Thesis Ref. No. _____

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND VETERINARY PUBLIC HEALTH

INVESTIGATION OF MICROBIAL LOAD, SELECTED BACTERIAL PATHOGENS, ANTIMICROBIAL RESISTANCE PROFILE AND ANTIBIOTIC RESIDUES OF BACKYARD-SLAUGHTERED BROILERS MEAT FROM SELECTED FARMS IN BISHOFTU, ETHIOPIA

MSc Thesis

BY

MISRAK NETSERE

JUNE, 2023

BISHOFTU, ETHIOPIA

INVESTIGATION OF MICROBIAL LOAD, SELECTED BACTERIAL PATHOGENS, ANTIMICROBIAL RESISTANCE PROFILE AND ANTIBIOTIC RESIDUES OF BACKYARD-SLAUGHTERED BROILERS MEAT FROM SELECTED FARMS IN BISHOFTU, ETHIOPIA

A thesis submitted to Addis Ababa University College of Veterinary Medicine and Agriculture, Department of Veterinary Microbiology, Immunology, and Public Health in partial fulfillment for the requirements of the degree of Master of Science in Veterinary Public Health

By

Misrak Netsere

June, 2023

Bishoftu, Ethiopia

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE
DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND PUBLIC HEALTH

As MVSc research advisors, we certify that we have read and evaluated the thesis prepared under our guidance by **Misrak Netsere Haileyesus** entitled: **"Investigation of Microbial Load, Selected Bacterial Pathogens, Antimicrobial Resistance Profile and Antibiotic Residues of Backyard-Slaughtered Broilers Meat from Selected Farms in Bishoftu, Ethiopia"** We recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Public Health.

Submitted by:	Misrak Netsere Haileyesus	_____	_____
	Name of Student	Signature	Date
Approved for submission to assessment committee:			
1. **Professor Bekele Megersa** (DVM, MSc, PhD)		_____	_____
Major Advisor (AAU, CVMA)		Signature	Date
2. **Dr. Belachew Tefera** (DVM, MSc) _____		_____	_____
3. **Dr. Belachew Bacha** (DVM, MSc, PhD) ____		_____	_____
Co- Advisor (EAA, API-QTC)		Signature	Date

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology, and Veterinary Public Health

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by Misrak Netsere Haileyesus entitled **"Investigation of Microbial Load, Selected Bacterial Pathogens, Antimicrobial Resistance Profile and Antibiotic Residues of Backyard-Slaughtered Broilers Meat from Selected Farms in Bishoftu, Ethiopia"** we recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Public Health

Name	Signature	Date
_____	_____	_____
Chairman		
_____	_____	_____
External Examiner		
_____	_____	_____
Internal Examiner		
_____	_____	_____
Major advisor		
_____	_____	_____
Department Head		

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my original work and that all sources of information used in it have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree in Veterinary Public Health at Addis Ababa University College of Veterinary Medicine and Agriculture, School of Graduate Studies Department of Veterinary Microbiology, Immunology, and Public Health. It has also been deposited at the University library to be made available to borrowers under rules of the library.

I solemnly declare that this thesis is not being submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

Name: Misrak Netsere Haileyesus Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: _____

ACKNOWLEDGEMENTS

First and foremost, I would like to praise the almighty God and his mother Saint Marry for bestowing health, strength, patience and merciful assistance through all ups and downs of my life.

I would like to express my special gratitude to my academic advisers. Prof. Bekele Megersa, Head, department of veterinary microbiology immunology and public health for his excellent comments and suggestions on the scientific and editorial aspects of the thesis. My thanks also go to co-advisers Dr. Belachew Tefera, Head of the Animal products, and Input Quality Testing Center (API-QTC) and Dr. Belachew Bacha Animal products, and Input physico- chemical lab director for their constant supervision throughout the work and provision of necessary materials. They have been very generous with their time and expertise and have helped me overcome many challenges and difficulties in this study.

I owe a huge debt of gratitude to the Addis Ababa University College of Veterinary Medicine and Agriculture, Department of Veterinary Microbiology, Immunology, and Public Health for its financial support to pursue this study. My heartfelt gratitude goes to the Animal Products, and Input Quality Testing Center (API-QTC) for the provision of reference standards, chemicals and for granting me access to its laboratory facilities.

My appreciation goes to my teammates and all the staff from API-QTC for supporting me and for sharing laughter and memories. Special credits go to the residue testing team members, Drug microbiology quality testing team and animal product and feed microbiology testing team.

I am also indebted to Dr. Nardos Tefera for unreservedly laboring with me from the beginning to the end of the analytical work in this study.

Finally, I would like to express my gratitude to my loved ones, close friends, and my devoted husband, Dr. Shibabaw Tadesse, for their never-ending support and unconditional love.

LIST OF ABBREVIATION

ADI	Acceptable daily Intake
AMR	Antimicrobial resistance
APC	Aerobic Plate Count
API-QTC	Animal product and input quality testing center
AST	Antimicrobial susceptibility test
BPW	Buffer peptone water
BUG	Biolog Universal Growth
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
CSA	Central Statistical Agency
EAA	Ethiopian Agricultural Authority
EC	*E. coli count*
EHEC	Enterohemorrhagic *E. coli*
ES-ISO	Ethiopian Standard-International Organization for Standardization
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis Critical Control Point
HPLC	High Performance Liquid Chromatography
HUS	Haemolytic Uremic Syndrome
MC	Matrix Control
MDR	Multi Drug Resistant
MRL	Maximum residue level
NC	Negative control
NTS	Non Typhoidal *Salmonella*e
PDR	Pan Drug Resistant
RC	Reagent control
SPE	Solid Phase Extraction
STA	*S. aureus* count
STEC	Shiga toxin-producing *E. coli*
TC	total coliforms count
UHPLC-MS/MS	Ultra-high performance liquid chromatography coupled with a triple quadruple mass spectrometer
VTEC	Verocytotoxin *E. coli*
VTEC	Verotoxigenic *Escherichia coli*
WHO	World Health Organization
XDR	Extensively Drug Resistance

TABLE OF CONTENTS **PAGE**

LIST OF TABLES

LIST OF FIGURES

ANNEXES

ABSTRACT

Broiler meat is a popular animal source food that can pose risks to human health as it may be contaminated with harmful microorganisms and antimicrobial residues. Investigations of those risks of broiler meat are limited in the study area. Thus, a cross-sectional study was carried out on selected Bishoftu farms, from January to July 2022, to investigate the microbial load, to isolate and identify selected bacterial pathogens and their antimicrobial resistance profile as well as detection and quantification of antibiotic residues of backyard broilers meat. A one hundred twenty broiler carcass samples were randomly collected and analyzed using TEMPO® for enumeration of microbial load, 3M^{TM} Molecule for detection of Campylobacter, BIOLOG® for E. coli O157:H7 and Salmonella isolation and identification. The VITEK 2XL was used for antimicrobial resistance profiling, whereas a High-Performance Liquid chromatograph coupled with a triple quadrupole mass spectrometer (HPLC-ESI-MS/MS) was used for the detection and quantification of antibiotic residues. This study has revealed, 120 (100%) *E. coli* and total aerobic bacteria, 114 (95 %) coliform, and 105 (87.5%) *S. aureus* contaminations found in the samples were above the limit of Ethiopian standard requirements. In all the farms at least one bacteria pathogen was detected with an overall ratio of *Campylobacter* 75 (62.5%), *E. coli O157:H7* 27 (22.5%) *and Salmonella* 20 (16.7%). Of the isolates of *E. coli O157:H7* and *Salmonella,* 11 (40.7%) and 13 (65%) were multi-drug resistant with three extensively drug resistance (XDR) and one pan drug resistance (PDR) *Salmonella* isolates respectively. Regarding antibiotic residues, 4 (3.3%), 22 (18.3%), and 30 (25%) of the samples analyzed contained detectable levels of residues of sulfadiazine, oxytetracycline and enrofloxacin respectively, while none was detected with tetracycline residue. Among these, 26 (21.67%) and 1(0.8%) of the samples respectively had oxytetracycline and enrofloxacin residues above the EU Maximum Residue Limits. These indications of high pathogenic bacteria contamination and antibiotic residues with multi-drug resistance on broiler meats might be due to unregulated slaughter facilities and irrational veterinary antibiotic uses. To mitigate the health risks of consumers, the establishment of standard slaughterhouses, applications of food safety procedures and raising awareness in rational use of drugs for broiler meat producers are vital.

Key words: *Antibiotic residues, Antibiotic resistances,* B*acteria count, Campylobacter, Escherichia coli O157:H7 and Salmonella*

1. INTRODUCTION

Food borne diseases occur as a result of consuming contaminated foods, especially animal products (Abebe *et al.,* 2020). These diseases are major health problems both in developed and developing countries, but developing countries tend to suffer from the largest share of the burden of food borne diseases (Noori *et al.,* 2016). According to the WHO, 30% of the population suffers from foodborne diseases and spends billions of dollars for the treatment of food borne pathogens each year in developed countries. And up to 2 million deaths are estimated per year in developing countries (FAO/WHO 2006).

Broiler meat is one of the most consumed animal products, but it might include microbiological hazards and chemical hazard that are harmful to human health and cause deterioration when kept for an extended period. Special attention must be observed in broiler meat production because of possible contamination from alimentary tracts, fecal contamination of the carcasses during spilled gut content, water, packaging, utensils and handlers (Adeyanju and Ishola 2014; Hertanto *et al.*, 2018; Maharjan *et al.,* 2019; Enver *et al.,* 2021). Contamination of broiler meat by pathogenic microorganisms, has become one of the most challenging problems in the food industry worldwide (Wardhana *et al.,* 2021).

These contaminants can cause significant economic losses in livestock production, and they hamper the trade of animal and animal products, especially in developing countries like Ethiopia (Gurmu and Gebretinsae 2013). This is due to inadequate methods of food handling and sanitation, insufficient food safety lows, poor regulatory systems, inability to afford to purchase equipment that is less hazardous and shortage of training provided to food handlers which creates conducive environment for the propagation of foodborne pathogens. (Tadesse *et al.,* 2019).

Food safety is a critical consideration in the consumption of poultry products. Ensuring the microbiological quality and safety of slaughter facilities and the environment is essential to prevent contamination and maintain the safety of broiler products. While

specific literature on the microbiological quality of commercial broiler producers and slaughter facilities in Ethiopia limited, there are studies that have investigated the general microbiological aspects of poultry processing in the country and highlighted the low biosecurity practices and challenges in commercial poultry farms (Fufa *et al.*, 2021).

Another cause of concern is the failure of the poultry farms to observe antibiotic withdrawal periods and slaughtering broilers for consumption before the drug could be metabolized and excreted (Tilahun *et al.,* 2016). Worldwide, there has been an increasing public health concern due to occurrences of unsafe levels of antibiotic residues in animal source food. This is because of the adverse health effects on consumers such as allergic reactions (Kyuchukova, 2020; Onipede *et al.,* 2021), disturbance of the local human gut microbiome and selection and spread of antimicrobial resistant microbial strains (Menkem *et al.,* 2019; Onipede *et al.,* 2021)

Bishoftu, is one of Ethiopia's major broiler-producing, processing and marketing place under minimal biosecurity measures which may risk pathogenic zoonotic microorganism that potentially pose health risk consumers. A non-regulated poultry slaughter facility provide dressed carcasses to market of capital city supermarkets, kiosks, hotels, and home (Milkias 2016; Eguale, 2018). Besides, *Salmonella* and *Campylobacter* identified in broiler carcass in studies of (Molla *et al.*, 2004; Dadi and Asrat 2009). However, inadequate information is found on the safety and quality of broiler meat in the study area let alone the scarcity of organized, reliable, and accurate data on microbiological quality, antimicrobial resistance (AMR) and antibiotic residues combined with impacts of AMR and residues in broiler meat products of Ethiopia is limited or none. Therefore, this study has assessed and identified the level of quality indicator organisms, occurrence of food bacterial pathogens and their AMR profile and antibiotics residues status of backyard-slaughtered broiler's meat in Bishoftu, in order to provide insight on microbial quality and safety of meat in under regulated facilities, input on antibiotic residue regulations plan in edible broiler meat products and pointing out dimensional contributing factors of AMR in consumers and potential recommendations on food pathogen AMR surveillances accordingly interventions of one health one welfare approach in the country. Therefore,

the general objective of this study was to investigate microbial load, isolation and identification of selected bacterial pathogens and their antimicrobial resistance profile and antibiotic residues of backyard-slaughtered broilers meat from selected farms in Bishoftu, Ethiopia

The Specific objectives were:

- To determine the microbial load (total aerobic bacteria, total coliform, total E. *coli*, *S aureus*) of broilers meat.
- To isolate and identify bacterial foodborne pathogens (*Campylobacter*, *E. coli O157:H7* and *salmonella*) from broilers meat.
- To determine antimicrobial resistance profile of *E. coli O157:H7* and *salmonella* isolates against most commonly used antimicrobial drugs.
- To identify and quantify the level of tetracycline, oxytetracycline, enrofloxacin and sulfadiazine residues in broilers meat.

2. LITERATURE REVIEW

2.1 Broiler *Production* in Ethiopia

Poultry production is a common practice in Ethiopia. According to central statistics agency report, the country total poultry population is estimated to be about 48.96 million. Laying hens make up the majority of the flock (36.78%), followed by chicks (30.36%). Additionally, it is estimated that there are 5.22 million, 5.90 million, and 2.63 million Pullets, Cocks and cockerels are respective. The others are non-laying hens which account for 4.75% (2.32 million) of the total poultry population in the country. With regard to breed, indigenous, hybrid and exotic were reported to be 81.71 %, 10.86 % , and 7.43 % of the total poultry, respectively (CSA, 2020).

Ethiopia's poultry sector has experienced significant growth in recent years, driven by increasing urbanization, population growth, and changing dietary preferences. Studies have highlighted the significant growth of broiler production in Ethiopia examined the broiler value chain and reported that the industry has expanded rapidly in recent years, driven by increasing urbanization and changes in dietary preferences (Tadesse *et al.*, 2019). Another study assessed the factors influencing broiler production performance in Ethiopia and found that feed availability, breed selection, and disease management were crucial determinants of productivity (Birhanu *et al.*, 2018).

Ethiopia’s poultry industry can be divided into three primary production systems such as large-scale commercial poultry production systems, small-scale commercial poultry production systems, and village or backyard poultry production systems, based on a variety of factors, including breed, flock size, living conditions, nutrition and bio-security (FAO, 2007).

Small-scale commercial poultry production has been growing in Ethiopia, especially in peri-urban and urban areas. Farmers adopt semi-intensive or intensive production systems, with improved housing and management practices (Teshome *et al.*, 2016). The majority of these farms get their feed and day-old chickens from large commercial

poultry broiler farms, and they also participate in the supply of broiler meat to the capital city at various supermarkets, kiosks and hotels through middlemen. The number of broiler chicks in their flock that are kept for operation normally ranges from 2000 to 5000. Their production approach is defined by low biosecurity and a medium degree of nutrition, water, and veterinary service inputs (Milkias, 2016; Teshome *et al.*, 2016)

Broiler meat is a comparatively inexpensive and accessible source of protein for most consumers when compared to other foods that originate from animal sources like beef. Consumption of poultry meat is more common in urban than rural places. According to ILRI (2000), the average yearly consumption of poultry meat in the country is expected to be 69,000 tons. The projected per capita consumption of poultry meat in Ethiopia in the mid-1990s was 2.85 kg (Milkias, 2016).

2.2 Broiler Processing Facility in Ethiopia

In Ethiopia, there is no broiler abattoir established by the government however, limited privately owned broiler abattoirs which are located in and around Bishoftu town. According to Gezahegn and Karel (2010), identified three significant processing-related problems in the broiler sector. First, small-scale farmers cannot afford the significant investment needed to build processing facilities. Second, because slaughter requires its own distinct and special infrastructure, some farms do not have the room to conduct it. Third, almost all poultry farms including those in the cities and towns have extremely inadequate drainage and waste management systems, which is a necessary prerequisite for constructing such processing facilities. Apart from this, the lack of institutionalized formal broiler abattoir results in multifaceted challenges in one or another way that contributes to food quality and safety risk. In addition, Alemayehu, (2015) noted that the involvement of veterinary experts to inspect the health status of chickens and monitor the overall safety of the slaughtering process is limited.

Small-scale commercial broiler producers slaughter their chickens in the compound's open areas using local equipment. They process their broiler on a floor covered with a plastic mat. Mostly the carcasses are skinned and eviscerated, by hand. The method of washing is too unhygienic in which a carcass is immersion washing where water stored in a container may be used to wash a number of broiler carcasses one after the other (Alemayehu, 2015). The use of stagnant rather than running water for carcass rinsing is a potential cause of the microbial load that leads to cross-contamination of all subsequent carcasses (Gezahegn and Karl, 2010).

Ensuring the microbiological quality and safety of slaughter facilities and environment is essential to prevent contamination and maintain the safety of broiler meat. While specific literature on the microbiological quality of commercial broiler producers and slaughter facilities in Ethiopia limited, there are studies that have investigated the general microbiological aspects of poultry processing in the country and highlighted the low biosecurity practices and challenges in commercial poultry farms (Fufa *et al.,* 2021). Another study by Jemberu *et al.,* (2020) focused on biosecurity practices in poultry production in Ethiopia. The study identified various microbiological quality challenges, including poor waste management, a lack of appropriate disinfection procedures, and a lack of necessary personal protective equipment for workers. It emphasized the importance of implementing biosafety protocols in slaughter facilities to safeguard employees' health and safety and environmental contamination (Jemberu *et al.,* 2020).

2.3 Microbiological Quality Indicator Organism

Microbiological examination of foods is focused not only on causative agents of human diseases, but also on microorganisms causing spoilage and affecting the quality or shelf life (Yu et al., 2020). Indicator organisms have been used in meat and poultry products to assess microbiological safety, hygiene during processing, and the keeping quality of the product (Mpundu *et al.,* 2021).The uncontrolled growth of microorganisms in food causes spoilage and a serious problem accounting for sizable losses of food products (Zhongjia *et al.,* 2020;Wardhana *et al.,* 2021).

The effectiveness of the processing method, temperature, sanitary and hygienic conditions of the processing facilities, and the timing of feed withdrawal prior to slaughter are all factors that affect the microbiological quality of broiler meat (Mead 2004; Maharjan *et al.,* 2019). Broiler meat is very sensitive process from hygienic and safety aspect, given that it is related with a lot of manual manipulation of the raw broiler meat. Spoilage of fresh meat in most cases the result of the action of microorganisms, and rarely occurs as a result of physical and chemical problems (Enver *et al.,* 2021). The initial microflora significantly affects the viability of broiler meat, which indicates the importance of the control of the production process, manipulation and storage (Maharjan *et al.,* 2019).

There is a lack of information on the microbiological quality of broiler meat in Ethiopia, however few studies have been published in animal source food (Gurmu and Gebretinsae, 2013; Zerabruk *et al.,* 2019). Various types of microbiological quality indicator organisms have been advocated for use in particular applications including aerobic plate count, coliforms, *E. coli*, S. aureus and Enterobacteriaceae (Faruque *et al.,* 2019).

2.3.1 Aerobic plate count

Aerobic Plate Count (APC) is a widely used technique for determining the total viable count of microorganisms in food samples. It provides an estimation of the total number of viable bacteria present in a sample (Hertanto *et al.,* 2018). APC is an indicator of quality, not safety and cannot directly contribute towards a safety assessment of food. In addition, it can provide useful information about the overall product quality, handling, storage history and shelf life of products (Odwar *et al.,* 2014; Faruque *et al.,* 2019).

Microbial contamination of meat begins since bleeding of slaughter steps, especially if the tools used for blood removal are not sterile. Therefore, it is necessary to test the quality of microbiology for contamination that occurs can be controlled (Hertanto *et al.,* 2018). High aerobic mesophilic counts in foods indicate greater risks of pathogens being present in consumable products, poor implementation of sanitation procedures or

problems in process controls to which a test food item has been subjected (Shaltout *et al.*, 2021). Regarding to broiler carcass quality, the maximum number of aerobic bacteria in broiler meat was recommended that less than 10^5CFU/g according to Ethiopian Standard Agency (ES 3967:2015).

2.3.2 Coliform and E. coli count

Coliform is not a taxonomic classification but rather a working definition used to describe a group of Gram-negative facultative anaerobic rod-shaped bacteria that ferments lactose to create acid and gas at 48 h at 35°C (Faruque *et al.,* 2019). It must be emphasized that some coliforms are harmless bacteria found naturally in the environment and in both animal and human guts (Odwar *et al.,* 2014; Faruque *et al.,* 2019).

The presence of total coliform bacteria are considered as indicator organisms not likely to cause illness, but their presence indicates that your broiler meat supply may be vulnerable to contamination by more harmful microorganisms such as *E. coli,* Enterobacter and Citrobacter (Faruque *et al.,* 2019; Jhandai *et al.,* 2020). It is also used as an indicators of sanitary quality of broiler meat or as a general indicator of sanitary condition in the food manufacturing facilities (Hertanto *et al.,* 2018; Jhandai *et al.,* 2020).

E. coli is the only member of the total coliform group of bacteria, which are used as indicators of fecal contamination and unsanitary processing. High levels of E. coli indicate poor sanitation practices and the potential presence of enteric pathogens (Odwar *et al.,* 2014). However, the majority of E. coli strains are not thought to be pathogens, they can be opportunistic pathogens that infect immune-compromised hosts and cause illnesses. There are also pathogenic strains of *E. coli* such as *E. coli* O157:H7, that may cause gastrointestinal illness in healthy humans (Odwar *et al.,* 2014; Liur and Veerman 2021).

2.3.3 Staphylococcus aureus count

Staphylococcus aureus is a gram-positive bacterium that is part of the normal microbiota on human skin and mucous membranes. It is characterized by its ability to produce coagulase enzyme and form yellow or golden colonies on agar plates. It is a facultative anaerobe and can grow under various environmental conditions (Bhandari and Nepali 2013). *S. aureus* can cause a range of infections, ranging from minor skin infections to more severe conditions such as pneumonia, bloodstream infections, and food poisoning. It generates several virulence factors, including toxins and enzymes, which increase its pathogenicity (Ibrahim *et al.*, 2018).

S. aureus can contaminate broiler meat and eggs, either through external sources during processing or from carrier animals. Poor hygiene practices during slaughter, handling, and storage can contribute to contamination. *S. aureus* in poultry products can pose a risk of foodborne illness if consumed (Bhandari and Nepali 2013). High levels of *S. aureus* indicate poor hygiene practices during processing (Mead 2004; Maharjan *et al.*, 2019).

2.4 Foodborne Bacteria Pathogens

2.4.1Campylobacter

Campylobacter is the most important of foodborne pathogen, which cause gastrointestinal disease in humans in both developed and developing countries (Nzouankeu *et al.,* 2010; Zendehbad *et al.,* 2013). Food animals, mainly poultry, cattle, sheep, and pigs, may act as asymptomatic intestinal carriers of *Campylobacter* and animal food products can become contaminated by this pathogen during slaughter and carcass dressing (Thomas *et al.,* 2020). Cross contamination of ready to eat foods during preparation by food handlers and direct interaction with animals have also been noted (Dadi and Asrat, 2009).

One of the major significant dietary sources for these organisms has been found to be poultry meat (Adesiji *et al.,* 2011; Kuria *et al.,* 2018). Indeed, studies have shown that preparation of raw poultry and consumption of contaminated broiler meat or broiler products in general have been identified as the main risk factor for human infection with *Campylobacter* (Cardinale *et al.,* 2002; Adeleye *et al.,* 2018). Contamination of poultry carcasses by *Campylobacter* can occurs during processing directly via intestinal contents or indirectly from bird to bird, via equipment and water. *Campylobacter jejuni* and *Campylobacter coli* are the species most commonly associated with human infections and cause clinically similar illnesses (Zendehbad *et al.,* 2013).

Campylobacter related infections in Ethiopia may be underestimated due to a lack of adequate laboratory facilities, a sustainable supply of consumables, expensive expenses, a scarcity of well-trained human resources, and a well-designed surveillance system. Even though the spread of *Campylobacter* spp. in Ethiopia is not well organized and reported from a national perspective, there is limited studies have been done on prevalence, distribution of *Campylobacter* spp. in poultry meat (Dadi and Asrat, 2009).

Table 1: Studies of *Campylobacter* in food of animal origin in Ethiopia

Sample type	No. of examined	No. of positive (%)	Study area	Source
Beef	227	14 (6.2)	Addis Ababa and Bishoftu	(Dadi and Asrat, 2009)
Mutton	114	12 (10.5)		
Goat meat	92	7 (7.6)		
Pork	47	3 (6.4)		
Broiler	60	12 (20)		
Total	**540**	**48 (8.9)**		
Poultry cloacal swab pools	20	15 (70%)	Bishoftu and Mojo, Central Ethiopia	(Wayou *et al.,* 2022)
Fecal sample	171	22(57.9)	Jimma Town	(Debelo *et al.,* 2022)
Carcasses swab	171	7(18.4)		
Knives swab	171	6(15.8)		
Hand swabs	171	3(7.9)		
Total	**684**	**38 8.9)**		

2.4.2 Escherichia coli (E. coli)

E. coli are a normal part of the intestinal micro-flora of many healthy animals, including humans (Hiko *et al.,* 2008). They are pathogenic and non-pathogenic *E. coli*, non-pathogenic *E. coli* strains described as commensal and present in the normal microflora of intestine while Pathogenic *E. coli* can cause diseases (Odwar *et al.,* 2014; Liur and Veerman, 2021). Pathogenic *E. coli* strains, which cause enteric disease, are enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), diffuse-adherent (DAEC) and the recently emerged, adherent invasive *E. coli* (AIEC) (Liur and Veerman, 2021; Zarei *et al.,* 2021).

Among the EHEC strains, *E. coli* O157: H7, commonly known as verocytotoxin *E. coli* (VTEC), also referred to as Shiga toxin-producing *E. coli (STEC),* is an emerging foodborne bacteria pathogen that has sparked global concern after causing numerous foodborne outbreaks around the world (Kuria *et al.,* 2018; Abebe *et al.,* 2020). Several outbreaks of bacterial food-borne disease due to the consumption of undercooked or raw meat contaminated with STEC strains have been reported (Hossainpour and Alikhani 2019; Zarei *et al.,* 2021). The hemolytic-uremic syndrome (HUS) is a serious condition, and up to 50% of HUS patients can develop long-term renal dysfunction or blood pressure related complications. Children aged two to six years have an increased risk of developing HUS. Antimicrobial agents should be avoided in the acute phase of the disease since studies showed that antimicrobial agents may increase the risk of HUS in children (Gambushe and Zishiri, 2022)

E. coli can easily contaminate animal-derived meats at the time of slaughter and during some stages of the food production process (Olatoye *et al.,* 2012; Hamid *et al.,* 2018). The potential contamination of edible carcass tissue poses the greatest risk to food safety and the degree and nature of this contamination are connected to the methods used in abattoir killing as well as any processes that could transmit the organism between or inside carcass dressing operations (Hamid *et al.,* 2018; Abebe *et al.,* 2020).

Transmission of *E. coli* serotype O157:H7 is via fecal-oral route, due to improperly washed hands or following consumption of contaminated foods from animal origin harboring the organism specially meat and the meat products as well as milk and dairy products which are not treated well by heat (Hessain *et al.,* 2015). Other causal factors include exposure to contaminated water from potable drinking sources, swimming pools and lakes (Abebe *et al.,* 2020).

People from any age group can be affected by *E. coli O157:H7* infection; while old people and young children can be more susceptible. Low infective dose as 10-100 cells, stress resistance mechanisms, and production of toxins contribute to the severity of the *E. coli O157:H7* infection. In addition, efficient acid resistant methods facilitate survival and colonization of the organism under the acidic conditions in the gastrointestinal tract and food with low pH value. Therefore, *E. coli O157:H7* is considered as a serious threat to consumers (Abdissa *et al.,* 2017).

In Ethiopia, data on presence of *E. coli O157:H7* in broiler meat is scarce. However, few studies have been done on prevalence, distribution and associated virulent genes of *E. coli O175: H7* in foods of animal origin indicate below in table 2.

Table 2: Studies of *E. coli O157:H7* in animal source food in Ethiopia

Sample type	No. of examined	No. of positive (%) *E. coli O157:H7*	Study area	Reference
Beef	250	20 (8)	Debre Zeyit and Modjo	(Hiko *et al.,* 2008)
lamb and mutton	243	6 (2.5)		
Goat meat	245	5 (2)		
Total	**738**	**31 (4.2)**		
Beef	128	17 (13.3)	Addis Ababa	(Bekele *et al.,* 2014)
Sheep meat	128	12 (9.4)		
Goat meat	128	10 (7.8)		
Total	**384**	**39 (10.2)**		
Fecal sample	370	7 (2)	Addis Ababa and Debre Birhan	(Abdissa *et al.*, 2017)
Skin swab	370	2 (0.5)		
Intestinal mucosal swab	370	3 (0.8)		
Carcass internal swab	370	2 (0.5)		
Cutting board	125	1 (0.8)		
Carcass swab	110	5 (4.5)	Central Ethiopia	(Beyi *et al.,* 2017)
Cutting board swab	110	4 (3.6)		
Minced beef		0		
Cloacae samples	194	13.4	Eastern Ethiopia	(Shecho *et al.,* 2017a)
Cheese	35	2 (5.7)	Bishoftu, Central Ethiopia	(Bedasa *et al.,* 2018)
Raw milk	25	3 (12)		
Pasteurized milk	40	0 (0)		
Yogurt	35	0 (0)		
Meat	65	2 (3.1)		
Total	**200**	**7 (3.5)**		
Raw beef	384	14 (3.64)	Addis Ababa	(Haile *et al.,* 2022)

2.4.3 Salmonella

Salmonella is a facultative anaerobic, gram-negative, non-spore forming rods genus belonging to the Enterobacteriaceae family (Asfaw *et al.,* 2020). There are two species, *S. enterica and S. bongori,* each have several serovars. More than 2500 serovars have been described (Ha and Nguyen, 2012). Non-typhoidal *Salmonella* (NTS) are among the most important causes of diarrheal in humans, contributing to an estimated 230,000 deaths annually (Rortana *et al.,* 2021).

Salmonella infection has been linked to numerous foodborne human illnesses in recent years, and it is one of the most widely spread causes of foodborne bacterial

diarrhea infections globally (Ha and Nguyen 2012; Asfaw *et al.*, 2020). Much of the published reports indicated that the major sources of human infection mainly associated with the consumption of raw or undercooked poultry meat or dairy products (Ha and Nguyen 2012; Dagnew *et al.*, 2020; Belachew *et al.*, 2021).

Contamination could be from the actual infection of food animals at the farm, cross contamination during slaughtering, distribution and subsequent handling and processing are important in transmitting *salmonella* species (Eguale 2018; Rortana *et al.,* 2021).The other probable source of cross contamination could be from *salmonella* carrier slaughterhouse personnel (Abdi *et al.,* 2017).

In Ethiopia despite attempts to study on prevalence, distribution and associated of *salmonella* mainly at farm level. However, few studies have been done on prevalence, distribution and associated virulent genes of *salmonella* in animal source food indicate below in table 3.

Table 3: Studies on salmonella in foods derived from animals in Ethiopia

Sample type	No. of examined	No. of positive (%)	Study area	Reference
Broiler meat	104	16(15.4)	Debre Zeyit and Addis Ababa	(Molla *et al.*, 2004)
Skin	104	8 (7.70		
Liver	55	19 (34.5)		
Gizzard	56	23 (41.1)		
Heart	59	14(23.7)		
Total	**378**	**80 (21.1)**		
Burger	35	1 (2.9)	Gondar	(Ejo *et al.*, 2016)
Cottage cheese	20	0 (0)		
Cream cake	50	0 (0)		
Egg sandwich	35	0 (0)		
Minced meat	25	2 (8)		
Pasteurized milk	45	0 (0)		
Pizza	24	0 (0)		
Raw egg	50	9 (18)		
Raw meat	50	6 (12)		
Raw milk	50	3 (6)		
Total	**384**	**21 (5.5)**		
Bovine carcass swab	70	4 (5.7)	Addis Ababa Abattoirs Enterprise	(Kebede *et al.*, 2016)
Bovine lung	35	2 (5.7)		
Bovine liver	35	2 (5.7)		
Ovine carcass swab	70	1(1.4)		
Ovine lung	35	1(2.8)		
Ovine liver	35	3(8.5)		
Total	**280**	**13(4.64)**		
Farm egg content	83	2 (2.4)	**Jimma**	(Taddese *et al.*, 2019)
Farm eggshell	83	0		
Cloacae	83	2 (2.4)		
Market eggshell	83	4 (4.8)		
Market egg contents	83	3 (3.6)		
Total	**415**	**11(2.65)**		
Feces	259	38(14.67)	Bishoftu and Adama	(Asefa *et al.*, 2022)
Eggs	56	14(25.00)		
Meat	69	10 (14.49)		
Total	**384**	**62 16.15)**		

2.5 Antimicrobial Resistance Profile of Bacterial Foodborne pathogens

Antimicrobial resistance (AMR) remains a growing threat for human and animal health, lessening the ability to treat bacterial infections and furthering the risk associated with morbidity and mortality caused by resistant bacteria (Hedman *et al.,* 2020).

According to CDC's 2019 Antibiotic resistance threats report, antimicrobial resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019. In the U.S., more than 2.8 million antimicrobial-resistant infections occur and more than 35,000 people die each year. AMR has become a significant issue in recent years, and numerous programs have been established in both human and veterinary medicine to monitor it. These programs primarily target human infections, zoonoses, and indicator bacteria of animal-derived normal gut flora (Hamid *et al.,* 2018; Hedman *et al.,* 2020).

In Ethiopia livestock sector is under rapid development and the sector serves as a source of food, income, and foreign exchange to the country's economy. There will be an increased demand for quality sources of animal protein. Further promotion of intensive poultry farming could address issues of food security, but it may also increase risks of AMR due to regular use of antibiotics (Thanthirige *et al.,* 2016; Hedman *et al.,* 2020; Rau *et al.,* 2021). The healthcare, veterinary, and agricultural sectors, as well as individuals at any stage of life, could be impacted by antimicrobial resistance. Because of this, it is difficult to determine the extent of the public health burden caused by resistant food-borne diseases (WHO, 2000).

Livestock, and their products such as meat, milk and egg may serve as reservoirs for human infections by AMR pathogen microorganisms, thus allowing these microorganisms to persist and spread in the community (Molla *et al.,* 2004; Tefera *et al.,* 2022). In addition, the diagrammatic presentation of the spread of antibiotic resistance bacteria among the community, environment livestock is shown in figure 1. To combat this effectively, it is recognized that a multidisciplinary approach including human and

animal health, food production and environmental factors is necessary, in alignment with the World Health Organization One Health concepts (WHO, 2015).

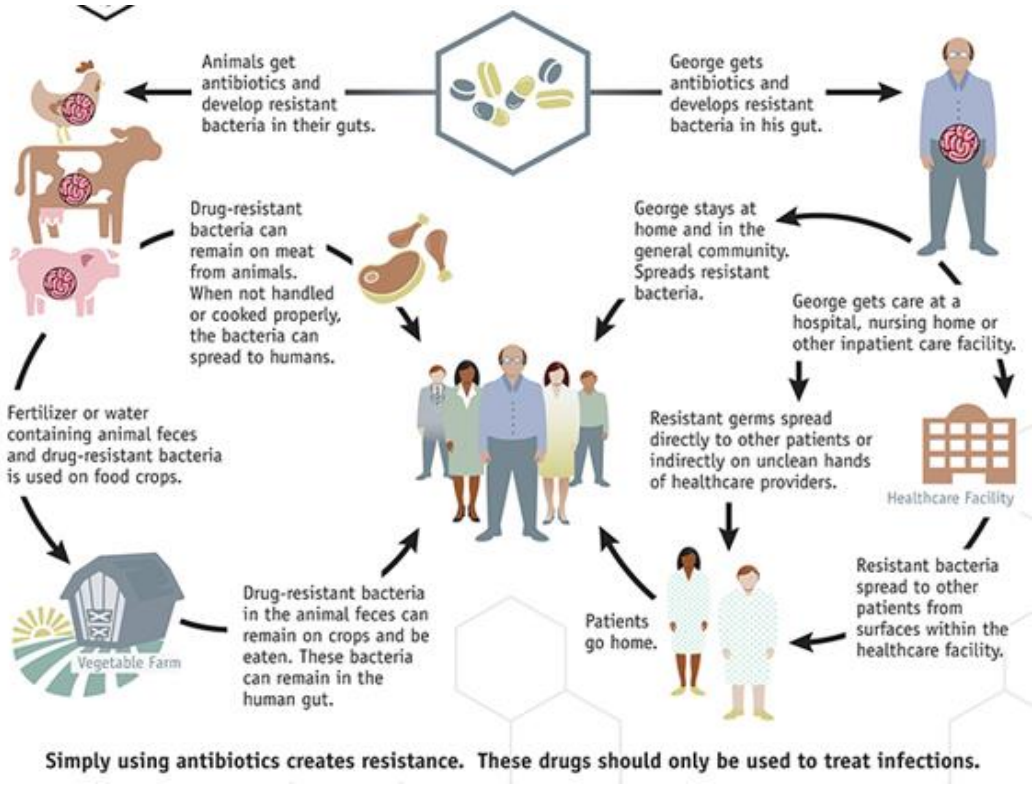


Figure 1: Spread of antibiotic resistance bacteria pathogen (WHO, 2015).

2.5.1 Antimicrobial resistance of E. coli O157:H7

Multidrug resistance in *E.coli* has become a worrying issue that is increasingly observed in human and veterinary medicine worldwide (Haile *et al.,* 2022). In the enterobacterial gene pool, *E. coli* acts as a donor and as a recipient of resistance genes and thereby can acquire resistance genes from other bacteria but can also pass on its resistance genes to other bacteria (Tuem *et al.,* 2018). *E. coli* is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a high ability for acquiring resistance genes, primarily through horizontal gene transmission (Poirel *et al.,* 2004). In general, antimicrobial resistance to *E. coli* is regarded as one of the most

difficult problems for both people and animals on a global scale and it should be viewed as a concern to public health (Haile *et al.*, 2022).

E. coli O157:H7 strains have developed resistance to several antimicrobial agents due to improper use of antimicrobials. In developing countries particularly Ethiopia, the emergence of antibiotic resistance *E. coli O157:H7* is a key contributor to the ineffectiveness of the treatment of infectious diseases (Gambushe and Zishiri, 2022). *E. coli O157:H7* has developed varying degrees of resistance against a number of commonly used human and animal antibiotics including Erythromycin, Amoxicillin-Clavulanic acid, Sulfonamide, Ampicillin and Tetracycline, Nitrofurantoin and there are also strains developed MDR according to several investigations conducted in Ethiopia from diverse animal product and clinical sources (Hiko *et al.*, 2008; Abdissa *et al.*, 2017; Beyi *et al.*, 2017; Shecho *et al.*, 2017; Bedasa *et al.*, 2018; Hamid *et al.*, 2018; Haile *et al.*, 2022).

2.5.2 Antimicrobial resistance of Salmonella

The emergences and spread of antimicrobial resistant *Salmonella* infection are global challenges, especially in developing countries where there is an increased misuse of antimicrobials in humans as well as in animals (Abate and Assefa, 2021). The increasing proportion of single and multiple antibiotic-resistant *salmonella* strains isolated from human salmonellosis cases has been associated with the widespread use of antibiotics in food animals (Kebede *et al.*, 2016; Woyessa *et al.*, 2020).

Salmonella strains resistant to various antimicrobial agents, particularly resistant to ampicillin β-lactams, extended-spectrum β-lactams, fluoroquinolones, aminoglycosides, tetracycline, chloramphenicol, tetracycline, and Kanamycin (Nair, 2018) and third generation cephalosporins, are considered as an emerging problem worldwide (WHO, 2014), resulting in higher morbidity and mortality rates and higher overall treatment costs. This may represent a public health risk by transfer of resistant *Salmonella* strains

to humans through the consumption of contaminated food and food products (Abebe *et al.*, 2020).

In Ethiopia, a high frequency of resistance *Salmonella* spp. against different antimicrobial agents have been reported (Kebede *et al.*, 2016; Eguale 2018; Dagnew *et al.*, 2020, Belachew *et al.*, 2021; Mohammed and Dubie 2022). Multidrug resistance (MDR) *salmonella* also reported in Ethiopia (Molla *et al.*, 2004, Abunna *et al.*, 2016).

2.6 Antibiotics Use Practice

Modern approaches applied for efficient chicken production have resulted in wide-spread disease havoc across the world. For example, intensive rearing conditions with high stock densities in poultry houses have provided ideal conditions for the manifestation and transmission of bacterial diseases. In addition, due to poor management conditions, the disease incidences are not only becoming more frequent, pronounced, unmanageable but also difficult to control in the long run (Muhammad *et al.*, 2009; Mund *et al.*, 2017). These diseases not only influence poultry growth and production, but also contribute considerably to economic losses due to high mortality among flocks (Chapman and Jeffers, 2014).

Furthermore, high disease loads call for the heavy use of antibiotics, in order to prevent and treat microbial infestation (Mund *et al.*, 2017). Antibiotics are among the most widely used veterinary drugs in the poultry industry. Antibiotics application in poultry is favorable to farmers and the economy as well because it has generally improved poultry performance effectively and economically (Agyare *et al.*, 2019). However, the benefit of improved productivity from the use of antibiotic drugs in poultry industry is not obtained without risks. The risk can be associated with the accumulation of antibiotic residues in edible poultry tissue and egg and the development of drug resistance intended for human consumption. Human health is directly related to the environment, and in particular, the nature and quality of food (Bai and Ogbourne, 2016). Antibacterial drugs are among the

main veterinary medicinal products that can potentially contaminate foods of poultry origin (Beyene, 2016).

For years, the use of antibiotics was seen as a hallmark and allowed to be used without any restrictions, regulations, or supervision in various commercial poultry enterprises. However, the negative effects of these remained undetected until resistance was acquired against antibiotics. Concerns about antibiotic therapeutic and growth promotion deployments aroused mainly due to the emergence of an increased number of resistant bacteria (Butaye *et al*., 2003; Marshall and Levy, 2011).However, extensive use of antibiotics and growth hormones has been banned in developed countries (Mund *et al.*, 2017), but the situation is quite contrary in developing countries including Ethiopia due to inadequate safety standards and regulations or in some cases these standards or regulations even do not exist (Lawal *et al*., 2015; Temesgen and Abdisa, 2015).

Ethiopia is seeing rapid increase in both commercial and smallholder/semi-intensive poultry enterprises. Despite their poor management and lack of biosecurity measures the risk of infection is higher on these farms. Farm owners frequently use antibiotics without veterinary prescription and supervision based on their own diagnosis and recommendation to combat the issues of infectious diseases (Woyessa *et al.*, 2020). Antibiotics were commonly utilized in poultry farms for therapeutic, prophylactic, and growth promoters purpose (Beyene *et al*., 2018; Woyessa *et al*., 2020). Some of the common antibiotics used as preventative and/or control of bacterial infections in poultry farms include oxytetracycline, amoxicillin, ciprofloxacillin, and sulfa medicines. The farms also utilize amprolium and sulfa medications to address piperazine and coccidiosis. (Beyene *et al*., 2015; Gemeda *et al*., 2020).

The misuse, excessive use of antibiotics and failure to adhere strictly to the withdrawal period of antibiotics in poultry production is reported to be the primary cause of antibiotic residues in edible poultry tissues (Lawal *et al*., 2015; Mund *et al*., 2017; Agmas and Adugna, 2018; Ma *et al*., 2021). Existence of the antibiotic residues in broiler meat can pose hazards to human health. The risk can be expansion of antibiotic resistance among

bacterial pathogens that have been known as a worldwide health difficulty, allergic reactions, imbalance of intestinal microbiota, carcinogenicity, mutagenicity and loss of hearing (Bai and Ogbourne, 2016; Alewy *et al.*, 2018).

Resistant microorganism can get access to human, either through direct contact or indirectly via meat. The animal fed with the low prophylactic level of antibiotic may develop bacteria evolving resistance to this antibiotic during the preparation or consumption of food of animal origin. It has been documented that human develop drug resistant bacteria such as *Salmonella, Campylobacter* and *E. coli* from food of animal origin (Landers *et al*., 2012; Beyene *et al*., 2018; Gemeda *et al*., 2020).

2.7 Antibiotics Residues

Antimicrobial residues in food have received much attention in recent years because of growing food safety and public health concerns (Tilahun *et al.*, 2016). Their presence in food of animal origin constitutes socioeconomic challenges in international trade in animal and animal products (Okocha *et al.,* 2018). Antibiotics are widely used in the poultry industry for the treatment and prevention of several diseases, as well as to increases the effectiveness of animal production (Hussein and Khalil, 2013). In addition, assist in converting stress due to environmental changes, vaccination, debeaking and other management practices (Shareef *et al.,* 2009). On the other hand, non-restrictive usage of antimicrobials in food animals may have unintended consequences, such as the emergence of resistant bacteria or a higher chance of detecting antibiotic residues in edible tissues (Hind *et al.,* 2014; Ramatla *et al.,* 2017).

Human health can either be affected through residues of drugs in foods of animal origin, which may cause direct side effects or indirectly through selection of antimicrobial resistance determinant that may spread human pathogen (Beyene, 2015; Lawal *et al.,* 2015; Tilahun *et al.,* 2016). Risks to human health from consuming foods of animal origin that contain unacceptable levels of antimicrobial residues include the emergence of antimicrobial-resistant strains (Darwish *et al.,* 2013; Ma *et al.,* 2021), imbalance of

intestinal micro-flora (Cotter *et al.,* 2012), and carcinogenicity (Aiello *et al.,* 2005). Other effects include reproductive disorders, bone marrow toxicity and anaphylactic reaction in individuals with known hypersensitivity to penicillin (Berends *et al.,* 2001; Maria and Mary, 2012).

Antibiotics used according to the label instructions should not result in residues in edible poultry tissues and products. The occurrence of residues above established safe or tolerances levels leading to noncompliant results in edible tissues from food animals have been associated with inadequate farm management practices. These include failure to properly follow the withdrawal periods, extra-label drug use which means the use of a drug in a manner that is inconsistent with its approved labeling, use of unapproved antibiotics, prolonged drug excretion (Beyene, 2015; Lawal *et al.,* 2015; Tilahun *et al.,* 2016). However, failure to adhere to the withdrawal period, including the use of overdosing and long-acting medicines, may be the most obvious cause of inappropriate residues (Beyene and Tesega, 2014; Beyene, 2015).

Drug residues is all active ingredients or metabolites of those ingredients that remain in foodstuffs obtained from animals to which the medicinal product in question has been administered (Jank *et al.,* 2017). The concept of drug residues in food was developed during the second half of the 20^{th} century, resulting in the definition of a no observed effect level, an acceptable daily intake (ADI), and maximum residue level in food (MRL) (CAC, 2017).

Maximum Residue Limits (MRLs) of veterinary drugs is the maximum concentration of a residue resulting from the registered use of an agricultural or veterinary drugs to be legally permitted or recognized as acceptable in or on a food (CAC, 2017). It is derived from acceptable daily intake (ADI) that is set based on the assessment of human health hazard and other relevant public health risks as well as food technological aspects to determine a dose that produces no-observed-effect level of the most sensitive effect in the most sensitive species (FAO/WHO, 2015).

The Withdrawal Period is the time between the last doses given to the animal and the time when the level of residues in poultry edible tissues and products like meat and egg is lower than or equal to the MRL. Poultry tissue and products must not be used for human consumption until the withdrawal period has elapsed (Tansakul *et al.,* 2007; Goetting *et al.,* 2011).

Table 4: Recommended MRLs of target antibiotics in broiler meat

Pharmacologically active substance	Target Poultry tissue	MRL (µg/Kg)		ADI
		CAC	EU	
Oxytetracycline and Tetracycline	Muscle	200	100	0-30 µg/kg body wt.
	Liver	600	300	
	Kidney	1200	600	
	Egg	400		
Sulfadiazine	Muscle	100	100	0-50 µg/kg body wt.
	Liver	100	100	
	Kidney	100	100	
	Egg	-	100	
Enrofloxacin	Muscle	-	100	
	Liver	-	200	
	Kidney	-	200	
	Egg	-	-	

Source: (EC/EU, 2010; CAC, 2018)

Antimicrobial residues are spreading swiftly, regardless of geographical, economical, or legal differences between countries (Darwish *et al.,* 2013). Because of this, the concerns over food residues are becoming more economic as well as public health related. Data on presence of antibiotic residues in broiler meat in Ethiopia are limited. The few investigations that have been done on the presence of antibiotic residues in animal source food are indicate below in table 5.

Table 5: Studies of *antibiotic residues* in animal sourced foods in Ethiopia

Sample type	No of examined	Antibiotic detected	No of positive (%)	Method for detection	Study area	Reference
Beef	128 128 128	OTC	48.3 0 48.1	HPLC	Addis Ababa, Bishoftu Nazareth	(Bedada and Zewde, 2012)
Milk	400	OTC Penicillin G	83.33 16.66	HPLC	Nazareth	(Abebew *et al.*, 2014)
Beef	250	Qualitative test	76.4	Premi® test Kit	Bahir Dar and Debre Tabor	(Agmas and Adugna, 2018)

2.8 Detection Methods of Bacteria Foodborne Pathogens and Antibiotic Residues

2.8.1 Foodborne bacteria pathogens

Detection of pathogenic microbes in food is therefore essential for public health and food safety reasons. There are several techniques available for the detection of pathogenic bacteria in food. These include different biological detection techniques such as culture-dependent microbiological methods (ISO-6579, 2002) (Ha and Nguyen 2012) and culture-independent detection techniques (Abebe *et al.*, 2020), nucleic acid sequence-based detection techniques including different types of polymerase chain reaction (PCR) methods (Nataro and Kaper, 1998; Seyum, 2019), immunological detection techniques (Nagaraj *et al.*, 2016) and different biosensor-based methods including electrical and optical biosensors (Brindha *et al.*, 2018).

2.8.2 Antibiotics residues detection methods

Several analytical methods have been developed to determine levels of antibiotic residues in poultry meat and egg. The analytical methods for antibiotics detection can be divided into two groups, namely confirmatory and screening. Screening methods are generally performed by microbiological, enzymatic and immunological methods and primarily used to obtain semiquantitative measurements. This approach is viable because of easy

operation, quick analysis period, cost effectiveness and good selectivity. Confirmatory methods provide full or complementary information, unequivocal identification and/or quantification of analysts. It is indispensable part of routine analysis in regulatory laboratories around the world for surveillance and food safety testing (Pikkemaat *et al.,* 2009; Mungroo and Neethirajan, 2014; Aytenfsu *et al.,* 2016).

Microbiological methods used to detect antibiotic residues in poultry meat and eggs based on inhibiting microbial growth, microbial receptor activity, and enzymatic reactions (Aytenfsu *et al.,* 2016). Microbial inhibition assays involve the culture of a microorganism from a standard strain, usually *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *E. coli*, *Bacillus megatherium*, *Sarcina lutea*, and/or *Streptococcus thermophilus* (Tilahun *et al.,* 2016). Two types of microbiological test are employed: one using test tubes (Delvo test-X-Press test, Charm I/Charm II, Eclipse/Zeu-Inmunotech) and the other using combinations of Petri dishes (Pikkemaat *et al.,* 2009).

Immunochemical methods represent an important tool for determining drug residues, given their high specificity. These methods are based on the reaction of an antigen binding to a specific primary antibody or, for each antigen, analogously to an enzyme substrate reaction. The most common immunochemical methods, such as enzyme-linked immunosorbent assay (ELISA), direct competitive ELISA test, radioimmunoassay, and receptor binding, are also used with different instruments for measurement (Ferguson *et al.,* 2002; Gaudin *et al.,* 2005).

Biosensors use a semi quantitative approach, which makes them a very practical solution in the large-scale detection of antibiotic residue in animals including at farms and slaughterhouses. Biosensors have been developed in recent years as an alternative approach to screen veterinary drugs in meat. In general, a biosensor is an instrument that combines, in close contact, cognition elements with an antibody/antigen, enzyme/substrate, receptor and its specific ligand, or living cells and an analyte that binds specifically to them. The biochemical signals of biosensors are converted by a transducer

to an electronic signal. Then, these signals are processed by a microprocessor that gives the final result (Toldrá and Reig, 2006; Mungroo and Neethirajan, 2014).

Physical-chemical methods are used mainly to isolate, separate, quantify, and confirm the presence of dangerous residues in samples. Separation methods based on the principles of chromatography are generally coupled with high-sensitivity and selectivity detection techniques leading to quantifying an analyte with a high level of precision and exactitude and its unequivocal identification at very low concentration levels. A number of analytical techniques such as Gas Liquid Chromatography, Gas Chromatography-Mass Spectrometry, High Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry-Mass Photometry, etc., are established for detection and quantification of residues in animal tissues (Gaudin *et al.,* 2005; Nisha, 2008; Mensah *et al.,* 2014; Aytenfsu *et al.,* 2016; Lakew *et al.,* 2022)

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was carried out in Bishoftu town of East Shewa zone; Oromia regional state, Central Ethiopia and located 47 Kms southeast of Addis Ababa. It is located at 08°45' North latitude and 38°59' East longitude. Its average altitudes are1850 meter above sea level in the central high land of Ethiopia. The mean relative humidity is 61.3% and the average yearly temperature ranges from 12.3 °C to 27.7 °C (Fisseha, 2017). Bishoftu is one of the principal chicken-producing areas in the nation. The large integrated poultry farms like ELFORA, ALEMA, and ELERE farms in the study region are also advantageous to the regional market for small scale commercial broiler farms.

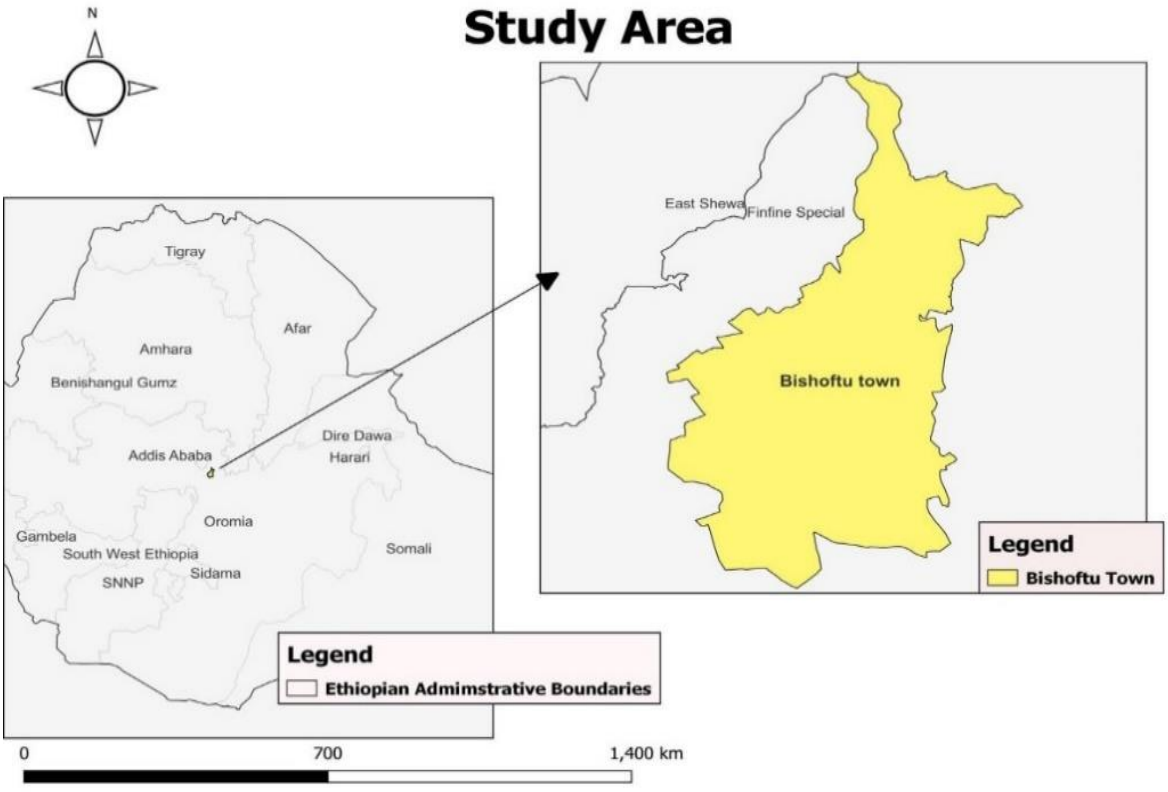


Figure 2: Map of study area, (using QGIS version 3.16.8- Hannover)

3.2 Study Design and Units

A cross-sectional study was carried out between January 2022 and July 2022 in selected Bishoftu town, Ethiopia. The study area was selected based on the Ethiopia Agriculture Authority (EAA), Animal Product and Input Quality Testing Center (API-QTC) regular surveillance program alignment preference in poultry products. The broiler farm data at Bishoftu Livestock and Fisheries office was not legally established by the municipal administration. Thus, the productions are informal in the residential areas of shared homesteads without a trade license as a matter the list was not available. The studied poultry farms were purposively selected because of (1) the availability of broiler meat providers for commercial use (2) suitability of slaughtering plan at time of sample collection (3) accessibility of farms who did not feed medicated feeds (antibiotics or feed additives) to their flocks within 3 weeks of slaughtering and (4) farm owners' consent for permission to enter their backyard slaughtering area.

3.3 Sample Size and Sampling techniques

A one hundred twenty fully dressed broiler carcass samples were collected from seven broiler chicken farms. The number of broiler meat samples per total slaughter in the farm at the time of collection counted and a minimum of two percent proportional number of samples were purchased per the market price rates. Finally, using a systematic random sampling technique, the individual fully dressed broiler carcass collected from the different corners of the plastic mat that was arranged to be loaded in a box for the market sale.

3.4 Sample Collection, Transportation and Storage

Sample collection and transportation was conducted according to API-QTC meat sample collection transportation and storage SOP (QMS_MBL_APF_SOP_001; Rev-01: 2018). Each final package of dressed carcass was collected aseptically and differently stored in appropriate sterile plastic bags, the date and place of sample collection properly recorded, each samples assigned a unique identification number and labeled with permanent ink.

The samples then immediately transported using an ice box to the testing laboratory called Animal Product and Input Quality Testing Center (API-QTC), Akaki-Kality Sub city, Addis Ababa. Samples processed for the microbial load enumeration and pathogen detection before 24 hrs of collection except for antibiotic residues tests that was kept in a deep freezer at a temperature of -20 °C until analysis.

3.5 Sample Preparation and Processing

Samples (breast, wing, thigh, neck, back skin, and muscles) were pooled together and weighed 25 g for microbiological analysis. While the samples (breast, thigh and neck muscles) were pooled together and weighed 500 g (CAC, 2009) for antibiotic residue analysis. The antibiotic residue samples were minced and homogenized using a meat blender. From each homogenized samples, 4.0 g was accurately weighed, in duplicates, in a 50 ml falcon tubes and kept frozen (-20 °C) until the time for extraction. While the microbial load enumeration and identification of selected bacterial foodborne pathogen were determined within 24 hours using appropriate standard microbiological test procedures.

3.5.1 Enumeration of quality indicator microorganisms

Total aerobic bacteria (AC), total coliforms (CC), total E. coli (EC) and total S. aureus (STA) counts were enumerated using the TEMPO® system guidelines, Initial dilutions were made using 25 g of pooled samples from breast, wings, thighs, neck and back skin and 225 ml ISO standard buffered peptone water (BPW) in 1:9 ratios. Used as initial inoculum for the next specific microbiological tests to avoid multiple enrichment process. The mixtures were homogenized using a laboratory smasher (AESAP1064) for 5 minutes. Each vial holding the dehydrated media received 0.1 ml of the homogenized sample mixture and 3.9 ml of sterile distilled water in order to enumeration the AC, CC, EC and STA. 4 ml of inoculated medium that was obtained corresponds to a 1/4000 dilution. Dilutions were increased based on the expected level of contamination and higher level of contamination of suspect samples.

All TEMPO® vials containing the dehydrated media (AC, CC, EC and STA) and a specific test card were scanned with a barcode scanner. Following scanning, the straws supplied with a specific test card were inserted into corresponding inoculated medium vials in the slots of the filling rack. All the required test sample information had been entered into the TEMPO ® software and the filling rack was loaded into the TEMPO filler. The TEMPO filler carried out an automatic transfer of the inoculated medium from the vial into each set of three wells (volumes 2.25 µl, 22.5 µl, and 225 µl) of the test cards. The cards were removed from the filling station and incubated following the manufacturer's directions at a specific temperature for the specified time. After that the test cards were inserted into the TEMPO® reader station, where the machine immediately began reading the findings.

3.5.2 Isolation and identification of bacterial foodborne pathogens

3M Molecular Detection of Campylobacter

To prepare samples for *Campylobacter* detection, 25g of pooled broiler samples (breast, wings, thighs, neck, back skin and muscles) were rinsed in 225 ml of ISO standard BPW and massaged by hand. 30 ml of 3M *Campylobacter* Enrichment Broth was added to 30 ml of the broiler meat mixture in the sterile bag and the bag was gently massaged for about10 ± 2 seconds. The bag was rolled up to minimize headspace and keep the enrichment from being exposed to air and incubated the bag aerobically at 41.5 ± 1 °C for 22–26 h. 20 µL of each enriched samples were transferred to an individual lysis tube and heated at 100 ± 1^0C for 15 min in a heating block. Removed the lysis tube from the heating block and placed on the chilling block for 10 minutes. These lysis tubes were left on a bench at room temperature (20 to 23 °C) for 5 min, after which 20 µL of each sample lysates were transferred into *Campylobacter* reagent tube and loaded with 3M MDS. Presumptive positive results were reported in real time while negative results were displayed at the end of the 75 min of default run.

Isolation and identification Salmonella and E. coli O157:H7

Isolation of *Salmonella* and *E. coli O157:H7* performed by using previously prepared initial inoculum and incubated at 37 °C Isolation of *Salmonella* and *E. coli O157:H7* performed pre-enrichment by adding 25 g of pooled broiler meat to 225 ml of buffer peptone water and incubated at 37 °C. *Salmonella* was selectively enriched with the addition of 0.1 ml pre-enriched sample to 10 ml of Rappaport Vassiliadis (RVS) broth at 41.5°C and 1 ml pre-enriched sample to 10 ml of tetrathionate (TT) broth (supplement with iodine) (SRL) at 37°C. To selectively enrich E. coli O 157:H7, add 0.1 ml of pre-enriched sample to 10 ml of EC broth at 44 °C. *Salmonella* with typical colonies were observed 24 hours after plating on XLD and SS agar plates. Aside from that, CT- SMAC (Sorbitol MacConkey with cefixime and tellurite) and EMB (Eosin methylene blue) agar plates were plated for the presence of distinctive colonies of *E. coli O157:H7 Salmonella* and *E. coli O157:H7* were further confirmed using GEN III Omnilog system.

GEN III Omnilog determination of Salmonella and E. coli O157:H7

Suspicious colonies of *Salmonella* and *E. coli O157:H7* were further confirmed using the GEN III Omnilog system (Lot number 3003241, BIOLOG, USA) fully automated coated microplate based bacterial identification system. *E. coli O157:H7* and *Salmonella* isolates were sub-cultured on Biolog Universal Growth (BUG) agar and incubated at 37°C for 24±2 hr. For each isolate, a single bacteria colony was picked from cultured Biolog Universal Growth (BUG) agar and emulsified with Inoculating Fluid A (IF A). Using a turbidity meter, the cell density of the bacterial inoculum was determined and adjusted to the desired transmittance (90-98%). Using an automated multichannel pipette, 100 µl of bacterial cell suspension was inoculated into each coated 96-well microplate and incubated aerobically at 33°C for 22 hrs. Cultured microplates were read on a BIOLOG Microstation reader that identifies species/subspecies.

Microbiological analysis Quality Control

Confidence in the reliability of test results was supported by the application of adequate quality assurance procedures and the routine use of quality control strains. Thus, *E. coli* ATCC-25922 (susceptible to all tested drugs) was taken as an important part of quality control for culture and antimicrobial susceptibility tests. Matrix control (MC), a reagent control (RC), and a negative control (NC) were taken as part of quality control for each test run for detection of *Campylobacter*. The sterility of sample collecting materials was checked randomly by culturing on nutrient agar and sterility of culture media was checked by incubating from each batch of prepared media for 24 hrs. Moreover, the whole procedures and result interpretation were done following standard operating procedure (SOP).

*3.5.3 Antimicrobial Susceptibility Test (*VITEK®2XL)

Antimicrobial resistance test of isolated *Salmonella* and *E. coli O157:H7* were performed using the automated VITEK®2XL method. *E. coli O157:H7* and *Salmonella* isolates were sub-cultured using nutrient agar and incubated at 37^0C for 24±2 hrs. For each isolate, 2–3 pure fresh colonies from cultured nutrient agar bacteria were suspended in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0) using different clear polystyrene tubes for each target bacteria and thoroughly mixed. The cell density of the bacterial inoculum was measured and adjusted to the specified turbidity (0.47-0.6%) using a Densi Chek Plus (turbidity meter). An AST card was then selected (AST GN card for *E. coli* and *Salmonella*), scanned with a barcode scanner, and the card straw inserted into an empty tube in the slot of the cassette next to the bacterial suspension.

All required test sample information was entered into the Vitek 2 FLEX Prep Data Entry and identified preferred bacteria were selected from the database. Cassettes containing suspension tubes with cards were loaded into the Vitek XL Compact System. The instrument was automatically continued processing and bacterial susceptibility results were observed within 19 hours.

3.5.4 Antimicrobial residue analysis (LC-ESI-MS/MS)

Preparation of mobile phases

Mobile phase A or aqueous mobile phase (water with 0.1% FA) was prepared by adding 1.0mL of FA into a half-filled volumetric flask of 1.0 L capacity, then brought to volume with deionized water. The flask was degassed offline using Sonicator for 10 minutes, and transferred to aqueous reservoir of the machine. Then Mobile phase B or organic mobile phase Acetonitrile (ACN) with 0.1% (FA) was prepared by mixing 1.0mL of FA pipetted into a 1.0L volumetric flask and brought to the volume using ACN. The mobile phase degassed and transferred to organic mobile phase reservoirs.

Preparation of extraction solutions

Extraction solution (Acidified ACN + McIlvaine Buffer + 0.1 mol/L Na_2EDTA) was prepared as follow: 0.1% FA in acetonitrile: About 0.4mL of formic acid was pipetted and mixed with 400.00 mL of acetonitrile in a graduated cylinder and then transferred to a dispenser bottle for use. McIlvaine buffer (mixed citrate-phosphate): 14.2 g anhydrous dibasic sodium phosphate and 9.6 g anhydrous citric acid were separately dissolved well each in 500mL de-ionized water. Then 308.0 mL citric acid solution (0.1M) and 192.0mL phosphate solution (0.2M) were mixed carefully in Duran bottle (pH was maintained at 400 ± 0.05). McIlvaine Buffer 0.1 mol/L Na_2EDTA: 18.6g dissolution EDTA dihydrate added in 500.0mL McIlvaine buffer and sonicated.

Preparation of standard solutions

The stock standard solutions were prepared at concentrations corresponding to 1.0 mg/mL (1000µg/mL) taking the stability and solubility of the standards in the solvents into account. Standard solutions were prepared separately by transferring 10.0mg equivalent of the base materials quantitatively in to 10.0 mL volumetric flasks. Sulfadiazine, enrofloxacin and two of the tetracyclines, were dissolved in methanol and diluted to the volume accordingly.

Intermediate standard solutions for sulfadiazine, enrofloxacin and the tetracyclines were prepared by pipetting 400 µL aliquot of stock and diluting in a 10.0 mL volumetric flask with methanol to 40ng/µL. when they are not in use, all the stock and intermediate standards were stored in amber vials at ≤ -20℃. Then a working standard was made by pipetting 1.0 mL of intermediate solutions into a 10.0 mL volumetric flask and diluting to the mark with diluent (80:20 water/Acetonitrile) giving final concentrations of 4ng/µL.

Table 6 Spiking procedure and volume and target tissue concentrations

SDZ, ENR TTC and OTC		
Spiked Level	Spiked volume (µl)	Concentration in 4g muscle tissue (µg/kg)
0.25 x MRL	25	25
0.5 x MRL	50	50
1.0 x MRL	100	100
1.5 x MRL	150	150
2.0 x MRL	200	200
2.5 x MRL	250	250

Solid-phase sample extraction and clean-up procedure

4g of each of the test samples were weighed, ten milliliters of the extraction solution (2.0 mL Na_2EDTA-McIlvaine buffer and 8mL of acidified ACN with 0.1% FA) was added in sequence to the falcon tubes. The tubes were capped tightly and mixed briefly for 30.0 seconds. Subsequently, the sample mixtures were shaken vigorously for 15.0 minutes using a wrist-action mechanical shaker. After shaking, the 50.0 mL sample tubes were centrifuged for 15.0 minutes at 4500 RPM (2268xg) at 4℃. Then samples purified by Solid Phase Extraction (SPE) techniques using 12 ports SPE vacuum manifold (Supelco, Germany). After carefully mounting Oasis PRiME HLB cartridges on the vacuum manifold, the supernatant was loaded from the 50.0 mL falcon tube via Oasis PRiME HLB cartridges. The process does not require cartridge conditioning and equilibration steps were not performed. Then the eluted solutions were directly collected into 15.0 mL scaled conical plastic centrifuged tubes, which were preplaced in the manifold. About 5

mL of the clean extracts were collected into another sample tubes and evaporated at 40 ℃ under a gentle stream of nitrogen gas nearly to dryness (0.1 mL) using MultiVap 54 Lab Tech a nitrogen gas streamed sample concentrator with a 2/3 filled water bath. The sample concentrator was coupled with an online nitrogen gas generator.

Afterwards, the concentrated residues were reconstituted with 1 mL initial mobile phase, recapped and vortexed for 30.0 seconds, and centrifuged for 15' at 4500 RPM at 4 ℃. Finally, clear supernatant supposed to contain antibiotic residues of interest, was transferred into auto sampler vials and closed tightly to make them ready for injection. Finally, a 10 µL injection volume was injected into the UHPLC-ESI_MS/MS.

LC-MS/MS Method of analysis

The analysis was performed by UHPLC of an Agilent 1290 Infinity II system (Agilent Technologies Ltd., USA) interfaced to an Agilent 6470 LC/TQ/ triple-quadruple mass spectrometer (MS/MS) equipped with an Agilent jet steam electrospray ionization source, which was operated in positive mode (AJS-ESI +) and controlled by Mass Hunter software. Antibiotics separation was chromatographically achieved on phenomenex Synergi hydro-RP, (4.6 mm X 150 mm; 4µm, 80 Å) column with a guard cartridge system (4 X 3.0 mm). The mobile phase was a binary gradient mobile phase with a flow rate, which was set at 1.0 mL/min for a total run time of 17 min. Methanol with 0.1% FA (Mobile Phase-A) and acetonitrile with 0.1% FA (v/v) (Mobile phase-B) were used.

Liquid Chromatography Mass Spectrometer Condition (LC-ESI-MS/MS)

The column compartment was operated at 30 ℃, while the auto-sampler temperature was set at 10℃. The injection volume was 10µL. the auto sampler was rinsed after each injection using a solution of H_2O; MeOH (50:50, v/v). The system was conditioned with a mobile phase for more than an hour prior to actual analysis. In this study, gradient elution of the mobile phase was used for separation of multi-class antibiotics.

The Electrospray ion source was used in a positive mode (AJS-ESI +) with data acquisition in multiple reaction monitoring (MRM) mode and analyzed using Mass Hunter software. The mass spectrometer (MS/MS) parameters were optimized and the source parameters were as follows: gas temperature. 350 ℃; gas flow rate, 12 L/min; sheath gas temperature, 250 ℃; sheath gar flow, 11 L/min; nebulizer pressure, 40 psi; capillary voltage, 4KV; nozzle voltage, 500V.

Antibiotic Residue Analysis Quality Control

During analysis of each batch of all the samples, quality control samples like blank control, positive control and calibration samples were run together. A six-point calibration scale was used to check linearity of the method and to quantify of the residues. Besides, system-suitability test was performed on daily bases using a mixed antibiotic standard solution of five replicate injections prepared at the concentration of MRLs. The performance was checked before starting the actual sample analysis activity and the required parameters were evaluated.

3.6 Data Management and Statistical Analysis

Laboratory data were entered into a Microsoft Excel spreadsheet to analyze descriptive data. For the enumeration of microbial load and antibiotic residues data mean and average value calculated and for the identification of food bacterial pathogens, proportional ratio was estimated per samples and farms.

3.7 Ethical Consideration

Prior to data collection, ethical approval was obtained from the Animal Research Ethical Review Committee (ARERC) with reference number VM/ERD/25/04/15/2023, (Annex X). It was possible to obtain consent from the owners of the broiler farms, facilities and potential participants by casually asking them to volunteer for the study. Privacy and confidentiality of the sample sources was maintained.

4. RESULTS

4.1 Enumeration of Microbial Quality Indicators

In this study all broiler farms of backyard-slaughtered carcass samples (n=120) were found to be highly contaminated in aerobic bacteria, coliform bacteria, *E. coli* and *S. aureus* bacteria. Of this the microbial load count were n=120 (100%) for aerobic count (AC) and *E. coli* count (EC), Coliform count (CC) n=114 (95%) and *S. aureus* (STA) n=105 (87.5%) were above the limit of Ethiopian standard (ES) (ES 3967:2015) contamination level. The results are summarized within each farm and the four quality indicators count in table 7.

Table 7: Summary of positive samples found in broiler meat against Ethiopian Standard

Farm Code	No. Sample tested	Positive samples above ES limit /farm (%)			
		AC:1 x 10^5 (cfu/g)	**EC:1x10^2** (cfu/g)	**CC:5 x 10^3** (cfu/g)	**STA:1 x10^2** (cfu/g)
Farm 1	15	15 (100)	15 (100)	14 (93.3)	14 (93.3)
Farm 2	16	16 (100)	16 (100)	16 (100)	13 (81.3)
Farm 3	10	10 (100)	10 (100)	10 (100)	9 (90)
Farm 4	29	29 (100)	29 (100)	28 (96.6)	29 (100)
Farm 5	20	20 (100)	20 (100)	20 (100)	18 (90)
Farm 6	15	15 (100)	15 (100)	15 (100)	15 (100)
Farm 7	15	15 (100)	15 (100)	11 (73.3)	7 (46.7)
Total	**120**	**120 (100)**	**120 (100)**	**114 (95)**	**105 (87.5)**

The average mean values of the carcass tested samples were $2.9x10^7$cfu/g, $2.5x10^6$, $1.8x10^6$ and $6x10^3$cfu/g for AC, CC, EC, and STA respectively (Table 8).

Table 8: Summary of bacterial contamination found in broiler meat samples

Quality indicators	ES acceptance limit (cfu/g)	Minimum (cfu/g)	Maximum (cfu/g)	Mean (cfu/g)
AC	1 x 10^5	8.5 x 10^5	2.7 x 10^8	2.9 x 10^7
CC	5 x 10^3	1.3 x 10^3	9.1 x 10^6	2.5 x 10^6
EC	1 x 10^2	1.0 x 10^3	4.9 x 10^6	1.8 x 10^6
STA	1 x 10^2	<10^2	1.1 x 10^5	6 x 10^3

* AC =Aerobic count, EC= *E. coli* count, CC= Coliform count and STA= *S. aureus* count

4.2 Isolation and Identification Foodborne bacterial Pathogens

Out of 120 broiler meat samples examined for isolation and identification of selected foodborne pathogens n=100 (83.3%) was positive for the foodborne bacteria pathogens. Of this n=78 (65%) was positive for at least one pathogen and n=22 (18.3%) for two pathogens. In all of the 7 (100%) farms found positive samples. The total contamination figure of N=120 sampled carcass in species is n=75 (62.5%) for *Campylobacter, E. coli O157:H7* n=27 (22.5%) and *Salmonella* n=20 (16.7%) all detailed in Table 9.

Table 9: Summary of foodborne pathogens found in broiler meat samples

Farm code	Sample tested	No of negative (%)	At least one pathogen (%)	Two pathogens (%)	No. of positives/samples / farm (%)		
					Camp.	E. coli	Salm.
Farm1	15	12(80)	3(20)	0(0.0)	0 (0.0)	0 (0.0)	3 (20)
Farm2	16	0(0.0)	9(56.3)	7(43.8)	16 (100)	7 (43.7)	0 (0.0)
Farm3	10	5(50)	5(50)	0(0.0)	0 (0.0)	5 (50.0)	0 (0.0)
Farm4	29	0(0.0)	29(100)	0(0.0)	29 (100)	0 (0.0)	0 (0.0)
Farm5	20	3(15)	10(50)	7(35)	0 (0.0)	15 (75)	9 (45)
Farm6	15	0(0.0)	15(100)	0(0.0)	15 (100)	0 (0.0)	0 (0.0)
Farm7	15	0(0.0)	7(46.7)	8 (53.3)	15 (100)	0 (0.0)	8 (53.3)
Total	**120**	**20 (16.7)**	**78 (65)**	**22 (18.3)**	**75 (62.5)**	**27 (22.5)**	**20 (16.7)**

*Camp= *Campylobacter*, E. coli = *E. coli O157:H7*, Salm= *Salmonella*

4.3 Antimicrobial Resistance Profile of Identified Bacterial Pathogens

In this investigation, 27 *E. coli O157:H7* and 20 *Salmonella* isolates strain tested for AMR profile with 18 selected antimicrobial agents were 100% susceptible against gentamicin, amikacin, ceftiofur, cefpodoxime, and amoxicillin/clavulanic acid to *E. coli O157:H7* isolates while cefpodoxime and amoxicillin/clavulanic acid were completely susceptible to *Salmonella*. On the other side, most of them were also observed with high resistance to tetracycline, doxycycline, ampicillin; pradofloxacin, enrofloxacin, marbofloxacin; while low results were observed in trimethoprim-sulfamethoxazole, neomycin, cefovecin and cefalexin to both isolates. The result is presented in Figure 2.

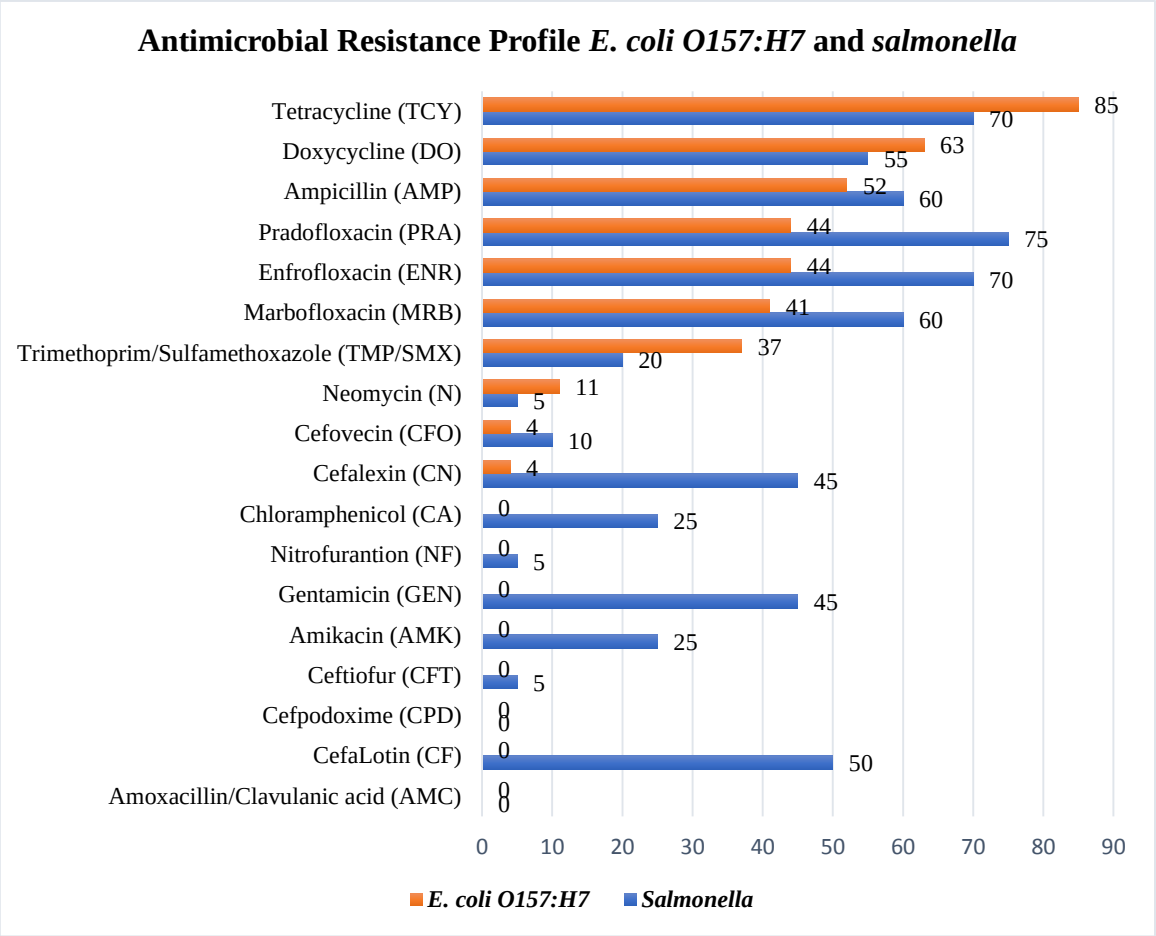


Figure 3: Antimicrobial Resistance profile of *E. coli* O157:H7 and *Salmonella*

In this investigation, 20 *Salmonella* isolates and 27 *E. coli O157:H7* isolates were evaluated for AMR profiles; at least one class of antibiotic was detected in 19 (95%) *salmonella* isolates and 26 (96.3%) *E. coli O157:H7* isolates. Of which 13 (65%) of *salmonella* isolates and 11 (40.7%) of *E. coli O157:H7* isolates were determined to be multidrug resistant (MDR), on the *salmonella* isolates, 3(15%) extensively drug-resistant (XDR) and 1(5%) pan drug-resistant (PDR) were observed. The result was summarized in table 10.

Table 10: Multiple drug resistance profiles of *E. coli O157:H7* and *Salmonella*

S/N	Level of resistance to antibiotic classes	AMR profiles of bacterial isolates	
		E. coli O157:H7 (n=27)	***Salmonella (n= 20)***
1	Susceptible to all classes of antibiotics	1 (3.7%)	1 (5.0%)
2	Resistance to < 3 antibiotic classes	15 (55.6%)	2 (10.0%)
3	Resistance to >= 3 antibiotic classes (MDR)	11 (40.7%)	13 (65.0%)
4	Resistance to all but susceptible to <= 2 antibiotic classes (XDR)	0.0%	3 (15.0%)
5	Resistance to all antibiotic classes (PDR)	0.0%	1(5.0%)
	Total	**26 (96.3)**	**19 (95.0%)**

In this study 27 *E. coli O157:H7* isolates were tested for AMR profile, n=26 /27 (96.3%) of the isolates were resistance to one or more antimicrobials. Of which n=11(40.7%) *E. coli O157:H7* isolates were found multidrug resistance (MDR). The result was summarized in Table 11.

Table 11: Summary of multiple drug resistance profile of *E. coli O157:H7*

Antimicrobial classes	Antimicrobial agents	Frequency
Sulfonamides, tetracycline, and penicillin	TMP/SMX, TCY, AMP	1
Sulfonamides, tetracycline, and penicillin	TMP/SMX, TCY, DO, AMP	1
Tetracycline, fluoroquinolones, cephalosporin, and penicillin	TCY, DO, PRA, ENR, CN, AMP	1
Sulfonamides, tetracycline, fluoroquinolones, and penicillin	TMP/SMX, TCY, PRA, MRB, ENR, AMP	2
Sulfonamides, tetracycline, and fluoroquinolones,	TMP/SMX, TCY, DO, PRA, MRB, ENR	2
Sulfonamides, tetracycline, fluoroquinolones, and penicillin	TMP/SMX, TCY, DO, PRA, MRB, ENR, AMP	2
Sulfonamides, tetracycline, fluoroquinolones, penicillin, and aminoglycoside	TMP/SMX, TCY, DO, PRA, MRB, ENR, AMP, N,	2
Total MDR (%)		**11(40.7)**

* TCY= Tetracycline, DO= doxycycline, AMP= Ampicillin, PRA= pradofloxacin, ENR= Enrofloxacin, MRB= Marbofloxacin, TMP/SMX= Trimethoprim-sulfamethoxazole, N= Neomycin and CN = Cefalexin,

Out of 20 *Salmonella* isolated strain tested for AMR profile, 19 (95%) of the isolates were resistant to at least one antibiotic. 13(65%) of them were MDR, 3(15%) were extensively drug-resistant (XDR) and 1(5%) was pan drug resistant (PDR) (Table 12).

Table 12: Multi drug resistance, extensively drug resistance and pan drug resistant, profile of *Salmonella*

Resistance profile	Antimicrobial classes	Antimicrobial agents	Frequency
MDR	Sulfonamides, phenicol, Tetracycline, fluoroquinolones, aminoglycoside, cephalosporin, and penicillin	ENR, GEN, CF, CN	1
		PRA, GEN, AMK, CF, CN	1
		TCY, PRA, MRB, ENR, AMP	5
		PRA, MRB, ENR, GEN, CF, CN, AMP	1
		TCY, DO, PRA, GEN, CFO, CF, CN	1
		TMP/SMX, CA, TCY, DO, PRA, MRB, ENR, AMP	1
		TMP/SMX, CA, TCY, DO, PRA, MRB, ENR, CF	1
		TCY, DO, PRA, GEN, AMK, CF, CN, AMP	1
		DO, PRA, MRB, ENR, N, GEN, CF, CN, AMP	1
		Total (%)	**13(65)**
XDR	Sulfonamides, phenicol, nitrofuran, tetracycline, fluoroquinolones, aminoglycoside, cephalosporin, and penicillin	CA, DO, ENR, GEN, AMK, CF, CN, AMP	1
		TMP/SMX, CA, TCY, DO, PRA, MRB, ENR, GEN, AMK, CF, CN, AMP	1
		TMP/SMX, CA, NF, TCY, DO, PRA, MRB, ENR, GEN, AMK, CFT, CFO, CF, CN, AMP	1
		Total (%)	**3(15)**
PDR	Sulfonamides, phenicol, nitroforan, tetracycline, fluoroquinolones, aminoglycoside, cephalosporin, and penicillin	TMP/SMX, CA, NF, TCY, DO, PRA, MRB, ENR, GEN, AMK, CFT, CFO, CF, CN, AMP	1
		Total (%)	**1(5)**

*TCY= Tetracycline, DO= doxycycline, AMP= Ampicillin, PRA= pradofloxacin, ENR= Enrofloxacin, MRB= Marbofloxacin, TMP/SMX= Trimethoprim-sulfamethoxazole, N= Neomycin, CFO = Cefovecin, CN = Cefalexin, CA= Chloramphenicol, NF= Nitrofurantion, GEN= Gentamicin, AMK= Amikacin, CFT= Ceftiofur, and CF= Cefalotion

4.4 Identification and Quantification of Antibiotic Residue

In this study, four antibiotic residues from three different classes (sulfonamides, quinolones, and tetracyclines) were examined in 120 samples of broiler meat from various farms. The results showed that n=47/120(39.2%) samples contained detectable level of residues. Of this 38/120 (31.8 %) were contaminated with one type of antibiotics residues and 9/120 (7.50%) were positive for more than one type of antibiotics residues and no detectable residues in 73/120 (60.8%) of the samples. The total contamination level of broiler meat sampled in targeted antibiotic n=30(25%) for Enrofloxacin (ENR), Oxytetracycline (OTC) n=22(18.3%), and Sulfadiazine (SDZ) n=4(3.3%) from higher to lower level of occurrence. However, tetracycline (TTC) residues were not found in the study area. The complete result and analysis were summarized in Table 13.

Table 13: Summary of antimicrobial residues found in broiler meat samples

Farm code	No. of samples tested	No. of Antibiotic residues detected/%			Number of positive /samples / (%)			
		Samples ND*	Single residue detected	More than one residue	SDZ	OTC	TTC	ENR
Farm1	15	2 (13.3)	12 (80)	1 (6.7)	ND	13(86.7)	ND	1 (6.7)
Farm2	16	15 (93.75)	1 (6.25)	0 (0.00)	ND	ND	ND	1 (6.25)
Farm3	10	10(100)	0 (0.00)	0 (0.00)	ND	ND	ND	ND
Farm4	29	0(0.00)	21 (72.4)	8(27.6)	ND	9(31.0)	ND	28(96.6)
Farm5	20	20(100)	0(0.00)	0 (0.00)	ND	ND	ND	ND
Farm6	15	11(73.3)	4(26.7)	0 (0.00)	4(3.3)	ND	ND	ND
Farm7	15	15(100)	0(0.00)	0 (0.00)	ND	ND	ND	ND
Total	**120**	**73(60.83)**	**38(31.67)**	**9(7.50)**	**4(3.3)**	**22(18.3)**	**0(0.0)**	**30(25)**

ND* = Nondetectable residues samples found to contain zero or below the method LOD

In this study, the levels of antimicrobials found in broiler meat samples depicted that, SDZ ranges from 9.25 to 13.29 µg/kg, OTC from 9.92 to 135.36, ENR from 17.38 to 943.5 and no detectable TTC. The result indicates in table 14.

Table 14: Antimicrobial residues contamination level in broiler meat samples

Concentration of residues (µg/kg)	Antimicrobial Types and Residue Levels			
	SDZ (µg/kg)	OTC (µg/kg)	TTC (µg/kg)	ENR (µg/kg)
Minimum	9.25	9.92	ND	17.38
Maximum	13.29	135. 36	ND	943.50
Mean	11.09	27.33	ND	315.17
Range	9.25-13.29	9.92-135.36	ND	17.38-943.50

* SDZ= Sulfadiazine, OTC = Oxytetracycline, ENR = Enrofloxacin and TTC= Tetracycline

In the poultry meat samples, all residue concentrations of SDZ, and TTC quantified were lower than the MRLs established by either EU (100 µg/kg) (EC, 2010) or Codex (200 µg/kg) (CAC, 2015 and CAC, 2018) guidelines. However, from broiler meat samples 1 (0.8%) and 26 (21.7%) were quantified to contain antimicrobial residues of OTC and ENR above EU MRLs respectively. Thus, a total of 27 (22.5%) poultry meat samples were unsafe and unacceptable for human consumption.

Table 15: Positive samples with above and below the maximum residue limits

Antibiotics	MRL (EU) (µg/kg)	Positive samples above and below MRLS	
		Above	Below
ENR	100	26 (21.7)	4 (3.3)
OTC	100	1 (0.8)	21 (17.5)
SDZ	100	0 (0)	4 (3.3)
Total (n= 120)		**27/ (22.5)**	**29/ (24.2)**

* ENR= Enrofloxacin , OTC= Oxytetracycline and SDZ =Sulfadiazine

5.DISCUSSION

Ethiopian standard agency has set acceptable concentration limits of microorganisms in broiler meat to ensure quality of mat and safeguard human health. The recommended concentration limits were total aerobic bacteria less than 10^5CFU/g, total coliform less than $5x10^3$ CFU/g, *E. coli* less than 10^2 CFU/g, and *S. aureus* less than $1x\ 10^2$ CFU/g (ES 3967:2015). The microbiological quality indicator tests of the current study counts were 95% CC, and 87.5% STA though failed to meet the criteria of Ethiopian standard (ES 3967:2015). While the EC and AC results were (100%) deemed to be unacceptable. Microbial quality indicators give an idea about the hygienic measures applied during processing to maintain quality of the poultry carcasses.

The current quality indicator organisms count in readily packed carcass for the market were higher than raw broiler meat sold in Nairobi, Kenya (Odwar *et al.,* 2014), Bangladesh (Faruque *et al.,* 2019), Egypt (Shaltout *et al.,* 2020), Indonesian (Wardhana *et al.,* 2021; Jhandai *et al.,* 2020), in STA count (Joseph *et al.,* 2021) and Ambon City Market broiler meat EC counts (Liur and Veerman, 2021). As anticipated the elevated numbers might be due to sample type, sampling method, spatial and hygiene sanitation practice differences compare to the current scenario.

Broiler meat producers in the study area slaughter their broiler in open spaces and manual operational processes and cross contamination during processing pose potential risk of high contamination level. Poor quality water utilization and fecal contamination with own feces during evisceration are possible factors of increased hygiene indicator organisms in broiler meat processing (Faruque *et al.,* 2019; Wardhana *et al.,* 2021).

High quantity of hygiene indicator bacteria that contaminated broiler meat were unsafe for human consumption and considered as unhealthy, based on the prescribed limits (ES 3967:2015). Despite numbers varied from certain studies in the current findings; unhygienic working environments, contamination in meat processing, equipment and poor hygiene and sanitation practice might be the reasons to these level of contamination

(Gezahegn and Karel 2010; Mohamed-Noor *et al.,* 2012; Omer 2018; Shaltout *et al.,* 2021).

Campylobacter, *E. coli O157:H7* and *Salmonella* are considered one of significant animal origin food borne pathogens that cause human infections through handling in slaughtering process, cross contamination or ingestion of undercooked meat (Zendehbad, *et al.,* 2013). Here in this study all seven farms have positive samples and frequency in total sampled were 83% contaminated by food borne bacteria pathogen could be due to scalding skinning, evisceration and washing. The findings of 62.5% *Campylobacter*, 22.5% *E. coli O157:H7* and 16.7% *Salmonella* were indistinguishably comparable with the finding of 56% (Cardinale *et al.,* 2002) in Senegal, similarly *salmonella* results obtained in Ethiopia (Molla *et al.,* 2004), in South Africa (Van Nierop *et al.,* 2005) and in Egypt (Abd-Elghany *et al.,* 2015). These might be due to sampling, spatial, hygiene and sanitation practice similarity in slaughter facilities. This study further strengthened the contributing factors; lack of proper slaughter facilities, backyard open space slaughtering within the rearing compound, unsafe local equipment, lack inspection of broiler meat health status during antemortem and postmortem and food safety procedures in slaughtering process.

In *Campylobacter* frequency slightly higher figure compare to reports in Ethiopia 21.7%, in South Africa 32.3% in overall fresh and frozen but 49% in fresh supermarket broiler meat and Kenya 50% (Dadi and Asrat, 2009;Van Nierop *et al.,* 2005 ;Joseph *et al.,* 2021). Different sample sizes, types and study area difference might be the reasons. Moreover high numbers found in *salmonella* frequency than Egypt 2% (Al-Hazmi *et al.,* 2013), 3.75% (Svobodová *et al.,* 2012) and 1.6% in Morocco (Cohen *et al.,* 2007) and *E. coli O157:H7* significantly higher than studies in Ethiopia that found from animal source of food 13.4% , 12%, and 9.1% respectively (Shecho *et al.,* 2017b ; Hamid *et al.,* 2018; Tadese *et al.,* 2021). Signifcantly high figures obtained compare to Saudi Arabia 2.5%, China 3.28 % ,Turkey 1.94% and Kenya 11.42 % respectively (Hessain *et al.,* 2015 ; Zhang, Zhu, and Wu 2015Din, 2016 ;Kuria *et al.,* 2018). The reasons could be clear and cut that broiler carcasses may contaminated during slaughtering process with

other broiler batches cross contamination due to nature of processing, workers, scalding tanks, feather removal procedures, or during evisceration intestinal contents spills that contaminate the muscle and organs of the broiler these idea has strong agreement with (Mohamed *et al.,* 2012; Zendehbad, *et al.*, 2013; Adeyanju and Ishola 2014; Messad *et al.*, 2014; Noori, 2016).

Although direct association impact of high prevalence of food pathogens with food poisoning hospitalized patients' number is out of the study scope, attribution to public health risks are predictable as CDC, 2011 estimated a million infections with 378 deaths every year in US due to *Campylobacter, E. coli O157:H7* and *Salmonella*. Moreover, *E. coli O157:H7* can survive and multiply even in 0°C and 12°C hence consumers risks to infection are undeniable (Arias *et al.,* 2001). Less figures found in this study compare to some prevalence studies of *Campylobacter* in Algiers 80%, Nigeria 96% and in Cameroon 90%, Senegal 62.5% and *E. coli O157:H7* in Egypt 50%, Iran 38.7%, Iraq 22%,39%, Nigeria 32.1%, Senegal 32% (Cardinale *et al.,* 2002; Alambedji *et al.,* 2006; Nzouankeu *et al.,* 2010; Adeyanju and Ishola 2014; Ahmed *et al.,* 2014; Noori *et al.,* 2016; Adeleye *et al.,* 2018; Mustafa, 2018; Elwaraqi *et al* 2019; Zarei *et al.,* 2021; Elsharawy *et al.,* 2022). These could be due to spatial, sampling, sample size and technique differences.

Antimicrobial resistance in *E. coli O157:H7* and *Salmonella* isolated from various livestock and products have been reported in Ethiopia (Molla *et al.,* 2004; Hiko *et al* 2008; Eguale, 2018; Hamid *et al.,* 2018 Dagnew *et al.,* 2020, Belachew *et al.,* 2021, Tadese *et al.,* 2021). The presence of widespread AMR can adversely affect public health, as treatment of illness caused by *E. coli O157:H7* and *Salmonella* becomes difficult (Thung *et al.,* 2016 Castro-Vargas *et al.,* 2020*).* High resistance of *E. coli O157:H7*and *Salmonella* to tetracycline, doxycycline, ampicillin; pradofloxacin, enrofloxacin, marbofloxacin and chloramphenicol in our study suggest that these antibiotics are widely used in broiler farm. Resistance to ampicillin, tetracycline and chloramphenicol has been reported resistance profile in broiler carcass and giblets in Ethiopia (Molla *et al.,* 2004). In another case, Belachew *et al* have found that

tetracycline, and chloramphenicol resistance in small hold Broiler Supply Chains in Central Ethiopia (Belachew *et al.,* 2021). The findings of our study, which revealed that 39.2% of the samples tested positively for antibiotics residues, support the idea that antibiotic residues may contribute in the emergence of resistance.

MDR profile in broiler has been reported in Ethiopia but curiously our findings demonstrated MDR strains of *E. coli O157:H7* and *Salmonella,* XDR *and* PDR strains of *Salmonella*. Despite the small count still is a risk to concern as its found in readily packed broiler meat to be provided for consumers (Molla *et al.,* 2004; Hamid *et al.,* 2018). The occurrence of MDR, *XDR and PDR* in this study might be due to the administration of multiple antimicrobials for prophylaxis or infection control and indiscriminate use of antimicrobials in the farms and/or public health sector, thereby selecting for resistant populations of *E. coli O157:H7 a*nd *Salmonella* (Gambushe *et al.,* 2022).

Antibiotic residues from various animal products have been reported in Ethiopia (Abebew *et al.,* 2014; Agmas and Adugna, 2018) and elsewhere in the world (Shareef *et al.,* 2009; Jammoul and Darra, 2019; Bartkiene *et al.,* 2020) suggesting the global occurrence of antibiotic residues. In our finding, from total tested samples 47 (39.2%) of the samples were detected antibiotic residues. High proportion of positive samples in this study suggests that there is high risk of public exposure to antimicrobial residues through consumption of broiler meat (Thi Huong-Anh *et al.,* 2020).

The current result showed that 3.3% ,18.3% and 25% samples analyzed contained residues of SDZ, OTC and ENR respectively while tetracycline residue was not detected in any of the samples. Of these, 0.8% and 21.67% of the samples had residues of OTC and ENR, respectively, at levels that have exceeded the MRLs recommended by European Union (100 µg/kg for both drugs) (EC/EU, 2010) and the CAC (200 µg/kg for OTC and 100 µg/kg for ENR) (CAC, 2018**)**. This percentage is considerably alarming and indicates an extensive abuse of veterinary drugs in the local poultry industry because the residues cannot be reduced by Freezing (-70°C for 90 days) or heat treatments (up to 90°C for 60 mins) (Al Aboudi et al., 2022) Therefor Violative levels of antimicrobial

drug residues in different poultry tissues are still a public health issue.

Compared to other previous studies, the residues levels of OTC, and ENR in broiler meat samples in present study were lower than that obtained in India (2.9%) OTC, TTC (2.9%) and (0.6%) ENR (Al Aboudi et al., 2022). Similarly higher result of OTC (10%) residues was reported in lebane, even though lower residues level of ENR (12.5%) were recorded by the same author (Jammoul and Darra 2019).This might be due to difference in sample size, sampling area and antibiotic use practices in the poultry farm.

The occurrence of antibiotic residue in the study areas might be due to improper usage of veterinary drugs, failure to follow withdrawal period of the antimicrobial and antibiotics use as feed additive (Agmas and Adugna 2018).The questionnaire survey reported by Beyene, *et al. (*2015) in Bishoftu and Modjo also strengths that 70.0% of poultry farmers were not respected drug withdrawal period, and 65% drugs were given by non-professionals.

This paper summarized the investigations of microbiological indicators count, three bacterial food pathogens identification and MDR that pose major public health risk and environment welfare since the backyard slaughters performed in open and under regulated disposals. Moreover, here the antibiotic residues performed for only four drugs due to resource accessibility many major drug residues might be found as irrational use of drugs is common in the poultry industry of the area. Limitations in resource could not limit prediction of health risk concerns.

5. CONCLUSION AND RECOMMENDATIONS

In conclusion, the present study indicated that backyard-slaughtered broiler meat samples obtained from all the investigated farms residing in Bishoftu town were found unsafe for human consumption due to the presence of high level of bacterial load (aerobic bacteria, coliform *E. coli*, and *S. aureus* counts), foodborne bacterial pathogens (*Campylobacter*, *E. coli* O157:H7, and Salmonella), antibiotic residues above acceptable limits and the associated multidrug resistance pathogen bacteria pose a significant public health risk. The findings of this study also provide novel insights into the presence of MDR, XDR, and PDR bacteria in broiler meat in the study area. To the best of our knowledge, this is the first report on XDR and PDR bacteria in broiler meat in the country. Furthermore, the present study yields noteworthy results concerning the identification and quantification of antibiotic residues exceeding maximum acceptable limits in broiler meat within the standard. Overall, this study significantly contributes to the understanding of food safety risks associated with broiler meat consumption in Ethiopia. It adds to the limited scientific literature on MDR, XDR, and PDR bacteria in poultry meat, underscoring the importance of ongoing surveillance, prudent antimicrobial use, and adherence to regulatory standards. These findings emphasize the need for continued monitoring and regulation of antibiotic use in the poultry industry to ensure food safety and safeguard public health. Based on the above mention findings the following recommendation were forwarded.

- It is recommended to undergo training and educational programs on implementing good slaughtering practices, encompassing appropriate handling, storage, and transportation methods for broiler meat to prevent cross contamination.
- Farmers should strictly adopt responsible and prudent use of antibiotics and strictly adhere to recommended withdrawal periods to mitigate the risk of antibiotic resistance and the occurrence of antibiotic residues in broiler meat.
- It is essential to enhance consumer awareness regarding the significance of safe food handling and appropriate cooking practices to mitigate the potential risks of foodborne illnesses. Moreover, it is recommended to promote consumer

preference for poultry products sourced from reputable suppliers that adhere to stringent hygiene and safety practices.

- Establishment of well-equipped standard broiler slaughtering infrastructures and meat processing facilities in Bishoftu town to prevent broiler backyard killing practices that will help chicken meat inspection to guarantee public health and consumer protection.
- It is advised to develop and implement legislative guidelines and regulatory enforcement measures concerning food safety and antibiotic usage within the poultry industry, establish comprehensive surveillance programs to monitor foodborne pathogens and antibiotic residues in poultry meat, allocate support and resources for research and development aimed at controlling foodborne pathogens and antibiotic resistance in the poultry sector.
- One health approaches is the opportunity to implement control programmers that reduce the multiple impacts of zoonosis in both human and animal populations.
- Further detailed epidemiological and molecular studies are essential to identify sources of acquisition of resistant bacteria pathogen strains and underlying factors contributing to the presence of antibiotic residues surpassing acceptable limits in broiler meat of Bishoftu town and develop effective strategies to mitigate this concern.

6. REFERENCES

Abate, D., and Assefa, N. (2021). Prevalence and antimicrobial resistance patterns of Salmonella isolates in human stools and animal origin foods in Ethiopia: A systematic review and meta-analysis. *International Journal of Health Sciences*, ***15***(1), 43–55. http://www.ncbi.nlm.nih.gov/pubmed/33456442%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC7786440

Abd-Elghany, S. M., Sallam, K. I., Abd-Elkhalek, A., and Tamura, T. (2015). Occurrence, genetic characterization and antimicrobial resistance of Salmonella isolated from chicken meat and giblets. *Epidemiology and Infection*, ***143***(5), 997–1003. https://doi.org/10.1017/S0950268814001708

Abdi, R. D., Mengstie, F., Beyi, A. F., Beyene, T., Waktole, H., Mammo, B., Ayana, D., and Abunna, F. (2017). Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia. *BMC Infectious Diseases*, ***17***(1), 1–12. https://doi.org/10.1186/s12879-017-2437-2

Abdissa, R., Haile, W., Fite, A. T., Beyi, A. F., Agga, G. E., Edao, B. M., Tadesse, F., Korsa, M. G., Beyene, T., Beyene, T. J., De Zutter, L., Cox, E., and Goddeeris, B. M. (2017). Prevalence of Escherichia coli O157: H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. *BMC Infectious Diseases*, ***17***(1), 1–6. https://doi.org/10.1186/s12879-017-2372-2

Abebe, E., Gugsa, G., and Ahmed, M. (2020). Review on Major Food-Borne Zoonotic Bacterial Pathogens. *Journal of Tropical Medicine*, *2020*. https://doi.org/10.1155/2020/4674235

Abebew, D., Belihu, K., Zewde, G., and Zeit, D. (2014). Detection and determination of Oxytetracycline and Penicillin G antibiotic residue levels in bovine bulk milk from Nazareth dairy farms, Ethiopia. *Ethiopian Veterinary Journal*, ***18***(1), 1–15.

Abunna, F., Bedasa, M., Beyene, T., Ayana, D., Mamo, B., and Duguma, R. (2016). Isolation and antimicrobial susceptibility tests on isolates collected from poultry farms in and around Modjo, Central Oromia, and Ethiopia. *Japsc*, ***5***(2), 21–35.

Adeleye, S. A., Braide, W., Chinakwe, E. C., Esonu, C. E., and Uzoh, C. V. (2018).

Chicken Meat, Beef and Vegetables: Potential Sources of *Campylobacter jejuni* Contamination in Imo State, Nigeria. *Sustainable Food Production*, ***3,*** 63–71. https://doi.org/10.18052/www.scipress.com/sfp.3.63

Adesiji, Y., Alli, O., Adekanle, M., and Jolayemi, J. (2011). Prevalence of Arcobacter, Escherichia coli, Staphylococcus aureus and Salmonella species in Retail Raw Chicken, Pork, Beef and Goat meat in Osogbo, Nigeria. *Sierra Leone Journal of Biomedical Research*, ***3***(1), 8–12. https://doi.org/10.4314/sljbr.v3i1.66644

Agmas, B., and Adugna, M. (2018). Antimicrobial residue occurrence and its public health risk of beef meat in Debre Tabor and Bahir Dar, Northwest Ethiopia. *Veterinary World*, ***11***(7), 902–908. https://doi.org/10.14202/vetworld.2018.902-908

Agyare, C., Etsiapa Boamah, V., Ngofi Zumbi, C., and Boateng Osei, F. (2019). Antibiotic Use in Poultry Production and Its Effects on Bacterial Resistance. *Antimicrobial Resistance - A Global Threat*. https://doi.org/10.5772/intechopen.79371

Al-Hazmi, M., Al-Arfaj, A., Mostafa., A., and Ihab, M. (2013). Molecular Detection of Salmonella enteric Serovar Enteritidis in Chicken-Related Samples Collected from Egypt. *Life Science Journal*, ***10***(3), 2645–2649.

Alewy, D., Mashhadany, A., Aljabar, A. A. A., and Zaki, A. M. (2018). Detection of Antibiotic Residues Among Poultry Meat in Erbil City and Impact of Thermal Processing On Remnants Dhary. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, ***3***(237), 237–247.

Arias, M. L., Monge-Rojas, R., Chaves, C., and Antillón, F. (2001). Effect of storage temperaturas on growth and survival of Escherichia coli 0157: H7 inoculated in foods from a neotropical environment. *Revista de Biología Tropical*, ***49***(2), 517–524.

Asefa Kebede, I., and Duga, T. (2022). Prevalence and Antimicrobial Resistance of Salmonella in Poultry Products in Central Ethiopia. *Veterinary Medicine International*, *2022*. https://doi.org/10.1155/2022/8625636

Asfaw Ali, D., Tadesse, B., and Ebabu, A. (2020). Prevalence and Antibiotic Resistance Pattern of Salmonella Isolated from Caecal Contents of Exotic Chicken in Debre Zeit and Modjo, Ethiopia. *International Journal of Microbiology*, *2020*(i).

https://doi.org/10.1155/2020/1910630

Aytenfsu, S., Mamo, G., and Kebede, B. (2016). Review on chemical residues in milk and their public health concern in Ethiopia. *J Nutr Food Sci*, ***6***(4), 1–11.

Bai, S. H., and Ogbourne, S. M. (2016). Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. *Environmental Science and Pollution Research*, ***23***(19), 18988–19001.

Bedada, A. H., and Zewde, B. M. (2012). Tetracycline residue levels in slaughtered beef cattle from three slaughterhouses in central Ethiopia. *Global Veterinaria*, ***8(***6), 546–554.

Bedasa, S., Shiferaw, D., Abraha, A., and Moges, T. (2018). Occurrence and antimicrobial susceptibility profile of Escherichia coli O157:H7 from food of animal origin in Bishoftu town, Central Ethiopia Segni. *International Journal of Food Contamination*, 2–9. https://doi.org/10.1186/s40550-018-0064-3

Bekele, T., Zewde, G., Tefera, G., Feleke, A., and Zerom, K. (2014). *Escherichia coli O157 : H7 in Raw Meat in Addis Ababa , Ethiopia : Prevalence at an Abattoir and Retailers and Antimicrobial Susceptibility*. ***7***, 2–9.

Belachew, T., Mulusew, E., Tolosa, Y., Asefa, Z., Negussie, H., and Sori, T. (2021). Prevalence and antimicrobial-susceptibility profiles of salmonella in smallhold broiler supply chains in central Ethiopia. *Infection and Drug Resistance*, *14*(September), 4047–4055. https://doi.org/10.2147/IDR.S331249

Beyene, T. (2015). Veterinary Drug Residues in Food-animal Products: Its Risk Factors and Potential Effects on Public Health. *Journal of Veterinary Science and Technology*, ***07***(01), 1–7. https://doi.org/10.4172/2157-7579.1000285

Beyene, T. (2016). Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. *J Vet Sci Technol*, *7*(1), 1–7.

Beyene, T., Gurmu, F., Beyi, A. F., Hogeveen, H., Beyene, T. J., Ayana, D., Woldemariyam, F. T., Hailemariam, E., Gutema, F. D., and Stegeman, J. A. (2018). Veterinary medicinal product usage among food animal producers and its health implications in Central Ethiopia. *BMC Veterinary Research*, ***14***(1), 1–7. https://doi.org/10.1186/s12917-018-1737-0

Beyene, T., Kemal, A., Jibat, T., Tadese, F., Ayana, D., and Feyisa, A. (2015).

Assessment on Chemicals and Drugs Residue in Dairy and Poultry Products in. https://doi.org/10.4172/2155-9600.S13

Beyene, T., Kemal, A., Jibat, T., Tadese, F., Ayana, D., Feyisa, A., Takele Beyene, Abdulkaf Kemal, Tariku Jibat, Fanos Tadese, D. A. and A. F., and Abdulkaf Kemal, T. B. (2015). Assessment on Chemicals and Drugs Residue in Dairy and Poultry Products in Bishoftu and Modjo, Central Ethiopia. *Journal of Nutrition and Food Sciences*, *s13*. https://doi.org/10.4172/2155-9600.s13-002

Beyi, A. F., Fite, A. T., Tora, E., Tafese, A., Genu, T., Kaba, T., Beyene, T. J., Beyene, T., Korsa, M. G., Tadesse, F., Zutter, L. De, Goddeeris, B. M., and Cox, E. (2017). *Prevalence and antimicrobial susceptibility of Escherichia coli O157 in beef at butcher shops and restaurants in central Ethiopia*. 1–6. https://doi.org/10.1186/s12866-017-0964-z

Bhandari N, Nepali DB, P. S. (2013). Assessment of bacterial load in broiler chicken meat from the retail meat shops in Chitwan, Nepal. *Int J Infect Microbiol*, ***2***(3), 99–104. https://doi.org/OI: http://dx.doi.org/10.3126/ijim.v2i3.8671

Butaye, P., Devriese, L. A., and Haesebrouck, F. (2003). Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. *Clinical Microbiology Reviews*, ***16***(2), 175–188.

Codex Alimentarius Commission. (2012). Maximum residue limits for veterinary drugs in foods. *Updated as at the 35th Session of the Codex Alimentarius Commission, CAC/MRL*, 1–40.

Codex Alimentarius Commission:(2017). Maximum residue limits (MRLs) and risk management recommendations (RMRs) for residues of veterinary drugs in foods. *CAC/MRL*, *2*, 8.

Cardinale, E., Gros-Claude, J. D. P., Tall, F., Cissé, M., Guèye, E. F., and Salvat, G. (2002). Prevalence of Salmonella and Campylobacter in retail chicken meat in Spain. *International Journal of Food Microbiology*, ***72***(1–2), 165–168. https://doi.org/10.1016/S0168-1605(01)00638-9

Castro-Vargas, R. E., Herrera-Sánchez, M. P., Rodríguez-Hernández, R., and Rondón-Barragán, I. S. (2020). Antibiotic resistance in Salmonella spp. isolated from poultry: A global overview. *Veterinary World*, ***13***(10).

https://doi.org/10.14202/vetworld.2020.2070-2084

Chapman, H. D., and Jeffers, T. K. (2014). Vaccination of chickens against coccidiosis ameliorates drug resistance in commercial poultry production. *International Journal for Parasitology: Drugs and Drug Resistance*, ***4***(3), 214–217.

Cohen, N., Ennaji, H., Bouchrif, B., Hassar, M., and Karib, H. (2007). Comparative study of microbiological quality of raw poultry meat at various seasons and for different slaughtering processes in Casablanca (Morocco). *Journal of Applied Poultry Research*, ***16***(4), 502–508. https://doi.org/10.3382/japr.2006-00061

Central Statistical Agency. (2020). Agricultural sampling survey. *Report on Livestock and Livestock Characteristics*, *II*(March), 1–215.

Dadi, L., and Asrat, D. (2009). Prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat products in Ethiopia. *Ethiopian Journal of Health Development*, ***22***(2). https://doi.org/10.4314/ejhd.v22i2.10072

Darwish, W. S., Eldaly, E. A., El-Abbasy, M. T., Ikenaka, Y., Nakayama, S., and Ishizuka, M. (2013). Antibiotic residues in food: The African scenario. *Japanese Journal of Veterinary Research*, ***61***(SUPPL.).

Debelo, M., Mohammed, N., Tiruneh, A., and Tolosa, T. (2022). Isolation, identification and antibiotic resistance profile of thermophilic Campylobacter species from Bovine, Knives and personnel at Jimma Town Abattoir, Ethiopia. *PLoS ONE*, *17*(10 October), 1–14. https://doi.org/10.1371/journal.pone.0276625

Eguale, T. (2018). Non-typhoidal Salmonella serovars in poultry farms in central Ethiopia: Prevalence and antimicrobial resistance. *BMC Veterinary Research*, ***14***(1), 1–8. https://doi.org/10.1186/s12917-018-1539-4

Ejo, M., Garedew, L., Alebachew, Z., and Worku, W. (2016). Prevalence and Antimicrobial Resistance of Salmonella Isolated from Animal-Origin Food Items in Gondar, Ethiopia. *BioMed Research International*, *2016*. https://doi.org/10.1155/2016/4290506

Enver, K., Senita, I., Sabina, O., Saud, H., Almir, T., Nermina, Đ., and Samir, M. (2021). Microbiological Contamination of Fresh Chicken Meat in the Retail Stores. *Food and Nutrition Sciences*, ***12***(01), 64–72. https://doi.org/10.4236/fns.2021.121006

European Parliament and the Council of the European Union. (2010). Commission Regulation (EU) No 37/2010. *Official Journal of the European Union, L 15/1*.

FAO, OMS, and CODEX. (2015). *Maximun Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for residues of veterinary drugs in foods CAC/MRL 2-2015*. *July*, 22.

Faruque, M. O., Mahmud, S., Munayem, M. A., Sultana, R., Molla, M. T., Ali, M. F., Wasim, M., Sarker, S., and Evamoni, F. Z. (2019). Bacteriological Analysis and Public Health Impact of Broiler Meat: A Study on Nalitabari Paurosova, Sherpur, Bangladesh. *Advances in Microbiology*, ***09***(07), 581–601. https://doi.org/10.4236/aim.2019.97036

Ferguson, J. P., Baxter, G. A., McEvoy, J. D. G., Stead, S., Rawlings, E., and Sharman, M. (2002). Detection of streptomycin and dihydrostreptomycin residues in milk, honey and meat samples using an optical biosensor. *Analyst*, *127*(7), 951–956.

Gambushe, M, S., and Zishiri, O. T. (2022). *Review of Escherichia coli O157 : H7 Prevalence , Pathogenicity , Heavy Metal and Antimicrobial Resistance , African Perspective*. *June*, 4645–4673.

Gaudin, V., Cadieu, N., and Sanders, P. (2005). Results of a European proficiency test for the detection of streptomycin/dihydrostreptomycin, gentamicin and neomycin in milk by ELISA and biosensor methods. *Analytica Chimica Acta*, ***529***(1–2), 273–283.

Gemeda, B. A., Amenu, K., Magnusson, U., Dohoo, I., Hallenberg, G. S., Alemayehu, G., Desta, H., and Wieland, B. (2020). Antimicrobial Use in Extensive Smallholder Livestock Farming Systems in Ethiopia: Knowledge, Attitudes, and Practices of Livestock Keepers. *Frontiers in Veterinary Science*, *7*(February), 1–15. https://doi.org/10.3389/fvets.2020.00055

Goetting, V., Lee, K. A., and Tell, L. A. (2011). Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: A review of the literature. *Journal of Veterinary Pharmacology and Therapeutics*, **34**(6), 521–556. https://doi.org/10.1111/j.1365-2885.2011.01287.x

Gurmu, E., and Gebretinsae, H. (2013). Assessment of Bacteriological Quality of Meat Cutting surfaces in selected Butcher shops of Mekelle city, Ethiopia. *Journal of*

Environmental and Occupational Science, ***2***(2), 61. https://doi.org/10.5455/jeos.20130416114711

Ha, M., and Nguyen, A. (2012). *Prevalence of Salmonella in retail whole chicken carcasses in Hanoi, Vietnam par*. 1–123. https://papyrus.bib.umontreal.ca/xmlui/bitstream/handle/1866/12614/Nguyen_Man_Ha_Anh_2012_memoire.pdf?sequence=6andisAllowed=y

Haile, A. F., Alonso, S., Berhe, N., Atoma, T. B., Boyaka, P. N., and Grace, D. (2022). Prevalence, Antibiogram, and Multidrug-Resistant Profile of E. coli O157: H7 in Retail Raw Beef in Addis Ababa, Ethiopia. *Frontiers in Veterinary Science*, *9*(February), 1–7. https://doi.org/10.3389/fvets.2022.734896

Hamid, M., Tefera, Y., Tadese Eguale, and Worku, Y. (2018). Escherichia coli O157:H7: Prevalence, Identification and Antimicrobial Resistance in Cattle Slaughter at Addis Ababa Municipal Abattior, Ethiopia. *International Journal of Advanced Research in Biological Sciences*, ***5***(10), 136–146. https://doi.org/http://dx.doi.org/10.22192/ijarbs.2018.05.10.014

Hedman, H. D., Vasco, K. A., and Zhang, L. (2020). A review of antimicrobial resistance in poultry farming within low- resource settings. *Animals*, ***10***(8), 1–39. https://doi.org/10.3390/ani10081264

Hertanto, B. S., Nurmalasari, C. D. A., Nuhriawangsa, A. M. P., Cahyadi, M., and L R Kartikasari. (2018). The physical and microbiological quality of chicken meat in the different type of enterprise poultry slaughterhouse : a case study in Karanganyar District In *IOP Conference Series: Earth and Environmental Science* **102**(1)012051. https://doi.org/doi :10.1088/1755-1315/102/1/012051

Hessain, A. M., Al-arfaj, A. A., Zakri, A. M., El-jakee, J. K., Al-zogibi, O. G., Hemeg, H. A., and Ibrahim, I. M. (2015). Molecular characterization of Escherichia coli O157 : H7 recovered from meat and meat products relevant to human health in Riyadh , Saudi Arabia. *Saudi Journal of Biological Sciences*, ***22***(6), 725–729. https://doi.org/10.1016/j.sjbs.2015.06.009

Hiko, A., Asrat, D., and Zewde, G. (2008). Occurrence of Escherichia coli O157:H7 in retail raw meat products in Ethiopia. *Journal of Infection in Developing Countries*, ***2***(5), 389–393. https://doi.org/10.3855/jidc.203

Hind, A. E., Adil, M. S., and Rade, S. A. El. (2014). Screening of Antibiotic Residues in Poultry Liver , Kidney and Muscle in Khartoum State , Sudan. *Journal of Applied and Industrial Sciences*, ***2***(3), 116–122.

Hussein, M. A., and Khalil, S. R. (2013). Screening of Some Antibiotics and Anabolic SteroidsResidues in Broiler Fillet Marketed in El-Sharkia Governorate. *Life Science Journal*, ***10****(1)*(May 2014).

Ibrahim, H. M., Hassan, M. A., Amin, R. A., Shawqy, N. A., and Elkoly, R. L. (2018). The Bacteriological Quality of Some Chicken Meat Products. *Benha Veterinary Medical Journal*, ***35***(1), 50–57.

Jammoul, A., and Darra, N. El. (2019). Evaluation of antibiotics residues in chicken meat samples in lebanon. *Antibiotics*, ***8***(2). https://doi.org/10.3390/antibiotics8020069

Jank, L., Martins, M. T., Arsand, J. B., Motta, T. M. C., Feijó, T. C., dos Santos Castilhos, T., Hoff, R. B., Barreto, F., and Pizzolato, T. M. (2017). Liquid chromatography–tandem mass spectrometry multiclass method for 46 antibiotics residues in milk and meat: development and validation. *Food Analytical Methods*, ***10***(7), 2152–2164.

Jhandai, P., Sciences, A., Gupta, R., and Sciences, A. (2020). *Detection of Escherichia Coli From Food of Animal Origin in Hisar*..

Joseph, K. N. K., Esther, W. N., and Lucy, W. K. (2021). Microbiological quality of free-range chicken carcasses from a non-regulated slaughter facility in Kenya. *Journal of Veterinary Medicine and Animal Health*, ***13***(1), 22–27. https://doi.org/10.5897/jvmah2020.0870

Kebede, A., Kemal, J., Alemayehu, H., and Habte Mariam, S. (2016). Isolation, Identification, and Antibiotic Susceptibility Testing of Salmonella from Slaughtered Bovines and Ovines in Addis Ababa Abattoir Enterprise, Ethiopia: A Cross-Sectional Study . *International Journal of Bacteriology*, *2016*, 1–8. https://doi.org/10.1155/2016/3714785

Kuria, J. K. N., Ngethe, E. W., Kabuage, L. W., and Gathura, P. B. (2018). Isolation of campylobacter sppand escherichia coli 0157: H7 from free-range indigenous chicken value chain in Kenya. *East African Medical Journal*, ***95***(1), 1116–1124.

Kyuchukova, R. (2020). Antibiotic residues and human health hazard – review.

Bulgarian Journal of Agricultural Science, *26*(3), 664–668.

Lawal, J. R., Jajere, S. M., Geidam, Y. A., Bello, A. M., Wakil, Y., and Mustapha, M. (2015). Antibiotic Residues in Edible Poultry Tissues and Products in Nigeria: A Potential Public Health Hazard. *International Journal of Animal and Veterinary Advances*, ***7***(3), 55–61. https://doi.org/10.19026/ijava.7.5241

Liur, I. J., and Veerman, M. (2021). Level of escherichia coli contamination of broiler chicken meat in Ambon City *IOP Conference Series: Earth and Environmental Science PAPER*, ***883***(1), 012036. https://doi.org/10.1088/1755-1315/883/1/012036

Ma, F., Xu, S., Tang, Z., Li, Z., and Zhang, L. (2021). Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosafety and Health*, ***3***(1), 32–38. https://doi.org/10.1016/j.bsheal.2020.09.004

Maharjan, S., Rayamajhee, B., Chhetri, V. S., Sherchan, S. P., Panta, O. P., and Karki, T. B. (2019). Microbial quality of poultry meat in an ISO 22000:2005 certified poultry processing plant of Kathmandu valley. *International Journal of Food Contamination*, ***6***(1). https://doi.org/10.1186/s40550-019-0078-5

Marshall, B. M., and Levy, S. B. (2011). Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews*, ***24***(4), 718–733.

Mead, G. (2004). Microbiological quality of poultry meat: a review. *Brazilian Journal of Poultry Science*, ***6***(3), 135–142. https://doi.org/10.1590/s1516-635x2004000300001

Menkem, Z. E., Ngangom, B. L., Tamunjoh, S. S. A., and Boyom, F. F. (2019). Antibiotic residues in food animals: Public health concern. *Acta Ecologica Sinica*, ***39***(5), 411–415. https://doi.org/10.1016/j.chnaes.2018.10.004

Mensah, S. E., Koudandé, O. D., Sanders, P., Laurentie, M., Mensah, G. A., and Abiola, F. A. (2014). Antimicrobial residues in foods of animal origin in Africa: public health risks. *Rev Sci Tech*, ***33***(3), 987–996.

Milkias, M. (2016). Chicken Meat Production, Consumption and Constraints in Ethiopia. *Issn*, ***54***, 2224–6088. www.iiste.org

Mohamed-Noor, S. E., Shuaib, Y. A., Suliman, S. E., and Abdalla, M. A. (2012). Study of microbial contamination of broilers in modern abattoirs in Khartoum state. *Annals of the University Dunarea de Jos of Galati, Fascicle VI: Food Technology*, ***36***(1), 74–80.

Molla, B., Mesfin, A., and Alemayehu, D. (2004). Multiple antimicrobial-resistant Salmonella serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. *Ethiopian Journal of Health Development*, ***17***(2), 131–139. https://doi.org/10.4314/ejhd.v17i2.9854

Mpundu, P., Mbewe, A. R., Muma, J. B., Sitali, G. M., Mubita, C. M., and Munyeme, M. (2021). Comparison of Bacterial Cross-Contamination among Broiler Carcasses between Commercial and Non-Commercial Processed System and Its Public Health Implications. *Open Journal of Veterinary Medicine*, ***11***(01), 1–13. https://doi.org/10.4236/ojvm.2021.111001

Muhammad, F., Akhtar, M., Zia-ur-Rahman, J. I., and Anwar, M. I. (2009). Role of veterinarians in providing residue-free animal food. *Pakistan Veterinary Journal*, ***29***, 42–46.

Mund, M. D., Khan, U. H., Tahir, U., Mustafa, B. E., and Fayyaz, A. (2017). Antimicrobial drug residues in poultry products and implications on public health: A review. *International Journal of Food Properties*, ***20***(7), 1433–1446. https://doi.org/10.1080/10942912.2016.1212874

Noori, T.E, and Alwan, M. J. (2016). Isolation and Identification of Zoonotic Bacteria from Poultry Meat. *International Journal of Advanced Research in Biological Sciences*, ***3***(8–2016), 57–66.

Nzouankeu, A., Ngandjio, A., Ejenguele, G., Njine, T., and Wouafo, M. N. (2010). Multiple contaminations of chickens with campylobacter, escherichia coli and Salmonella in Yaounde (Cameroon). *Journal of Infection in Developing Countries*, ***4***(9), 583–586. https://doi.org/10.3855/jidc.1019

Odwar, J. A., Kikuvi, G., Kariuki, J. N., and Kariuki, S. (2014). A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Research Notes*, ***7***(1), 1–8. https://doi.org/10.1186/1756-0500-7-627

Okocha, R. C., Olatoye, I. O., and Adedeji, O. B. (2018). Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Reviews*, ***39***(1), 1–22. https://doi.org/10.1186/s40985-018-0099-2

Onipede, O. J., Nwankwo, B., Adewuyi, G. O., and Nwachukwu, C. U. (2021). Levels of antibiotics residues in chicken and catfish sold in some parts of Lagos state and Ota

local government Ogun state south-western Nigeria. *Scientific African*, *12*, e00768. https://doi.org/10.1016/j.sciaf.2021.e00768

Pikkemaat, M. G., Rapallini, M. L. B. A., Oostra-van Dijk, S., and Elferink, J. W. A. (2009). Comparison of three microbial screening methods for antibiotics using routine monitoring samples. *Analytica Chimica Acta*, ***637***(1–2), 298–304.

Poirel, L., Madec, J.-Y., Lupo, A., Schink, A.-K., Kieffer, N., Nordmann, P., And Schwarz5, S. (2004). Antimicrobial Resistance in Escherichia coli. *Microbiology Spectrum American Society for Microbiology Press*, ***23***(10), 979–980. https://doi.org/10.1097/01.inf.0000141751.73083.7a

Ramatla, T., Ngoma, L., Adetunji, M., and Mwanza, M. (2017). Evaluation of antibiotic residues in raw meat using different analytical methods. *Antibiotics*, ***6***(4), 1–17. https://doi.org/10.3390/antibiotics6040034

Rau, R. B., Ribeiro, A. R., Dos Santos, A., and Barth, A. L. (2021). Antimicrobial resistance of Salmonella from poultry meat in Brazil: results of a nationwide survey. *Epidemiology and Infection*, ***149***, 26–39. https://doi.org/10.1017/S0950268821002156

Rortana, C., Nguyen-Viet, H., Tum, S., Unger, F., Boqvist, S., Dang-Xuan, S., Koam, S., Grace, D., Osbjer, K., Heng, T., Sarim, S., Phirum, O., Sophia, R., and Lindahl, J. F. (2021). Prevalence of salmonella spp. And staphylococcus aureus in chicken meat and pork from Cambodian markets. *Pathogens*, ***10***(5), 1–17. https://doi.org/10.3390/pathogens10050556

Shaltout, F. A., Afify, A., Sayed, E., and I. M, Z. (2020). Aerobic Plate Count of Contaminants and Molecular Characterization of Eschereichia Coli in Raw Chicken Meat in Ismailia, Egypt. *Journal of Veterinary Healthcare*, ***2***(2), 23–30. https://doi.org/10.14302/issn.2575-1212.jvhc-20-3477

Shaltout, F. A., Lamada, H. M., and Edris, E. A. (2021). Bacteriological Examination of Cooked Meat and Chicken Meals. *Cohesive Journal of Microbiology and Infectious Disease, **3**(5)*(April 2020). https://doi.org/10.31031/CJMI.2020.03.000575

Shareef, A. M., Jamel, Z. T., and Yonis, K. M. (2009). Detection of antibiotic residues in stored poultry products. *Iraqi Journal of Veterinary Sciences*, ***23***(3), 45–48.

Shecho, M., Thomas, N., Kemal, J., and Muktar, Y. (2017). Cloacael Carriage and

Multidrug Resistance Escherichia coli O157:H7 from Poultry Farms, Eastern Ethiopia . *Journal of Veterinary Medicine*, *2017*, 1–9. https://doi.org/10.1155/2017/8264583

Svobodová, I., Bořilová, G., Hulánková, R., and Steinhauserová, I. (2012). Microbiological quality of broiler carcasses during slaughter processing. *Acta Veterinaria Brno*, ***81***(1), 37–42. https://doi.org/10.2754/avb201281010037

Taddese, D., Tolosa, T., Deresa, B., Lakow, M., Olani, A., and Shumi, E. (2019). Antibiograms and risk factors of Salmonella isolates from laying hens and eggs in Jimma Town, South Western Ethiopia. *BMC Research Notes*, ***12***(1), 1–7. https://doi.org/10.1186/s13104-019-4516-5

Tadese, N. D., Gebremedhi, E. Z., Moges, F., Borana, B. M., Marami, L. M., Sarba, E. J., Abebe, H., Kelbesa, K. A., Atalel, D., and Tessema, B. (2021). *Occurrence and Antibiogram of Escherichia coli O157 : H7 in Raw Beef and Hygienic Practices in Abattoir and Retailer Shops in Ambo Town , Ethiopia*. *2021*.

Tadesse, T., Elfinesh, B., Birhanu, M., H, S., and Tekeba, E. (2019). Assessment of Broiler Production; Processing and Marketing Practices in Ethiopia: Identifying the Root Causes for Poultry Products Importation and Way Forward. *Novel Techniques in Nutrition and Food Science*, ***4***(4). https://doi.org/10.31031/ntnf.2019.04.000591

Tansakul, N., Niedorf, F., and Kietzmann, M. (2007). A sulfadimidine model to evaluate pharmacokinetics and residues at various concentrations in laying hen. *Food Additives and Contaminants*, ***24***(6), 598–604. https://doi.org/10.1080/02652030601182870

Tefera, B., Tilki, T., Tefera, N., Bayene, Z., Belew, S., Haftu, R., Tolasa, F., Nuguse, Y., Ararso, S., Getachew, D., Soyum, B., and Andualem, T. (2022). Antimicrobial resistance status of selected bacteria isolated from animal source foods and feed in Ethiopia. *Ethiopian Veterinary Journal*, ***26***(2), 18–37. https://doi.org/10.4314/evj.v26i2.2

Temesgen, M., and Abdisa, M. (2015). Food standards, food law and regulation system in Ethiopia: a review. *Public Policy and Administration Research*, ***5***(3), 58–73.

Teshome, T., Bekele, E., Million, B., Hagos, S., and Eshetie, T. (2016). *Assessment of Broiler Production ; Processing and Marketing Practices in Ethiopia : Identifying*

the Root Causes for Poultry Products Importation to Ethiopia And Way Forward. 620–625. https://doi.org/10.31031/APDV.2019.07.000657

Thi Huong-Anh, N., Van Chinh, D., and Thi Tuyet-Hanh, T. (2020). Antibiotic Residues in Chickens and Farmers' Knowledge of Their Use in Tay Ninh Province, Vietnam, in 2017. *Asia-Pacific Journal of Public Health*, ***32***(2–3), 126–132. https://doi.org/10.1177/1010539520909942

Thomas, K. M., de Glanville, W. A., Barker, G. C., Benschop, J., Buza, J. J., Cleaveland, S., Davis, M. A., French, N. P., Mmbaga, B. T., Prinsen, G., Swai, E. S., Zadoks, R. N., and Crump, J. A. (2020). Prevalence of Campylobacter and Salmonella in African food animals and meat: A systematic review and meta-analysis. *International Journal of Food Microbiology*, ***315***(October 2019), 108382. https://doi.org/10.1016/j.ijfoodmicro.2019.108382

Thung, T. Y., Mahyudin, N. A., Basri, D. F., Wan Mohamed Radzi, C. W. J., Nakaguchi, Y., Nishibuchi, M., and Radu, S. (2016). Prevalence and antibiotic resistance of Salmonella Enteritidis and Salmonella Typhimurium in raw chicken meat at retail markets in Malaysia. *Poultry Science*, ***95***(8), 1888–1893. https://doi.org/10.3382/ps/pew144

Tilahun, A., Jambare, L., Teshale, A., and Getachew, A. (2016). Review on Chemical and Drug Residue in Meat. *World Journal of Agricultural Sciences*, ***12***(3), 196–204. https://doi.org/10.5829/idosi.wjas.2016.12.3.23739

Toldrá, F., and Reig, M. (2006). Methods for rapid detection of chemical and veterinary drug residues in animal foods. *Trends in Food Science and Technology*, ***17***(9), 482–489.

Tuem, K. B., Gebre, A. K., Atey, T. M., Bitew, H., Yimer, E. M., and Berhe, D. F. (2018). Drug Resistance Patterns of Escherichia coli in Ethiopia: A Meta-Analysis. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/4536905

Van Nierop, W., Dusé, A. G., Marais, E., Aithma, N., Thothobolo, N., Kassel, M., Stewart, R., Potgieter, A., Fernandes, B., Galpin, J. S., and Bloomfield, S. F. (2005). Contamination of chicken carcasses in Gauteng, South Africa, by Salmonella, Listeria monocytogenes and Campylobacter. *International Journal of Food Microbiology*, ***99***(1), 1–6. https://doi.org/10.1016/j.ijfoodmicro.2004.06.009

Wardhana, D. K., Haskito, A. E. P., Purnama, M. T. E., Safitri, D. A., and Annisa, S. (2021). Detection of microbial contamination in chicken meat from local markets in Surabaya, East Java, Indonesia. *Veterinary World*, ***14***(12), 3138–3143. https://doi.org/10.14202/vetworld.2021.3138-3143

Wayou, B. A., Kassa, G. M., Sori, T., Mondin, A., Tucciarone, C. M., Cecchinato, M., and Pasotto, D. (2022). Molecular Survey and Identification of Campylobacter spp. in Layer Farms in Central Ethiopia. *Tropical Medicine and Infectious Disease*, ***7***(2). https://doi.org/10.3390/tropicalmed7020031

Woyessa, M., Agga, G. E., Gumi, B., Ayana, D., and Mamo, G. (2020). Antibiotic Use in Poultry Production in Selected Districts of East Showa Zone , Central Ethiopia : From Antibiotic Stewardship Perspective College of Veterinary Medicine and Agriculture ,. *American-Eurasian Journal of Scientific Research*, ***15***(3), 101–111. https://doi.org/10.5829/idosi.aejsr.2020.101.111

Yu, Z., Joossens, M., and Houf, K. (2020). Analyses of the Bacterial Contamination on Belgian Broiler Carcasses at Retail Level. *Frontiers in Microbiology*, *11*(September). https://doi.org/10.3389/fmicb.2020.539540

Zarei, O., Shokoohizadeh, L., Hossainpour, H., and Alikhani, M. Y. (2021). *The Prevalence of Shiga Toxin-Producing Escherichia coli and Enteropathogenic Escherichia coli Isolated from Raw Chicken Meat Samples*. *2021*.

Zendehbad, B., Ali, A., and Alipour, A. (2013). Identi fi cation and antimicrobial resistance of Campylobacter species isolated from poultry meat in Khorasan province , Iran. *Food Control*, ***32***(2), 724–727. https://doi.org/10.1016/j.foodcont.2013.01.035

Zerabruk, K., Retta, N., Muleta, D., and Tefera, A. T. (2019). Assessment of Microbiological Safety and Quality of Minced Meat and Meat Contact Surfaces in Selected Butcher Shops of Addis Ababa, Ethiopia. *Journal of Food Quality*, *2019*. https://doi.org/10.1155/2019/3902690

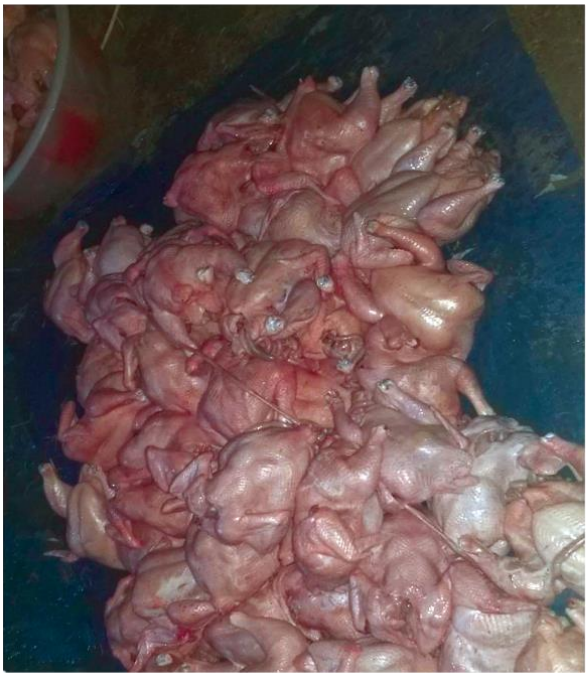
7. ANNEXES

ANNEX I: Backyard Slaughtering House Working Experiences

ANNEX I Snapshot of backyard sloughtering house

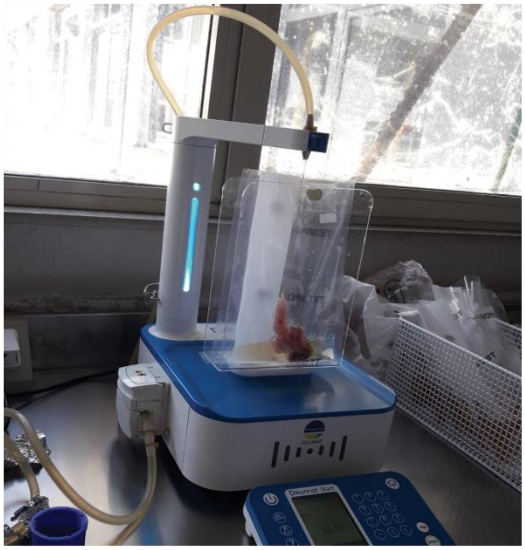


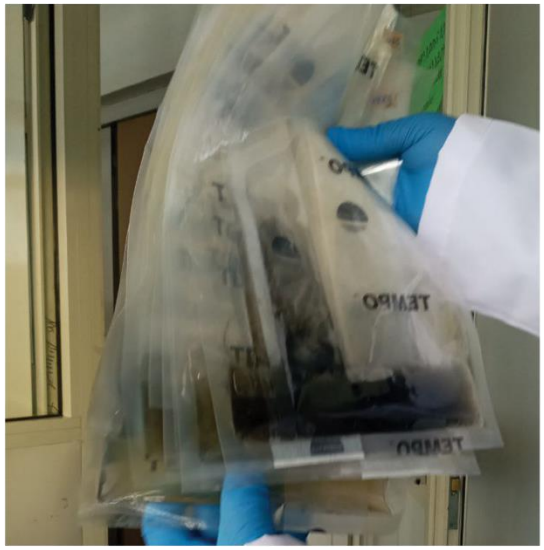





ANNEX II: Picture of Sample Preparation and Campylobacter Enriched Samples

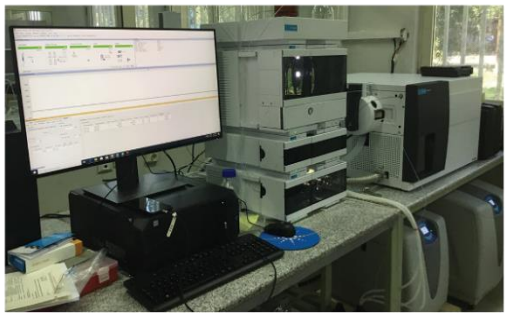
ANNEX II: Sample preparation and sample enriched for campylobacter





ANNEX III: Some of Instrument Used for Microbiological and Antimicrobial Analysis

ANNEX III: Instruments used for microbiological and antimicrobial residue analysis

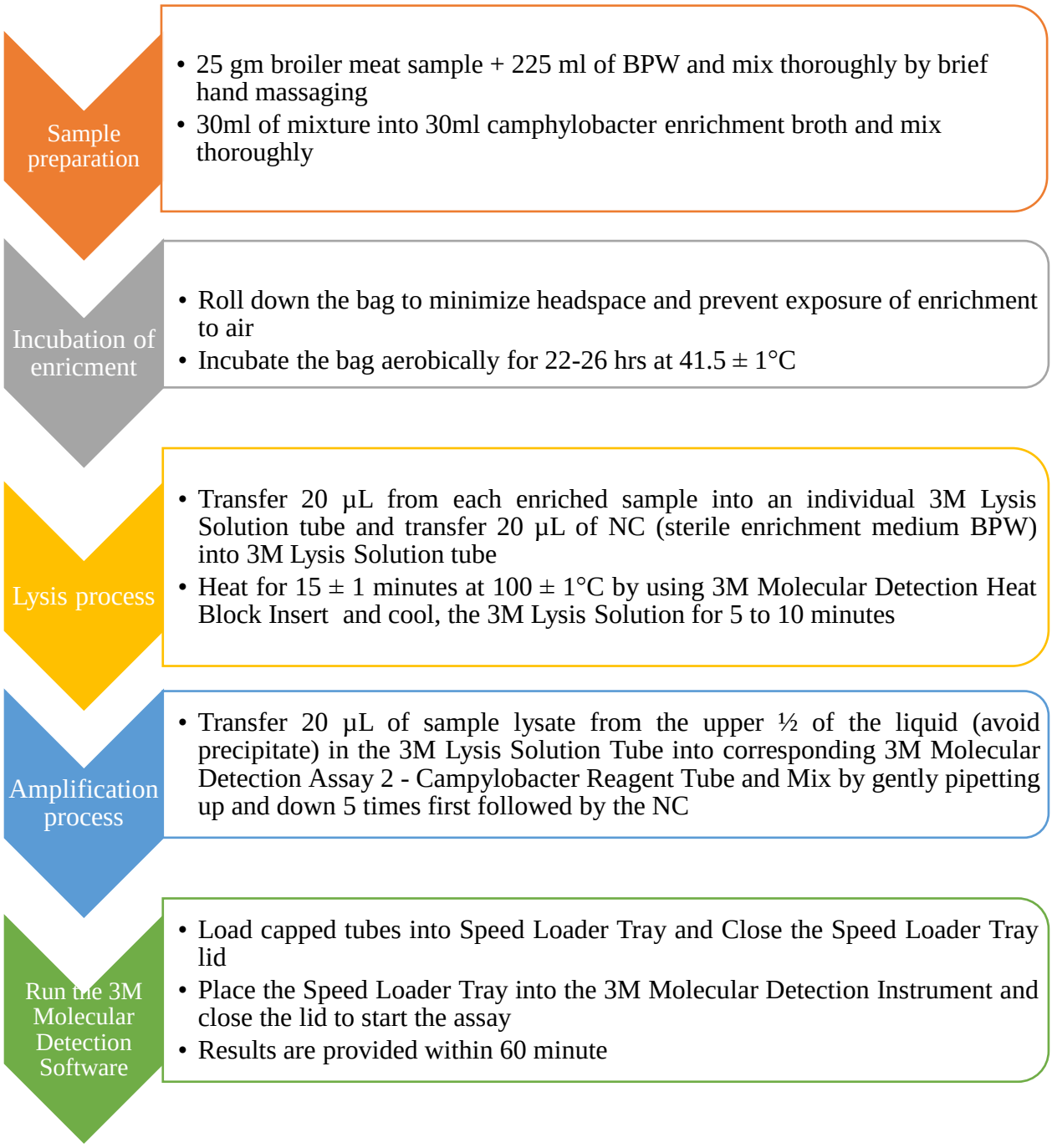


ANNEX IV: Laboratory Analysis Procedure

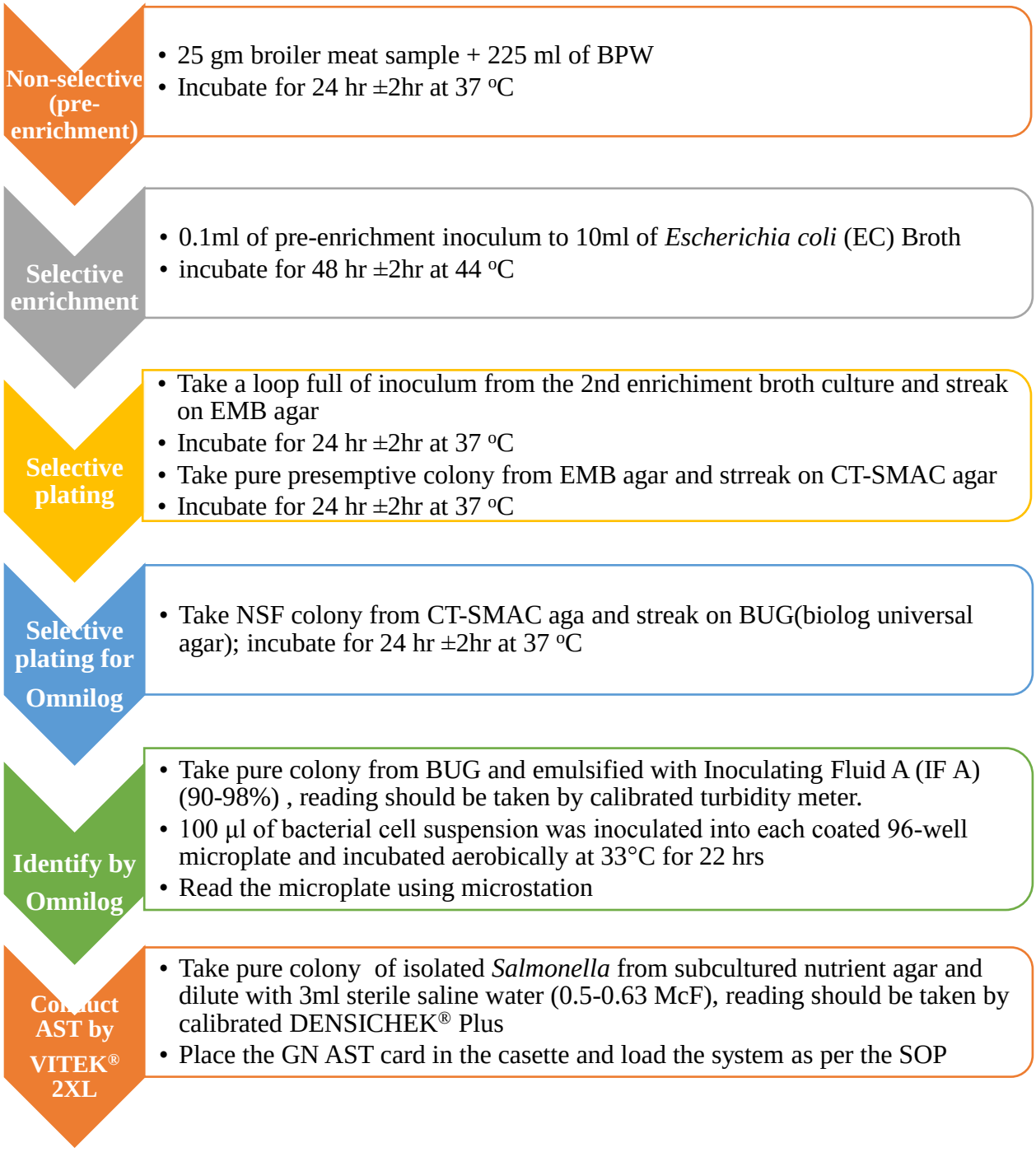
A. Analysis of microbial load summary

Test Parameter	Tempo Dilution	Enumeration range	Tempo vial		Incubation	Incubation time
			Primary dilution of matrix	ml of primary dilution+ ml of secondary diluent		
CC	1/400	10^2-4.9*10^5	10^{-1}	0.1ml +3.9ml	35^{o}C	22-27hrs
	1/4000	10^3-4.9*10^6	10^{-2}			
EC	1/400	10^2-4.9*10^5	10^{-1}	0.1ml +3.9ml	37^{o}C	22-27hrs
	1/4000	10^3-4.9*10^6	10^{-2}			
STA	1/400	10^2-4.9*10^5	10^{-1}	0.1ml +3.9ml	37^{o}C	24-27hrs
	1/4000	10^3-4.9*10^6	10^{-2}			
AC	1/40000	10^4-4.9*10^7	10^{-3}	0.1ml +3.9ml	35^{o}C	40-48hr
	1/400000	10^5-4.9*10^8	10^{-4}			

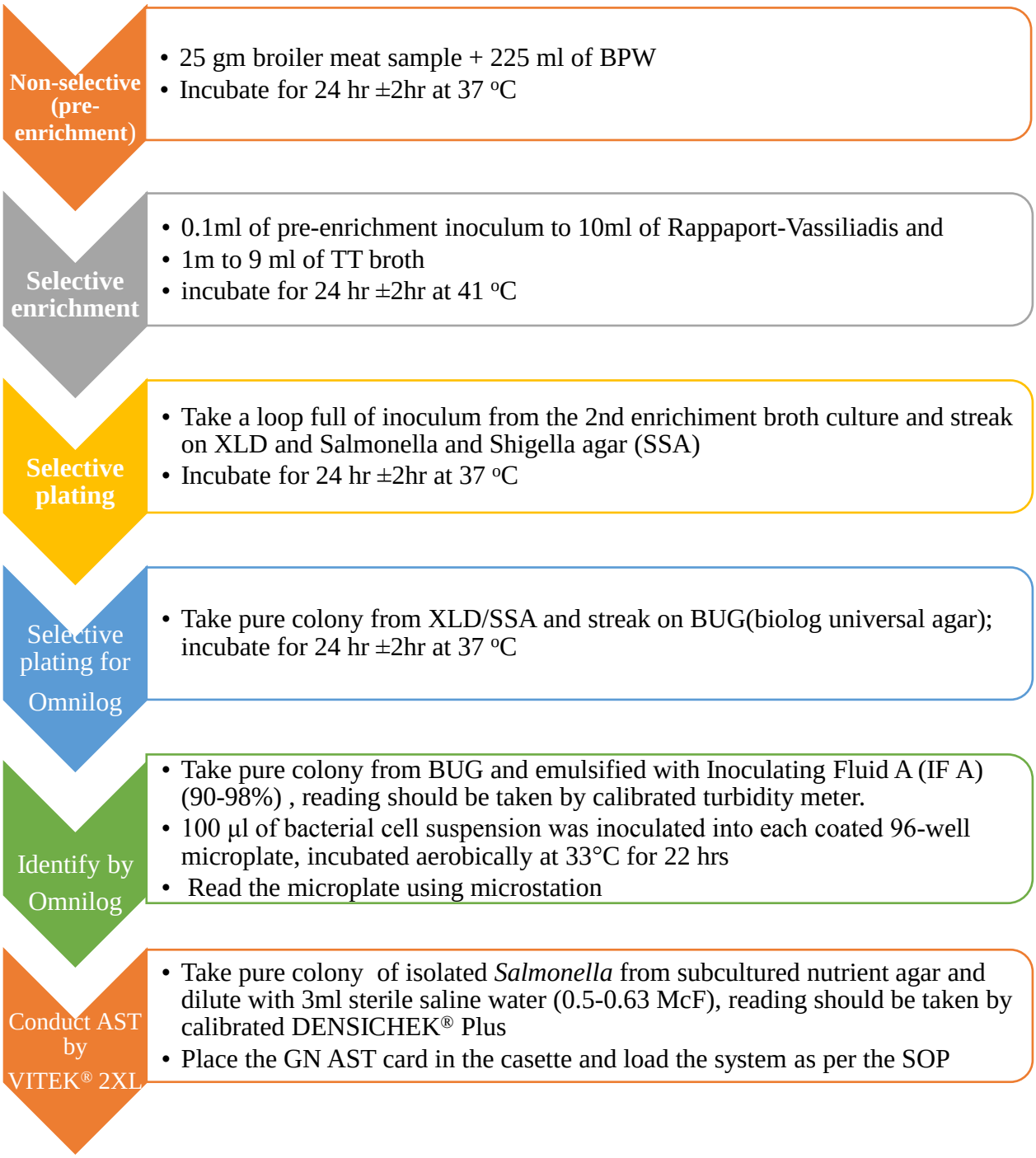
B. Test flow Chart for detection of Campylobacter



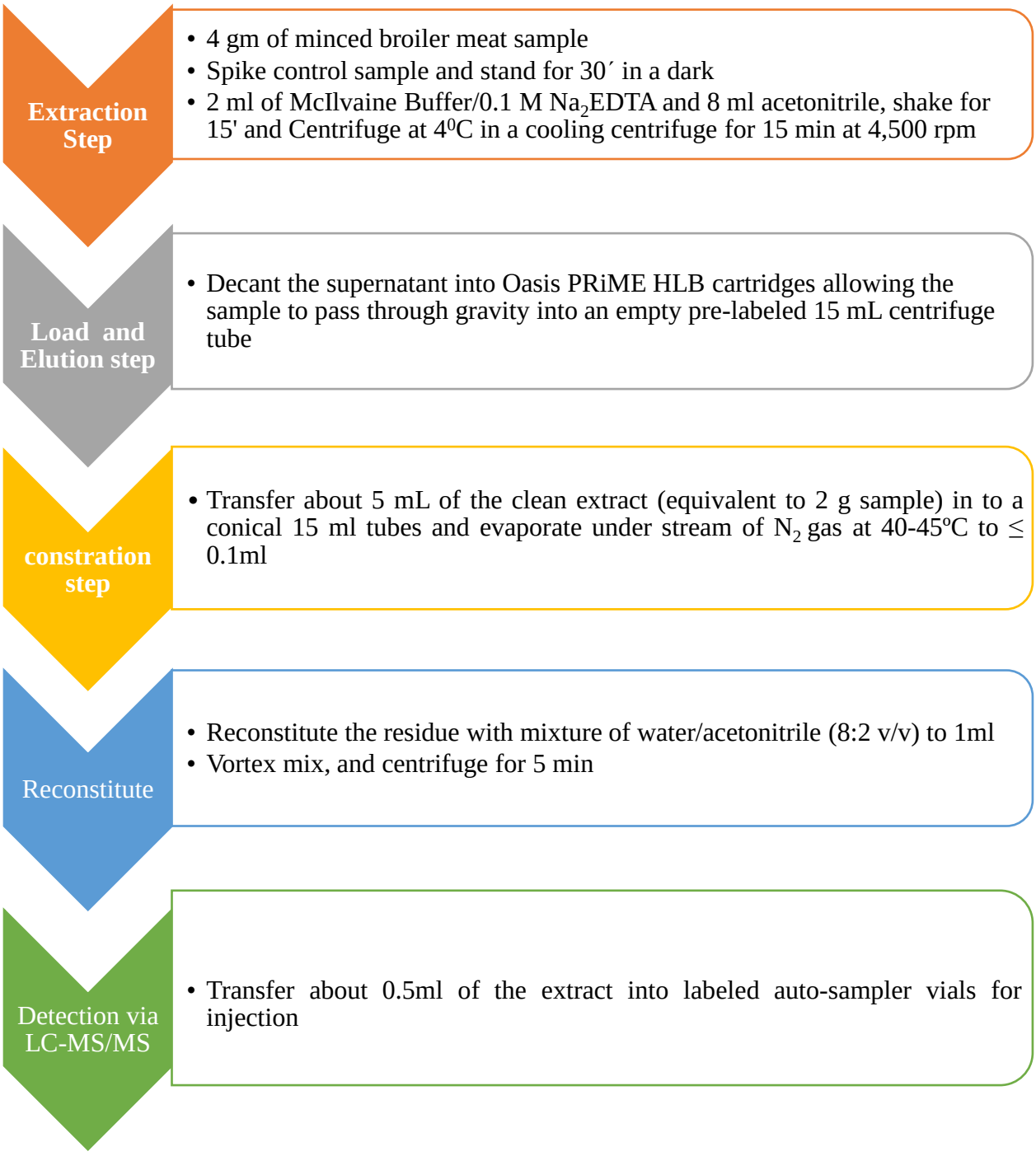
C. Test flow Chart to isolate and identify E. coli O157:H7 and conduct AST.



D. Test flow Chart to isolate and identify Salmonella and conduct AST.



E. Test flow Chart for sample extraction and cleanup producer



ANNEX V: Microbial Load Result by TEMPO

TEMPO 5.5.1.0

TEST RESULTS REPORT

Filter on Tests & Samples

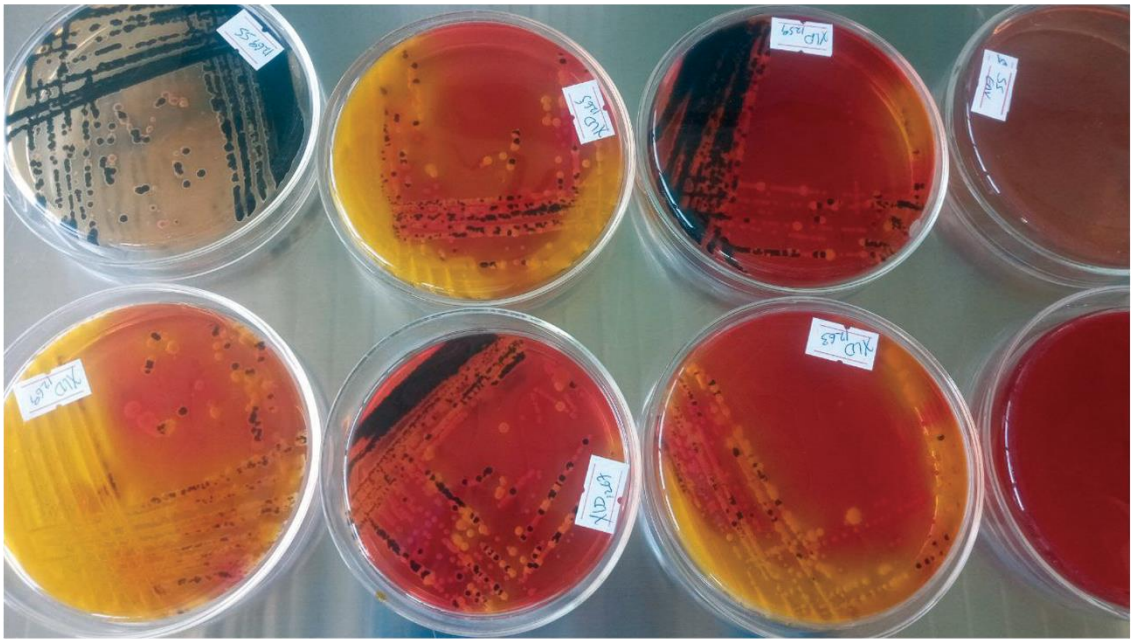
Display tests with or without final result
Display tests validated or not

Incomplete list: 10 items printed out of 319.

Sample	Test	Dilution	Mode	Prepared on	By	Result	✲	Status	Annotations	Validated
QMS_APF_MTA_1434_2022	CC	1/400	22-27h	Fri 04/15/2022 02:18 PM	Labtech	> 4.9 E5 CFU/g		☐		✔
QMS_APF_MTA_1434_2022	CC	1/4,000	22-27h	Fri 04/15/2022 02:18 PM	Labtech	= 1.1 E6 CFU/g		☐		✔
QMS_APF_MTA_1435_2022	CC	1/400	22-27h	Fri 04/15/2022 02:21 PM	Labtech	= 4.9 E5 CFU/g		☐		✔
QMS_APF_MTA_1435_2022	CC	1/4,000	22-27h	Fri 04/15/2022 02:23 PM	Labtech	= 1.2 E6 CFU/g		☐		✔
QMS_APF_MTA_1436_2022	CC	1/400	22-27h	Fri 04/15/2022 02:27 PM	Labtech	> 4.9 E5 CFU/g		☐		✔
QMS_APF_MTA_1436_2022	CC	1/4,000	22-27h	Fri 04/15/2022 02:28 PM	Labtech	= 4.9 E6 CFU/g		☐		✔
QMS_APF_MTA_1437_2022	CC	1/400	22-27h	Fri 04/15/2022 02:33 PM	Labtech	> 4.9 E5 CFU/g		☐		✔
QMS_APF_MTA_1437_2022	CC	1/4,000	22-27h	Fri 04/15/2022 02:35 PM	Labtech	> 4.9 E6 CFU/g		☐		✔
QMS_APF_MTA_1438_2022	CC	1/400	22-27h	Fri 04/15/2022 02:37 PM	Labtech	> 4.9 E5 CFU/g		☐		✔
QMS_APF_MTA_1438_2022	CC	1/4,000	22-27h	Fri 04/15/2022 02:38 PM	Labtech	> 4.9 E6 CFU/g		☐		✔

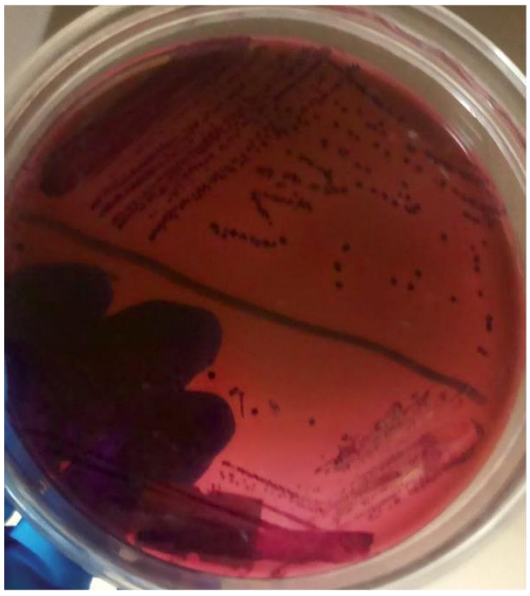
ANNEX VI: Colony Morphology of Some Isolated Pathogenic Bacteria

ANNEX VI: Colony morphology of some isolated pathogenic bacteria



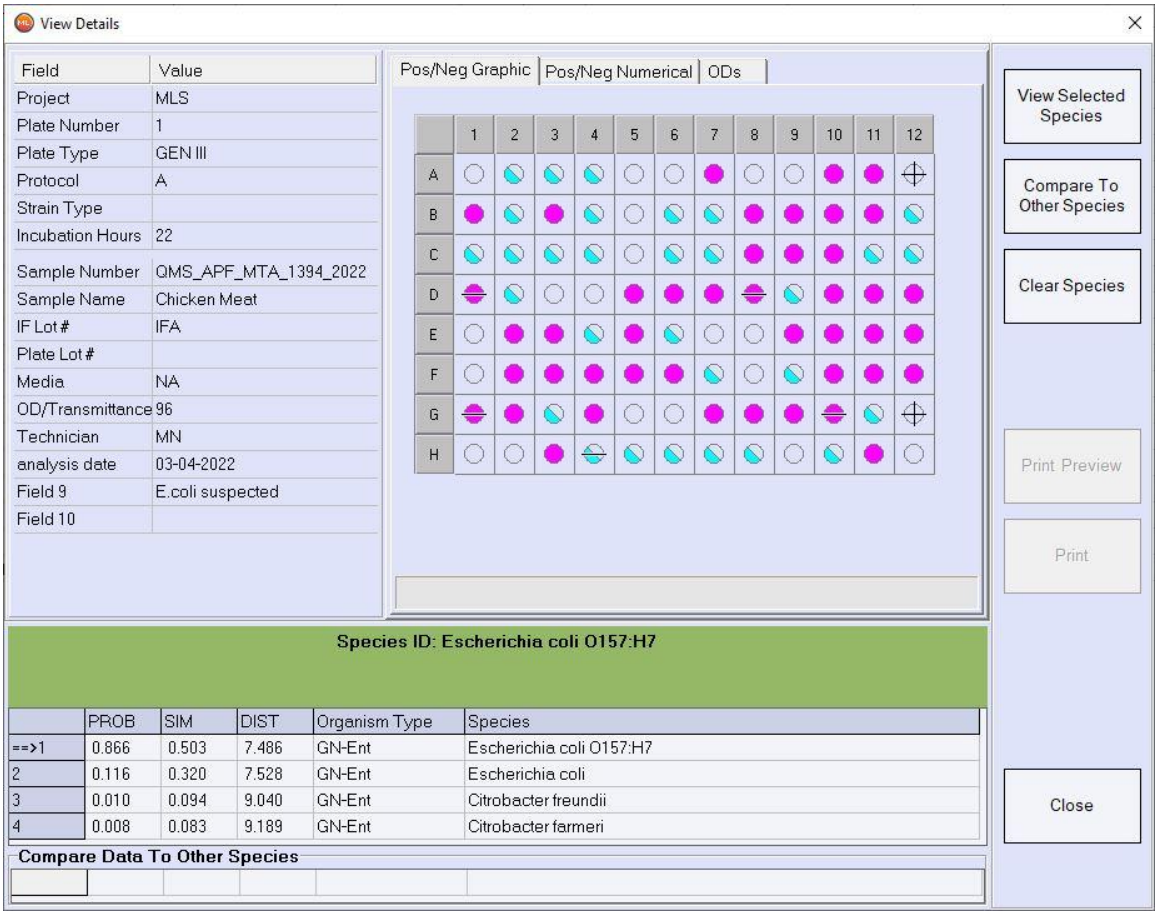
Presumptive *Salmonnella* colony, reddish pink colony with black cntter on XLD agar

Presumptive *E. Coli O157: H7,* non sorbitol fermenters, cloudy white colony on SMAC agar



Presumptive *E. Coli O157: H7,* blue black colony, with green metallic sheen on EMB agar

ANNEX VII: Identification Result by BIOLOG



ANNEX VIII: Antimicrobial Resistance Profile Result by VITEK 2XL

APVDFQAC

bioMérieux Customer:
System #: VITE_002

Laboratory Report

Printed by: LabTech
Report Version: 1 of 1

Isolate: APF2MTA1534-1 (Approved)
Card Type: AST-GN97 Bar Code: 687181910308511 9 Testing Instrument: 000019E1E59C (Serial No. 4903)
Setup Technologist: Laboratory Technician(LabTech)

Organism Quantity: **Selected Organism: Escherichia coli**

Comments:	

Identification Information			
Organism Origin	Technologist		
Selected Organism	Escherichia coli		
	Entered: May 9, 2022 12:17 CDT	**By:** LabTech	
Analysis Messages:			

Card is for veterinary use only

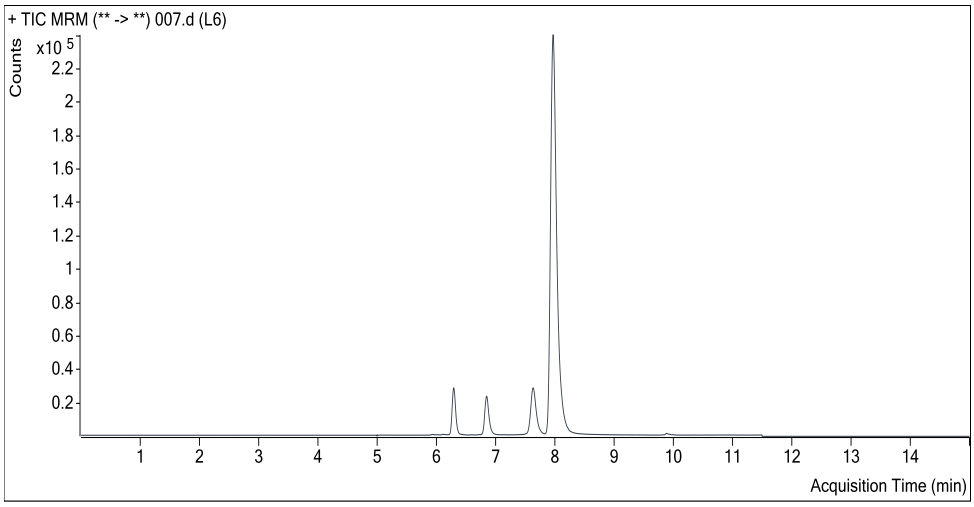
Susceptibility Information	**Card:**	AST-GN97	**Lot Number:**	6871819103	**Expires:**	Nov 19, 2022 12:00 CST
	Status:	Final	**Analysis Time:**	9.68 hours	**Completed:**	May 9, 2022 22:14 CDT

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Gentamicin	<= 1	S
Ampicillin	<= 2	S	Neomycin	<= 2	S
Amoxicillin/Clavulanic Acid	<= 2	S	Enrofloxacin	>= 4	R
Cefalexin	8	S	Marbofloxacin	>= 4	R
Cefalotin	4	S	Pradofloxacin	>= 4	R
Cefpodoxime	<= 0.25	S	Doxycycline	4	S
Cefovecin	<= 0.5	S	Tetracycline	>= 16	R
Ceftiofur	<= 1	S	Nitrofurantoin	<= 16	S
Imipenem			Chloramphenicol	<= 2	S
Amikacin	<= 2	S	Trimethoprim/ Sulfamethoxazole	<= 20	S

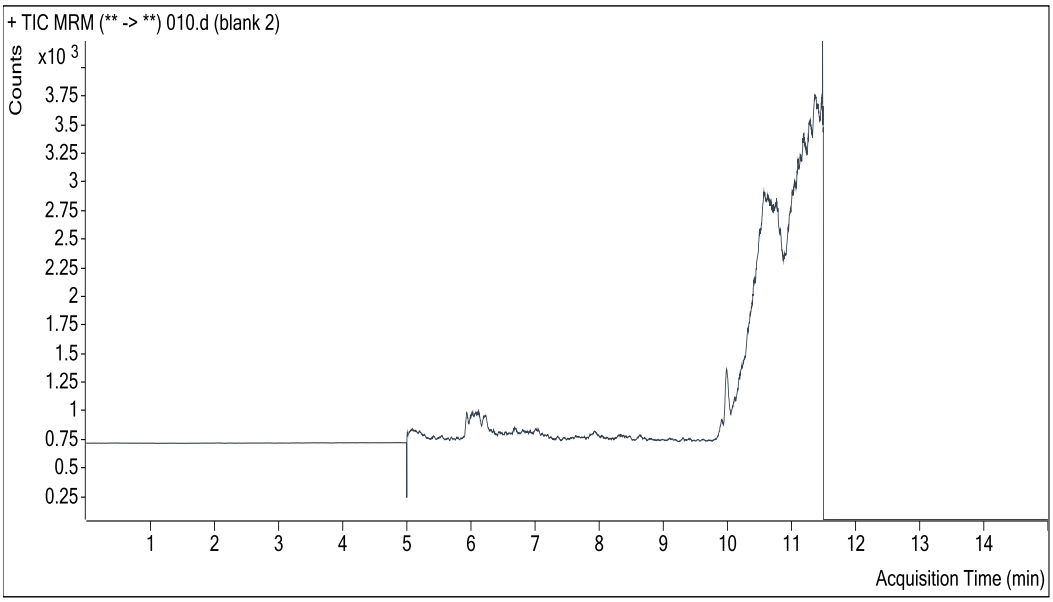
AES Findings:	**Last Modified:** Feb 15, 2021 14:22 CST	**Parameter Set:** VET:CLSI-based+Natural Resistance
Confidence Level:	Consistent	

ANNEX IX: VITEK 2XL Antibiotic Residues Chromatogram Result

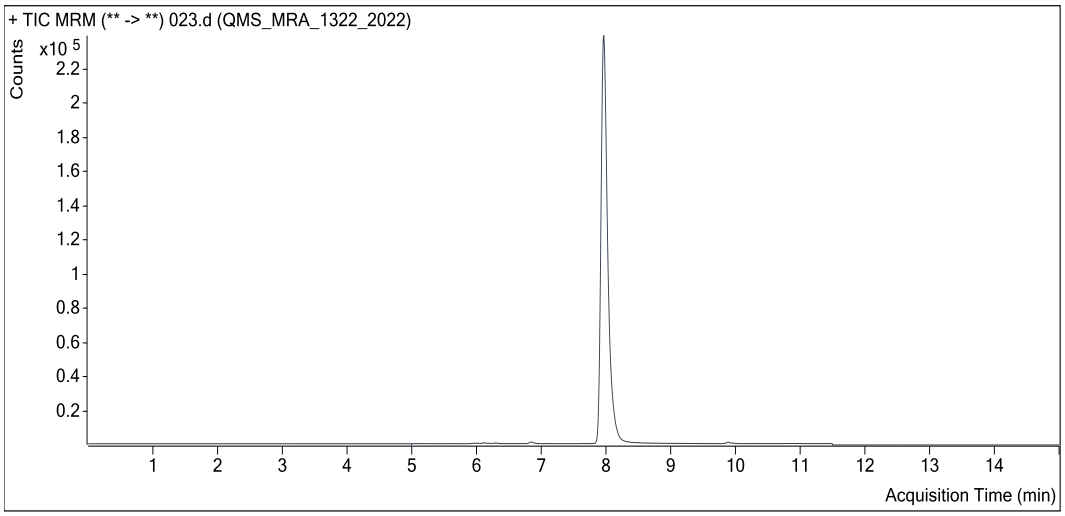
A. Representative chromatograms result of four standards sample spiked

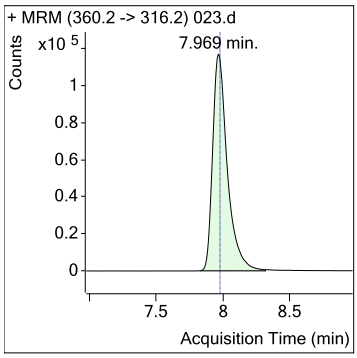


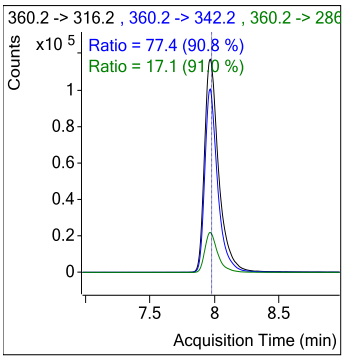
B. Representative chromatograms of blank sample

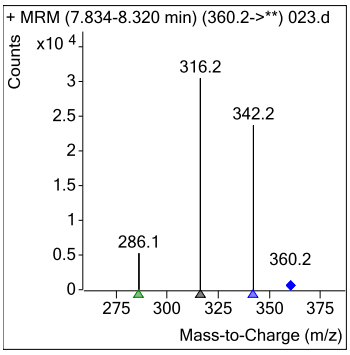


C. Chromatograms of Enrofloxacine positive broiler sample

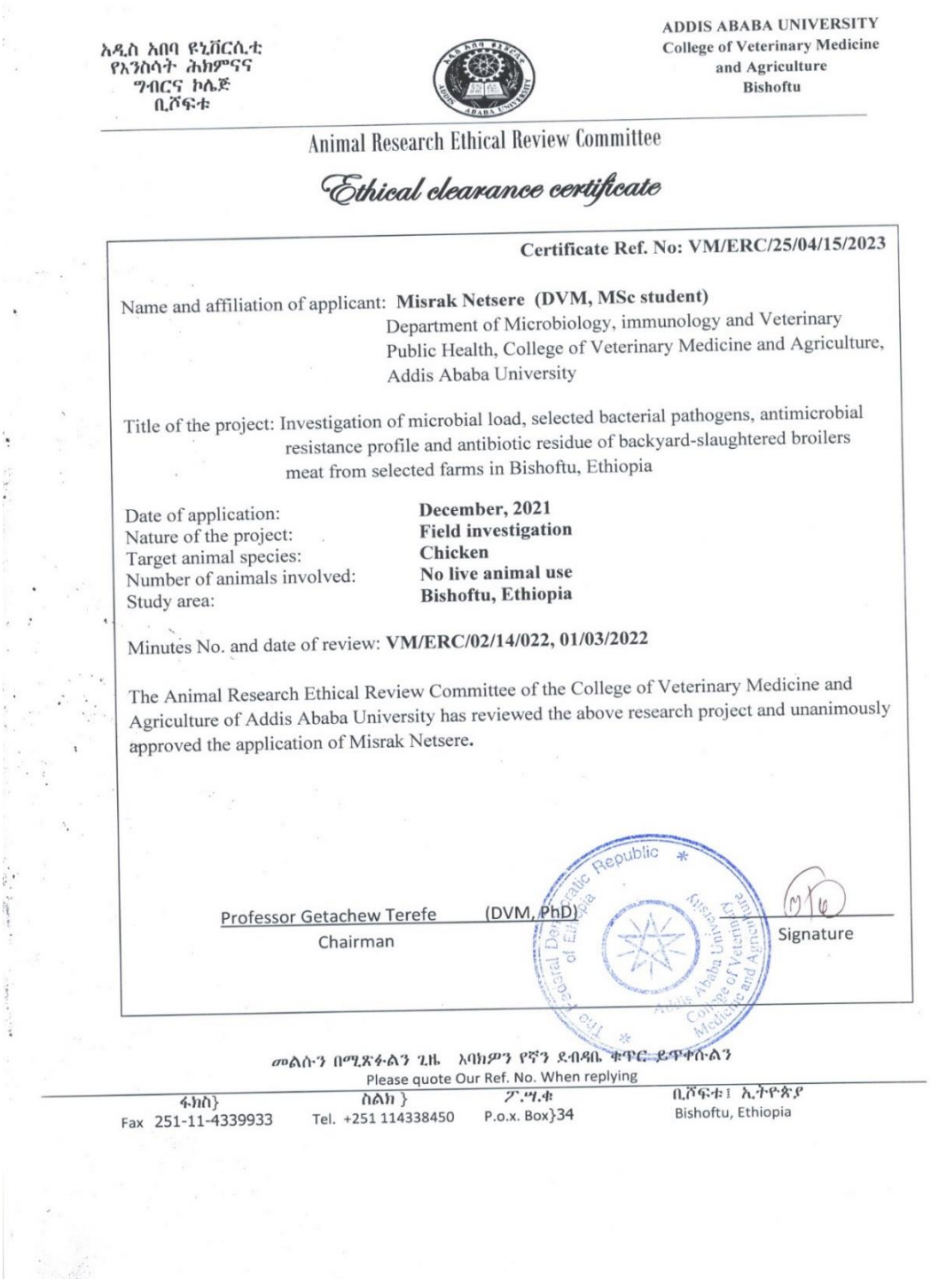








ANNEX X: Ethical Clearance



አዲስ አበባ ዩኒቨርሲቲ
የእንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ

ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/25/04/15/2023

Name and affiliation of applicant: **Misrak Netsere (DVM, MSc student)**
Department of Microbiology, immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: Investigation of microbial load, selected bacterial pathogens, antimicrobial resistance profile and antibiotic residue of backyard-slaughtered broilers meat from selected farms in Bishoftu, Ethiopia

Date of application:	**December, 2021**
Nature of the project:	**Field investigation**
Target animal species:	**Chicken**
Number of animals involved:	**No live animal use**
Study area:	**Bishoftu, Ethiopia**

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

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

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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

መልሱን በሚጽፉልን ጊዜ እባክዎን የኛን ደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When replying

ፋክስ Fax 251-11-4339933
ስልክ Tel. +251 114338450
ፖ.ሣ.ቁ P.o.x. Box}34
ቢሾፍቱ፤ ኢትዮጵያ Bishoftu, Ethiopia