4%), 4(14.8%) and 5(18.5%) isolates exhibited MDR to 8, 7, 6, 5, 4 and 3 antimicrobial classes understudy, respectively. From the E. coli O157:H7 isolates, 16(80.0%) exhibited MDR, from which 1(5.0%), 2(10.0%), 6(30.0%), 5(25.0%) and 1(5.0%) of isolates were resistant to 7, 6, 5, 4 and 3 antimicrobial classes tested, respectively. The remaining 20% were not multidrug-resistant (Figure 4).

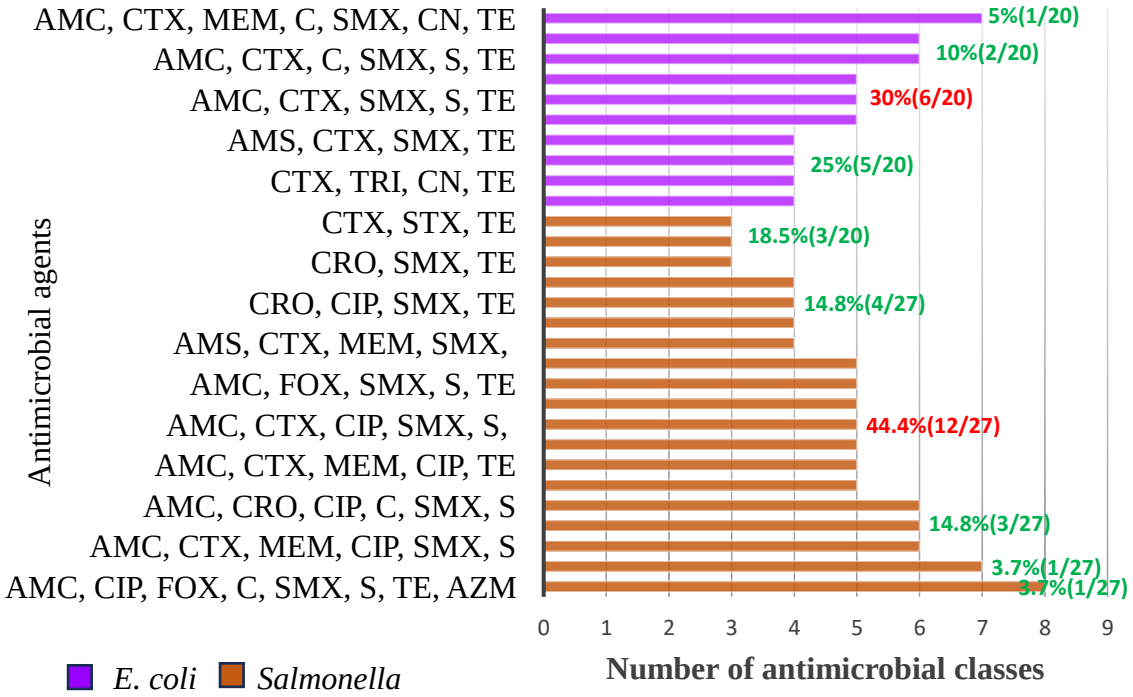
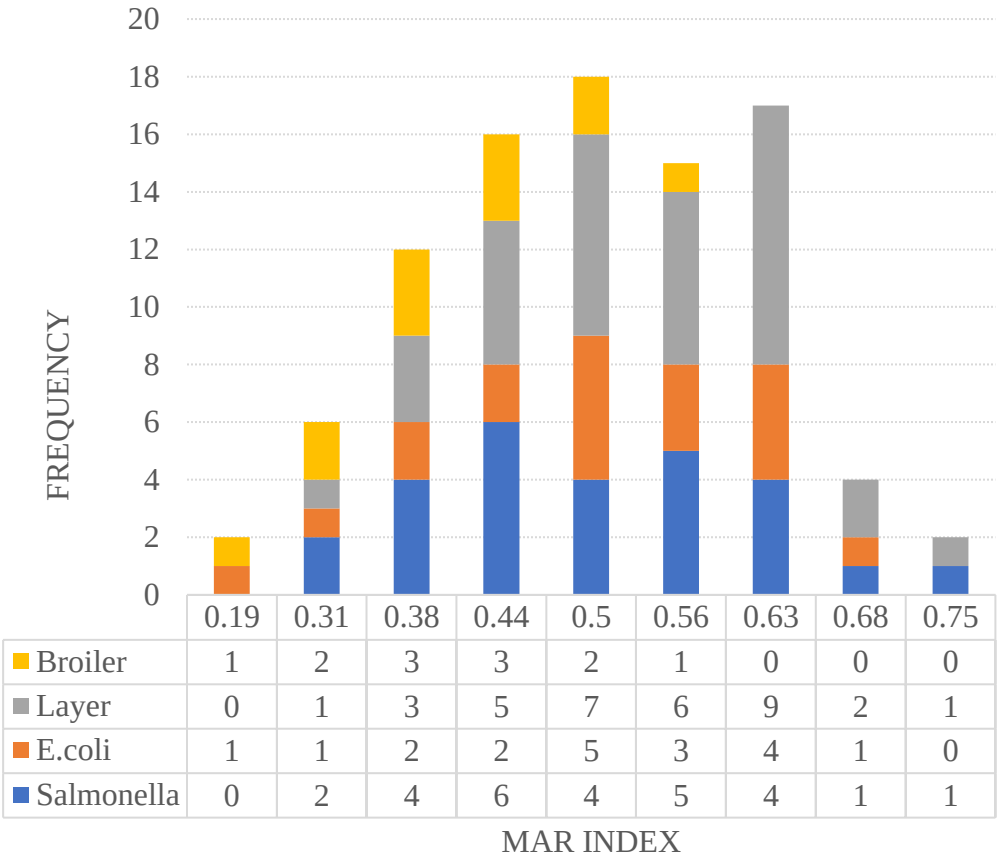


Figure 2. MDR pattern of *Salmonella* and *E. coli*. Note: isolates showing the repeated MDR pattern to the same antimicrobial class was removed from the list of antimicrobial agents.

For current study, multiple antimicrobial resistance (MAR) index for both bacteria was computed by dividing the number of antimicrobials to which each bacteria isolate was resistant by the total number of antimicrobials tested (n=16). All the *Salmonella* and 95% of *E. coli* isolates exhibited MAR index higher than 0.2. The MAR index of *Salmonella* tested ranges from 0.31(5/16) to 0.75 (12/16) with an average of 0.53 and that of *E. coli* was recorded as 0.19 (3/16) to 0.55 (11/16) with an average of 0.35. MAR index for broiler chicken ranges from 0.19 to 0.56

with an average of 0.38 and for layers was from 0.31 (5/16) to 0.75 (12/16) with an average of 0.53. In bacteria isolated from layers, the most frequent MAR index recorded was 0.63 (10/16), 0.56 (9/16), 0.5 (8/16), 0.44 (7/16) and 0.38 (6/16) with respective rate of 26.47% (9/34), 17.65% (6/34), 20.59% (7/34), 14.71% (5/34) and 8.82% (3/34). MAR index of bacteria isolated from broiler chickens was 0.56, 0.5, 0.44, 0.38, 0.31 and 0.19 in 7.69% (1/13), 15.38% (2/13), 23.08% (3/13), 23.08% (3/13) and 7.69% (1/13) of isolates, respectively. Highest MAR index 0.75 (12/16) was recorded in bacteria isolated from layer chicken. Only single isolate from broiler chicken exhibited MAR index (0.19) lower than 0.2 (Figure 5). The difference in MAR index between broiler and layer type of chicken was statistically significant (P= 0.001, X^2=9.559) (Table 6).

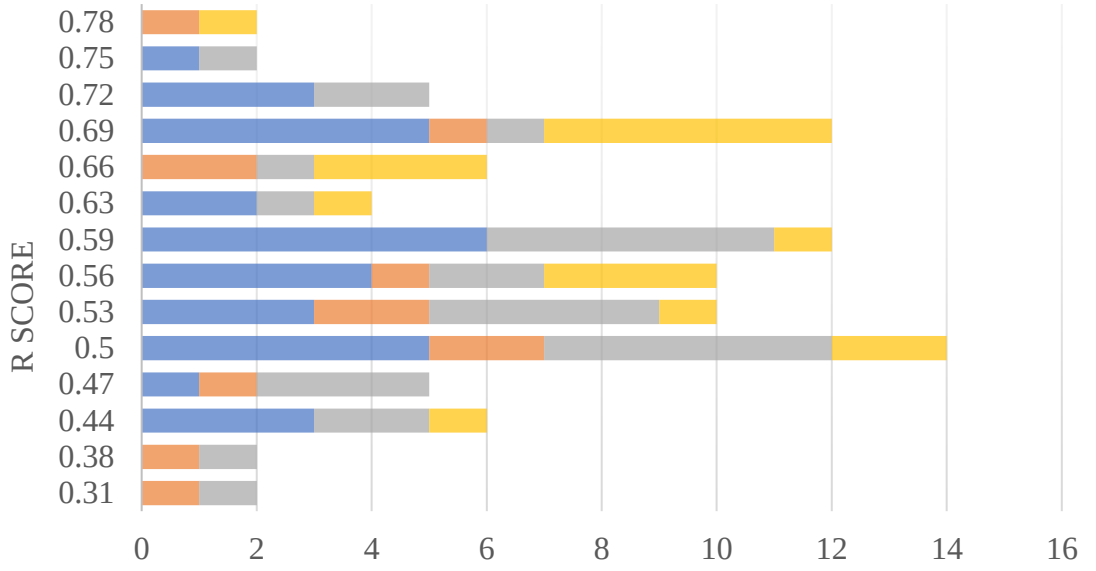


	0.19	0.31	0.38	0.44	0.5	0.56	0.63	0.68	0.75
Broiler	1	2	3	3	2	1	0	0	0
Layer	0	1	3	5	7	6	9	2	1
E.coli	1	1	2	2	5	3	4	1	0
Salmonella	0	2	4	6	4	5	4	1	1

Figure 3. Graphical presentation of MAR index

Most (78.72%) of the isolates under investigation exhibited R score ≥ 0.5 (high/very high AMR) and the remaining 22.28% has shown R score lower than 0.5 (low/very low AMR). Regarding genus of bacteria, highest R score (0.78) was documented in *E. coli*. About 81.48% of the

Salmonella exhibited high/very high AMR and 85.0% of *E. coli* revealed high/very high AMR (R score ≥ 0.5) (Figure 6). The difference in R score between the two genus of bacteria was statistically significant (P=0.002, X^2= 9.204) (Table 4). R score in relation to the type of chicken, high/very high AMR R score ≥ 0.5) was observed in 85.2% of the isolates from layer and 69.23% of isolates from broiler chicken (Figure 6). Most frequent R score fall under the region greater than 0.5 showing high/very high AMR pattern. The difference in R score between the two types of chicken was statistically nonsignificant (P= 0.2032, X^2= 1.619) (Table 4).



	0.31	0.38	0.44	0.47	0.5	0.53	0.56	0.59	0.63	0.66	0.69	0.72	0.75	0.78
Layer	0	0	3	1	5	3	4	6	2	0	5	3	1	0
Broiler	1	1	0	1	2	2	1	0	0	2	1	0	0	1
Salmonella	1	1	2	3	5	4	2	5	1	1	1	2	1	0
E.coli	0	0	1	0	2	1	3	1	1	3	5	0	0	1

Figure 4. Graphical presentation of Resistance score

Table 4. Correlation between the chicken type and genus of bacteria with MAR index and R score

Variables		**MAR index**			**R score**		
		X^2	P-value	df.	X^2	P-value	df.
Chicken type	Layer Broiler	9.559	0.001	1	1.619	0.2032	1
Genus of bacteri	*Salmonella* *E. coli*	0.245	0.6207	1	9.204	0.002	1

X^2-Kruskal Wallis chi square, df. -degree of freedom

There was concurrent resistance among cefotaxime, ceftriaxone, meropenem and amoxicillin-sulbactam, cefoxitin, ceftriaxone, ciprofloxacin, and meropenem; sulfamethoxazole, sulfamethoxazole-trimethoprim, amoxicillin, ciprofloxacin and sulfamethoxazole, gentamicin and azithromycin. There was a significantly strong correlation between the cefotaxime and amoxicillin-sulbactam (r_s = 0.353*), cefoxitin and meropenem (r_s = 485*) and chloramphenicol and ciprofloxacin (r_s = 0.321*), sulfamethoxazole and meropenem (r_s = 0.324*). Isolates resistant to the sulfamethoxazole-trimethoprim showed resistance to the ceftriaxone with a significantly strong correlation (r_s = 0.431*). There was also a significant correlation between the azithromycin resistance and amoxicillin-sulbactam resistance (r_s = 0.341*). A significant negative correlation was observed between trimethoprim and cefoxitin (r_s = **-0.268***), tetracycline and ceftriaxone (r_s = **--0.424***) and azithromycin and meropenem (r_s = **-0.321***) (Figure 7).

	AMC													
AMC	1.000	**AMS**												
AMS	0.013	1.000	**CTX**											
CTX	-0.171	**0.353***	1.00	**CRO**										
CRO	-0.061	0.074	0.124	1.00	**MEM**									
MEM	-0.052	0.087	0.142	-0.060	1.00	**CIP**								
CIP	0.079	-0.061	-0.009	-0.013	0.07	1.00	**FOX**							
FOX	0.091	-0.063	-0.153	0.183	**0.485***	0.130	1.00	**C**						
C	-0.221	0.025	0.027	0.085	0.08	**0.321***	0.122	1.00	**SMX**					
SMX	0.227	0.250	-0.156	0.114	**0.324***	-0.076	00.095	0.003	1.00	**STX**				
STX	0.121	0.057	0.033	**0.431***	-.13	-0.015	-0.043	0.042	0.187	1.00	**TRI**			
TRI	-0.103	-0.069	0.013	-0.237	0.00	-0.060	**-0.268***	0.079	-0.116	0.207	1.0	**S**		
S	0.222	0.072	0.088	-0.084	-0.08	0.026	-0.121	0.060	-0.144	-0.042	-0.079	1.00	**CN**	
CN	-0.084	0.091	0.171	0.168	0.05	0.107	0.195	0.032	-0.077	-0.023	-0.188	-0.032	1.00	**TE**
TE	0.169	-0.094	-0.173	**-0.424***	0.28	-0.008	-0.237	-0.074	-0.132	-0.275	-0.155	0.204	-0.030	1.00	**AZM**
AZM	0.137	**0.341***	-0.185	0.123	**-0.321***	0.004	-0.105	0.041	0.072	-0.071	-0.156	-0.041	-0.137	0.080	1

Figure 5. Pairwise correlation amongst different antimicrobials tested against both bacteria

*P-value < 0.05, r_s= Spearman coefficient.

4.7 KAP of the Conventional Poultry Producers Towards the AMU and AMR.

4.7.1 Sociodemographic Characteristics of the respondents

A total of 36 respondents including farm owners, managers and any permanent employee engaged in conventional poultry production were included and interviewed to assess their KAP towards the AMU and AMR up on full consent for participation. From this, the majority 80.6% were males, aged ≥ 40, around 40% had high school educational level followed by vocational 27.78%, elementary 13.9%, university degree 11.1% and illiterate 8.3%. About 44.44 have three years of poultry production experience followed by 1 year 16.67%. Most of producers raise layer type of chickens (67.7%) and the remaining 33.3% raise broilers. About 52.78%, 25.00% and 22.22% of the respondents were from 10 farms raising <1000 chickens per farm, 4 farms raising 1000-1500 chickens per farm and 2 farms raising >1500 chickens per farm. Three forth (75%) of the poultry producers have no prior information, exposure or training on veterinary practices as well as poultry production.

4.7.2 KAP of respondents and its association with sociodemographic characteristics

The level of KAP of the poultry producers included in the current KAP assessment was evaluated by scoring the consistency of their responses to the questions forwarded to them. About 50.0% (18/36) of the respondents have poor knowledge about AMU and AMR. The lowest portion 22.20% (8/36) of the respondents have good knowledge of the AMU and AMR. About 47.22% (17/36) of the poultry producers interviewed during the current study has poor attitude towards the AMU and AMR and the lowest portion 19.44% (7/36) revealed good attitude on AMU and AMR. Regarding their practice towards the AMU and AMR, 38.90% (14/36) and 41.60% (15/36) of the respondents have fair and poor practice towards AMU and AMR. Good practice towards the AMU and AMR was observed in lowest portion 25% (9/36) of the respondents (Figure 8).

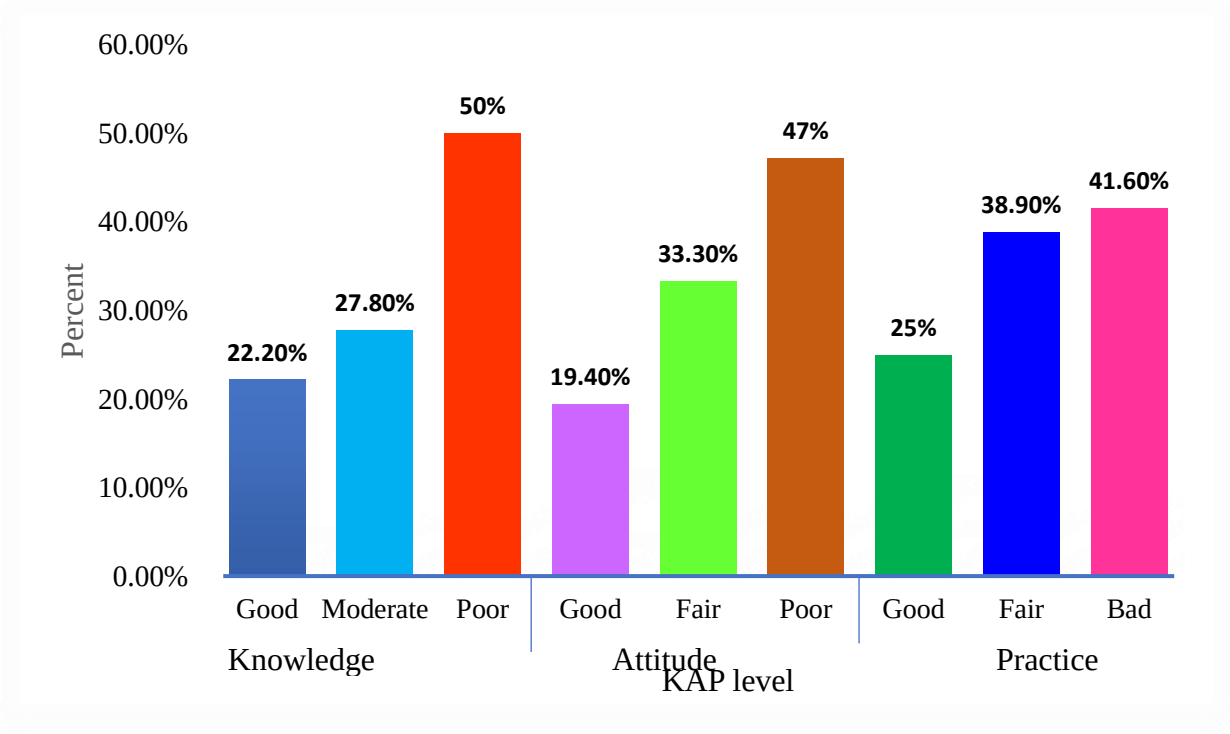


Figure 6. Graphical representation of poultry producers' KAP level

The Pearson pairwise correlation revealed that there was a strong correlation between knowledge and attitude (r = 0.5506, p= 0.0005), between knowledge and practice (r = 0.6420, p = 0.0000) and between attitude and practice (r = 0.5337, p = 0.0008) (Table 8).

Table 5. Correlation among Knowledge, attitude and practice

Variables	Knowledge	Attitude	Practice	Number
Knowledge				
Pearson correlation coef.	1			36
Sig. (2-tailed)				
Attitude				
Pearson correlation coef.	0.5506*	1		36
Sig. (2-tailed)	0.0005			
Practice				
Pearson correlation coef.	0.6420*	0.5337*	1	36
Sig. (2-tailed)	0.0000	0.0008		

coef. - Coefficient

The educational level of the poultry producers in the current study has a statistically significant (P<0.005) effect on their knowledge, attitude and practice on AMU and AMR. While keeping those having no formal education constant, KAP increased by 1.75, 2 and 2 units in those producers who studied university respectively. The KAP level increase by factor of 0.7, 0.45

and 0.05 in those having four years of poultry production experience while keeping those with zero-year constant but the difference was statistically insignificant. The level of KAP increases by 1, 1, and 1.054 units respectively, in those raising layer chicken while keeping those producing broilers constant with high statistical significance (p=000). The level of KAP increases by 0.483, 0.526 and 1.041 units respectively, in those raising 1000-1500 and 0.769, 0.528 and 0.505 units in those raising >1500 chickens per flock but the effect was not statistically significant (p>0.05). KAP level increased by 0.963, 0.519 and 0.333 units respectively, in those taken training before but the effect was not statically significant (p>0.05). Level of knowledge, attitude and practice significantly (p<0.05) decreases by -1.484, -1.026, and -1.323 factors respectively, in those having non-health jobs (Table 9).

Table 6. Association of sociodemographic characteristics and KAP of poultry producers

Variables	**Knowledge**			**Attitude**			**Practice**		
	Coef.	**P-value**	**95% CI**	**Coef.**	**P-value**	**95% CI**	**Coef.**	**P-value**	**95% CI**
Educational level									
No formal education	Ref.								
Elementary	0.2	0.611	-0.594-0.994	5.35e-16	1.000	-0.851-0.851	0.2	0.653	-0.698-1.09
High school	0.286	0.406	-0.406-0.977	0.857	0.087	-0.098-1.384	0.072	0.104	-0.0676-1.496
Vocational	1.4	0.000	0.684-2.116	1.3	0.023	0.133-1.667	1.2	0.005	0.391-2.009
University	1.75	0.000	0.920-2.580	2	0.000	1.110-2.889	2	0.000	1.061-2.939
Year of experience									
0yr	Ref.								
1yr	-0.133	0.782	-1.110-0.842	-0.467	0.334	-1.436-0.503	0.233	0.613	-1.163-0.697
2yrs	-0.6	0.239	-1.620-0.419	-3.13e-16	1.000	-1.013-1.013	-0.6	0.217	-1.571-0.371
3yrs	-0.113	0.783	-0.939-0.713	-0.113	0.782	-0.933-0.708	-0.575	0.026	-1.687--0.113
4yrs	0.7	0.196	-0.381-1.781	0.45	0.400	-0.624-1.524	0.05	0.769	-1.180-0.880
Purpose of chicken									
Broiler	Ref.								
Layer	1	0.000	0.481-1.518	1.0	0.000	0.5148-1.485	1.054	0.000	0.564-1.544
Stocking size									
<1000	Ref.								
100-1500	0.483	0.003	0.367-1.432	0.526	0.036	0.062-1.310	1.041	0.048	0.147-1.062
>1500	0.769	0.005	0.283-1.236	0.528	0.049	0.003-1.054	0.505	0.067	-0.038-1.048
Prior training									
No	Ref.								
Yes	0.963	0.001	0.410-1.515	0.519	0.084	-0.072-1.109	0.333	0.284	-0.289-0.956
Job type									
Health-related	Ref.								
Non health	-1.484	0.000	-2.107--0.861	-1.026	0.009	-1.781--0.271	-1.323	0.000	-1.968--0.67

Ref.- reference value; Coef. -coefficient; 95%CI-95% confidence interval

5 DISCUSSION

Today, the emergence of mono-drug and multi-drug resistant bacteria in poultry production is serious to poultry productivity and one health threat across the world (Hamed *et al.*, 2021). This is further fueled by the fact that poultry is among the quick-growing per capita meats produced globally with a 5% international annual growth rate (Hedman *et al.*, 2020). Determination of the antimicrobial resistance pattern of *Salmonell*a and *E. coli* O157:H7 in poultry farms is very crucial in order to implement prevention strategies to tackle the effect of antimicrobial-resistant *Salmonella* and *E. coli* O157:H7 on poultry health and production as well as environmental and public health (Grzinic *et al.*, 2023). In the current investigation, from a total of 228; 200 cloacal swabs and 28 organ samples cultured, 47 (20.61%) were found positive for both *Salmonella* 11.84% (27/228) and *E. coli* 8.77% (20/228).

The positivity rate of the sample for *Salmonella* was also lower than the report of (Sarba *et al.*, 2020) 19.0% (39/205) from East Shoa Ethiopia, (Belachew *et al.*, 2021) 24.3%, (131/539) in Central Ethiopia, (Elshebrawy *et al.*, 2022) 39% (78/200) Mansoura city, Egypt and (Al-Hindi *et al.*, 2023) 31.25% (35/112) from Saudi Arabia and. It was higher than the previous report by (Taddese *et al.*, 2019) 2.65% from Jimma Town, South Western Ethiopia, (Asfaw *et al.*, 2020) 2.65% from Debre Zeit and Modjo, Ethiopia and (Abda *et al.*, 2021) (9.27%) from Kafa zone, Southwest Ethiopia. The positivity rate of *E. coli* O157:H7 is lower than the report of 13.4% (26/194) in Eastern Ethiopia and but higher than the report of (Abebe *et al.*, 2023) 6.5% in Dessie and Kombolcha towns, Ethiopia. From these *E. coli* O157:H7 isolates, 75% (15/20) were from cloacal swabs and 25% (5/20) were from postmortem samples. Of the 27 (11.84%) *Salmonella* isolates, 70.37% (19/27) were from cloacal swabs and 29.63% (8/27) were from postmortem samples. From 15 *E. coli* O157:H7 and 19 *Salmonella* cloacal swab isolates, 10 (66.7%) and 15 (78.95%) were isolated from healthy-looking chicken and 5 (33.3%) and 4 (21.05%) were from diseased chicken, respectively. Relatively higher frequency of these bacteria in healthy-looking chickens than from diseased further exacerbate the effect of the AMR on the poultry health and production, environmental and public health (Sabry *et al.*, 2020; Assoumy *et al.*, 2021).

This is due to that this apparently healthy chicken harboring drug-resistant *Salmonella* were left unchecked and not treated with alternative antimicrobials or not isolated from the production. In addition, as people contact with chickens while husbandry and use their products directly and indirectly without caution as the chickens are considered healthy, they contract this drug-resistant bacteria from the chickens.

In the current investigation, both *Salmonella* and *E. coli* isolates showed higher resistance to penocillins (ampicillin 100%), third-generation cephalosporins (85.1%-90%), tetracycline (77.8%-90%), and folate synthesis inhibitors (70-85.1%). This higher resistance rate to penicillins, tetracyclines and folate synthesis inhibitors may be owing to the frequent use of these antimicrobials in veterinary medicine. According to (Beyene *et al.*, 2015) from Bishoftu, Central Ethiopia and (Etefa *et al.*, 2021) from Rift Valley areas of Ethiopia oxytetracycline, sulfonamides and penicillin combinations are the most frequently used antimicrobials. This is hardly shocking considering how widely available, inexpensive, and prescription-free these antimicrobials were in use in poultry. According to (Gharieb *et al.*, 2015) these medications are given to chickens either for therapeutic purposes or as growth boosters in the feed, which causes the enteric bacterial flora of the chicken to become resistant. This intestinal flora may then confer resistance on harmful bacteria, including *Salmonella* and *E. coli* making it multidrug resistant. In addition, this higher resistance to penicillin group antimicrobials may be ascribed to their frequent use both in animal and human health care.

Even if frequent use of cephalosporins in poultry production at this study site was not observed, a higher resistance rate for these antimicrobial groups may be attributed to the cross-resistance from biocides used in farms, entry of resistant bacteria due to poor biosecurity practices on farms or through poultry feed and water (Endale *et al.*, 2023). In addition to this, it may also be ascribed to cross-resistance between them and penicillins as bacteria resist both through communal mechanisms of resistance beta-lactamase production and alterations in penicillin-binding proteins. Furthermore, (Bhattarai *et al.*, 2024) stated that some antimicrobials might elicit a co-selection for other antimicrobials even for antimicrobials classes not related at all.
Conversely, a higher rate of susceptibility was recorded to gentamicin (66.7%) and (75%), chloramphenicol (66.7%) and (60%) azithromycin and meropenem (81.5%) and (50%) in *Salmonella* and *E. coli*, respectively.

As a result of the current investigation revealed, that all the *Salmonella* isolates were resistant to the antimicrobial agents tested at a different rate. The highest resistance rate was recorded against ampicillin (100%) followed by sulfamethoxazole and cefoxitin (85.1%), tetracycline (77.8%), trimethoprim/sulfamethoxazole (74.1%), amoxicillin-clavulanic acid 66.6% (18), ceftriaxone and streptomycin (55.6%), ciprofloxacin (51.9%), ampicillin-sulbactam (48.1%), trimethoprim (44.4%), cefotaxime (40.7%), meropenem (18.5%), gentamicin (11.1%), chloramphenicol (7.4%), and only one isolate was nonsusceptible to azithromycin. In contrast, (Gharieb *et al.*, 2015) from Zagazig City, Egypt reported higher (100%) resistance against erythromycin and tetracycline and (Al-Hindi *et al.*, 2023) recorded the highest (65.7%) resistance to ampicillin and cefotaxime in Saudi Arabia. Highest resistance (100%) to erythromycin was reported by (Kim *et al.*, 2012) from Korea. The overall AMR rate of the present study was similar to the finding of (Hai *et al.*, 2020) from Nanjing, Jiangsu Province, China and of (Abda *et al.*, 2021) from Kafa zone, Southwest Ethiopia who stated all the *Salmonella* isolates were resistant to at least one drug but higher than the report of (Waghamare *et al.*, 2018) who reported 73.8% overall resistance from Mumbai city, India. In another study, (Taddese *et al.*, 2019) from Jimma Town, South Western Ethiopia reported the antimicrobial resistance rate of *Salmonella* as 90.09%, which is lower than the current finding.

This higher resistance rate of bacteria to the above antimicrobial may indicate that they are frequently and irrationally used in the farms where the study was undertaken. In addition, it may be attributed to possible antimicrobial cross-resistance in bacteria resistant to one drug may also become non susceptible to another as stated by (Pulingam *et al.*, 2022). Furthermore, it may also be associated with cross-resistance arising from the use of sanitizers even if the drug was not common on the farm (Bland *et al.*, 2022). In previous study, (Amsalu *et al.*, 2020) found cross resistance between the antimicrobials and biocides. This variation in the antimicrobial resistance rate of *Salmonella* reported from different study sites might be attributed to the difference in sample type, poultry producers' practice of antimicrobial use (irrational vs rational use), and farm management (as good and hygienic management reduces infection and subsequent antimicrobial use). It may also be attributed to the poultry production system whether it was mostly antimicrobial dependent or more organic production and the type of antimicrobial agents commonly used for the chicken therapeutically or non-therapeutically in each study site.

All *Salmonella* isolates exhibited nonsusceptibility against ampicillin which was similar to the report of (Abda *et al.*, 2021) from Kafa zone, Southwest Ethiopia. Almost the same pattern, 97.8%, 85.2%, 81.8%, 70%, 45% and 28.8% was reported by (Abdi *et al.*, 2017) from Southern Ethiopia; (Molla *et al.*, 2003) from Debre Zeit and Addis Ababa, Ethiopia; (Bushen *et al.*, 2021) from Jimma University poultry farm, Southwest Ethiopia; (Asfaw *et al.*, 2020) from Debre Zeit and Modjo, Ethiopia; (Yildirim *et al.*, 2011) from middle Anatolia, Turkey, and (Tsegaye *et al.*, 2016) from Alage, Ziway and Shashemene, Ethiopia, respectively. Of the total tested *Salmonella* isolates, (66.6%) from which (44.4%) were cloacal swab isolates and (22.2%) postmortem isolates and (48.1%) were found nonsusceptible to the β-lactams and β-lactamase inhibitors combinations amoxicillin-clavulanic acid and ampicillin-sulbactam, respectively. This was lower than the report of (Gharieb *et al.*, 2015) 91.7 % to amoxicillin-clavulanic acid and 75 % to ampicillin-sulbactam; and (Bushen *et al.*, 2021) from Southwest Ethiopia who recorded 54.5% of the tested *Salmonella* were resistant both amoxicillin-clavulanic acid and ampicillin-sulbactam. On the other hand, (Asfaw *et al.*, 2020) documented a lower 24.0% resistance rate against amoxicillin-clavulanic acid in Debre Zeit and Modjo, Ethiopia; (Al-Hindi *et al.*, 2023) from Saudi Arabia 11.4% against ampicillin-sulbactam and 45.7% to amoxicillin-clavulanic. In addition (Kim *et al.*, 2012) documented a resistance rate of 45.0% to amoxicillin-clavulanic and 23.0% to ampicillin-sulbactam. This varied resistance rate to amoxicillin-clavulanic and ampicillin-sulbactam may be owing to the reasons stated in previous paragraph of this manuscript. In addition to this, it may also due to the variation in consumption rate and frequency of this antimicrobial agents in farms of each study site.

With regard to the cephalosporins, about (40.7%), (55.61%) and (74.1%) of the *Salmonella* were found non susceptible to cefotaxime, ceftriaxone, and cefoxitin, respectively. The resistance rate of *Salmonella* to ceftriaxone in the current study was lower than the report of (Qiao *et al.*, 2017) (81.3%), but higher than the resistance rate (36.4%) recorded by (Bushen *et al.*, 2021) and (Gharieb *et al.*, 2015) who recorded (16.7 %). In another study, (Noda *et al.*, 2015) documented 6.4%, and 5.1% resistance rates of *Salmonella* against cefoxitin and cefotaxime, respectively, which was lower than the resistance pattern recorded in the current investigation. Before five years, (Jeon *et al.*, 2019) from Korea reported that none of the 57 *Salmonella* isolates exhibited resistance to third-generation cephalosporin and cefoxitin.

This higher resistance rate observed in the present study than the previous findings reported several years ago instigates that the resistance to these drugs in poultry production is increasing over time. In addition to this, (Aljindan and Alkharsah, 2020) documented that through time *Salmonella* has established broad resistance to some cephalosporins. WHO (WHO, 2024) categorized *Enterobacterales* resistant to third-generation cephalosporins as the critical pathogens. Thus, *Salmonella* isolated in this study falls under this category of pathogens. Pathogens under this group are considered as those that cause the maximum menace to public health owing to restricted treatment opportunities, high ailment burden and snowballing trends in ABR, with few or no promising candidates in the therapeutic pipeline.

Particularly the third- and fourth-generation cephalosporins are critically important antimicrobials for treating infections brought on by *Salmonella* and *E. coli* in both humans and animals (WHO, 2021). In spite of this, third-generation cephalosporin-resistant isolates of *Salmonella* have been recorded to be emerging quickly all over the world recently including current studies. This is a grave health risk since cephalosporins are clinically very critical drugs to treat different infections in humans and instigate that *Salmonella* in poultry production is producing extended spectrum β lactamases conferring resistance against this antimicrobial class. other authors (Shigemura *et al.*, 2018) stated by ceasing the application of antimicrobial agents in this group in poultry production, it is possible to counter back the emergence and spread of extended-spectrum cephalosporin-resistant *Salmonella*. They recorded a steady decline in occurrence of *Salmonella* extended spectrum cephalosporin resistance after the intentional cessation of ceftiofur use by the Japanese poultry industry. The findings of present study necessitate enactment of the same strategies for these antimicrobial class in current study area.

In an attempt made to identify the resistance of *Salmonella* against aminoglycosides, about (55.6%) and (11.1%) exhibited resistance to streptomycin and gentamicin, respectively. The majority (66.7%) and (44.4%) of the *Salmonella* were found susceptible to gentamicin and streptomycin, respectively. Other authors, (Ibrahim *et al.*, 2021) from the East Coast of Peninsular Malaysia and (Abda *et al.*, 2021) from Kafa, Ethiopia reported zero and (19%) and (32.14%) resistance against streptomycin, respectively. In addition, (Asfaw *et al.*, 2020) recorded a lower (30.0%) rate in Debre Zeit and Modjo, Ethiopia.

Conversely, (Kim *et al.*, 2012) and (Gharieb *et al.*, 2015) documented a higher (28%) and (66.7%) resistance rate than the current study. As stated formerly in this manuscript, this varied resistance rate may be ascribed to variation in the usage of these antimicrobials in each study site. Within the context of One Health, *Salmonella* resistant to aminoglycosides represents a serious risk because of their potential effects on the health of people, animals, and the environment. As aminoglycosides are frequently used to treat bacterial infections, including *Salmonella* infections in both humans and animals.

In relation to the folate synthesis inhibitors, about (85.1%), (74.1%) and (44.4%) of total *Salmonella* isolates exhibited nonsusceptibility to the sulfamethoxazole, trimethoprim/sulfamethoxazole and trimethoprim, respectively. The resistance against trimethoprim/sulfamethoxazole was lower than the report of (Abdi *et al.*, 2017) (100%) resistance in poultry production in Southern Ethiopia. In contrast, (Dagnew *et al.*, 2020) documented zero rate resistance to trimethoprim/sulfamethoxazole and trimethoprim in Adama and Modjo towns, Ethiopia. Also (Abda *et al.*, 2021) from Kafa, Ethiopia reported lower (46.42%) resistance than in the current study. Other authors (Gharieb *et al.*, 2015) and (Al-Hindi *et al.*, 2023) recorded (70%) and (68.6%) resistance to trimethoprim-sulfamethoxazole. As stated, in the previous portion of this manuscript, sulfa drugs and their combinations are one of the most frequently used drugs by the poultry producers of current study site. This could be the possible contributing factor for the occurrence of relatively higher resistance rate in present study than some other reports.

Regarding ciprofloxacin, (51.9%) of the total *Salmonella* isolates exhibited resistance, which was almost similar to the resistance rate documented by (Abd El-Aziz *et al.*, 2021) and higher than the previous report of (Abdi *et al.*, 2017) (31.1%) in poultry industry southern Ethiopia. Conversely, (Sharma *et al.*, 2019) reported higher (82.86%) resistance from northern India. Ciprofloxacin is the frontline antimicrobial for salmonellosis in both animals and human beings, but increasing resistance in current and other studies in poultry production instigates its therapeutic futility in the future. As poultry production is the main source of foodborne salmonellosis in humans, the existence of *Salmonella* resistant to first-line antimicrobials further fuels the public health hazard of the bacteria.

According to (Aljindan and Alkharsah, 2020) *Salmonella* resistant to antimicrobials like ciprofloxacin is responsible for 33,000 morbidities annually in the United States. A high incidence of *Salmonella* resistant to fluoroquinolones has been documented even after some of these antimicrobials were taken out of production, which poses a serious risk to the safety of poultry products and public health.

In an attempt made to determine the serotype level antimicrobial resistance pattern of *Salmonella*, the most frequent and public health important isolates *S. Enteritidis* and *S. Typhimurum* exhibited high resistance to the most drugs tested. *S. Enteritidis* was resistant to ampicillin, cefoxitine and sulfamethoxazole (100%), amoxicillin/clavulanic acid (77.8%), trimethoprim/sulfamethoxazole and streptomycin (66.7%), and ceftriaxone, tetracycline and ciprofloxacin (55.6%). The majority (77.8%), (66.7%) and (55.6%) of the *S. Enteritidis* were susceptible to azithromycin, meropenem and chloramphenicol and Gentamicin, respectively, which was in line with the report of (Waktole *et al.*, 2024) from Central Ethiopia. *S. Typhimurium* exhibited (100%) susceptibility to chloramphenicol, azithromycin and Gentamicin and the majority (85.7%) were susceptible to meropenem. In contrast, before 14 years, (Yang *et al.*, 2010) from Shaanxi, China reported none of the *S. Typhimurium* and *S. Enteritidis* isolates were resistant to trimethoprim, gentamicin, tetracycline, and amoxicillin/clavulanic acid were found.

In contrast, (Siddiky *et al.*, 2021) *S. Typhimurium* exhibited the highest resistance against ciprofloxacin and streptomycin (100%) then to tetracycline and gentamicin (86.66%), ampicillin (66.66%) and amoxicillin-clavulanate (40%). At the same time *S. Enteritidis*, showed the highest nonsusceptibility against streptomycin (100%) then to ciprofloxacin, tetracycline, and gentamicin (80%). In another study by (Yu *et al.*, 2021) 95.3% and 72.7% of the *S. Enteritidis* were nonsusceptible to ampicillin and tetracycline respectively which was almost higher resistance rate as in this study. Nontyphoidal *Salmonella* resistant to the fluoroquinolones are ranked under high priority pathogens which are notably difficult to cure and contribute significantly to the burden of disease, exhibit rising resistance patterns, difficult to prevent, highly transmissible, and for which there are limited treatment alternatives.

This relatively higher resistance rate along with higher isolation rate of *S. Enteritidis* and *S. Typhimurium* in the poultry, which is growing hotspot for AMR is an alarming finding necessitating effective control strategies in poultry production and associated food chains. It is documented that *S. Enteritidis* and *S. Typhimurium* are the most frequent serotypes that acclimate to diverse hosts and transmission niches and they have noteworthy epidemiological significance across the globe. Poultry products are well-thought-out important reservoirs of many *S. Enteritidis* and *S. Typhimurium* which can reach the human being through chicken product consumption (Elshebrawy *et al.*, 2022; Carneiro *et al.*, 2024). The higher resistance of poultry-specific serovars *S. Gallinarum* and *S. pullorum* in the current study reveal their health, productivity and welfare hazards on the poultry. From *S. pullorum* tested, all and 66.7% exhibited non-susceptibility to ampicillin, cefoxitine and sulfamethoxazole, and amoxicillin/clavulanic, ampicillin/sulbactam, cefotaxime, streptomycin and tetracycline, respectively. Interestingly, all and 80% of *S. pullorum* were susceptible to azitomycin and ciprofloxacin, meropenem, cefotaxime and gentamicin, respectively. The single isolate *S. Dublin* exhibited resistance to ampicillin, ceftriaxone, sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim and tetracycline.

Regarding the antibiogram of *E. coli* O157:H7, all the isolates were found resistant to at least three antimicrobials tested with highest resistance rate against ampicillin (100%) followed by cefotaxime and tetracycline (90%), cefoxitin and trimethoprim (70%), sulfamethoxazole (65%), amoxicillin-clavulanic acid and ceftriaxone (55%), ampicillin-sulbactam (50%), trimethoprim-sulfamethoxazole (40%), streptomycin and meropenem (35%). Interestingly, all the *E. coli* O157:H7 isolates were susceptible to azithromycin. In line with current findings, (Hailu *et al.*, 2021) from Ohio State America reported, 100% resistance to ampicillin. Conversely, (Shecho *et al.*, 2017) from eastern Ethiopia and (Ibrahim *et al.*, 2021) East Coast of Peninsular Malaysia reported the highest (96.15%) and (100%) resistance to azithromycin, respectively.

In another study by (Tegegne *et al.*, 2024) from Addis Ababa chicken slaughterhouses the highest resistance rate recorded was 47.5% against streptomycin and zero resistance to gentamicin and ciprofloxacin. In the same study, the lowest (2.5%) and (3.1%) resistance was observed against chloramphenicol and sulfamethoxazole-trimethoprim, respectively.

This varied resistance rate among different studies could be owing to the difference in the sample type, health status and overall management of source chicken in each site. According to (Rahman *et al.*, 2020; Zhang *et al.*, 2022), poultry and their products are the most important source and dissemination vehicle for mono-drug and multidrug resistant *E. coli* O157:H7 and associated public or one health hazards. This demonstrates that a higher antimicrobial resistance rate with a higher isolation rate of *E. coli* O157:H7 in chicken is of great concern as it reaches human beings through direct and indirect contact and the food chain.

In relation to the penicillin groups of the tested antimicrobials, like our finding different studies have reported a high resistance rate of *E. coli* O157:H7 against ampicillin, (Shecho *et al.*, 2017) (92.30%), (Davis *et al.*, 2018) (62%), (Fuh *et al.*, 2018) (77.78%), (Ibrahim *et al.*, 2021) (87.5%), (Koju *et al.*, 2022) (60.0%) and (Tegegne *et al.*, 2024) (100%). This high degree of resistance demonstrates that resistance by *E. coli* O157:H7 in poultry production is rendering ampicillin therapeutically futile for the *E. coli* O157:H7 infected animals and humans as the resistance can disseminate and circulate within one health interface. Almost the same (51%) and lower (3.3) resistance to amoxicillin-sulbactam and amoxicillin-clavulanic acid was documented by (Davis *et al.*, 2018) and (Yassin *et al.*, 2017) in unspecified strains of *E. coli*, respectively. Conversely, (Benameur *et al.*, 2021) documented higher (83%) resistance to amoxicillin-clavulanic acid. These all instigates that, *E. coli* was developing resistance to the penicillin groups in an upward fashion making use of these antimicrobials therapeutically futile.

In relation to the tested cephalosporins, *E. coli* O157:H7 exhibited a high level of resistance with which highest resistance recorded in cefotaxime. A slightly higher resistance (84.61%) than the current result against cefoxitin was reported by (Shecho *et al.*, 2017). The resistance pattern of *E. coli* O157:H7 to ceftriaxone is almost in agreement with the report of (Fuh *et al.*, 2018) (55.56%). In another study by (Kaushik *et al.*, 2018) 28.2% resistance to ceftriaxone was documented which is relatively lower than the current. As compared to the result of this study, relatively lower resistance rate to cefotaxime (21.3%) and ceftriaxone (18.2%) was also reported by (Yassin *et al.*, 2017) from China and cefoxitin (12.6%), ceftriaxone (13.8%) and cefotaxime (17.2%) by (Tigabie *et al.*, 2023) from Gondar City, Northwest Ethiopia in non-serotyped *E. coli*.

It is obvious that third-generation cephalosporins are the highest priority and critically important beta-lactam antimicrobial commonly used for the chemotherapy of infections caused by Gram-negative bacteria *E. coli* (de Been *et al.*, 2014), ominously the resistance is rendering it challenging over time. Furthermore, the multidrug resistance in these bacteria further bottleneck the therapeutic opportunities.

As the antibiogram result revealed, more than half of *E. coli* O157:H7 isolates exhibited resistance against tested folate synthesis inhibitor/sulfonamide antimicrobial agents, 70% (14/20), 65% (11/20) and 40% (7/20) to trimethoprim, sulfamethoxazole and trimethoprim-sulfamethoxazole, respectively. This was lower than the previous report of (Hailu *et al.*, 2021), 100% against trimethoprim-sulfamethoxazole (Ejeh *et al.*, 2017) from Zaria, Nigeria 85.7% against trimethoprim-sulfamethoxazole and of (Yassin *et al.*, 2017) 78.9% to sulfamethoxazole and 77.8% to trimethoprim/sulfamethoxazole. Conversely, (Shecho *et al.*, 2017) documented that most (92.30%) of the test isolates were susceptible to trimethoprim. The resistance rate (78%) against trimethoprim-sulfamethoxazole documented by (Benameur *et al.*, 2021) was notably higher than in the present study. This demonstrates there was a high level of resistance to sulfonamides even if it was varied in region and time of the study.

Among the tested antimicrobials, aminoglycosides, carbapenems, phenicol, quinolone and macrolides class showed good invitro effect on the *E. coli* O157:H7 in which above half of the test isolates were susceptible. This finding corroborates the previous report of (Shecho *et al.*, 2017) who documented higher susceptibility to ciprofloxacin (100%), chloramphenicol (96.15%) gentamicin (88.46%) and streptomycin (65.38%). Conversely, the same author and (Ibrahim *et al.*, 2021) reported higher (96.15%) and (100%) resistance against erythromycin, respectively. In addition, (Hailu *et al.*, 2021) noted that all (100%) isolates were susceptible to chloramphenicol, ciprofloxacin and meropenem. In contrast, this author reported lower susceptibility to gentamicin (6.7%) and streptomycin (46%). Carbapenems are the drug of choice to treat severe infections with ESBL *Enterobacteriaceae* (Gutierrez and Rodriguez, 2019), current finding indicates interesting invitro activity of these critical drugs against *E. coli* O157:H7 but there is resistance.

In an attempt at MDR determination, all the *Salmonella* isolates possessed multidrug resistance to a minimum of 3 and maximum of 8 antimicrobial classes out of 9 antimicrobial groups tested. This finding corroborates the report of (Gharieb *et al.*, 2015; Sarba *et al.*, 2020; Hailu *et al.*, 2021) who documented MDR in all (100%) of the *Salmonella* isolates. In addition, (Abdi *et al.*, 2017) reported all (100%) the *Salmonella* isolated from the poultry industry of Southern Ethiopia were multidrug resistant. In contrast, (Al-Hindi *et al.*, 2023) recorded MDR in 65% of the isolates which is lower than in the current study. MDR recorded in the present study was higher than the MDR *Salmonella* isolates human (82.9%) reported by (Wei *et al.*, 2023).

All the *Salmonella* isolates exhibited MDR against antimicrobial classes understudy, most (44.4%) were multidrug resistant to six antimicrobial classes. From the *E. coli* O157:H7 isolates, 80% exhibited MDR, to maximum of seven antimicrobial classes tested. Other authors (Shecho *et al.*, 2017), (Koju *et al.*, 2022) and (Yassin *et al.*, 2017) reported high levels of MDR 92.30%, 71.1% and 83%, respectively. The remaining 20% (4/20) were not multidrug-resistant. According to (Gontijo *et al.*, 2024) MDR bacterial infections are the leading cause of death. The most recent antimicrobial resistance report reveals that over half of the bacterial species commonly linked to fatal diseases caused by multidrug resistance are Gram-negative bacteria, including *Salmonella enterica*, and *Escherichia coli*. These bacteria accounted for nearly three million deaths globally in 2019, which is equivalent to roughly 60% of all MDR-related deaths. MDR microorganisms have posed a risk to public health, particularly. In this spectacle, a higher level of MDR was documented in both bacteria isolated from the main hotspot and vehicle poultry production distributing it to the public as well as the one health interface. This necessitates timely intervention to tackle further rise MDR to the serious level as the poultry production is becoming most common protein source and income source with higher and uncontrolled use of antimicrobials.

In the current study, a higher MAR index (the ratio of the number of antimicrobial agents to which the bacteria were resistant to the total number of antimicrobials against which the bacteria were tested) was documented for both bacteria. All the *Salmonella* and 95% of *E. coli* isolates exhibited a MAR index higher than 0.2.

This result was in line with the report of (Adegoke *et al.*, 2020) who recorded a MAR index ≥ 0.2 in 86.8% of isolates and (Elshebrawy *et al.*, 2022) who recorded a MAR index ≥ 0.2 in 94% *of E. coli* isolates. Similarly (Mir *et al.*, 2022) reported that 100% of the *Salmonella* isolates MAR index value higher than 0.2. This MAR index value higher than 0.2 suggests these isolates were identified from farms where there is a high menace of bacterial contamination where there was frequent higher level antimicrobial consumption and higher antimicrobial abuse (Hossain *et al.*, 2019). The MAR index of *Salmonella* ranges from 0.31 to 0.75 with an average of 0.53 and that of *E. coli* was recorded as 0.19 to 0.55 with an average of 0.35. Nevertheless, the difference in MAR between the two genera of the bacteria under investigation was statistically non-significant (P-value=0.8610, X2=0.031, d.f. 1) (P-value=). This result in conjunction with the result of MDR pattern instigates, *Salmonella* was exhibiting higher antimicrobial resistance than *E. coli*. Interestingly, one isolate from broiler chicken exhibited MAR index (0.19) lower than 0.2 indicating antimicrobial use in the farm from which the isolate was isolated was infrequent and rational (Karim *et al.*, 2023).

In converse, (Ibrahim *et al.*, 2021) documented a higher MAR index for *E. coli* than for *Salmonella*. MAR index of bacteria isolated from broiler chicken ranges from 0.19 to 0.56 with an average of 0.38 and for layers was from 0.31 to 0.75 with an average of 0.53. The highest MAR index 0.75 was recorded for bacteria isolated from layer chicken. This result shows the MAR index was higher in bacteria isolated from layer chickens, suggesting that there was higher and more frequent consumption of antimicrobials in layer farms than in broiler farms. The difference in MAR index between broiler and layer type of chicken was statistically significant (P= 0.000, X^2=9.559). In contrary, (Bhattarai *et al.*, 2024) MAR index was significantly lower bacteria isolated from in layers. Since these bacteria were resistant to multiple antimicrobials and isolated from the areas where there was high antimicrobial abuse, infection by this bacteria pose greater challenge in treating as stated by (Ranasinghe *et al.*, 2022).

Regarding the R score, most (78.72%) of the bacterial isolates under investigation exhibited R score ≥ 0.5 (high/very high AMR) and the remaining 22.28% showed R score lower than 0.5 (low/very low AMR). This was lower than the R score recorded by (Bhattarai *et al.*, 2024) who recorded 0.5 to 0.7 in all isolates in selected districts of Nepal.

About 81.48% of the *Salmonella* isolates exhibited high/very high AMR and 85.0% of *E. coli* isolates were revealed high/very high AMR (R score ≥ 0.5). From both genera of bacteria, the highest R score (0.78) was documented in *E. coli*. A statistically significant (P=0.002, X^2= 9.204) difference was observed between the two genera of bacteria under study. A higher (85.2%) R score ≥ 0.5 (high/very high AMR) was recorded in bacteria isolated from layer than from broiler chicken (69.23%) and the difference in R score between the two types of chicken was statistically nonsignificant (P= 0.2032, X^2= 1.619). In contrast, (Bhattarai *et al.*, 2024) reported a highly significant difference in R score with chicken type. The result suggests that a majority (78.72%) of bacterial isolates displayed high/very high levels of antimicrobial resistance, particularly in *E. coli* strains compared to *Salmonella* strains.

R scores are crucial in guiding clinical decision-making, especially in cases where drug-resistant infections are prevalent. Quantifying resistance levels helps to inform healthcare providers to tailor treatment regimens to ensure optimal patient outcomes and minimize the spread of resistant pathogens within healthcare settings and communities. In addition to this, in combatting AMR, the R score serves as a quantitative indicator of the level of AMR exhibited by pathogens to specific antimicrobial agents, aiding healthcare professionals in selecting appropriate treatment strategies and combating drug-resistant infections effectively.

In the current study, concurrent phenotypic resistance was observed to different antimicrobials including cefotaxime, ceftriaxone, meropenem and amoxicillin-sulbactam, cefoxitin, ceftriaxone, ciprofloxacin, and meropenem; sulfamethoxazole, sulfamethoxazole-trimethoprim, amoxicillin, ciprofloxacin and sulfamethoxazole, gentamicin and azithromycin. Concurrent resistance against the antimicrobials of the same class could be due that they share the same mechanism of action but unrelated classes may be due to cross selection effect or plasmid carriage of different resistance genes (Shariati *et al.*, 2022; Teng *et al.*, 2022). In line to this study, (Lazar *et al.*, 2014; Varga *et al.*, 2019a; Bhattarai *et al.*, 2024) reported concurrent resistance to the antimicrobials of the same and different classes. The resistance correlation between amoxicillin-sulbactam and cefotaxime, cefoxitin and meropenem, chloramphenicol and ciprofloxacin, sulfamethoxazole and meropenem, sulfamethoxazole-trimethoprim and ceftriaxone, and azithromycin and amoxicillin-sulbactam was significantly strong (P<0.05).

The strongest correlation was recorded between cefoxitin and meropenem (r_s = 0.4847). In another study, the strongest correlation was documented between chloramphenicol and trimethoprim-sulfamethoxazole resistance (r_s = 0.60) (Lunha *et al.*, 2020). In addition (Gajdacs *et al.*, 2021) reported a strong correlation in *E. coli* resistance to gentamicin and ciprofloxacin.

Conversely, a significant (P<0.05) negative correlation was observed between trimethoprim and cefoxitin, tetracycline and ceftriaxone, and azithromycin and meropenem resistance. This means that, as the *Salmonella* and *E. coli* attain resistance to trimethoprim, tetracycline and azithromycin, they become more susceptible to cefoxitin, azithromycin and azithromycin and vice versa, respectively. This may be attributed fact that on some occasions, there is bacterial hyper-susceptibility in which strains resistant to certain antimicrobial agents become more susceptible than the parental strain (Suzuki *et al.*, 2014).

As the KAP assessment result evaluated by scoring the consistency of their response to the questions forwarded to them revealed, the poultry producers addressed in the current KAP assessment have lower levels of KAP. The majority (50.0%), (47.22%) and (41.60%) of the respondents have lower levels of knowledge, attitude and practice regarding AMU and AMR. A good level of the KAP was recorded for the lowest portion (22.20%), (19.44%) and (25%) of the respondents interviewed. This finding agreed with the KAP trend reported by (Gebeyehu *et al.*, 2021) from the Oromia zone, northeastern Ethiopia who documented the majority of the respondents (80.2%, 85.3% and 78.5%) have poor knowledge and attitude and bad practice towards AMU and AMR. Conversely, (Geta and Kibret, 2021) from the Amhara region, north western Ethiopia reported that the majority 50.5%, 52.5% and 60.4% of the animal (including poultry producers) have good KAP on AMU and AMR, respectively. The relatively better trend observed in the practice of the respondents despite their lower record of knowledge may be ascribed to bias in their response while interviewing. This finding suggests a concerning gap in knowledge, attitude, and practice among poultry producers regarding antimicrobial use and resistance. This phenomenon in the area in which higher resistance levels are recorded further fuels the emergence and spread of AMR locally as well as globally. Addressing these gaps through targeted education and awareness programs is crucial to mitigate the hazards emanating

from unfortunate antimicrobial use and AMR in poultry production and health, environmental health, food security and public health.

In an attempt made to identify intercorrelation among knowledge, attitude and practice, a highly strong correlation was observed among the knowledge, attitude and practice of the respondents on AMU and AMR. The correlation between knowledge and attitude was ($r = 0.5506$, $p= 0.0005$), between knowledge and practice was ($r = 0.6420$, $p = 0.0000$) and between attitude and practice was ($r = 0.5337$, $p = 0.0008$). This finding corroborates the previous report of (Geta and Kibret, 2021; Hassan *et al.*, 2021) who reported a strong correlation among the KAP of animal producers. In addition (Tufa *et al.*, 2023) also recorded a positive correlation between the knowledge and attitude of the livestock producers. This strong correlation among the KAP posited that individuals with good knowledge about AMU and AMR, are inclined to have better attitudes towards responsible antimicrobial use. It also suggests that an increase in levels of knowledge will bring about a positive change not only in attitude but also in practices. These improved levels may finally result in decreased misuse of antimicrobials with less antimicrobial resistance. Thus, targeted educational campaigns and interventions, mainly to enhance public knowledge about the proper use of antimicrobials, instill a positive attitude toward responsible antimicrobial use, and promote practices that reduce antimicrobial resistance.

In an attempt made to elevate the association between the respondents' demographic characteristics and the KAP level, educational level, the type of chicken they raise and the job type have shown statistically significant ($P<0.005$) effects on the KAP of the poultry producers on AMU and AMR. in the current study has a statistically significant effect on their knowledge, attitude, and the practice. Level their KAP towards the AMU and AMR increases as their educational level increases, while keeping those having no formal education constant, KAP increase by 1.75, 2 and 2 factors in those producers have vocational and university educational level, respectively. This finding corroborates the fact stated by (Hassan *et al.*, 2021; Kalam *et al.*, 2021; Kalam *et al.*, 2022) that the educational level of poultry farmers has a positive association with their KAP. The KAP level increases by factor of 0.7, 0.45 and 0.05 in those having four years of poultry production experience while keeping those with zero-year constant. However, the effect of years of experience in poultry production on their KAP was statistically

insignificant. In converse, (Hassan *et al.*, 2021; Kalam *et al.*, 2021; Kalam *et al.*, 2022) reported year of experience in poultry production is a key determining factor of KAP of poultry producers.

The level of KAP increases by 1, 1, and 1.054 units respectively, in those producing layer chicken while keeping those producing broilers constant with high statistical significance (p=000). Another author (Subedi *et al.*, 2023) recorded the same trend with regard to the effect of the type of chicken on the KAP on AMU and AMR in which layer chicken producers have significantly higher KAP levels. This higher level of in-layer raisers than in broiler raisers may be owing to that layer producers stay in production for a longer time than broiler raisers because broiler production is mostly seasonal. The level of KAP increased by 0.769, 0.528 and 0.505 respectively, in those raising >1000 chickens per flock but the effect was not significant ($p>0.05$) on the practice towards AMU and AMR. KAP level increases by 0.963, 0.519 and 0.333 units respectively, in those taken training before but the effect was not statically significant ($p>0.05$) on attitude and practice towards AMU and AMR. The level of KAP decreases by -1.484, -1.026, and -1.323 factors in those having non-health jobs with high statistical significance ($p<0.05$). This result could be supported by the report of (Jairoun *et al.*, 2019) stating that medical students have significantly better KAP towards AMU and AMR than non-medical students. Thus, job type is the determining factor for good awareness and judicious use of antimicrobials.

6 STUDY LIMITATIONS

In current investigation, molecular characterizations such as PCR, gel-electrophoresis and whole genome sequencing were not performed as evidenced by a lack of consumables at CVMA and left for another research. Due to the small number of the *Salmonella* at serovar level, the antimicrobial sensitivity pattern is almost related and this precludes the strict deduction of the antibiogram at the serotype level. In addition, there may be discrepancies between the KAP survey's findings and actual actions; a survey of this kind can only reveal what is stated; it cannot confirm what was actually done by the respondents.

7 CONCLUSION AND RECOMMENDATIONS

The current study exposes the AMR pattern of *Salmonella* and *E. coli* isolated from chickens of conventional poultry farms and the KAP of the poultry producers on AMU and AMR in Hossana town, central Ethiopia. The result of the antibiogram test, MAR index and resistance score revealed, that antimicrobial resistant *Salmonella* and *E. coli* were serious threats in the area. In addition, a high rate of MDR was recorded for both *Salmonella* and *E. coli* isolates, which further bottlenecks the therapeutic pipeline for these pathogens. Poultry production centers in the study site were recognized as very important hotspots for the emergence and distribution of antimicrobial resistant pathogens like *Salmonella* and *E. coli* to humans and other ecosystems. The poultry producers in the current study site have low knowledge, poor attitudes and inappropriate practices on antimicrobial use and resistance. In addition, this low level of KAP on AMU and AMR may further contribute to the antimicrobial misuse and subsequent propagation of AMR. Thus, the following recommendations were forwarded:

- Implementation of effective, incessant and targeted monitoring and regulations on irrational antimicrobial dispensing and use along with collaborative and multilevel stewardship campaign.
- Awaring of poultry producers about the rational antimicrobial use, proper management of the pharmaceuticals in the farm and while discarding and AMR and its preventive methods.
- Improving poultry producers' KAP on AMU and AMR through regular training.
- Further characterization of the genetic profiles of the isolates characterization of the bacterial isolates from the study area.

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9 ANNEXES

Annex 1. Collection and transportation to the laboratory; (a) cloacal swabbing (b) sample transportation to the laboratory.

Figure 7. Sample collection and transportation to the laboratory; (a) cloacal swabbing (b) sample transportation to the laboratory.

Annex 2. Antimicrobial susceptibility interpretation cut points with respective disc concentration

Table 7. Antimicrobial susceptibility interpretation cut points with respective disc concentration

Antimicrobial agents	**Symbols**	**Disc model**	**Discs Conc. (µg)**	**Zone diameter cut points for susceptibility (mm)**		
				R	**I**	**S**
Ampicillin	AMP	CT223B	10	≤13	14–16	≥17
Amoxicillin/ clavulanic acid	AMC	CT003B	30	≤13	14-17	≥18
Ampicillin/ Sulbactam	AMS	CT1653B	30	≤11	12-14	≥15
Cefotaxime	CTX	CT166B	30	≤22	23-25	≥26
Ceftriaxone	CRO	CT417B	30	≤19	20-22	≥23
Cefoxitin	FOX	CT0119B	30	≤14	15–17	≥18
Meropenem	MEM	CT0047B	10	≤19	20-22	≥23
Ciprofloxacin	CIP	CT024B	10	≤21	22-25	≥26
Chloramphenicol	C	BD231539	30	≤12	13-17	≥18
Sulfamethoxazole	SMX	BD231539	300	≤12	13-16	≥17
Trimethoprim/ Sulfamethoxazole	SXT	CT052B	23.75	≤10	11-15	≥16

Trimethoprim	TRI	CT013B	5	≤13	14–17	≥17
Streptomycin	S	CT0774B	10	≤11	12–14	≥15
Gentamicin	CN	BD231682	10	≤12	13-14	≥15
Tetracycline	TE	CT054B	30	≤11	12-14	≥15
Azithromycin	AZM	CT1615B	15	≤12	-	≥13

R = resistant; I = intermediate; S = susceptible
Adopted from the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020).

Annex 3. Media Preparation Procedures for Bacteriological Tests

Preparation of buffered peptone water medium

1. First, the required amount (grams) of dehydrated medium (at a rate equivalent to 20.07 grams/1000 ml) was suspended in laboratory-grade water.
2. Then heated along with frequent agitation to dissolve the medium completely.
3. After that, it was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and allowed to cool.
4. Finally, 10 ml of the medium was poured into the sample collection tubes.

Preparation of MacConkey Sorbitol agar medium

1. First, a sufficient amount (grams) of MacConkey Sorbitol Agar medium (at a rate equivalent to 25.06 grams/495 ml) was suspended in laboratory-grade water.
2. Then heated with frequent agitation to boil until the medium dissolved completely.
3. Afterward, the dissolved suspension was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and allowed to cool to 45-50°C.
4. Then, shake to mix well and pour into sterile Petri plates and stand until it solidifies for the inoculation of bacteria.

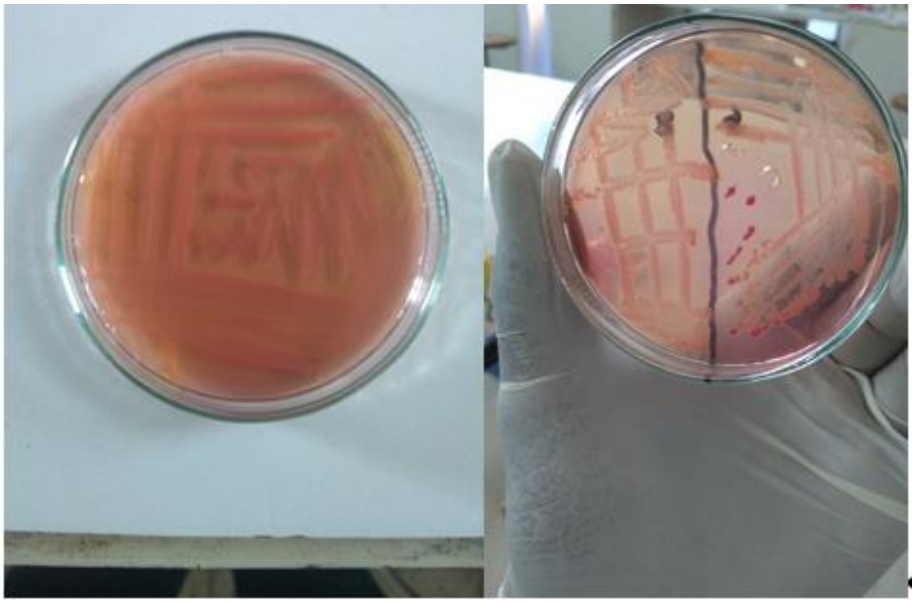

Figure 10. *E. coli* on MacConkey Sorbitol Agar

Preparation of nutrient agar medium

1. On-time intended amount (grams) of nutrient agar medium (at a rate equivalent to 28.0 grams/1000 ml) was suspended in laboratory-grade water.
2. Heated to boil until the medium dissolved completely.
3. Sterilized by autoclaving at 15lbs pressure (121°C) for 15 minutes and cooled to 45-50°C in a water bath.
4. Finally, the suspension was mixed well and poured into sterile Petri plates.

Preparation of Rappaport Vassiliadis soya broth medium

1. The needed amount (grams) of the dehydrated Rappaport Vassiliadis soya broth medium (at a rate equivalent to 27.11 grams/1000 ml) was suspended in laboratory-grade water.
2. Then, boiled along with recurrent agitation until all the powder was dissolved completely.
3. After that, the medium was dispensed into glassware test tubes and sterilized by autoclaving at 115°C for 15 minutes.

Preparation of xylose lysine deoxycholate agar medium

1. The required amount (grams) of the XLD medium (at a rate equivalent to 28.34 grams/1000 ml) was suspended in laboratory-grade water.

2. Then heated while agitating frequently until the medium dissolved completely.
3. Immediately after completely dissolving, it was transferred to a water bath at 50°C.
4. As it cooled down, it was poured into sterilized Petri plates

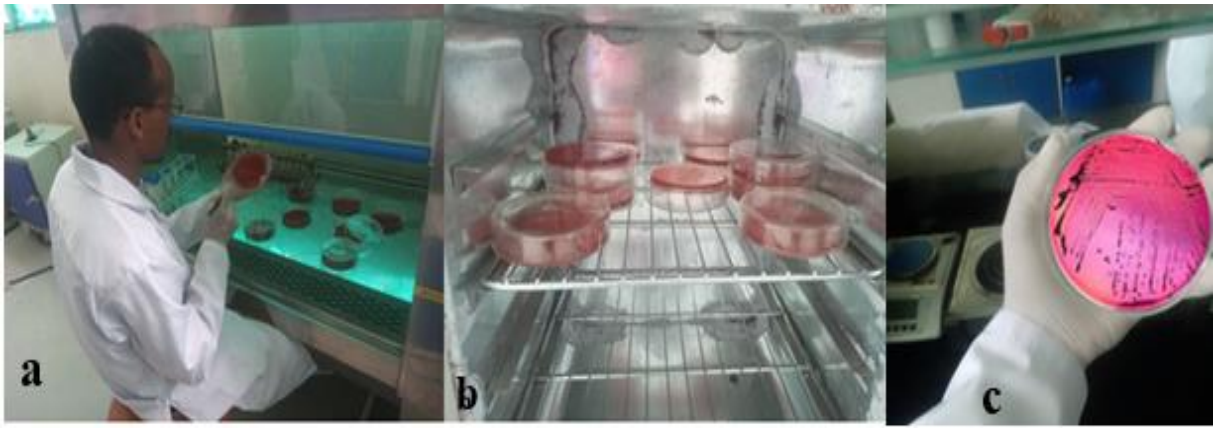

Figure 11. Bacterial inoculation on the XLD media; (3a) and incubation at incubator (3b) and *Salmonella* on the XLD agar (3c).

Preparation of the Salmonella Shigella agar media

1. Intended amount (by ratio of 60gm/1L) was suspended in distilled water.
2. Mixed thoroughly and heat with frequent agitation and boiled for one minute.
3. Poured into plates and stand to solidify and stored in the refrigerator.
4. The plate was brought to room temperature and allowed agar surface to dry.
5. The inoculum from RV broth was heavily inoculated by cotton tipped swab.
6. Incubated aerobically at 35-37°C for 18-24 hours.
7. Observed for the colorless colony with blacken center.

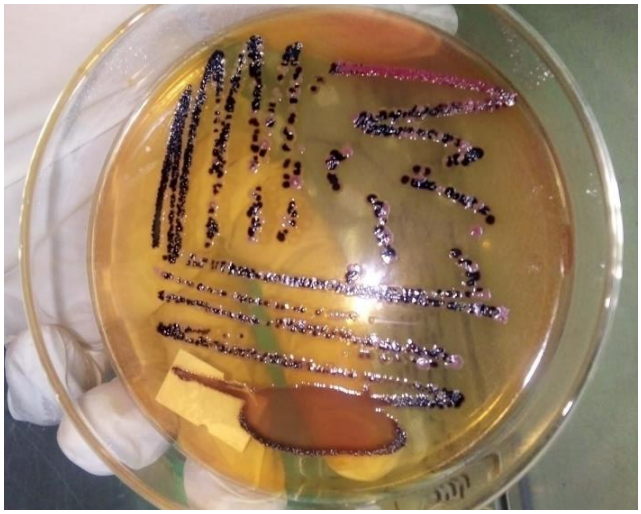

Figure 12. *Salmonella* on Salmonella shigella agar

Preparation of MacConkey agar

1. The intended amount (grams) of MacConkey agar (at a rate equivalent to 49.53 grams/1000 ml) was suspended laboratory-grade water.
2. Then boiled for about 1 minute with frequent stirring.
3. Following that, sterilized by autoclaving at 15lbs pressure (121°C) for 15 minutes.
4. Then cooled to 45-50°C, mixed adequately by shaking and poured into sterilized Petri plates.

Preparation of Mueller Hinton agar

1. The required amount (grams) of Mueller Hinton agar (at a rate of 21.00 grams/1000ml) was suspended in laboratory-grade water and mixed thoroughly.
2. Then boiled gently with frequent swirling until the powder was dissolved completely
3. Autoclaved at 15 psi pressure and 121°C for 15 minutes.

Figure 13. Disk diffusion antibiogram test; Muller Hinton agar (a), antimicrobial discs (b) used and disc diffusion on Muller Hinton agar (c and d).

Annex 4. Media Preparation Procedures for Biochemical Tests

Preparation of Triple sugar iron agar and bacterial inoculation

1. The required amount (grams) TSI agar medium (at a rate of 59.4 grams/1000ml) was suspended in laboratory-grade water.
2. Then, heated to boil with recurrent agitation until the powder was dissolved completely.

3. Then, about 5 to 7 mL of the medium was dispensed in each glassware test tube and closed by cap loosely.
4. It was autoclaved at 121°C for 15 minutes and allowed to cool in a slanted position (about 30°).
5. The pure bacteria were taken with a sterile straight inoculation wire from the top of a well-isolated 18-24 hrs old colony on nutrient agar.
6. Inoculated by first stabbing through the center of the medium to the 3 to 5 mm high to the base of the tube and streaking on the surface of the TSI slant.
7. The culture was incubated at 37°C in ambient air for 18 to 24 hrs leaving the cap loose.

Figure 14. Triple sugar iron test of bacterial isolates. *E. coli* (red arrow) and *Salmonella* (yellow arrow) on TSI.

The procedure of the indole production test

1. About three drops of Kovac's reagent were added to the sterile filter paper
2. With inoculating wire, a loopful of 24 hours old colony from nutrient agar was taken and rubbed on the filter paper reagent-saturated area
3. Observed for the appearance of a blue color within 3 minutes indicating a positive.

The procedure of the Simon Citrate test

1. Enough amount of medium (at a rate of 24.28 grams/1000 ml) was suspended in distilled water.

2. Then boiled with frequent swirling until the medium was dissolved completely.
3. Mixed well and distributed in glassware test tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes
4. Cooled down as slants
5. Inoculated by a 24-hour-old colony with inoculating wire streaking the slant back and forth.
6. The tubes were incubated aerobically with a loosened cap at 37°C for 4 days.
7. Observed for the color change from green to deep blue

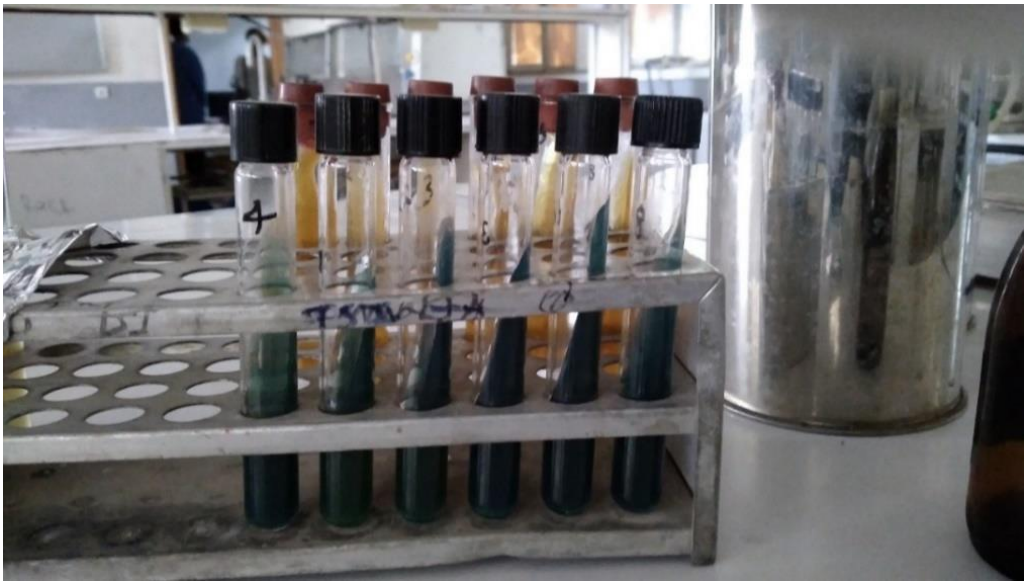

Figure 15. Simon citrate test for *Salmonella*

The procedure of the Sulfide Indole Motility (SIM) test

1. The intended amount of the medium (at a rate of 30.0 grams/1000 ml) was suspended in laboratory-grade water and boiled until the medium was dissolved completely
2. Then dispensed into the test tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and cooled in an erect position.
3. With a sterilized inoculating wire several colonies of 24-hour-old bacteria were picked and stabbed about halfway of the SIM medium tube.
4. Incubated with a loosened cap (aerobically) 37°C for 24 hours.
5. Finally, observed for the radiating growth (*E. coli* and *Salmonella*) appearance of black color (*Salmonella*) on the medium.

Figure 16. Sulfide indole motility test of *Salmonella.* (H_2S positive =red arrow)

Annex 5. Postmortem sampling

The procedure of postmortem sample collection from freshly dead chicken

1. The chicken was placed on its back with its feet facing me
2. Their wings were reflected backward
3. The skin was cut between the legs and the breast so the legs could be completely abducted and lie flat against the table
4. The skin was removed from the ventral surface of the bird by cutting across at the caudal edge of the keel and then pulling the skin cranially and caudally peeling away from the muscle to expose the muscular body wall.
5. A small cut was made into the body cavity using scissors or a scalpel blade just behind the breastbone, and then the abdominal muscle was pulled caudally to expose some abdominal viscera.
6. The cut was extended up through the cervical area and the beak was cut open at the angle of the jaw. The oral cavity, esophagus, trachea, and crop were all visible.
7. The keel bone and breast muscles were then removed by incising the pectoral muscles on each side of the keel and cutting through the ribs using the heavy poultry shears.

8. The small cut was made into the body cavity using scissors or a scalpel blade just behind the breastbone and then the abdominal muscle was pulled caudally to expose some abdominal viscera.
9. The cut was extended up through the cervical area and the beak was cut open at the angle of the jaw. Now the oral cavity, esophagus, trachea, and crop were all visible.
10. The keel bone and breast muscles were then removed by incising the pectoral muscles on each side of the keel and cutting through the ribs, using the heavy poultry shears.
11. The keel and breast muscles were removed entirely
12. Finally, the intended internal viscera were removed and transferred to the universal bottles containing buffered peptone water and transported to the laboratory.

Figure 17. Postmortem sampling of freshly dead chickens and processing

Annex 6. Bacterial Serotyping

The *Salmonella* isolates were serotyped by following the procedures depicted by the kit manufacturer and the result was interpreted accordingly.

I. Slide agglutination Test for *Salmonella* Isolates

1. A loopful of 24 hours old pure colonies from nutrient agar were picked up by a sterile stick and transferred to a clean microscopic glass slide

2. About one drop (0.5ml) of normal saline was added and emulsified to check autoagglutination of the isolates (interestingly none of the isolates has shown autoagglutination).
3. After that, about 1-2 drops of Poly 'O' antisera was added to the microscopic slides and a pure colony from the nutrient agar was transferred to these antisera and mixed well and observed for agglutination within 1 minute.
4. Those positive for the Poly-O-antisera were tested with a monovalent O and H antisera including *S. Typhimurium* (1,4,[5],12 i 1,2), *S. Enteritidis* (3 1,9,12), *S. Dublin* (1,9,12[Vi]), *S. Gallinarum* (1,9,12), and *S. Pullorum* (1,9,12).
5. Those appeared positive for *Salmonella* polyvalent antisera and none of the monovalent antisera were classified as other serotype.
6. As a negative control: one drop of 0.85% sterile NaCl solution was dispensed on one side of the microscopic glass slide and bacteria was mixed thoroughly.

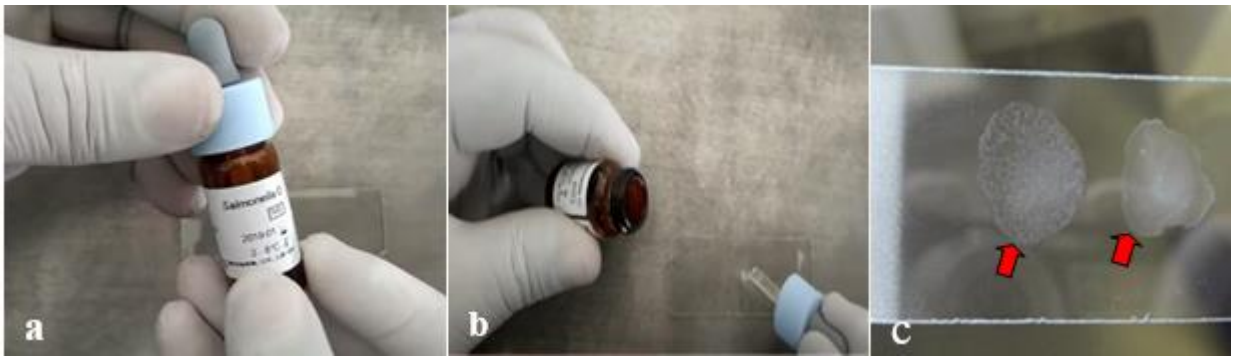

Figure 18. Slide agglutination test of *Salmonella. Salmonella* antisera (a), dropping of antisera to the glass slide; and slide showing agglutination of the test isolates (c).

II. Dulcitol fermenting and ornithine decarboxylation test for *S. Gallinarum* and *S. Dublin* identification

a. Dulcitol fermentation test

1. About 5 grams of Phenol Red Dulcitol Broth was suspended in 250 ml of distilled water.
2. Dissolved by heating to dissolve and allowed to cool
3. Then dispensed into test tubes containing and sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes and allowed to cool
4. An inoculum from a pure colony was inoculated to the tube with phenol red dulcitol broth.

5. The inoculated tube is incubated at 35-37ºC for 24 hours and observed for the color change, (positive -red to yellow= *S. Gallinarum*)

b. Ornithine decarboxylation test

1. Ornithine decarboxylase broth was prepared by adding 5% to the nutrient agar
2. Afterward, the pure colony was inoculated into the tube with ornithine decarboxylase broth.
3. The inoculum was incubated at 37 ºC for 24 hours and observed for color change from purple to yellow.
4. Then the culture was incubated for an extra 24 hours at 37 ºC.
5. Finally, the culture was observed for color change from yellow to purple original color (positive= *S. Pullorum*).

III. Slide agglutination test of *E. coli* O157:H7 isolates

For the current investigation, OK O antisera were applied for slide agglutination of live *E. coli* O157:H7 isolates as follows.

1. The reagents were brought to room temperature
2. About 20 µL of the O antisera was dropped on a glass in a circular manner
3. A loopful of the 24 hours old *E. coli O157:H7* isolates were picked up from the nutrient agar with a sterile inoculating loop and emulsified with the antisera
4. Then the glass slide was rocked gently and observed for agglutination for 5-10 seconds
5. The slides showing cloudy agglutination within 5-10 seconds were confirmed as *E. coli* O157:H7.

Annex 7. Questionnaire for the Assessment of KAP of Poultry Producers Towards Antimicrobial Use and Resistance

Dear all respondents, I am **Dr Habtamu Endale,** MSC student in Veterinary Pharmacology at Addis Ababa University, College of Veterinary Medicine and Agriculture. Now I am requesting you to participate in the interview with great cordiality. This questionnaire was aimed only at my MSc thesis work and the data will not be used for other purpose that affects your personality. So, please feel free to respond correctly and accordingly to the questions raised by the data collector. I like to show my heartfelt gratitude to you for your consent and participation.

Data collector: _____ Sign: _____ Date: _____

I. Respondent bibliography: Name: _____

Sex___ Age_____, Educational status: Illiterate☐ Elementary ☐ Highschool ☐ Diploma ☐ University degree ☐Year of experience in poultry production _____Prior training or information on veterinary practices and poultry production___ Job type, whether it is health (medical and veterinary) related or not. Related ____ Not related_____ Purpose of chicken they are raising ____ Stocking size _____

II. Knowledge-related questions on antimicrobial use and AMR

1. Is there a difference between bacterial and viral infection of chicken? Yes__No
2. Diseases caused by both viruses and bacteria can be treated by antimicrobials. Correct---Neutral---incorrect
3. Do you know which antimicrobials are effective against which disease? Yes__No__
4. Antimicrobials are given to the chicken by distinctive route and route of administration affect its effectiveness. Agree __strongly agree___neutral___Disagree ___ strongly disagree.
5. Do you think single dose of any antimicrobials can't cure any type of bacterial disease? Agree __strongly agree___neutral___Disagree ___ strongly disagree.
6. Do you know antimicrobials given to chickens can exist in their egg and meat and reach human beings?
7. Do you know AMR? Yes/No
8. Does long-time administration of antimicrobials for poultry result in AMR? Correct---Neutral---incorrect
9. Giving a lower dose of antimicrobials to poultry leads to the development of antimicrobial resistance. Agree __strongly agree___ neutral___Disagree ___ strongly disagree.
10. Therapeutic use of antimicrobials causes antimicrobial resistance. Agree _strongly agree_
11. neutral___Disagree ___ strongly disagree.
12. Do you know another factor that causes antimicrobial resistance? Yes__No

III. Attitude-related questions on antimicrobial use and AMR

1. Is identifying the cause of the disease needed before prescribing antimicrobials for diseased chicken? Agree _strongly agree___neutral___Disagree ___ strongly disagree.
2. Do you think giving antimicrobials through food at any time to the chicken to prevent disease in the chicken is not recommended? Agree _strongly agree___neutral___Disagree ___ strongly disagree.
3. Do you think expired drugs lose their efficacy and do not treat the infection and thus should discarded? Agree _strongly agree_neutral___Disagree ___ strongly disagree.
4. Do you think frequent nontherapeutic use of antimicrobials can contribute to the AMR? Agree _strongly agree___neutral___Disagree ___ strongly disagree.

5. Do you think stopping medication when the chicken looks better results in AMR and subsequent failure of the drugs? Agree _strongly agree__neutral_Disagree _strongly.
6. Do you think free access to antimicrobials at any place by anybody is good? Correct___ neutral ____ Incorrect
7. Does using antimicrobials for growth promotion and egg-laying improvement cause antimicrobial resistance? Agree _strongly agree___neutral___Disagree ___ strongly disagree.

IV. Practice related questions on antimicrobial use and AMR

1. From where do you buy drugs for the chicken? **i**. From veterinary pharmacy **ii**. Market **iii**. From animal health workers
2. Who administers the drug for your chicken? **i**. Yourself **ii**. Registered veterinarian **iii**. Animal health workers
3. Do you change the drug if the chicken does not recover from the drug you used? Yes/No_
4. How do you, and others giving the medication select a drug for the treatment of diseased chicken? **i**. Randomly **ii**. After appropriate diagnosis **iii**. Emperatively
5. Do the drug vendors write prescriptions while you buy drugs for the chicken? Yes/No
6. If antimicrobial are prescribed for a longer period, do you follow and give them for that much time? Agree _strongly agree___neutral___Disagree ___ strongly disagree.
7. If your chicken gets sick, do you consult a veterinarian? Yes--No
8. Utensil and equipment sharing with other farms transfer drug-resistant bacteria from farm to farm. Agree _strongly agree___neutral___Disagree ___ strongly disagree.
9. Do you keep an antimicrobial withdrawal period to use and sell their eggs or them for meat? Yes/No
10. How do you discard unused or leftover antimicrobials and disinfectants? Incorrect/Correct
11. Do you use specific syringes or other equipment for each drug to administer the drug to the chicken? Yes___No

Figure 19. Questionnaire face-to-face interview with poultry producers

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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/02/10/16/2024

Name of Applicant: **Habtamu Endale (DVM, MSc student)**

Address: Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Antibiogram studies of postmortem and fecal isolates of Salmonella and E.coli O157 H7 in conventional poultry farms in Hossana town, Central Ethiopia*

Date of application:	**December, 2023**
Nature of the project:	**Field investigation and questionnaire survey**
Target animal species:	**Chicken**
Number of animals involved:	**207**
Study area:	**Hossana, Central Ethiopia**

Minutes No. and date of review: **VM/ERC/02/16/024, 26/03/2024**

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

The Federal D... of E...
Addis Ababa Uni... College of Vet... Medicine and ...

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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MSC THESIS ON ANTIBIOGRAM STUDY OF SALMONELLA AND ESCHERICHIA COLI ISOLATES IN CONVENTIONAL POULTRY FARMS IN HOSSANA TOWN, CENTRAL ETHIOPIA

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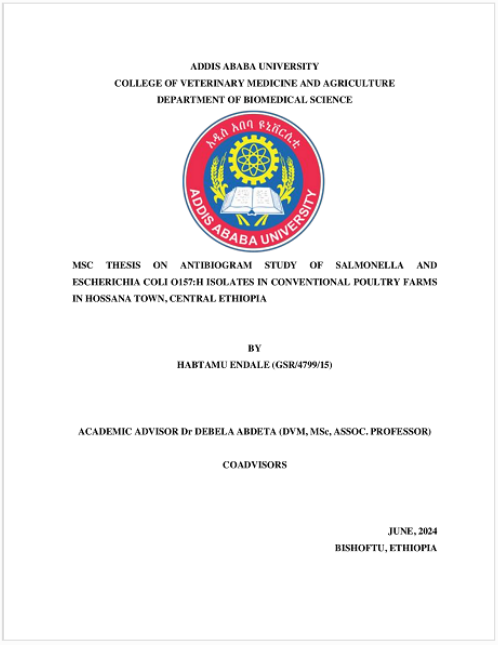

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE
DEPARTMENT OF BIOMEDICAL SCIENCE

MSC THESIS ON ANTIBIOGRAM STUDY OF SALMONELLA AND ESCHERICHIA COLI O157:H ISOLATES IN CONVENTIONAL POULTRY FARMS IN HOSSANA TOWN, CENTRAL ETHIOPIA

BY
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JUNE, 2024
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