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EPIDEMIOLOGY OF *Escherichia coli* O157:H7, AND ASSESSMENT OF POSTHARVEST LOSS ASSOCIATED WITH FISH HANDLING IN SELECTED LAKES OF NORTHERN ETHIOPIA

MVSc THESIS

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JUNE, 2018

BISHOFTU, ETHIOPIA

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

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Final approval and acceptance of the thesis dissertation is contingent upon the submission of its final corrected copy to the candidate's major department.

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DEDICATION

This thesis is dedicated in memory of my late grandparents Abebaw Asfaw and Yeshihareg Fentaw, for their unreserved love, passion, and affection throughout my childhood.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my genuine work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

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LIST OF ABBREVIATIONS

ARGs	Antibiotic resistance genes
BPW	Buffered peptone water
CAF	Chloramphenicol
cfu	Colony forming unit
DACA	Drug authority and control agency
DNA	Deoxy-ribonucleic acid
eae	effacing gene
ETB	Ethiopian birr
FAO	Food and agriculture organization
Gb3	Globotriaosylceramides
GI	gastrointestinal tract
GLM	General linear model
HUS	Hemolytic-uremic syndrome
IFLAM	Informal Fish Loss Assessment Method
ISO	International standards organization
Kg	Kilogram
KIA	Kligler Iron Agar
Km	Kilometer
LT	Load Tracking
MDR	Multi drug resistance
PA	Participatory appraisal
PCR	Polymerase chain reaction
PHFL	Post-harvest fish loss
PHL	Post-harvest loss

LIST OF ABBREVIATIONS (*Continued*)

QLAM	Questionnaire Loss Assessment Method
RNA	Ribonucleic acid
SD	Standard deviation
SLTEC	Shiga like toxin producing *Escherichia coli*
SMAC	Sorbitol MacConkey agar,
STEC	Shiga toxin producing *Escherichia coli*
*Stx*s	Shiga toxins
TSB	Trypton soya broth
TTP	Thrombotic thrombocytopenic purpura
VTEC	Vero toxigenic *Escherichia coli*

ABSTRACT

A cross-sectional study with a simple random sampling approach was conducted from October 2017 to May 2018. The objectives of the study were; isolating and estimating the prevalence of *Escherichia coli* O157: H7, evaluating antimicrobial susceptibility pattern of isolates and assessing post-harvest loss in fish in selected Lakes of Northern Ethiopia. All the microbial identification and isolation procedures were conducted based on ISO 6887-3:2017 recommendations. Antimicrobial susceptibility test was also performed following the standard procedure of Kirby-Bauer disk diffusion protocol. Post-harvest losses assessments were conducted based on FAO recommendations of qualitative and quantitative field assessment methods. From the total of 410 fish samples examined, six (1.46%) of them were contaminated with Shiga toxin producing *Escherichia coli* O157: H7 strain. The antibiotic susceptibility test revealed that the isolates were resistant to Ampicillin and Streptomycin. On the other hand, Ciprofloxacin, Gentamycin and Nalidixic acid were found effective in inhibiting the growth of most of the isolates. Fishermen believed that high environmental temperature, absence or delayed marketing, harvesting immature fish, predators, and flood are the major causes of post-harvest loss of fish in the two Lakes. The monetary value of post-harvest loss was estimated to be 10,934,000 ETB for the last 4-6 years in both study areas. The occurrence of such a pathogenic organism, and huge product loss associated with fish indicates the need for intervention by stakeholders. Supports like refrigerators, electricity generators, boat, legal net and on job training about proper handling practices may play a tremendous role in decreasing microbial contamination and PHL in fisheries sector.

Keywords: Antibiotic susceptibility test; *Escherichia coli* O157; Fish*;* Northern Ethiopian lakes; Prevalence; Post-harvest loss

1. INTRODUCTION

Fisheries play a significant role in food security, livelihood, source of income and social development in developing countries (Hossain *et al.,* 2015). New technological advances and increased demands for fish as a source of animal protein are the main reasons for the industry's growth. Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world (Garcia-Rodríguez and De La Cruz-Aguero, 2011). World per capita fish supply has outpaced population growth in the past five decades and reached a new record high of 20 kg in 2014 due to its vigorous growth. In the last two decades, dramatic growth in aquaculture production has boosted average consumption of fish and fishery products at the global level (FAO, 2016).

Fish can spread diseases caused by pathogenic micro-organisms they may contain. They can acquire pathogens by direct contact with water. Bacteria of the family *Enterobacteriaceae* are widely distributed in aquatic ecosystems and can be detected in fish skin, gills and intestines (Ribeiro *et al.,* 2015). Fish are generally considered as vehicles for several bacterial disease transmissions (Elsaidy *et al.*, 2015; Zhao *et al.*, 2018). The unhygienic conditions of the landing sites, storage, and domestic retail markets are causing a great public health risk. The fecal contamination of natural water bodies has emerged as a major challenge in developing and densely populated countries. The fish harvested from such areas often contain human pathogenic microorganisms. *Escherichia coli* have been traditionally recognized as an indicator organism of fecal contamination of water and fish (Albuquerque, 2013).

Foodborne pathogens are the leading causes of foodborne human illness and death in the world (Agüeria *et al.*, 2018). The reason for the increased risk can be attributed to many reasons; changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are few of them (Lopez-Campos *et al.*, 2012; Bevilacqua *et al.*, 2017). *Escherichia coli* O157 is one of the virulent strain under the species *Escherichia coli.* It is the leading cause of hemorrhagic colitis,

hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in man. These illnesses may lead to death due to improper absorption of nutrients and destruction of certain tissues in the target organs (Earley and Leonard, 2006; Cobbaut *et al.*, 2009).

Epidemiology of foodborne pathogens especially that of *Escherichia coli* O157: H7 was not well studied in Ethiopia in the past years. However, recently there is an increasing trend of reporting occurrence level of the organism in beef and dairy products (Hiko *et al.*, 2008; Tassew *et al.*, 2010; Haileselassie *et al.*, 2013; Taye, 2013; Abebe *et al.*, 2014; Desta *et al.*, 2016). This may be due to the fact that beef and dairy products are considered the risk for *E. coli* due to close contact with cattle manure. However, studies showed that the organism has been isolated from mutton, chicken, fish, and vegetables. *Escherichia coli* O157: H7 genes have been reported from fish in India and USA. A study conducted in the USA showed that *E. coli* O157: H7 genes have been detected in gastrointestinal sample from fish with a prevalence of 6.95% (Ribeiro *et al.*, 2015). In India, O157 genes were identified from fish samples taken from street market (Sanath Kumar *et al.*, 2001).

Fishes and fish products can be contaminated by Enterobacterial species that can cause severe foodborne outbreak (Ribeiro *et al.*, 2015). The risk can be worse in developing countries like Ethiopia due to many reasons which include but not limited to; lack of awareness of fishermen about hygienic handling of fish, extensive cattle production around water bodies and landing sites, suitable weather condition for the flourishing of microorganism in feces, widespread open defecation by rural population, high flooding that drain contaminants to Lakes and landing sites, and unavailability of fish processing plants can be few of them. All those listed reasons indicate that consumption of raw and undercooked fish and fish products in Ethiopia should not be practiced. Even though fish pose a great risk of foodborne pathogens, to the best of my knowledge, there is no a single study conducted in Ethiopia reporting *Escherichia coli* O157:H7 associated with fish. In addition to problems related to poor handling practices of fish and fish products, post-harvest loss and causes are not yet studied in the study areas. If fishermen and policymakers are being able to make informed decisions in attempt to reduce contamination and losses, significance of these problems need to be scientifically studied and recommendations should be generated.

General objective

- ✓ To assess the level of contamination of fish with Shiga toxin producing *Escherichia coli* O157: H7 and to investigate post-harvest losses in fish in selected Lakes and their near-by markets of Northern Ethiopia.

Specific objectives

- ✓ Estimating the prevalence of *Escherichia coli* O157: H7 in fish.
- ✓ Determining *in vitro* susceptibility pattern of the isolates to antibiotics.
- ✓ Identifying causes of post-harvest loss associated with fish.
- ✓ Estimating the monetary value of post-harvest loss associated with fish.

2. LITERATURE REVIEW

2.1. Characteristics and nomenclature of *Escherichia coli* O157:H7

Escherichia coli O157: H7 is a well-known Shiga toxin producing bacteria that belongs to the family *Enterobacteriaceae*. They are reasonably tolerant to different extreme conditions like; minimum pH for growth, heating, irradiation, antimicrobials, ruminant gastrointestinal tract fluids, and even cool nutrient-dilute water as well (Yoon *et al.*, 2004; Karmali *et al.*, 2010). The organism has gained recognition as an important food-borne pathogen in recent years. Most strains of *E. coli* isolated from natural sources are harmless commensals, but the O157:H7 serotype has a highly virulent character and regarded as the ugliest *Escherichia coli* strain by WHO (Ayscue *et al.*, 2009; Berry and Wells, 2010). Nomenclature of *Escherichia coli* O157: H7 has been variously described as verotoxigenic *E. coli* (VTEC), Shiga like toxin producing *E. coli* (SLTEC), and currently, Shiga toxin producing *E. coli* (STEC) (Saeedi *et al.*, 2017). Unlike any other non-pathogenic strains of *E. coli*, *E. coli* O157 has no the ability to ferment sorbitol, unable to produce β- glucuronidase, has an attaching and effacing gene (*eae*) and, produces Shiga toxins (*Stx*s) that inhibit host protein synthesis (Duffy *et al.*, 2006; Pennington, 2010; Wilson *et al.*, 2018).

2.2. Epidemiology of *E. coli* O157:H7

2.2.1. Global occurrence

Escherichia coli O157 is not responsible for most prevalent foodborne diseases in many places compared to other gram negative bacteria (Pennington, 2010). However, there are still concerns regarding complicated clinical infections caused by this serotype and known to have higher hospitalization rate than other organism (Worley *et al.*, 2017). The geographical distribution pattern of the outbreak shows an extent prevalence through many countries from USA to African, Europe, Far East and Australia. Fewer reports were recorded in the Middle East countries(Saeedi *et al.*, 2017). Meta-analysis of published researches indicated the prevalence of the organism in cattle was estimated to be 10.68% in fed beef, and 1.79% in

adult dairy. Seasonally, Fed beef fecal prevalence was 10.65% during summer and 9.17% during the winter months (Ekong *et al.*, 2015). Prevalence of *E. coli* O157: H7 in sheep farms also range from 0% to 9.3% (Saeedi *et al.*, 2017). *E. coli* O157:H7, has strongest association worldwide with HUS among Shiga-toxin-producing *E. coli* strains (STEC). The most HUS cases distributed in South America with prominently Argentina. However, at least during certain periods, non O157: H7 STEC appears to be more common causes of HUS in Australia, Germany, and Austria (Kiermeier *et al.*, 2015; Perrin *et al.*, 2015).

2.2.2. Risk factors

There are several risk factors for occurrence of the organism in food animals. Few of them include seasons (Spring and Summer), warm climate, urban industrialized lifestyle, rainfalls, wet livestock, high temperature, long time periods of sunshine predispose the proliferation of *E. coli* O157:H7 which can lead to high rates of incidence and prevalence. However, these infections are lower in comparison with the bacterial dissemination within their cattle reservoirs (Ongeng *et al.*, 2014; Ekong *et al.*, 2015). *E. coli* is a commensal bacterial population within gastrointestinal tract among cattle. Generally, cattle are natural carriers of *E. coli* O157 with no clinical demonstrations. The level of human infection is in relation to cattle density and cattle to human ratio (Bruyand *et al.*, 2017).

2.2.3. Growth and survival

Viability and growth of bacteria depends primarily on availability of essential nutrients including organic carbon, phosphate, and nitrogen (Peterson et al., 2005). However, *E. coli* O157 survives even at low density of oligotrophic environments such as surface water or groundwater that may be used as drinking water (Chekabab *et al.*, 2013). Farm environments, particularly cattle farms, may become readily contaminated with a variety of strains of *E. coli* including *E. coli* O157 from feces of animals. Once present, the organism may survive in environments for some time (Eppinger and Cebula, 2015). The organism then spread directly or indirectly from the farms to the wider environment and present a risk of contamination to other animals and growing crops. Levels of pathogens remaining viable in the soil are subject to a variety of factors, including exposure to sunlight, drying, etc. However, *E. coli* O157 can

survive for very long periods in animal wastes and soil (Hussein and Sakuma, 2005; Pennington, 2010; Saeedi *et al.*, 2017).

2.2.4. Reservoirs and mode of transmission

The most important reservoir for the organism are cattle which also considered as the main source of human infections. Colonization of this microorganism in adult ruminants is asymptomatic (Smith *et al.*, 2014). Some cattle shed 104 CFU *E. coli* O157: H7 per one gram of feces which are called super shedders (Munns *et al.*, 2015). These animals account for more than 95% of all excretion of the organism in feces. Super-shedders have prominent outcomes for the distribution of *E. coli* O157: H7 in cattle as main reservoir, and thereafter, increase the risk of human infection by environmental exposure (Arthur *et al.*, 2010). Other investigations indicated that the presence of *E. coli* O157: H7 within a unicellular amoeba of Acanthamoeba. Therefore, Acanthamoeba is an important reservoir within freshwater and soil to spread *E. coli* O157: H7 to other hosts, including human and grazing cattle (Saeedi *et al.*, 2017). In addition to the above reservoirs, flies are also known to carry *E. coli* O157:H7 with 2.7 times higher recovery rate than manure, hence causing spread of the infection to human by wandering in restaurants and kitchens (Burrus *et al.*, 2016). In farms, troughs are important means for transmitting the microbial agent of *E. coli* O157: H7 from an animal to others. Furthermore, diarrheal feces of infected animals have considerable effects on the spread of contaminations. As the organism persists in the environment for a long period (for more than seven weeks in bovine manure), the risk for transmission increases through contaminated grass and stockyards (Smith *et al.*, 2014).

E. coli O157: H7 can infect human hosts via contaminated foods, water, and even soil (Van Elsas *et al.*, 2011; Ongeng *et al.*, 2014). In accordance with several surveys, contaminated foods are the first factor to spread of infections caused by *E. coli* O157: H7 among human consumers. It is followed by contaminated dairy products, contact with animal reservoirs, contaminated water, environmental conditions and uncertain resources respectively. Some investigations indicate that *E. coli* O157: H7 can spread among human populations throughout person-to-person contacts. The other reported transmission route are

unpasteurized apple drinks, vegetables, non-ruminant meat and meat products, direct contact with ruminants and laboratory related (Williams *et al.*, 2008; Farrokh *et al.*, 2013; Saeedi *et al.*, 2017).

2.3. Diagnostic approaches to detect *E. coli* O157

Development of a rapid microbial detection methods with high sensitivity and specificity for pathogen identification allows the prompt notification of outbreaks and prevents more cases (Baker *et al.*, 2016). For most foodborne pathogens, the frequency and total numbers of microorganisms on a particular food product are relatively low. This represents a challenge for detection since sampling protocols need to be designed to account for infrequent and non-uniform distributions of a pathogen. Consequently, most detection methods include steps to increase (enrich) the initial number of the target pathogen to population levels for consistent and reliable detection. Thus, the first step for STEC detection is to enrich the sample to be analyzed. Enrichment media vary in composition but generally provide an environment appropriate to increase a bacterial cell population. Such constituents provide a supportive nutritional matrix for growth of microorganisms (Sanath Kumar *et al.*, 2001). Bacteriological testing of feces for *E. coli* O157 in animals generally is conducted for research purposes as *E. coli* O157 is not associated with clinical illness. Culture methods to identify the presence or absence of fecal shedding typically involve the use of an enrichment, immunomagnetic separation, and the use of selective and differential media such as sorbitol MacConkey agar containing cefixime and tellurite, with latex agglutination used to detect the presence of the O157 antigen (Karmali *et al.*, 2010). Some of the essential and feasible approaches to detect the organism are discussed in brief.

2.3.1. Conventional culture methods

Enrichment, colony isolation and confirmation are the three general phases of the standard method recommended by the US Department of Agriculture for detection and identification of *E. coli* O157:H7. The first phase is a 24h enrichment, which provides conditions that promote growth of *E. coli* but are inhibitory to other species. The USDA enrichment culture

medium includes lactose as a carbohydrate source, bile salts for suppression of certain Gram-positive species, and novobiocin to suppress Gram-negative species other than *E. coli*. In the second phase, the enrichment culture is plated onto a selective medium to obtain isolated colonies. The medium is a modification of MacConkey agar, in which the lactose is replaced by sorbitol (Sorbitol MacConkey agar, SMAC) and a chromogenic indicator for 13-glucuronidase activity is included. Sorbitol-fermenting colonies appear red, but E.coli O157:H7 colonies are color less owing to lack of sorbitol fermentation (Smith *et al.*, 2014). The other recommended confirmatory tests be H2S production and carbohydrate fermentation in triple-sugar-iron agar, indole production, methyl red-Voges-Proskauer test, citrate utilization, lysine decarboxylase, and antigen agglutination (Hunt, 2010; Bryan *et al.*, 2015; Zelyas *et al.*, 2016).

2.3.2. Detection by latex agglutination techniques

The use of latex reagents in slide agglutination assay provides a rapid screening procedure for the presumptive identification of *Escherichia coli* O157:H7 (Baker *et al.*, 2016). These assays are used for serotyping non-sorbitol fermenting colonies, generally isolated on Sorbitol MacConkey (SMAC) agar (Wang *et al.*, 2013). The assays are designed for use with pure cultures and perform best when using freshly isolated organism. Isolates may be analyzed for the presence of the somatic O157 antigen. In all instances where a positive serological result is obtained, further biochemical characterization is required to confirm that the suspected organism is *E. coli*. In these procedures latex particles coated with the *E. coli* somatic O157 antisera (antibodies) are mixed on a slide with a suspension of bacteria and are observed for agglutination reactions. The tests are designed to provide a color differentiation between the surface of the slide and the particles. It is essential that proper positive and negative controls to be employed in the assay procedure (Baker *et al.*, 2016).

2.3.3. Molecular detection methods

Molecular approaches involving the isolation, detection, and in some cases quantitation of either DNA or RNA are instrumental in the emergence of rapid detection systems for

Escherichia coli O157 (Otero *et al.*, 2017; Yokoyama *et al.*, 2018). A key component to the process of any PCR assay is to successfully isolate DNA from samples, which can subsequently be detected with nucleotide specific primers. Primers have been developed to detect virulence genes such as *stx1*, *stx2* and *eae*, and distinguish *E. coli* pathotypes, as well as common STEC serotypes. In theory, with proper DNA extraction techniques and sufficient DNA purity level, PCR methods can detect a single DNA molecule, which can be amplified to obtain a greater amount of DNA for further analysis (Clermont *et al.*, 2000; Fortin *et al.*, 2001).

2.4. Prevention and control strategies

Optimum control of *E. coli* O157:H7 needs to involve all stages of food production, from farm to fork. Quantitative risk assessments and simulation models are available which describe stages in the farm-to-fork that contribute to an increased risk of foodborne illness and allow potential control measures to be assessed (Saxena *et al.*, 2015; Gonzales-Barron *et al.*, 2017).

2.4.1. Control in cattle and farms

Controlling *E. coli* O157:H7 at farm level would be highly desirable not only to reduce the risk of contamination of beef carcasses at slaughter, but also to reduce human illness associated with direct animal contact, environmental contamination, contamination of crops following manure application and contamination of water used for irrigation, recreation, for aquaculture and as a drinking water source (Kieckens *et al.*, 2017; Martorelli *et al.*, 2017; Zhilyaev *et al.*, 2017). Most studies on the control of VTEC on farms have been directed toward serotype *E. coli* O157:H7 Identifying and modifying management strategies associated with fecal shedding could reduce animal exposure and transmission (Karmali *et al.*, 2010).

Since direct contact with the cattle or their environment is the main root of contamination, reducing *E. coli* O157: H7 at the farm level should decrease the risk of human illness through

both direct and indirect routes (Otero *et al.*, 2017; Wang *et al.*, 2017). There are several approaches to on-farm control that theoretically could be performed to reduce fecal shedding of *E. coli* O157: H7 in cattle. Pre-harvest control of *E. coli* O157: H7 is a control strategy which improves human health, food and water safety and cattle pathogen-reduction by good sanitation procedures during food preparation, practices and transport management, cattle water and feed controlling and animal treatments (Rantala and Hyvo, 2001; Gomes *et al.*, 2016). Another option for controlling *E. coli* O157 in cattle is to increase herd resistance to infection (Berry and Wells, 2010; Ekong *et al.*, 2015). Examples of interventions using this approach include vaccination, probiotics, antimicrobials, and bacteriophages and discussed briefly in the following sections.

2.4.1.1.Vaccination as a control measure in cattle

Vaccination is the most promising approach in reducing cattle carriage of the organism. The reduction of *E. coli* O157 prevalence in cattle potentially could cause a significant decline in the incidence of related diseases in human(LeJeune and Wetzel, 2007; Soon *et al.*, 2011). Immunization commonly prevents bacterial infection or pathologic effects of the bacterial toxin. Preventing infection typically requires utilizing antigens that promote bacterial colonization, whereas pathologic effects of the toxin can be neutralized with toxoids(Baker *et al.*, 2016). A number of O157 antigens are being investigated as potential vaccine targets. Some vaccine products have demonstrated efficacy to reduce the prevalence of cattle carrying STECO157 by making the gut environment unfavorable to colonization (Arthur *et al.*, 2010; Wang *et al.*, 2017). Although cattle vaccines against STEC O157 have gained either full or preliminary regulatory approval in Canada and the United States, it is not yet clear if they will be widely adopted by cattle feeders because there is not yet an economic signal to indicate that cattle vaccinated against STECO157 are valued over non vaccinated cattle (Snedeker *et al.*, 2012; Varela *et al.*, 2013; Smith, 2014).

2.4.1.2.Probiotics supplementation in feed

Probiotics are live bacteria fed to a host to elicit a beneficial response, and are typically, but not limited to, Lactobacillus spp. strains. In cattle, numerous probiotics have been identified and tested for efficacy against *E. coli* O157:H7. Some effective probiotics include, *Streptococcus faecium, Lactobacillus acidophilus, L. casei, L. fermentum, L. gallinarum, L. plantarum, Propionibacterium freudenreichii*, and *Streptococcus bovis* as a direct-fed microbial alone or in combination with others have been the most thoroughly studied and often are very effective at reducing the prevalence of fecal shedding of *E. coli* O157: H7 (Farrokh *et al.*, 2013). Feeding Lactobacillus-based direct-fed microbial also have been shown to reduce the prevalence of *E. coli* O157: H7 on cattle hides (Wisener *et al.*, 2015). Commensal *E. coli*, including colicinogenic strains, have also been tested for probiotic potential against *E. coli* O157: H7 in inoculated calves, but results have been limited to date. Laboratory studies indicated that *E. coli* O157:H7 can become resistant to individual colicins, so effective treatments may require a cocktail of strains producing different colicin types (Berry and Wells, 2010).

2.4.2. Prevention in food processing plants

Food safety in the processing sector is based on a hazard analysis and critical control point (HACCP) approach. This approach is not specific to the control of STEC, but broadly addresses biological, chemical, and physical hazards. HACCP programs in the processing sector include microbial testing of product for indicator bacteria and/or specifically for *E. coli* O157 (Van Elsas *et al.*, 2011). Most of the regulatory focus on the prevention of STEC has been in the processing sector. Many nations and municipalities have guidelines and regulations setting standards for internal cooking temperatures and sanitary measures at the retail level, that, when properly enforced, also serve as important barriers to human infection. Public health surveillance of STEC infections can play an important role in devising and implementing control measures. Whereas reporting of human cases of STEC infection (or only O157:H7 infection) to public health authorities is mandatory in some countries, it is not so in others (Baker *et al.*, 2016).

A variety of food processing technologies have been proposed for control of *E. coli* O157:H& in foods. Heating, e.g. pasteurization, is an effective method of killing the pathogen. Steam-vacuuming and organic acid sprays are capable of reducing populations of the pathogen on the surfaces of animal carcasses (Bai *et al.,* 2015; Ahlstrom *et al.*, 2017). New technologies such as the use of high hydrostatic pressure, pulsed power electricity, ohmic heating, bright light pulsing and ozonation also show promise. Gamma irradiation has also been shown to be an effective method of control. These and other control procedures need to be evaluated alone and in concert to arrive at effective pathogen reduction strategies for a particular food. Cost is a consideration and is often prohibitive in the implementation of the new processes, and more research is needed to develop cost-effective control measures (Wilson *et al.,* 2018).

2.5. Prevalence of *Escherichia coli* O157:H7 in foods of animal origin in Ethiopia a systematic review and meta-analysis of published researches

2.5.1. Literature search strategy

The literature search was conducted to identify all published studies reporting the prevalence of *Escherichia coli* O157 in foods of animal origin. It was conducted in electronic databases of PubMed, google scholar, and African Journals Online from April to May 2018. The specific search medical subject heading (MeSH) terms include "*Escherichia coli* and Ethiopia", "*Escherichia coli* prevalence and Ethiopia", "*Escherichia coli* O17: H7 and Ethiopia", "*Escherichia coli* O157 and meat in Ethiopia", "*Escherichia coli* O157 in milk in Ethiopia", "*Escherichia coli* O157" AND "Ethiopia" AND "prevalence". Based on the intensive literature search, a total of 187 pieces of literature that report the prevalence of *Escherichia coli* in Ethiopia were retrieved. However, only 30 reports on the prevalence of *Escherichia coli* in meat, milk, and related working environmental samples were screened and nine of them reported the pathogenic strain *E. coli* O157 made it to the final meta-analysis procedure. Study screening strategy and exclusion reasons are presented in figure 5.

2.5.2. Eligibility criteria and data extraction procedure

All Articles that report the prevalence of *Escherichia coli* O157 in meat and milk in Ethiopia were downloaded and added to Mendeley reference manager. Duplicates were rigorously checked and removed. Quality criteria were developed prior to starting the review of full papers. Inclusion/exclusion criteria were defined regarding the relevance of the articles to the research questions of interest. The inclusion criteria include an observational study that reports prevalence of the diseases, published article or MSc thesis in University online repositories, reporting the prevalence of the organism only in foods of animal origin from 2000 to 2017, diagnostic methods that employed one of the diagnostic approaches (culture, Latex agglutination, and molecular methods). Articles that met the above criteria were considered for the final meta-analysis and systematic review. Titles were checked twice in both excluded and included databases of the Mendeley reference manager before the start of data extraction process to avoid missing a valuable report. Those papers considered relevant were retained and their results were extracted to a pre-prepared data extraction excel sheet. Data extracted from valuable papers include study area, study year, sample size, number of positives, food type examined, diagnosis method used, author's name, article title, and year of publication.

2.5.3. Statistical analysis

Statistical analyses were done using Stata 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX). A simple summary of reports with prevalence of *Escherichia coli* O157 were done with descriptive statistics. Meta-analysis of prevalence data was analyzed, pooled the estimates and the 95% confidence intervals. Due to the natures of studies, substantial heterogeneity was expected and a random-effects meta-analysis was done with the estimate of heterogeneity being taken from an inverse-variance model (DerSimonian and Laird, 1986). Between-study heterogeneity was assessed using Higgin's I^2 and Cochran's Q method. I^2 values of 25%, 50%, and 75% were considered as low, moderate and high heterogeneity, respectively (Higgins and Thompson, 2002). Subgroup analysis was also conducted by food type sampled, location of study and diagnosis method used.

2.5.4. Descriptive results of eligible studies

From all screened studies, 9 articles were eligible for the final systematic review and meta-analysis. Literature were heterogeneous, had inappropriate study designs, unrepresentative sample size, and unreliable diagnostic methods that cannot identify pathogenic strain O157. This diversity, together with the lack of data on other required variables, reduced the datasets substantially. Descriptive summary statistics were calculated to determine the total number of sampled foods and the range of prevalence estimates in different foods. Accordingly, the overall apparent prevalence in all studies estimated 4.91% in all samples examined. A detailed summary of the studies can be found in Table 1.

2.5.5. Meta-analysis

Due to the expected variation between studies, a random-effects meta-analysis was carried out using the total sample size and number of positives (effect size and standard error of the effect size). The result of meta-analysis indicated that individual study prevalence estimates ranged from 0% to 10% with an overall random pooled prevalence of **4% (95% CI =3% – 5%)**. Figure 1 presents the Forest plot derived from the meta-analysis. Between-study variability was high ($\tau 2 = 0.01$; $I^2 = 89.06\%$ with Cochran's Q statistics value of 146.28). Studies weighted approximately equal with weights on individual studies due to high heterogeneity between studies.

2.5.6. Subgroup analysis

Subgroup analyses were done for sample types (beef, milk, mutton, chevon, and environmental swabs like hand swab, knife swab and working water samples), study location (Eastern, Central, Southern and Western Ethiopia), and diagnosis method used (latex agglutination (serological test) and molecular techniques). Results of the subgroup analysis are depicted in figures 2,3, and 4 respectively with forest plot and in table 2 for the overall statistics.

Table 1: Summary of studies included in the systematic review and meta-analysis

Study (references)	SS	NP	AP (%)	Region	Diagnosis method	Sample Type
(Abdissa *et al.*, 2017)	1235	6	0.49	Central Ethiopia	Serological	Beef
(Abdissa *et al.*, 2017)	1247	10	0.80	Central Ethiopia	Serological	Environmental samples
(Atnafie *et al.*, 2017)	300	8	2.67	Southern Ethiopia	Serological	Beef
(Atnafie *et al.*, 2017)	330	7	2.12	Southern Ethiopia	Serological	Environmental samples
(Bekele *et al.*, 2014)	384	39	10.16	Central Ethiopia	Serological	Beef
(Beyi *et al.*, 2017)	195	5	2.56	Central Ethiopia	Serological	Beef
(Beyi *et al.*, 2017)	330	4	1.21	Central Ethiopia	Serological	Environmental samples
(Dulo *et al.*, 2015)	235	6	2.55	Eastern Ethiopia	Serological	Chevon
(Haile *et al.*, 2017)	150	14	9.33	Western Ethiopia	Serological	Beef
(Haile *et al.*, 2017)	150	11	7.33	Western Ethiopia	Serological	Environmental samples
(Hiko *et al.*, 2008)	243	6	2.47	Central Ethiopia	Serological	Mutton
(Hiko *et al.*, 2008)	245	5	2.04	Central Ethiopia	Serological	Chevon
(Hiko *et al.*, 2008)	250	20	8.00	Central Ethiopia	Serological	Beef
(Mersha *et*	120	8	6.67	Central	Molecular	Chevon

al., 2010)				Ethiopia		
(Mersha *et al.*, 2010)	224	21	9.38	Central Ethiopia	Molecular	Mutton
(Mersha *et al.*, 2010)	344	13	3.78	Central Ethiopia	Molecular	Environmental samples
(Bedasa *et al.*, 2018)	200	24	12	Central Ethiopia	Serological	Milk

SS sample size, NP number of positives, AP apparent prevalence

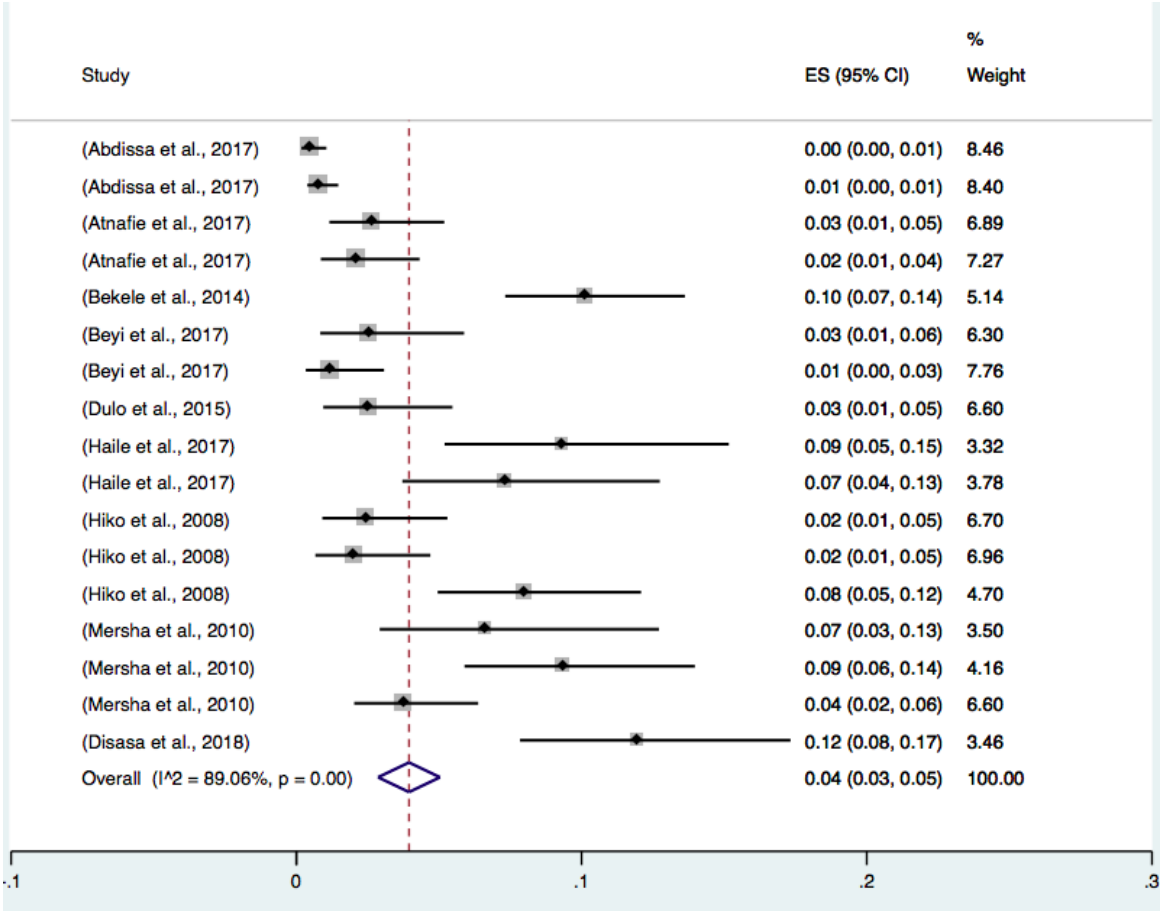

Figure 1: Forest plot on *Escherichia coli* O157 prevalence estimates in Ethiopia.

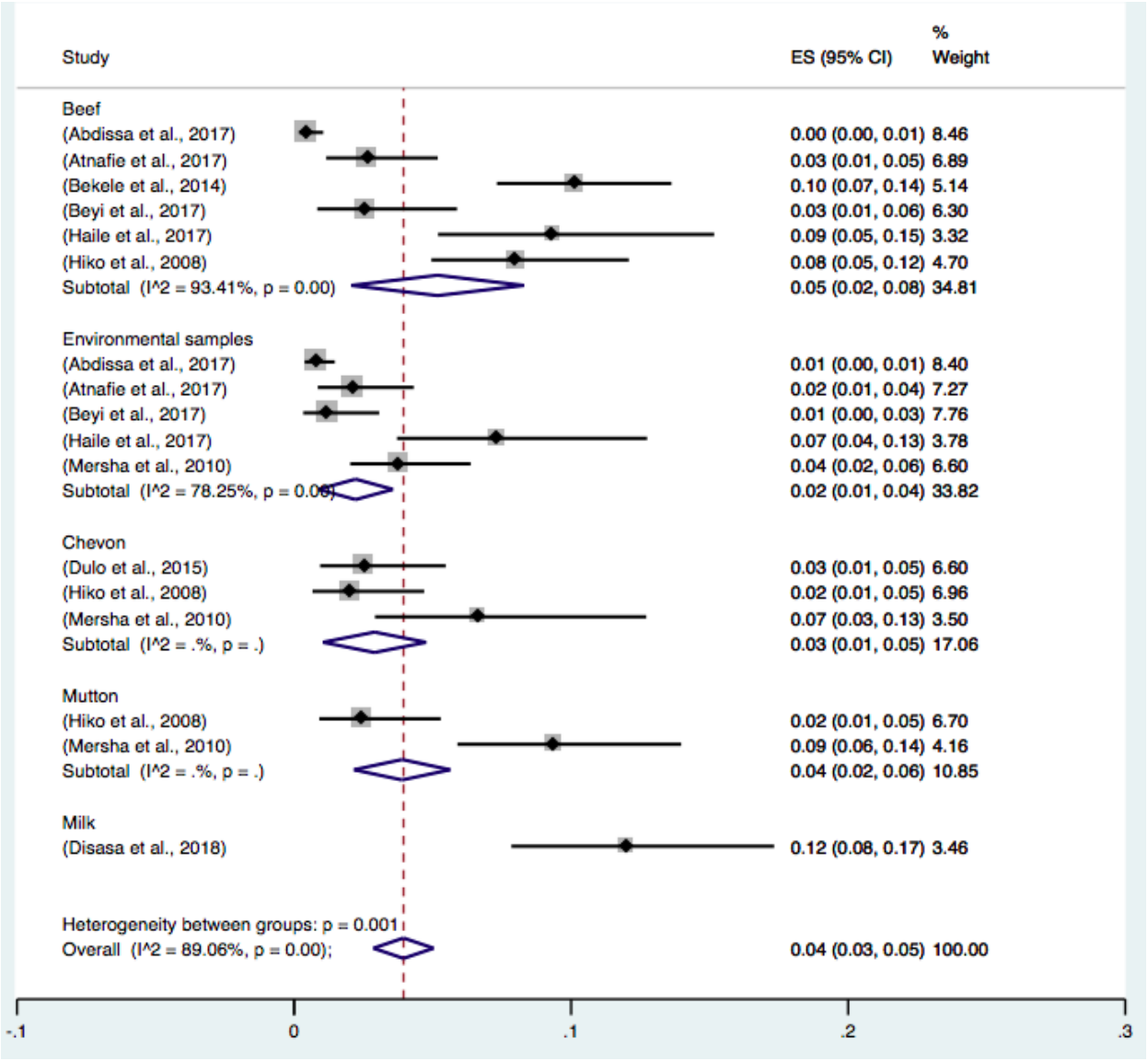

Figure 2: Forest plot of subgroup analysis by sample type on prevalence of *Escherichia coli*.

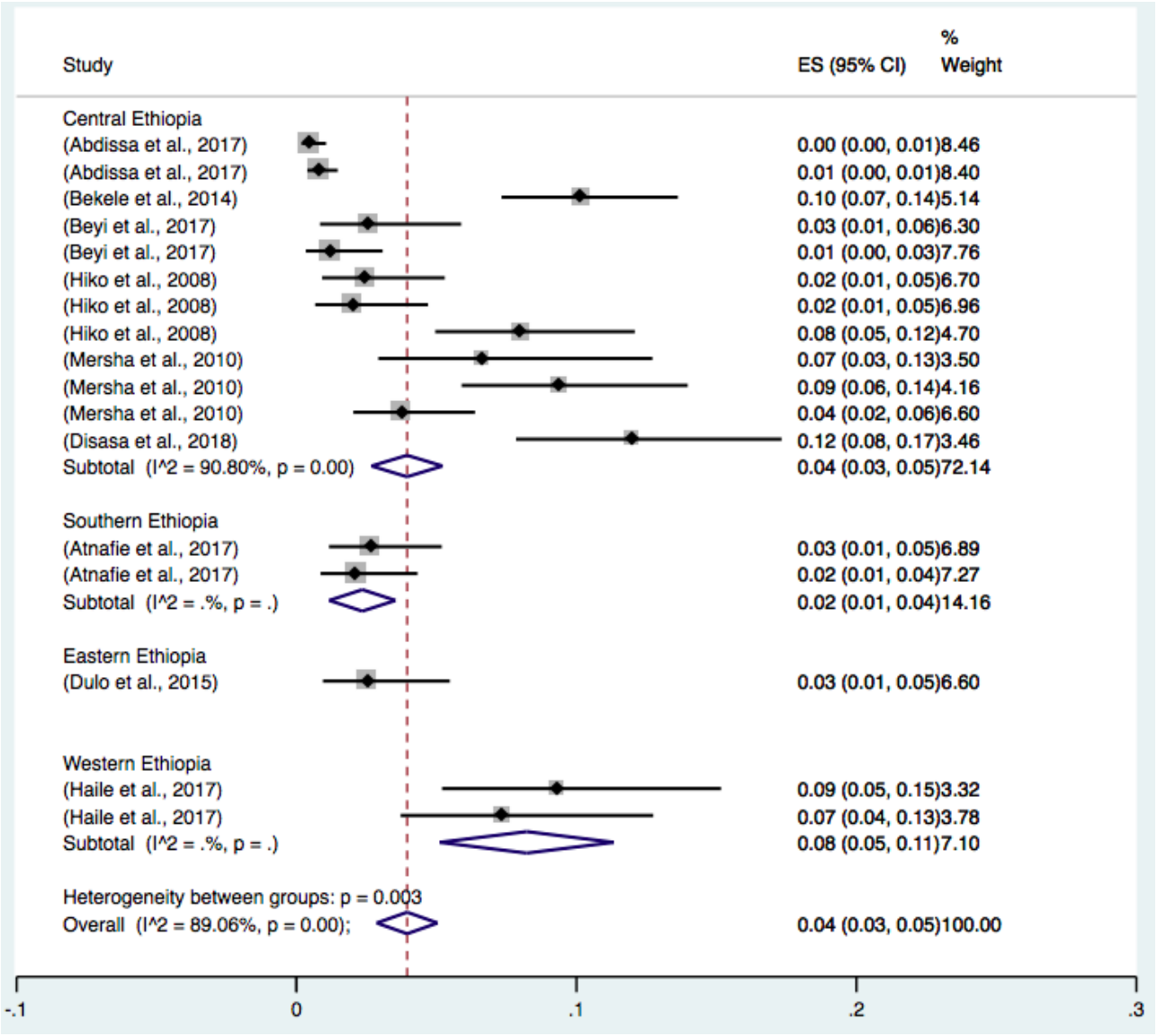

Figure 3: Forest plot of subgroup analysis by study location

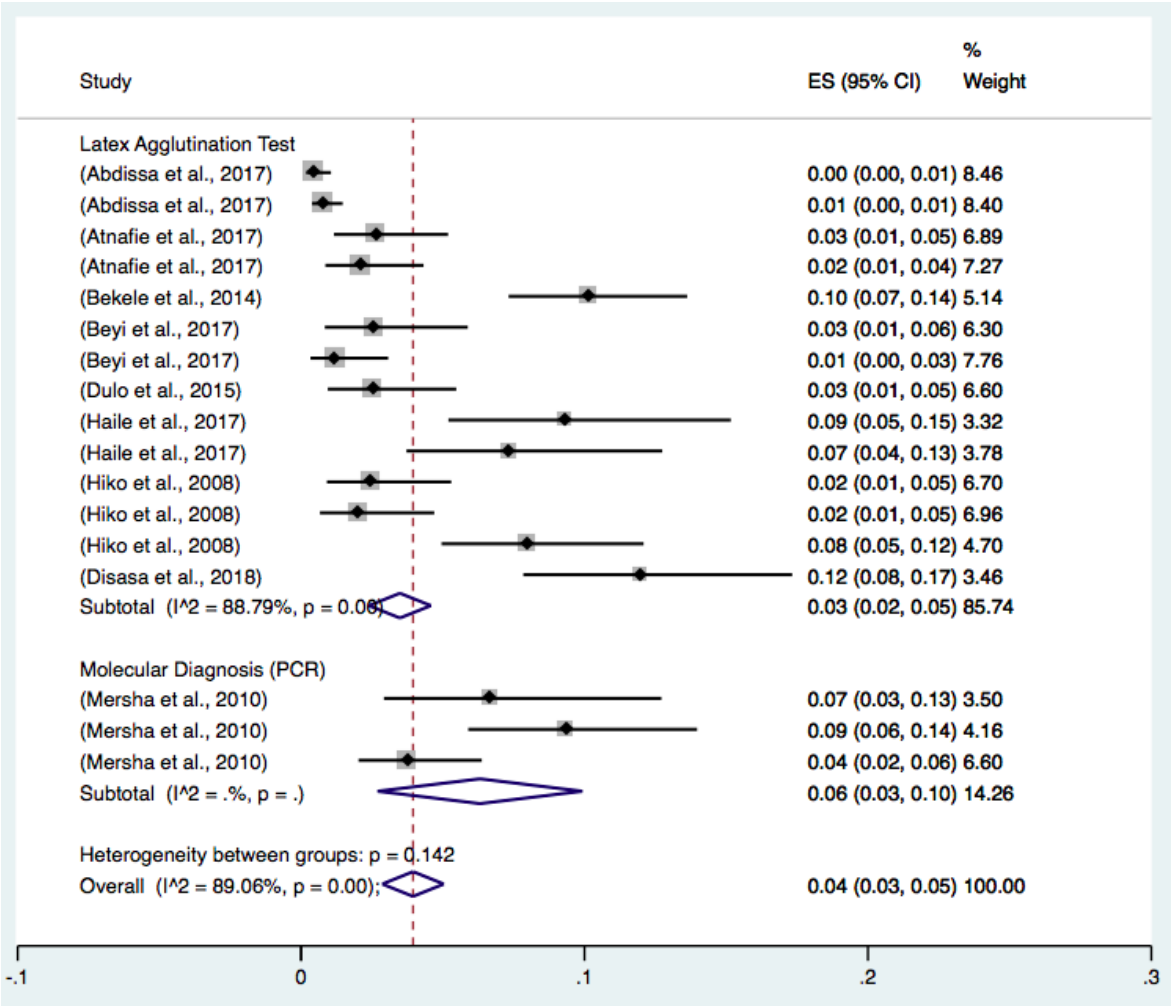

Figure 4: Forest plot of subgroup analysis by diagnosis method

Table 2: Subgroup analysis for comparison of prevalence of *Escherichia coli* O157

	Prevalence (95%CI)		**Heterogeneity test**		
Region		I^2	**Q**	**DF**	***P***
Eastern Ethiopia	3(1-5)	*	*	1	0.00
Southern Ethiopia	21-4)	*	*	1	0.00
Central Ethiopia	4(3-5)	90.8%	119.61	11	0.00
Western Ethiopia	8(5-11)	*	*	1	0.00
Overall	**4(3-5)**	**89.06%**	**146.28**	**16**	**0.00**

Sample type					
Beef	5% (2 - 8)		75.93	5	0.00
Chevon	3% (1-5)		*	*	0.00
Environmental sample	2% (1-4)		18.39	4	0.00
Milk	12(8-17)		-	0	*
Mutton	4% (2-6)			2	*
Overall	**4% (3-5)**				**0.00**
Diagnosis method					
Latex Agglutination	3(2-5)	88.79%	115.98	13	0.00
Molecular Diagnosis	6(3-10)	*	*	2	*
Overall	**4(3-5)**	**89.06**%	**146.28**	**16**	**0.00**

***** indicates omitted results due to lack of enough observations (few studies)

2.5.7. Significance of findings of the systematic review and meta-analysis

This is the first systematic review and meta-analyses on the status of *Escherichia coli* O157 in foods of animal origin in Ethiopia. The review process was limited to foods of animal origin in order to lower heterogeneity between studies and absence of enough studies in other foodstuffs. This report was from analysis of data obtained through a systematic review of scientific publications on the organism between 2008 to 2017. Literature was very heterogeneous, had inappropriate study designs, unrepresentative sample size, and different diagnostic methods. Lack of data on required variables and other factors reduced the number of studies to be included in the final meta-analysis substantially. The final systematic review and meta-analyses were done with 9 studies. According to this review, the first published result that reported *Escherichia coli* in foods of animal origin was in 2008 (Hiko *et al.*, 2008). However, from that year onwards an incredible effort has been noted to report the organism`s occurrence in different foods. Studies increased these days in terms of number and quality by applying latest diagnostic techniques like molecular (PCR) methods. The other qualities of studies were sampling not only the food but also working environment like swabs of workers

hand, knife and handling equipment. Theses sampling approaches are appreciated because they are helpful in identifying sources of contamination.

The overall pooled prevalence of *Escherichia coli* O157 was 4% across all samples. This prevalence might seem low however, the organism is responsible for severe infections and series attention should be given. Ethiopians have a controversial raw meat and milk consumption habit (Avery, 2004). The occurrence of the organism in these foods coupled with these habits can be a factor in causing infections in the country. Raw meat consumption like (*birndo, dulet, and kitfo*) should be handled with caution.

Most of eligible studies retrieved were conducted in central Ethiopia. This can be due to most of milk and meat processing plants are found in this area which can attract the attention of researchers and funders in investigating the occurrence of the organism. Even though this approach may not be wrong at all, it is wise to involve a broader area of research as the condition be worse in rural households where poor hygienic practice and raw food consumption is imminent. According to studies included, the overall pooled prevalence of the organism in central Ethiopia was 4% (95% confidence interval of 3% - 5%).

Based on diagnostic techniques used, the prevalence of the organism was lower in molecular diagnostic technique than latex agglutination test. This can be attributed to serologically reactive organisms yet may fail to possess toxins unique to O157 strain detectable by molecular diagnosis approach.

In light of the above findings planning of mitigation strategies to reduce the impact of the organism is mandatory. If the country wants to achieve the long-awaited membership to world trade organization, it needs to reduce the occurrence of this and other foodborne pathogens to an acceptable level. *Escherichia coli* O157: H7 and other known pathogenic strains should be well studied by applying modern methods of diagnosis to precisely estimate the true status in

foods of animal origin. In addition, future studies need to bear in mind to include rural households of the country where the level of hygienic practices is expected to be low.

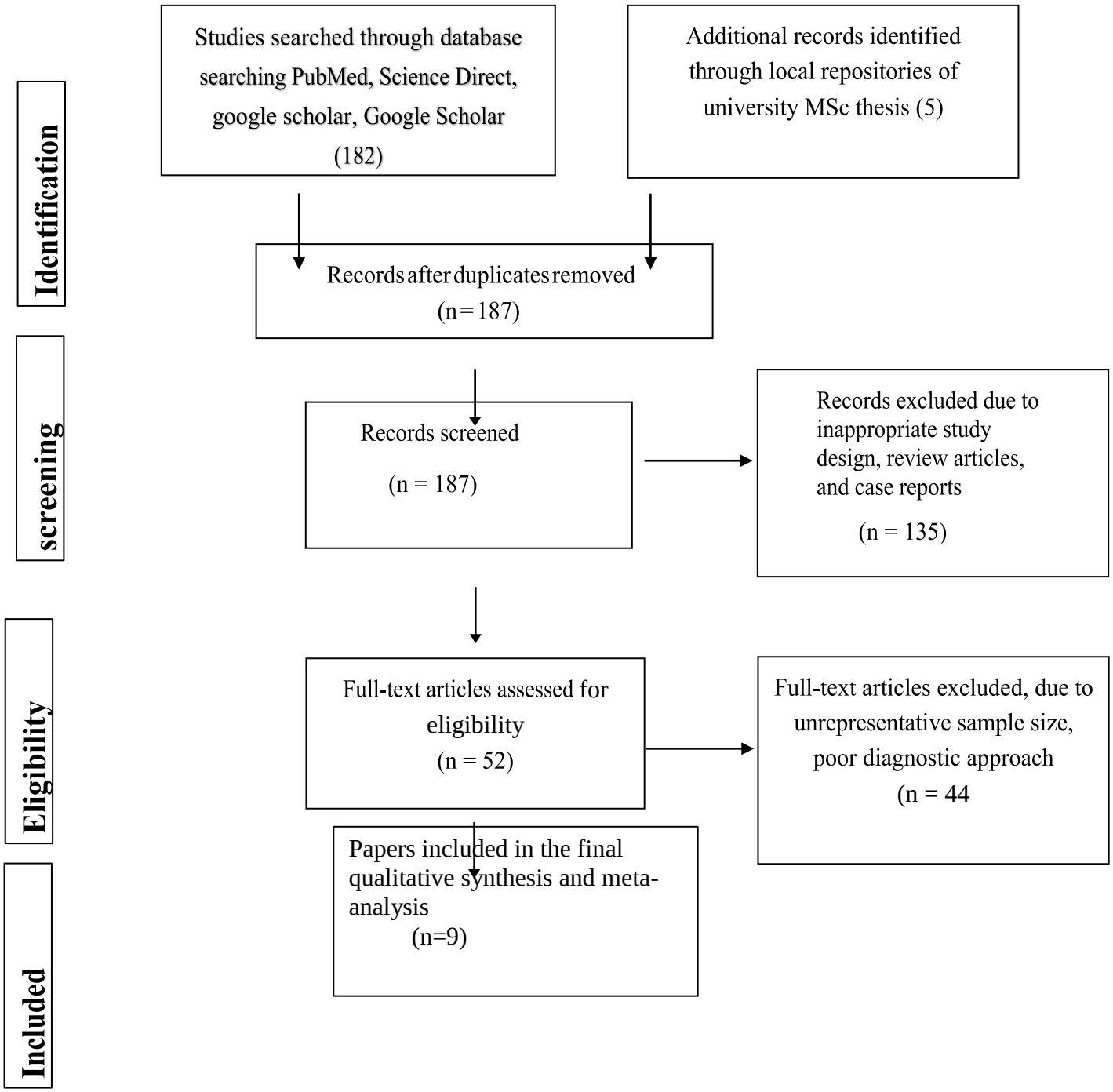

Figure 5: Flowchart of literature search and inclusion/exclusion process

2.6. Status *of Escherichia coli* O157 in fish and fish products: a brief overview

The quality of fresh fish is strongly determined by bacterial microbiota. In this context, the use of *E. coli* as a sanitary indicator for fish samples has been first reported long time ago and widely applied as a microbiological quality parameter, especially on what fecal contamination is concerned. Fish is part of the human diet in many countries and is an important source of nutrients, especially of high digestible proteins. However, it is also known that fish can be a source of foodborne toxinfections, which emphasizes the need of a thorough control of its bacteriological characteristics. A bacterial species associated with infection via ingestion of edible products of marine origin is Escherichia coli. The occurrence of this bacterium in food is directly related to fecal contamination. On what seafood is regarded, the occurrence of *E. coli* is related to water contamination and/or unhygienic conditions during the handling process. Often cited as potential cause for *E. coli* contamination are: The quality of the ice used for conservation, and also the food processing plants (Albuquerque, 2013).

Shiga toxin producing *Escherichia coli* especially that of *E. coli* O157 is not common in fish and fish products provided that contamination of waterbodies, aquarium and production line kept safe from contamination with feces and manures from farms and irrigation sites. However, keeping strict hygienic measures will not be met in every production site. A few studies reported the occurrence of the organism in fish in different part of the world. In India Shiga-toxigenic *E. coli* in fish has been detected and reported to be prevalent (Sanath Kumar *et al.*, 2001). In united states, *E. coli* O157 has been reported to be prevalent in fish products (Ribeiro *et al.*, 2015). In Brazil, researchers isolated only one strain of STEC from shellfish, and evidence that preventive measures especially during harvest and post-harvest are of major importance to avoid contamination of any nature (Ayulo *et al.*, 1994). The organism has been also isolated not only from fish but also a shellfish in India. The authors suggested that the isolation of EHEC strains from shrimp samples indicates a severe adherence to hygienic handling methods, also stating the major importance that proper cooking and processing has for a safe consumption of the product (Surendraraj *et al.*, 2010).

E. coli occurrence in fish is considered a sanitary case and may represent a risk to the consumers if related to pathogenic strains of *E. coli*. However, the presence of non-pathogenic *E. coli* in fish and shellfish should also alert to public health, since this bacterium is recognized as an indicator of fecal contamination, possibly indicating the presence of other enteric pathogens. In order to ensure that the seafood is not a vehicle for *E. coli*, some key measures should be considered, namely: 1) maintaining the microbiological water quality of local capture; 2) post-harvest care; 3) adequate hygiene conditions in the handling process; 4) in cases of processed foods, measures should be taken to ensure the bacteriological safety during all the process. Besides that, it is extremely not recommended to consume raw or undercooked seafood (Albuquerque, 2013).

2.7. Antibiotic resistance in *E. coli*

In Gram-negative bacteria and all *Enterobacteriaceae* chromosomally encoded β-lactamases confer resistance. β-lactamases are enzymes found in most bacterial species, which are able to hydrolyze the β-lactam ring of β-lactam antibiotics (Otero *et al.*, 2017). Although these enzymes have protected bacteria against naturally occurring β-lactams long before the introduction of synthetic antibiotic agents, the numbers and varieties of β-lactamases have increased dramatically since the introduction of modern penicillin and cephalosporin. A single base change in the gene for a β-lactamase can change the substrate specificity of the enzyme. Such changes occur frequently, especially in the *Enterobacteriaceae* (Sunde and Norström, 2005; Von Baum and Marre, 2005).

Multidrug-resistant *E. coli* strains are commonly isolated from animals and food products. The use of antibiotics in animals contributed to the emergence and spread of the number of antibiotic-resistant strains, including *E. coli*, which can also infect humans through either direct contact with animals or through consumption of contaminated food. *E. coli* is able to survive and adapt in various extra intestinal habitats and to spread resistances between humans, animals, their products and the environment through several transmission pathways. Environment plays a key role in the spread of antimicrobial resistance serving as an unlimited reservoir of antimicrobial resistance genes (Von Baum and Marre, 2005; Dib *et al.*, 2018).

Therefore, *E. coli* may acquire other drug resistance traits from environmental bacteria and conversely it can spread its resistance genes to potential pathogens in different habitats. Several studies have reported the presence of multi-resistant strains in hospital effluents (Ho *et al.*, 2012). There is good evidence that *E. coli* O157 colonies farm animals and are transmitted to man via the food chain or directly. Antimicrobial resistance in such bacteria has not been an issue since there is evidence that antibiotic chemotherapy might release more toxin and thus lead to a greater risk of hemolytic uremic syndrome and most but not all strains are antibiotic susceptible. Nevertheless, such *E. coli* can carry multi-drug resistance plasmids although some of the large plasmids in EHECs cannot be transferred(Panos *et al.*, 2006).

2.8. Post-harvest losses in fisheries: types, causes and possible ways of mitigation

Post-harvest fish lose (PHFL) refers to fish that is either discarded or sold at a relatively low price because of quality deterioration. This means that fish operators lose potential income. It also means that less fish is available to consumers, or consumers are supplied with low quality fish and fish products. Fish is a very perishable commodity and hence susceptible to high post-harvest losses. Both physical (material) and quality losses are high in fisheries sector. And these translate into losses in nutritional contribution of fish to the total diet and health's of populations. Very high levels of post-harvest loss occur during pre-processing, processing, storage and transportation of fishery products (Ahmed, 2008; Diei-Ouadi and Mgawe, 2011).

2.8.1. Factors that affect post-harvest fish loss

A high ambient temperature is the most important factor that contributes to the production of biogenic amines during post-harvest handling. Both the postmortem formation of amino acids and their rapid decarboxylation biochemically or microbiologically are temperature dependent that leads to spoilage. Long and unreliable transport with Lack of/ inadequate preservation and lack of markets due to imbalance between supply/demand also site as factors for the loss. In addition to this, Species of fish, Gears used and the storage time affect post-harvest fish lose (Tesfay and Teferi, 2017).

2.8.2. Types of post-harvest fish losses

Many kinds of post-harvest loss have been implicated in fisheries. The major types of post-harvest loss in fish includes nutritional loss, physical loss, quality loss, and market force loss are the main types which are discussed in the next session.

2.8.2.1.Physical loss

Physical fish loss refers to fish that, after capture or landing, is not used. It is either thrown away accidentally, voluntary or as authorized. Physical losses of fish after harvest can be regarded in two distinct ways; First, there is what might be termed complete physical loss. Quantities of fish may spoil completely and become inedible. Related to these losses is the under-utilization of resources when small fish are converted into fish meal instead of being used for human food. Also, there are many less popular fish which are seldom used for human consumption. The second type of physical loss, which can be regarded as a loss of material, is a result of poor handling and processing of both fresh and cured fish. The physical loss of material is caused by, poor handling and preservation or the discarding of by catch. Physical loss can be also caused by theft, by insects eating the fish, or by bird or animal predation. Poor packaging and rough handling can also be a direct cause of physical loss(Getu *et al.*, 2015).

2.8.2.2.Quality loss

Quality loss refers to fish that has undergone changes owing to spoilage or physical damage and has suffered quality deterioration. Such fish is sold for a lower price than that which would have been achieved if the fish were of best quality. This is the most common PHFL in many areas. It is the difference between the potential value of fish/fish product (best quality) and actual value of the fish after it has undergone changes due to spoilage (lower quality) and sold at a lower price. Examples of quality loss include damaged dried fish sold at a reduced price, fresh fish sold several hours after catch without preservation, rejected fish sold for another purpose (animal feeds). High ambient temperatures poor transport long period's

storage inadequate market information reduces quality and leads to low selling prices (Diei-Ouadi and Mgawe, 2011). Quality loss is directly related to nutritional loss. Decomposition of fish to such a degree that it is unfit for human consumption can be considered a nutritive loss. Fresh fish is perishable and is subject to bacterial spoilage. As fish spoils, its nutritional value decreases, as the bacteria causing the spoilage degrade the protein which is intended for human consumption. The major factors that affect the nutritive value of fish products are related to how fish is handled, processed or preserved and stored (Kumolu-Joh and Ndimele, 2011). Traditional practices such as exposing fish for long periods to weather elements coupled with traditional methods of preservation and poor storage are subjecting fish to different kinds of degradation. High temperatures of about 50°C as are encountered in smoking especially in Africa where hot smoking is preferred, affect the availability of lysine, one of the amino acids found in fish protein. Loss of available lysine and other essential monoacids could also occur at much lower temperatures, such as 0°C. This raises the possibility that nutritional losses can occur when fish is sun dried. Other nutrients present in fish muscle which can be affected by the heat used in traditional processing methods include methionine and other sculpture amino acids and vitamins K (Akande and Diei-Ouadi, 2010; Kumolu-Joh and Ndimele, 2011).

2.8.2.3.Market force loss

Market force losses are due to inadequacy between demand and supply leading to changes in price of fish. If the price of fish falls because of oversupply, the seller may incur a market force loss. Market force loss is difficult to measure accurately, because it usually sets the ground for quality and physical losses (Hossain *et al.*, 2015).

2.8.3. Methods of reduction of post-harvest loss in fisheries

Reducing post-harvest losses requires wiser use of resources, reducing spoilage and discards and converting low-value resources, which are available on a sustainable basis, into products for direct human consumption (Kebede and Gubale, 2016; Olusegun and Mathew, 2016). The need for assessment of losses is the first step towards overcoming losses, ways of identifying

losses and defining solutions to the various post-harvest losses are the other steps that needs to be taken to solve the problem and the final stage is to describe various ways of reducing post-harvest losses in fish (Bengwe and Kristófersson, 2012).

2.8.3.1. Chilling and freezing of fish

Chilling with ice is an effective means of reducing spoilage in fish. Ice is an ideal cooling medium; it is harmless, it has a very large cooling capacity for a given weight or volume, it is comparatively cheap and it is able to cool the fish quickly by intimate contact with the fish. However, ice alone is not effective for long preservation, because melting water brings about a sort of leaching of valuable flesh contents which are responsible for the flavor. Freezing means removal of heat from the body. To check the enzymal, bacterial action and putrefaction it is preferred to store the fish under lower temperatures. The fishes are chilled in ice when they are to be stored for a few days. Ice is put inside the body cavity in large fishes. The fishes are arranged in tiers in shelves or boxes and stacked and should not be dumped in heaps in cold storage. It is preferred to store at a temperature below 6.6^{o}C to prevent microbial spoilage of fish (Garcia-Rodríguez and De La Cruz-Aguero, 2011).

2.8.3.2. Drying of fish

Drying involves dehydration i.e. the removal of moisture contents of fish, so that the bacterial decomposition or enzymic autolysis does not occur (Akande and Diei-Ouadi, 2010; Kumolu-Joh and Ndimele, 2011). When moisture contents reduce up to 10%, the fishes are not spoiled provided they are stored in dry conditions. Fish drying is achieved either naturally or by artificial means. Spoilage and loss of quality of fish can often he reduced by simple improvements in drying practices. Drying can be done on the earth, but then the fish is bound to get contaminated with dirt. Drying on mats on the ground, or on hard surface such as concrete is better. It is better still to put the fish on some sort of racks above the ground so that it is more exposed to breeze. If the rack is not too solid, say wire mesh or an old fishing net, then both sides of the fish can dry. Also, if the fish is away from the ground it is less

vulnerable to domestic animals. Fish can be smoked in any equipment which suspends the fish above a fire or in the smoke from a fire (Adesehinwa *et al.*, 2005).

2.8.3.3. Reducing insect infestation

Use of salt is known in many parts of the world as an effective deterrent to blowfly infestation. Investigations have also shown that salted dried fish tends to be less susceptible to beetle infestation than non-salted fish. Although, it is generally understood that high levels of salt will be effective in reducing infestation it is difficult to determine the minimum effective concentration required (Bengwe and Kristófersson, 2012). Moreover, it is difficult to assess whether this is a practicable method in the long term. Most of the trials have been carried out in areas where salt is not normally used and there is usually some unsalted fish nearby. It could be that blowflies merely prefer the latter, given a choice. In areas where most or all fish is salted, like Indonesia, blowflies attack salted fish very readily. Also, in areas where salting is not normally used, consumers are unfamiliar with the salty produce and may not accept it (Garcia-Rodríguez and De La Cruz-Aguero, 2011).

2.8.3.4. Use of fumigants

Cured fish can be protected from beetle infestation during storage by the use of fumigants. These are insecticides existing in the gaseous form at ambient temperatures. As gases, they can diffuse into the dried fish and kill any insect infestation. It is important to remember that they do not provide lasting protection and the product can become re-infested unless suitable precautions are taken (Hossain *et al.*, 2015). Two of the most effective fumigants for cured fish have been found to be phosphine and methyl bromide. It is crucial to remember that as fumigants are toxic gases, they are very dangerous, so fumigation should only be carried out by trained personnel (Adesehinwa *et al.*, 2005).

3. MATERIALS AND METHODS

3.1. Description of the study areas

The study was conducted in two Lakes of Northern Ethiopia. Namely, Tekeze hydroelectric power Dam and Lake Hayq. Tekeze hydroelectric power Dam is found in Amhara region, and some part in Tigray region. The Dam is built on the Tekeze river which is a tributary of the Nile. The Dam has an average area and depth of 160.4km^2 and 58m respectively (Teame *et al.*, 2016). It has a tremendous resource in terms of fish production that reach an average 5,065 tons of fish annually which created invaluable job opportunities for the surrounding community. Lake Hayq is a freshwater lake located in south Wollo zone of Amhara Regional State. It is one of the highland Lakes of Ethiopia, measuring an altitude of 2,030 meters above sea level. It receives water from many small seasonal streams and one perennial river named Ankerka. The catchment area of the lake is 65 km^2. A maximum depth of 88.2m and 81.44m, were recorded in 1941 and 2013, respectively. Lake Hayq provides economical support via tourism and fishery, and most importantly it provides drinking water to the local inhabitants (Seid, 2016). This lake is well known for its fishery resource for the community and nearby towns. It has a potential of an average 500 tone of fish production per year (Tesfaye and Wolff, 2014). The two study Lakes are illustrated in figure 6.

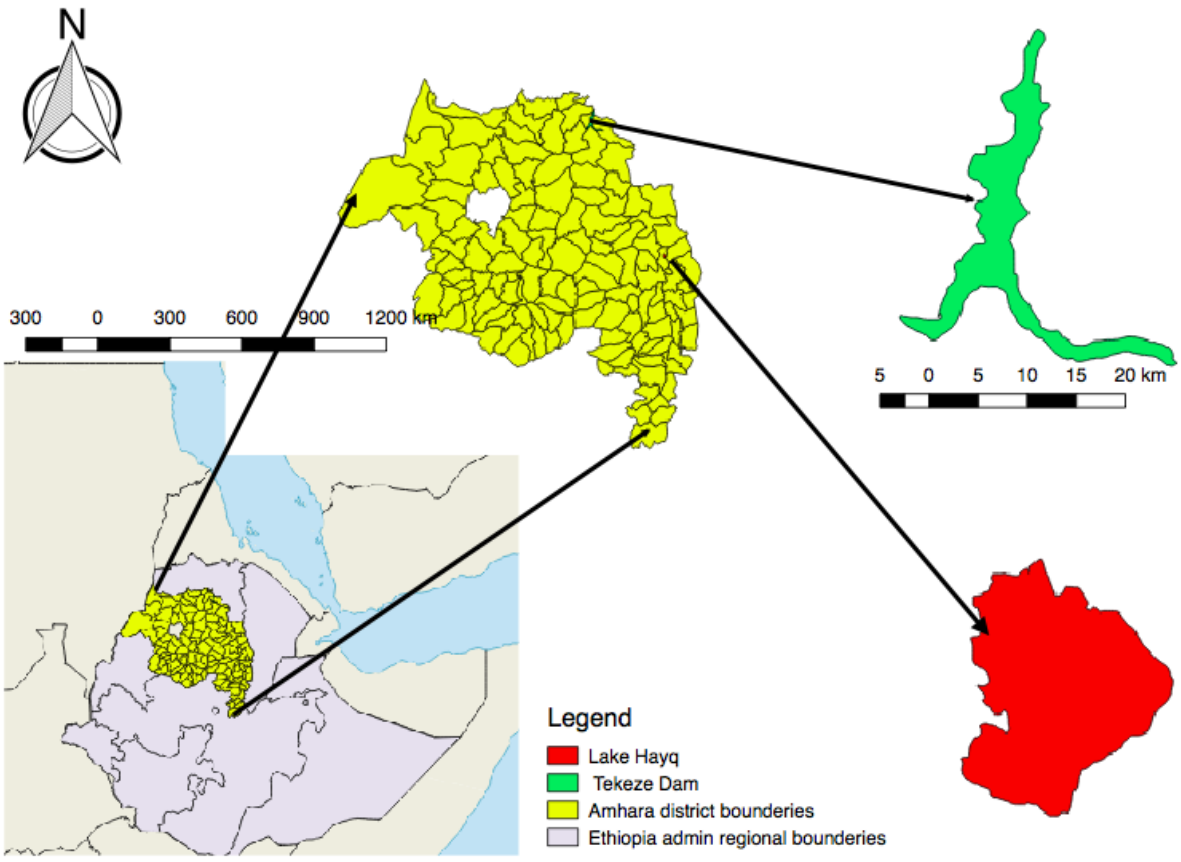

Figure 6: A map that shows study sites

3.2. Study design, sampling method and sample size determination

A cross-sectional study design with simple random sampling approach was implemented from October 2017 to May 2018. Fish harvested from the two Lakes were sampled in different locations (landing site, local retail market and, restaurants). From these sampling locations, samples were taken as follows; in the study areas, fish is sold in a jerry can (casa) and sac containers. Containers (casa and sacs) were randomly selected in simple random sampling method. From a selected jerry can or sac container, few swabs from different fish contained in that container were taken and pooled together in to a single transport media which represents one sample of the whole sample size. In addition, for fish tissue samples, a container was

randomly selected as well. From that container, fish were randomly selected and approximately 20g of the muscle was cut and added to stomacher bags. In local markets, samples were collected with simple random sampling approach as well. All restaurants were included in the study sites of Hayq city because there were no enough restaurants that serve fish. However, from these restaurants, fish samples and working environment samples were taken in simple random sampling approach.

To estimate the prevalence of *E.coli* O157: H7 in fish the sample size was determined by (Thrusfield, 2007) as follows;

$$n = \frac{1.96^2 * pexp\ (1 - pexp)}{\mathrm{d}^2}$$

Where *n*, is the required total sample size, *pexp* is the expected prevalence of the organism in fish and d, stands for the desired absolute precision of 5%. Because there was no prior information regarding the prevalence being estimated, a more conservative expectation of 50% prevalence of *Escherichia coli* O157: H7 was considered. Hence, the sample size was estimated to be a minimum of 384 fish as well as other expected sources of contamination samples from the landing sites and restaurants and we sampled 410 fish and working environment samples from the two Lakes. From Lake Hayq, 250 samples were taken while from Tekeze dam 160 samples were taken. This sample size imbalance was due to the absence of local retail markets and restaurants around Tekeze dam. Samples from local retail markets and restaurants were taken only from Lake Hayq and nearby Hayq city.

The sample size for the postharvest loss interview process was estimated based on (Coe, 1996; Israel, 2012).

$$n = (N)/(1 + N(e^2))$$

Where n, is the sample size, N is the population size, and *e* is the level of precision. Based on this formula, the total fishermen are estimated to be (N=1000) in the two Lakes which make the sample size 290 fishermen from all study sites with a confidence level of 95%, a precision of 5%. The sample size was further reduced by finite population correction formula; n'=1/(1/N+1/n). Where n, is the sample size, N is the population size and n' is the final sample size corrected.

Based on this formula, the final sample size for the questionnaire was 75 individuals involved in fishing and marketing. However, to generate a more comprehensive data about post-harvest loss and handling practices, the number of participants increased to 140. The sample size was distributed proportionally between the two Lakes based on their production potential and number of individuals involved in fishing activities. Hence, 85 participants were interviewed from Tekeze dam while in Lake Hayq 55 individuals were involved in the interview process.

3.3. Sample collection procedure

Filleted fish swab, filleted fish muscle (tissue), whole fish (skin) swab, knife and cutting board swab, ready to eat fish from restaurants, workers hand swab, and container swab were collected by following recommended procedures. Approximately 20g of fish was cut and inserted into a stomacher bag that contained 200 ml of transport media (buffered peptone water). In addition, swabs from fish, hand of worker, container and, knife were collected in a universal bottle that contain 10ml buffered peptone water as a transport media. Samples collected from the respective sites were transported to the nearest laboratory in shortest possible time and processed upon arrival.

3.4. Microbiological analysis procedures

3.4.1. Sample preparation and identification procedure of Escherichia coli O157:H7

All the isolation and identification procedures of the organism were performed based on ISO 6887-3:2017 recommendation for microbiological analysis of fish samples (ISO6887-3:2017, 2017). Samples collected from study sites were transferred appropriately to the nearby animal health laboratories (Sekota Dryland Agricultural Research Center for samples taken from Tekeze dam, and Kombolcha Regional Animal Health laboratory for samples taken from Lake Hayq) with a transport media. After reaching the laboratory, samples were incubated at 41.5^{o}C for 6hrs to increase the recovery rate of stressed cells. Muscle samples of fish placed in a plastic bag were homogenized using a homogenizer. From each incubated suspension (swabs in 10 ml of BPW, and homogenized muscle samples) approximately, two hundred

microliters of the samples were streaked onto Sorbitol MacConkey agar plates and incubated at 37°C for a maximum of 24 hrs. Following incubation, sorbitol negative (colorless) colonies were identified by their color and further streaked onto sorbitol MacConkey agar plates again to get a clear colorless typical *E. coli* O157 isolates. From the pure culture, isolates were subcultured onto nutrient agar for further preservation and transportation of isolates with glycerol stocks.

Final serological confirmation of positive samples was not carried out in the above laboratories. For that matter, isolates were prepared for transportation to Addis Ababa University-CVMA laboratory as follows; isolates from the nutrient media were subcultured onto Trypton soya broth (Oxoid, Ltd., Basingstoke, UK) for 24h at 37^{o}C. One ml of the Trypton soya broth (TSB) bacterial suspension was mixed with equal volume of sterilized 50% glycerol in sterilized Cryovial tubes. These bacterial-glycerol stocks were stored at -80^{o}C deep freezer and transported with ice box packed with ice to AAU-CVMA lab. Samples were stored in deep freezer and when Latex kit is secured, bacterial stocks were revived by culturing onto TSB and further sub culturing onto nutrient agar. From nutrient agar colonies were further subjected to biochemical tests. Indole test and dextrose/lactose fermentation test by culturing onto TSB and streaking onto Klinger iron agar (KIA) media respectively were carried out to screen positive samples. Based on these tests, red ring formation by indole test, and yellow slant and butt formation for sugar fermentation test were presumed to be positive for *Escherichia coli* O157: H7. Positive cultures were further subjected to a serological test using latex kit for the confirmation of *E. coli* O157: H7 strain. Bacterial colony was picked and subjected to slide agglutination test using an *E. coli* O157 latex kit (Oxoid Ltd., Hampshire, UK). A drop of test latex and sterile saline water were dispensed into the reaction card separately. Up to five presumptive E. coli O157: H7 colonies were picked by lightly touching the center of the colony with sterile inoculating needle. The picked colonies were thoroughly emulsified with the saline on latex card and then finally with the test latex reagent. Formation of agglutination within one minute was regarded as positive.

3.4.2. Antibiotic susceptibility test

All *E. coli* O157 isolates were subjected to susceptibility testing against eight antibiotics. Gentamycin, Kanamycin, Chloramphenicol, Ciprofloxacin, Streptomycin, Tetracycline, Nalidixic acid, and Ampicillin disks were used to assess the susceptibility pattern of the isolates. The method used for the susceptibility test was the Kirby-Bauer disk diffusion on Muller-Hinton agar plates prepared according to the manufacturer's recommendation. The isolated bacterial colonies from pure fresh culture were transferred to a test tube of 5 ml Trypton soya broth (TSB) and incubated at 37^{o}C for 6hrs. The turbidity of the culture broth was adjusted using sterile saline solution to obtain a turbidity comparable to 0.5 McFarland turbidity standard. A sterile cotton swab was immersed to the suspension and swabbed uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic disks were placed and gently pressed using sterile forceps and incubated at 37^{o}C for 24hrs. The diameter of inhibition zone formed around each disk was measured using a digital caliper. The results were interpreted according to clinical laboratory standard protocols (CLSI, 2015).

3.5. Post-harvest loss (PHL) assessment methods

Post-harvest losses assessment was carried out using qualitative and quantitative field assessment methods. Informal fish loss assessment method (IFLAM), load tracking (LT) and the questionnaire loss assessment method (QLAM) methods were used to assess the causes and to estimate the amount of PHL of fish, (adapted from FAO (Diei-Ouadi and Mgawe, 2011)).

3.5.1. Informal fish loss assessment method

Secondary data from district agricultural office reports about fish production and losses in the study areas were collected and the estimates were calculated.

3.5.2. Load tracking (LT)

Load tracking was used to measure weight losses at different stages along the distribution chain specifically at transport and marketing. A random fishing vessel was sampled for measurements and at least 30% fish weight was measured in balance at landing site and trader's store with nineteen replications. The physical loss was calculated by subtracting final weight from initial weight.

3.5.3. Questionnaire loss assessment method

A questionnaire was translated to Amharic and administered to fishermen to generate information about losses and handling practices and to validate information generated by the informal fish loss assessment and load tracking methods. Types of loss, reasons for loss, frequency of loss, variables that affect losses, fishing gear type, seasonality, livelihood activities and many more variables were recorded.

3.6. Data management and analysis

All data generated in field and laboratory were entered, coded, and filtered in Microsoft Excel® version 2016 software. From the excel sheet, data were further exported and analyzed using Stata 14 (StataCorp. Stata, Statistical Software: Release 14. College Station, TX) for statistical handling purpose. Descriptive statistics (frequency tables and graphs) were used to visualize the findings, while logistic regression, Fisher's exact test and survey analysis function of stata's GLM model with robust standard errors were used to make statistical inference about the findings. A p-value ≤ 0.05 was considered as significant.

4. RESULTS

4.1. prevalence of *Escherichia coli* O157: H7 in fish

The overall prevalence of *Escherichia coli* O157: H7 in fish was found to be 1.46% (6/410). The occurrence of the organism was numerically higher in Lake Hayq than Tekeze Dam. In addition, it was also higher in filleted fish than whole fish swabs. However, these differences were not found statistically significant. The organism was not isolated from ready to eat fish sampled from restaurants, knife and cutting board sab, workers hand, and container swabs. The prevalence of organism was numerically higher in samples taken from landing sites than local retail markets and restaurants. The prevalence of the organism in different sample types, sampling location, and study Lakes is presented in Table 3.

Table 3: Prevalence of *E. coli* O157 in different sample types, study Lake and sampling sites

Variables	Total sampled	Positives(n)	prevalence	Fishers exact
Sample type				
Filleted fish swab	125	3	2.4%	0.894
Filleted fish muscle(tissue)	89	2	2.24%	
Whole fish (skin) swab	125	1	0.8%	
Knife and cutting board swab	10	0	-	
Ready to eat fish	13	0	-	
Workers hand swab	24	0	-	
Container swab	24	0	-	
Study Lake				0.565
Hayq	250	4	1.65	
Tekeze	160	2	1.01%	
Sampling Sites				1.0
Landing site	293	5	1.7%	

Retail market	75	1	1.3%
Restaurants	42	0	-
Total	**410**	**6**	**1.46%**

4.2. Antibiotic susceptibility test

Based on the susceptibility test result, Ampicillin and Streptomycin performed poorly against the isolates. On the other hand, Ciprofloxacin and Nalidixic acid were found to be effective in preventing the growth of most of the isolates. Tetracycline, Gentamycin, and kanamycin also performed reasonably well (Table 4).

Table 4: Antibiotic susceptibility test result

Antibiotic disk	**Disc concentration**	**Observed performance against isolates**		
		Susceptible	**Intermediate**	**Resistant**
Gentamycin	10 µgm	6(100%)	0(0.00%)	0(0.00%)
Kanamycin	30 µgm	4(66.7%)	2(33.3%)	0(0.00%)
CAF	30 µgm	5(83.3%)	1(16.7%)	0(0.00%)
Ciprofloxacin	5 µgm	6(100%)	0(0.00%)	0(0.00%)
Streptomycin	10 µgm	0(0.00%)	1(16.7%)	5(83.3%)
Tetracycline	30 µgm	4(66.7%)	1(16.7%)	1(16.7%)
Nalidixic acid	30 µgm	6(100%)	0(0.00%)	0(0.00%)
Ampicillin	10 µgm	0(0.00%)	0(0.00%)	6(100%)

4.3. Antibiotic resistance pattern and resistance index of the isolates

The maximum number of resistance recorded was only being resistant to two drugs in five of the isolates. This indicates there were no multi drug resistant isolates. The overall resistance and resistance index pattern was calculated and depicted in table 5.

Table 5: Antimicrobial resistance pattern of the isolates

Isolate code	Antibacterial resistance profile	Sample type	Source (study Lake)	Number of resisted antibiotics	MDR index
P1	S&AMP	Whole fish	Hayk	2	0.25 (2/8)
P2	S&AMP	Filleted fish swab	Hayq	2	0.25 (2/8)
P3	S&AMP	Filleted fish swab	Hayk	2	0.25 (2/8)
P4	S&AMP	Filleted fish flesh	Hayq	2	0.25 (2/8)
P5	S&AMP	Filleted fish swab	Tekeze	2	0.25 (2/8)
P6	AMP	Filleted fish flesh	Tekeze	1	0.125 (1/8)

S=Streptomycin, AMP=Ampicillin

4.4. Post-harvest loss assessment

4.4.1. Demographic characteristics of participants selected for the interview

Randomly selected fishermen were interviewed at landing sites and nearby retail markets to assess the extent and causes of PHL in fisheries. A total of 140 participants were interviewed from the two study Lakes. Almost all of the participants were males with fishing experiences ranging from 1 to 30 years. Eighty-nine of the participants were married while the rest were single. All demographic related information is depicted in the next tables and figure (Table 6)

Table 6: Demographic characteristics of participants

Variables	Mean	Min	Max
Age	28.2 $\pm$ 7.7	15	55
Fishing experience	5 $\pm$ 3.5	1	30
Family size	3 $\pm$2	1	10
Annual income from fisheries (ETB)	22268 $\pm$ 109.75	5000	50000

4.4.2. Information on fisheries and related activities

Fishing activities in the two Lakes were studied to understand causes for PHL and recommend actions needed to reduce postharvest loss and poor handling practices. These activities measured based on a questionnaire on randomly interviewed fishermen involved in fishing and marketing. Data generated include how fishermen participate in fishing (private, cooperative, or share), what equipment possessed, type of net used, distance from fishing site to landing site/market, where they set gears, when they set gears, how much time they wait to allow nets to catch before hauling, how many days they involved in fishing per week, season of good and poor marketing, season of high loss and many more variables were generated (Table 7 and figures 7-10).

Table 7: Fishing activity variables generated by questionnaire

Variables	**Mean**	**Min**	**Max**
Distance to market (km)	13.9 ±6.7	1	70
Number of gillnets owned	6.9±4.4	0	30
Number of longlines owned	26.45 ±3.6	0	300
wait time spent before marketing	2.4±1.6	.5	6
Fishing days in a week	6.0±1.0	3	7
Wait time before hauling	8.25 ±4.1	2	12
Maximum catch/person/day	25.1±9.8	3	105
Minimum catch/person/day	1.8+1.5	1	8
Daily loss incurred in kg	4.5+1.3	0	25

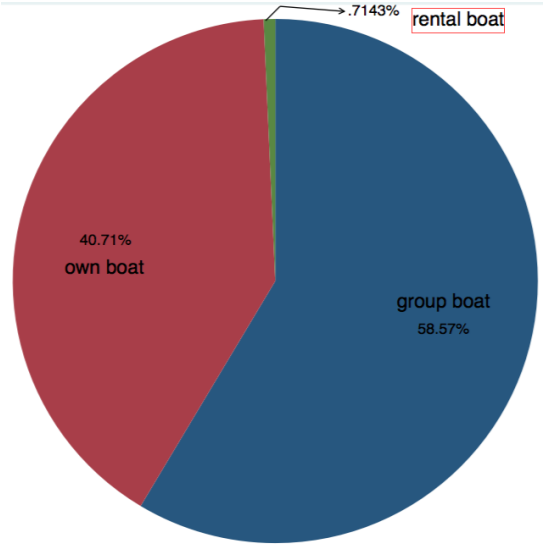

Figure 7: Boat ownership of participants

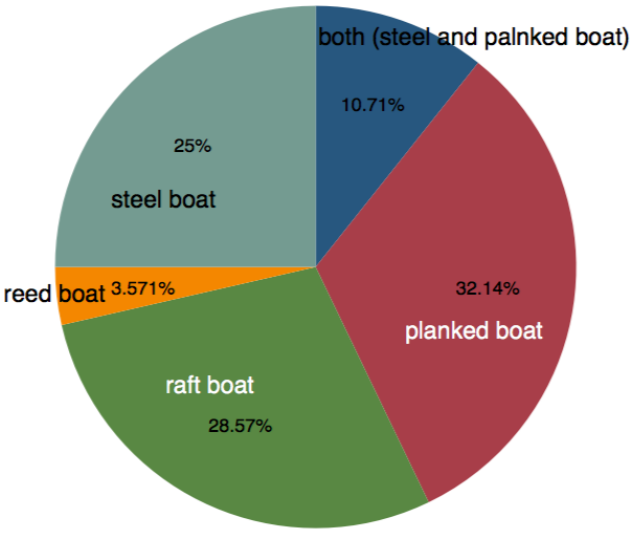

Figure 8: Type of Boat used by participants

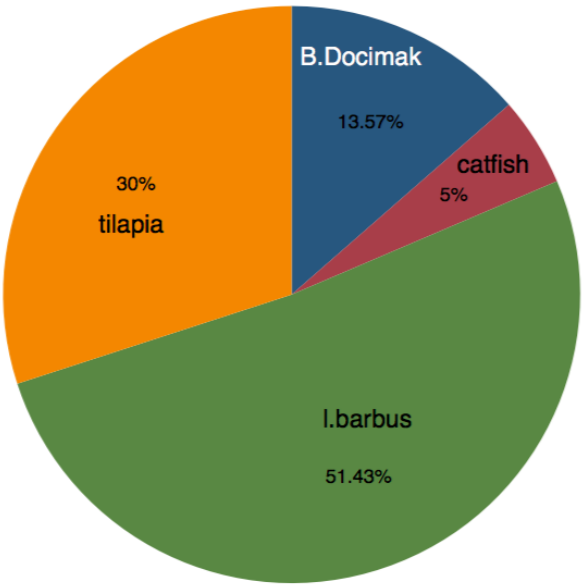

Figure 9: Species of fish mostly spoiled

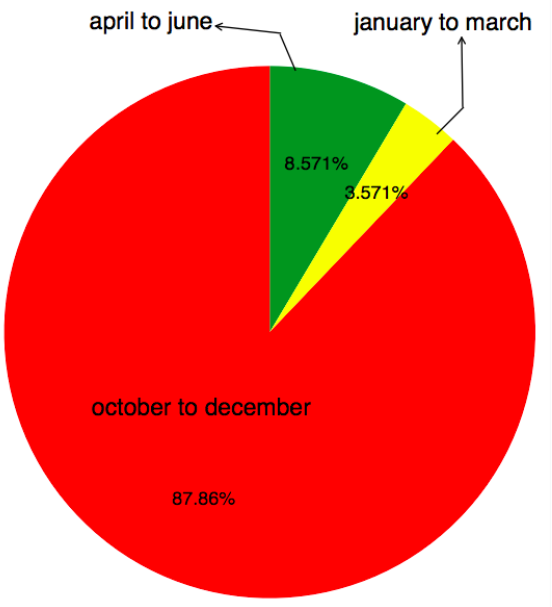

Figure 10: Season of high loss

4.4.3. Causes and extent of PHL associated with fisheries

Fishermen believed that high environmental temperature, absence (delayed) marketing, harvesting immature fish, presence of predators, and flood are the major reasons why they incur loss in the two Lakes (Table 8). Based on FAO recommendations for study PHL, a matrix scoring was applied for every interviewee by having the list of probable suspected causes in order to observe how much fishermen do agree or disagree with these causes. These methods were found appropriate at Tekeze Dam only and applied there. Fishermen strongly agreed that long hours of setting gear before hauling causes high post-harvest quality loss, while agreed to the causes that Fishermen from distant fishing grounds land large quantities of spoiled fish. Other and above-listed causes of PHL at Tekeze dam were depicted in tables 9.

Table 8: Causes of PHL according to informants

Cause of PHL	**Hayq**	**Tekeze**	**Both Lakes**
	N=55	**N=85**	**N=140**
	Frequency (%)	**Frequency (%)**	**Frequency**
Harvesting immature fish	5 (9.09)	1 (1.18)	6 (4.29)
High environmental temperature	15 (27.27)	39(45.88)	54(38.57)
Delayed marketing	23 (41.82)	24(28.24)	47(33.57)
Losing with the net in the Lake	4 (7.27)	-	4(2.86)
Predators	8 (14.55)	-	8(5.71)
Distance from market	-	15(17.65)	15(10.71)
Flooding	-	6(7.06)	6(4.29)
Total	**55** (**100**)	**85** (**100.00**)	**185**(**100**)

Table 9: Causes suggested by a graduate response (Likert scale)

Variables	SA	A	NAND	D	SD
Long hours of setting gear before hauling causes high post-harvest quality loss	81.18	15.29	0	3.53	0
Fishers from distant fishing grounds land large quantities of spoiled fish	20.00	45.88	17.65	15.29	1.18
On average, two crates of fish are found spoiled on landing	4.71	21.18	45.88	27.06	1.18
High post-harvest fish loss occurs during the rainy season	3.53	42.35	22.35	17.65	14.12

SA- strongly agree A- agree, NAND- neither agree nor disagree, D- disagree, SD- strongly disagree

Despite visualization of findings by graphs and tables, we developed a GLM model consisting of different variables. The analysis was done by using the daily loss of fish in kilograms claimed by fishermen as a response variable, and other important variables as predictors for loss. By using this model, we found that some of the variables were significantly associated with daily loss incurred. A predictor model for PHL of fisheries in the study Lakes consisting of all significant variables are depicted in Table 10.

Table 10: Multivariable GLM model that show association between the amount daily loss and predictors

Variables	Coefficients	P value	95% CI
Lake			
Hayq	Reference		
Tekeze	5.76631	0.01	4.5, 6.99
Boat type used			
Both (planked &steel)	Reference		
Planked boat	0.4	0.728	1.85, 2.65

Raft boat	-4.09	0.001	-6, -2.17
Steel boat	2.5	0.101	0.50, 5.65
Boat ownership			
Group boat	Reference		
Own boat	-4.6	0.001	-6, -3.2
Rental boat	13.6	0.001	12, 14.8
Species of fish			
Bagrus documak	Reference		
Catfish	-0.078	0.7	-0.6, -0.48
Labeo barbus	4.97	0.01	3.8, 6.1
Tilapia	3.04	0.002	1.07, 5.0
Preservation method used			
No preservation	Reference		
Salting	-5.2	0.000	-6.7, -3.6
Sun drying	-2.34	0.016	-4.2, -0.43
Distance to market (Km)	1.18	0.042	0.06, 0.156
Maximum catch/day (Kg)	0.19	0.01	0.145, .23
Minimum catch/day (Kg)	-1.01	0.01	-1.4, -0.60
Fishing experience (years)	-0.17	0.01	-.27, -0.077
Constant	**-3.89**	**0.054**	**-7.85, -0.062**

The other studied parameter about PHL was the monetary value of loss incurred by fishermen. It was calculated from the data generated by the interview. As indicated in table 7, at Lake Hayq, a fisherman works for 4 days in a week on average and incur 1.04 kg loss of fish per day (see table 7). Legal fishing season of the year in that particular area ranges from October to April (7 months) while the rest is breeding season for the fish. In Hayq one kilogram of fish is sold for 120 Ethiopian Birr (ETB). With simple multiplication, the estimated total loss per fisherman in that particular Lake is found to be 13,977.6 ETB per year. Based on similar calculations for Tekeze Dam, a fisherman works actively for 6 days in a week and sell fish 90 ETB per kg. The total monetary loss per individual in Tekeze dam was found to be 68,544 ETB per year.

4.4.4. PHL estimation based on secondary data

Based on secondary data generated from district agricultural office, the total annual fish production and loss were depicted (Table 11).

Table 11: Total Fish production and loss between 2004 to 2010 (Ethiopian calendar)

Year (EC)	Hayq		Tekeze		Total	
	Production (ton)	**Loss (ton)**	**Production (ton)**	**loss**	**Production (ton)**	**Loss (ton)**
2004	341	15	NA	NA	NA	NA
2005	413	8	NA	NA	NA	NA
2006	292	12	NA	NA	NA	NA
2007	213	6	813.1	38.7	1026.1	44.7
2008	381.5	5	1570.53	40.9	1952.03	45.9
2009	120	4.5	3661.84	24.6	3781.84	29.1
2010*	46.2	5	189.52	4.7	235.72	9.7
Total	**1806**	**55.5**	**6234.99**	**108.9**	**8040.99**	**164.4**

NA, data not available, * data available till February 2010 EC (Ethiopian calendar)

The amount of loss in terms of money was estimated with a simple calculation. One kilogram of fish has been sold 60 and 80 ETB (average fish price of the production years) at Tekeze and Hayq respectively for the last six years. Accordingly, a total of 6,534,000 and 4,400,000 Ethiopian birr was lost at Tekeze dam and Lake Hayq respectively.

4.4.5. PHL estimation by load tracking

One of FAO recommendations for the estimation of PHL in fisheries is to track fish weight loss across its value chain. This measurement involved measuring fish at harvesting time and in the market with a minimum fifteen different days (replications) weighing up to 30% of the fish harvested. Based on this we measured weight loss of different species of fish at Tekeze

dam for nineteen replications in forty-five days gap. The amount of loss was generated by subtracting final weight in measured in store from initial weight measured at landing sites (Table 12). Since our measurements included measuring before and after filleting of fish, it was not possible to estimate monetary value of the loss.

Table 12: Fish loss generated by load tracking

Species of fish	**Initial weight (Kg)**	**Final weight (Kg)**	**Loss (difference) (Kg)**
Tilapia	60.01	16.96	43.05
Bagrus documac	145.34	89.3	56.04
Catfish	156.85	89.7	67.15
Labeo barbus	9.95	4.215	5.735
Total loss	**372.15**	**200.175**	**171.975**

5. DISCUSSION

5.1. Prevalence and antibiotic sensitivity test of *Escherichia coli* O157:H7

Escherichia coli O157: H7 is one of the most feared foodborne pathogens worldwide. It is a reportable organism in the USA and Europe. The organism has been investigated for its occurrence in different foodstuffs as well as cattle globally. In Ethiopia, there is a limitation in estimating and identifying the occurrence of the organism in foods as well as cattle and human patients. However, one can argue that recently there is a good trend of studying the organism in different foods of animal origin. Reports in milk and meat estimated the overall prevalence of the organism to be 4.91% (see systematic review and meta-analysis section in literature review part of this document). The present study reported that the organisms' occurrence in fish was 1.46% in the two study sites. This prevalence is, however, lower than reported elsewhere from fish and related species. A study conducted in the USA reported a prevalence of 6.95% in fish (Ribeiro *et al.*, 2015) while in India from shrimp samples the prevalence was found to be 5.8% (Surendraraj *et al.*, 2010). In France, the organism was isolated from shellfish with a prevalence of 4.16% (Gourmelon *et al.*, 2006). The prevalence of the organism in this particular study was somehow lower than other foods of animal origin in Ethiopia as well. This difference can be attributed to the fact that fish is a cold blood animal in which the organism cannot flourish in it. The source for the occurrence of the organism can be poor handling practice by fishermen and retailers operating in the study sites. As noted during the study which is depicted in figures of annex part of this manuscript (annex 4), fishermen fillet fish in soil, grass, and stones without any clean bedding material. This unhygienic handling practice can be one of the sources of contamination. In addition to this, fishermen don't prevent or reduce the activity of flies that wonder in the fish during processing and marketing. A study showed that *Escherichia coli* O157 occurrence was 2.7 times higher in house flies (*Musca domestica*) than manure from dairy farms (Burrus et al., 2016), in which the authors recommended the diagnosis of flies that wander in restaurants can give a better result than foods sold. In light of this, the occurrence of *Escherichia coli* O157: H7 in fish can also be attributed to uncontrolled activity of flies in the study locations.

The occurrence of *Escherichia coli* O157: H7 was numerically higher in Lake Hayq than Tekeze dam harvested fish. This difference can be due to 1) the sample size is relatively higher in Lake Hayq, 2) higher agricultural practices around Lake Hayq were noted. Farmers that live near Hayq water their cattle in the Lake, graze around the Lake where fish is gutted and filleted, they also use cattle manures to crop papaya, sugarcane, banana, onion and many more crops around the Lake. The remaining of the manure drain to the landing site where fish is filleted and arranged for sale, 3) Lake Hayq is relatively humid than Tekeze (Mulugeta *et al.*, 2017) which makes it a favorable environment for the organism to thrive and for increased activities of flies than the hot climate Tekeze area. The prevalence of the organism was also found numerically higher in filleted fish (swab and flesh) than whole fish samples. This may indicate that the processing environment (filleting and gutting) can be a source of contamination. A whole fish harvested from the Lakes may have no enough time to be contaminated from the environment than filleted fish. During the process of filleting, almost all parts of the body will come in contact with the ground. Consequently, causing higher contamination rate than whole fish indicating unhygienic processing my expose fish to this and other pathogenic organisms.

In regard to the sampling sites, the organism's prevalence was numerically higher at landing sites than nearby retail markets and restaurants. In Tekeze there were no local markets as well as restaurants in the nearby district (Abergelle) that serve fish. Fish harvested in that Dam is directly sold in landing sites for whole sellers who store in freezer and took it to Addis Ababa and Mekelle cities. Due to this reason samples from restaurants and retail market were taken only from Hayq city. Hence, sample size from the landing site, retail market and restaurants may not be proportional. This situation further can lead us to the conclusion that the higher occurrence of the organism in landing sites can be due to higher number of fish samples taken from landing sites. The other conclusion can be, in landing sites fish filleted and handled improperly exposed to dust, dung, and flies. However, fishermen wash their harvest after finishing the filleting process which may lower the chance of recovery of the organism further in the value chain.

Due to cost-related issues, though we didn't sample enough ready to eat fish from restaurants, the occurrence level of the organism was zero out of thirteen samples examined. This is

somehow a relief that the organism may not reach to end users. However, this argument may need further detailed investigation with representative sample size in fish serving restaurants to have a sound conclusion. In addition to restaurants, the organism was not also isolated from other working environment samples. Containers, knife, and workers hand swabs were free from the organism. This suggests that the need for further investigation of environments of landing sites that pose a risk of contamination during processing.

As *E. coli* are indicator organism for the presence of contamination in food samples, the existence of other pathogenic organisms in fish harvested from those areas is imminent. Future researches may focus on detecting other pathogenic bacteria and their level of concentration in fish. In addition, risk analysis of contracting infection due to E. coli O157 and other pathogenic organism from fish consumption should be analyzed based on data generated from every value chain.

The antibiotic sensitivity test result showed that important drugs in the field of veterinary and human medicine performed reasonably well. While drugs like Streptomycin and Ampicillin were poor in inhibiting the growth of the isolates. Even though some illness conditions caused by this organism may not be treated by antibiotics at all, this situation is an indication that Streptomycin and Ampicillin should not be used as a treatment option for infections caused by this organism. Recent studies showed that the organism demonstrated a high level of resistance in Ethiopia as well as elsewhere for these drugs (Sunde and Norström, 2005; Abdissa *et al.*, 2017; Bedasa *et al.*, 2018). It is also said that Streptomycin and Ampicillin are the two most frequently co-transferred resistance phenotypes among sulfonamide-resistant *E. coli* isolates recovered (Sunde and Norström, 2005). Chloramphenicol, one of the most frequently and widely used drug, performed reasonably well in inhibiting the growth of 4 the isolates. However, one isolate was found to be an intermediately resistant to this drug.

Even though there were no resistant isolates for at least to three drugs, yet the multidrug resistance index calculated showed a relatively higher index. From the total six isolates, five of them were resistant to two drugs (Ampicillin and Streptomycin), while one isolate was resistant to ampicillin only. Hence the MDR index of the isolates was found between 0.125 and 0.25. This value is somehow higher than the acceptable international standard for multi-

drug resistance index of $\leq$ 0.2. This can be due to a widespread irrational drug use in both animals and humans in the country. Irrational drug use can result in resistant gens to develop and further transfer to susceptible organisms from the environment (Von Baum and Marre, 2005). This situation may need attention sooner than later before losing effective drugs in terms of cost and efficiency. Even though it is out of the scope of this paper, it is wise that responsible bodies like drug authority and control agency (DACA) should identify actors involved in irresponsible drug use; develop and implement strict control measures to the adhesion of rational drug use at national level.

5.2. Causes and extent of PHL at Lake Hayq and Tekeze Dam

As the demographic characteristics result indicated, almost all of the participants from the two water bodies were males. This condition may not be surprising because most of the agricultural practices and fishing activities are dominated by men in Ethiopia. Regarding the age of participants involved in the fishing activities, most of them were from the youth group with a mean age of 28 years. Recently the government gave an attention to the youth in order to lower the unemployment level. Youth cooperative establishment was seriously taken at Tekeze, in which most of the participants were a member of cooperatives. Participants from Tekeze had a relatively lower fishing experience than Hayq counterparts, apparently, this is due to the fact that Tekeze hydroelectric power Dam was built recently.

An extensive data was generated on fishing-related activities in order to observe if these activities had relation to the loss that fishermen incur on a daily basis. Most of the participants actively involved in fishing during the months of October to March. According to the informants, fish is surplus in months of October to March at both Lakes. The reason for fish abundance was fishing prohibition in months of May to September as a breeding season which makes the next seasons to be abundant in terms of production. Participants said that months from October to December are also seasons of high loss because of increased production. In these month absences of a market is high and price of fish can be much lower than other seasons. This condition is recognized as market force loss which is one of the challenges of the fisheries sector. This situation was much severe at Tekeze Dam than Lake Hayq because;

Tekeze fishermen sell their harvest only to whole sellers who deliver fish to Addis Ababa or Mekelle cities. If whole sellers fail to receive producers in every single day due to their own reasons, fishermen may not have any option of selling their harvest around Tekeze. We noticed that there was no a single restaurant and local retail markets in Abergelle as well as Sekota town. During high production months of October to December, producers incur a huge loss in a daily basis. Informants said that they threw their harvest in the wild if a whole day awaited buyer does not have the capacity to receive all the product. Participants said that fish loss is much lower and expensive in terms of price in Ethiopian orthodox church fasting seasons. In these months fish fetches a good price and they may not encounter market force loss due to 1) fish is not surplus in these months because exploited in previous high production season 2) during the fasting season other animal products are prohibited and fish can be served in some restaurants so that traders actively buy fish and take it to AA and Mekele cities.

As suggested by fishermen, the most important causes of loss in the fishery sector were high environmental temperature, harvesting immature fish, presence of predators, loss of fish in the Lake with the net, high flooding, and delay before marketing. All these causes can lead to a physical loss of product which further leads to a penalized market value. As the temperature reaches more than 35^{o}C at Tekeze in mid-day, in which fishermen reach landing site from different districts where the waterbody boarders. By that time, their product could have been lost due to high temperature and the distance they traveled. Harvesting immature fish was also the other reason for higher PHL. In the study areas, fishermen use a monofilament net which is suitable for catching immature fish. This can be one of the causes of increased postharvest loss in the two areas. If a very small fish is captured which may fail to be marketed, it is going to spoil in the process. Hence, increasing postharvest loss as well as overexploitation of the resources of the Lakes. This condition was much higher in Lake Hayq than Tekeze Dam. Predators like alligators and crocodiles at Tekeze and, Pelicans and other birds in Lake Hayq, were also the other reasons listed out by interviewees as causes for fish loss. These animals were found in abundance in the study areas. The graduated response matrix result indicated that Long hours of setting gear before hauling and distance to landing sites are important causes of loss at Tekeze Dam. The average time for setting gear by fishermen was 6hrs. participants believed that, if a fisherman forgot to check the net in shortest possible time, it can

result in the death of fish and further spoilage during transportation. Which is why participants agreed to the matrix of long hours of setting gears can result in increased fish PHL. Fishermen from far districts travel a maximum of 70 km by boat to reach marketing site (landing site). During this time fish may undergo spoilage due to environmental and spoilage bacteria. The other matrix result that had a strong agreement between participants was flooding (rainy season). Since Tekeze Dam has many tributaries, if there is unexpected rain in the highland areas, the flood can reach the Dam and reduce the shelf life of the fish harvested by contaminating the skin, gills, and GIT of fish with environmental bacteria. Even if fishermen did not understand the reason behind, they claimed that if there is a flood fish undergo spoilage in short time. However different kinds of literature stated that fish quality is in direct relation to water quality (Boyd, 2017; Zhao *et al.*, 2018).

We developed a GLM model based on the information generated by the questionnaire which may be able to predict PHL in fisheries. Continuous predictors like distance traveled to reach the market, and maximum catch/day had a positive association with the amount of loss, while minimum catch/day, and fishing experience had an inverse relation to the amount of loss. This is crystal clear that traveling much higher distance to sell and catching plenty of fish where there is no enough buyer in the market may cause higher losses. However, Fishing experience and minimum catch/day were inversely associated with the amount of PHL incurred by a fisherman. This can be due to the fact that if a fisherman is experienced enough, he might be able to manage to reduce loss, and also if a man catches few kilograms of fish, he may not incur loss because either he manages it well and sell to the whole seller, or he might take it home to consume with family. The other important predictors found to have an association with fish loss were; species of fish harvested, type of boat used by fishermen, ownership of a boat, using preservation methods, and study Lakes where participants were involved in fishing. As observed from the model, if a fisherman had his own boat than a group boat, he may manage to reduce PHL. This may be true that having own property by fishermen may be well managed to reduce loss. In addition, if a fisherman uses preservative methods like sun drying, he can manage to reduce loss significantly. Regarding species, mostly harvested as claimed by fishermen, they may incur higher loss in Tilapia and *Labeo barbus* than *Bagrus documak.* Tilapia is the most abundant fish species in the two Lakes than any other species

which may increase the amount of loss relatively than other scarce species. The amount of PHL was found to be higher at Tekeze Dam than Lake Hayq. The reason for this difference can be due to many factors. One of the most important reason can be 1) traveling a long distance (a maximum of 70 km) to reach the market in Tekeze, and 2) the production volume of Lake Hayq is gradually decreasing time to time (Vijverberg *et al.*, 2012). The decreased production forced fishermen and consumers to utilize the catch effectively without incurring a loss. During the interview process participants from Hayq replayed that "*what amount I am going to lose from this? we produce few kg of fish per day here, even not enough for resorts found around the Lake*". 3) Lake Hayq fishermen do have many options of selling their product, unlike Tekeze Dam fishermen. In lake Hayq, there are many restaurants that serve fish in nearby cities of Hayq, Dessie, and Kombolcha. This chance of selling directly to restaurants is not available in Tekeze Dam. These reasons might have contributed to a much higher rate of loss in Tekeze than Lake Hayq.

PHL in fisheries is one of the challenges in food security struggle for developing countries (Ahmed, 2008). We tried to estimate the amount of loss by different approaches. Secondary data utilized from agricultural offices indicated that the loss is much higher than acceptable level. The country loses a total of 164.4 ton of fish from the two Lakes in the last 4-6 years. The loss of resources in terms of money was found to be 6,534,000 and 4,400,000 Ethiopian birrs at Tekeze Dam and Hayq respectively for the last 4-6 years. A study conducted in Lake Tekeze (different landing site from this study was taken), and in Hashenge Lake (found around Michew city) estimated a total monetary loss of 4,822,560 ETB (Tesfay and Teferi, 2017), which is considerably lower than our estimate. This lower estimation can be due to 1) price of fish increased by 100% between the two studies, 2) the year gap between the two study in which we included losses recorded after their measurement, 3) we included production and loss data of Abergelle, Ziquala, and Sahala districts (Amhara region). The previous study estimated loss only from Tanqua Abergelle district (Tigray region). Another study conducted in Amerti and Fichawa reservoirs also estimated a relatively lower estimate than ours. They reported a loss of annual 6.81 tons of fish (Teklu, 2014). This difference can be due to these reservoirs have considerably lower fish production potential than Tekeze and Hayq.

Load tracking of fish was conducted from landing site to Abergelle district where whole sellers store their fish indicated that a total of 171.9 kg of fish weight was lost. Since our measurements included measuring before and after filleting of fish, it was not possible to estimate the monetary value of the loss. However, we witnessed that the gutted bone and other by-products of fish have been wasted at field. These discarded products are known for their enormous source of protein in poultry and other farm production systems. The amount of loss can be worse if it was done for the whole production season. In addition to the above measurements, the loss estimated from questionnaire indicated much higher loss in terms of money than secondary data estimates and load tracking. A questionnaire loss assessment was conducted as FAO recommends a questionnaire loss assessment is a validation for results of LT and secondary data utilization. The amount of loss generated by questionnaire indicated that a total of 13,977.6 and 68,544 ETB per year/ per fishermen at Lake Hayk and Tekeze Dam respectively have been lost. This calculation is an annual loss incurred by a single legally registered fisherman. As the number of fishermen may not be clearly figured because there are illegal fishermen as well who sell their product in secret, estimating the exact annual loss may not be possible. The number of illegal fishermen fluctuates time to time depending on the security level implemented by local militia. However, the result is a clear indication that there is a significant amount of resource being lost in these areas.

Post-harvest loss in fisheries due to poor handling practices is one of the most important challenges for developing countries. A study showed that if Ethiopia put measures to reduce PHL in fisheries, the sector can contribute a minimum of 1.7% to the national GDP (Tesfaye and Wolff, 2014). These identified and many other reasons made the contribution of the fisheries sector to the country's economy to be negligible. The sector recently draws the attention of governmental and non-governmental Organizations involved in fisheries and related activities. United nation agricultural department (FAO) has been involved to reduce PHL in fisheries in developing countries recently. A country needs to understand causes and extent of loss of fish in their water bodies in order to develop mitigation strategies. The findings of this study can be a basis for planning of intervention measures to reduce resource loss in these and other Lakes of the country as well.

6. CONCLUSION AND RECOMMENDATIONS

This study is the first of its kind in investigating *Escherichia coli* O157:H7 from fish in Ethiopia. The level of occurrence of the organism may be low but should be taken seriously because of severe disease-causing capabilities of *Escherichia coli* O157:H7 with very low infective dose. Handling practices by fishermen during filleting, and gutting might be the cause of contamination. Ready to eat fish sampled from restaurants were found free of the organism indicating that it may not reach end users provided that proper cooking is in place. Since this study has a limitation of including enough sample size from restaurants, this argument may need further investigation of fish serving restaurants with enough sample size that can be extrapolated to the whole study area. Drugs like Ciprofloxacin, Tetracycline, and Nalidixic acid were effective in inhibiting the growth of the isolates while Streptomycin and Ampicillin performed poorly. Regarding the PHL in the two water bodies, high environmental temperature, harvesting immature fish, presence of predators, high flooding, and delay before marketing were the most determining reasons for loss in fisheries sector. The calculated monetary loss was found to be 10,934,000 ETB for the last 4-6 years from both Lakes. This figure may need intervention by responsible bodies to overcome such a huge asset loss.

Based on the above conclusion the following recommendations are drawn:

1. *Escherichia coli* O157: H7 was isolated from fish indicating that raw and undercooked fish consumptions may result in contracting infections.
2. Drugs like Nalidixic acid and Ciprofloxacin should be used as a first line treatment for infections caused by *Escherichia coli* O157: H7.
3. As this study identified a single bacterium, other important foodborne pathogens associated with fish should be routinely examined in Ethiopia.
4. Future studies may focus on identifying sources of contamination by examining water quality, landing site, and flies by applying latest diagnostic approaches.
5. Fishermen need developmental interventions by responsible organizations. Supports like freezers, generators, boats, net and on job training about proper fishing practices may play a tremendous role in decreasing loss in the two study Lakes.

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8. ANNEXES

Annex 1: Media used in the isolation, identification and susceptibility test

1.1. Sorbitol-MacConkey Agar (Oxoid Ltd., England)

Composition (g/l): Peptone (10 g/l), Sorbitol (10 g/l), bile salts No.3 (1.5 g/l), Sodium chloride (5g/l), Neutral red (0.03 g/l), crystal violet (0.001 g/l) Agar (15 g/l) and final pH of 7.1±0.2 at 25°C. Preparation: Dispersed 48.5g in 1 liter of distilled water. Soaked for 10 minutes, mixed and sterilized by autoclaving at 121oC for 15 minutes. Cooled to 47^{o}C and mix before pouring into Petri dishes and then dried the agar surface.

1.2. Tryptone Soya Broth (TSB) (Oxoid Ltd., England)

Composition: Pancreatic digest of casein (17.0 g), peptic digest of soyabean meal (3.0 g), sodium chloride (5.0 g), Di-Base potassium phosphate (2.5 g), Glucose (2.5 g). Preparation: -Suspend 30g of powder in 1 liter of purified water. Mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. Autoclaved at 121oC for 15 minutes.

1.3. Buffered peptone water (Oxoid Ltd., England)

Composition (g/l): Peptone 10.0; Sodium chloride 5.0. Disodium phosphate 3.5, Potassium dihydrogen phosphate, 1.5, pH 7.2 ± 0.2 @ 25°C. Preparation: Added 20g to 1 liter of distilled water. Mixed it well and sterilized by autoclaving at 121^{o}C for 15 minutes.

1.4. MacConkey agar (Oxoid Ltd., England)

Composition (g/l): Peptone 20.0g, Lactose 10.0, Bile salts, 5.0, Sodium chloride, 5.0, Neutral red 0.075, Agar 12.0. Preparation: -Suspend 52g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Dry the surface of

the gel before inoculation.

1.5. Mueller-Hinton agar (Oxoid Ltd., England)

Composition: Beef extract (2.0g), acid hydrolysate of casein (17.5g), starch (1.5g), Agar (17g). Final pH: 7.3± 0.1. Preparation: Suspended 38g of powder in 1 liter of purified water mix thoroughly. Heated with frequent agitation and boiled for 1 minute to completely dissolve the powder. Autoclaved at 121^{o}C for 15 minutes.

1.6. KIA media (Oxoid Ltd., England)

Composition (g/l): Peptone 15.0gm, lactose 10.0gm, Proteose peptone 5.0gm, sodium chloride 5.0gm, beef extract 3.0gm, yeast extract 3.0gm, dextrose 1.0gm, sodium thiosulfate 0.3gm, ferrous sulfate 0.2gm, phenol red 0.024gm, agar 12.0gm, final pH 7.4 +/- 0.2 at 25ºC.
Preparation: Suspend 55g in 1 liter of distilled water. Bring to the boil to dissolve completely. Mix well and distribute into containers. Sterilize by autoclaving at 121°C for 15 minutes. Allow to set as slopes with 1-inch butts.

Annex 2: Biochemical and serological tests used to isolate E. coli O157:H7

2.1. Indole test procedures

Inoculate the tryptophan broth with bacterial culture or emulsify isolated colony of the test organism in tryptophan broth. Incubate at 37°C for 24-28 hours in ambient air. Add 0.5 ml of Kovac's reagent to the broth culture. results: Positive: Pink colored rink after addition of appropriate reagent. Negative: No color change even after the addition of appropriate reagent.

2.2. Sugar fermentation test procedures (lactose and dextrose fermentation)

Procedure: stab the center of the KIA medium into tube butt. Withdraw the needle, and streak surface of the slant. Loosen caps to allow a free exchange of air before incubating at 35°C for 18 – 38 hours. Read tubes for acid production on slant/butt, gas production, and hydrogen

sulfide production. Results: An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose and lactose. An alkaline slant-alkaline butt (red/red) indicates dextrose and lactose did not ferment (non-fermenter). Cracks, splits, or bubbles in the medium indicate gas production. A black precipitate in butt indicates hydrogen sulfide production

2.3. *E. coli* O157:H7 Latex test principle and procedure

The polystyrene latex particles provided in the kit are coupled to antibodies against E. coli serotype O157:H7. When the latex particles are mixed on a test card with fresh colonies of E. coli O157, the bacteria will bind to the antibody causing the latex particles to agglutinate (positive reaction). Bacteria that are not E. coli O157:H7 will not bind to the antibody and will not agglutinate the latex particles (negative reaction).

procedure:

1. Dispense one drop (30µL) of sample diluent on to two wells of a clean, dry agglutination slide.
2. Using an inoculating loop, remove up to 5 suspected E. coli colonies from the sorbitol MacConkey agar plate. Only select colorless colonies whose morphology resembles that of E. coli.
3. Emulsify the colonies in the two drops of sample diluent on the test slide to produce a heavy, smooth suspension. Spread the suspension, over the entire surface of the wells.
4. Rock the slide gently for 30 seconds and observe for auto agglutination or clumping. Of the suspensions remain smooth, proceed to section 5. If the suspension is “stringy” or “granular”, the sample is unsuitable for testing with E. coli O157:H7 Rapid Latex Test Kit since it may give a falsely positive agglutination when latex is added. Here, alternative test method should be used

Annex 3: Antibiotic sensitivity test procedure and decision criteria for *E. coli*

1. The Kirby-Bauer Disc Method

This method is also called the agar diffusion method or the disk diffusion method. The procedure followed is simply that an antibiotic disk is applied to the surface of an agar plate containing the organism to be tested and the plate is incubated at 37°C for 24-48 hours.

Procedures:

- Obtain culture broths of the test bacteria.
- Obtain a swab and dip it into the E. coli broth culture. Roll the swab against the inside of the tube to remove excess liquid.
- Streak the plates with the swab in even strokes to obtain a uniform growth pattern across the entire surface of the plate. Rotate the plate 90 degrees and using the same swab, streak the plate again. Rotate the plate 45 degrees and re-swab.
- Replace the lid. Discard the swab. Label the plate. Allow the plates to dry for 2-5 minutes.
- Remove the forceps from the alcohol beaker and pass through the flame of a bunsen burner. When all the alcohol has burned off, use the sterile forceps to aseptically remove one of each antibiotic disc from the dispenser and place it on each plate.
- Repeat the alcohol-flame sterilization of the forceps and tap each disc gently onto the plate.
- Replace the lid, and invert the plate. Complete the label at the bottom of plates and incubate at 37°C for 24hrs.
- Record the results by measuring the diameters of the zone of inhibition (ZOI). The data is recorded and interpreted using CLSI manual for the *Enterobacteriacea* (CLSI, 2015). The interpretation criteria is summarized in the next table.

Antibiotic disk	Disc concentration	Zone of inhibition to the nearest mm		
		Susceptible	Intermediate	Resistant
Ampicillin	10 µgm	≥17	14-16	≤13
CAF	30 µgm	≥18	13-17	≤12
Ciprofloxacin	5 µgm	≥21	16-20	≤15
Gentamycin	10 µgm	≥15	13-14	≤12
Kanamycin	30 µgm	≥18	13-17	≤13
Nalidixic acid	30 µgm	≥19	14-18	≤13
Streptomycin	10 µgm	≥15	12-14	≤11
Tetracycline	30 µgm	≥15	12-14	≤11

CAF, chloramphenicol

Annex 4: Pictures depicting, fish handling practices, interview process, and sampling

Pic 1. Sampling process for microbiological analysis (Tekeze landing site)

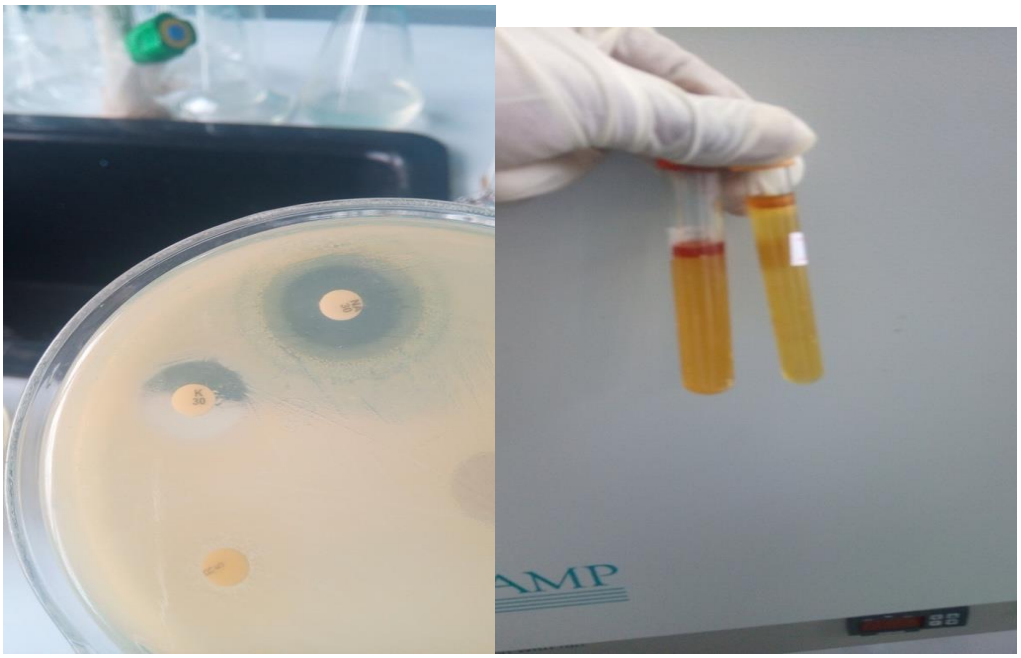
Pic 2. Careless handling during marketing (Tekeze landing site)

Pic 3. Unhygienic filleting and the interview process(Tekeze)

pic 4. Careless handling during transportation (Tekeze)

Pic 5. Load tracking

Pic 6. Unhygienic processing at landing site (lake Hayq)



Pic 7. Antibiotic sensitivity test and indole test (pink colors indicate positive result (left))

Annex 5: Data collection sheet

Sn.	Date	Study site	Sample id	Sample type	Collection site	Growth on SMAC	Growth on MacConkey	Indole test	KIA	Latex test
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Annex 6: Questionnaire format

Questionnaire format for Survey on fish post-harvest losses and handling techniques in Tekeze Reservoir and lake Hayq

****

Interviewer name _____ date _____ lake_____

I. **Personal profile /demographic data**

1. *Woreda* _____ *Kebele* _____ *village* _____

Respondents'	Religion (√)	Marital status	Educational level (√)
Name _____	Orthodox	Single	Illiterate
Age _____	Muslim	Married	Read and write
Sex _____	Protestant	Widowed	Primary (1 Secondary (9-
Family size M ____ F ____ T _____		Divorced	College/university gradu

2. Source of income/livelihood of the household. 1. Crop cultivation 2. Livestock rearing 3. Fisheries & related activities 4. Petty trade 5. Mixed farming (crop &livestock)
3. How much is the average income of house hold annually from fisheries? _____

II. Fishery Activity

4. How long did you engage in fishing and related activities? _____years
5. Are you working (1) in cooperative or (2) in private? If (1) please fill the following

The name of the cooperatives _____

Date of establishment_____ number of members M_____F_____

6. Distance of working place to reach the market _____ (km) or walking time _____ (hr/minutes)

III. Fishery Equipment and Handling

7. Types and amounts of boat owned in cooperative or privet (in number)
   a. Steel boat _____ b. Planked boat _____ c. Raft (boffofe boat) _____ other (specified) _____
8. What type of ownership of fishing boat do you have? 1. Own boat 2. group boat 3. Rental
9. Which type of fishing net is used? 1. Monofilament 2. Multifilament
10. How long fishing take per trip? _____(hours)
11. How many times do you go for fishing per a week? _____ (days)
12. At which month do you actively participate in fishing? _____ & not actively participate? _____
13. What time of the day you choose to set your gear? 1. Morning 2. Day time 3. Evening 4. Midnight
14. How long to set the gear in the water (in hours or day) before actual fishing?
    _____
15. Do you know where to set fishing gear to get the fish type you like to catch? 1. Yes 0. No
    If yes, where do you like? 1. Open water 2. River mouth 3. Shoreline 4. Vegetation free shallow area
16. How much do you catch per trip or per day? 1. Maximum _____Kg 2. Minimum _____Kg
17. What fish species/type you most commonly harvest or you like to harvest currently?
    1. Tilapia 2. L. Barbus 3. Catfish 4. Bezo 5. Common carp 6. Other spp _____

IV. Fish Post Harvest Loss and Market Related Questions

18. Where do you sell your daily catch (customers mostly use your product)? 1) Local market 2) Landing site 3) Door to door
19. Do you have a market problem to sell the fish product and is it a factor for post-harvest loss? 1. Yes 2. No

20. When do you get good market /income? Season_____ months _____ days_____
21. When do you get poor market / income? Season _____ months _____ days____ Why? _____
22. When do you get high FPHL? Season _____ months _____ days____
23. have you ever encountered 100 percent fish spoilage which failed to be marketable? ___
24. How many freezers do you have in your cooperatives? _____
25. Who decides/control/ the fish price? 1) The fishers (producers) 2) The consumer 3) whole seller 4) The retailer 5) middlemen 6) processors 7) Other (specify)_____
26. From the total harvest per kg, how many losses do you incur before marketing it?

    _____
27. Average price of fish of last year (2016/17) in kg by fish species and percentage of catch?

    Tilapia _____ estimated percentage from total catch _____

    Catfish _____ estimated percentage from total catch _____

    Barbus _____ estimated percentage from total catch _____

    Other (specify)_____
28. To sell fish, which types of product processing are used for your customers?
    1. Whole fish 2. Filleted fish 3. Gutted fish 4. Dried 5. Other form (specify)_____
29. What are the modes of transportation used to sell fish? 1. Trekking with carrying by shoulder 2. Some part in shoulder 3. Trucking /open car/ 4. Refrigerated truck 5. Other (specify) _____
30. Where do you store your fish product after harvest before marketing?

    _____
    1) Sell on the day immediately it is harvested the fish. 1. Yes 0. No 2. If no how long to attend? _____hr
31. How to identify the spoiled fish compared to fresh fish during and after harvesting? Explain the method and its sign. _____
32. Do you know the action of fish spoilage/lost was started? 1. Yes 0. No if yes where?

    _____
33. Do you have discard fish during the fish is spoil? 1. Yes 0. No, If yes

1. Which types of fish are more discarded? _____ Why? _____
2. Where is the place that you are discarding the fish? _____

34. What types of techniques are used to reduce the fish product lost? Explain

_____

35. Do you use the following fish processing and preservation methods?

A. Smoking B. Salting C. Sun drying D. Frying E. Canning F. Chilling G. Other (specify) _____

If the product is already lost, how do you use the by-product? Explain

_____

36. Do you sell fish by-product for recycling purpose? / use your own / do you see others? 1. Yes 0. No If yes, for who? _____. Which part of fish? _____

37. what are the major causes of FPHL in your area?

_____delay before marketing of harvested fish

_____ high environmental temperature

_____distance from market

_____harvesting immature fish

_____I don't know

_____other (specify)_____

v. **Scale for graduated responses**

	SA	**A**	**NAND**	**D**	**SD**
Long hours of setting gear before hauling causes high post-harvest quality loss					
Fishers from distant fishing grounds land large quantities of spoiled fish					
On average, two crates of fish are found spoiled on landing					
High post-harvest fish loss occurs during rainy season					