ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND PUBLIC HEALTH

Antimicrobial susceptibility of *Escherichia coli* isolates from feaces of slaughtered cattle, beef carcass and abattoir environment at Karalo abattoir and surrounding butcher shops, Addis Ababa, Ethiopia

MVSc THESIS

BY

GEBREMICHAEL TILAHUN

VETERINARY MICROBIOLOGY PROGRAMME

SEPTEMBER, 2020

BISHOFTU, ETHIOPIA

Antimicrobial susceptibility of *Escherichia coli* isolates from feaces of slaughtered cattle, beef carcass and abattoir environment at Karalo abattoir and surrounding butcher shops, Addis Ababa, Ethiopia

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

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Title: **Investigation on antimicrobial susceptibility of *Escherichia coli* isolates from feaces of slaughtered cattle, beef carcass and abattoir environment at karalo abattoir and surrounding butcher shops, Addis Ababa, Ethiopia**

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STATEMENT OF THE AUTHOR

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

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LIST OF ABBREVIATIONS

ABC	ATP-binding cassette
AST	Antimicrobial susceptibility testing
ASTM	American Society for Testing Materials
AMR	Antimicrobial Resistance
BPW	Buffered Peptone Water
CFU	Colony Forming Units
CLSI	Clinical Laboratory and Standard Institute
DNA	Deoxy-ribonucleic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
EMB	Eosin methylene blue
FAO	Food and Agriculture Organization
FBD	Food Borne Diseases
IFA	Immunofluorescent Antibody
IFT	Institute of Food Technologists
ISO	International Standard Organization
KOH	Potassium Hydroxide
LPS	Lipo Poly Saccharide
MDRs	Multidrug resistance proteins
MIC	Minimum inhibitory concentration
mTSB	Modified Trypticase Soy Broth
NCCLS	National committee for clinical laboratory standards
NFGNB	Nonfermenting Gram Negative bacilli
NSF	Non-Sorbitol Fermenting
OIE	Office for International des Epizootics
PCR	Polymerase Chain Reaction
SMAC	Sorbitol MacConkey agar
TSI	Triple sugar iron agar
ZOI	Zone of inhibition.

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ABSTRACT

The wide spread occurrence of food borne bacterial pathogens and antimicrobial resistance are currently becoming the growing concern for animal and public health authorities. A cross-sectional study was conducted at Karalo area in Addis Ababa from December 2019 to May 2020 to investigate the occurrence and antimicrobial susceptibility profile of *E .coli* isolates from faeces of cattle brought for slaughter, beef carcass, floor of slaughter hall and meat transporting vehicles at Karalo abattoir and the surrounding butcher shops. A total of 175 samples were systematically collected and examined to isolate and identify *E.coli* by using standard bacteriological technique. The overall occurrence of *E.coli* was 51/175 (29.1%; 95% CI: 22.8-36.37) and isolation of *E.coli* was the highest in faeces (13/35; 37.1%) followed by floor swab (12/35; 34.3%), vehicle swab (10/35; 28.6%), carcass swab in butcher shops (9/35; 25.7%) and carcass swab in abattoir (7/35; 20%). All *E.coli* isolates from different sample sources were 100% susceptible to Nalidixic acid , Ciprofloxacin and Cefitriaxone . On the other hand, 84.3% (43/51) of *E.coli* isolates showed resistance to two or more antimicrobial agents. The highest level of resistance was observed to Sulfiso xasole (76.5%) in all samples followed by Cephalotine (64.7%), Ampicillin (47.1%), Amox icillin clavulanic acid (43.1%), Tetracycline (41.2%) and Streptomycin (33.3%). Over 69% of isolates from fecal samples and 90% of isolates obtained from vehicle swab were resista nt to 3 or more antimicrobials of which multidrug resistance as high as seven antimicrobials was recorded in a single isolate from feacal sample. Considerable proportions of feaces, carcass and premises in the present study harbored drug resistant *E.coli* which has a significant risk to public health by passing through food of animal origin. Therefore, appropriate intervention program and awareness creation on best practice of hygiene and sanitary measures on abattoirs as well as best handing practice of carcass in butcher shops are essential to minimize the risk associated with consumption of contaminated meat. Education on the surveillance and rational use of antimicrobial agents in animals and animal products are crucial. Furthermore, investigation on sources of contamination of meat in abattoirs, their environment and associated butcher shops play an important role in decreasing microbial contamination to ensure consumer safety.

Key words: *Abattoir, Antimicrobial susceptibility, Butcher shop, Carcass, E.coli, Karalo*

1. INTRODUCTION

Antimicrobial agents are natural or synthetic chemicals that kill or inhibit the growth of susceptible microorganisms. They had been used to treat and prevent microbial infections in animals and human for decades (Silbergeld, *et al*. 2008). Inappropriate application of antimicrobials in livestock and humans has led to the emergence of antimicrobial resistant bacterial strains that affects the effectiveness of antimicrobial therapy (Davies, 2010 and Cantas, 2013).

Advancing in accurate and effective methods of rapid antimicrobial susceptibility testing is indispensable for both animal and human health and this antimicrobial susceptibility test information about pathogens may significantly contribute to reduction in morbidity and mortality, cost of treatment, and duration of hospitalization by providing the information to animal health professionals and physicians in a rapid and timely manner. To find out the *in vitro* antimicrobial susceptibility, a variety of methods are commercially available, and veterinary microbiology laboratories select a manual or instrument based method to carryout routine antimicrobial susceptibility testing (Chiang ,et al. 2009).

Antimicrobial resistance currently becomes growing concern for animal and public health. Threats to public health could arise from the transfer of harmful microorganisms from animals to people via direct or indirect contact with animals through food. Besides to this fact concern has been raised for the potential transfer of resistance determinants from animals to humans through commensal bacterial flora like *Escherichia coli*. (Bartoloni et al., 2006). As a result, antimicrobial resistance monitoring of pathogenic and commensal bacteria has been put in place in many countries (Brasil 2008, ; EFSA 2008, ;NARMS 2013). In a number of surveillance and monitoring programs, commensal bacteria such as .*coli* species adopted as indicators of the selection pressure exerted in the gut flora following antimicrobial administration (EFSA 2008). Due to this reason, *E.coli* is considered a suitable pathogen for comparing the effects of selective pressure in all related populations and useful as an early alert system for tracking emerging resistant bacteria in livestock and their possible spread to humans through animal products (EFSA 2008). Other concern is the transfer of antimicrobial resistance genes carried

by commensal bacteria from the animal to humans through food processing or unhygienic environment (FAO/WHO/OIE 2008).

The wide spread and unwise use of antimicrobials in livestock is thought to be responsible for the emergence and wider spreading of antimicrobial resistant (AMR) bacteria in humans. Selections for drug resistant bacteria in humans have also been reported in the normal micro biota of exposed individuals populations or individuals.This shows that antimicrobial resistance can be developed in both pathogenic and commensal bacterial strains and can even be transferred to other bacterial strains, including other pathogenic and environmental bacteria (Lindsey, 2011). Consumption of uncooked and/or contaminated meat poses the risks of acquiring food borne *E.coli* strains causing a serious public health concern (Frye, And Jackson. , 2013). *E.coli* strains easily harbors antimicrobial resistant genes from one another.Ths is due to genes encoding AMR determinants that are carried on mobile genetic -elements mainly plasmids and transposons of some bacterial strains could be transmitted to other bacteria strains during contact causing a threat to cure acute infections in man and animals (Van den Bogaard and Stobberingh, 2000). Antibiotic resistance in *E. coli* has been reported worldwide and wide range of resistance among *E. coli* is a growing agenda in both developed and developing countries (Bell, *et al* 2002).

The occurrence and antimicrobial susceptibility profiles of *E. coli* show a considerable geographic variations and significant differences in various populations and environments were human and animal interacts (Erb , *et al* 2007). In Ethiopia, a number of studies have were carried out on the occurrence. A research conducted in Addis Ababa municipal abattoir indicated that the prevalence of *E. coli* to be 13.3% of Beef carcass (Tizita *et al*., 2014). Similar results revealed that the prevalence of *E. coli* as a carcass contaminant in sheep of goat and cattle slaughtered at Modjo export abattoir and Debre Zeit municipal abattoir (Mersha *et al*., 2009; Hiko *et al*., 2008).This indicates unhygienic practices of food processing of animal origin is at risk in developing countries than developed ones and antimicrobial resistance in *E. coli* from various clinical sources and food samples are indicators(Gebre-Sellassie 2007, Endalafer et al 2011, Yismaw , 2010).

A review done by several researchers showed that the *E.coli* has established resistance against oxytetracycline drugs (Alemu, *et al*., 2000). In the same way, penicillin + streptomycin and oxytetracycline are the main prescribed antimicrobials in Ethiopia (Beyene , 2015). Up to now,

few studies have been conducted to identify the antimicrobial resistance genes of *Escherichia coli* in Ethiopia. Regarding study on *E.coli* from all types of food samples of bovine origin in some districts of Tigray region has reported high resistance rates (≥ 80%) to cephalotine, chloramphenicole, tetracycline and (≥ 60%) to gentamycin and could be a potential threat to public health (Abebe *et al*., 2014). Even if there are different studies performed in Addis Ababa area on livestock especially cattle slaughtered in different slaughter houses in the city administration, little or no information is available on occurrence and antimicrobial resistance of *E.coli* at Karalo abattoir and surrounding butchers shops in beef cattle ready for slaughtering as well as abattoir environment. In this respect, there is a need for documented information on occurrence and antimicrobial resistance on some food borne pathogens circulating in the area to undertake relevant interventions to minimize contamination of animal products and environment in the study area.

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General objective

- To study antimicrobial susceptibility status of *E.coli f*rom beef cattle, slaughter h ouse,and beef at different stage of its production and dissemination and associate d environment so as to assess possible public health consequences of disseminati on of resistant bacteria

Specific objectives

- To assess the occurrence of *E.coli* from cattle carcass, feaces, Karalo abattoir en vironment and beef at slaughter house and butcher shops in the study area, and

- To determine the antimicrobial susceptibility profile of *E. coli* isolates

2. LITERATURE REVIEW

2.1. Occurrence and Characteristics of *Escherichia coli*

Escherichia coli is a normal inhabitant of the intestines of animals and humans but its recovery from food of animal origin may be of public health significance due to the presence of Entero pathogenic and/or Entero toxygenic strains which lead to sever gastrointestinal disorder (Soomro *et al*., 2002). *E.coli* is considered as the major indicator of fecal pollution in food production and processing. Its existence in processed foods results from recontamination , due to this phenomenon bacterium usually does not survive food preservation processes (Law, 2000).

The majority of *E. coli* isolates do not constitute a serious health hazard, but some serotypes result in food poisoning and alimentary disturbances. The most dangerous strains among them are enterohemorrhagic *E. coli* has become a major pathogen in food industry mainly in meat and dairy industries, as well as to the public, due to its ability to cause severe illness such as ,hemorrhagic colitis ,hemolytic uremic syndrome and thrombocytopenic purpura (Reuben *et al.,* 2013).

Escherichia coli are Gram negative, facultative anaerobic bacteria that belong to the family *Enterobacteriaceae* and the genus *Escherichia.* Mainly they are motile in liquid media by means of their peritrichous flagella. Some of their strain have acquired Virulence factors that have favors them to settle in to new niches and in some cases to cause serious disease (Farrokh *et al*., 2012). It is characterized by antigenic structure on its surface such as O-antigen, a polysaccharide domain in the bacterium's lipopolysaccharide (LPS) in the outer membrane, and the H-antigen containing flagella protein (Ratnam *et al*., 1988).

The bacteria has been isolated from the feaces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs, and a range of wild animal species (Heuvelink *et al*. 1999). It has also been isolated from water bodies like ponds, streams as well as wells, water troughs and abattoir effluent. It has been found to survive for months in manure and water trough sediments (Sargeant *et al*., 2000). Therefore *E.coli* is considered as a consistent indicator of contamination by abattoir waste matter, manure, soil, and contaminated water (Oliver *et al*.,

2009; Disassa *et al*., 2017). Although detection of *E. coli* in meat, milk reflects fecal contamination, abattoir environment coli forms have also been isolated in meat. The existence of fecal coli form bacteria may not necessarily indicate a direct fecal contamination of meat but it is a precise indicator of unhygienic practices during carcass dressing and further handling processes in food industry (Hayes *et al*., 2001).

2.2. Bacterial isolation

Conventional methods for detecting microorganisms in foods of animal origin often involve culturing the organism in selective and differential Medias and identifying isolates according to their morphological, biochemical, immunological and/or serological characteristics. This includes enriching in a liquid enrichment media that allows for the recovery, resuscitation and multiplication of a particular microorganism. The universally used enrichment media is buffered peptone water (BPW) either with or without supplements that are enriched with 8 mg/litre vancomycin, 10 mg/litre cefsulodin and 0.05 mg/litre cefixime (BPW-VCC) to hamper the growth of Gram-positive microorganisms like *Aeromonas* spp. and *Proteus* spp. Modified trypticase soy broth also supplemented with 20 mg/litre novobiocin or 10 mg/litre acriflavin and modified *E. coli* broth supplemented with 20 mg/litre novobiocin to reduce the growth of Gram positive microorganisms are also familiar enrichment media (Radostits *et al*., 2000).

The commonly used solid plating medium for the isolation of non-sorbitol fermenting *E. coli* is sorbitol MacConkey agar and selectivity is also improved by the addition of selective supplements cefixime and potassium tellurite (Piccozi et al.,2005). In recent years a range of chromogenic media have also become accessible commercially for the isolation of *E. coli* in humans ,animals ,animal feed stuffs and food of animal origin . The media contain a mixture of artificial chromogenic conjugates made up of a substrate for an *E.coli*-specific enzyme together to a chromophore that produce a visible and qualitative color change when digested by specific microbial enzymes. Whenever the *E. coli* enzyme cleaves the colorless conjugate, one or more insoluble chromophores are released, resulting a specific color for the *E.coli* colonies (Bettelheim, 1998b).

A 100-fold increase in sensitivity of detection of *E.coli* by IMS was achieved when compared to direct subculture from enrichment broth (Wright *et al.* 1994). However to analyze large numbers of samples, manual IMS is labor intensive. There for, an automated IMS in combination with an integrated ELISA would increase efficiency and reduce the workload. (Reinders *et al*., 2002).

Real time and conventional PCR technique allows not only detection of *E.coli* irrespective of their serotypes, but also detects all different variants of verotoxins (Gilgen *et al*., 1998). Well known specific PCR procedures are described for detection of genes encoding the virulence factors. Because the direct detection of the genes encoding the virulence factors by PCR in most laboratories is most convenient (Global Salm-Surv, 2003). PCR can be used on pure or mixed culture plate or broth cultures, and extracts from food or feaces (OIE, 2004).

2.3. Antimicrobial use in food animals

Antimicrobials are substances produced by wide range species of microorganisms such as bacteria, fungi, actinomycetes that hinders the growth of other microorganisms and may eventually wipeout them.However, the term "antimicrobials" comprises of synthetic or semi s ynthetic antimicrobial agents, such as metronidazole and sulfonamides, which are not produced by microbes (Chambers and Sande, 1996).

From a medical point of view, the history of humankind can be regarded as a struggle against infectious diseases. Infections were the leading cause of discomfort and death among humans and animals worldwide at the beginning of the 20th century. Since the discovery of penicillin by Alexander Fleming (1929) and the first introduction of the sulpha drugs by Domagk (1932), the number of new antibiotics available has increased enormously between 1940 and 1960. 'The era of antimicrobials' led to optimism until the early 1970s that infectious diseases can be prevented and controlled and mankind felt confident that modern medicine would succeed (Katsumata and Yoneyama , 2006).

The discovery of antimicrobial agents radically changed the outcome of common human and animal diseases. Antimicrobials are essential in human and veterinary medicine to treat infections caused by pathogens. Antimicrobials are applied for many purposes that include the treatment of clinically sick animals, for prevention or disease prophylaxis during periods of

high risk of infection, as a growth promoter and feed efficiency (McEwen and Fedorka, 2002). Many illnesses that were deadly before the discovery of antibiotics are now readily curable. However, infections are the second-leading cause of death worldwide sofar, causing over 13 million deaths each year. This is the result of the emergence of new diseases, the re-emergence of diseases once controlled and more specifically of the development of antimicrobi al resistance. There is also a substantial debate in veterinary medicine regarding the application of antibiotics in food animals raised for human consumption. Th potential threat to human heal th resulting from reckless use of antimicrobials in food animals is important. Resistant microor ganisms which are pathogenic propagated in these food animals are poised to enter the food supply and could be widely circulated in food products (Garofalo *et al.,* 2007).

The different antimicrobial drugs have a range of targets on the bacteria that include cell wall and cell membranes, ribosomes, nucleic acids, bacterial cellular enzymes and cellular metabolis m. The different mechanisms by which these antimicrobials inhibits the multiplication ,growth, and the destruction of bacteria include Inhibition of cell wall synthesis, Disruption of cell mem brane function, Inhibition of protein synthesis (both 50S and 30S), Inhibition of nucleic acid synthesis both the DNA and RNA synthesis and action as antimetabolites. The difference in mammalian and bacteria cells especially the structural and metabolic differences enables the antimicrobials to cause selective toxicity to the bacterial organisms without causing any damag e to the concerned host cells (Brunton *et al*, 2013).

2.4. Bacterial resistance strategies

The incredible ability of bacteria to adapt unfavorable environmental conditions is an example of the ancient law of nature, survival of the fittest. Microbial resistance is a natural biological phenomenon of microorganisms to a selective pressure, such as weather conditions, food, oxygen or water availability, or the presence of antimicrobial agents. A newly invented class of antibiotic is effective at first when introduced but will ultimately select for survival of the small fraction of microbial populations that have an intrinsic or acquired resistance mechanism (Walsh, 2003).

The mechanisms by which bacteria resist the action antimicrobials were recognized soon after the widespread operation of the first antibiotics (Angulo *et al.*, 2004). Increasing prevalence of resistance in many pathogens has been reported over the years in different regions of the world that include developing countries (Byarugaba, 2005).Currently scientists have begun to underst and at the molecular level the sophisticated mechanisms that enable bacteria to neutralize antimicrobials. In addition to this method pharmaceutical companies are developing fewer new antibiotics to replace those drugs that are no longer effective (Silbergeld *et al*., 2008).

Regarding bacterial populations, individual organisms may carry mutations that render drugs ineffective, conveying a survival advantage to the mutated strain due to normal genetic variation. Useful mutations can also be transferred by plasmid exchange within the bacterial colony in the presence of antimicrobials resulting in proliferation of the resistance trait (Courvalin, 2008). Drug resistance has been observed following the introduction of each new class of antimicrobials, and the threat is accompanied by a sluggish drug development pipeline and lack investment in the discovery and development of new antibiotic agents (Spellberg *et al*., 2004).

Resistance to antimicrobials can be classified into three groups: intrinsic, mutational and acquired resistance. Intrinsic resistance refers to an inherent resistance to drug that is a naturally occurring characteristic of the microorganism. For instance, certain oral bacteria like many streptococci lack the nitro reductases enzyme necessary to convert metronidazole to its active metabolites as the result of this action the microbes are not affected by the drug (Walker, 1996). Mutational resistance occurs as the result of spontaneous chromosomal mutation that gives a genetically-altered bacterial population which is resistant to the antimicrobial. Mutations resulting from the alteration of a single nucleotide base can result in resistance, as indicated and well documented for aminoglycosides and for rifampin (Walker, 1996). Acquired resistance denotes to the horizontal acquisition from another microorganism of a genetic eleme nt that encodes antibiotic resistance in the process occurred by transduction, transformation or conjugation (figure 1). Transduction is a process in which exogenous DNA is transferred from one bacterium to another by the involvement of a bacteriophage, while transformation is the process by which bacteria attain segments of DNA that are free in the environment. In of co of genetic material occurs by direct cell-to-cell contact(conjugation), through a sex pilus, the most common mechanism of transferring antibiotic resistance genes (Furuya and Lowy, 2006).

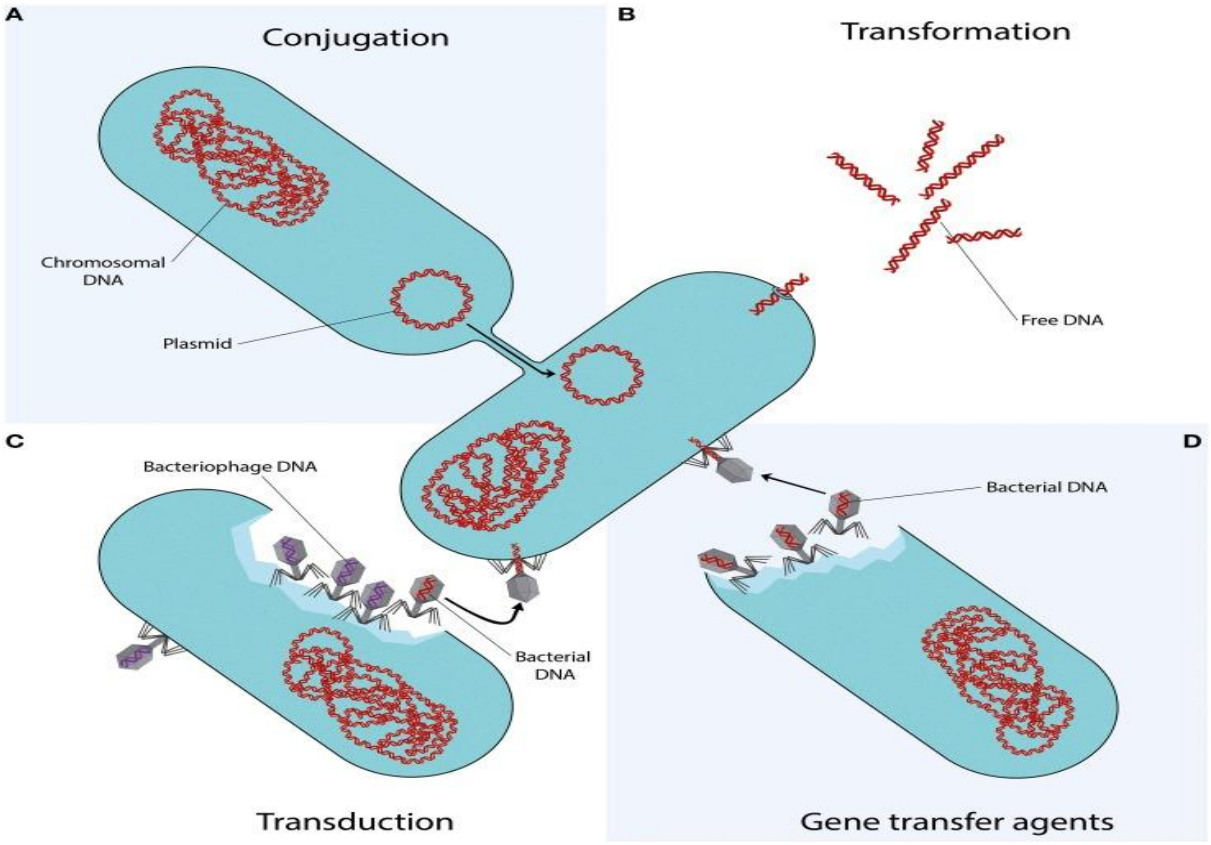

Figure 1. Process of acquisition of genetic material by bacterium for acquired resistance

Resistance can be caused by the presence of an enzyme that inactivates the antimicrobial agent as well as the presence of an alternative enzyme for the enzyme that is inhibited by the drug which reduces the binding of the antimicrobial agent that depends on posttranslational or posttr anscriptional modification of the antimicrobial agent's target which reduces binding of the antimicrobial agent, reduced uptake of the antimicrobial agent, active efflux of the antimicrobia l agent and overproduction of the target of the antimicrobial agent. In addition, resistance may be caused by a previously unrecognized mechanism (Maarten, 2001). *Escherichia coli* isolates acquire the capacity to transfer DNA via bacterial conjugation or transduction, which favors ge netic material to spread horizontally through an existing population (Brussow *et al.*,2004).Gene rally,microorganisms such as bacteria use three main strategies to become resistant to wide range of antimicrobials: Inactivating the antibiotic ,Preventing the drug from reaching its target and Altering the target (Hooper and Ince, 2003).

2.4.1. Inactivating the antimicrobial

Some of the bacteria isolates produce modifying enzymes that are inherent within or near the cell surface, selectively target and inactivate the antimicrobial agent. Main feature of the target sites for antimicrobial agents is their essential role in the growth and survival of microorganisms (Lambert, 2005).

2.4.2. Preventing the drug from reaching its target

Increasing the efflux plays main role with hydrophobic compounds that most likely enter the cell *via* diffusion (Silver, 2003). With the similar speed where these antimicrobial agents are entering the cell, efflux mechanisms are pumping them out again, before they reach their target. Members of the The ATP-binding cassette (ABC) members of super family are primary transporters using energy liberated by ATP hydrolysis. A mutation resulting in over expression of a multidrug efflux pump leads to resistance of different structurally unrelated antibiotics. Multidrug resistance proteins (MDRs) are widespread in bacteria (Langton, 2005).

2.4.3. Altering the target

The presence of genes of microbes affording resistance to self-produced antibiotics, the outer membrane of Gram-negative bacteria, general absence of the target or reaction hit by the antimicrobial or absence of an uptake transport system for the antimicrobial (Wright, 2005).

In general there is variation in the types of mechanisms used by gram negative bacteria versus gram positive bacteria, usually because of differences in structure, etc. (Figure 2). Gram negative bacteria are more likely to make use of all the mechanisms, whereas gram positive bacteria sometimes use limiting the uptake of a drug (no LPS), and don't have the capacity for certain types of drug efflux mechanisms (Martinez, JL. and Baquero, F. 2014)

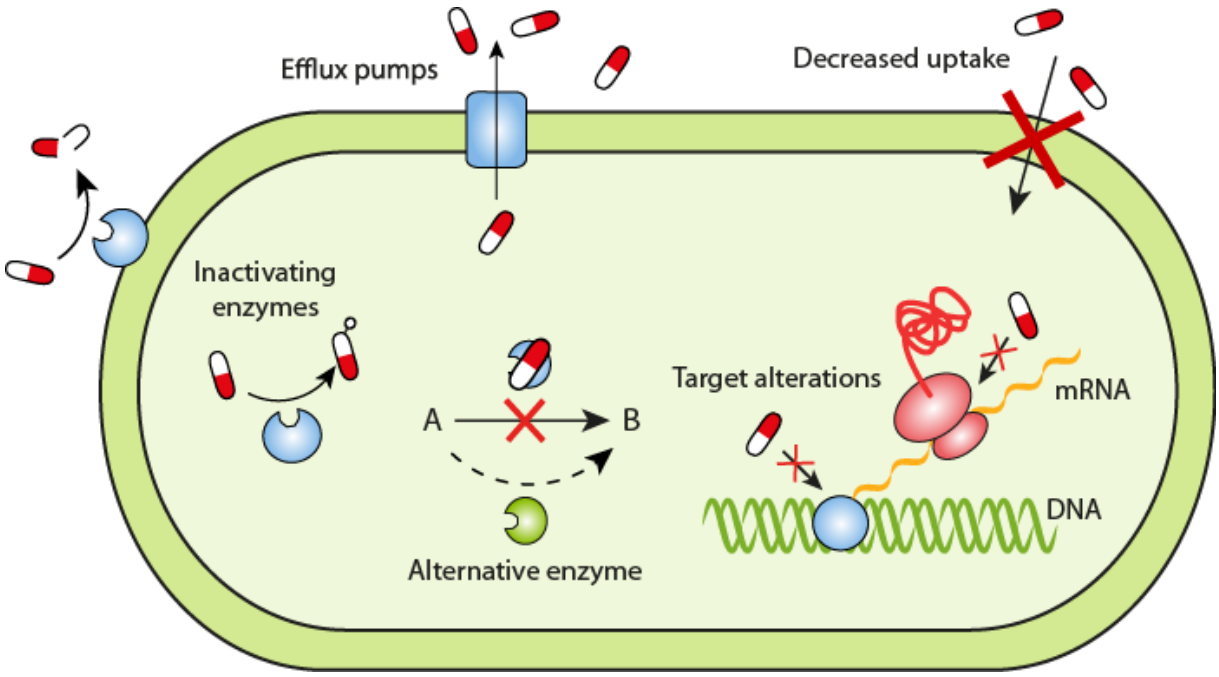

Figure 2. Antimicrobial resistance strategies in bacteria

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) has its own benefit in laboratory to establish how effective antibiotic therapy is against a bacterial infection, can control the use of antibiotics in clinical practice, assists the clinicians in both human and animal medicine in the choice of drug for the treatment of infection; help the local pattern of antimicrobials prescriptions and to reveal the changing trends in the local isolates (Wayne, PA. 2009).

2.5.1. Conventional antimicrobial susceptibility test

The commonly used disk diffusion method or Kirby Bauer disk method (Figure 2) is the practical and easy method which uses antibiotic impregnated disks to test particular bacteria whether it is susceptible or resistant to specific antibiotic agent (Jorgensen JH, and Turnidge JD. 2007) . The bacterial inoculums uniformly spread using sterile cotton swab on a prepared sterile Mueller-Hinton agar plates. The antibiotic disks were placed on top of the previously inoculated Mueller Hinton agar medium surface by the sterile forceps. Each of the disc must

pressed down to guarantee absolute contact with the agar surface. The plates were incubated f or 18–24 hr at 35-37 ^{0}C temperature before an interpretation of the result. If the bacteria were killed or inhibited by the antimicrobial used on plates of agar, there will be no growth in the immediate area around the disks indicated as zone of growth inhibition. The diameter of the zone of inhibition is directly proportional to the sensibility of the organism isolated and to the diffusion rate of antimicrobial agent through the agar medium. A zone of inhibition was measured in millimeters: The radius and diameter measure in which half the distance of the zone and then multiply by2 and measures the entire length of the zone of inhibition and subtract the disk diameter (Standard disk size 5-6mm). The result of the test can be interpreted by using the criteria published by Clinical and Laboratory Standard Institute (Wayne, PA. 2009).

The outcome of the disk diffusion test are qualitative and will be reported out as Susceptible, Intermediate and Resistant .The term susceptible denotes that the bacterial isolates are inhibited by the usually recommended dosage of an antibiotic. The intermediate group consists the bacterial isolates with antimicrobial MICs that approach usually possible blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated and the category of resistant indicates that isolates are not inhibited by the usually achievable concentrations of the antimicrobial agents with normal dosage schedules, which demonstrate an existence of the specific microbial resistance mechanisms (Rakesh KA. 2013). The susceptibility of the bacterial isolates to each antimicrobial agent is measured and the result is interpreted in accordance with criteria provided by (CLSI, 2012).

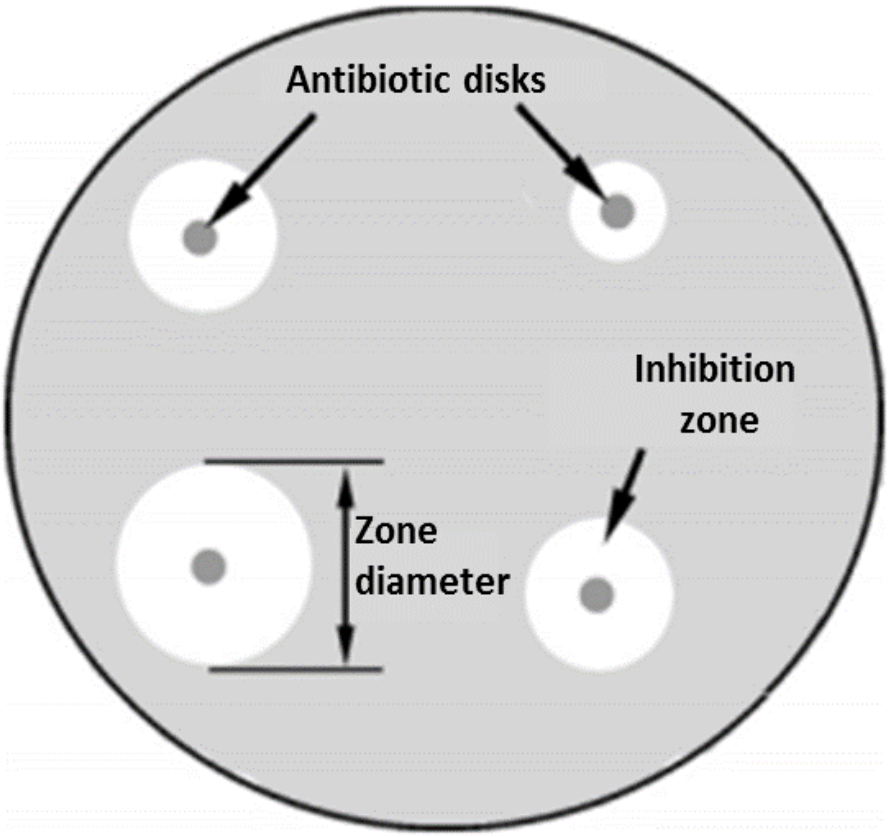

Figure 3. Kirby Bauer disk diffusion method

2.6. Prevention and control of *E.coli*

The evolution of antibiotic resistance feature of bacteria, its spread and emergence represent one of the most threatening health care problems with global consequences (Hawkey, 2008). The rise of multi-drug resistant bacteria urgently requires a better understanding of the factors and hot spots involved in diffusion and development of drugs. Horizontal gene transfer actions are accountable for the acquisition of resistance mechanisms among species and from antimicrobial producers to pathogenic and commensal bacteria. Humans, Animals, hospitals, farms, agriculture and aquacultures are reactors where the practice of antibiotics selects for resistant bacteria and promotes gene exchange (Lupo *et al.*, 2012). Effective control program to reduce *E.coli* infections substantially will require the implementation of intervention strateg ies throughout the food continuum, from farm to fork. The intervention measures at the farm include targeted animal management practices addressing common points of contamination, competitive exclusion bacteria and bacteriophage. In addition consumers have a role in imple

menting intervention controls in food handling and preparation to reduce risk of contaminatio n. (Sanchez *et al*., 2002).

Meat from cattle, like that of other mammalian and avian species, can be contaminated by feces during slaughter and processing and care should be taken not to contaminate the products. Thus, all precautions should be taken to minimize the risk, and foods of animal origin should be well cooked before they are eaten. Sanitation of plant, working apparels, Sterilization of equipments and Personal hygiene particularly hand washing after relieving oneself, is also important (Pal, 2007).

To improve quantity and quality of food, FAO is promoting good management practices in the food processing particularly in beef and dairy sector in collaboration with the private sector. This effort includes the preparation of manuals such as the FAO Guide to Good Farming Practice, development of training material and capacity building interventions in relation to hygienic handling and processing of animal products as well as testing and quality control (Sargeant and Smith, 2003).

3. MATERIALS AND METHODS

3.1. Study area

The present study was conducted at Karalo Abattoir and surrounding butcher shops from December 2019 to May 2020. The abattoir is found in Yeka sub city in the north eastern part of Addis Ababa, Ethiopia (Figure 3). Geographically, the sub city is located between $9^0$2'14.28"N and $38^0$50'6" E .with an area covering 85.98 square kilometers and human population of 424,217 . The area experiences a bimodal rainfall pattern with an average of 1100 mm, a short rainy season from February to April and long rainy season from the middle of June to the end of September. The remaining months are dry periods. Its annual average minimum and maximum temperature are 10.7^0C and 23.4^0C, respectively (CSA, 2016). Karalo abattoir slaughters on average of 200 cattle per day that includes different breeds of cattle which are mostly local zebu and very few exotic breeds of which adult cattle were dominated during slaughtering. The sources of cattle for slaughter are mostly from the north eastern part of Ethiopia mainly Northern Showa, Afar, and western Ethiopia like Wollega and Jimma areas. Few are brought from Harar and Borena areas as well as from dairy farms in and around the city. (Beshada, 2012.)

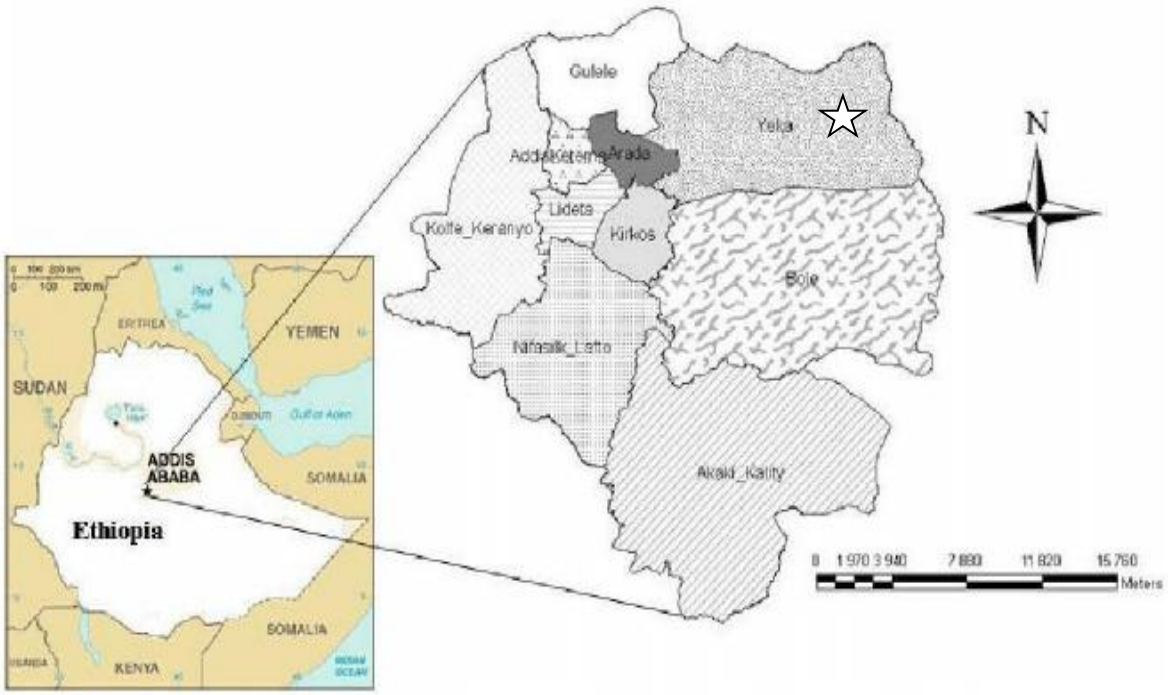

Figure 4. Map of Addis Ababa City and Study area (CSA , 2016)

3.2. Study design and population

A cross-sectional study was conducted from December 2019 to May 2020 to investigate antimicrobial susceptibility of *Escherichia coli* isolates from beef carcass abattoir environment and butcher shops in Karalo abattoir and surrounding areas.

3.3. Sample size and sampling

The number of study animals were determined based on the expected prevalence of *E. coli* and the desired absolute precision according to the formula stated on Thrusfield (2007). Accordingly, the sample size was 172 as determined by expected overall prevalence of E.coli 12.9% at Addis Ababa Abattoir enterprise. (Asmelash, 2015)

$$n=Z^2*Pexp\ (1-Pexp)/d^2$$

Where :- n = required sample size

Z^2= statistic for level of confidence is 1.96

Pexp = Expected prevalence of 12.9%

d = desired absolute precision of 0.05

The samples were taken from slaughtered cattle, abattoir environment as well as butcher shops from Karalo area. Accordingly 172 sample size is obtained but for convenience 175 samples (35 for each five type of samples) consisting of feacal samples from live cattle, carcass swab from abattoir and butcher shops, swab samples from slaughter hall floor and meat transport vehicles collected aseptically using sterile gauze and plastic falcon tubes filled with appropriate volume of transport media (10 ml BPW) and transported immediately using icebox with ice packs to Microbiology Research laboratory at Aklilu Lemma institute of pathobiology of the Addis Ababa University.

3.4. Sampling technique and sample collection procedure

Samples were collected aseptically using systematic random sampling technique from cattle slaughtered at Karalo abattoir by recording every 10 cattle from the total slaughtered ones that includes fecal, carcass and environmental pooled swab samples and recorded with appropriate format (Annex.1). Selected carcasses and environmental samples had been swabbed using the method for sampling beef carcasses and surface swabbing. (Capita, R.M. et al 2004.) Briefly, a sterile gauze pad with size of 7.6 cm x 7.6 cm, Johnson & Johnson Medical, Inc. (Annex 2) has been placed on specific sites of a carcass and environmental pooled samples like floor of slaughter hall ,wall of meat transport vehicles in addition to feacal samples (ASTM,2006). A sterile gauze first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses and environmental samples. The abdomen (flank), thorax (lateral) and breast (lateral) which are sites with the highest rate of contamination and appropriate for sampling (ISO 17604, 2005).

All samples were labeled with necessary information including the date of sampling; sample code and sample type and placed aseptically in sterile falcon tube with sterile gauze socked with 10ml BPW (Annex 2). Feacal samples were collected from randomly selected cattle and kept in cool box transported immediately to lab and 5gm of each sample was diluted in 45 ml of BPW. All types of the samples were kept in an icebox containing ice packs and immediately transported to Microbiology Research laboratory of Aklilu Lemma Institute of Pathobiology for microbiological analysis. Up on arrival, the samples are incubated at 37°C for 18-24 hrs until being processed for isolation for microbiological analysis. Each feacal samples were homogenized with vortex mixer before culturing on Medias. *E.coli* species isolated according to the techniques recommended by the International Organization for Standardization (ISO1 1290-1).The bacteriological media in different stages were prepared according to the manufacturer's recommendations.

3.4.1. Isolation of *E. coli*

Isolation of bacteria was done using techniques recommended by International Organizations for Standardization (ISO-6579, 2000). Up on arrival on the laboratory, all fecal, carcass, and environmental swab samples will be incubated firstly with buffered peptone water for recovery at 37°c for 24hrs. And then the samples collected were individually inoculated on M acConkey agar for primary isolation of *E.coli* (Difco laboratories,USA) incubated at 37°c for 24 hrs. A single, isolated colony of *E.coli* which revealed characteristics smooth, circular, pink colony was picked and sub-cultured into Eosin methylene blue (EMB) agar for 24 hours at 37°c. The bacterial colony appeared green metallic sheen will be regarded as *E.coli.*(Holt *et al*., 1994). The pure colonies taken from EMB agar was inoculated into nutrient agar (OXOID) a non selective media which was used to perform different biochemical test (Annex 6).

3.4.2. KOH test

The 3% Potassium Hydroxide (KOH) test works on the same principle as the Gram stain were it utilizes the differences in cell wall composition. Gram-negative bacteria cell walls are broken down in the presence of the weak alkali solution. Homogenizing Gram-negative bacteria in 3% KOH causes cell wall disruption and colonies transferred to potassium hydroxide solution become viscous and formed a stringy 'slimy' precipitate that adhered to the loop giving positive reactions (Annex 7). While Gram-positive colonies transferred to potassium hydroxide solution with no reaction observed and remain unchanged in the KOH suspension and giving negative reactions. (Jennifer, and Tim, .2008)

3.4.3. Biochemical tests

The selected colonies were streaked onto the surface of pre-dried Nutrient agar plates in a manner that allow isolated colonies to develop and incubated at 37±1ºC for 24 hr. for further confirmation with biochemical tests. Biochemical tests of *E. coli* isolates were done according to (ISO-6579, 2002) by using different biochemical tests such as triple sugar iron (TSI) agar, indole production, citrate utilization test, tests. Pure colonies from nutrient agar culture were picked and inoculated into biochemical test tubes containing TSI agar, Simon's citrate agar and

Tryptone broth and then incubated for 24 hr. at 37 ^{0}C (for citrate utilization test more than 24 hr. incubation period was conducted). *E.coli* isolates that are positive for indole test, negative for citrate utilization, and positive for TSI test were considered as *E.coli*.(Annex 8).

3.4.4. Antimicrobial susceptibility tests

The antimicrobial susceptibility test was performed according to the Clinical Laboratory Standard Institute (CLSI, 2017) by using the Kirby-Bauer disk diffusion method. A sterile cotton swab stick was dipped into the suspension of pure colonies that are taken from general purpose media (Nutrient agar) which were cultured on Muller Hinton broth for 24hrs and rubbed over the surface of sterile Muller Hinton agar plate (Oxoid, England) which were prepared according to the manufacturer guidelines and allowed for 15 minutes to dry. After the plates dried, 12 antimicrobial disks such as Gentamycin (10 µg), Chloramphenicole (30 µg), Ciprophloxine (5 µg),Sulfisoxasole (25 µg),Streptomycin (10 µg),Tetrracycline (30 µg), Nalidixic acid (30 µg) and Ampicillin (10 µg), Cefitriaxone (30µg), Cephalotine (30 µg), Amikacine (30µg) and Clavulanic acid (30µg) were placed on the inoculated Muller Hinton agar plates using sterile forceps and gently pressed onto it to ensure firm contact with the agar surface and allowed for 15 min for better diffusion in prepared media then incubated at 37°C for 24 hrs. After incubation for 24 hrs, the diameter of the zones of inhibition of each isolate was measured and compared with interpretative guideline as resistant, intermediate or susceptible described by Clinical Laboratory Standard Institute (CLSI, 2017) (Table 1).

Table 1. List of antimicrobials used and the interpretative cut off points for susceptibility of isolates

S. N	Antimicrobials	Strength in µg	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
1	Tetracycline	30	≤11	12-14	≥15
2	Chloramphenicole	30	≤12	13-17	≥18
3	Ampicillin	10	≤13	14-16	≥17
4	Gentamycin	10	≤12	13-14	≥15
5	Streptomycin	10	≤11	12-14	≥15
6	Sulfisoxasole	25	≤12	13-16	≥17
7	Nalidixic acid	30	≤13	14-18	≥19
8	Ciprofloxacin	5	≤15	16-20	≥21
9	Cefitriaxone	30	≤13	14-20	≥21
10	Cephalothine	30	≤14	15-17	≥18
11	Amikacine	30	≤14	15-16	≥17
12	Amoxicillin and Clavulanic acid	30	≤13	14-17	≥18

Source**:** adapted from Clinical Laboratory Standards Institute (CLSI, 2017)

3.5. Data management and analysis

All the data was entered in Microsoft Excel and screened for any entry errors and analyzed by using STATA version 14. Descriptive statistics such as frequencies, chi-square and Fishers exact test were used to compare difference in the occurrence of *Escherichia coli* in carcass, abattoir environment and feacal samples. A p-value < 0.05 was considered as indicative of a statistical significance.

3.6. Ethical considerations

Before any attempt to collect sample for the current study, Ethical clearance was obtained from the Research Ethics Review Committee of College Veterinary Medicine and agriculture of Add is Ababa University with Ref. No. of VM/ERC/26/03/12/2020 (Annex 10).In addition verbal consent was obtained from the Abattoir and butcher shops managers or owners at the time of sample collection.

4. RESULTS

4.1. Occurrence of *E.coli*

Out of 175 samples collected from carcass swab, abattoir environment and feaces, 51 (29.1%) were positive for *E.coli* with (95% CI = 22.8–36.37%) (Figure5). Of these positive cases, the isolation of *E.coli* was the highest in feaces, 13 (37.1 %), followed by 12 (34.3%) in floor, 10 (28.6%) in vehicle, 9 (25.7) from butcher carcass and 7 (20 %) in abattoir carcass (Table 2). However, the difference in isolation rates of *E.coli* from different type of samples was not statistically significant (P>0.05).

Table 2. Occurrence of *Escherichia coli* from different sample types

Type of sample	Total examined	Number positive	(%) positive	95% CI	X^2	p-value
Carcass swab at Abattoir	35	7	20	0.96-36.8		
Carcass swab at Butcher	35	9	25.7	13.7-42.8	3.2	0.53
Floor swab	35	12	34.3	20.3-51.5		
Vehicle swab	35	10	28.6	15.9-45.8		
Feaces	35	13	37.1	22.6-54.3		
Overall	175	51	29.1	22.8-36.3		

CI =confidence interval

With regard to slaughtered animals cattle are the primary interest for the study: age, sex and breed were considered. Out of the total *E.coli* isolates, 29(28.43%) were isolated from male and no positive isolate from female. Based on breed type 28(27.18%) were from zebu and 1(50%) were exotic breeds. In addition to this 28(27.45%) isolates were from adult and 1(33.3%) young cattle (Table 3). Accordingly, the predisposing factors like age ,sex and breed did not show any significant association (P>0.05) for the presence of *E. coli*.

Table 3. Distribution of *E.coli* isolates among different risk factors

Factor	Category	Total	Positive N (%)	Fisher test (p.value)
Sex	F	3	0(0)	0.559
	M	102	29(28.43)	
Breed	Zebu	103	28(27.18)	0.478
	Exotic	2	1(50)	
Age	Adult	102	28(27.45)	0.331
	Young	3	1(33.3)	

4.2. Antibiogram profiling

4.2.1. Overall antimicrobial susceptibility test

The result of antimicrobial susceptibility test of all 51 *E. coli* isolates from carcass in both abattoir and butcher shops, slaughter hall floor, meat transport vehicle and feaces samples to the selected 12 different antimicrobial disks is indicated in the current finding (Table 4).

Table 4. Over all antimicrobial susceptibility test profile of *E. coli* isolates for selected antimicrobial agents.

Antimicrobial agent	Concentration in µg/ml	Susceptibility status			Mean ± SD
		Resistant N (%)	Intermediate N (%)	Susceptible N (%)	
Tetracycline	30	21 (41.2)	3 (5.9)	27 (52.9)	15.47 ± 8.9
Chloramphenicole	30	3 (5.9)	3 (5.9)	45 (88.2)	22.07 ± 4.6
Ampicillin	10	24 (47.1)	15 (29.4)	12 (23.5)	10.82 ± 7.7
Gentamycin	10	11 (21.60)	17 (33.3)	23 (45.1)	14.19 ± 1.8
Streptomycin	10	17 (33.3)	19 (37.3)	15 (29.4)	12.06± 5.3
Sulfisoxazole	25	39 (76.5)	7 (13.70	4 (7.8)	5.13 ± 6.6
Nalidixic acid	30	0 (0)	0 (0)	51 (100)	23.04 ± 3.5
Ciprofloxacin	5	0 (0)	0 (0)	51 (100)	31.82± 4.2
Cefitriaxone	30	0 (0)	0 (0)	51 (100)	29.86 ± 4.2
Cephalotine	30	33 (64.7)	15 (29.4)	1 (2)	12.04 ± 4.5
Amikacine	30	0 (0)	21 (41.2)	30 (58.8)	18.16 ± 2.6
Amoxicillin and Clavulanic acid	30	22 (43.1)	17 (33.3)	12 (23.5)	13.33 ± 5.8

Key: N=numbers, SD=standard deviation

In the current study, the bacterial isolates showed varying degree of susceptibility to the antimicrobial agents used (Table 4).Accordingly, all E. coli isolates were highly susceptible to Nalidixic acid (100%) with mean and SD 23.04±3.5 zone of inhibition,Ciprofloxacin (100%) with 31.82± 4.2 mean and SD of the zones of inhibition and Cefitriaxone (100%) with mean m ean and SD 29.86 ± 4.2 diameter of the zones of inhibition. In addition,E.coli isolates were 88 % susceptibleChloramphenicole ,Amikacine (58.8%),Tetracycline (52.9%),Gentamycin(45.1), Streptomycin(29.4%),Ampycilline (23.5%),Amoxicillin Clavulanic acid(23.5%),Sulfisoxasole (7.8%) and Cephalotine (2%).In contrast to this, the present study revealed that *E.coli* isolates were developed resistance to eight drugs namely Sulfisoxasole(76.5%),Cephalotine (64.7%), A mpicillin (47%), Amoxicillin Clavulanic acid (43.1%),Tetracycline (41.2), Streptomycin (33.3 %), Gentamycin (21.6%) and Chloramphenicole (5.9%). On the other hand, Ampicillin and Streptomycin relatively showed higher proportion of intermediate values with 29.4% and

37.3%, respectively to *E.coli* isolates. The details of susceptibility pattern of the isolates are presented in Table 4.

Multi Drug Resistance is defined as resistance of an isolate to more than 2 antimicrobials tested (Dominic *et al*., 2005). In the present study forty three of 50 (86%) resistant isolates were multi-drug resistance (MDR) ,resistant to two or more antimicrobials. The study also revealed multi-drug resistance to *E.coli* isolates in 10/50 (19.6%), 12/50 (23.53%), 6/50(11.8%), 9/50(17.6), 5/50(9.8%) and 1/50(0.2%) for two, three, four, five, six and seven antimicrobials, respectively. The highest multi-drug resistance pattern was recorded for seven drugs in a single isolate of feacal sample. Besides, about 7 (12.9%) isolates were identified as resistant for one drug (Table 5).

Table 5. Overall of multi-drug resistance pattern of *E.coli* isolates

Resistance	Patterns of drugs	No. of isolates (%)
One drug	Te(2), G,AMC(2)&CF(2)	7(12.9)
Two drugs	Te& CF, Te&G, G&AMC,Te&CF,Te&G, G&CF,GM&CF,S&G,G&AMC, S&G	10(19.6)
Three drugs	Te, G&CF; S,G&CF;Te,AM& G ;GM,G&CF; G, CF& AMC;GM,G&CF;G,CF&AMC; AM,CF&AMC;G,CF&AMC;AM,G&CF;AM,S&G;AM,GM&G	12(23.53)
Four drugs	Te,AM,G&AC; AM,GM,G&CF;AM,G,CF&AMC; AM,GM,G&CF; C,AM,G&AMC;Te,AM,G&CF	6(11.8)
Five drugs	Te,AM,S,G&AMC; Te,AM,G,CF&AMC;Te,GM,S,G&AMC;Te,S,G,CF&AMC; Te,AM,S,CF&AMC;C,AM,S,G&CF;AM,S,G,CF&AMC; AM,S,G,CF&AMC	9(17.6)
Six drugs	Te,AM,S,G,CF&AMC;Te,AM,S,G,CF&AMC;Te,AM,S,G,CF&AMC; AM,GM,S,G,CF&AMC;Te,C,AM,GM,G&CF	5(9.8)
Seven drugs	Te,AM,GM,S,G,CF&AMC	1(1.9)

Key: Te=Tetracycline, C=Chloramphenicol, AM=Ampicillin, S=Streptomycin, GM=Gentamycin, G=Sulfisoxazole, CF=Cephalotine, AMC=Amoxicillin clavulanicacid

4.2.2. Antimicrobial resistance across sample types

Table 6 shows antimicrobial resistance pattern of *E. coli* strains isolated at different stages. Resistance to as high as 7 antimicrobials was detected in one of the *E.coli* isolate from fecal sample of slaughtered cattle. However, none of the isolates from carcass at slaughter house and butcher house were found to exhibit this resistance pattern. Overall, rate of occurrence of MDR to several antimicrobials was recorded in isolates from vehicle swab and fecal sample of slaughtered cattle. For instance, over 69% of isolates from fecal samples and 90% of isolates obtained from vehicle swab were resistant to 3 or more antimicrobials. In some of the isolates, highly related resistance patterns were observed in isolates from different sources.

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Table 6 . . Antimicrobial resistance pattern of *E. coli* isolates based on different types of samples

Source of sample	Resistant to how many antimicrobials	No. of isolates (%)	Resistance pattern (No.)
carcass swab at abattoir (n=7)	-	2(28.6)	Pan susceptible
	2	1(14.3)	Te, CF
	3	1(14.3)	Te, G, CF
	5	2(28.6)	Te, AM, S, G, AMC(1); Te, AM, G, CF, AMC(1)
	6	1(14.3)	Te, C, AM, GM, G, CF
Floor swab (n=12)	1	3(25)	Te (2) G (1)
	2	4(33.3)	Te, G (2), Te, CF(1); G, AMC (1)
	3	2(16.7)	S, G, CF (1); TE, AM,G (1)
	5	3(25)	Te, GM, S, G, AMC (1); Te, AM, S, G, CF(1); Te, S, G, CF, AMC(1)
Carcass swab at Butcher shop (n=8)	-	1(12.5)	Pan susceptible
	2	2(25)	GM, CF(1; G, CF(1)
	3	3(37.5)	GM, G, CF (2); G, CF, AMC (1)
	4	2(25)	C, AM, G, AMC(1); Te, AM, G, CF(1)
Feacal sample (n=13)	1	1(7.7)	CF
	2	3(23.1)	S, G (2) G, AMC(1)
	3	2(15.4)	AM, CF, AMC (1) ;G, CF, AMC(1)
	4	3(23.1)	AM, G, CF, AMC(1), Te, AM, G, AMC(1), AM, GM, G, CF
	5	1(7.7)	Te, AM, S, CF, AMC
	6	2(15.4)	Te, AM, S, G, CF, AMC(2)
	7	1(7.7)	Te, AM, GM, S, G, CF, AMC
Swab from Vehicle (n=10)	1	1(10)	CF
	3	3(30)	AM, G, CF(1); AM, S, G(1); AM, GM, G(1)
	4	1(10)	AM, GM, G, CF
	5	3(30)	AM, S, G, CF, AMC(2) C, AM, S, G, CF(1)
	6	2(20)	Te, AM, S, G, CF, AMC(1), AM, GM, S, G, CF, AMC(1)

Key: Te=Tetracycline, C=Chloramphenicole, AM=Ampicillin, S=Streptomycin, GM=Gentamycin, G=Sulfisoxasole, CF=Cephalotine, AMC=Amoxicillin clavulanicacid

5. DISCUSSION

Detection of high rate of *E. coli* in bovine carcasses Abattoir environment and feaces is not surprising as it is one of the commensal organisms commonly available in gastrointestinal tract of humans and animals. Contamination of carcass in abattoir and butcher shops, slaughter hall floor, meat transport vehicle and feaces can reduce production of safe food and cause great economic loss. This is particularly important when bacterial isolates obtained from this samples are resistant to antimicrobials commonly used in humans. These resistant bacterial strains may predispose persons consuming beef to infection which cannot be difficult to treat or they may transmit their resistant genetic markers to other pathogenic bacterial strains.

The current study revealed that the overall occurrence of *E.coli* was 29.1%. The result is similar with the previous report of 29.55% by Kibrom (2017) at Addis Ababa and relatively comparable with the abattoir based finding of 30.9% of Taye *et al* (2013) and 30.97 % by Mekonen *et al.* (2012). However, it was much lower than the prevalence report of Balcha *et al* (2014) with the outcome of 62.5% at Mekele as well as 35.2% and 37.74% reported by Abebe, *et al* (2019) and Wassie, *et al* (2017) from the study of abattoir and butcher shops at Bishoftu a nd Gondar Town respectively. While higher than the result of (Ousman *et al.*,2014) with preva lence of 15.89% from meat samples at Dire Dawa ELFORA and Municipal abattoir and Asm elash,(2015) with prevalence of 12.9% at Debre Zeit ELFORA export abattoir and Addis Abab a Abattoirs Enterprise.The observed difference in occurrence and prevalence among various studies might be due to difference in sampling and isolation procedures, variability in sampled populations and numbers of animals, storage and processing condition, hygienic measures of slaughter house and different rates of carcass contamination during slaughtering procedure as well as cross-contamination in abattoir environment and butcher shops. The present 29.1% occurrence of *E.coli* is lower when compared to the previous reports of Soepranianondo, *et al* (2019) with contamination rate 32.5% at city slaughterhouses in East Java Province, Indonesia, 35.21% prevalence by Shivani Chaudhary *et al* (2014) in India and Ishmael *et al* (2019) from formal meat sector (49%) as well as informal meat sectors (73%) that harboured *E.coli* at South Africa. The difference among the reported findings might be due to hygienic status of the meat plants, sanitary measures taken by the respective authorities and storage and processing, different rates of carcass contamination during slaughtering procedure as well as

seasons, wet seasons create favorable conditions for higher rate of contamination of carcass and environment by feacal matter (Itelima, and Agina 2011)

Regarding the risk factors associated with the occurrence of *E.coli*, Estimates among animal breed, sex and age of animals showed n significant difference (P>0.05) in which *E. coli* was isolated with considerable proportions in occurrence in male 29(28.43%) and female 0(0%) , on breed type 28(27.18%) were isolated from zebu and 1(50%) from exotic breeds. In addition to this 28(27.45) were isolated from adult and 1(33.3%) young cattle. This disagrees with the findings of Asmelash (2015), in which the prevalence of *E. coli* among animal species and age varies considerably and showed significant difference (P<0.05)

Antimicrobial resistance emerges from the maltreatment of antimicrobials in animals and human, and the successive transfer of resistance genes and bacteria among humans ,animals, animal products and the environment (Schroeder *et al*., 2002). Multi Drug Resistance (MDR) is defined as resistance of one isolate to two or more antibiotics used for antimicrobial susceptibility test which has been common problem among Gram negative bacterial species (Dominic *et al*., 2005). In Ethiopia, there have been studies regarding drug resistance of *E. coli* isolates from food of animal origin, (Abraham *et al*.,2019; Bedasa *et al*., 2018; Asmelash ,2015 and Bekele *et al*., 2104).

In the present study, the result of in-viro antibiotic sensitivity test to *E.coli* isolates showed different degree of sensitivity against the tested antibiotics which ranges from 2% to 100%. The high susceptibility was observed against Nalidixic acid, Ciprofloxacin and Cefitriaxone. This result is similar with the findings of Diriba (2018) in cattle who reported 100% susceptibility to the two drugs, Nalidixic acid and Ciprofloxacin, and Atnafe *et al* (2017) on Cefitriaxone and Naladixic acid 100% effective for clearing the organism in abattoir and butcher shops of Hawassa, Ethiopia. In addition, *E.coli* isolates were also susceptible to drugs Chloramphenicole (88%), Amikacine (58.8%), Tetracycline (52.9%), Gentamycin (45.1%), Streptomycin (29.4%), Ampicillin (23.5%), Amoxicillin Clavulanic acid(23.5%), Sulfisoxasole (7.8%) and Cephalotine (2%) which agrees with the reports of Bagre *et al.* (2014) from Burkina Faso , in which all *E.coli* isolates were 82% susceptible to Chloramphenicole and 23% Gentamycin. On contrary, they were resistant to Ampicillin (47.1%), Cephalotine (64.7%) and Sulfisoxasole (76.5%). This might be as a result of

suboptimal, prolonged and misuse use of antimicrobials for prophylaxis and treatment of infection. Antimicrobial resistance emerges from the use of antimicrobials in animals and human, and the subsequent transfer of resistance genes and bacteria among animals, humans, animal products and the environment (McEwen *et al.*, 2002).

Regarding multi drug resistance (MRD) facts of the current study, 86% (43/50) of *E. coli* isolates in all examined samples showed resistance to two or more antimicrobial agents. This finding is much higher than the reports of 36.6% by Asmelash (2015) at Debre zeit ELFORA export abattoir and Addis Ababa abattoir Enterprise, 17.9% findings of Bekele *et al.,* (2014) in beef, 39.7% in the study of Yohannes *et al.,* (2017) and the report of Taye *et al*, (2013) with result of 66.6%. However it is lower than the study of 92.86% of Diriba (2018) and Abebe et al (2014) with MDR findings of 93.2% at Tigray, Ethiopia. When compared to other countries, it is analogous with the finding of 82.5%, by Marfa *et al* (2019) that showed resistance two to eight drugs in raw beef preparation at Spain and lower than the report 52.5% in Nepal by Govinda *et al* (2019) and the findings of Rahman et al (2019) with 50% MDR *E.coli* isolates from milk beef and chicken at Bangladesh. The difference in antimicrobi al resistance to different drugs is due to antibiotic application in farming industry (Smolander, et al.,2009),in particular animal husbandry responsible for most of the world's antibiotic usage.In addition use of drugs for growth promoters, prevention ,therapeutic and nutritional purposes are the causes of increased magnitude of drug resistance (McEwen and Fedorka-Cray,2002).

Generally, the development of drug resistant *Escherichia coli* strains might be associated to various reasons including the indiscriminate use of antibiotics in food producing animals (Cosgrove and Carmeh, 2003) and due to the selective pressure to rampant use of antibiotics in the animal industry (Mohammed *et al*., 2014).

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6. CONCLUSION AND RECOMMENDATIONS

In the present study, considerable proportions of carcass in both abattoir and butcher shops, feacal, slaughter hall floor and meat transport vehicle samples were found contaminated with *E. coli* species. The presence of these microbes in the collected samples of carcass, floor vehicle and feaces from animals and abattoir environment indicated that it might be contaminated from either infected animal brought to abattoir or unsanitary conditions during slaughtering processes , transportation of the product at primary production level and handling at butcher shops . This is predominantly significant in causing several health impacts in consumers who have a tendency of eating raw, undercooked meat and meat products. In addition, the higher prevalence of multidrug resistant *E. coli* isolates in carcass and feaces of animals as well as abattoir environment in particular is a serious issue which can have a risk to public health. Hence, based on the present study the following recommendations are forwarded:

- Appropriate intervention programs and awareness creation on best practice of hygiene and sanitary measures on Abattoirs as well as best handling practice of meat in butcher shops are essential to minimize the risk associated with consumption of contaminated meat
- Education on the surveillance and control program on rational use of antimicrobial agents in animals and animal products are recommended to ensure consumer safety
- Further investigation on source of contamination of meat in Abattoirs ,their environme nt and associated butcher shops should be studied.

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8. APPENDICES

Annex 1. Sample collecting sheet for bacteriological analysis

S.N	Type of samples collected	Date of collection	Sample code	Area	Positive	Negative	Remark

Annex 2. Sample collection methods

Figure 1. Carcass swab sampling(A) ,Abattoir floor swab sampling (B) , Meat transport vehicle swab sampling (C) and Feacal sample taking (D)

Annex 3. Samples collection using transport media.

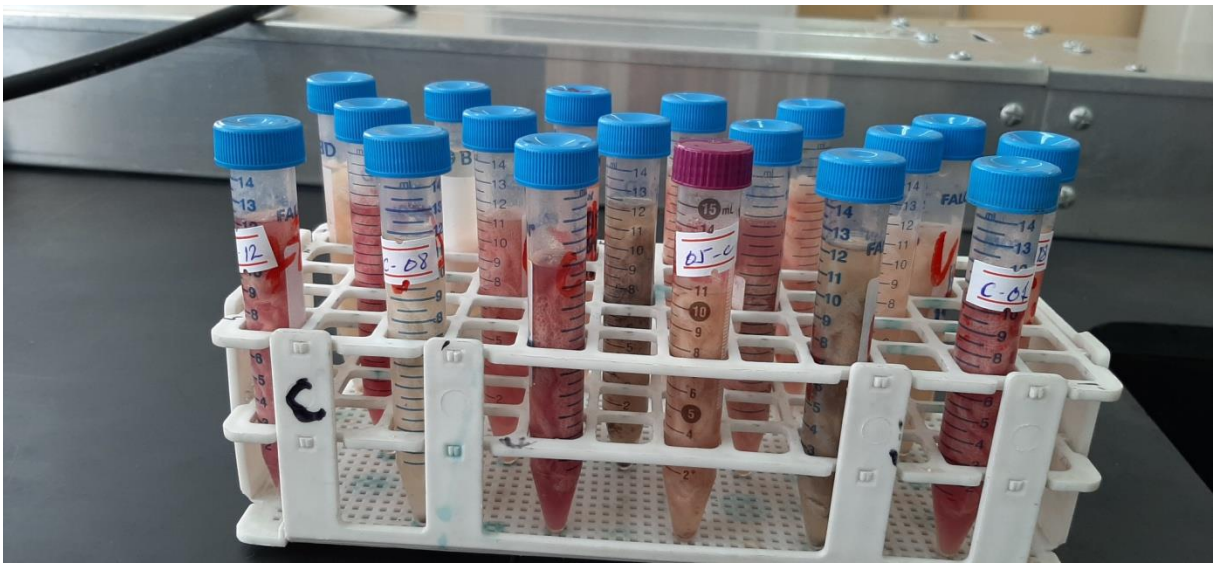

Figure 1. Swab samples with BPW in 14 ml falcon tubes

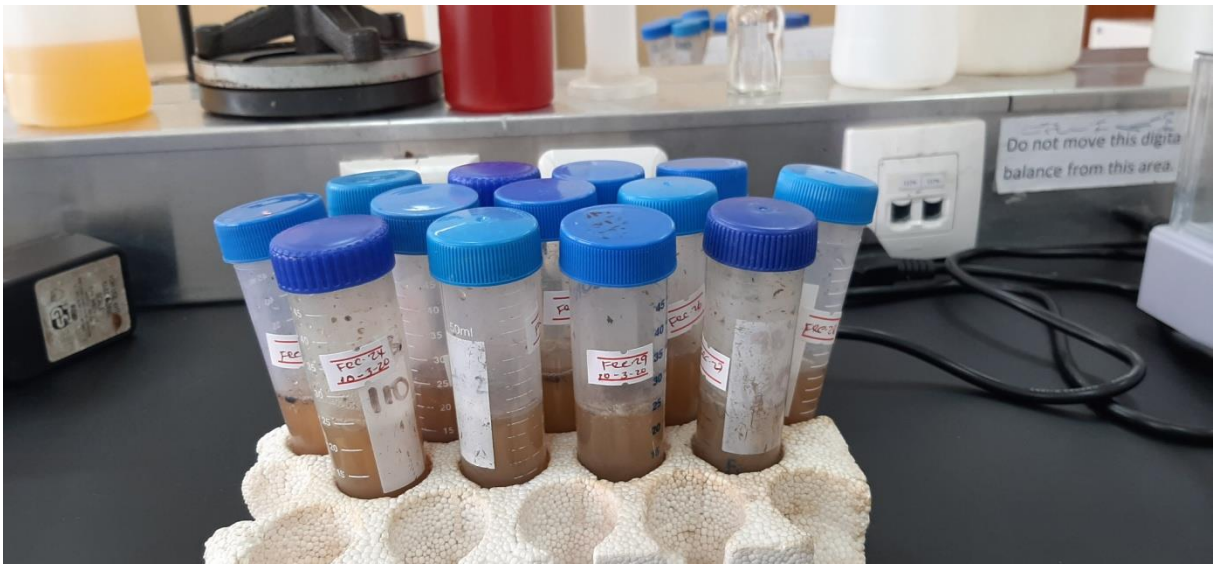

Figure 2. Feacal samples with BPW in falcon tubes of 45 ml size

Annex 4. Laboratory data collecting sheet for Isolation and confirmation of *E.coli*

S.N	Sample code	Wet mount	Motility test	KOH test	Oxidise test	Indole test	Citrate test	TSI test

Annex 5. Procedures of the laboratory Media preparation

1. **Specimens**
   Faecal samples from live animals, carcass, meat transport vehicles, slaughter hall floors and butcher shop swab samples can be taken and processed following appropriate laboratory Procedures.

2. **Buffered peptone water (CM0509, Oxoid)** Composition g/l Peotone 10.0 Sodium chloride 5 Disodium phosphate 3.5 Potassium dihydrogen phosphate 1.5 Final PH 7.2 + 0.2 @ 25°C Preparation: Add 20g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. It is extremely important that the distilled water used is of a high quality with a low mineral content/conductivity.

3. **Growth media**

   - Nutrient agar
   - MacConkey agar

4. **Selective and differential media**
   - Eosine methylene blue (EMB) agar

5. **Colony appearance**

   - **On MacConkey agar :** Colonies are medium size and bright pink to red with flat or elevated surface and complete with edges.
   - **On EMB agar :** Colonies show a characteristics colour of green metallic sheen appearance on the media indicating the growth of *E.coli*

   The inoculated plates are incubated at 37^0c for 24-48 hrs incubation.

6. **Composition and preparation of medias**

   <u>Nutrient agar</u>

   - **Composition of Nutrient Agar**

Beef Extract……..……..3.0 g
Peptone……..…….……5.0 g
Agar…….15.0 g
Distilled Water…….1000 ml
Final pH 6.8 /- 0.2.
Composition of Nutrient Broth: Nutrient broth contains same ingredients except agar.

❖ Preparation of Nutrient Agar

Nutrient agar and broth are available commercially in powdered (free-flowing, homogeneous) form.

1. Dissolve the dehydrated medium in the appropriate volume of distilled water i.e., 23 gm dehydrated nutrient agar (*see the manufacturer instruction*) in 1000 ml distilled water.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
3. Sterilized the medium by autoclaving (121°C for 15 min)
4. Dispense the medium into tubes *(i.e. 3 ml to make nutrient agar slopes, 5 ml to make nutrient agar deeps)* or plates. Left the agar medium to solidify.
5. Date the medium and give it a batch number.
6. Store in a cool dark place.

MacConkey agar

Ingredients	Amount
Peptone (Pancreatic digest of gelatin)	17 gm
Proteose peptone (meat and casein)	3 gm
Lactose monohydrate	10 gm
Bile salts	1.5 gm
Sodium chloride	5 gm
Neutral red	0.03 gm
Crystal Violet	0.001 g
Agar	13.5 gm
Distilled Water	Add to make 1 Liter

NB. Final pH 7.1 +/- 0.2 at 25 degrees C.

Preparation of MacConkey Agar

Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water.

Heat to boiling to dissolve the medium completely.

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

Cool to 45-50°C. Mix well before pouring into sterile Petri plates.

Eosine methylene blue

❖ Composition of EMB

- Enzymatic digest of animal tissue
- Dipotassium phosphate
- Lactose
- Sucrose
- Agar
- Eosin Y: Indicator ***(7)***
- Methylene Blue: pH indicator
- Final pH (at 25°C)

❖ EMB Agar procedure

- Suspend the agar in a 1000 ml distilled water. Mix it well in order to achieve a uniform suspension.
- Bring to boil so that the medium will be dissolved completely.
- Sterile the medium through autoclaving at 121 degree Celsius for about 15 minutes. Do not overheat the medium.
- Allow the medium to cool and shake to oxidize the ethylene blue and suspend the precipitate.
- If the agar is inoculated on the same day, then don't necessarily need to autoclave it.

Note: Make sure not to store the medium in the direct light as doing so could lead to photo-oxidation.

Mueller Hinton Agar

Intended Use: Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

Ingredients Gms / Litre

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

Formula adjusted, standardized to suit performance parameters

Directions:

Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Note: The performance of this batch has been tested and standardized as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

Annex 6. Culture Medias used for Isolation of *E.coli*

Figure 4.Growth of *E.coli* on MacConkey agar shows pink colonies

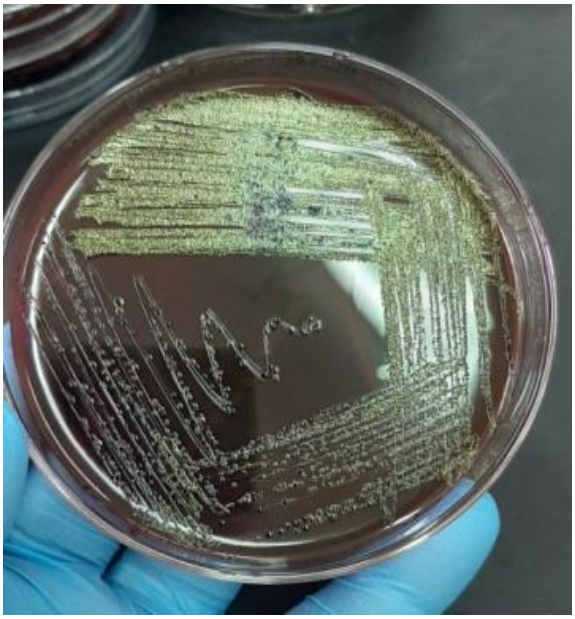

Figure 5. Growth of *E.coli* on EMB agar with green metallic sheen colonies

Figure 6.Pure colonies of *E.coli* on Nutrient agar

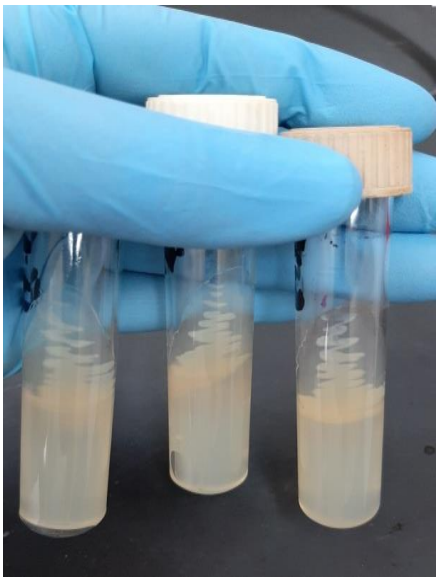

Figure 7. Growth of *E.coli* on Nutrient agar slant

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Annex 7. KOH test Procedure

Procedure: One drop of 3% KOH solution was placed onto a clean microscope slide labelled with the micro-organism for testing. Sufficient colonies of microorganism were collected from the culture plate to make a dense suspension. The colonies were emulsified into the potassium hydroxide for 60 seconds and any reaction was observed.

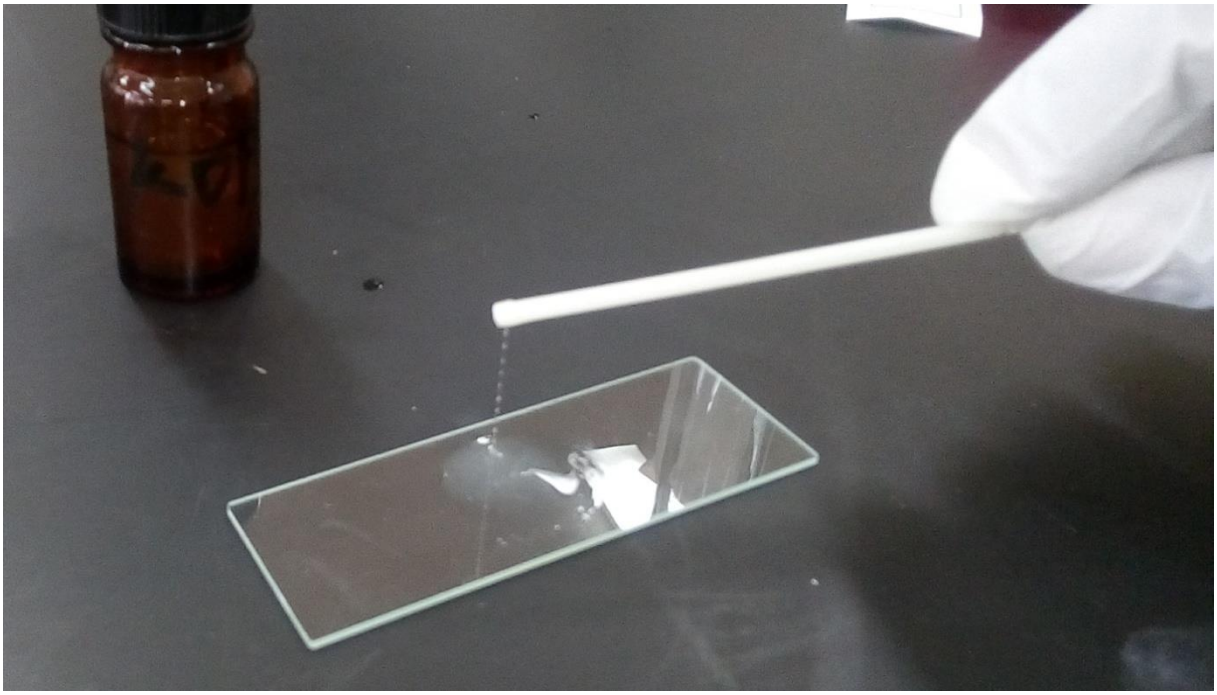

Figure 7. Dense suspension of emulsified peptidoglycan layer of gram negative bacteria by KOH test

Annex 8. Pictures of Biochemical tests

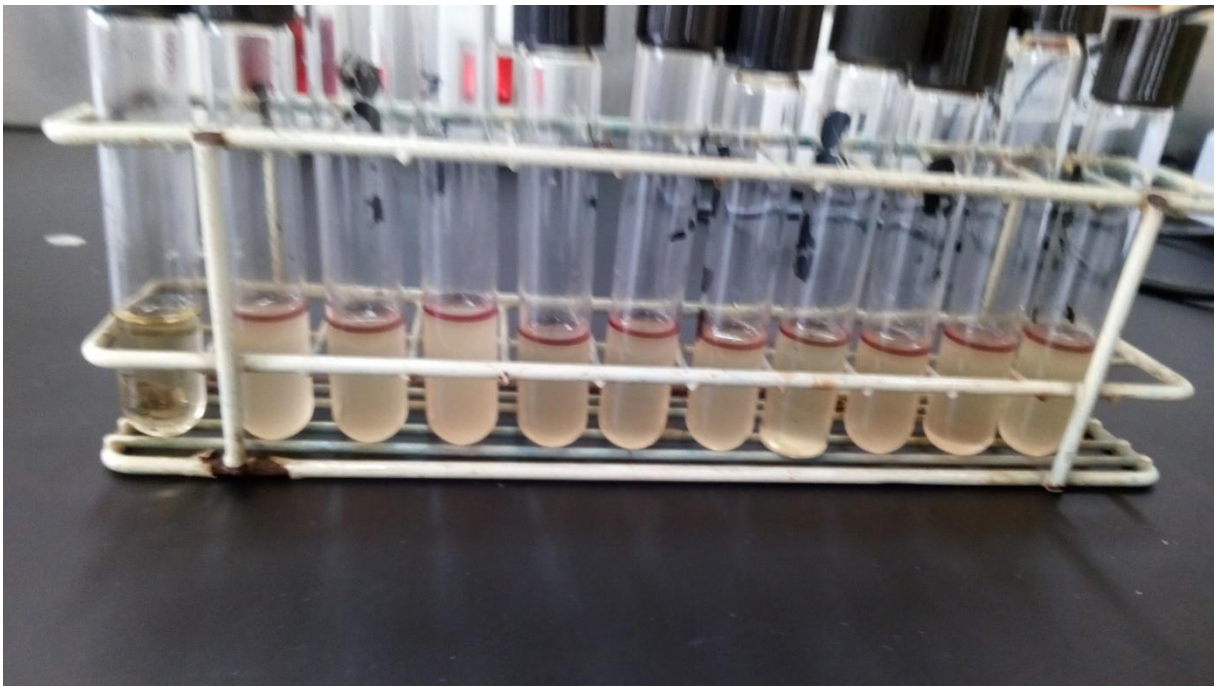

Figure.1 Indole test

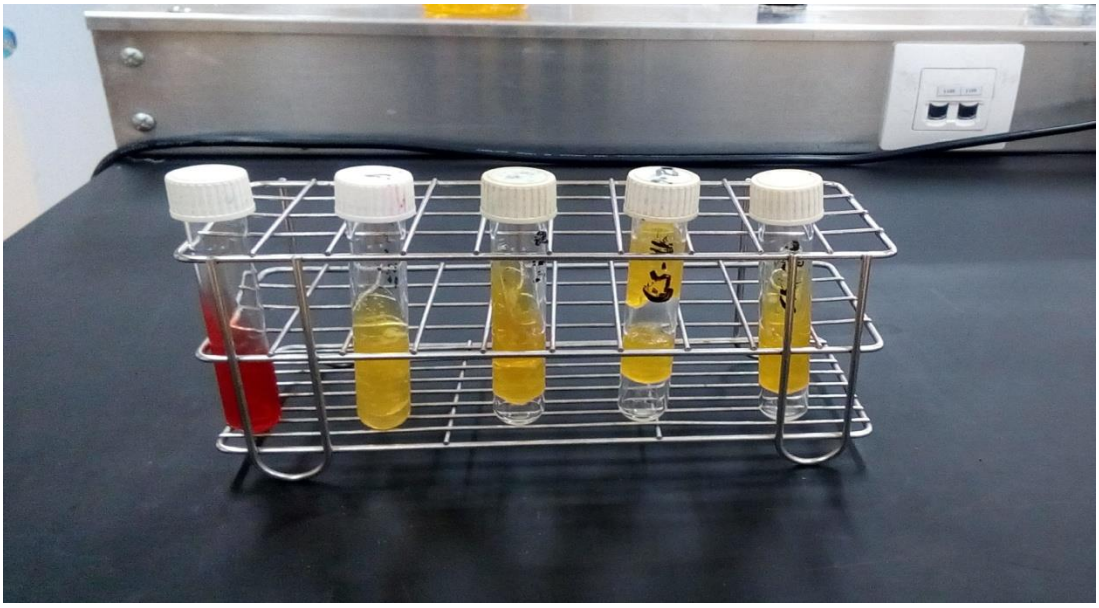

Figure.2 TSI test

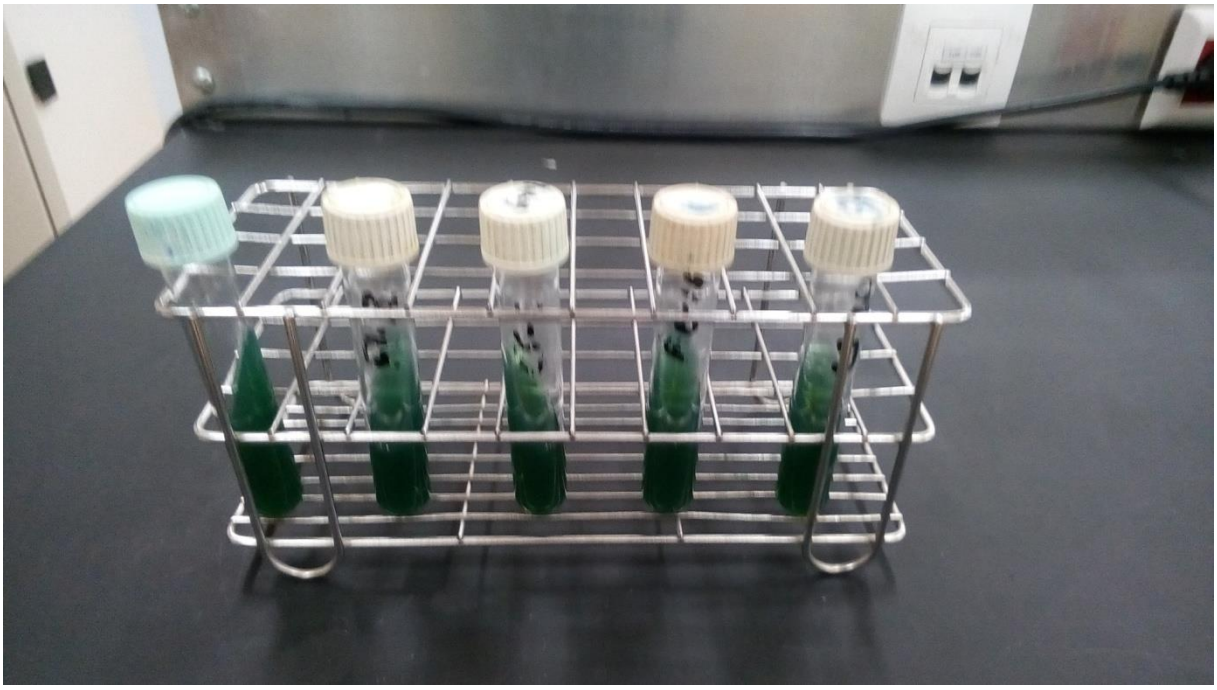

Figure 3. Citrate test

Annex 9. Antimicrobial susceptibility testing record in the lab

Figure 1. Placing of antimicrobial disks on the inoculated Muller Hinton Agar plates using sterile forceps (A), Measuring zone of inhibition for AMS test (B) recording of the diameter of zone inhibition (C), Different antimicrobial susceptibility patterns of *E. coli* on prepared media plates(D &E).

Annex 10. Ethical clearance form approved by Research Ethics Review Committee of CVMA

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/26/03/12/2020

Name of Applicant: Gebremichael Tilahun (BVSc, MVSc fellow)

Address: College of Veterinary Medicine and Agriculture (Addis Ababa University)

Title of the project: *Investigation on antimicrobial susceptibility of Escherichia coli isolates form feces of slaughtered cattle, beef carcass and abattoir environment at Karalo abattoir and surrounding butcher shops, Addis Ababa, Ethiopia*

Date of application:	**06/02/2020**
Nature of the project:	**non-invasive**
Target animal species:	**Cattle**
Number of animals involved:	**175**
Study area:	**Addis Ababa, Ethiopia**

Minutes No. and date of review: **VM/ERC/03/12/020, 11/03/2020**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when this is deemed necessary
3. A separate clearance is required for any work (except questionnaire) on human subjects

Dr Getachew Terefe
Chairman

Signature