ISOLATION AND ANTIMICROBIAL RESISTANCE PATTERN OF *ESCHERICHIA COLI* O157: H7 FROM LAYER POULTRY FARMS IN BISHOFTU TOWN, ETHIOPIA

MVSc THESIS

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

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As MVSc research advisors, we certify that we have read and evaluated the thesis prepared under our guidance by **Sena Zinabu Ababungul** entitled: "Isolation and antimicrobial resistance pattern of *Escherichia coli* O157:H7 from layer poultry farms in Bishoftu town, Ethiopia." We recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Public Health.

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STATEMENT OF AUTHOR

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LIST OF ABBREVIATIONS

A/E lesion	Attaching and effacing lesion
A/EEA	Attaching and Effacing *Escherichia coli*
AMR	Antimicrobial Resistance
CDC	Center of Disease Control and Prevention
CI	Confidence Interval
CLSI	Clinical Laboratories and Standard Institute
CSA	Central Statical Agency
DNA	Deoxyribonucleic Acid
Eae	Intimin gene
EAEC	Enteroaggregative *Escherichia coli*
E. coli	*Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EMB	Eosin Methylene Blue
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
F	Fimbriae antigen
FAO	Food and Agricultural Organization
H	Flagellar antigen
HC	Hemorrhagic Colitis
HlyA	Enterohaemolysin
HUS	Hemolytic Uremic Syndrome
K	Capsular antigen
LEE	Locus of Enterocyte Effacement
MDR	Multi-drug Resistance
MR	Methyl Red
O	Somatic antigen
OR	Odds Ratio
PCR	Polymerase Chain Reaction

Continued…

PO157	Plasmid O157
SMAC	Sorbitol MacConkey
STEC	Shiga Toxin Producing *Escherichia coli*
Stx	Shiga toxin
Tir	Translocated intimin receptor
TSI	Triple Sugar Iron
TTP	Thrombotic Thrombocytopenic Purpura
VP	Voges-Proskauer
WHO	World Health Organization

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ABSTRACT

Escherichia coli O157: H7 (*E. coli* O157:H7) is a zoonotic pathogen responsible for the majority of severe cases of human enterohemorrhagic *E. coli* disease. However, the precise attribution of chickens and their food products as the sources of this zoonotic serotype has not yet been thoroughly investigated in Bishoftu town. Therefore, a cross-sectional study was conducted from November 2021 to May 2022 on different sizes of layer poultry farms found in Bishoftu town to isolate, characterize, and determine the antimicrobial resistance profile of *E. coli* O157: H7. For this study, a total of 420 samples were collected from cloacae, eggs shells, the contents of eggs, personnel hands and the chicken environments. *E. coli* O157: H7 was isolated and confirmed using bacteriological, biochemical, and the latex-agglutination methods. All of the isolates were subjected to molecular characterization and susceptibility testing against twelve selected antimicrobials. Farm level information on the potential risk factors was collected using semi-structured questionnaires. Of a total of 420 samples examined, *E. coli* was detected in 66 (15.71 %; 95 % CI: 12.37, 19.55) whereas *E. coli* O157:H7 was confirmed in 16 (3.81%; 95 % CI: 2.19, 6.11) of the samples using the latex method. Of the 16 isolates, 9 (56.25 %) were from cloacae samples, 3 (18.75 %) were from litter samples, 1 (6.25 %) was from personnel hands, and 3 (18.75 %) were from egg shell swabs. The study showed that there was a statistically significant difference in the prevalence of *E. coli* O157: H7 among the different farm sizes and between chicken age groups. The odds of isolation of *E. coli* O157: H7 were 5.5 times greater in small-scale farms than in large-scale poultry farms and 4.1 times higher in adult chickens than in their younger counterparts. PCR amplification of intimin (*eae)* and shiga toxin (*stx1)* genes specific for *E. coli* O157: H7 using specific primers identified *eae* gene from 7 (43.75%) *E. coli* O157:H7 isolates, but *stx1* was not detected. The results of antimicrobial sensitivity showed that 75% and 62.50% of the isolates had developed resistance against ampicillin and amoxacillin, respectively. On the other hand, 87.50% of the isolates were susceptible to gentamycin. Resistance to three or more drugs was detected in 68.7% of the isolates, which signifies a public health risk. The results of this study revealed the circulation of *E. coli* O157:H7 in poultry, their products and environ. Therefore, further research is highly encouraged to investigate the distribution of *E. coli* O157: H7 at human poultry interface and the driving factors of antimicrobial resistance in this setting.

Keywords: *Antimicrobials, Bishoftu, Characterization, E. coli O157:H7, Isolation, Poultry,*

1. INTRODUCTION

The poultry sector continues to grow and industrialize in many parts of the world. An increasing population, greater purchasing power and urbanization have been strong drivers of the growth (FAO, 2014). In 2020, there were some 33 billion chickens in the world, up from 14.38 billion chickens in 2000. Industrially raised chickens tend to fall into egg laying hens and broiler chickens (Shahbandeh, 2022). Figures released by Food and Agriculture Organization (FAO) show that, world poultry meat production soared from 9 to 133 million tonnes between 1961 and 2020, and egg production shot up from 15 to 93 million tonnes. The United States of America is the world largest poultry meat producer, with 17 percent of global output, and China is by far the world largest egg producer, with 38% of global production (FAO, 2022).

As an important farm species, poultry is raised by approximately 80% of rural households and is used as a significant source of revenue generation for small farmers in developing countries (Attia *et al*., 2022). In Ethiopia, as one of the developing countries, it has been setting up new businesses for more than 10 years. Next to cattle, chicken is the largest livestock group in Ethiopia, estimated to be about 57 million (CSA, 2021). An egg is a high-protein-rich poultry product used in a number of traditional Ethiopian dishes for decades (Kralik *et al*., 2017). However, several studies have declared that enteric bacteria like *Salmonella*, *E. coli*, *Campylobacter*, and others could contaminate these products and cause food-borne diseases (Abebe *et al*., 2020; Belina *et al*., 2021).

The nutritive value of eggs makes them vulnerable to microbial contamination, which results in food-borne diseases (Okorie-kanu *et al*., 2016). According to WHO (2015); Hannah and Max (2018) data, about 7.69% of the world's population suffers from food-borne diseases every year, and 7.50% of all deaths annually are due to food-borne illnesses. This could be higher in developing countries like Ethiopia, where little or no control measures are put in place. *Salmonella* species, *Campylobacter*, and pathogenic *E. coli* are among the most common and leading causes of food-borne outbreaks worldwide (WHO, 2017).

E. coli is a Gram-negative highly motile bacterium that belong to the family Enterobacteriaceae (Ejeh *et al*., 2017). It is one of the common microbial floras of the gut of farm animals, poultry, and human beings. Most isolates are harmless, but some strains are pathogenic and cause severe food poisoning in humans (Ramos *et al*., 2020). In egg laying hens infections of the reproductive tract with *E. coli* is considered as the most common bacterial infection (Poulsen *et al*., 2020), and higher mortality rates (28.4–31.4%) in chicks due to yolk sac infection were also found to be significantly correlated with *E. coli* isolation (Amare *et al*., 2013; EL-Sawah *et al*., 2018).

E. coli have several types of strains that are divided into six groups of pathotypes based on the mechanism of disease causes (Kaper *et al*., 2004). Among them, Enterohaemorrhagic *E. coli* (EHEC) serotype *E. coli* O157: H7 is one of the most potent zoonotic pathogen that produces a powerful toxin responsible for the severe causes of hemolytic uremic syndrome (HUS), end-stage renal failure, and death in humans (Fatima and Aziz, 2021). Other important characteristics of this serotype include the production of attaching and effacing lesions as well as the presence of a virulence plasmid known as plasmid O157 (PO157) (Lim *et al*., 2010). *E. coli* O157:H7 is responsible for about 20% of food-borne outbreaks worldwide (Holmes *et al*., 2017).

In humans, globally, Shiga toxin-producing *E. coli* (STEC) causes 2,801,000 acute illnesses annually. Among those, a total of 10,200 cases of STEC infections occur in Africa with an incidence rate of 1.4 cases per 100,000 person/years, in which *E. coli* O157:H7 contributes 10% to this burden (Majowicz *et al*., 2014; Lupindu, 2018). Large numbers of this serotype are also maintained in the poultry house, environment, and fecal contamination, as on other farms (Guabiraba and Schouler, 2015). Several studies in different countries found *E. coli* O157: H7 levels ranging from 0.0% to 14.5% on poultry farms and their products (Jo *et al*., 2004; Doane *et al*., 2007; Olatoye *et al*., 2012; Shecho *et al*., 2017).

Detection of *E. coli* O157: H7 is dependent on distinguishing the pathogenic serotypes from normal fecal flora containing commensal strains of *E. coli*. Identification and confirmation of this pathogenic serotype are attained through bacterial culture, serological examination, and molecular detection of its virulence genes (Bouzari *et al*., 2012; Abreham *et al*., 2019). The administration of antibiotics in STEC infection is controversial because they can activate the

phage-mediated lytic cycle in STEC, which lyses bacterial host cells to release toxins (Panos *et al*., 2006). However, an increase in antibiotic resistance (AMR) has been noted over the last 20 years (Disassa *et al*., 2017; Shecho *et al*., 2017; Bedasa *et al*., 2018).

According to WHO (2017), *E. coli* O157: H7 could contaminate poultry products and cause food-borne zoonotic diseases. The major public health concern about food-borne illness is infection by antimicrobial-resistant strains, which leads to more intractable and severe disease. Ethiopia ranks second in Africa in terms of zoonotic disease health burden after Nigeria (Kemunto *et al*., 2018), where *E. coli* O157:H7 is recognized as one of the major causes of foodborne diarrheal disease (Minda and Shimelis, 2021). A study by Shecho *et al*. (2017) indicated 13.4% of *E. coli* O157: H7 isolated from cloacae samples taken from poultry farms, Eastern Ethiopia. A study conducted by Taddese *et al*. (2020) in Jimma town showed that, Out of 415 total samples, 156 (37.59%) were identified as *E. coli* from farm egg shell 21/83 (25.30%), farm egg content 12/83 (14.46%), cloacae swab 42/83 (50.60%) and market egg shell 45/83 (54.23%). Therefore, the accurate and consistent attribution of chicken and their food products as the sources of this zoonotic serotype need to be thoroughly investigated.

In developing countries like Ethiopia, where childhood diarrheal disease is common and eggs represent an important food in consumers' nutrition as well as a source of income for producers, it has not yet been determined to what extent chickens serve as sources of *E. coli* O157: H7, particularly for egg contamination in the study area. Rapid identification and characterization of this zoonotic pathogen are important for food hygiene management and prompt epidemiological investigations. Hence, the purpose of this study is to provide information on the epidemiology and public health risk of *E. coli* O157:H7 from commercial layer poultry farms in Bishoftu, Ethiopia.

Specific objectives

- To isolate, identify, and characterize *E. coli* O157:H7 from cloacae, eggs, personnel hand swabs and environmental samples of layer poultry farms found in Bishoftu town.
- To determine the antimicrobial resistant patterns of *E. coli* O157:H7 isolates to selected antimicrobials.
- To investigate the risk factors associated with the occurrence of *E. coli* O157:H7 in Bishoftu commercial poultry farms.

2. LITERATURE REVIEW ON *ESCHERICHIA COLI* O157: H7

2.1. General Overview of *E. coli*

Escherichia coli is a gram-negative, facultative anaerobic, non-spore-forming, motile, rod-shaped bacteria (WHO, 2018). They are classified under the family Enterobacteriaceae, known to be normal inhabitants of the gastrointestinal tract of animals, humans and birds, but some of its strains have become highly adapted to cause diarrhea and a range of extra-intestinal diseases, mainly in immuno-compromised hosts (Guabiraba and Schouler, 2015). It was first isolated by a German pediatrician, Theodore Esherichin in 1884 from feces of human neonates. Since then numerous outbreaks have been documented (Mainil, 2011). For the genus *E. coli*, there are hundreds of serotypes which are classified on the bases of various surface antigens referred to as Somatic (O), Capsular (K), Flagellar (H) and Fimbrial (F) (indicated in Figure 1 below) (Stenutz *et al*., 2006).

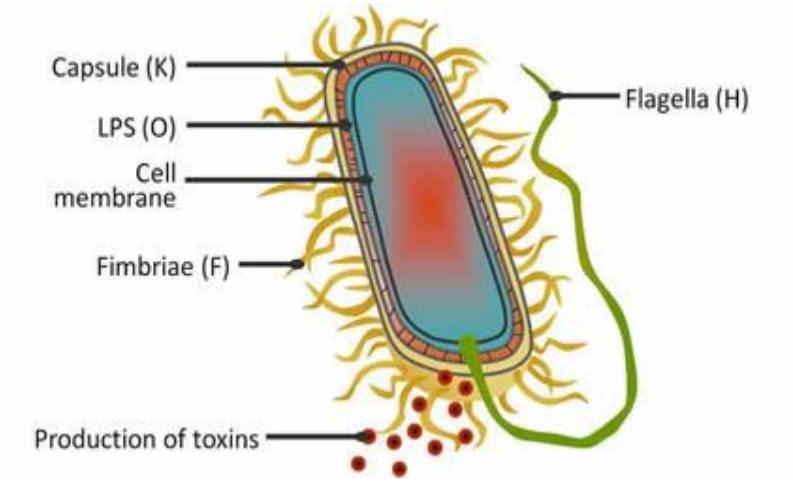


Figure 1: Schematic presentation of *E. coli* cell surface antigens.

Source: (*Escherichia coli* Laboratory, 2004)

There are two types of *E. coli*, pathogenic and non-pathogenic. The non-pathogenic strains of *E. coli* are harmless, hinder the growth of harmful bacteria and produce vitamins. Additionally, they play several important roles in humans, such as performing specific metabolic functions which

are absent in humans, modulating the morphology and physiology in the gut as well as assisting in development of the immune system (Shah *et al*., 2018). On the other hand, there are three clinical syndromes caused by pathogenic *E. coli*: (i) sepsis/meningitis; (ii) urinary tract infection and (iii) diarrhea. Furthermore, *E. coli* causing diarrhea is divided into different 'pathotypes' depending on the type of disease they causes (Nataro and Kaper, 1998; Stenutz *et al*., 2006), *i.e*. Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Attaching and effacing *E. coli* (A/EEC), Enteroinvasive *E. coli* (EIEC) and Enterohaemorrhagic *E. coli (*EHEC) strains. Lately, Enteroaggregative *E. coli* (EAEC) has also been found to be a diarrhoegenic strain (Gomes *et al*., 2016).

Among EHEC strains, *E. coli* O157:H7 is one of the most studied food-borne pathogens, because of its widespread diffusion, peculiar tolerance to some physical and chemical treatments, severity of illness and low dose infectiveness (Beneduce *et al*., 2003). It was first identified as a human enteric pathogen in 1982, and then it is a major cause of hemorrhagic colitis (HC) and HUS (Mead and Griffin, 1998; Sheikh *et al*., 2013).

2.2. Antigenic Structure of *E. coli* O157: H7

A serological classification scheme for *E. coli* was first described in the 1940s and was based on the immunogenicity of the bacterial surface structures. Serotyping is widely used and is a well-established type of *E. coli* classification for pathogenecity detection and epidemiological studies (Deborah and Frankel, 2005). Serotyping based on the O and H antigens are considered the 'gold-standard' as only limited laboratories have the ability to type the K-group (DebRoy *et al*., 2011). Serogroup O is related to the somatic antigen; capsular antigen refers to K; serogroup H encompasses flagella antigen (Orskov and Orskov, 1992; Sarowska *et al*., 2019). Overall, serotyping is complex as currently there are 186 different O-groups as well as 53 H-groups among *E. coli* (Fratamico *et al*., 2016; Park *et al*., 2022) . Major antigenic structures in *E. coli* O157:H7 includes:

2.2.1. E. coli O-antigen

The outer membrane of *E. coli* is composed of lipopolysaccharides that include lipid A, core oligosaccharides, and a unique polysaccharide, referred to as the O-antigen (Reeves and Cunneen, 2010). Loss of the O antigens results in attenuated virulence suggesting their importance in host–pathogen interactions (DebRoy *et al*., 2011). There have been O-groups numbered from O1-O188, except for O31, O47, O67, O72, O94, and O122 that have not been designated, and four groups have been divided into subtypes O18ab/ac, O28ab/ac, O112ab/ac, and O125ab/ac, giving a total of 186 O-groups (Scheutz *et al*., 2004; Fratamico *et al*., 2016). The variability in O antigen structure provides the major basis for the serotyping schemes of many Gram-negative bacteria and is the most widely used method for identifying strains for epidemiological purposes, making O serotyping one of the most important components in typing organisms (Sun *et al*., 2011).

The O157 is an important *E. coli* O serogroup because it is the most frequently reported O serogroup of EHEC strains associated with outbreaks and sporadic cases of diarrhea, HC and HUS worldwide. This O antigen, O157 is characteristically found in pathogenic clones, with the O157:H7 clone being particularly significant in human disease (Yoon and Hovde, 2008). All EHEC strains produce stx, but STEC strains possessing O antigen 157 are the most frequently isolated from humans and are the predominant cause of HUS, because of the very low infective dose of this organism, bacteria entering the human food chain can still pose a health problem after undergoing enormous dilution (Hunt, 2010).

2.2.2. E. coli H-antigen

Another antigen, the flagellin, designated as antigen ‘H’, is a protein that encodes the scourge of *E. coli*. Serology has defined 53 H-flagellar antigens that are numbered from H1 to H56, but H-types 13, 22, and 50 are not in use (Wang *et al*., 2003; Fratamico *et al*., 2016). H antigens are not often used in antigenic identification of *E. coli* isolates and are not correlated with pathogenecity or virulence of *E. coli*. They are proteins that are destroyed by heating to 100°C (Lim *et al*, 2013).

2.3. Virulence Factors and Mechanism of Bacterial Pathogenecity

The ability to produce one or more stx is a hallmark of *E. coli* O157: H7 infection and it is the major virulence factor for this serotype (Rahal *et al*., 2012). However, toxin production is not sufficient to cause disease. Two other factors are indicted in contributing to the virulence of *E. coli* O157:H7. The first of these two factors is harboring a 60 megadalton virulence pO157, which encodes hemolysin. The other factor is the locus of enterocyte effacement (LEE), which encodes intimin (*eae*) (Figure 2) (Ogierman *et al*., 2000; Perera *et al*., 2015).

As one of the major virulence factor for *E. coli* O157:H7, stx have two forms, stx1 and stx2 encoded by stx1 and stx2 genes, which are known and reported to be responsible for HUS (Lv *et al*., 2010). Stx of EHEC cleaves ribosomal RNA, thereby disrupting protein synthesis and killing the intoxicated epithelial or endothelial cells (Kaper *et al*., 2004). Stx is produced in the colon and travels by the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production, resulting in renal inflammation (Obrig and Diana, 2012). This damage can lead to HUS, which is characterized by haemolytic anaemia, thrombocytopoenia and potentially fatal acute renal failure (Kaper *et al*., 2004; Melton-Celsa, 2014).

Initial profiling of the plasmids present in *E. coli* O157:H7 demonstrated the presence of multiple plasmids and the high prevalence of the pO157. The pO157 was found in 99% of 107 clinical isolates of *E. coli* O157:H7 from humans (Ratnam *et al*., 1988; Yoon and Hovde, 2008). The subsequent epidemiological studies suggest that almost all *E. coli* O157:H7 strains possess this plasmid. A pO157-like plasmid is also present in O26:H11 strains and in most STEC isolates from humans and animals. However, its biological significance in infection is unknown (Lim *et al*., 2013).

Formation of attaching and effacing lesions (A/E lesion) is another unique characteristic of EHEC/EPEC pathogenesis (Garmendia *et al*., 2005). It is proposed that the formation of A/E lesion results in a reduction in the absorptive capacity of the intestinal mucosa, which inevitably

leads to disruption of the electrolyte balance and subsequently to diarrhea. A/E lesion formation is dependent on a number of physiological and environmental conditions (Clarke *et al*., 2003). The genetic element responsible for the lesions is called the LEE, and it is a well-known pathogenicity island present in EPEC, EHEC and other A/E *E. coli* that are pathogenic in human and animal species. The central portion of this LEE encodes intimin, which mediates intimate attachment to the host cell, and Tir (Translocated intimin receptor). Tir-intimin interaction mediates tight binding of EPEC and EHEC to the intestinal epithelia, resulting in the formation of effacing lesions on intestinal epithelia (Figure 2) (Yoon and Hovde, 2008).

There are many requirements for an *E. coli* O157:H7 infection to occur, involving complex interactions between bacterial and host factors. This pathogen has shown the ability to survive in harsh environments. In addition to its ability to infect humans through the consumption of contaminated foods, *E. coli* O157: H7 can survive in water, making it an environmental threat to humans (Chekabab *et al*., 2013).

To establish intestinal colonization, the pathogen must survive in the acidic environment of the stomach and compete with other gut microflora. Following colonization, bacteria produce stxs in the intestinal lumen, which must be absorbed by the intestinal epithelium and transported to the bloodstream (Lim *et al*., 2010) . A three-stage model for EHEC has been proposed, including (i) initial adherence, (ii) signal transduction, and (iii) intimate adherence. Intimin is type III secretion system effector protein that facilitates the intimate adherence of *E. coli* O157: H7 cells to the intestinal epithelium. This adhesive factor may lead to bacterial colonization and proliferation. After colonization, stxs produced by STEC pass through intestinal epithelial cells into the bloodstream, allowing them to reach target organs, including the kidneys, brain, and eyes, and causing diseases such as HUS (Paton and Paton, 1998; Yoon and Hovde, 2008).

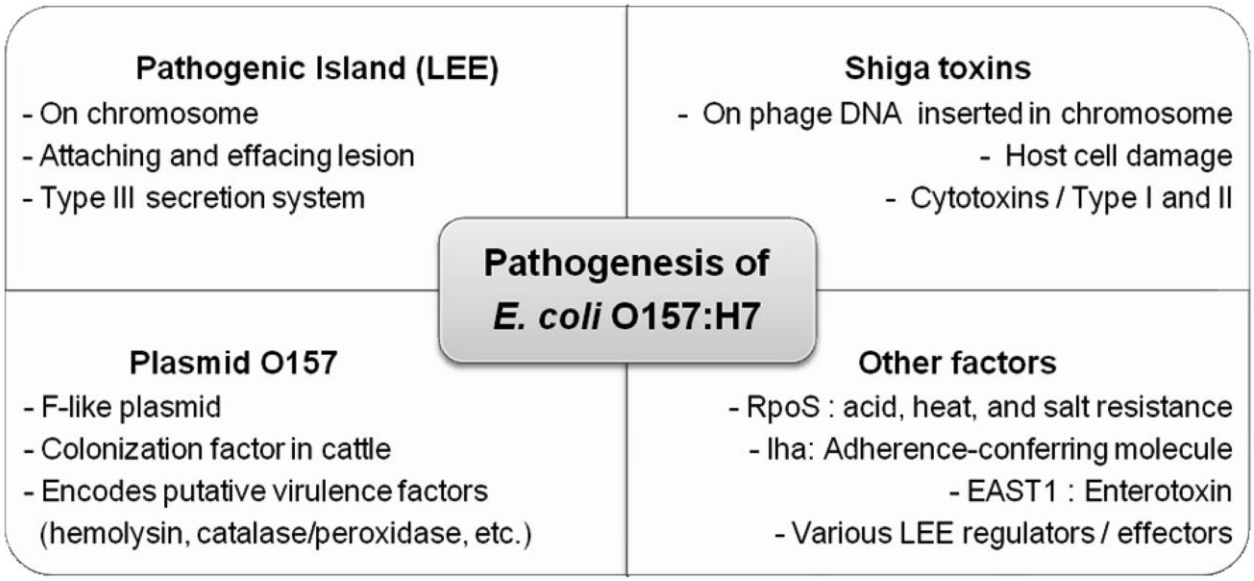


Figure 2: Virulence factors of *E. coli* O157: H7.

Source: (Lim *et al*., 2010)

2.4. Epidemiology

2.4.1. Distribution

The first *E. coli* O157: H7 infections were reported as a human pathogen in 1982, when *E. coli* O157:H7 was involved in outbreaks associated with two fast food chain restaurants and with eating any of three sandwiches containing three ingredients in common (beef patty, rehydrated onions, and pickles) in Oregon and Michigan, Unites States of America (Riley *et al*., 1983), and it is also linked to sporadic cases of HUS in 1983. Since then, many outbreaks associated with EHEC have been reported in the United States, and *E. coli* O157:H7 has become one of the most important foodborne pathogens (Lim *et al*., 2010). For instance, *E. coli* serotype O157:H7 has been isolated in outbreaks in Canada, Great Britain, Argentina, Australia, Belgium, China, Germany, Italy, Japan, and South Africa (Bell *et al*., 1994; Ackers *et al*., 1998; Effler *et al*., 2001).

Cattle feces are the most important source of *E. coli* O157:H7, however, the presence of *E. coli* O157:H7 in the feces of other animal species has been well recognized. Thus, it is distributed

globally in soil, water, vegetation, decaying matter, and the large intestine of most animals and humans (Chapman *et al*., 1997). The highest incidence rates occur in children, in elders and immunocompromised peoples. *E. coli* O157:H7 has been also reported in feces of different animal and animal products in Ethiopia. For instance, from cloacae samples taken from poultry farms, Eastern Ethiopia (Shecho *et al*., 2017), beef cattle at processing plants and at retail shops in Ethiopia (Abdissa *et al*., 2017), in faeces, skin swabs and carcasses before and after washing, from sheep and goats in Ethiopia (Mersha *et al*., 2010). These generally show the worldwide distribution of the organism.

2.4.2. Source of infection and route of transmission

E. coli O157:H7 is found in the intestines of healthy cattle, goats, deer, chicken and sheep. According to WHO (2018), the transmission of these bacteria to humans may occur in different manners. As indicated in Figure 3 below, the most frequent route of transmission for *E. coli* O157:H7 infection is via consumption of contaminated food and water. Intestinal tract of animals, including poultry is the most important reservoir of *E. coli* (Ameer *et al*., 2021). Farm fecal contamination of the egg shell was reported as source of infection (EL-Sawah *et al*., 2018). Transmission of pathogenic *E. coli* through egg is common and can result in huge mortality in chicks. The most important source of egg infection seems to be fecal contamination of the egg surface with subsequent penetration of the shell and membranes (Kabir, 2010).

Pathogenic serotypes can also be introduced into poultry flocks through contaminated well water (McGee *et al*., 2002). Water trough sediments contaminated with feces can serve as a long-term (>8 months) reservoir of *E. coli* O157:H7, and the surviving bacteria in contaminated troughs is another source of infection (LeJeune *et al*., 2001). Johnson *et al*. (1999) outlined many possible sources of *E. coli* O157:H7 in the farm environment, such as manure heaps, ponds, bedding, feed and feed troughs, water and water troughs, farm equipment, ground and pasture, and noted that once present in the environment, this organism can be transferred to other sites by wind as well as by animals and humans (Figure 3).

The infectious dose of *E. coli* O157: H7 that has caused disease symptoms in humans has been reported to be as low as 4 to 24 organisms (Stein and Katz, 2017). 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 humans (Premarathne *et al*., 2017). Reports of *E. coli* O157: H7 in other animal species and birds confirm the existence of non bovine animal reservoirs or vehicl es of *E. coli* O157:H7 (Bach *et al*., 2002).

Bacteria in infected people's stools can be passed from person to person if hygiene or hand washing habits are poor or from soiled fingers of one person to the mouth of another. This is especially likely in toddlers who have not yet been toilet trained. Bacteria can also be passed from person to person in day care centers and nursing homes, in public restrooms, and by touching objects, particularly food. (Ameer *et al*., 2021; Mueller and Tainter, 2022).

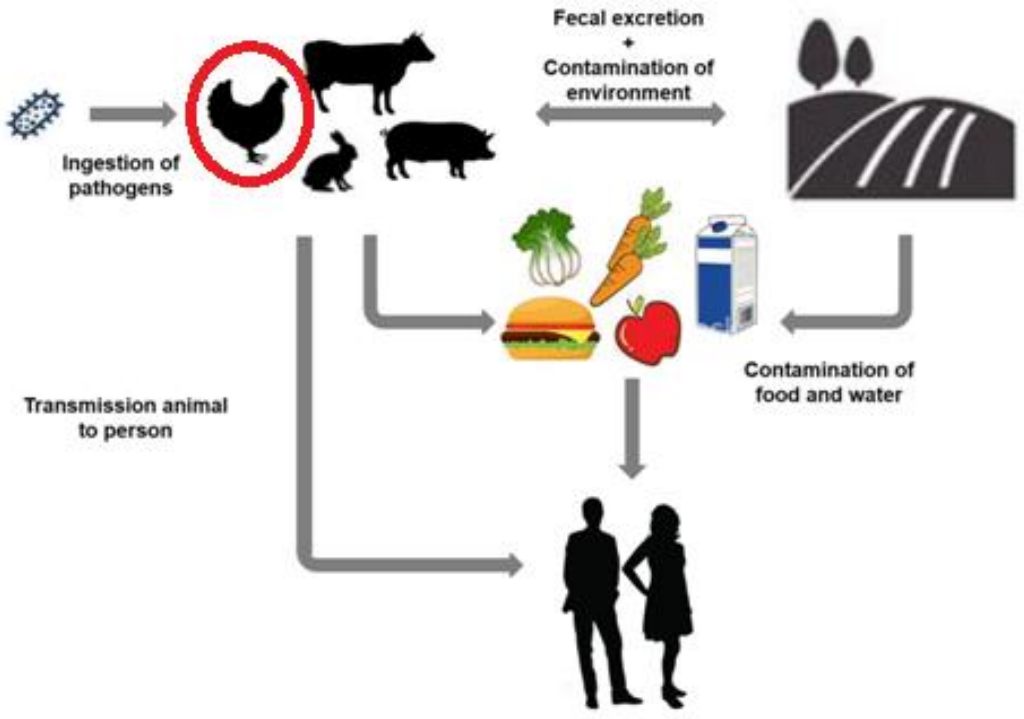


Figure 3: Schematic representation of different contamination sources and transmission of STEC infection.

Source: (Hwang *et al*., 2021)

2.4.3. Susceptible animals/humans and epidemic characteristics

Healthy colonized cattle and other ruminants are the most significant animal reservoir harboring *E. coli* O157:H7 (Munns *et al*., 2015), and studies have linked ~75% of the human *E. coli* O157:H7 outbreaks to food products of bovine origin (Callaway *et al*., 2009). It naturally colonizes their gastrointestinal tracts, and the lymphoid follicle-dense mucosa at the terminal rectum, called the recto-anal junction mucosa, is known as a principal site of colonization in cattle (Lim *et al*., 2007). Other susceptible animals that may impact transmission includes pigs, gulls, geese, and pets (Stein and Katz, 2017). Chickens of all ages and breeds are susceptible to *E. coli* O157:H7 infections, but it has been found that the severity of the disease is greater in younger chickens (Ferens and Hovde, 2011).

People from any age group can be affected by *E. coli* O157:H7 infection; while old people and young children can be more susceptible. Infective dose, stress resistance mechanisms, and production of toxins contribute to the severity of the *E. coli* O157:H7 infection (Lim *et al*., 2010). The disease can occur in all seasons of the year but is more common during the summer months, with the majority of cases occurring during the months of May through September (Oliver and Page, 2016).

Increased shedding of *E. coli* O157:H7 by cattle during summer months may contribute to a similar seasonal pattern of *E. coli* O157:H7-associated foodborne illness in humans. The seasonality of fecal shedding of the organism indicates that environmental replication may play a key role in its ecology in the farm environment (Bach *et al*., 2002). If the breeding density is high, the disease occurs at any time. The occurrence of a specific serotype and its role in disease production depends upon the health status of the birds, climatic conditions, geographical situations and managemental strategies (Srinivasan *et al*., 2013).

2.4.4. Growth and environmental survival of E. coli O157:H7

A number of factors have a significant influence on the survival and growth of *E. coli* O157: H7 in food and environment, including temperature, pH, salt, and water activity (Chauret, 2011). The optimum growth temperature for *E. coli* O157: H7 is 37^{o}C, the minimum and maximum

temperatures are 7-8^{o}C and 41^{o}C in selective *E. coli* broth respectively (Raghubeer and Matches, 1990; Han and Linton, 2004). The upper growth temperature for *E. coli* O157: H7 is culture medium dependent. The organism survives well in chilled and frozen foods. For example, only little change was noted in number of bacteria in hamburgers stored at -20^{o}C for 9 months (Han and Linton, 2004). The effect of environmental stress and food production processing on the growth, survival and inactivation of *E. coli* O157: H7 are well recognized.

E. coli O157:H7 can survive and persist in numerous environments such as soil, water, and food as well as in animal reservoirs (Yuk and Marshall, 2004). To survive in varied environments, *E. coli* O157:H7 requires the ability to adapt to variations or extreme changes in temperature, pH, and osmolarity conditions commonly encountered in nature. These environmental adaptations of *E. coli* O157: H7 play an important role in the persistence and dissemination of this microorganism on farms (Lim *et al*., 2010).

The organism has been observed to survive for days (at physiological (>30 °C) temperature, aerobic and under nutrient-limiting conditions) to almost a year in the nutrient rich habitats (Jiang *et al*., 2002). *E. coli* O157: H7 can survive for a long time in water, especially at cold temperatures or in dry, dusty conditions, and it has been shown that wetting the litter can reduce the incidence of colisepticaemia. Barker *et al*. (1999) showed that *E. coli* O157:H7 survives and replicates in *Acanthamoeba polyphaga. A. polyphaga* is a common environmental protozoan found in soil, water, and fecal slurry. As a result, it may be an effective *E. coli* O157: H7 transmission vehicle in these environments (Lim *et al*, 2013).

E. coli O157: H7 is dangerous due to its resistance to low pH (2.5), which allows passage through the stomach, its low infective dose, and its high pathogenicity (Van-Elsas *et al*., 2011). Growth efficiency and competitiveness under conditions of low available nutrient levels likely represent the most important physiological factors leading to the successful persistence of *E. coli* in nutrient-limited open environments. That is why Pathogenic *E. coli* strains such as *E. coli* O157:H7 pose a threat to the food chain and represent a still underestimated environmental risk (Ihssen and Egli, 2005).

2.4.5. Risk factors associated with E. coli O157:H7 infection

All ages are susceptible to *E. coli* O157:H7, but young birds are more frequently affected. Severity of the disease is greater in young birds (4 to 9 weeks old) including developing embryos than adults (Shecho *et al*., 2017). Zhao *et al*. (2005) described young animals tend to carry *E. coli* O157:H7 more frequently than adults, because young chicks are not fully immunocompetent and have lost protection from maternal antibodies. It is also responsible for a considerable number of various diseases at different ages including neonatal infection of chicks, oophoritis or salpingitis in laying hen, air sacculitis and septicemia in all ages of chickens (Kabir, 2010). However, there are an equal chance of *E. coli* O157:H7 infection among different breeds of chickens (Shecho *et al*., 2017).

Faecal contamination of egg may result in the penetration of *E. coli* through the shell and may spread to the chickens during hatching and is often associated with high mortality rates, or it may give rise to yolk sac infection. So, quality of the egg at the hatchery can play a crucial part in relation to the bird's susceptibility to the infection (Kabir, 2010). The severity of the disease in chickens depends on the pathogenicity of the *E. coli* serotypes, the chicken's immune status and the presence of predisposing factors, such as poor chicken welfare, immunosuppression and co-infections that can increase host susceptibility (Awawdeh, 2017).

Various intrinsic and extrinsic factors like damage to mucosal and skin barriers, impaired mononuclear phagocytic system, extreme environmental temperature, stress have also been correlated with pathogenicity of *E. coli* O157:H7 in chickens (Rind *et al*., 2016). Various management factors can also have an influence on the occurrence of diseases: ventilation and temperature, litter quality, stocking density and housing conditions or an all-in-all-out-system (Caffrey *et al*., 2017; Becker *et al*., 2021). In general, biosecurity system of the farm is one of the major risk factor for the dissemination of pathogenic *E. coli* in poultry farms. Internal biosecurity is used to prevent the spread of pathogens within the farm, for example through hygiene measures such as protective clothing, hand washing or cleaning, and disinfection of the farms (Maertens *et al*., 2018).

The infection is aggravated by poor hygiene in breeding farms and faulty management at the hatchery (Kim and Kim, 2010). Other factors that favor rapid bacterial growth include the fact that the yolk contains a lot of fat and water, favored nutrients for bacteria (EL-Sawah *et al*., 2018). Generally, the risk factors for STEC O157: H7 infections include contact with animals, their environment and poor personal hygiene, such as not washing hands after handling animals or prior to eating (Howie *et al*., 2003; Lupindu, 2018).

2.4.6. Prevalence of E. coli O157:H7 in Poultry farms

The prevalence of *E. coli* O157:H7 among poultry farms varies considerably (Onyango *et al*., 2009). Several studies showed different level of *E. coli* O157: H7 on poultry farms and other animal products in different countries. Olatoye *et al*. (2012) reported 13% and 14% of *E. coli* O157: H7 from Lagos and Ibadan poultry farms, Southwest Nigeria, respectively. Aibinu *et al*. (2007) also isolated *E. coli* O157:H7 from chicken in Lagos and Ogun State in Nigeria and found 14.5%. Joseph (2018) collected swab samples from poultry farms and free-ranged chicken, 24 (6.67%) strayed and 7 (1.94%) poultry farm samples were found to be positive for *E. coli* O157:H7.

A study by Shecho *et al*. (2017) indicated 13.4% of *E. coli* O157: H7 isolated from cloacae samples taken from poultry farms, Eastern Ethiopia. A study conducted by Taddese *et al*. (2020) in Jimma town showed that, Out of 415 total samples, 156 (37.59%) were identified as *E. coli* from farm egg shell 21/83 (25.30%), farm egg content 12/83 (14.46%), cloacae swab 42/83 (50.60%) and market egg shell 45/83 (54.23%) with varied degree of susceptibility to antimicrobials. In another study, the prevalence of *E. coli* O157:H7 was determined from meat and meat products in Riyadh, Saudi Arabia, and the prevalence were found to be 2.5% and 5% in raw chicken and ground chicken respectively (Hessain *et al*., 2015).

2.5. Clinical Manifestations of *E. coli* O157: H7 Infection

Infection with *E. coli* O157:H7 can be asymptomatic shedding or may manifest as non-bloody diarrhea. Specially, in humans, there are three principal manifestation of illness called HC, HUS, and thrombotic thrombocytopenic purpura (TTP) in all age groups (Ameer *et al*., 2021). Unless

infection with *E. coli* O157:H7 is asymptomatic, following an incubation period of 3–4 days, the illness starts with severe abdominal cramps accompanied by a non-bloody diarrhea. In most patients the watery diarrhea becomes grossly bloody after two or three days (Rahal *et al*., 2012).

Gastrointestinal symptoms due to infection with *E. coli* O157:H7 usually resolve within a week. Patients then mostly recover with no major sequelae. Nevertheless, 5–10% of patients develop the HUS approximately one week after onset of HCs (Karmali *et al*., 1983; Rahal *et al*., 2012). The release of stx is believed to play a central role in the development of HUS. HUS is a potentially life-threatening complication that can arise from STEC infection. The production of stx is a key factor contributing to the development of HUS (Varrone *et al*., 2021). According to report by Minnesota Department of Health (2007), HUS occurs when the *E. coli* O157 toxin destroys red blood cells. HUS can lead to kidney failure, neurologic damage, and in some cases, death. Approximately 5 – 10% of HUS cases are fatal.

Hemorrhagic colitis caused by *E. coli* O157:H7 is a clinical syndrome that consists of abdominal cramps, diarrhea that progresses to become bloody, radiologic evidence of clonic mucosal edema, erosion, or hemorrhage; and the absence of conventional enteric organisms in the stool (Rahal *et al*., 2012). TTP is similar to HUS, except that it more commonly afflicts adults instead of children. In addition to the renal attack characteristic of HUS, TTP has an associated fever with the addition of neurological symptoms (Petridis *et al*., 2002).

2.6. Public Health and Economic Significance of *E. coli* O157: H7

Enterohemorrhagic *E. coli* (O157 and other serotypes) are zoonotic pathogens linked with severe human illnesses (Muniesa *et al*., 2006). Following its identification as the causative agent of human illness, numerous outbreaks and sporadic cases of illness associated with *E. coli* O157:H7 has been reported from Argentina, Australia, Belgium, Canada, China and many other countries (Bach *et al*., 2002). During July–October 2014, an outbreak of 119 cases of *E. coli* O157:H7 infections associated with exposure to contaminated pork products occurred in Alberta, Canada (Honish *et al*., 2017). Esumeh *et al*. (2017) Screened 20% of *E. coli* O157:H7 from diarrheic Patients, Benin City, Nigeria.

According to Getaneh *et al*. (2021), the prevalence of *E. coli* O157: H7 related diarrhea was 15.30% in 378 under-five-year-old children recruited at random from hospitals in Eastern Ethiopia. A Hospital-based cross-sectional study by Adugna *et al*. (2015) at Bahirdar indicated 28.90% isolation rate of *E. coli* serotype O157:H7. In another study, the prevalence of *E. coli* O157: H7 was determined from food handlers in food-handling establishments in Southern Ethiopia and the prevalence was found to be 6.03% (Wada *et al*., 2017). Number of human infections peaks during the summer months and this may be due to that Houseflies and blow flies can carry relatively high concentrations of potentially virulent *E. coli* O157:H7 during summer (Bach *et al*., 2002).

Person-to-person spread of *E. coli* O157:H7 has been the primary mode of infection in many outbreaks especially in day care facilities particularly where there have been lapses in hygiene (Lim *et al*., 2010). Transmission usually occurs through consumption of undercooked or contaminated foods of bovine origin, faecal contamination of other food products or direct contact with infected animals (Kiranmayi *et al*., 2010). Getaneh *et al*. (2021) reported that, children from households with livestock were four times more likely to contract *E. coli* O157:H7 than households without livestock.

The severity and long-term sequelae of infection with *E. coli* O157 and other verocytotoxin-producing *E. coli* result in high costs. Abe *et al*. (2002) assessed the impact of direct economic losses and indirect economic consequences due to *E. coli* O157: H7 outbreak in Japan from elementary school lunches in 1996. The economic impact of the outbreak was estimated to be about 82,686,000 yen. The laboratory costs showed the highest ratio of the total cost of this outbreak (about 26%). In 2003, the annual cost of illness due to O157 STEC in United States was $405 million, including $370 million for premature deaths, $30 million for medical care, and $5 million in lost productivity (Frenzen *et al*., 2005).

In addition to the direct human costs due to *E. coli* O157:H7 infection, cattle and dairy producers, meat packers and dairy processors, meat and milk distributors etc incur direct and indirect costs associated with this foodborne disease threat. The cost of *E. coli* O157:H7 to the food industry as a result of recalls, destroyed food, control measures and lost demand associated

to loss of consumer confidence is estimated to be in the billions of dollars in the united States alone (Roberts *et al*., 2000; Frenzen *et al*., 2005). So far there is no study shows the economic impact of *E. coli* O157:H7 under Ethiopian condition.

2.7. Diagnostic Techniques

Identification of *E. coli* is based on the traditional diagnostic methods composed of clinical signs, characteristic macroscopic lesions found during post-mortem examination (Nolan *et al*., 2013), and isolation and identification of *E. coli* from a given samples (Kabir, 2010). Culture methods are labour intensive, nonspecific and time consuming in comparison with molecular methods such as PCR, which can be used to detect the unique nucleic acids (DNA) of pathogens directly from a given samples (Sibley *et al*., 2012).

2.7.1. Clinical signs and pathological lesions

The severity of the disease depends on the presence of predisposing factors, the immune status of the bird, the pathogen's route of entry, virulence of the *E. coli* strain and the duration of exposure (Dziva and Stevens, 2008). Clinical signs appeared by 1-day post infection in the untreated inoculated group. These signs are dullness, depression, dropping of wings, off food, ruffled feather and inability to stand and gradually developed to brown diarrhea and gasping. Later on, sneezing and coughing. Mortalities recorded by the 7^{th} day post-infection in infected chickens (2 out of 30 birds). Upon necropsy of died or sacrificed, birds congestion of liver, lung, spleen and kidneys and the 2 ceci filled with yellowish to greenish or brownish contents with gas. Later on, severe pericarditis, perihepatitis and gas distended ceci were observed (EL-Sawah *et al*., 2018).

2.7.2. Bacteriological isolation and biochemical tests

Escherichia coli is isolated using selective media such as MacConkey agar and/or Eosin-Methylene Blue (EMB) agar, and then transferred to nutrient agar (Quinn *et al*., 2011). The bacterium grows on ordinary nutrient media at temperature of 18-44°C. On nutrient agar plates, incubated for 24 hours at 37°C, colonies are low, convex, smooth and colorless. A presumptive diagnosis of *E. coli* infection can be made if most of the colonies are characteristically black

center with metallic sheen on EMB agar, bright pink with precipitate surrounding colonies on MacConkey agar (Zinnah *et al*., 2007; Shecho *et al*., 2017). *E. coli* is identified as gram-negative rods by Gram staining techniques (Mushtaq *et al*., 2015), and can be further differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar-fermentation and other biochemical tests (indicated in Table 1 below).

Further identification of the isolated colonies is based on its unique features of delayed sorbitol fermentation (>24 h) and inability of producing β-glucuronidase, which can hydrolyze a synthetic molecule, 4-methylumbelliferyl- D-glucuronide (MUG). Thus, Sorbitol MacConkey (SMAC) agar supplemented with MUG has been used for detection of *E. coli* O157:H7. To increase the selectivity for *E. coli* O157:H7, cefixime, potassium tellurite, and vancomycin have been added to SMAC agar plates to inhibit other Gram-negative flora. The serotypes O157 and H7 can be further confirmed by a commercially available latex agglutination assay (Lim *et al*., 2013; Dulo *et al*., 2015; Shecho *et al*., 2017).

Table 1: Some of the biochemical characterization used for identification of *E. coli* O157:H7 (OIE, 2008; Islam *et al*., 2014)

Biochemical tests	***E. coli* O157:H7 reaction**
Citrate test	-
Triple Sugar Iron test (TSI)	+
Indole test	+
Methyl red test (MR)	+
Voges-Proskauer test (VP)	-
Catalase test	+
Urea hydrolysis test	+
Sorbitol MacConkey	-

2.7.3. Molecular characterization

Molecular genetic methods remain the most popular and most reliable techniques for differentiating pathogenic strains from non-pathogenic members. It can be used following phenotypic identification of *E. coli* isolates using species-specific PCR and DNA hybridization to detect pathogenic *E. coli* (Stenutz *et al*., 2006). PCR is performed to characterize *E. coli*

strains by targeting different virulence genes coding for different virulence factors (Casey and Bosworth, 2009). Previous studies have used molecular techniques to identify pathogens and/or genes directly from samples. These methods have proven to be reliable, cost-effective, user friendly, sensitive and specific as well as providing a distinct advantage in regards to time saving over conventional culture methods (Gioffré *et al*., 2004; Stenutz *et al*., 2006).

PCR for the detection of stx-producing genes in *E. coli* O157:H7 remains a gold standard detection method. Detection of the bacteria or toxins may take more than 24 hours (Gould, 2012). DNA is extracted by boiling of the isolates or using DNA extraction kit. According to technique previously indicated by Wasilenko *et al*. (2012); Firoozeh *et al*. (2014), each suspect colony is inoculated on SMAC and incubated for 24 h at 37 °C to get fresh colony. Few colonies are then selected and suspended separately in 100 µL of sterile distilled water in eppendorf tubes; the suspensions are then boiled at 92.5 °C for 17 min in a water bath. After centrifuging at 13000 rpm for 10 min, the supernatant containing the template DNA is transferred into nuclease-free eppendorf tubes, and are stored at -20 °C until use.

The entire extracted DNA of the standard strains and of the recovered *E. coli* O157:H7 isolates by bacteriological examination were examined using multiplex-PCR for molecular typing of the toxic and virulence genes (stx1, stx2, *eae*, and *hlyA*) using specific oligonucleotide primers. The sequence of the primers and the size of the amplified fragments are need to be identified (Hessain *et al*., 2015). Detection of the stx1, stx2 and *eaeA* genes is performed according to the protocol previously indicated by Inat and Siriken (2010).

Amplification of DNA is conducted using initial denaturation at 94- 98 °C for 1min to 3 min, 25-35 cycles of denaturation at 94 °C for 20 to 60s, annealing at 58 °C for 40 s, extension at 72 °C for 1 min, and final extension at 72 °C for 8 min. Finally, gel electrophoresis conducted at 125 V for 1 h. Molecular weight marker of 100 up to 1000 bp used to identify the amplified products as a ladder, which was visualized by UV illumination (Lorenz, 2012).

2.8. Treatment

The use of antibiotics in the treatment of STEC infection is controversial (Panos *et al*., 2006). Some authors reported an increase in the level of stx production and a greater risk of fatal complications following administration of antibiotics in STEC infection (Zhang *et al*., 2000). However, others suggest that some antimicrobials, if administered early in the course of infection, may prevent disease progression to HUS (Schroeder *et al*., 2002).

In vitro studies showing most strains are susceptible to various antibiotics, although certain antibiotics, at sub-lethal concentrations may increase the release of shiga-like toxin which has been associated with the development of HUS (Collins and Green, 2010). Antimicrobials may lyse bacterial cell walls, thereby liberating stx, and/or cause increased expression of stx genes in vivo (Schroeder *et al*., 2002). As a result, treatment of infection with EHEC strains, including *E. coli* O157:H7 is mainly based on fluid and electrolyte replacement (Rahal *et al*., 2012).

In light of the difficulties in treating this agent, alternate treatment approaches were investigated. A study by Ogawa *et al*. (2001) illustrates the use of natural products for the treatment of *E. coli* O157:H7. The study was performed on infant rabbits and it indicates that the administration of *Lactobacillus casei* had a protective effect against the toxins of *E. coli* O157:H7. It enhances the local immune responses to STEC cells and stxs, and leads to elimination of STEC and thus decreases stx concentrations in the intestines.

2.10. Control and Prevention of *E. coli* O157:H7 Infection

The key points in controlling and preventing *E. coli* infections are management interventions, infections control and vaccination strategies (Kabir, 2010). In cattle, control of *E. coli* O157:H7 is possible through vaccination. The goal of vaccination is either to reduce the susceptibility of cattle to colonization by *E. coli* O157:H7 or to decrease the duration of such colonization. *E. coli* O157:H7 adheres to the wall of the large intestine by secreting virulence factors directly into host cells. The adherence of the organism could be prevented by a vaccine that induces

production of antibodies against these virulence factors and result in its elimination from the gastrointestinal tract (Bach *et al*., 2002).

Prevention and control measures for *E. coli* O157:H7 infection need to be applied at all stages of the food chain, from farm to fork. So, effective control of the agent requires a multi-faced approach (Kiranmayi *et al*., 2010). In addition to reducing the frequency and intensity of *E. coli* O157:H7 fecal shedding by animals exposed to the organism, control of *E. coli* O157:H7 in environmental sources such as water troughs, feed and manure is critical in breaking the cycle of infection and re infection of livestock (Bach *et al*., 2002).

During STEC outbreaks in the community or hospitals, people infected should be isolated to contain the outbreak. Infections that may be complicated by HUS may be spread through person-to-person contact. So hand washing is the most effective intervention (Dagne *et al*., 2019). Animals should also be kept healthy, visitors should be informed about measures to prevent catching diseases from animals, food should be prohibited in areas where animals are kept, and areas for food consumption should be separated from animals by a transition area (National Associate of State Public Health Veterinarians, 2011).

To control the risk of human infection through direct contact with farm animals, strict hygiene practices should be established, including controlling the movement of visitors to farms, restricting access to farm animals, making washing facilities readily available, providing a means of disinfection in case visitors come into contact with the animals (Fairbrother and Nadeau, 2006). It is also very important to cook and reheat food thoroughly, especially meat, poultry, eggs and seafood, to ensure food reaches boiling temperature (≥70°C) to kill *E. coli* O157:H7 (Bach *et al*., 2002).

2.9. Antimicrobial Resistance

Antimicrobial resistance (AMR) is one of the most serious global public health threats in this century (Murray *et al*., 2022). AMR, especially of pathogenic bacteria has been partly attributed to the misuse of antibiotic agents in medicine and agriculture (Sharma *et al*., 2018). *E. coli*

O157:H7 is an emerging and major zoonotic foodborne pathogen with an increasing concern for the emergence and spread of antibiotic-resistant strains which may results in sporadic cases to serious outbreaks in the whole world (Tadesse *et al*., 2012; Minda and Shimelis, 2021). The antimicrobial susceptibility test of the isolates can be performed using disc diffusion method according to CLSI (2018) using different numbers of antibiotic discs (Ayenew *et al*., 2021).

According to the study by Disassa *et al*. (2017), most of the *E. coli* O157:H7 (≥50%) isolates are resistant to tetracycline (81.8%), streptomycin (81.8%), kanamycin (63.6%), cefoxitin (54.5%), and norfloxacin (54.5%). Another study by Minda and Shimelis (2021) on food of animal origin at different catering establishments in the selected study settings of Arsi Zone indicated that, of the four *E. coli* O157 : H7 isolates subjected to 10 panels of antimicrobial discs, 3 (75%) were highly resistant to kanamycin, streptomycin, and nitrofurantoin. According to study by Abreham *et al*. (2019) on sheep and goat carcass at an export abattoir, only three antibiotics namely Norfloxacin, Ceftazidime and Polymyxin B were seen to be effective against this bacteria, but all the rest of the antibiotics used (Amoxicillin (25 μg), Clindamycine (10 μg), Cloxacillin (5 μg), Doxycycline (30 μg) and others) has no effect on it.

According to Shecho *et al*. (2017), of the total 14 antimicrobials included in the panel of study, the susceptibility results varied with 96.15% and 0% of *E. coli* O157:H7 isolates expressing resistance to erythromycin, clindamycin, spectinomycin, and ciprofloxacin, respectively. Resistance to more than two antimicrobial agents was detected in 24 (92.30%) of the isolates. The results of antimicrobial susceptibility test of *E. coli* isolated from backyard chicken in and around Ambo by Sarba *et al*. (2019) show that, high susceptibility (100%) of *E. coli* to ciprofloxacin, sulfamethoxazole-trimethoprim and norfloxacin followed by gentamicin (89%), streptomycin (85%), ceftazidime (84.6%), nalidxic acid (83%), nitrofurantoin (76.8%), kanamycin (75%), amikacin (69.6%) and chloramphenicol (63.4%). *E. coli* were resistant to 12 of the 17 antimicrobials tested. Higher resistance (100%) was observed to cloxacillin, cefuroxime and amoxicillin followed by cefotaxime (92.7%), tetracycline (46.3%), nitrofurantoin (23.2%) and chloramphenicol (17.1%).

The results of antimicrobial susceptibility test of *E. coli* O157:H7 isolated from chicken droppings produced by free - ranged and poultry birds in cross river, Nigeria by Joseph (2018) showed that, out of the 9 isolates screened, 8 (88.89%) were resistant to tetracycline, 7 (77.78%) to ampicillin and nitrofurantoin and 6 (66.67%) to chloramphenicol. All isolates were resistant to at least one antibiotic. Development of antibiotic resistance among bacteria such as *E. coli* poses an important public health concern. Effectiveness of treatments and ability to control infectious diseases in both animals and humans may be severely hampered (Thaker *et al*., 2012).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted from November 2021 to May 2022 in Bishoftu town, which is located at 90^0N and 40^0E with an altitude of 1880 m.a.s.l in the central highlands of Ethiopia, 47 Km South East of Addis Ababa (Figure 4). It has annual rainfall of 1151.6mm of which 84% falls down during the long rainy season that extends from June to September and the remaining during the short rainy season that extends from March to May. The mean annual minimum and maximum temperatures are 8.5ºC and 30ºC, respectively, and the mean humidity is 61.3% (National Metrological Service Agency, 2005). Small, Medium, and Large-scale intensive layer farms are located in Addis Ababa, Bishoftu, Modjo and Adama (FAO, 2019). The study comprised commercial layer poultry farms in Bishoftu town. There are 21 commercial layer poultry farms, including ELFORA, Maranatha and Alema, located in Bishoftu town (Dawit *et al*., 2011; Ismael *et al*., 2021).

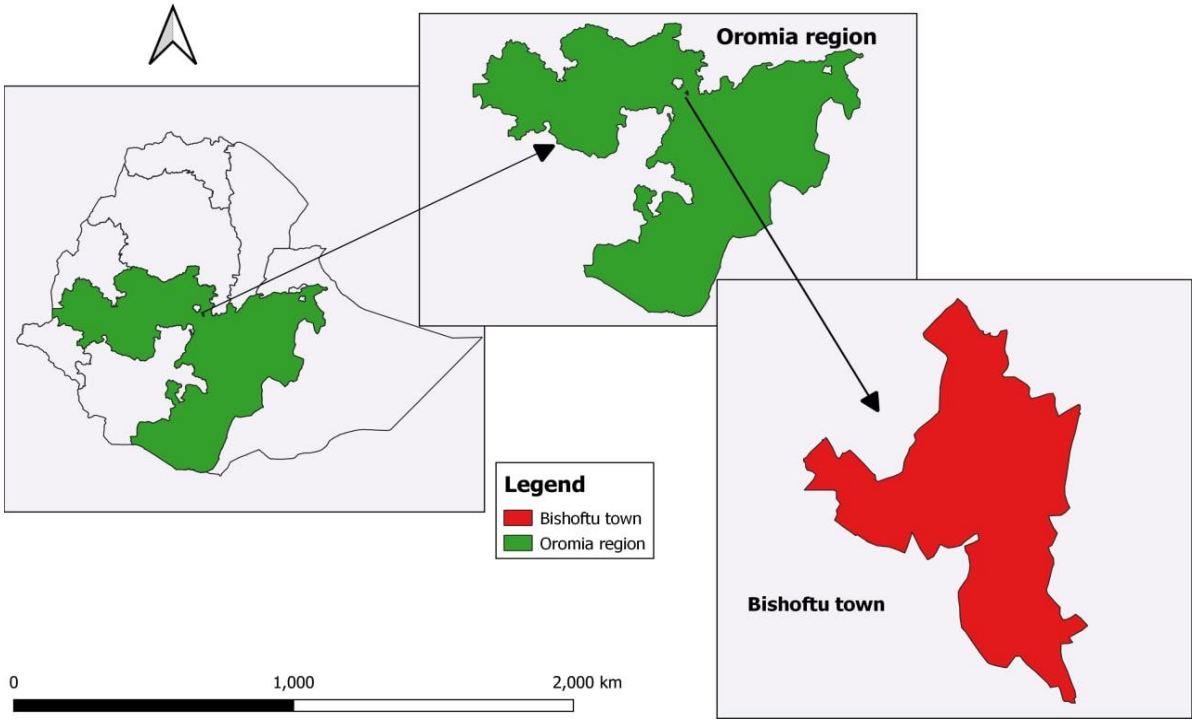


Figure 4: Map of Bishoftu town, East Showa zone, Oromia region, Ethiopia.

3.2. Study Population and Study Samples

The study on isolation, characterization and determination of the antimicrobial resistance profile of *E. coli* O157: H7 was conducted on randomly selected chickens from layer poultry farms found in Bishoftu town, Ethiopia. The study predominantly comprised chickens from 4-70 weeks of age and exotic breeds kept under small, medium and large scale farms. The farms were first categorized according to Dawit *et al*. (2011); Vernooij *et al*. (2012) in to a small scale (<1000 chickens), medium scale (1001–10,000 chickens), and large scale (>10,001 chickens) farms. The age was conveniently classified into young growers up to six months (26 weeks) of age and adult layer chickens according to previously indicated by Shecho *et al*. (2017). The target population of the study comprised 11 commercial layer poultry farms in Bishoftu town *i.e.* 3 large scale, 4 medium scale and 4 small scale poultry farms. A total of 420 samples were collected and processed from the target population. Of the 420 samples collected, 124 were from Large-scale farms, 144 were from Medium-scale and 152 were from Small-scale layer commercial poultry farms. From each large, medium, and small-scale layer poultry farm, 41, 36, and 38 samples were collected, respectively. The study samples include; cloacae swab, egg shell swab, hand swab from farm attendants, content of the egg and environmental samples like water, feed and poultry litter samples.

3.3. Study Design

All through the study period, a cross-sectional study design was followed to isolate, characterize and determine the antimicrobial resistance profile of *E. coli* O157: H7 from all study samples collected from different layer commercial poultry farms in Bishoftu town, Ethiopia.

3.4. Sample Size Determination

Sample size was determined using the formula given by Thrusfield, (2005).

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

Where, n=number of sample size,

P_i= expected prevalence of *E. coli* O157:H7 and,

d=desired precision level.

Therefore, using 50% expected prevalence, at a confidence level of 95% and required absolute precision of 5%, the minimum calculated sample size was 384. But 420 samples (115 cloacae swabs. 115 egg shell swabs, 25 hand swabs, 85 environmental samples and 80 egg internal contents) were collected to maximize the precision of the study. The number of samples from each sample types was distributed in to small, medium and large scale poultry farms proportionally.

3.5. Sampling Techniques

Stratified random sampling was carried out to categorize layer poultry farms into small, medium, and large scale farms based on the number of chickens raised on the farms. Then, systematic random selection was used for individual layer farms from each stratum, and again, Simple random sampling was used to collect all study samples from each selected farm. Data regarding different risk factors were collected by using semi-structured questionnaires and observational check lists for farm level data.

3.6. Sample Collection and Transportation

The cloacae, egg shell swabs, personnel hand swabs, and environmental samples were collected from layer poultry farms using pre-sterilized cotton swabs and plastic bags. The cloacae samples were collected according to the method previously indicated by Shecho *et al*. (2017), by inserting moistened sterile swabs into the cloacae of the chickens and gently swabbing the mucosal wall, taking any fluid or fecal material around the cloacae. Egg shell swabs were taken by gently swabbing the surface of eggs collected from the site. Following the observation of personnel responses from the consent note, hand swabs were collected from interested farm workers by swabbing both hands. All of the environmental samples (poultry litter, feed and water) were collected by using sterile plastic bags and labeled. The collected swab samples were immediately transferred into screw-capped test tubes containing buffered peptone water and labeled accordingly using markers. For the contents of the eggs, randomly selected eggs were taken carefully using plastic bags and labeled accordingly. Then labeled samples were kept in an ice box containing ice packs. Finally, the collected samples were transported in icebox from the

collection site to the Veterinary Microbiology Laboratory of Addis Ababa University, College of Veterinary Medicine and Agriculture for examination.

3.7. Questionnaire Survey

A pretested semi-structured questionnaire and observational checklists were used to collect the necessary farm-level data. They were designed mainly to obtain hygienic status and biosecurity practices in the farms. The questionnaire interviews were used to collect data from 11 farm owner/managers (one from each farm). It was developed to collect data about socio-demographic characteristics, training on chicken handling, farm management and biosecurity status, hygienic status of the workers and others. Observational checklist was used to collect data regarding housing, storage of chicken feed and water, feces and litter management, hygienic status, utilization of on farm cloths and footwear, utilization of footbath dip at entry gate, rearing animals with suitable density and possible sources of contamination (Appendix 2).

3.8. Bacteriological Methods

3.8.1. Sample preparation and isolation procedures

The properly collected cloacae and hand swab samples were placed in sterile tubes containing 9 mL buffered peptone water and incubated aerobically at 37^{o}C for 24 hours. Environmental samples such as litter and feed were streaked on modified tryptic soy broth by mixing 25 grams of each sample in 225 mL of modified tryptic soy broth using homogenizer at 3000 rpm and incubated aerobically at 37 ^{o} C for 24 hours (Ethelberg *et al*., 2009). The water samples were enriched by mixing 10 mL of each water samples in 90 mL of modified tryptic soy broth (HiMedia, India) and incubated at 37°C for 24 hours.

Samples of egg were processed according to Loongyai *et al*. (2011) in which external shell of eggs were swabbed with sterile cotton swabs dipped in 9 mL of sterile buffered peptone water and subsequently incubated for 24 h at 37^{o}C. In order to prepare the contents of eggs, first egg surfaces were sterilized by immersion in 75% alcohol for 2 min, air dried for 10 min, and then cracked with a sterile spoon. Each egg contents was mixed thoroughly by vortex and 1 mL of the

mixed egg contents was inoculated into 9 mL of buffered peptone water and incubated at 37ºC for 24 hours.

Isolation of *E. coli* was carried out according to ISO 16654 (2001); Hamisi *et al*. (2014). A loop full of the inoculated peptone water culture was transferred (sub cultured) onto the MacConkey and EMB agar to observe the colony morphology (shape, size, surface texture, edge, color and opacity). Then, a pink colony from MacConkey agar was picked and sub-cultured onto EMB agar until a pure culture with homogenous colonies was obtained. Colonies with a metallic green sheen on EMB (characteristic of *E. coli*) were later characterized microscopically using Gram stain (Sarba *et al*., 2019).

3.8.2. Biochemical tests and Serotyping of E. coli O157:H7

After isolation of the organism on the selective media, the selected pure colonies were streaked onto the surface of pre-dried Nutrient agar (Oxoid Ltd, UK) plates in a manner that allow isolated colonies to develop and incubated at 37ºC for 24 hours for further identification and confirmation with biochemical tests (Jorgensen *et al*., 2015). Then the isolates were subjected to different biochemical tests according to Prescott (2003) such as TSI agar (HiMedia, India), indole production, citrate utilization test, methyl red and voges-proskauer.

Pure colonies from nutrient agar culture were picked and inoculated into biochemical test tubes containing TSI agar, Simon's citrate agar, MRVP and tryptone broth, and then incubated for 24 hours at 37^0C (for the citrate utilization test, 72 hours of incubation was used). Colonies producing yellow slant, yellow butt, presence of gas bubbles, and absence of black precipitate in the butt were observed, which indicates growth of *E. coli* on TSI agar (Islam *et al*., 2014). No growth and no color change on Simon's citrate agar indicated by the green color of the slant were considered as *E. coli*. A few drops of Kovac's reagent were added to the mixture containing tryptone broth and the formation of a red or pink colored ring at the top was a positive reaction, which was considered *E. coli* for indole test. On MRVP broth, red color development after addition of three to five drops of MR reagent (MR test) and no color change after 1 hour of addition of 15 drops of alpha-naphthol and five drops of 40% potassium hydroxide to the test

broth (VP test) were considered *E. coli* (All test principles and procedures are written in Appendix 1) (Lupindu, 2017).

The bacterium that was confirmed as *E. coli* was sub-cultured onto SMAC agar (Oxoid Ltd., UK) supplemented with 0.05 mg/l Cefixime and 2.5 mg/l potassium tellurite. The colorless colonies obtained from the culture (non-sorbitol fermenter) were again sub-cultured onto nutrient agar. Non-sorbitol-fermenting *E. coli* (pale colonies) were considered presumptive *E. coli* O157: H7, whereas pinkish colored colonies (sorbitol fermenters) as non-O157:H7 *E. coli*.

For confirmation of *E. coli* O157: H7, latex agglutination test was performed using an *E. coli* O157:H7 latex test kit (Oxoid Ltd, UK, DRO620M). The kit consists of four components, namely, the latex test reagent, a latex particles sensitized with specific rabbit antibody against O157 antigen, the latex control reagent consisting of latex particles sensitized with pre-immune rabbit globulin, positive and negative controls which are suspension of inactivated *E. coli* O157:H7 cells and a suspension of inactivated non-specific *E. coli* cells and reaction slides. The agglutination test was performed to determine strains using polyvalent antisera (Manyi-Loh *et al*., 2018; Haile *et al*., 2021). The test was carried out in accordance with the manufacturer's instructions (Appendix 1). First, the latex kit's performance was evaluated using the kit's control suspensions. The test was continued after the positive control reacted positively to the test latex and the negative control reacted negatively.

3.9. Molecular Characterization of *E. coli* O157:H7

Conventional PCR assay was conducted to assess the presence of virulence genes (*stx1*and *eae*) in *E. coli* O157:H7 colonies following the previously described methods by (Hasan *et al*., 2016). DNA was first extracted from *E. coli* isolates by using standard boiling method of DNA extraction, according to the technique described previously by Wasilenko *et al*. (2012); Firoozeh *et al*. (2014). Thus, suspected fresh colonies were selected and suspended separately in 100 µl of sterile distilled water in eppendorf tubes; the suspensions were then boiled in a water bath at 92.5 °C for 17 minutes. After centrifuging at 13000 rpm for 10 min, the supernatant containing the template DNA was transferred into nuclease-free eppendorf tubes. The extracted DNA was used

as a template for conventional-PCR to amplify the virulence genes using specific *eae* and *stx1* primers.

Amplification of DNA was conducted using initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and 30 seconds, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min, and finally putting at 4 °C until the machine turned off. Visualization of the amplified product was done by mixing the 4μL gel red with loading dye, 10μL PCR products and 10-μL markers (ladder) onto a 2% agarose gel. Electrophoresis was conducted at 125 V for 1:20 hour. A 100-1000 base pair molecular weight marker was used to identify the amplified products as a ladder, which was visualized by UV illumination. The sequences of the primers and amount of reagents required for one reaction master mix preparation are shown in Table 2 below.

Table 2: Primers sequence used in conventional PCR for amplification of stx1 and *eae* genes

Types of reagent	**For one reaction**
RNase free water	3μL
Primer **EAE-forward**-5pm/μl (5'-AAACAGGTGAAACTGTTGCC-3')	
Primer **EVT1forward**-5pm/ μl (5'-CAACACTGGATGATCTCAG-3')	2μL
Primer **EAE-reverse**-5pm/μl (5'-CTCTGCAGATTAACCTCTGC-3')	
Primer **EVT1-reverse**-5pm/ μl (5'-CCCCCTCAACTGCTAATA-3')	2μL
IQ super mix	10μL
DNA template	3μL
Total volume	**20μL**

EAE (intimin), EVT (Shiga toxin1)

3.10. Antimicrobial Susceptibility Test

Antimicrobial susceptibility of the *E. coli* isolates was determined by disk diffusion method (Kirby-Bauer) according to CLSI (2019) on Mueller-Hinton agar. For the susceptibility testing, the following twelve antimicrobial drugs and concentrations were used: ampicillin (10 μg),

amoxicillin (20 μg), cefotaxime (15 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), erythromycin (15 μg), gentamycin (10 μg), nalidixic acid (30 μg), streptomycin (10 μg), tetracycline (30 μg), and trimethoprim (25 μg) (Oxoid Ltd, UK).

Briefly, a bacterial suspension with a dilution equivalent to the opacity of 0.5 McFarland tubes was prepared. Then, the suspension was inoculated on the plates containing Mueller Hinton agar medium, and then antibiotic disks were placed on the medium. The inoculated plates were incubated at 37°C for 18 hours. After an incubation period, the diameters of inhibition zones of bacterial growth around the disks were measured in millimeters and compared to the table provided in the standard described by CLSI and were determined to be sensitive, intermediate, and resistant (Table 3) (CLSI, 2013).

Table 3: Diameters of zones of inhibition used for interpretation of antimicrobial susceptibility for Enterobacteriaceae

Antimicrobial agents and symbols	**Disc potency (μg)**	**Zone diameter, nearest whole mm**		
		Resistance	Intermediate	Susceptible
Amoxicillin (AML)	20	≤13	14–16	≥17
Ampicillin (AMP)	10	≤13	14–16	≥17
Erythromycin (ERY)	15	≤13	14–22	≥23
Chloramphenicol (C)	30	≤12	13–17	≥18
Ciprofloxacin (CIP)	5	≤14	15–17	≥21
Ceftazidime (CTZ)	30	≤17	18-20	≥21
Cefotaxime (CTX)	15	≤13	14–17	≥18
Gentamycin (CN)	10	≤12	13-14	≥15
Sulfamethoxazole (SXT)	25	≤10	11-15	≥16
Nalidixic acid (NAL)	30	≤13	14-18	≥19
Streptomycin (S)	10	≤11	12–14	≥15
Tetracycline (TE)	30	≤11	12–14	≥15

Resistance to more than two antimicrobials was taken as multidrug resistant (MDR) isolates. MDR of individual isolate was calculated by dividing the number of antibiotics to which the isol

ate was resistant by the total number of antibiotics to which the isolate was exposed (Selim *et al*., 2013).

3.11. Inclusion and Exclusion Criteria's

3.11.1. Inclusion criteria's

Chickens from 4 to 70 weeks of age and exotic breeds kept on small, medium, and large-sized layer poultry farms, as well as poultry farm workers who were interested in taking part in the study, were included. Their willingness was known after they filled out the consent note (Appendix 2). The antibiotic discs were selected based on their availability, potential public health importance, frequent use in poultry farms, and recommendation from the CLSI guideline.

3.11.2. Exclusion criteria's

Broiler poultry farms, and layer poultry farms whose owners or managers were unwilling to participate in the study, as well as farm personnel who refused to provide hand swab samples, were excluded.

3.12. Quality Control

Confidences in the reliability of test results were increased by adequate quality assurance procedures. First sample collecting materials were sterilized using hot-air oven and their sterility was checked randomly by culturing on nutrient agar. Sterility of culture media was checked by incubating the prepared media for 24 hrs at 37^{o}C and then by observing growth of any contaminants. For the latex agglutination test, the quality and validity of the test kits was checked by using positive, negative and latex control. Moreover, the whole procedures and results interpretation were done following standard operating procedure. For PCR analysis, all the steps were conducted according to standard procedure, and validity of the test was checked by observing the correctness of positive and negative control. The questionnaire was daily checked by the principal investigator and advisor for its completeness.

3.13. Data Management and Analysis

Questionnaire and laboratory data were first coded and managed into Microsoft Excel and analyzed using R- statistical software version 4.0.3. Descriptive analysis such as sum and frequency distribution were computed. Chi-square (X^2) test and firth's bias-reduced logistic regression were used to measure the association between the different risk factors and occurrence of *E. coli* O157:H7 in poultry farms. The degree of associations was assessed using an odds ratio (OR) with univariable and multivariable logistic regression analyses. Before regression analysis, the data was checked for fulfillment of assumptions and stepwise logistic regression model was used to analyze and regress those factors having a significant putative effect on the occurrence of disease based on a *P*-value < 0.05 as the significance threshold for entries and removals. Goodness of fit for the model was checked by the Hosmer and Lemeshow goodness of fit test. The model appear fit, if the goodness of fit test *P*-value is greater than 0.05 and the calculated chi-square value is less than the table value. To map location of study area, QGIS version 3.20 GIS software was used. In all the analyses, the confidence level was held at 95% and P<0.05 was set for significance. The percentages of AMR of each pattern (Susceptible, Intermediate and Resistance) were calculated and discussed in percentages.

3.14. Ethical Clearance

Ethical clearance was obtained from animal research ethical review committee of Addis Ababa University College of Veterinary Medicine and Agriculture (Certificate Ref. No: VM/ERC/08/02/14/2022).

4. RESULTS

4.1. Prevalence of *E. coli* O157: H7 in Poultry Farms

The overall prevalence of *E. coli* O157: H7 in all sample types was 16 (3.81%; 95% CI: 2.19, 6.11). Of which, 9 (56.25%) were from cloacae, 3 (18.75%) were from litter samples, 1 (6.25%) was from worker hand swabs, and 3 (18.75%) from egg shell swabs. However, there was no *E. coli* O157: H7 positive isolate from samples of the contents of the eggs, feed, and water. Cloacae were found to be the most common source of contamination. The test statistics among study samples with $P > 0.05$ (Table 4) indicated that there was no significant difference in prevalence rate among all types of the study samples.

Table 4: *E. coli* O157: H7 distribution in different study samples in the study area

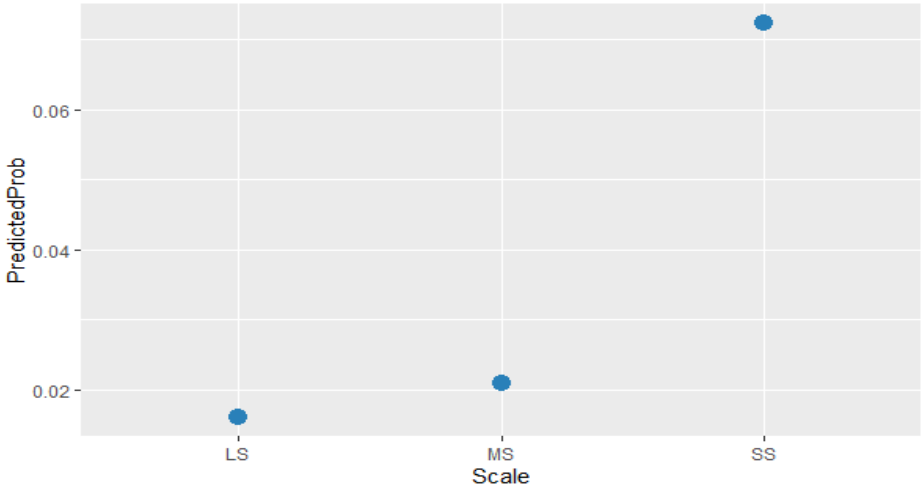
Sample types	**Number of positive**	**Proportion (%)**	**OR [95% CI]**	***P*- value**
Cloacae swab	9	56.25	Ref	
Egg shell swab	3	18.75	0.35 [0.09-1.22]	0.100
Egg content	0	0.00	0.07 [0.06-1.21]	0.068
Feed sample	0	0.00	0.27 [0.02-4.49]	0.378
Water sample	0	0.00	0.25 [0.01-4.44]	0.344
Litter sample	3	18.75	0.95 [0.26-3.39]	0.931
Hand swab	1	6.25	0.72 [0.12-4.24]	0.712
Total	**16**	**1.00**	**[2.19-6.11]**	

OR (odds ratio), *P*-value (Probability value), 95% CI (95% Confidence interval), Ref (reference)

Out of the 16 serologically confirmed isolates, 2 (12.50%) were from large-scale, 3 (18.75%) were from medium-scale, and 11 (68.75%) were from small-scale poultry farms. Small-scale farms had a higher prevalence of *E. coli* O157:H7 than medium and large-scale layer poultry farms. The higher prevalence of *E. coli* O157:H7 in small-scale farms was statistically significant with $P = 0.045$ and OR = 4.8 [95% CI = 1.25–31.14] when compared to large and small-scale poultry farms (Table 5). However, there was no discernible difference in the prevalence of *E. coli* O157:H7 between medium-scale and large-scale farms.

Table 5: Distribution of serologically confirmed *E. coli* O157: H7 across the different farm sizes

Farm sizes	Number of positive	Proportion (%)	OR (95%CI)	*P*- value
Large scale	2	12.50	Ref	
Medium scale	3	18.75	1.3 [0.21-9.97]	0.777
Small scale	11	68.75	4.8 [1.25-31.14]	0.045
Total	**16**	**1.00**		



LS=Large scale (0.016), MS=Medium scale (0.02), SS= Small scale (0.072)

Figure 5: ggplot for prevalence of serologically confirmed *E. coli* O157: H7 across the different poultry farm sizes

Out of the 16 serologically confirmed isolates, 3 (18.75%) were from young chickens and 13 (81.25%) were from adult chickens. As presented in the Table 6 below, a higher prevalence of *E. coli* O157: H7 was found in adult than in young chickens. There was a marginally significant difference in prevalence of *E. coli* O157:H7 between young and adult age groups of chickens with $P = 0.050$ and OR = 3.55 [95% CI=1.12-15.7].

Table 6: Distribution of *E. coli* O157:H7 in different age groups of chicken studied

Age group	Number of positive	Proportion (%)	OR [95%CI]	*P*- value
Young	3	18.75	Ref	
Adult	13	81.25	3.55 [1.12- 15.7]	0.050
Total	**16**	**1.00**		

OR (odds ratio), *P*-value (Probability value), 95% CI (95% Confidence interval), Ref(Reference)

The prevalence of *E. coli* O157:H7 was assessed at different sizes of poultry farms, considering different operational and structural biosecurity measures as risk factors for the occurrence of the bacteria (Table 7**).** *E. coli* O157: H7 was more prevalent in farms with personnel who had an animal health educational compared to farms with personnel who had no an animal health education (P=0.003, OR=19.5 at 95% CI=2.68-141.7). The test statistic also indicated a significant difference in the prevalence of *E. coli* O157:H7 between farms with personnel who disinfected their hands before and after handling poultry when compared with farms with personnel who didn't disinfect their hands (P=0.013, OR=6.6, 95% CI=1.48-29.4).

There was a significant difference in the prevalence (*P*= 0.05) of *E. coli* O157: H7 between farms that had litter or cage disinfected after being emptied and those that had no litter disinfected after being emptied (P = 0.033, OR = 3.63 at 95% CI of 1.11–11.9). The prevalence of *E. coli* O157: H7 was also assessed in different layer farms, taking into account factors such as disinfection of materials directly used for chickens, utilization of footbath dip at entry gate, record keeping, and level of litter management. However, there was no statistically significant difference among those risk factors in the prevalence of *E. coli* O157: H7.

Table 7 : Results of statistical analysis showing association between hypothesized risk factors and occurrence of *E. coli* O157:H7

Risk factors	Prevalence (%)	OR (95% CI)	*P*-value
Educational level of the respondent			
9-12	4(10.50)	19.5 [2.68 -141.7]	0.003
>12	10 (5.40)	7.76 [1.53 - 39.4]	0.013
Animal health	1 (1.00)	Ref	
Training on biosecurity for employees of the farm			
Yes	4(2.50)	Ref	
No	12 (4.90)	1.34 [0.46 - 3.93]	0.045
Hand hygiene before and after handling poultry			
Yes	2(1.00)	Ref	
No	14 (6.3)	6.6 [1.48 - 29.4]	0.013
Disinfection of materials directly used for chickens			
Yes	1 (1.2)	Ref	
No	15 (4.4)	3.76 [0.48 - 28.9]	0.203
Disinfection of a litter after being emptied			
Yes	12 (3.1)	Ref	
No	4 (10.5)	3.63 [1.11 - 11.9]	0.033
Record keeping			
Yes	14 (4.7)	Ref	
No	2 (1.6)	3.03 [0.68 - 13.5]	0.147
Utilization of footbath dip at entry gate			
Yes	6 (2.5)	Ref	
No	10 (5.5)	2.22 [0.79 - 6.24]	0.128
Faeces and litter management			

Good	5 (3.1)	Ref	
Poor	5 (6.8)	2.28 [0.64 - 8.11]	0.205
Moderate	6 (3.3)	1.06 [0.32 - 3.54)	0.926

Ref (Reference)

In the multivariable logistic regression only farm size, age of chicken, and training on biosecurity remain significantly associated with prevalence of *E. coli* O157: H7 (Table 8). Even though there was no statistically significant difference in prevalence among the different sample types, it was included in the multivariable logistic regression below to clarify the discussion more.

Table 8: Results of multivariable logistic regression analysis of risk factors in relation to occurrence of *E. coli* O157: H7

Predictors	**Prevalence (%)**	**OR [95% CI]**	***P*-value**
Study sample			
[Cloacae swab]	7.83%	Ref	
[Egg shell swab]	2.61%	0.35 [0.09 - 1.22]	0.100
[Content of the egg]	0.00%	0.07 [0.06 - 1.21]	0.068
[Feed sample]	0.00%	0.27 [0.02 - 4.49]	0.378
[Water sample]	0.00%	0.25 [0.01 - 4.44]	0.344
[Litter sample]	6.82%	0.95 [0.26 - 3.39]	0.931
[Hand swab]	4.17%	0.72 [0.12 - 4.24]	0.712
Farm Size			
[Large]	1.61%	Ref	
[Medium]	2.08%	1.36 [0.22 – 10.50]	0.737
[Small]	7.23%	5.50 [1.42 – 36.21]	**0.030**
Age			
[Young]	1.62%	Ref	
[Adult]	5.53%	4.13 [1.29 – 18.38]	**0.022**
Biosecurity Training			
[No]	4.90%	Ref	
[Yes]	2.50%	0.75 [0.23 – 2.09]	**0.041**

Ref (factor that used as a reference in a statistical analysis)

4.2. Molecular Detection of Virulence Genes from *E. coli* O157: H7 Isolates

From the total of 16 serologically confirmed *E. coli* O157:H7 isolates that were submitted to the Animal Health Institute (AHI), Sebeta, Ethiopia, seven (43.75%) had the *eae* virulent gene and were negative for the stx1 virulent gene, using conventional PCR analysis. Due to the absence of specific primers for stx2 and *hlyA* virulence genes, *E. coli* O157:H7 isolates were detected only for *eae* and stx1 virulence genes. The *eae* gene was detected in *E. coli* O157:H7 isolates from cloacae, egg shell swabs and poultry litter sample. Table 9 shows the distribution of *eae* virulence gene among *E. coli* O157:H7 isolates. The results of molecular analysis amplifying the 470bp fragment of the *eae* gene are shown in Figure 6**.**

Table 9: Distribution of intimin virulence gene among *E. coli* O157:H7 isolates

Sample type	**No of isolates examined**	**No of intimin gene detected**
Cloacae swab	9	4
Egg shell swab	3	2
Poultry litter sample	3	1
Workers hand swab	1	0
Total	**16**	**7**

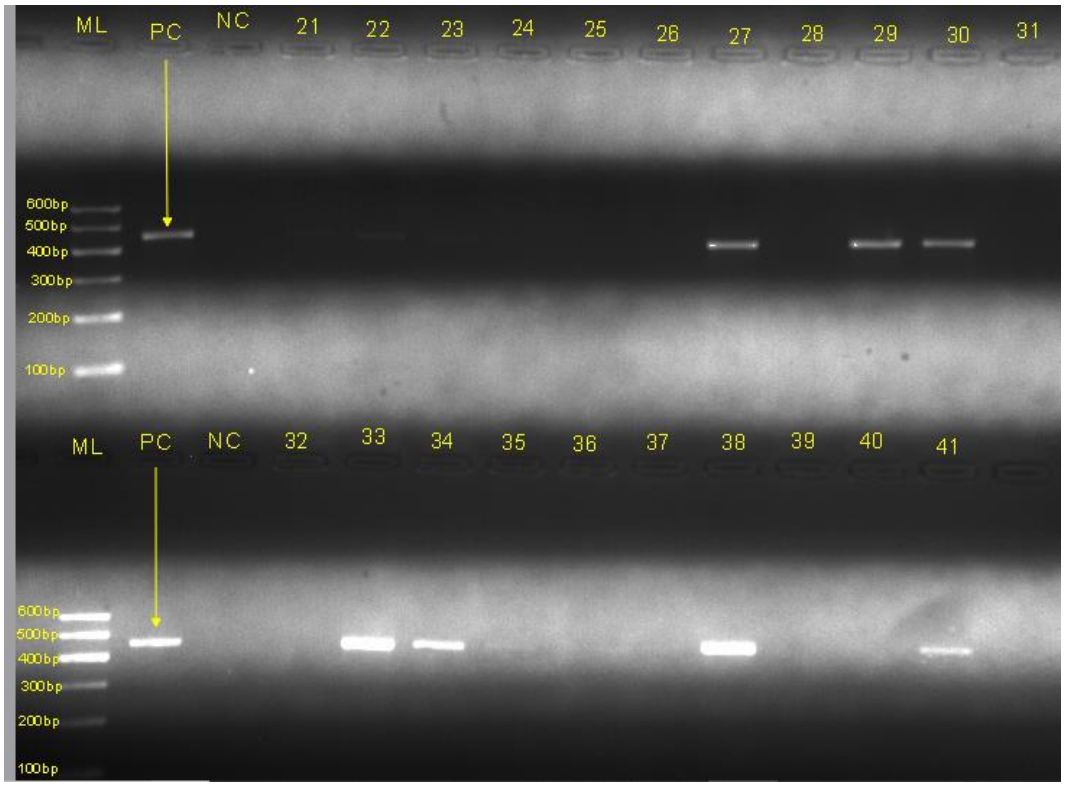


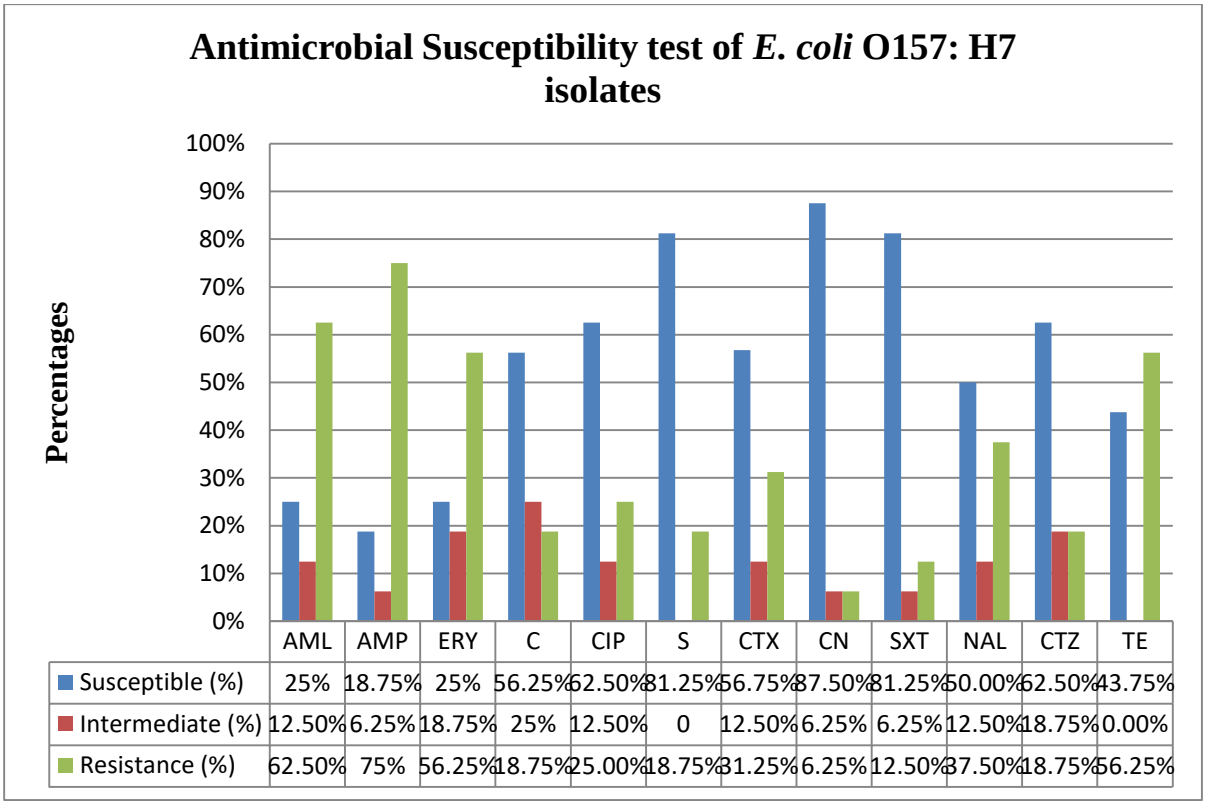
Figure 6: Gel electrophoresis of amplified products of *eae* genes in *E. coli* O157:H7 strains isolated from layer poultry farms in Bishoftu, Ethiopia.

***Key**: Lane ML (DNA ladder), Lane: PC (Positive control), Lane NC (negative control), and Lane 21-41 (PCR products), Lane 27, 29, 30, 33, 34, 38, and 41 are positive for eae gene.*

4.3. Results of Antimicrobial Susceptibility Test of *E. coli* O157: H7

In this study, all the 16 serologically confirmed *E. coli* O157: H7 isolates were subjected to an antimicrobial susceptibility test, using twelve different antimicrobial agents. Among the 12 antimicrobial agents used, maximum resistance (≥ 50.00%) was observed against ampicillin (75.00%), amoxicillin (62.50%), erythromycin (56.25%), and tetracycline (56.25%). Furthermore, *E. coli* O157: H7 isolates were resistant to nalidixic acid (37.50%), cefotaxime (31.25%), and ciprofloxacin (25.00%). In contrast, gentamycin was the most effective agent of all, with only 6.25% resistance (resistant to only one isolate). In the present study, trimethoprim-sulfamethoxazole was the second most effective antibiotic after gentamycin, with 12.50%

resistance. The results of antimicrobial susceptibility test against all antimicrobial agents are given in Figure 7 below.



	AML	AMP	ERY	C	CIP	S	CTX	CN	SXT	NAL	CTZ	TE
Susceptible (%)	25%	18.75%	25%	56.25%	62.50%	81.25%	56.75%	87.50%	81.25%	50.00%	62.50%	43.75%
Intermediate (%)	12.50%	6.25%	18.75%	25%	12.50%	0	12.50%	6.25%	6.25%	12.50%	18.75%	0.00%
Resistance (%)	62.50%	75%	56.25%	18.75%	25.00%	18.75%	31.25%	6.25%	12.50%	37.50%	18.75%	56.25%

Amoxicillin (AML), Ampicillin (AMP), Erythromycin (ERY), Chloramphenicol (C), Ciprofloxacin (CIP), Streptomycin (S), Cefotaxime (CTX), Gentamycin (CN), Sulfamethoxazole (SXT), Nalidixic Acid (NAL), Ceftazidime (CTZ), Tetracycline (TE)

Figure 7: The results of antimicrobial resistance pattern of *E. coli* O157:H7 isolates against 12 selected antimicrobial agents.

4.4. Multidrug Resistance of *E. coli* O157: H7

In this study a total of 11 (68.75%) isolates of *E. coli* O157: H7 were resistant to three or more classes of antimicrobials. The most frequent combinations of antimicrobials where the *E. coli* O157: H7 developed MDR were ampicillin, amoxicillin, nalidixic acid, and tetracycline, which were observed in 27.30% of the *E. coli* O157: H7 isolates. Three isolates of *E. coli* O157: H7 recovered from cloacae samples showed MDR against eight of the twelve antimicrobials used. A considerable number of the isolates were resistant to six or more different classes of antimicrobial agents (Table 10).

Table 10: Biogram of multidrug resistant *E. coli* O157:H7 from layer poultry farms in Bishoftu town.

Type of drugs registered as MDR	Source of MDR isolates #				Total (%)
	Cloacae swabs	Egg shell swabs	Litter samples	Hand swabs	
AMP, ERY, C	1	0	0	0	1 (9.1%)
AMP, AML, ERY	0	1	0	0	1 (9.1%)
ERY, SXT, CTX	0	0	0	1	1 (9.1%)
AMP, AML, NAL, TE	2	0	1	0	3 (27.3%)
AMP, AML, ERY, NAL, S, TE	0	1	0	0	1 (9.1%)
AML, ERY, SXT, NAL, C, CN, TE	0	0	1	0	1 (9.1%)
AMP, AML, ERY, CIP, CTZ, CTX, S,TE	2	0	0	0	2 (18.2%)
AMP, ERY, NAL, CIP, CTZ, CTX, C, TE	1	0	0	0	1 (9.1%)
Total MDR (%)	**6 (54.5%)**	**2 (18.2%)**	**2 (18.2%)**	**1 (9.1%)**	**11 (68.75%)**

MDR (Multidrug Resistance), Amoxicillin (AML), Ampicillin (AMP), Erythromycin (ERY), Chloramphenicol (C), Ciprofloxacin (CIP), Streptomycin (S), Cefotaxime (CTX), Gentamycin (CN), Sulfamethoxazole (SXT), Nalidixic Acid (NAL), Ceftazidime (CTZ), Tetracycline (TE)

Multi-drug resistance profiles against three, four, six, seven and eight antimicrobial classes are 3 (18.75%), 3 (18.75%) 1 (6.25%), 1(6.25%) and 3 (18.75%), respectively. The frequency of resistant phenotype was more common for cloacae swab samples 6 (54.50%). Out of eleven isolates developed MDR; eight (72.70%) were isolated from small scale layer poultry farms. In terms of age group, 8 (72.70%) of isolates developed MDR were isolated from adult chickens. Statistically significant difference was not observed in MDR profiles based on sample types, farm size and age.

Table 11: Analysis of MDR profiles based on sample type, farm size and age

Category		No of antibiotic classes					MDR/ Total	Proportion of MDR[95%CI]	*P*- value
		R3	R4	R6	R7	R8			
Sample type	Cloacae	1	2	0	0	3	6/9	-	
	Egg shell swab	1	0	1	0	0	2/3	0.67[0.05,1.43]	0.170
	Litter sample	0	1	0	1	0	2/3	0.67[0.12,3.93]	0.862
	Hand swab sample	1	0	0	0	0	1/1	1.0 [0.04,4.93]	0.831
Total MDR [95%CI]		3	3	1	1	3	11/16	0.69[0.42,0.98]	0.083
Farm size	Large	1	1	0	0	0	2/2	-	
	Medium	0	0	1	0	0	1/3	0.33[0.02,4.51]	0.489
	Small	2	2	1	1	3	8/11	0.72[0.70,16.3	0.127
Total MDR [95%CI]		3	3	1	1	3	11/16	0.69[0.96,5.9]	0.059
Age	Young	0	1	0	0	2	3/3	-	
	Adult	3	2	1	1	1	8/13	0.62[0.61,9.87]	0.267
Total MDR [95%CI]		3	3	1	1	3	11/16	0.69[0.61,9.87]	0.267

95% CI (95% Confidence Interval), MDR (Multidrug resistant), R3 (Resistant to three antibiotics), R4 (Resistant to four antibiotics), R6 (Resistant to six antibiotics), R7 (Resistant to seven antibiotics), R8 (Resistant to eight antibiotics)

5. DISCUSSION

Escherichia coli O157: H7 infection has been recognized as an important global public health concern (Islam *et al*., 2014). It has been isolated from dairy cattle, calves, chicken, swine, sheep, and their products. In these, chickens have been considered as vehicles of transmission of *E. coli* O157: H7, because once chicks can be colonized by small populations of this pathogen, they will continue to be long-term shedders (Dinçoğlu and Gönülalan, 2016). Though, information on the prevalence of *E. coli* O157: H7 in chickens and their products at the global and wider geographical levels is limited. In Ethiopia, the overall pooled prevalence of *E. coli* O157: H7 was 4% across all samples (Assefa, 2019).

In this study, different samples such as cloacae swabs, egg shell swabs, contents of the eggs, swab samples collected from the hands of chicken handlers, and different environmental samples were tested for the presence of *E. coli* and *E. coli* O157: H7. Accordingly, *E. coli* was detected in 66 (15.71%) of the samples tested, whereas *E. coli* O157: H7 was detected in 16 (3.81%) of the samples. The proportion of positive samples in this study was lower than in the previous study by Shecho *et al*. (2017), who reported 13.40% of *E. coli* O157:H7 isolated from cloacae samples collected from poultry farms in Eastern Ethiopia. Aibinu *et al*. (2007) also isolated 10.00% of *E. coli* O157:H7 from chicken in Lagos and Ogun State, Nigeria, and Gbadamosi *et al*. (2018) reported 6.67% of *E. coli* O157:H7 strain isolated from unfrozen and frozen raw chicken in Ibadan, Nigeria. In another study, Olatoye *et al*. (2012) confirmed 13.00% and 14.00% levels of *E. coli* O157:H7 from Lagos and Ibadan poultry farms, respectively.

The lower prevalence in the present study might be due to the sampling techniques, sample type, geographical location, or the sensitivity of the microbiological technique used for isolation of the bacteria. For instance, the use of immuno-magnetic separation may improve the sensitivity of the detection, which was not used in this study methodology. Moreover, the methods and techniques used in the laboratory identification of STEC in this study could also be responsible. Seasonal distribution of *E. coli* O157:H7 has been reported previously by Cagney *et al*. (2004) with highest prevalence in summer and lowest in winter so it is possible that the contamination rate of present study lower than previous studies (Hamzah *et al*., 2013).

Moderately comparable levels of *E. coli* O157:H7 (2.70%) was reported by Doane *et al*. (2007) from cloacae swabs and environmental samples collected from three chicken farms in Tennessee, North Carolina, and Washington. On the other hand, the finding of the present study was higher than 1.94% and 1.11% reported by Dinçoğlu and Gönülalan (2016); Joseph (2018) from chickens in Sanliurfa region, Southern Turkey and from layer poultry farms in Cross River State, Nigeria, respectively. The higher prevalence of *E. coli* O157: H7 in the present study may probably be due to sample size (high number of sample were collected in the present study when compared with those studies), hygienic status of poultry farms, sampling techniques, geographical location and the methods and techniques used in the laboratory identification of STEC in this study could also be responsible.

The prevalence of *E. coli* from cloacae in this study was 24.40%, which is lower than a previous study by Taddese *et al*. (2020), who reported *E. coli* as the most prevalent bacteria (50.60%) isolated from poultry cloacae in Jimma town, Ethiopia. Despite the fact that the highest prevalence of *E. coli* O157: H7 was found in a faecal sample (7.83%) among the various sample types from poultry farms in this study, the difference was not statistically significant ($p > 0.05$). Similar findings were reported by Aibinu *et al*. (2007); Shecho *et al*. (2017), who reported a higher prevalence of 10.00% and 13.60% of *E. coli* O157: H7 from chicken cloacae in Lagos and Ogun State, Nigeria and Eastern Ethiopia, respectively. Amir *et al*. (2021) also reported 19.00% of STEC, which is a higher number of isolates than the present study from feces of chickens in Pakistan.

Conversely, in this study the prevalence in chicken cloacae is higher than the reports by Kadhim and Kareem (2021), who isolated 1.00% of *E. coli* O157: H7 from cloacae swabs in 100 samples of laying chickens and 5.00% from 100 samples of broilers in the holy Karbala city, Iraq. Observed variations in prevalence of *E. coli* O157: H7 among studies could be attributed to variable methodological modus-operandi to identify the organism, such as sampling and isolation procedures, sampling area and time, culture media of choice, cross contamination with other principal reservoirs and lack of strict hygienic measures among the farms.

In this study, the egg shell prevalence of *E. coli* and *E. coli* O157:H7 were 14.00% and 2.61%, respectively. The present study revealed a prevalence which is lower than previous studies by

Islam *et al*. (2018); Taddese *et al*. (2020), who reported 34.64% and 25.30% of *E. coli* from Dhaka city, Bangladesh and Jimma town poultry farms, respectively. The prevalence of *E. coli* O157: H7 in this study was significantly lower than the prevalence of 9.80% reported by Atoyebi *et al*. (2019) in eggs shell from poultry farms in Ibadan, Oyo State Nigeria. In contrast, Chaemsanit *et al*. (2015) reported 0.00% prevalence of *E. coli* O157: H7 in egg shells, which is lower than in the present study. These variations may probably be due to sampling place (market or farm), number of egg samples examined, contact of eggs with feces and poultry litter, hygienic condition of poultry farms, and methods of eggs collection.

The present study finding strongly suggests that the presence of *E. coli* O157: H7 on egg shells could be due to cross contamination with feces and the poultry environment because positive egg shell swabs were found from farms that where cloacae swabs and litter samples were positive for *E. coli* O157: H7. In fact, the passage of an egg through the chicken cloacae is expected to contribute to the contamination of the egg shell. Furthermore, eggs may also be contaminated after deposition when they come in contact with environmental bacteria (Spitzer, 2015). This is the major reason why hygienic practice is of major importance in poultry farming.

In the present study, contents of the eggs contains 3 (3.75%) of *E. coli* but it is devoid of *E. coli* O157:H7 which is in line with the findings of Chaemsanit *et al*. (2015); Atoyebi *et al*. (2019), who reported 0.00% prevalence of *E. coli* and *E. coli* O157:H7 in contents of the eggs. The absence of *E. coli* O157: H7 in egg contents observed in this study could be due to a natural protective mechanism that makes contents of the eggs contamination difficult. The egg shell and the outer shell membrane that separate the shell from the albumen serve as a major barrier to bacteria. In addition, the albumen gives a basic environment which discourages the proliferation of many bacteria (Spitzer, 2016).

In this study, 25.00% and 6.82% of *E. coli* and *E. coli* O157: H7 were confirmed from poultry litter samples, respectively. This is totally inconsistent with a previous report by Doane *et al*. (2007) who reported 0.00% of *E. coli* O157: H7 from 48 poultry litter samples collected from three chicken farms in Tennessee, North Carolina, and Washington. The present study suggests that poultry litter could be a possible vehicle of *E. coli* O157:H7 on poultry farms.

The poultry farm workers and staff could be exposed to the infected poultry or their contaminated products, thereby acting as bacterial carriers in the community. The most important pathogens in poultry could be transmitted directly or indirectly to humans, including *E. coli* O157:H7 (Sackey *et al*., 2001). The prevalence of 1 (4.17%) from personnel hands swab in the present study was comparable to a previous study done in Iraq 3 (6.00%) by Kadhim and Kareem (2021). In contrast, the prevalence of *E. coli* O157:H7 in this study was lower than the 10.00% and 12.50% prevalence reported by Jan (2018) and Sadeqi *et al*. (2019) in Pakistan and Iran, respectively. The presence of bacteria in the hands of farm workers strongly suggests the circulation of pathogen's in the farms and could be regarded as the primary cause of egg shell contamination, which has public health implication.

Consumption of contaminated feed and water is the most common route of transmission for *E. coli* O157: H7 infections (Rangel *et al*., 2005). In the current study, none of the feed and water samples were positive for *E. coli* O157: H7. On the other hand, (Doane *et al*., 2007; Al-Yasiri and Mohammed, 2018) isolated 4 (8.30%) out of 48 fresh and trough feed samples and 5 (3.33%) out of 150 poultry feed samples, respectively. Suggesting that feed and water may not be a source of *E. coli* O157:H7 contamination on those selected poultry farms.

The higher prevalence of *E. coli* O157:H7 was found at small-scale poultry farms (7.23%) than at medium-scale (2.08%) and large-scale farms (1.61%). Statistical analysis of this result showed significant variation ($P< 0.05$) in the prevalence rate between small-scale and large-scale poultry farms. However, chickens in medium and large-scale poultry farms had an equal chance of being infected by *E. coli* O157: H7. According to this study, chickens in small-scale poultry farms had 5.5 times higher chance of being infected by *E. coli* O157: H7 compared to chickens in large-scale poultry farms. The present study was in line with the study by Elmi *et al*. (2021), who reported a high prevalence of *E. coli* in small-scale farms. Another study by Amare *et al*. (2021) indicated that the average morbidity and mortality of layer chickens in the small-scale farms (7.4±7.2) significantly ($p<0.05$) higher than in the medium-scale farms (4.7±4.1).

The variation in prevalence of *E. coli* O157:H7 among different sizes of poultry farms might be due to biosecurity level differences. The existing evidences by Hailemariam *et al*. (2017)

depicted failure to fully practicing biosecurity measures in the integrated and larger commercial-scale types while virtually no or minimal routine application of biosecurity measures in the small-scale poultry production system. In another study, Amare *et al*. (2021) indicated as the biosecurity score of medium-scale commercial poultry farms (0.69±0.108) significantly ($p<0.001$) higher than the small-scale farms (0.58±0.120) in Ethiopia. Kwoji *et al*. (2019) noted that most small-scale farmers entrust farm management to individuals with little or no formal education or experience in poultry farm practice, and thus pay little attention to bird and environmental hygiene. Furthermore, workers at small-scale poultry farms lacked the necessary equipment to maintain general biosecurity measures.

In this study, a statistically significant difference was observed in the prevalence of *E. coli* O157: H7 between young and adult chickens, with a *P*-value of 0.022. The higher prevalence of *E. coli* O157: H7 was found in adult chickens (5.53%) than in young chickens (1.62%). Their odds ratio value indicted that, adult chicken's had 4.13 times higher chance of being infected by *E. coli* O157: H7 than young chickens. The findings of the present study differ greatly from those reported by Zhao *et al*. (2005); Shecho *et al*. (2017); Joseph (2018), described young animals tend to carry *E. coli* O157:H7 more frequently than adults. The present study finding was in agreement with previous studies by Sarba *et al*. (2019), who reported a high prevalence of *E. coli* in adult chickens. Accordingly, the high prevalence of *E. coli* O157: H7 in adult chickens rather than young ones could be attributed to the fact that adult chickens have a much longer exposure time to infection.

In the present study, the prevalence of *E. coli* O157:H7 was assessed at different sizes of poultry farms, considering different operational and structural biosecurity measures as risk factors for the occurrence of the bacteria. As indicated in Table 9, the simple logistic regression analysis reveals that there was no significant relationship between *E. coli* O157: H7 and disinfection of material directly used for chickens, record keeping, and utilization of footbath dip at entry gate, and feces and litter management. During stepwise multiple logistic regression, only the needs of biosecurity training for farm employees was found to be statistically significant. Accordingly, the odds ratio indicates that *E. coli* O157: H7 was more prevalent on farms with personnel who had no poultry biosecurity training than on farms with personnel who had biosecurity training.

As indicated in table 6, a higher prevalence of *E. coli* O157: H7 was reported in small-scale farms. The result was consistent with farm-level data obtained by semi-structured questionnaires and observational chick lists. Even though there were no statistically significant differences among different sizes of poultry farms, the survey data indicated that poor hand hygiene, lack of footbath dip at the entry gate, poor litter management, and poor record-keeping habits were common in small-scale farms. Consequently, these create conducive environments for bacterial growth and colonization. So, mainly small-scale commercial poultry farmers need training on poultry diseases control and prevention; poultry handling skills; special routine operations; record keeping; poultry housing and feeding.

It has been indicated that the virulence of *E. coli* O157:H7 has been mainly associated with the production of stx encoded by stx1 or stx2 genes, the presence of the pathogenicity island LEE, responsible for A/E lesion, and the *hlyA* gene, encoding enterohaemolysin (Chahed *et al*., 2006; Wu *et al*., 2011). The present study showed that, out of 16 samples detected by stx1 and *eae* virulence gene specific primers, 7 (43.75%) of the isolates harbored the *eae* gene but there was no stx1 gene detected.

In the present study, four isolates from cloacae, two isolates from egg shell swab and one isolate from poultry litter had the *eae* gene. Even though the virulence gene detection was not done for all specific primers, the current result is consistent with the previous study by Kalin *et al*. (2012), who determined that all the isolates from human (1) and broilers (5) were positive for the *eae* gene but none were positive for stx1, stx2, or *hlyA* genes. Wang *et al*. (2014) also detected the highest percentage (63.30%) of *eae* gene from animal fecal (including chickens) and food samples in Eastern China. A similar results of the *eaeA* gene has also been found in other studies (Blanco *et al*., 2004). Blanco *et al*. (2004) reported that *eae* alone can lead to diarrhea in humans by A/E ability. Additionally, many investigators have underlined the strong association between carrying *eae* gene and the capacity of *E. coli* O157: H7 to cause severe human disease, especially HUS (Oswald *et al*., 2000; Blanco *et al*., 2004; Yousif, 2015). In the present study, this important virulence gene was detected in most isolates suggesting a public health risk.

Antimicrobial drug administration to food animals at low doses for extended periods of time for growth promotion and disease prevention has been linked to the global health crisis of AMR, which poses a threat to public health. In the present study, all serologically confirmed isolates (n=16) were subjected to 12 different antimicrobial agents, and the isolates were characterized as susceptible, intermediate, and resistant based on the size of zone of inhibition. Accordingly, large number of *E. coli* O157:H7 isolates were found to be sensitive to gentamycin (87.50%), trimethoprim- sulfamethoxazole (81.25%) and streptomycin (81.25%).

The result of the present study go hand to hand with the previous studies of Shecho *et al*. (2017), who reported *E. coli* O157: H7 isolates as susceptible to Trimethoprim (92.30), gentamycin (88.46%), and streptomycin (65.38%). Nearly similar results were detected by Mustafa and Inanc (2018); Kmetova (2009) in which *E. coli* O157:H7 isolates were found to be sensitive to gentamycin. Similar to the current finding Sebsibe and Asfaw (2020) had reported susceptible *E. coli* O157:H7 isolates to gentamycin (92.60%), streptomycin (92.60%) and trimethoprim-sulfamethoxazole (59.30%). Whereas Gbadamosi *et al*. (2018); Altalhi *et al*. (2010); Abd El Tawab *et al*. (2015) found 100% of gentamycin and streptomycin, 48.60% of streptomycin, and 46.60% of gentamycin resistance against *E. coli* O157: H7, respectively.

The majority of the *E. coli* O157:H7 isolates in this study were resistant to ampicillin (75.00%), amoxicillin (62.50%), erythromycin (56.25%), and tetracycline (56.25%) (Figure 3). This result is consistent with the findings of Mustafa and Inanc (2018) in which *E. coli* O157: H7 isolates were resistant to tetracycline, amoxicillin, and ampicillin. The present study also agree with report of Shecho *et al*. (2017) in which *E. coli* O157: H7 isolates from poultry farms were resistant to erythromycin (96.15%), ampicillin (92.30%), and tetracycline (76.92%). In line with the present study, high level of *E. coli* isolates resistant to ampicillin, erythromycin, and amoxicillin were reported by Zinnah *et al*. (2008). Abd El Tawab *et al*. (2015) reported 80.00% and 63.30% of *E. coli* isolates resistant to tetracycline and erythromycin, respectively. According to study by Zeryehun and Bedada (2013) on AMR pattern of fecal *E. coli* in selected broiler farms of Eastern Hararge Zone, Ethiopia, *E. coli* isolates were resistant to tetracycline (90.00%), ampicillin (60.00%), and amoxicillin (56.00%).

Small-scale intensive operations typically raise broiler or layer chickens with antimicrobials administered in commercial feed and water (FAO, 2016). In this case, administration of antimicrobials through medicated feed or drinking water on a farm or flock-wide basis leads to imprecise dosing when chickens can choose what quantity of feed or water to consume, potentially enhancing opportunity for AMR (Love *et al*., 2011). Various studies have reported that inappropriate use of antimicrobial agents remains common among small-scale poultry farms due to a lack of AMR awareness and access to quality veterinary services (Alhaji *et al*., 2018).

In fact, the frequent misuse of antibiotics in humans and food animals is closely linked to the recent emergence of MDR bacteria. In the present study, resistance to more than two antimicrobial agents was detected in 11/16 (68.75%) of the isolates. The prevalence of MDR isolates has been reported in many previous studies. Gbadamosi *et al*. (2018), Bedasa *et al*. (2018); Zeryehun and Bedada (2013); Shecho *et al*. (2017); Hamid *et al*. (2018) reported 100%, 92.50%, 92.30%, 92.30%, and 75.00% of MDR isolates, respectively, which are greater than the present study. In this study, the resistance patterns most frequently observed were ampicillin, amoxicillin, nalidixic acid, and tetracycline, which have 27.30% of the *E. coli* O157:H7 isolates. In this study, three isolates were found to be resistant to eight different antimicrobials used, which has a serious public health implication.

The higher resistance rate might be due to inappropriate and excessive use of these antibiotics for therapeutic and prophylactic purposes in chicken infections. The variation in prevalence may be due to difference in utilization of antimicrobials and infection prevention and control techniques. In other ways, change in resistant genes of *E. coli* O157:H7 which may be as a result of natural resistance in which the pathogen possess characteristics that inhibit the action of the antibiotics or acquired resistance in which there is a change in the genetic characteristics of the pathogen plays a crucial role in variation and formation of MDR (Arber, 2014). The monitoring and treatment of drug-resistant bacteria in the poultry industry will be a long and difficult task, and one which will require collaborative efforts. The rational use of antibiotics, in conjunction with the improvement of the breeding environment during the entire production cycle, will be helpful in the development of the poultry industry and the protection of public health (Liu *et al*., 2021).

6. CONCLUSION AND RECOMMENDATIONS

E. coli O157: H7 is one of the most significant food-borne pathogen that causes life-threatening food-borne illnesses. The present study shows a substantial presence of *E. coli* O157: H7 in layer poultry farms found in Bishoftu town. The presence of positive isolates in egg shells and worker hands provided strong evidence of cross contamination from poultry feces and their environments. So, chicken eggs produced under unhygienic conditions have threatening effects on public health with regard to *E. coli* O157: H7 serotype. Furthermore, the presence of the *eae* gene in positive *E. coli* O157: H7 isolates suggests a significant public health risk if these pathogen are transmitted to humans through the food chain. Unfortunately, the egg shell isolates and the only positive isolate from a worker hand swab were among the isolates that developed multidrug resistance. So, there is a potential for these multidrug-resistant bacteria to be transferred to humans through contaminated poultry products. Therefore, the developed multidrug resistance in bacteria from chicken eggs and farm personnel hands should be considered a public health concern. Generally, this high rate of increase in multidrug resistance in *E. coli* O157: H7 is quite alarming, coupled with the fact that all these isolates harbor plasmids on which these genes may be located and which are highly transferable.

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

- ✓ Poultry farms' safety practices should be improved.
- ✓ Training on biosecurity issues regarding the possible ways of prevention of *E. coli* O157: H7 should be given to all poultry farms.
- ✓ Appropriate hygienic practices for eggs and interventions to reduce the spread of *E. coli* O157:H7 through poultry products should be implemented.
- ✓ Continuous surveillance of AMR patterns, particularly among organisms resident in the gastrointestinal tract of chicken and other farm animals should be needed.
- ✓ Antimicrobial use strategies at all levels of the poultry industry should be implemented.

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7. APPENDICES

Appendix 1: Principles and procedures of media preparation, isolation and identification tests

1. Enrichment and Transportation Media

1.1. Buffered Peptone Water (BPW) (HIMEDIA, India)

Composition	**g/liter**
Ingredients	
Proteose peptone	10.00
Sodium chloride	5.00
Disodium hydrogen phosphate	3.5
Potassium dihydrogen phosphate	1.5

Preparation

Suspend 20 gram in 1000ml of distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amount in to tubes or flasks as desired and sterilize in autoclave at 121^{o}C for 15 minutes.

1.2. Tryptone Soya broth (TSB) (HIMEDIA, INDIA)

Composition

Ingredients	**g/liter**
Tryptone	17.00
Soya peptone	3.00
Sodium chloride	5.00
Dipotassium hydrogen phosphate	2.5
Dextrose(Glucose)	2.5

Preparation

Suspend 30 gram in 1 liter of distilled water. Heat if necessary to dissolve the medium completely. Mix well, dispense in tubes or flasks as desired and sterilize for 15 minutes in the autoclave set at 121^{0}C.

2. Primary Isolation Media

2.1. MacConkey Agar used to isolate *E. coli*

Purpose: is a selective and differential medium designed to isolate and differentiate bacterium based on their ability to ferment lactose.

Principle: Used as Selective medium- Gram positive bacteria are inhibited by the presence of bile salts and crystal violet inhibitors in the medium. Most of gram negative bacteria will grow. Differentiate- Between Gram negative bacteria by their ability to ferment lactose. Pink colonies- Bacteria that ferment lactose (These reactions are due to the acid produced by the fermentation of lactose. The acid end-products act on bile salts, and neutral red is absorbed by the precipitated salts). Pale colonies- non fermenters are no colored and transparent

2.2. Eosin Methylene Blue Agar (EMB agar)

Purpose: is a selective and differential medium used to isolate fecal coliforms.

Principle: It contains aniline dyes (methylene blue and eosin), which inhibit the growth of Gram-positive bacteria selecting for Gram-negative bacteria. EMB also contains lactose which makes the media differential based on an organism's ability to ferment lactose. Sucrose is also included in the medium because certain members of the Enterobacteria or coliform group ferment sucrose more readily than they ferment lactose. These sugars provide favorable conditions for the growth of fecal coliforms.

3. General Growth Media

3.1. Nutrient agar media preparation and sub-culturing technique (Oxoid, UK)

Purpose: To obtain pure cultures for use in identification tests

Principle: The survival and growth of microorganisms depend on available nutrients and a favorable growth environment. Nutrient agar is capable of sustaining growth of less fastidious bacteria.

Procedure:

- Weigh 1.12 grams of dehydrated nutrient agar medium, place in a flask and add 40ml distilled water to it. Cover the flask with aluminum foil.
- Dissolve the dehydrated media by using a hot plate and then place on a water bath until the autoclave is adjusted.
- Sterilize the dissolved dehydrated media in an autoclave at 121^{o}C for a holding time of 15 minutes
- Cool media in water bath at 50^{o}C (because agar solidifies at 42^{o}C)
- Pour 15ml of the medium into a petridish inside the safety cabinet
- Allow poured plates to dry thoroughly and incubate at 37^{o}C for 24hrs to check whether contamination has occurred or not.
- After 24 hours, arrange all the materials for sub culturing inside safety cabinet
- Heat the wire loop till red hot in the blue part of the Bunsen flame heat and cool for few seconds.
- Touch a single bacterial colony with the wire loop and transfer to the nutrient agar. After a well formed it will be distributed by quadrant streaking method.
- After inoculation, incubate the cultures at 37oc for 72 hours; place the Petridish upside down to prevent condensation dropping on to cultures.

Appendix 2: Questionnaire and Consent Form

Informed consent for poultry farm owner, manager or professional

My name is Sena Zinabu. I am a second-year veterinary public health master student at Addis Ababa University, College of Veterinary Medicine and Agriculture. Now I am going to do research on one of the zoonotic (disease that is transmitted from animal to human) poultry diseases, and you are being selected to take part in this research as a poultry farm owner, manager, or professional eligible for the research on *E. coli* O157: H7 and its antimicrobial susceptibility pattern in poultry and human health. About eleven poultry farms will be included in this study, and from your farm I will collect about 30 samples (cloacal swab, egg swab, and environmental samples like litter, water, and feed samples). Furthermore, if your farm workers are interested in participating in this investigation, I will collect hand swabs from interested workers solely to determine the pathogen's circulation on the farm. If properly manipulated, it does not have any risk to your chicken/farm other than psychological discomfort to the chicken. So, in order to minimize this stress, I will collect the samples with the help of an experienced individual in proper handling of the chicken or with the help of your experienced workers. Before you decide, it is important for you to understand that the purposes of the research are to isolate, identify, and characterize *E. coli* O157: H7 from cloacae, egg, and environmental samples; to determine antibiotic susceptibility patterns of identified isolates; and to investigate the risk factors associated with the occurrence of *E. coli* O157: H7 in order to provide input for a better intervention and recommendations to prompt rationale antibiotic use in poultry farms in Bishoftu, Ethiopia. Please feel free to ask if it is not clear or to discuss it with anyone you wish. Please take time to decide whether or not you want to take part in this research.

We would like to stress that taking part in this study is entirely voluntary and responses that are confidential will be used only for the purpose of the study.

Do you consent to participating in this study? _____

If yes, please put your signature here: _____

Thank you!

Date _____

Questionnaire to Collect Farm Level data and Observational Check List

Farm: Name_____ Kebele _____ Farm size_____

Age of chickens_____ weeks, Breed_____ No of chickens in the farm_____

Respondent Background

1. Name of the respondent _____Sex _____ Age _____
2. Educational level of the respondent a) >8 b) 9-12 c)>12 d) Animal health (DVM)

Farm management and Antimicrobial resistance

1. How often do you attend the farm?

A) Everyday B) Sometimes C) Every week D) Every month

2. Did all personnel of the farm received training on biosecurity? A. Yes B. No

3. Is there equipment exchange with other farms? A. Yes B. No

4. Are multiple age-groups not kept together? A. Yes B. No

5. Disinfection of vehicles A. Yes B. No

6. Disinfection of hands and shoes of those who enter to the farm building A. Yes B. No

7. Provision of clothes and shoes only for hygiene control area A. Yes B. No

8. Cleaning or disinfection of materials directly used for chickens when carry them in hygiene control area A. Yes B. No

9. Prohibition of carrying clothes and shoes used abroad into the farm A. Yes B. No

10. Quarantine of animals under segregation from other animals for certain period when introducing into the farm A. Yes B. No

11. Cleaning and disinfection of a litter or cage after being emptied A. Yes B. No

13. Record keeping for early identification of source of infection A. Yes B. No

14. Source of water for the farm? A) Pond B) pipeline C) Lake D) other, specify _____

15. Type of material used to provide water? A) Wooden made B) stone c) plastic made D) Clay

15. Presence of standing water A. Yes B. No

16. Source of supplementary feed? A) Market B) Farm C) Both D) Leftover

17. How do you feed chickens? A) Put feed in the feeder B) Throw on the ground C) both D) Other, specify_____

17.1. If you provide by the feeder, what is the type of feeder?

A) Wooden made B) stone made C) Ground D) clay made E) Metal made f) other

18. Do you process the feed? A) Yes B) No _____

19. Have you experienced serious disease occurrence in poultry? A) Yes B) No

20. How do you recognize sick birds? _____

21. Describe common disease you have experienced in the flock

Name of disease	Sign	Age affected	Season of occurrence	Severity of disease	Treatment given

22. Do you have any experience (knowledge) of *E. coli*? A) Yes B) No

22.1 If yes, how can you identify *E. coli* infection in chicken? _____

23. What do you think as the possible source of the infection _____

24. What managerial decisions you usually made to control *E. coli* infection in the farm?

_____

_____

25. Do you use antibiotics when only chickens are sick? A. Yes B. No

26. What are the common antibiotics you used for *E. coli* infection? _____

27. Are all treatments for cases prescribed by a veterinarian? A. Yes B. No

28. How many times you gave antibiotics for the previous batch? _____

29. Have you ever used leftover antibiotics to treat other chicken? A. Yes B. No

30. Antibiotic use in farms may influence human health? A) Yes B) No

31. Do you order shifting to use different antimicrobials during the course of treatment if they do not recover? A) Yes B) No

32. Do you increase dose and frequency when there are no recovery signs? A) Yes B) No

33. Do you know withdrawal periods of drugs? A) Yes B) No I don't

34. Are there experiences of treatment failure in the flocks? A) Yes, (possible reason: _____) B. No

35. Do antibiotics used as feed additives? If yes, for what purpose?

A) Yes, _____ B) No

36. What are the possible reasons of AMR to develop in your opinion?

_____

_____

37. Antimicrobials can pass to human through consumption of poultry meat and egg?

A) Yes b. No

38. Would you rather hold marketing before withdrawal period end to reduce residual effects on human health? A) Yes b. No

39. What are the common challenges of chicken production for you? _____

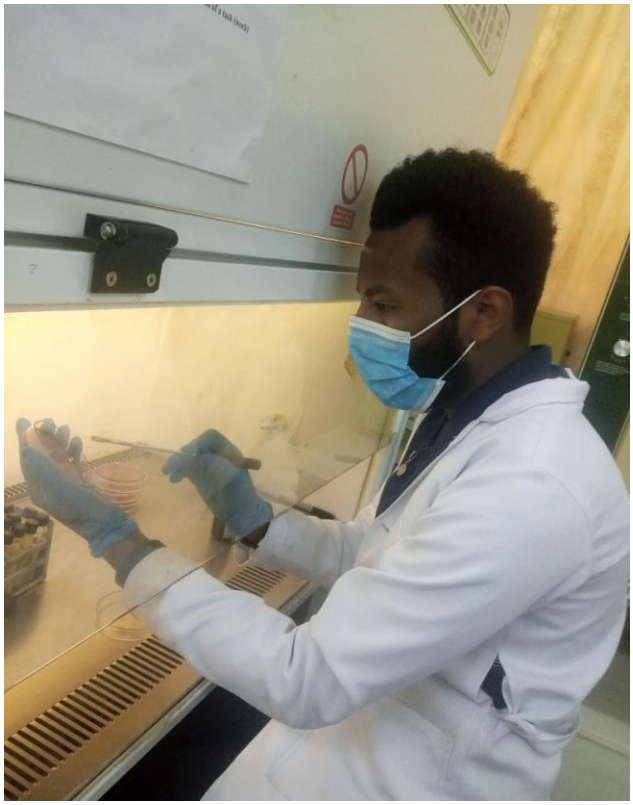
_____

Observational check list

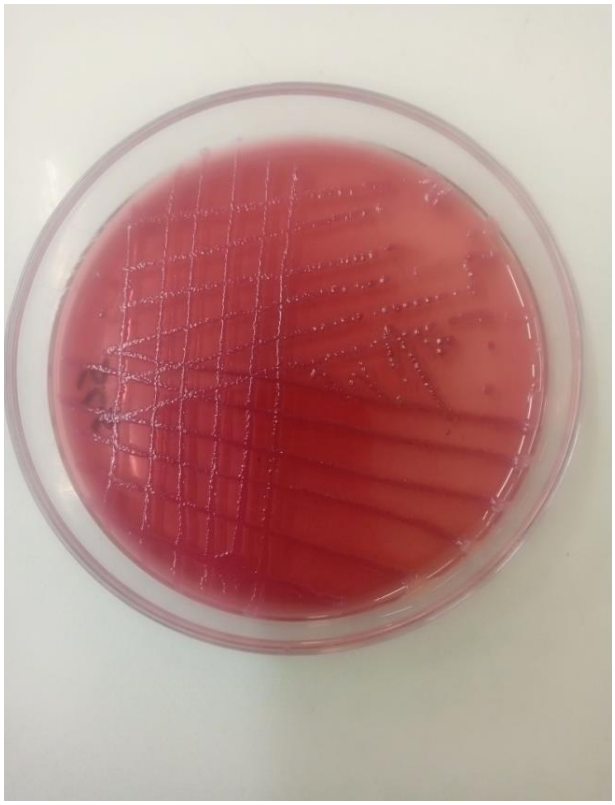
1. Hand hygiene before and after handling poultry A. Yes B. No
2. Utilization of on farm cloths and footwear. A. Yes B. No
3. Storage of poultry feed A. Yes B. No
4. Utilization of footbath dip at the entry gate A. Yes B. No
5. Cleaning and disinfection of foot wear before and after visits. A. Yes B. No
6. Feces and litter management A. Good B. Moderate C. Poor
7. Type of drinking water source _____
8. Pest control (Rodents and insects) A. Yes B. No
9. Workers contact with other flock A. Yes B. No
10. Rearing animals with suitable density A. Yes B. No
11. Distance from main road _____between farms _____from residential area _____
12. Possible source of contamination _____, _____

Appendix 3: Pictures during sample collection, laboratory work and test results

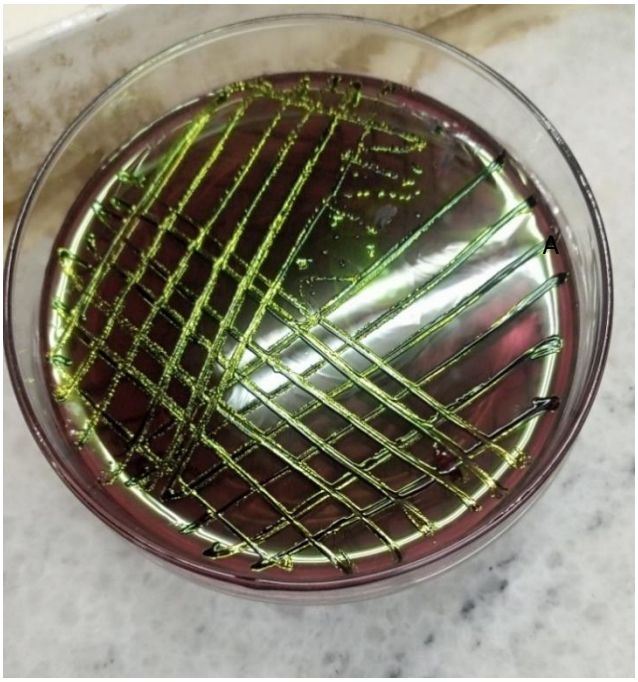
Cloacae swab sample collection (**A**), Egg shell swab sample (**B**), for egg internal content sampling (**C**)



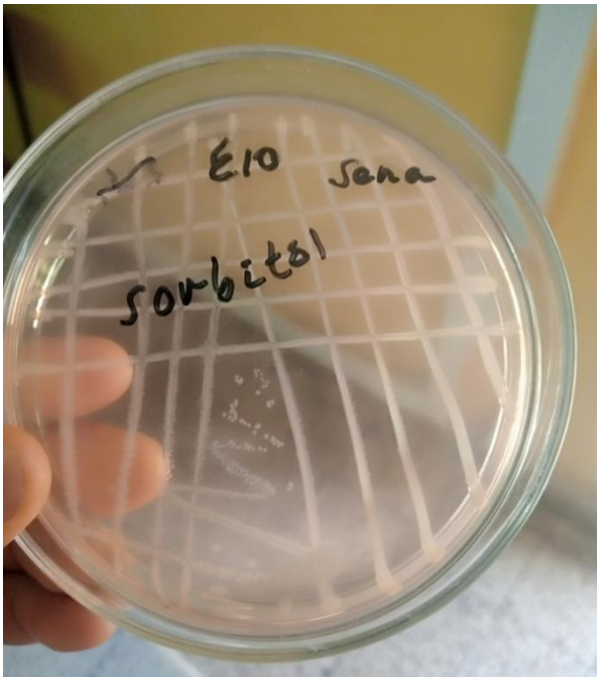
Culturing of bacteria (**Left**)



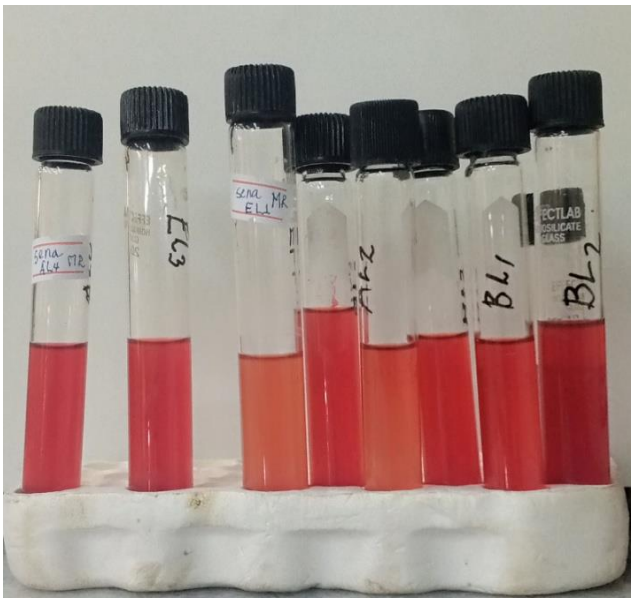
E. coli pink colony characteristics on MacConkey agar (**Right**)



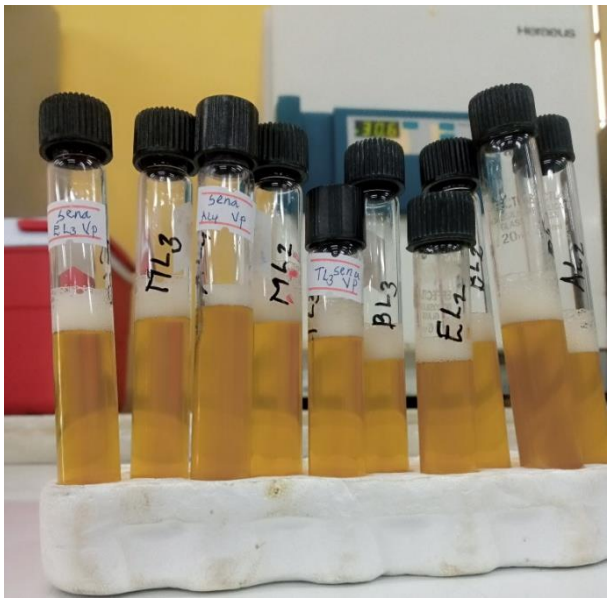
E. coli metallic green sheen on EMB agar (**Left**)



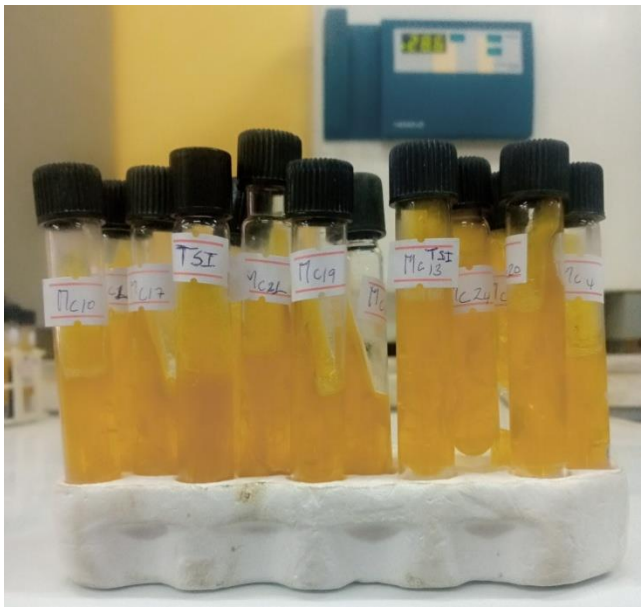
E. coli O157: H7 on sorbitol MacConkey agar (**Right**)
Non sorbitol fermenters



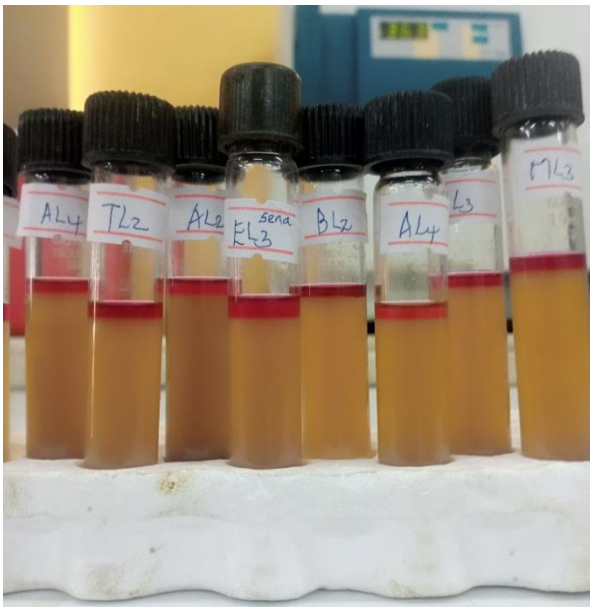
Methyl-red test result for *E. coli* (**Left**)
Formation of red color in the medium



Voges-proskauer test result for *E. coli* (**Right**)
Absence of any color change (yellow)

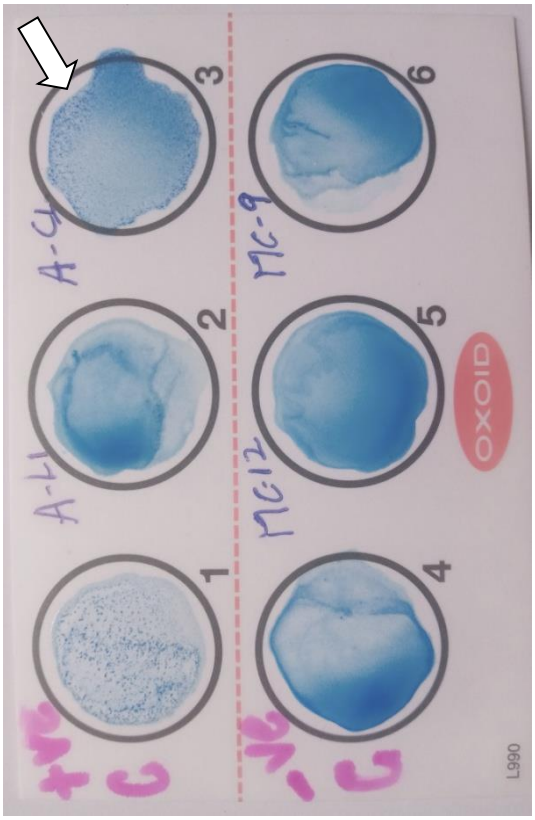


Triple-sugar iron test result for *E. coli* (**Left**)
Yellow slant, yellow butt and cracks in the agar



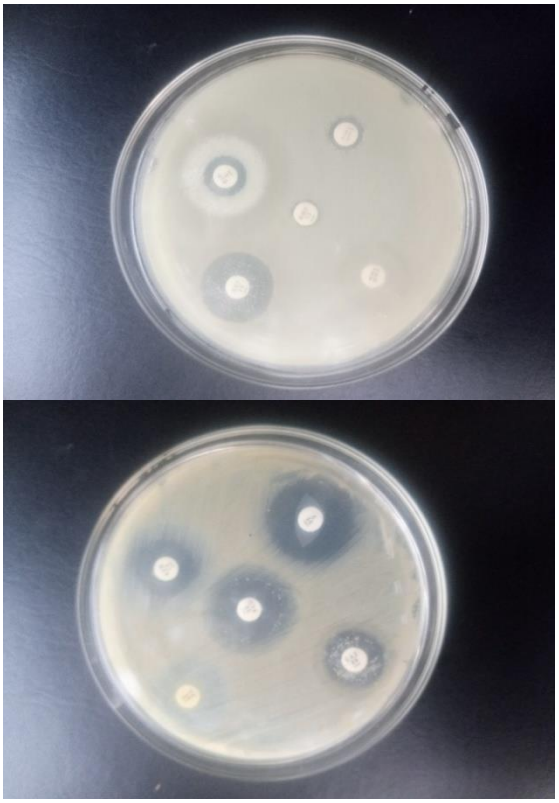
Indole test result for *E. coli* (**Right**)
cherry-red ring on top of the medium

Latex agglutination testing (**Left**)

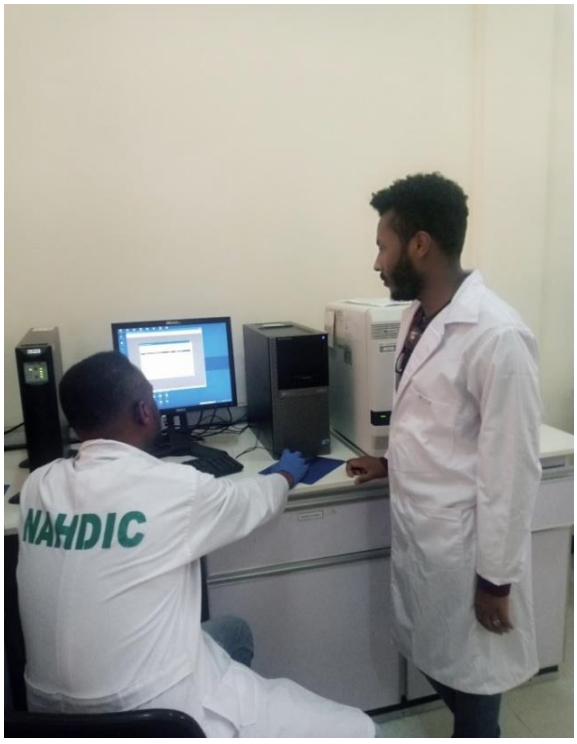


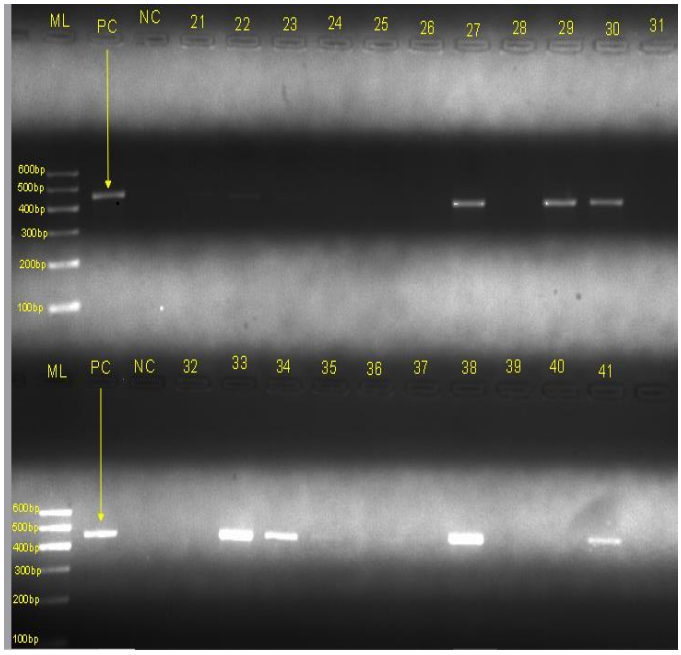
Formation of agglutination on circle # 3 indicated by arrow (**Right**)
Sample coded as A-C1 is confirmed as *E. coli* O157: H7

Antimicrobial Susceptibility testing (**Left**)



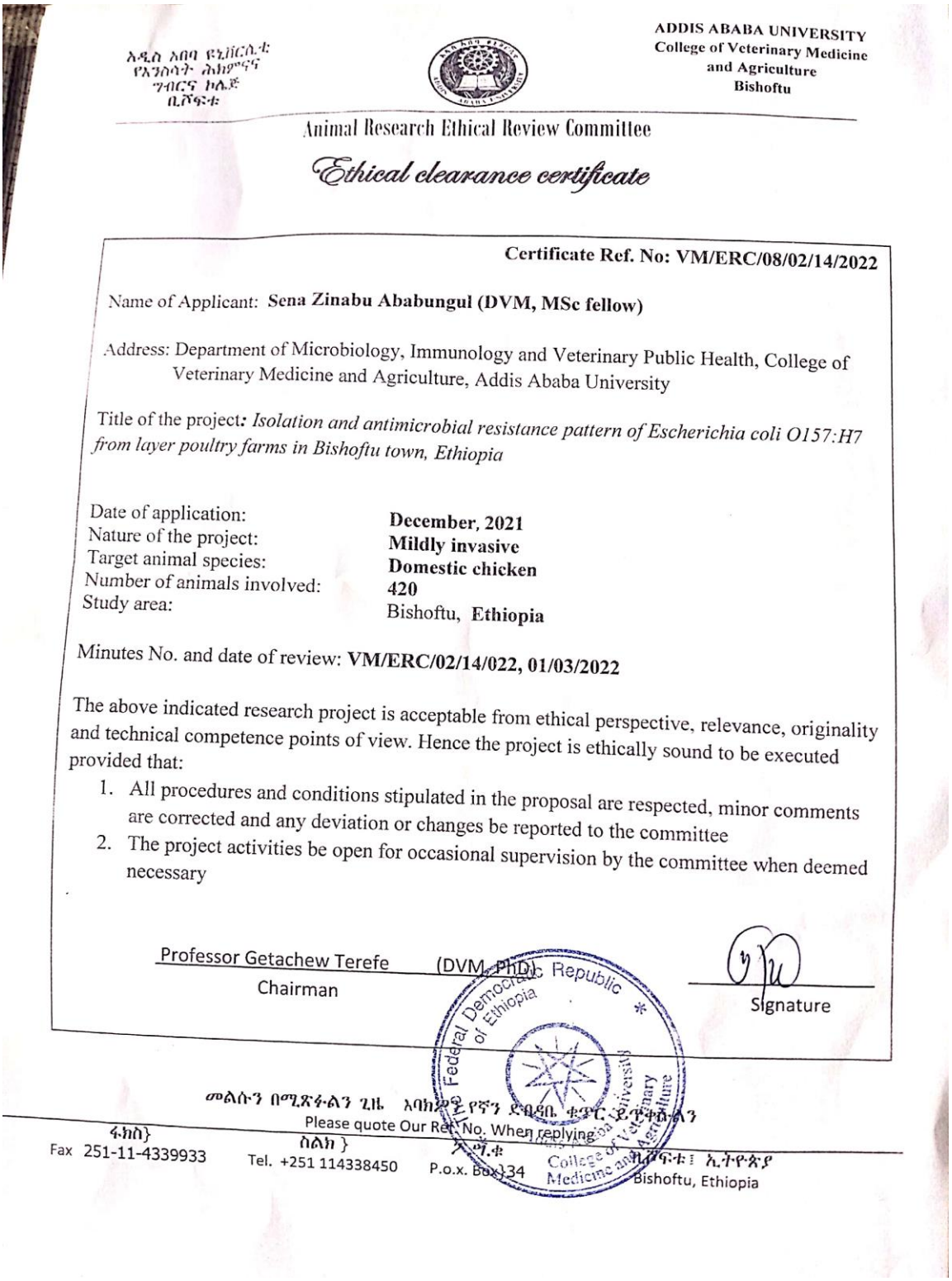
Antimicrobial Susceptibility test result (**Right**)





Molecular characterization of *E. coli* O157: H7 isolates at AHI molecular biology laboratory

Appendix 4: Ethical Clearance



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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/08/02/14/2022

Name of Applicant: **Sena Zinabu Ababungul (DVM, MSc fellow)**

Address: Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Isolation and antimicrobial resistance pattern of Escherichia coli O157:H7 from layer poultry farms in Bishoftu town, Ethiopia*

Date of application:	**December, 2021**
Nature of the project:	**Mildly invasive**
Target animal species:	**Domestic chicken**
Number of animals involved:	**420**
Study area:	Bishoftu, **Ethiopia**

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

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 deemed necessary

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

Federal Democratic Republic of Ethiopia
College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

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