HYGIENIC PRACTICE ASSESSMENT, ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *Escherchia coli* O157: H7 ALONG THE FISH SUPPLY CHAIN OF LAKES IN HARAMAYA, EASTERN ETHIOPIA

MSc THESIS

ABAS MOHAMMED HASSEN

NOVEMBER 2022

HARAMAYA UNIVERSITY, HARAMAYA

Hygienic Practice Assessment, Isolation and Antimicrobial Susceptibility Profile of *Escherchia coli* O157: H7 along the Fish Supply Chain of Lakes in Haramaya District, Eastern Ethiopia

A Thesis Submitted to the College of Veterinary Medicine,

Postgraduate Program Directorate

HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH

Abas Mohammed Hassen

November 2022

Haramaya University, Haramaya

HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

I hereby certify that I have read and evaluated this Thesis entitled "**Hygienic Practice Assessment, Isolation and Antimicrobial Susceptibility Profile of *Escherchia coli* O157: H7 along the Fish Supply Chain of Lakes in Haramaya District, Eastern Ethiopia**" prepared under my guidance by Abas Mohammed. I recommend that it will be submitted as fulfilling the thesis requirement.

Dinaol Belina (DVM, Msc, Assoc. Professor)	_____	_____
Advisor Name	Signature	Date
Adem Hiko (DVM, PhD, Proffessor)	_____	_____
Co Advisor Name	Signature	Date
Sisay Girma (DVM, Msc, Assist. Professor	_____	_____
Co Advisor Name	Signature	Date

As members of the Board of Examiners of the MSc Thesis Open Defence Examination, I certify that I have read and evaluated the Thesis prepared by Abas Mohammed Hassen and examined the candidate. I recommend that the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Veterinary Public Health.

_____	_____	_____
Chair Person	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the Thesis is contingent upon the submission of its final copy to the Council of Postgraduate Program (CPGP) through the candidate's College Graduate Committee (CGC).

DEDICATION

I dedicate this work to my Father and Mother

STATEMENT OF THE AUTHOR

By my signature below, First, I declare that this thesis is my own work. I have followed all ethical and technical principle of scholarship in the preparation, data collection, data analysis is and compilation of the thesis. Any scholarly matter that is included in thesis has been recognition through citation. This has been submitted in partial fulfillment of the requirements for the MSc, degree at Haramaya University. The thesis is deposited at the university library and is made available to borrowers under rules of the library. I solemnly declare that this thesis has not been submitted to any academic degree, diploma, or certificate. Brief question from this thesis may be made without special permission provided that accurate acknowledgement of the source is made, Requests for permission extended quotation from or reproduction of this thesis is in whole or in part may be granted the head of the school or department when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instance, however, permission must be obtained from the author of the thesis.

Name: Abas Mohammed Hassen Signature_____

Date of Submission: _____

School/Collage: Collage of Veterinary Medicine

BIOGRAPHICAL SKETCH

Abas Mohammed was born in 1991GC/ 1984 EC from his father Mohammed Hassen and his mother Nuriya Kasim Beker in Kombolcha woreda of East Hararghe zone, Oromia regional state, Ethiopia. Abas started and completed His primary Education from 1991 EC/1999 GC up to 1998 EC/ 2006 GC in Kombolcha District at Nicolas primary school. He continued his secondary and preparatory school in the same District, Kombolcha Senior Secondery and Preparatory school from the year of 1999 EC/ 2007 GC up to 2002 EC/2010 GC. Then after, joined his higher education from the year of 2003EC/2011GC and graduated since 2008 EC/ 2016 GC with DVM degree from Jigjiga University. He was employed in Kombolcha District in the year of 2009 EC/ 2017GC as Veterinary Clinician in Kombolcha Livestock and Fishery Resource Development. On a position named "Control and prevention of Livestock and Fishery Diseases". In academic year of 2011EC/ 2018-2019 GC, he started his Post-graduate program at Haramaya University, College of Veterinary Medicine by Veterinary Public Health. Currently, he accomplished his MSc research on the title of "**Hygienic Practice Assessment, Isolation and Antimicrobial Susceptibility Profile of *Escherchia coli* O157: H7 along the Fish Supply Chain of Lakes in Haramaya District, Eastern Ethiopia.** Funded by Haramaya University and Kombolcha Agriculture and Natural Resource Office.

ACKNOWLEDGEMENTS

This thesis work cannot be done without the hands and hearts of different individuals in their priceless suggestion and material contributions. Firstly I should have to thank Haramaya University and Kombolcha Agriculture office, for giving me an opportunity learn master's program. I want to extend special thanks to my advisor and co advisors for their different and equally important contribution in designing, reviewing analyzing up to finishing of the thesis. Even though my feeling cannot be expressed by single word, I cannot escape without giving gratitude to my major Advisor Dr Dinaol Belina for his endless material, moral and technical support in designing, analyzing and thesis write up. I should also thank My Co advisors Professor Adem Hiko and Dr Sisay Girma for their greater input starting from my first MSc class up to now, which enable me in understanding the scientific background in Veterinary Public Health. Their continuous kind advice in my day to day progress cannot be explained easily by words. However their greater input of knowledge they made could explain their role of responsible advisee in producing problem solving citizen. I should also thank my Mother and Father; my tour of strength, their contribution in provision of love and encouragement throughout my journey, brighten my future being themselves a ladder for my success. Wishing her joyful life time, I want to extend my gratitude to my pure hearted wife; Kasna Abibeker, for her own contribution in initiation and support through understanding most burden of research activities. Wishing them happy and improved life, I should also thank all Fisherman, Fish Retail worker and Fish consumer that participated in my study. Lastly I want to thank all my friends those helped me with their supportive idea in accomplishing the journey.

ACRONYMS AND ABBREVIATIONS

21CFR	Code of Federal Regulations Title 21
ARB	Antibiotic-Resistant Bacteria
ARGs	Antibiotic-Resistant Genes
BPW	Buffered Peptone Water
CEDRS	Catch and Effort Data Recording System
CFU/g	Colony forming Unit per gram
CLSI	International clinical laboratory standard protocols
DNA	Deoxyribo nucleic acid
ETEC	Entero toxigenic *Escherichia coli*
FDA	Food and Drug Administration
HC	Haemorrhagic colitis
HUS	Haemolytic uremic syndrome
IMS	Immunomagnetic separation
ISO	International standards organization
NGS	Next Generation Sequencing
PCR	polymerase chain reaction
PES	polyethersulfone
RNA	Ribose Nucleic acid
RTE	Ready to Eat
SMAC	Sorbitol MacConkey
SPSS	Statistical package for social science
STEC	Shiga-toxin-producing *E. coli*
Stx1 2	Shiga-toxin 2
Stx1	Shiga-toxin 1
TTP	Thrombotic Thrombocytopenic Purpura
VTEC	Vero-toxin-producing *E. coli*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACTS

Hygienic Practice Assessment, Isolation and Antimicrobial Susceptibility Profile of *Escherchia coli* O157: H7 along the Fish Supply Chain of Lakes in Haramaya District, Eastern Ethiopia

Fish is one of the nutritionally better source of food which require better handling. Good hygienic handling practice at any stake holder of fish chain may increase its quality which in turn increase demand for consumption. A cross-sectional study with the aim of assessment of hygienic handling practice with isolation of *Escherchia coli* O157:H7 along Fish Supply chain of Lakes in Haramaya District was carried out from October 2021 to May 2022. Among 420 total samples; 210 from lake and 210 from market, examined for presence of *Escherchia coli* O157:H7, 2.1% over all prevalence of organism were isolated. Prevalence per sampling site indicated that 3.8% and 0.5% from Lake and Market respectively. Similarly, 1.54%, 5.71% and 4.00% of this pathogen were isolated out of 210samples collected from Lake Adele, Haramaya Lake and Tinike respectively. Prevalence per sample type indicated that, 5.7 % of filleted Fish, 2.4 % of stored Fish, 0% from ready to eat sample, also 1.0% FES, 2.9% were isolated from freshly caught fish samples; visceral sample, fresh meat and skin. To consider intrinsic and extrinsic factor of contamination, prevalence per origin of sample revealed that 1.0%, 2.4% and 2.9% of this organism were isolated from Fish associated environmental sample, different fish sample and freshly caught sample respectively. The significant association exist among sample Site. However no significant association were observed among Lakes, origin of sample and sample types. The isolate were 100% susceptible to Enoxacin. Only 37.5% isolate susceptible to Nalidixic acid However 62.5 % isolate were shown intermediate susceptibility. However, the isolate has shown 100% resistance against Cefoxitine, Streptomycin, Ciprofloxacin, Amoxicillin and Ampicillin. The survey finding indicated that; most respondent show good hygienic practice, being that 100 % of participant do not reheat left over. 100% do not handle fish when they got sick .71.4% cover face during coughing or sneezing, 57.9% not wearing jewelry while working. However, except 68% consumer, out of all respondents, only 38.1% always dispose inedible fish part properly. beside this only 23.8% do cook fish with appropriate heating. In conclusion, Presence of this organism on freshly arrived sample could be resulted at early stage of autolysis, while isolation of this pathogen from viscera clearly indicate the organism obtained from water and resistance to antibiotic enable us

to estimate likely hood of contamination of water bodies with resistant strain of either livestock, human or environmental origin which could lead to public health threat. Proper fish cooking could reduce the public health risk, fisher man should feed the aquatic bird in better way that can minimize contamination of water body. Good hygienic practice behavior could achieve maximum food security and safety as well. Additionally, environmental sanitation and food safety regulation will increase the future development in increment of healthy and wealth of this particular community.

Key words: *Antimicrobial, Escherchia coli O157:H7, Fish, Isolation, Haramaya, Lakes*

1. INTRODUCTION

1.1. Back ground Information

World fish production has increased dramatically during the past 60 years, to around 179 million tons in 2018 with a value of $401 billion (FAO, 2020) Global fish consumption also increased from 9.0 kg per capita in 1961 to 20.5 kg in 2018. Aquaculture production presents 46% of the total production and 62% of the total sale value. Due to the increasing demand for high-quality protein, reduction of the wild fish catch, and advancement in fish farming technologies, global aquaculture production is expected to double by 2050 (Ibrahim *et al.,* 2020).

Fish is an important food for over 400 million African peoples, contributing essential proteins, minerals and micronutrients to their diets. Paradoxically, despite the high dependence on fish as a source of animal protein, fish consumption in sub-Saharan Africa is the world's lowest Ethiopia being a land locked country its fish production is entirely based on inland water bodies like lakes, reservoirs and rivers for fish supply for its population (World Fish Center, 2009). Ethiopia is one of the countries in the sub-Saharan Africa with the highest rates of malnutrition in children. Some of the underlying and basic causes for the problem could be low agricultural production, low and inadequate food consumption and malnutrition, and diseases (FAO, 2012).

In Ethiopia above 200 fish species have been identified; among those fish species, the most common are *Oreochromis niloticus*, *Clarias gariepinus*, *Barbus species* and *Lates niloticus* (FAO, 1995). The country has high potential of fish production in Lakes such as Tana (25%), Ziway and Langano (19%), Chamo (18%) and Abbaya (12%) of the national total population FAO (1995) Recently, the data of Ministry of agriculture and rural development MoARD (2011) stated that the total catch of fishes increase from 14,000 in 1998 to 24257 in year 2011.

Most of the *Escherchia coli* that are normal inhabitants in the small intestine and colon are non-pathogenic. Nevertheless; these non-pathogenic *E. coli* can cause disease if they spread outside from the intestine to other organs. It is worth noticing that this microorganism have pathogenic strains standing out as emerging zoonotic potential, as well as shigatoxigenic (STEC) and enteropathogenic (EPEC) *E. coli* (Scheutz *et.al.,* 2012). The pathogenic strains of *E. coli* may

cause diarrhea by producing and releasing toxins (called Enterotoxigenic *E. coli* or ETEC) and may be the cause of food spoilage in fish (Soliman *et al.*, 2010).

1.2. Statement of the Problem

Serving safe food to consumers is greatly dependent on the food handler's awareness and practices. Starting from production, hygienic practice conducted at each important point of stakeholder along supply chain could play inconsiderable role in the prevention of food born disease and food poisoning during which might likely to occur during food production and distribution phases. Foodborne disease could be obtained from consuming food having contaminants of viruses, bacteria, parasites, chemicals, and allergens, have been a seemingly never-ending threat to public health and significant hindrance to the development of socio-economy worldwide (Havelaar *et al.*, 2015).

Among major foodborne diseases, *Escherchia coli* O157:H7 is one of the most serious emerging disease. Starting from its discovery up to date, *Escherchia coli* O157:H7 remains as one of the most imperious foodborne pathogens, known to cause bloody diarrhea, abdominal pain, hemolytic uremic syndrome, and kidney failure particularly in humans almost everywhere on the globe (Bielaszewska, 2011). EHEC strains are capable of producing shiga toxin and can cause bloody diarrhea leading to severe dehydration. Human symptoms demonstrate a wide range from asymptomatic shedding, non-bloody diarrhea, hemorrhagic colitis and possible HUS (Hemolytic Uremic Syndrome) potentially leading to death (Beatty, 2006).

Escherchia coli O157:H7 is transmitted through direct contact with contaminated food and water. Throwing inedible fish part in water bodies for feeding aquatic birds, fecal contamination due to livestock grazing near to lakes, solid and liquid waste released to natural water bodies has emerged as a major challenge in developing countries. The progressive increase in antimicrobial resistance of *Escherchia coli* O157:H7 due to irrational uses of antibiotics to treat bacterial infection might enhance the development of drug-resistant bacteria. Moreover, improper use of first line antibiotic drug in fish pond, and improper disposal of unwanted antimicrobial drug that might enter the lake most likely by flood might increase in the development of emerging and antimicrobial resistance gene

Even though there is little consideration on animal source food, fishery practice is one of a greatly neglected problem in developing country. In Ethiopia, few study have been reported regarding Isolation of *Escherchia coli* O157:H7, in other animal source foods, but the study report regarding fish product along its chain is almost likely not exist. Despite its perishable nature and high chance of contamination along its marketing chain, there is limited well documented data regarding hygienic practice assessment and occurrence of *Escherchia coli* O157: H7 along fish marketing chain from site of production to consumption.

It is well known that there were no published research work conducted regarding hygienic practice assessment with isolation and antimicrobial susceptibility profile of *Escherchia coli* O157: H7 isolated along Fish Supply chain Lakes in particular study sites, even before recovery of Lake Haramaya up to today. Considering the above explained research gap, the current research was proposed to identify likely hood of occurrence, magnitude and intervention strategy throughout chain of fish in the target study area.

1.3. Objectives of Study

1.3.1. General Objective

To asses hygienic handling and utilization practice and determine occurrence of *Escherchia coli* O157:H7 with its antimicrobial susceptibility profile along Fish supply chain of lakes in Haramaya District, Eastern Ethiopia.

1.3.2. Specific objectives

- To assess hygienic handling and utilization practice of Fish along the supply chain of Lakes in the Haramaya District.
- To isolate *Escherichia coli* O157:H7 from selected sample sources and identify the likely hood source of contamination, along fish Supply chain of the target areas.
- To determine antimicrobial susceptibility profile of *Escherichia coli* O157:H7 isolated from fish and fish associated environmental samples including contact surfaces at the selected study sites.

2. LITERATURE REVIEW

2.1. General Over view of Fish and Hygienic Handlig practice of Food

2.1.1. Hygienic handling and common Fish born phathogen

Personal hygienic practices are vital to ensure production of harmless food for consumers. Good personal hygiene and food handling practices are the basis for preventing the transmission of pathogens from food handlers to the consumers Hashanuzzaman *et al.,* (2020). Based on the U.S. Food and Drug Administration (FDA) Code of Federal Regulations Title 21 (21CFR), fish is defined as freshwater and salt water finfish, crustaceans, mollusks, and other forms of aquatic animal intended for human consumption (21CFR123.3) (FAO, 2020).

Fish and fishery products are generally regarded as high risk commodities with respect to pathogen contents and other possible contaminants. These products are susceptible to a wide variety of potentially pathogenic bacteria and are major vehicles for transmission of several bacterial diseases (Maysoon, 2014). Fish is an important source of better quality protein consumed by human. However, fish are susceptible to a many varieties of disease causing organisms like bacterial pathogens, most of which are zoonotic, causing disease in human (Abisoye *et al.*, 2011).

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 (Bevilacqua *et al.,* 2017). Fish can be predisposed to a range of spoilages even if traditional preservation techniques have been used on Fishes, like other commercial commodities, are needed to be transported from landing sites to places where they can be sold or utilized by the consumers FAO (2012).

Because of their perishable nature, fish need very careful attention to maintain quality and avoid spoilage (FAO, 2012). Some data from both developing and developed countries indicate that at least 10% of the population may experience a food borne disease. This indicates that microbial food-borne illness still remains a global concern despite the extensive scientific progress and

technological developments achieved in recent years especially in developed countries (FAO, 2012).

It is well accepted that microorganisms are commonly present on fish surfaces, such as skin and gills, as well as inside of the fish in areas such as the digestive tract and internal organs, for example, the kidney, liver, and spleen. However, fish and fish products, especially raw or undercooked products, have been involved in outbreaks associated with bacterial pathogens, bio toxins, histamine, viruses, and /or parasites (Silva *et al.,* 2009). The incidence of these pathogens in sea foods, and the top pathogens have been reported to be *Salmonella* spp., *L. monocytogenes*, *Vibrio* spp., *Yersinia* spp., *C. botulinum*, *S. aureus*, and *Aeromonas* spp. (Amagliani *et al.,* 2012 ; Fernandes *et al.,* 2018).

2.1.2. Chemical Composition and Nutritional Constituents of Fish

Fish is an excellent source of high quality animal protein, essential fatty acids containing polyunsaturated fatty acid and a valuable micronutrients including; iodine, vitamin A and B. which are much greater in fishes than in terrestrial animal-source foods. Adebayo *et al*., (2012). Dried fish is an important source of crude protein, amino acids, water, lipids, fatty acids and ash or minerals. The proximate compositions such as moisture, protein, Lipid are important parameters of fishery product quality assessment, which influence the nutritive value, quality, functional properties and sensory properties of fishery product Kar *et al.* (2020).

In fresh fish, the flesh generally contains up to 65-90% moisture, 10-22% protein, 1-20% fat and 0.5-5% minerals (Radhika, 2018). In addition, small whole fish are among the most vital suppliers of micronutrients, such as vitamins, iodine, iron, zinc and calcium, which all play a critical role in cerebral development, immune system support and general health. Thus, the unique combination of high-quality protein and important micronutrients in small fish plays a significant role in combating the triple burden of hunger, micronutrient deficiency and non-communicable diseases (FAO, 2019).

2.1.3. Fish Preservation and Transportation Method in Ethiopia

Ethiopia mostly experiences a traditional stage in fish handling and preservation techniques. The traditional means of fish preservation and transportation contributed much to the low quality

of preserved fish and short life span of fish products. Fish is one of the most perishable foods that can be spoiled easily if not properly preserved, particularly in tropical and subtropical climates (FAO, 2012). The postharvest handling process of any products, including fish, is the procedure taken to ensure the quality of a product until handed over to the customer is drying technique. It is mostly performed by local fishermen on remote fishing sites where they could spend some time before bringing their catches to the nearby markets (Tut *et al.,* 2019).

The smoking technique is sparingly used, especially during the wet season when there is not enough sunlight. The smoked fish cannot be sold in the local market because of their low quality and can only be used for home consumption. Deep freezing and cold rooms are also used in some cases by Fish Production and Marketing Enterprise centers in the Gambella Region (Tut *et al.,* 2019). Dried fish is a rich source of protein, lipid and minerals that can serve as a promising source of nutrients to alleviate malnutrition in low-income countries. Nowadays, fish drying is becoming an increasing practice to extend the shelf life of excess catch in areas where other preservation mechanisms such as cold storage are scarce (Fikadu and Bezuayehu 2021).

Major factors contributing to a reduction in fish production in the region include inefficient fishing gears, poor transportation access, poor postharvest handling, low price at the landing site, and improper market place. Drying is the predominant postharvest technique and fishing methods are of a subsistence basis. All the fishing activities take place in the natural environment, and aquaculture is not yet established. Enough modern and efficient gears need to be made available. Other modern postharvest handling techniques need to be introduced to ensure a longer shelf life of fish after harvest (Tut, 2020).

2.1.4. Fish consumption patterns in Ethiopia

Ethiopia represents the world lowest country in annual per capita fish consumption in 2010 (FAO, 2015). In Ethiopia, current fish production potential is estimated around 94,500 tonnes per year. Tesfaye and wolf (2014)**,** indicated that high fish consumption expectations. But still in the country the dietary diversity is basically cereal crop. Since Ethiopia is a land locked country, the growing fish demand has largely been met through inland capture fishery and extensive aquaculture-reservoirs (Aseffa, 2014).

In Ethiopia, the research report of Yilma *et al.,* (2020), clearly stated that households relying on fishing for their livelihoods are assumed to consume more fish than none fish producing households. Thus, fish producing households are expected to have better nutritional status compared to none fish producing households. However, this assumption is not well studied and there is limited evidence about the consumption and the nutritional outcomes of fishing in and around Hawassa city (Yilma *et. al.,* 2020).

2.2. Public Health Importance of Food Born Pathogen

2.2.1. Common public health importance of food born pathogen

Food can be a vehicle for a number of pathogens belonging to bacterial, viral, and parasitic agents, including bacteria responsible for the majority of foodborne illnesses (Flecknstain *et al.,* 2010). Foodborne diseases and food poisoning are the widespread and great public health and well-being concerns of individuals and countries of the modern world. Especially, developing countries are largely affected by food-borne infections (Carbas *et al.,* 2012).

Among Bacterial species the most common food-borne zoonotic bacterial pathogen are *S. aureus, Salmonella species, Campylobacter species, L. monocytogenes,* and *E. coli*. These major zoonotic bacteria cause human infections which are characterized mainly by gastrointestinal symptoms including nausea, vomiting, diarrhea, abdominal cramps, and other agent-specific symptoms, while some bacteria may cause severe complications (Abebe *et al.,* 2020).

Escherchia coli is generally associated with seafood contamination in the tropics, where it is encountered in high numbers and isolated in finfish samples acquired at the retail market in Cochin (India) and, although typical *E. coli* O157:H7 or labile toxin-producing *E. coli* were not detected, the isolation of strains with the ability to produce hemolysis in human blood was a fact worth stating (Costa, 2013). *Escherichia coli* is one of numerous types of bacteria that usually inhabit the intestine of humans and animals. It has some strains are capable of producing disease when the immune system is compromised as a result from an ecological exposure Frehiwot and Fufa (2019).

2.2.2. Status of Foodborn Pathogen on Fish and Animal Source Food in Ethiopia

Poor sanitation practices, poor handling of food, weak regulatory systems, lack of resources and education for food-handlers, resulted in food-borne infections to happen frequently and pose a serious threat to human health in developing countries like Ethiopia (Tesfaye *et. al.*, 2020) .The finding of Tesfaye *et.al.*, (2020) stated that, the bacteriological examinations of the samples was compared with international guidelines of Public Health Laboratory Service; Gilbert *et. al.*, (2000). According to this guideline, the limits for aerobic colony count incubated for 48 hours at 30°C for satisfactory, acceptable and unsatisfactory categories which are <105, 105 - <106 and >106 CFU/g, respectively.

In central part of Ethiopia, Study conducted by Bezuayehu and Fikadu (2020), indicates the bacteriological quality of fish sold at supermarkets and fish shops in Addis Ababa. Based on information obtained from this study, fish from supermarkets are less contaminated by bacteria than fish from fish shops. *Protease* spp. and *Staphylococcus* spp. were found as the most prevalent species in fish shops while *Enterobacter* spp. were predominantly found in fish samples from supermarkets.

The study reported by Tesfaye *et al.* (2020) indicated that, bout 21% of the poultry, beef and mutton and fish samples had unsatisfactory microbial quality because of bacterial indicators or fungi based on international guidelines because the cold chain is not efficiently maintained across the fish supply chain in Ethiopia due to several challenges such as lack of basic inputs like ice and refrigerated trucks. Tesfaye *et al* (2020) recommended that; meats and fish should be processed under hygienic conditions and adding berbere spice to food and packaging enhances the quality of ready to eat to avoid public health risks.

2.2.3. Prevalence of *Escherchia coli* and *Escherchia coli* O157 in Ethiopia

Prevalence of *Escherchia coli* on fish and its body parts was reported by few researcher in Ethiopia. Among them; 2.4% by Nuru *et al.* (2012) on Lake Tana, 42.5% by Teka *et al.* (2017) on Lake Abaya and Chamo, 12% by Adanech and Temesgen, (2018) at Ziway and 9.4% by Awot *et al*. (2019) are among researcher which conducted their study on *E. coli* related with fish and its body parts. The prevalence of *E. coli* from fish samples in Ethiopia is relatively high ranging from 2.4 % to 53.30% which was conducted by Nuru *et al.* (2012) and Adem *et al.* (2018) respectively.

Escherchia coli O157:H7 strain is potentially harmful to human health. Study Conducted by Aynadis and Aweke (2019) is related with isolation, identification and antimicrobial susceptibility pattern of *E. coli* O157: H7, at Lake Hawasa with 2.33% over all prevalence of *Escherchia coli* O157:H7 were identified from the skin and muscle of Nile tilapia, Cat fish and Golden fish. Similar study was conducted by Ayalew *et al.* (2019) on Lake Hyke and Tekeze Dam. However, organisms' occurrence in fish was 1.46% in which was lowest reported prevalence from fish as well as from other animal source food in Ethiopia.

Table 1 Prevalence of *Escherichia coli*, and *Escherchia coli* O157:H7 in fish in Ethiopia

Place	**Site of recovery**	***E. coli* in %**	***E. coli* O157:H7 in %**	**References**
Lake Tana	Intestine	2.40	-	Nuru *et al.*, 2012
Lake Hayke	Skin, gill, intestine	25	-	Shimels *et al.*,2018
Lake Hyke and Tekeze Dam	Fish body part, equipments and Hand swab		1.46	Ayalew *et al.*, 2019
Lake Adelle	Various body part	53.30		Adem *et al.*, 2018
Tinike	Various body part	20.00		Adem *et al.*,2018
Lake Hawasa	Muscle and Skin	23.32	2.33	Aynadis and Aweke 2018
Mekele city	raw fish-meat	9.4		Awot *et al.*, 2019

2.3. General Characteristics of *Escherchia coli* O157:H7

2.3.1. Taxonomy and Nomenclature of *Escherchia coli* O157:H7

Escherchia coli is a Gram-negative, facultative anaerobe, within the family *Enterobacteriaceae*, and is normally a commensal bacterium that coexists with its human host in the intestines in a mutually beneficial relationship For the most part, *E. coli* is a group of harmless bacteria that are most often used as indicator organisms for fecal contamination and breaches in hygiene. However, several *E. coli* clones have acquired virulence factors that have allowed them to adapt to new niches and in some cases to cause serious disease (Tchaptchet and Hansen, 2011).

Among the pathogenic *E. coli* of greatest relevance to milk is *E. coli* O157:H7, an STEC serotype, which, because of its high virulence (it can cause disease at a dose of 5–50 cells), is of major concern to the dairy industry. According of review conducted by Mathusa *et al.* (2010). *Escherichia coli* are a rod-shaped bacillus having about 2μm long and 0.5μm in diameter with a cell volume of 0.6 to 0.7μm. It is a normal inhabitant in the intestines of animals and humans. Its recovery from food has public health concern due to the possible presence of pathogenic strains which lead to severe gastrointestinal disturbance. It is widely used as an indicator of the bacteriological contamination of food and environments most commonly from the fecal origin (Samuel *et al.,* 2011).

2.3.2. Epidemiology of *Escherchia coli* O157: H7

Epidemiology of foodborne pathogens especially that of pathogenic *Escherichia coli*, is not well studied in Ethiopia. The actual number of *Escherichia coli* O157 : H7 infections attributable to animal source food is difficult to assess accurately, because of the lack of diagnostic facilities and only a small proportion of illness cases are officially reported especially in developing countries (Scallan *et al.,* 2011). In Ethiopia, Prevalence report on Fish indicated that; only 1.46% and 2.35% of *Escherchia coli* O157: H7 had been isolated fish while were reported by Ayalew *et al.* (2019) and Aynadis and Aweke (2019).

2.3.3. Sign and Symptom of *Escherchia coli* O157: H7

Among the bacterial pathogens, *Escherichia coli* O157: H7 one of the most bacterial phathogen frequently been associated with food-borne illness with three types of syndromes; hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombolytic thrombocytopenic purpura (TTP). The bacteria initially causes nonbreeding diarrhea accompanied by abdominal cramps from which it may gradually develop into bloody diarrhea and hemolytic uremic syndrome (HUS), which causes kidney failure in humans (TTP) (Keene *et al.*, 1994; Amanda *et al.*, 2010; Pal and Raj 2016).

2.4. Diagnostic Aproach of *Escherchia coli* O157:H7

2.4.1. Conventional Culture Methods

To identify and differentiate the multiple types of diarrheagenic *E. coli* O157:H7 in the laboratory, a wide variety of methods are employed. Some of them include culturing on specific growth media, assessing biochemical profiles, serotyping and screening for the presence of virulence characteristics. However, these conventional methods are not rapid and reliable enough to distinguish such pathogenic strains which lead to the application of the molecular diagnostic technique for more effective detection and characterization of *E. coli* O157:H7 (Manning, 2010).

Diagnosing of *E. coli* O157:H7 is based on phenotypic differences from most other strains Initially testing for sorbitol fermentation has been suggested as a simple means to screen for *E. coli* O157:H7, because it lacks the β-glucuronidase enzyme Manafi and Kremsmaier (2001) and Müller and Ehlers (2005). Most of the existing culture methods were developed based on the above-mentioned feature, as well as the strain's inability to ferment rhamnose and its tolerance to tellurite (Bielaszewska *et al.*, 2005)

2.4.2. Molecular Detection and Genotyping Of *Escherchia coli* O157:H7

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:H7 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. Among the PCR-based methods, real-time PCR is promising for the rapid detection of bacteria in a quantitative manner (Kim *et al.,* 2020).

Restriction Endonuclease Analysis (REA) and Pulse Field Gel Electrophoresis (PFGE) are commonly used Genomic method for genotyping of *Escherchia coli* O157:H7. In case of Restriction Endonuclease Analysis, Restriction enzymes recognize a specific sequence of nucleotides and produce a double-stranded cut in the DNA, (Kessler and Manta 1990). The recognition sequences usually vary between 4 and 8 nucleotides, and many of them are inverted repeat palindrome, a sequence that reads the same forward and backwards in complementary DNA strands (Clark and Pazdernik. 2005).

The detection of *Escherchia coli* O157:H7 with the help of Pulse Field Gel Electrophoresis (PFGE) include; first bacteria were embedded in agarose, lysed, treated with protease and intact genomic DNA is released. Secondly, DNA was digested with restriction end nuclease. Then fragments of DNA were separated in agarose gels on a clamped homogenous electric field apparatus and Gels were stained with ethidium bromide. Finally, destained in water, and visualized with a Gel Doc gel analysis system (Avery *et al.,* 2002).

2.4.3. Serological Method for Detection of *Escherchia coli* O157:H7

The use of latex reagents in slide agglutination assay provides a rapid screening procedure for the presumptive identification of *Escherichia coli* O157:H7. This assays are used for serotyping non-sorbitol fermenting colonies, generally isolated on Sorbitol MacConkey (SMAC) agar Ye *et al.* (2013) and Manyi-loh *et al.,* (2018). 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 and flagellar antigen (Ye *et al.,* 2013).

2.4.4. Immunomagnetic Separation Based Automated Devices

Immunomagnetic separation (IMS) is a well-known method for the separation and concentration of target bacteria from a large volume of food samples. Magnetic beads functionalized with an antibody provide selectivity for target bacteria such as *Escherichia coli* O157:H7 (park *et. al.*, 2020), the magnetism of the magnetic beads can be used to rapidly separate and concentrate bacteria in a large sample volume (250 mL) using an external magnetic field, which can also eliminate PCR inhibitors. However, these challenges can be overcome or minimized by the incorporation of IMS during food sample pretreatment (Sharma and Mutharasan, 2013).

2.4.5. *Escherchia coli* O157:H7 Biochemical Identification Kit

The test is a qualitative micro method employing Biochemical approaches for the identification of *E. coli* O157:H7. It can also be used for validating known laboratory strains. *E. coli* O157:H7 produces numerous other putative virulence factors, the most notable of which is Shiga toxin (Stx). The *Escherchia coli* O157:H7 Biochemical Identification Kit contain fifteen dehydrated reactants, one Triple Sugar Iron (TSI) agar tube and one semi-solid agar biochemical test tube for the performance of either carbohydrate fermentation tests or other biochemical based tests, which allows simultaneous inoculation of each well with a predetermined amount of inoculum (Anonymus, 2017).

The reactions employed are a combination of conventional tests and chromogenic tests to detect the presence of specific enzymes and/or metabolic end-products produced by the bacteria. Identification of isolates is achieved by recoding the results visualized by a colour change after 18-24 hours incubation at 36°C±1°C and the addition of appropriate reagents in some cases (Anonymus, 2017).

2.5. Anti-Microbial susceptibility of *Escherchia coli* O157:H7

The prevalence of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs), two emerging food safety concerns. Pathogenic and spoilage microorganisms as well as ARB and ARGs can be introduced into fish and fish products in both preharvest and postharvest stages (Sheng and Wang 2021).The emergence of *Escherichia coli* O157:H7 serotype dates back to 1982 when it was first discovered in an outbreak traced to contaminated Hamburgers

(Riley *et al.,* 1983) Ever since its discovery to date *E. coli* O157:H7 remains as one of the most imperious foodborne pathogens, known to cause bloody diarrhea, hemolytic uremic syndrome and hemorrhagic colitis in humans almost everywhere in the world (Mersha *et al.,* 2010).

In Ethiopia the report conducted by Ayalew *et al.,* (2019) indicated that the antibiotic susceptibility test revealed that the isolates were resistant to Ampicillin and Streptomycin disks. However, Ciprofloxacin, Gentamicin and Nalidixic acid were found effective in inhibiting the growth of all of the isolates. Since pathogenic Escherichia coli strain was detected from fish, raw and undercooked fish consumption in Ethiopia may result in contracting infections.

The report of multi-drug resistance in *E. coli* that is rampant in, food sources, environment and human beings in Mekong Delta region is alarming (Dyar *et al.,* 2012; Nhung *et al.,* 2015). Antibiotic use in *E. coli* O157: H7 (STEC O157) infections is controversial because of the potential to increase production and secretion of Shiga toxins, thus promoting the onset of HUS in humans (Colello *et al.,* 2015). However, early administration of antibiotics such as Rifaximin, Fosfomycin, Azithromycin, and Meropenem was found to not stimulate the release of Shiga toxin from O157 and non-O157 strains in vitro. These antibiotics have been recommended for the treatment of early stages of STEC disease to prevent HUS (Bielaszewska *et al.,* 2012).

2.6. Control and prevention of *Escherchia coli* O157:H7

Survival of *Escherchia coli* O157:H7 in the environment can be affected by factors including PH, temperature, dehydration, indigenous microbial communities, moisture, and aerobic conditions (Abadias *et al.,* 2012). The ability of this phathogen to survive during food processing and subsequent recovery and growth during refrigerated storage was demonstrated experimentally (Oliveira *et al.,* 2010; Izquierdo *et al.,* 2013). An effective control program to substantially reduce *E. coli* O157:H7 infections will require the implementation of intervention strategies throughout the food continuum, from farm to table. Consumers also have a role in implementing intervention controls in food handling and preparation (Addis and Sisay, 2015).

Understanding factor affecting growth and multiplication of *Escherchia coli* O157:H7, the prevalence, ecology, concentration, and dynamics of pathogenic and spoilage microorganisms

present throughout the entire fish production chain will also contribute to the development and application of new intervention strategies. Various decontamination methods have been proposed, tested, and applied to ensure the microbiological safety of fish and fish products, including physical, chemical, and biological interventions (Cunha *et al.*, 2018). To control *E. coli* O157:H7 in foods, several physical and chemical methods have been widely used (Puligundla and Lim 2022). Some of them were summarized below

2.6.1. Physical and Chemical Method

Among physical method, the application of heat (Thermal method) has been an important technology to kill *Escherchia coli* O157:H7the pasteurization of milk at 72 ^{0}C can eliminate these pathogens. As alternatives to thermal processing, several studies have reported the potential of non-thermal treatments, such as irradiation technologies (which typically use ionizing radiation, such as gamma-rays, low-dose electron beam, and X-rays), UV irradiation (e.g., UV-C and pulsed UV light), high-pressure processing (HPP), and pulsed electric fields (PEFs) to eliminate *E. coli* O157:H7 in foods (Saldaña *et al.*, 2012).

Chemical method is the most commonly used relatively inexpensive chemical sanitizers, such as peroxy acetic acid, chlorine dioxide, sodium hypochlorite, acidified sodium chlorite, organic acids (e.g., acetic, lactic, and citric), and aqueous ozone have been popularly used to reduce the prevalence of *E. coli* O157:H7 and other food-borne pathogens on raw meat products Yoder *et al.* (2012). The robust survival exhibited by *Escherchia coli* O157:H7 under most conditions emphasizes the significance of having appropriate sanitation and disinfection methods to deal with it.

2.6.2. Biological Method for Prevention of *Escherchia coli* O157:H7

In recent years, bio control approaches are becoming increasingly attractive for the control of food-borne pathogens because of ever-increasing antimicrobial resistance, as well as consumer awareness of the health risks of chemical food additives and preservatives (Chauhan *,.et al* 2020). Study conducted by Lu and Breidt (2015) have showed that the use of phages to control pathogenic bacteria in foods is a promising novel strategy. Biocontrol agents are bioprotective microorganisms such as bacteriophages, probiotics, and other antagonistic bacteria, plant-

derived natural compounds, bacteriocins, endolysins, and enzymes, are rapidly emerging as effective, selective, relatively safe for human consumption and environmentally friendly alternatives against *Escherchia coli* O157:H7. Many studies reported success with these agents (Oliveira *et al.*, 2018; Puligundla and Lim 2022).

3.6.3. The Application of Next Generation Sequencing

The Application of Next Generation Sequencing (NGS) technologies has allowed in-depth insights into the fish micro biome the consortium of all microbial habitants in fish and the surrounding environment which facilitates better understanding of the sources of pathogenic and spoilage microorganisms (Brugman *et al.*, 2018). 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).

3. MATERIALS AND METHODS

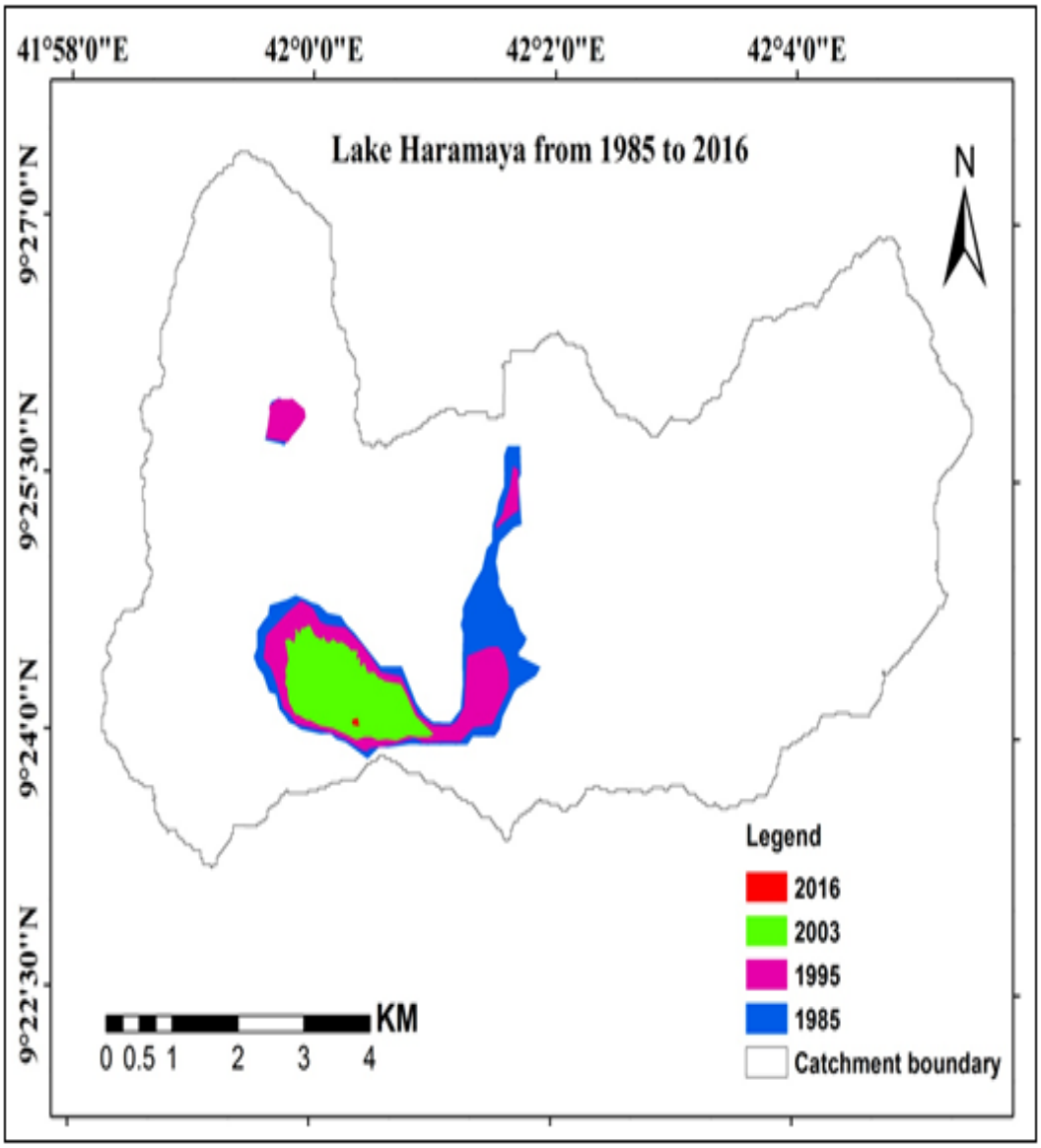
3.1. Description of the study areas

Haramaya Haramaya district is located in eastern Hararge Zone of Oromia Regional State, along the high way from Addis Ababa to Harar 508 km from Addis Ababa and 19 km ahead to reach Harar at an altitude of 1980 meters above sea level (m.a.s.l.), 9◦26’’N latitude and 42◦3”E longitude. The district has 35 kebeles or administrative districts. The mean annual rainfall is 780 mm. The mean annual minimum and maximum temperatures are 8.5 and 24.4◦C, respectively (HABD, 2014).

Lake Haramaya is situated on the main road from Addis Ababa to Harar town at a distance of 505 km from Addis Ababa and 20 km northwest of Harar town. It is situated at 9^0 22’ 30” 9^0 30’00” North of latitude and 41^0 58’ 30” - 42^0 6’ 30” East of longitude (Niway *et al.,* 2010; Google Earth, 2022) .The total area of the catchment is 5032 ha and encompasses a small part of Haramaya town, the Haramaya University Campus, three peasant associations (Setegn *et al.,* 2011). As reported by Brook (2003), Lake Haramaya provided fresh water for drinking, irrigation, fishing (*Oreochromis niloticus and Clarias gariepinus*), animal watering, general municipal uses and recreation to over 120,000 people of the region.

Having similar ecology, Lake Tinike lies between $9^0$22’03”–$9^0$27’12” north latitude and $41^0$58̓14”–$42^0$5̓26” east longitude, at an altitude range of 2010–2433 m above sea level Muleta *et al.,* (2006), While, Lake Adele is located at the latitude and longitude coordinates of 9.425833 N and 41.950833 E respectively. It has a length of about 3.2 kilometers and width of 1.15 km (Google Earth, 2022)

Lake Adele has a length of about 3.2 kilometers and width of 1.15 km. Lake Adele is one of the lakes giving incomparable economic benefits in Eastern Hararghe zone. It is being used for irrigation and water supply purposes, especially for animals (Haile, 2018). Lake Adele is one of the wintering areas for aquatic Palearctic wintering birds. Different wintering birds are commonly seen in the shallow parts of the lake (Kebede, 2013). As reported by Adem *et al.* (2018), the available Fish species in Adelle Lake was only *Clarias gariepinus* while, Tinike Lake has only *Oreochromis niloticus* fish species. The fish are caught by local fishermen for local consumption.



AB.

Figure 1: Map of study areas (Neway et *al.*, 2010)

3.2. Study design

A cross-sectional study design was conducted from October 2021 to May 2022 to asses' hygienic handling practice with isolation and antimicrobial susceptibility profile of *E. coli* O157:H7 along Fish supply chain of Lakes in Haramaya District.

3.3. Study Units

Study unit represent; different fish samples (filleted fish, stored meat and ready to eat fish) together with Fish associated environmental sample (FES) (lake water, wash water, storage equipment, transport equipment, serving equipment, pooled sample of knife and cutting board and Hand swab) selected fish part from freshly caught fish (fresh meat, Viscera and skin swab) Fisher man, Retail/ Restaurant worker and Consumer were also included for questionaire assessment of hygienic practices.

Inclusion criteria:

Viscera, Skin swab and fresh meat sample taken from freshly arrived whole fish to consider likely hood of occurrences of *E. coli O157:H7* as intrinsic Filleted fish meat, stored fish sample and Ready to Eat Fish sample and Fish associated environmental sample as extrinsic origin of this phathogen.

Exclusion Criteria:

Fish and Fish related Environmental sample coming from lakes other than lakes found in the Haramaya district and individuals who have no involvement in fishing, selling or consuming fish

3.4. Sample Size Determination

The sample size for isolation and antimicrobial susceptibility profile of *Escherchia coli* O157: H7 along Fish Supply chain was calculated using Thrusfield formula (2005) for single population proportion by considering the previous prevalence report, 2.35% *E. coli* O157: H7 isolated from Fish at Lake Hawassa, Southern Ethiopia by Aynadis and Aweke (2019).

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

Where *n* is the required total sample size, *Pep* is the expected prevalence of the organism along fish Supply chain and d, stands for the desired absolute precision of 5%. Thus, sample size were 35. Considering the value for each three Lakes and Retailers becomes 140 total fish sample. However the sample size was increased by three folds and a total of 420 samples were collected to consider likely hood of occurrence of *E. coli* O157:H7 from Fish associated environmental sample (FES) freshly caught fish, and different fish sample (Fillated fish, stored fish and ready to eat fish). Thus, a total of 420 consisting of 105 proportionally allocated sample of freshly caught fish (35 viscera, 35 fresh meat and 35skin), 105 Fish related environmental samples (FES) and 210 different fish sample (70 sample for each of Filleted fish, stored fish, Ready to eat sample)

For hygienic practice assessment along Supply chain; questionnaire survey and observation the sample size was determined purposively based on availability, willingness of the interviewees and ease for follow up of microbiological sample collection along supply chain. Accordingly, 63 participants consisting of 18 Fisherman, 20 retail and/or restaurant worker and 25 consumer were interviewed accordingly from each three lakes and seven fish serving installments found in Haramaya districts.

3.5. Sampling Techniques, Sample Collection and Preparation.

3.5.1. Sampling Method for Questionaire Survey

Each Lake and fish marketing site were purposively included based on the voluntary consent of fisher man and retail owner. Similarly, participant were selected purposively based on their willingness and based on their involvement in fishing or fish marketing chain including consumer. All questionnaires and observational assessment were administered via face-to-face interview to ensure the accuracy of the responses.

3.5.2. Sample Collection and Sampling technique for Microbiological Sample

Microbiological samples collected from lake sites were freshly caught fish, Filleted fish sample and swab sample of FES (Fish associated environmental sample) while; ready to eat (RTE), stored fish swab and swab of FES at marketing sites were sample collected from fish marketing sites. Each sample was proportionally allocated using simple random sampling from each Lake and Fish marketing site; freshly caught fish, filleted fish sample, RTE (approximately 10g)

samples were put in their respective sterile plastic bag (Sterillend TM 400) and tied separately and then labeled before collecting them into ice box. Similarly swab sample of stored fish and Fish associated environmental sample were taken in 10 ml of buffered peptone water. Then after, the collected sample was immediately transported to veterinary microbiology laboratory, CVM, Haramaya University.

Sampling site was selected purposively based on sample accessibility (availability of fish, the consent of the owner to offer related sample) and volunteer for questionaire survey. As a result, lake sites in Haramaya district such as Haramaya Lake, Tinike, Lake Adele and Fish retailing area were selected purposively. Proportional sample allocation and simple random sampling techniques were applied to select freshly caught fish, filleted fish, stored fish meat, RTE (ready to eat fish), Fish associated environmental sample.

3.5.3. Microbiological Sample Preparation

After reaching the laboratory, sample preparation and primary sample enrichment (using non-selective pre-enrichment media) were conducted according to ISO 21528-1:2004 Sample preparation and primary enrichment sample of ready to eat, filleted fish, stored fish and sample of recently caught fish (skin swab, Sample of viscera, raw meat sample) were prepared as follows. Approximately each 10g of filleted fish and RTE fish collected in plastic bag were incubated with 90 ml of peptone water. From each immediately caught fish, before evisceration, skin swab was taken from fish using sterile cotton swab and collected in screw capped test tube with 10ml buffered peptone water as a transport media. After taking skin swab, skin surface was disinfected using 75 % VV Alcohol. Then after, the fish was dissected on the back using sterile scalpel blade the meat sample was held with sterile forceps and placed in stomacher bag avoiding cross contamination.

The ventral line incision was per formed similarly to take visceral sample. After required amount of sample was collected in their respective plastic bag; approximately 10 g, for raw fish meat, filleted fish and ready to eat fish. Each of them was homogenized separately for two minutes using stomacher machine 400 (Seward Medical, England) and transferred into sterile plastic bag by adding 90 ml of buffered peptone water. Similar to skin swab, Filleted fish swab and FES swab were collected in screw capped test tube with 10ml buffered peptone water and all sample ware incubated at 37 ^{0}C for 24h.

3.6. Study Methodology

3.6.1. Microbiological Analysis

3.6.1.1. Isolation and identification of *Escherichia coli* O157:H7

Isolation and identification of *E. coli* O157: H7 was performed using techniques recommended by Quinn *et al.* (2002) with slight modification. From each incubated samples of fish and fish associated environmental surfaces (FES), loop full of the sample was streaked onto Macckonkey agar plates as differential media for the identification of *E. coli* and incubated at 37°C for 24 hours. After incubation at specified period of time, typical lactose fermenting colonies was taken and streaked on Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hrs.

From EMB Agar media, morphologically typical colonies were producing metallic sheen was transferred to Sorbitol Macckonkey agar plates and incubated at 37 ^{0}C for a maximum of 24h. Following incubation, sorbitol negative (colorless colonies) was identified by their color and further streaked onto Sorbitol Macckonkey agar plates supplemented with Cefixime (0.05mg/L) and Potassium tellurite (2.5 mg/L) again to get a clear colorless typical *E. coli* O157 isolates and to inhibit growth of some sorbitol non fermenting colonies. From the pure culture, isolates were sub-cultured onto nutrient agar for further preservation and it were further subjected to biochemical tests.

For Biochemical confirmation of *Escherchia coli* O157:H7, Indole test, Methyl red (MR) test, Voges-Proskauer (VP), and Citrate utilization test (IMViC) and Triple sugar iron (TSI) test were performed to check biochemical feature of *Escherchia coli* in the sample based on its presumptive biochemical property on IMVIC and TSI test. Similar to other member of *E. coli,* Red ring formation by indole test, red color on methyl red test, negative Voges-Proskauer, negative for citrate utilization and formation of acidic slant and acidic butt on TSI test was recorded as presumptive *E. coli* O157:H7 and stored on sterile 50% glycerol stock for further Latex agglutination test.(Appendix1)

For Latex agglutination test of *E. coli* O157: H, before bacterial colony was subjected to a latex agglutination test using an *E. coli* O157:H7 latex kit (Remel ^{TM}). A drop of control positive and

control negative latex for *E. coli* O 157: H7 was dropped on reaction card separately. Using plastic stick take pure colony on to the reaction card and test latex for both *E. coli* O 157 and H7 was dropped and dispensed into the reaction card separately. Then reaction card is rotated in circular motion for one minute and formation of precipitation for both test latex of E. coli O157 and latex test for H7 antigen was recorded as positive sample for *E. coli* O157:H7 for further anti-microbial susceptibility test.

3.6.1.2. Antimicrobial susceptibility test

The isolated bacterial colonies from pure fresh colonies was transferred to a test tube of 5 ml Trypton soya broth (TSB) and incubated at 37 ^{0}C for 6 h. The turbidity of the culture broth will be adjusted using a sterile saline solution to obtain turbidity comparable to 0.5 McFarland turbidity standard. A sterile cotton swab was immersed in the suspension and swabbed uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic disks was placed on the plate using disk dispenser and incubated at 37 ^{0}C for 24 h and diameter of the inhibition zone formed by each antibiotics; Cefoxitin, Nalidixic acid, Enoxacin. Ciprofloxacin, Streptomycin, Amoxicillin and Ampicillin were measured using a digital caliper and the results were interpreted according to clinical laboratory standard protocols (CLSI, 2015) as explained in the table below

Table 2: Antimicrobial susceptibility measurement criteria for *Ententrobacteriacea* (CLSI, 2015)

Antibiotic disk	**Discconcentration**	**Zone of inhibition to in mm**		
		Susceptible	Intermediate	Resistant
Ciprofloxacin	5 µg	≥21	16-20	≤15
Nalidixic acid	30 µg	≥19	14-18	≤13
Cefoxitin	30 µg	≥18	15 - 17	< 14
Enoxacin	10 µg	≥ 18	13-17	≤12
Streptomycin	10 µg	≥ 15	12–14	≤ 11
Amoxicillin	2 µg	-	-	-
Ampicillin	10 µg	≥ 17	14–16	≤ 13

3.6.2. Questionaire and Survey Assesment.

To asses Fish hygienic handling practice, pretested questionaire were prepared with few respondents starting from fisher man, Fish retail worker/ or owner and consumer were well discussed so as to prepare question which clearly explain and fit local context of society and

critical importance in Veterinary public health and food safety. From each lake and each purposively included retailing areas all volunteer and available respondent were interviewed.

Semi structured questionaire consist of three parts. The first part consist Demographic information, like Age, sex, job, level of education, location of respondents. The second and third part of questionaire consist of question related with personal hygienic practice and hygienic handling practice. Respondent's actual practice regarding four critical food safety factors (food borne diseases, contamination/cross contamination, personal health, and hygiene and temperature control) were interviewed at obtained free time to get relevant information. For unobservable hygienic practice and time limit, self-reported practice was considered as their usual practices.

Hygienic practice score was designed into two parts; the first part consisting question related to personal hygienic practices has two response; "Yes" and "No" being that the response Yes considered as "good practice" while No considered as "poor practices" weighting 1 and 0 respectively. The second part consist of question related with hygienic handling practices along Supply chain of fish consist of three response consisting of "Always, Some times and Never" was also scored good, moderate and poor and coded as (2, 1, 0) respectively.

Hygienic practice score was computed based on frequency percentages of respondent's answers for both question of personal hygienic practice and hygienic handling practice and their cumulative mean percentage. The frequency percentage of respondent's below 50% (cumulative mean percentage) is considered "Poor practice" while greater or equal to 50% is considered good practice.

3.7. Data Analysis and Management

The data of both questionaire survey and microbiological data were entered into a Microsoft excel program spread sheet and analyzed using SPSS 20 statistical software (version 20). Descriptive statistics were computed and expressed in terms of frequency percentages, while Cumulative mean percentage is used in questionaire survey part. The data was analyzed by Chi square test at 0.05 level of significance while, P-values ≤ 0.05 were used as point to assess for any significant association between variables.

3.8. Ethical Considerations

Ethical approval of the research was obtained from Haramaya University Animal Ethical Review Committee (HU AERC) of the College of Veterinary Medicine with Reference no AEC-01-021 for involvement of few fresh live fish sample. (Annex 4). The questionnaire survey and observational microbiological sample along supply chain was conducted based on verbal consent of participant for their willingness to participate and to give required sample and information. The questionary were anonymous to ensure confidentiality, being informed that, the objective of research without breaking Ethical consideration rule of particular area.

4. RESULTS

4.1. Occurrence of *Escherchia coli* O157:H7.

The present study indicated of the 420 bacteriologically examined samples, 2.10%, were harboring *Escherchia coli* O157:H7. The occurrence *E. coli* O157:H7 collected from the lake site and market site were, 3.80% and 0.5% respectively (Fig 1).

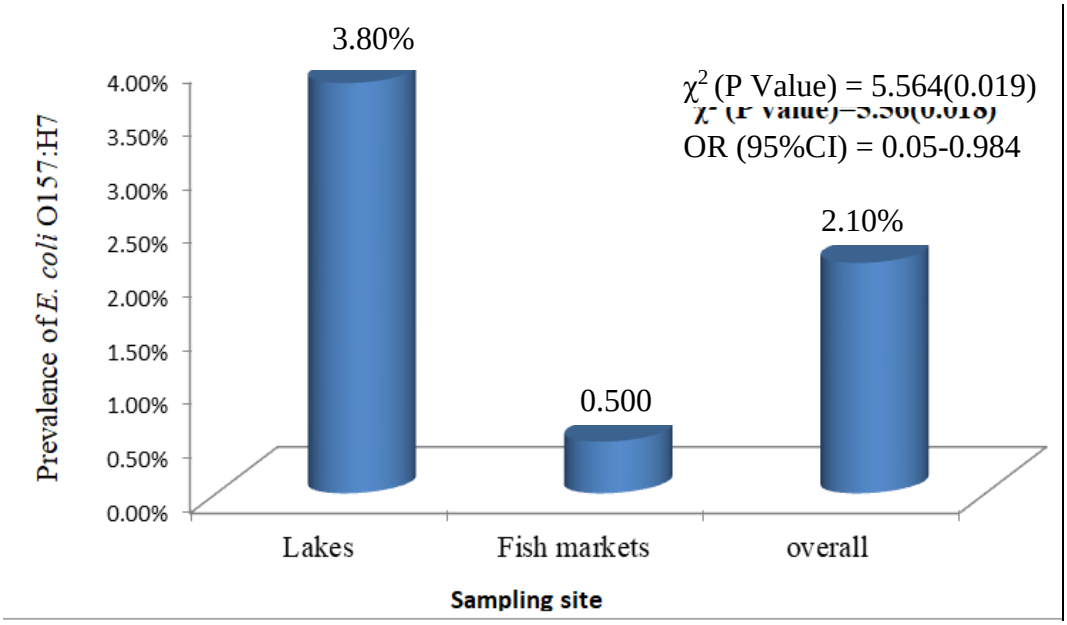


Fig.1 Overall prevalence of *E. coli* O157:H7 at fishing lakes and fish marketing sites in the study area

On the other hand, our study depicted 1.54%, 5.71% and 4% from Lake Adele, Lake Haramaya, and Tinike respectively with no statistical differences (P> 0.05) in prevalence of *E. coli* O157:H7 among samples collected from each lakes found in the Haramaya district (Table 2).

Table 3: Prevalence of *Escherichia coli* O157:H7 Lakes in the Haramaya district

Lake site	Total Examined	Positive (%)	χ^2 (P. value)	OR(95%CI)
Lake Adele	65	1(1.54)		
Lake Haramaya	70	4(5.71)	1.6(0.45)	0.385-0.520
Lake Tinike	75	3(4.00)		
Total	210	8(3.81)		

χ^2=Chi-square, P=Level of significance

In the current study, the highest *Escherchia coli* O157:H7 isolation rate was found in filleted fish 5.7%, followed by sample of freshly caught fish; Fresh meat 2.9%, Fresh viscera 2.9% and Fish skin 2.9% while, it was 1.4% from stored fish sample 1% from FES and 0% from ready to eat sample. In addition, prevalence per origin of sample indicated that; 2.4% was isolated from different fish sample, 2.9% from selected Fish part and 1% from Fish associated environmental sample (FES). Nevertheless, the study results showed both sample type and origin of sample are not significantly associated risk factors for occurrence of *Escherchia coli* O157:H7 at the study area (Table 3).

Table 4: Prevalence of Escherichia coli O157:H7 by sample types and origin of samples

	Total Examined	Positive (%)	χ^2 (P. value)	OR(95%CI)
1.Type of samples				
Filleted	70	4(5.7)	6.926(0.328)	0.274-0.364
Stored	70	1(1.4)		
RTE	70	0(0.0)		
FES	105	1(1.0)		
Fresh meat	35	1(2.9)		
Fresh viscera	35	1(2.9)		
Fish skin	35	1(2.9)		
2. Origin of sample				
Different fish sample	210	5(2.4)	1.022 (0.600)	0.704-0.787
Fish associated (FES)	105	1(1.0)		
Selected Fish part	105	3 (2.9)		
Total	420	9(2.1)		

FES= Fish associated Environmental sample, RTE=Ready to eat sample, X^2=Chi-square, P=Level of significance

The current finding reveals that, susceptibility profile of different isolate of *E. coli* O157:H7 performed on seven antibiotic indicated that; tested isolate were 100% susceptible to Enoxacin while, it was 100% resistant to Cefoxitine, Streptomycin, Ciprofloxacin, Amoxicillin and Ampicillin. However 62.5% isolate appeared with intermediate susceptibility to Nalidixic acid, while only 37.5 % were susceptible to Nalidixic acid (Table4).

Table 5: Antimicrobial susceptibility of *Escherchia coli* O157:H7

Antibiotics	**Susceptibility profile of *Escherchia coli* O157:H7 (n=8)**		
	Susceptible	**Intermediate**	**Resistant**
Cefoxitin 30 µg	0	0	8(100%)
Enoxacin 10 µg	8(100%)	0	0
Nalidixic acid 30 µg	3(37.5%)	5(62.5%)	0
Ciprofloxacin 5µg	0	0	8(100%)
Streptomycin 10µg	0	0	8(100%)
Amoxicillin 2µg	0	0	8(100%)
Ampicillin 10µg	0		8(100%)

4.2. Demography of Respondents

Socio-demographic characteristics total respondents participated in this study indicated that 63 participants consisting of 28.6% Fisherman, 31.7% Retail worker and 39.7% consumer. Most of participant included in the study were male 79.4%while female were very few 13(20.6%). Majority of respondent were ranged within 20-40 years of age followed by below 20 and over 40 years of age respectively. Regarding educational status and Experience, Majority were Secondery and Elementary followed by College and university and illiterate respectively, As it is shown on the table 5, most respondent are below 5 years of experience followed by 6-15 and above 15 years of experiences. Haramaya Lake constitute majority of respondent 71.4 %, while only 7.9% and 4.8 %were from Adele and Tinike respectively constituting only fisher man (table 5).

Table 6: Demographic information of Respondent

Variable	Category	Respondent (%)	Fisherman (%)	Retail worker No. (%)	Consumer No. (%)
Sex	Male	50(79.4)	18(100)	17(85)	15(60)
	Female	13(20.6)	0(0)	3(15)	10(40)
Age	Below 20	14(22.2)	6(33.3)	1(5)	7(28)
	20-40	44(69.8)	12(66.67)	17(85)	15(60)
	Over 40	5(7.9)	0(0)	2(10)	3(12)
Location	Tinike	5(7.9)	5(27.78)	0(0)	0(0)
	Adele	3(4.8)	3(16.67)	0(0)	0(0)
	Haramaya	55(71.4)	10(55.56)	20(100)	25(100)
Level of Education	Illiterate	9(14.3)	3(16.67)	3(15)	3(12)
	Elementary	20(31.7)	8(44.4)	9(45)	3(12)
	Secondary	23(36.5)	7(38.89)	7(35)	9(36)
	Collage, University	11(17.5)	0(0)	1(5)	10(40)
Experience	Below 5 years	25(39.7)	14(77.78)	11(55)	NA
	6-15 years	11(20.6)	4(22.2)	7(35)	
	Over 15 years	2(3.2)	0(0)	2(1)	
Job	Fishing	18 (47.4)	18(100)	-	NA
	Retail worker	20 (52.6)	-	20(100)	
Total		**63(100)**	**18(28.6)**	**20(31.70**	**25(39.7)**

4.3. Personal Hygienic practice Assessment along Fish Supply chain

Most respondents showed good Personal Hygienic practice indicating that; 100% of fisherman and retail worker do not handle fish when got sick. About 63.16 % of respondent do not touch mouth, tongue and nose during working, about 71.4% and 63.5% cover their nose while coughing or sneezing and avoid spitting at work place and wash hands before and after handling fish respectively. 57.9% were avoid Wearing jewelry while working. There is no significant association exist among respondents personal hygienic practices with P value (≥ 0.05).

Table 7: Personal hygienic practice of Respondent along Supply chain

Personal Hygienic practices	Responses	Frequency (%)	Fisherman (%)	Retail worker (%)	Consumer (%)	χ2 (p. value)
1. Regularly clean and wear clean, protective clothing	Yes No	11(28.9) 27(71.1)	3(16.6) 15(83.4)	8(40) 12(60)	NA	2.508 (0.133)
2. Use detergent to wash hands after going to the toilet	Yes No	28(44.4) 35(55.6)	6(33.3) 12(66.7)	11(55) 9(45)	11(44) 14(56)	1.804 (0.406)
3. Wash hands before and after handling fish	Yes* No	40(63.5) 23(36.5)	13(72.3) 5(27.7)	12(60) 8(40)	15(60) 10(40)	0.829 (0.661)
4. Cover face during coughing or sneezing and avoid spitting at work place	Yes* No	45(71.4) 18(28.6)	13(72.3) 5(27.7)	14(70) 6(30)	18(72) 7(28)	0.03 (0.985)
5. Avoid nails to grow long	Yes No	24(38.1) 39(61.9)	6(33.3) 12(66.7)	9(45) 11(55)	9(36) 16(64)	0.624 (0.732)
6. Avoid Wearing jewelry	Yes* No	22(57.9) 16(42.1)	10(55.6) 8(44.4)	12(60) 8(40)	NA	0.077 (0.782)
7. Fish handlers have a medical certificate	Yes No	0(0.0) 38(100)	0(0) 18(100)	0(0) 20(100)	NA	
8. Avoid Handling fish when you are sick	Yes* No	38(100) 0(0.0)	18(100) 0	20(100) 0	NA	
9. Avoid touch mouth, tongue, nose during working	Yes* No	24(63.2) 14(36.8)	12(66.7) 6(33.3)	12(60) 8(40)	NA	0.181 (0.671)
10. Training	Yes No	0(0.0) 38(100)	0(0) 18(100)	0(0) 20(100)	NA	

χ^2=Chi-square, P=Level of significance, Yes *= Good hygienic practice, NA= Not applicable
Note that for hygienic practice with NA; the frequency, chi square and P-value was computed out of 38 respondent which exclude consumer.

4.4. Hygienic handling practice of Participant along chain.

The current finding reveals that, hygienic handling practice of Fish along Supply chain revealed that, Out of total participant majority 55% use clean water and detergent for washing, 100% do not reheat fish indicating good hygienic handling practices. None of them perform Fish inspection. Except 68% consumer, out of all respondents, only 38.1% always dispose inedible fish part properly, besidethis . only23.8% do cook fish with appropriate heating. While significant association exist on hygienic practice activities such as using clean water and detergent, clean equipment working and serving area, dispose inedible fish part and waste properly and avoid waste material disposal in the lake with p value (≤ 0.05)

Table 8: Hygienic handling practice of fish, equipment and working place

Questionaire	Responses	Frequency (%)	Fisher Man (%)	Retail worker (%)	Consumer (%)	χ2 (P. value)
Inspect fish	Always	0	0(0)	0(0)	NA	
	Never	38(100)	18(100)	20(100)		
	S. time	0	0(0)	0(0)		
Use clean water and detergent	Always*	35(55.6)	7(38.9)	12(60)	16(64)	9.526 (0.049)
	Never	19(30.2)	7(38.9)	3(15)	9(36)	
	S. time	9(14.3)	4(22.2)	5(25)	0(0)	
Use ice during transportation	Always	0(0.00)	0(0.0)	0(0.0)	0(0.0)	
	Never	63(100)	18(100)	20(100)	25(100)	
	S. time	0(0.00)	0(0.0)	0(0.0)	0(0.0)	
Proper hand wash and equipment during working	Always	25(39.7)	6(33.3)	10(50)	9(36)	6.586 (0.159)
	Never	20(31.7)	8(44.5)	2(10)	10(40)	
	S. time	18(28.6)	4(22.2)	8(40)	6(24)	
Good fish handling, use refrigerator	Always	5(13.15)	2(11.1)	3(15)	NA	0.130 (0.937)
	Never	29(76.3)	14(77.8)	15(75)		
	S. time	4(10.52)	2(11.1)	2(10)		
Avoid cross contamination with visceral organ	Always	16(25.4)	4(22.2)	8(40)	4(16)	3.621 (0.460)
	Never	35(55.6)	10(55.6)	9(45)	16(64)	
	S. time	12(19)	4(22.2)	3(15)	5(20)	
Clean equipment working &serving area	Always	15(39.5)	4(22.2)	11(55)	0(0)	8.471 (0.014)
	Never	16(42.1)	12(66.7)	4(20)	0(0)	
	S. time	7(18.4)	2(11.1)	5(25)	0(0)	
Dispose inedible fish part and waste properly	Always	24(38.1)	3(16.6)	4(20)	17(68)	17.024 (0.002)
	Never	33(52.4)	12(66.8)	13(65)	8(32)	
	S. time	6(9.5)	3(16.6)	3(15)	0(0)	
Avoid waste material disposal in the lake	Always*	32(50.8)	2(11.1)	16(80)	14(56)	18.438 (0.000)
	S. time	31(49.2)	16(88.9)	4(20)	11(44)	
	Never	0.0	0.0	0.0	0.0	
Protect fish from contamination	Always	23(36.5)	6(33.3)	7(35)	10(40)	1.763 (0.779)
	Never	31(49.2)	8(44.5)	10(50)	13(52)	
	S. time	9(14.3)	4(22.2)	3(15)	2(8)	
Appropriately cook fish with adequate heating	Always	15(23.8)	4(22.2)	5(25)	5(20)	0.931 (0.920)
	Never	35(55.6)	9(50)	11(55)	15(60)	
	S. time	13(20.6)	5(27.8)	4(20)	5(20)	
Avoid reheating left over	Always*	63(100)	18(100)	20(100)	25(100)	
	Never	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	S. time	0(0.0)	0(0.0)	0(0.0)	0(0.0)	

χ^2=Chi-square, P=Level of significance, S. time= some time, Always*=good hygienic practices

5. DISCUSSION

Escherchia coli O 157:H7 is the most important emerging pathogen causing Hemorrhagic colitis, Hemorrhagic uremic syndrome, TTP in human. *Escherchia coli* O157:H7 controlled and prevented by ensuring food safety through maintaining sanitation and Personal hygienic practice. Over all prevalence of *Escherchia coli* O157:H7 in this finding were 2.1% out of 420 total sample. This finding is in line with the report of Aynadis and Aweke (2019), who reported 2.35% of *E. coli* O157:H7. However the finding is higher than 1.46% prevalence report of Ayalew *et al.* (2019), which might be due to agro ecological difference and inclusion of sample in different handling practices.

Occurrence of *E. coli*O157:H7 per sampling site indicated that it was higher in lake than market being 3.8% and 0.5% respectively showing significant association ($P \leq 0.05$), which agree with the report of Ayalew *et al.*, (2019), stating that the organism's prevalence was numerically higher at Lakes landing sites than retail market. The higher prevalence at the lake sites than at the market site might be due to the fact that inclusion of ready to eat (cooked) fish sample which could reduce the number organisms.

On the other hand, current finding depicted that there was no statistical association ($P > 0.05$) in prevalence of *E. coli* O157:H7 among samples collected from Lake Haramaya, Tinike, and Adelle respectively. Which agree with the finding of Ayalew *et al.* (2019). This might be due to similar agro ecology and similar environmental condition.

The current finding indicated among sample types, higher *Escherchia coli* O157:H7 isolated from filleted followed by each freshly caught samples; fresh meat, viscera and skin, stored Fish associated environmental sample, and ready to eat samples. Higher number of isolate was obtained from filleted than freshly caught samples corroborating the finding Ayalew *et al.*, (2019). However the finding do not agree with the finding of Aynadis and Aweke (2019) who reported higher prevalence in skin than fish muscle.

Occurrence of *Escherchia coli* O157:H7 by origin of sample indicate that, higher number of isolate was obtained from different fish sample, followed by selected fish part and from fish associated environmental sample respectively. This report was supported by Ayalew *et al.*, (2019), *Escherchia coli* O157:H7 were not isolated from ready to eat fish sample however, it was isolated from Fish associated environmental sample in lower magnitude. Occurrence

of this organism different fish sample indicate that likely hood of contraction from environment or fish itself.

The anti-microbial susceptibility of eight isolate was tested using seven antimicrobial; Cefoxitin, Enoxacin. Ciprofloxacin, Streptomycin, Amoxicillin and Ampicillin. Among them Except Enoxacin which showed 100% susceptibility and Nalidixic acid which revealed only 37.5% susceptibility. The rest anti-microbial such as Cefoxitin, Ampicillin, Amoxicillin-clavulanic acid, ciprofloxacin has shown 100% resistance. The finding is contrasting with the finding of Minda and Shimallis (2021) showing 100% susceptibility on ciprofloxacin, cefoxitin, amoxicillin-clavulanic, streptomycin. Resistance in the current finding might be due to low disk concentration.

The finding shows all eight isolate shows 100% resistance to Cefoxitin and Ampicillin. The finding corroborates with the finding of Aynadis and Aweke (2019) stating 100% were resistant to Cefoxitin and Ampicillin even with higher disc concentration, while it is slightly higher than the report of Ashenafi *et al.* (2020) and Nigatu *et al.* (2017), who reported 72.2% and 54.5%respectively from bovine milk, were resistant to Cefoxitin with, 30µg and lower disc concentration (5µg) respectively. This variation resulted due to; difference in disk concentration, sample source difference and difference time of the year study was conducted, which clearly suggest likely hood for development of complete resistance.

In contrast with the finding of Tizeta *et al.* (2014), who reported 71.8% total susceptibility to Nalidixic acid, 100% and 83.6% from isolate of cattle and sheep meat sample, while 40% in goat meat agree with current finding which shown 37.5% susceptibility, while 62.5% shows intermediate susceptibility. The isolate showed 100% resistance to Ciprofloxacin which is contrasted with the finding of Segni *et al.*(2018) who reported 39 (97.5%) isolate susceptible to the drug.

The current finding showed that; Ampicillin and Streptomycin were 100% resistance against the isolates this agree with the report of Ayalew *et al.* (2019) and Segni *et al.* (2018). However, it was contrasted with the report of Aynadis and Engdawork showing that only 37.5% were susceptible to streptomycin. The current finding stated that only 37.5% of isolate were found susceptible to Nalidixic acid, which is contrasting with the report of Aklilu *et al.,* (2021) and Ayalew *et al.* (2019), who respectively reported that Nalidixic acid has 100% effective and effective against most isolate.

In conclusion; occurrence of resistance may resulted due to low antibiotic disk concentration or it may be contraction of resistant isolate of other animal or human origin resulted due to cattle grazing lakeside area, and likely hood of contamination with resistant strain of human origin and other waste which might enter the lake through flood most likely during rainy season.

The current study indicated, 18(47.4%) fisherman and 20 (52.6%) Retail worker. Except for age and experience, statistically significant association were observed among sex, location, level of education and job with a (p-value < 0.05)The male were predominant 79 %(50) when compared to female which is only 13(20.6%). Being that gender of all fisherman participated were male, 85 %(17) and 60 %(15) of retail worker and consumer. This finding agree with Abdelrazig *et al.,* (2017), Akabanda *et al.,* (2017), Rabia *et al.,*(2017) being that majority of fisher man is male.

The current study reveals that female participant in fish retailing activity were only 15% of retailer which is contrasting with Esther *et al.* (2019) Grema *et al.,* (2019). Ahmed *et al.,* (2020), Lalit *et al.,* Tuglo *et al.,* (2021), where greater half majority of respondent is female This great variation might be due to the fact that most female of local area participate in child care, house wife related activity other small business activity such as chat and vegetable trade and other while only few of them participated in education.

In contrast with the finding of Hashanuzzaman *et al.,* (2020) conducted in Bangladesh, which states that majority of fish farmer (60%) were above 40 years of age, this finding states that; No participant over 40 year were participated, while majority 66.67% (12) of fisherman were ranged within 20-40 years of age and about 33.3%(6) of fisher man were below 20 years of age contradicting the report of Grema *et al,* (2019) reporting that respondent below 20 years of age were participated. However the finding agrees with the report of Hashanuzzaman *et al.,* (2020), being that majority 85% (17) of retail worker aged between 20-40 years.

Regarding Location and Educational status of participant, except consumer, which all of them were from Haramaya town and majority of them 40% (10) were attending college and university, while majority of fisher man 44.4%, 38.89% and Retail worker 45%, 35% participated were elementary and Secondery level respectively. Agree with Checkol *et al.,* (2021), which report that 40.91% had primary school. Contrasting with Abdelrazig *et al.,*

(2017) more than 50% of the interviewed food handlers attained primary education level while 17.5% of them had formal education.

Being that retail worker and consumer participated from Adele and Tinike, all consumer and retail worker participated were from Haramaya town while participant from Tinike and Adele were only 27.67% and 16.67% respectively. The dominant work experience Along Each stake holder were below 5 years constituting 77.8% and 55% of fisherman and Retail worker. While the fisherman and retail worker within the range of 6-15 years of experience were, 22.2% and 35% of fisherman and consumer respectively.

Respondent in study area have good hygienic practice since majority of participant wash hands before and after handling fish, corroborating with the report of Grema *et al.*, (2019) which states that about (91.9%) and (97.3%) fish handler wash their hands before and after handling raw fish. Similarly, majority of respondent cover face during coughing or sneezing not spitting at work place this indicate that the community have improved hygienic practice which might be expected after implementation of Covid 19 control and prevention.

The current finding indicated that majority 57.9% of respondent including 55.5% and 60% of fisher man and retail worker were not observed wearing their jewelry while handling fish. Which agree with the finding of Melese *et al.,* (2021) who reported 51.4% do not wear jewelry while working. All respondent never use ice during transportation because they do not travel long from harvesting site, using ice is not customary practice in the area. However most small retailer use Frothen fish from existing fish retailer that own refrigerator.

Regardless of their training status, medical certification, the current finding indicated good hygienic practice; that 100 % of participant do not handle when they got sick. Similarly, all of them never reheat fish left over which contrasted with report of Hashanuzzaman *et al.* (2020), who reported that (43.2%) keep left over fish for resale the next day, and some (27%) did reheat the fish before selling. Most of respondent do cook with adequate temperature however due to most consumer preference, moderately cooked fish were also common.

6. SUMMARY AND CONCLUSION

The current finding indicated that *Escherchia coli* O157:H7 isolate were higher on filleted fish, followed by selected fish part of freshly caught fish and stored fish respectively. While it was not isolated from Ready to eat fish. Filleting, evisceration and disposing inedible fish part like skin and visceral organ at lake and in the lake for feeding aquatic birds commonly practiced hence, presence of this organisms from of freshly caught fish sample might be a source of contamination for fish and lake areas. Fish in the natural water bodies and non-animal source food do not tend to antimicrobial therapy. However occurrence of resistance and intermediate susceptibility on fish sample enable us to consider likely hood of contracting the resistant strain from other non-fish source which could be either human or livestock with exposer history to organism and with prolonged history of irrational drug use living surrounding the lake area. Majority of participant in the study area revealed good hygienic practice like using clean water and detergent, avoiding reheating Fish left over, washing their hands before and after handling fish. However to ensure climax food safety, training and medical certification and other community based fish and food related training is very essential to prevent further development of *Escherchia coli* O157:H7 into other multi drug resistant strain which may not easily understood, for control and prevention. Based on the above conclusion the following recommendation was forwarded.

- Other predisposing factor for Contamination of lake and Fish with *E.coli* O157: H7 Such as Livestock grazing near the lake, Bird species should be investigated.
- All Community including should be aware on regular health checkup and risk of irrational drug use in control and prevention of food born pathogen.
- Fisher man should avoid disposing inedible fish part in the lake.
- Fish should be prepared with adequate heating.
- Prevalence per fish species and other food borne pathogens related with fish should be investigated.
- Good hygienic practice behavior could achieve maximum food security and safety as well.
- Additionally, environmental sanitation and food safety regulation will increase the future development in increment of healthy and wealth of this particular community.

7. REFERENCES

Abadias, M., Alegre, I., Oliveira, M., Altisent, R. and Viñas, I. 2012. Growth potential of *Escherichia coli* O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. *Food Control*. 27, 37–44.

Abdelrazig, A., Mustafa, K. and Mohamed, M. 2017. Hygienic Practices among Food Handlers in Restaurants of Al-nohod Locality Market-West Kordofan-Sudan. *Int J Pub Health Safe.* 2:3

Abebe, E., Gugsa, G. and Ahmed, M. 2020. Review on Major Food-Borne Zooic Bacterial Pathogens. *Journal of TropicalMedicine*. https://doi.org/10.1155/4674235 [Accessed on May 4, 2021]

Abisoye, B., Ojo S.K.S., Adeyemi, R. and Olajuyigbe, O. 2011. Bacteriological assessment of some commonly sold fishes in Lagos metropolis market Nigeria. *Prime Journal of Microbiology Research.* 1: 23-26.

Adanech Beshada Haile and Temesgen Kassa Getahun. 2018. Isolation and identification of Escherichia coli and *Edwardsiella tarda* from fish harvested for human consumption from Zeway Lake, Ethiopia. *African Journal of Microbiology Research.*12 (20) 476-480

Addis, M. and Sisay, D. 2015. A review on major food borne bacterial illnesses. *Journal of Tropical Diseases and Public Health,* 3(4): 1-5.

Adebayo, B.C., Odu, N.N., Igiwiloh, N.J. and Okonko. I.O. 2012. Microbiological and Physicochemical Level of Fresh Catfish from Different Markets in Akwa Ibom State, Nigeria. New York *Sci. J*. 5(4):46-52.

Adem Hiko, Kanani Tasisa and Getahun E. Agga. 2018. Helminthiasis and Gram Negative Enteric Bacteria in Freshwater Fish from Selected Lakes of Haramaya District, Ethiopia. *Fish Aqua J* 9 (2): 242.

Agüeria, D.A., Terni, C., Baldovino, V.M. and Civit, D. 2018. Food safety knowledge, practices and attitudes of fishery workers in Mar del Plata, Argentina. *Food Control* 91, 5–11.

Ahmed Mohammed Abdi, Abdella Amano, Abadir Abrahim, Mulusew Getehun, Sabit Ababor, Abera Kumie. 2020. Food Hygiene Practice and Associated Risk Factors Among Food Handlers Working in Food Establishment in Bole Sub City, Addis Abeba, Ethiopia. *Risk Management and Healthcare Policy* 2020:13 1861–1868

Akabanda, A., Hlortsi, E.H. and Kwarteng, J.O. 2017. Food safety knowledge, attitudes and practices of institutional food-handlers in Ghana Public Health. 17:40 DOI 10.1186/s12889-016-3986-9 (Accesed, May 24, 2022)

Aklilu Feleke Haile, Alonso, S., Nega Berhe, Tizeta Bekele Atoma, Prosper, N., Boyaka and Grace, D. 2021. *Escherichia coli* O157:H7 in Retail Lettuce (*Lactuca sativa*) in Addis Ababa City: Magnitude of Contamination and Antimicrobial Susceptibility Pattern. *Front. Microbiol*. 12:694506. doi: 10.3389/fmicb.2021.694506

Aklilu Feleke Haile, Silvia Alonso, Nega Berhe, Tizeta Bekele Atoma, Prosper N. Boyaka and Delia Grace. 2021. Escherichia coli O157:H7 in Retail Lettuce (Lactuca sativa) in Addis Ababa City: Magnitude of Contamination and Antimicrobial Susceptibility Pattern doi: 10.3389/fmicb.2021.694506.

Amagliani, G., Brandi, G. and Schiavano, G.F. 2012. Incidence and role of *Salmonella* in seafood safety. *Food Research International, 45*(2)780-788.

Amanda, L., Robinson, and John L.M.K. 2010. Biology of *Escherichia coli* O157:H7 in human health and food safety with emphasis on sub lethal injury and detection. *Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. A. Mendez-Vilas (ED) Department of Biology, Ball State University, Muncie, IN 47306 USA P1096-1105

Anonymus, 2017. *Escherchia coli* O157:H7 *Biochemical Identification Kit*. *Creative DiagNeverstic*. Ramsey Road Shirley, NY 11967, USA 45:1-3 on March 9, 2022)

Ashenafi Ababu, Dereje Endashaw and Haben Fesseha. 2020. Isolation and Antimicrobial Susceptibility Profile of *Escherichia coli* O157: H7 from Raw Milk of Dairy Cattle in Holeta District, Central Ethiopia. *Hindawi International Journal of Microbiology* Volume, Article ID 6626488, 8 pages https://doi.org/10.1155/2020/6626488.

Assefa Mitike Janko Janko, 2014. Fish Production, Consumption and Management in Ethiopia: International Journal of Economics and Environmental Management Sciences 3(9), pp. 460-466. DOI:10.4172/2162-1000183

Avery, SM., Liebana, E., Reid, CA., Woodward, MJ. and Buncic, S. 2002. Combined use of two genetic fingerprinting methods, pulsed-field gel electrophoresis and ribotyping, for characterization of *Escherichia coli* O157 isolates from food animals, retail meats, and cases of human disease. *J clin Microbiol* 40: 2806-2812

Awot Teklu, Tehetna Alemayhu, Shishay Assefa, Belayneh Getachew, Yohannes, Hagos, Tsegay Tkue, and Netsanet Berhe. 2019. Isolation and Antimicrobial Sensitivity Testing of Escherichia coli from Fish Mea Retailing Shops of Mekelle City, Ethiopia. *MEJS* 11(2): 229-238. *http://dx.doi.org/10.4314/mejs.v11i2.4*

Ayalew Assefa, Fikru Regassa, Dinka Ayana, Kebede Amenu, Fufa Abunna. 2019. Prevalence and antibiotic susceptibility pattern of *Escherichia coli* O157:H7isolated from harvested fish at Lake Hayq and Tekeze dam, Northern Ethiopia. https://doi.org/10.1016/j.heliyon.2019.

Aynadis Tilahun and Aweke Engdawork, A. (2019. Isolation, Identification and Antimicrobial Susceptibility Profile of *Escherchia coli* O157:H7 from Fish in Lake Hawassa, Southern Ethiopia. *International Journal of Veterinary Science and Technology* 3(1): 013-019.

Beatty ME., Adcock PM., Smith SW., Quinlan K, Kamimoto, L.A.2006. Epidemic diarrhea due to enterotoxigenic Escherichia coli. Clin Infect Dis 42: 329-334.

Bevilacqua, A., Corbo, M.R. and Sinigaglia, M. 2017. The Microbiological Quality of Food. Woodhead Publishing, Amsterdam. *Dis health* 11(1) 1-8

Bezuayehu Gutema and Fikadu Hailemichael. 2021. Microbial Quality of Traditionally Dried Fish Products from Selected Parts of Ethiopia. *Frontiers Environmental Microbiology.* 7 (1) 1-5. doi: 10.11648/j.fem.20210701.11

Bielaszewska, M., Idelevich, E.A. and Zhang, W. 2012. Effects of antibiotics on Shiga toxin production and bacteriophage induction by epidemic *Escherichia coli* O104: H4 strain .*Antimicrobial Agents and Chemotherapy*, vol. 56(6) 3277–3282

Bielaszewska, M., Mellmann, A., Zhang, W., Köck, R., Fruth, A. 2011. Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect Dis 11: 671-676.

Bielaszewska, M., Tarr, PI., Karch, H., Zhang, W. and Mathys W. 2005. Phenotypic and molecular analysis of tellurite resistance among Enterohemorrhagic Escherichia coli O157:H7 and sorbitol-fermenting O157: NM Clinical isolates. *Journal of clinical Microbiology*. 43:452–454.

Brook Lemma, 2003. Ecological changes in two Ethiopian lakes caused by contrasting human intervention. *Limnologica* 33: 44-53.

Brugman, S., Ikeda-Ohtsubo, W., Braber, S., Folkerts, G., Pieterse,C. M. J. and Bakker, P. 2018. A comparative review on macrobiotic manipulation. *Frontiers in Nutrition*, *5*, 80. https://doi.org/10.3389/f nut.2018.00080 [Accesed on 8 may 2021]

Carbas, B., Cardoso, L. and Coelho, AC. 2012. Investigation on the knowledge associated with foodborne diseases in consumers of north eastern Portugal. *Food Control*. 30(1):54–7.

Chauhan, R., Singh, N., Pal, G.K. and Goel, G. 2020.Trending Biocontrol Strategies Against Cronobacter Sakazakii: A Recent Updated Review. *Food Res. Int*. 137, 109385

Chekol, C., Andualem, M. and Hussien, M. 2021. Food Safety Practice and Associated Factors among Street Food Vendors in City Administrations of West Gojjam Zone, North west Ethiopia. *Austin Food Sci*. 2021; 6(2): 1046.

Clark, DP and Pazdernik NJ. 2005. *Molecular biology*. Amsterdam. Elsevier Academic Press, USA

Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards (CLSI).2015. *Performance Standards for Antimicrobial Susceptibility Testing*, Wayne, PA, USA, Tenth Edition vol. 35(3): 1–239.

Colello, R., Etcheverrı´a A.I., Conza, J.A., Gutkind, G.O. and Padola, N.L. 2015. Antibiotic resistance and integrons in Shiga toxin-producing *Escherichia coli* (STEC)" *Brazilian Journal of Microbiology*. 46:1-5

Constable, P.D., Hinchcliffe, K.W., Done, S.H., and Grundberg, W. 2017. Veterinary Medicine Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. UK. *Elsevier* pp: 1591.

Costa, R.A. 2013. *Escherichia coli* in seafood: a brief overview. *Advances in Bioscience an Biotechnlogy*, 4: 450.

Cunha, J. A., Heinzmann, B. M. and Baldisserotto, B. 2018. The effects of essential oils and their major compounds on fish bacterial pathogens. *A review. Journal of Applied Microbiology* 125(2), 328–344. https://doi.org/10.1111/jam.13911.

Dyar, O.J., Hoa, N.Q., Trung, N.V., Phuc, H.D., Larsson, M., Chuc, N.T. and Lundborg, C.S. 2012. High prevalence of antibiotic resistance in commensal *Escherichia coli* among children in rural Vietnam. *BMC Infectious Diseases,* 12(1) 12-92.https://doi.org/10.1186/ 1471-2334-12-92[Accessed on 8 February, 2021]

Esther, A.T., Taiwo, H.B. and Mumuni. 2019. Microbial Load in Bonga Shad Fish (*Ethmalosa fimbriata*), Preservation Methods and Hygiene Practices among Fishmongers in Epe Community, Journal of *Nigeria Agriculture Research and Technology: Open Access.* 20(1): DOI: 10.19080/ARTOAJ.2019.20.556112.

FAO (Food and Agricultural Organization). 2012. The state of world Fisheries and aquaculture department. Food and Agricultural organization of the United Nations, Rome.

FAO (Food and Agricultural Organization). 2012. The State of World Fisheries and Aquaculture 2012. Food and Agricultural Organization of the United Nations. Available at: http://www.fao.org/docrep/016/i272 e/i2727e.pdf [Accessed on 8 February, 2021]

FAO (Food and Agricultural Organization). 2015. Fishery and Aquaculture Country Profile. Retrieved fromhttp://www.fao.org/fishery/facp/ETH/en

FAO (Food and Agricultural Organization).2020. The state of world fisheries and aquaculture 2020. Retrieved from http://www. Fao.org/state-of-fisheries-aquaculture [Accessed on 8 February, 2021]

FAO/WHO (Food and Agricultural Organization /World Health Organization).2006. FAO/WHO guidance to governments on the application of HACCP in small and/or less-developed food businesses. Retrieved on March 7, 2015 from www.fao.org[Accessed on 8 February, 2021]

Fernandes, D.V., G.S., Castro, V.S., Neto, A.D. and Figueiredo, E.E.D. 2018.Salmonella spp. in the fish production chain*:* Areview. *Ciencia Rural,* 48(8): 0103-8478*:* [Accessed 8 February, 2021]

Fikadu Hailemichael and Bezuayehu Gutema. 2021. Assessment on the microbial safety status of fish products at fish retail areas in Addis Ababa, Ethiopia. *Journal of Entomology and Zoology Studies* 9(1): 41-46.

Fleckenstein, J.M., Bartels, S.R., Drevets, P.D., Bronze, M.S. and Drevets, D.A. 2010. Infectious agents of food - and water-borne illnesses. *American Journal of the Medical Science*. 340(3):238-46. DOI:10.1097/MAJ.0b013e3181e99893

Frehiwot Mesele and Fufa Abunna. 2019. *Escherichia coli* O157:H7 in Foods of Animal Origin and its Food Safety Implications: Review. *Advances in Biological Research* 13 (4): 134-145

Gilbert, RJ., de Louvois, J. and Donovan T. 2000. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Commun Dis Public Health* 3(3): 163-7.

Google Eearth, 2022. Location of Haramaya District and Lakes in Haramaya. https://www.google.com/earth/index.html.[accesed on July 18, 2022]

Grema, H.A., Kwaga, J.K.P., Bello, M. and Onimisi, H.U. 2019. Assessment of food hygiene knowledge, attitudes and practices of fish handlers in Kaduna state, Nigeria. *Adv. Anim. Vet. Sci.* 7(3): 131-137.

Guzman, MC., Bistoni, MA. and Tamagnini, LM. 2004. Recovery of *Escherichia coli* in fresh water fish, *Jenynsia multidentata* and *Bryconamericus iheringi*. *Water Res*. 38:2368–2374.

HABD, 2014. Haramaya Woreda Agricultural Development Berau

Haile Arefayne Shishaye. 2018. Diagnosing the Suitability of Lake Water for Domestic and Agricultural Uses: A Case Study in Eastern Ethiopia. *East African Journal of Sciences.* 12 (2) 101-110

Hashanuzzaman, M., Bhowmik, S., Shafiqur Rahman, Md., Abu Zakaria, MUM., Liton Chandra Voumik, L.C., Al Mamun, A. 2020. Assessment of food safety knowledge, attitudes and

practices of fish farmers and restaurants food handlers in Bangladesh. *Heyilon journal homepage*: www.cell.com/heliyon. https://doi.org/10.1016/j.heliyon.2020.e05485

Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N.,Bellinger, D.C., de Silva, N.R., Gargouri, N. 2015. World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. *PLoS Med*. 2015.

http://www.apexjournal.org/Nuru, A., Molla, B., Yimer, E. 2012. Occurrence and distribution of bacterial pathogens of fish in the southern gulf of Lake Tana, Bahir Dar, Ethiopia. *Livest. Res. Rur. Devel*. 24(149): 1-11.

Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M. and Akram, Q. 2020. Current trends of antimicrobials used in food animals and aquaculture. In M. Z.Hashmi (Ed.), Antibiotics and antimicrobial resistance genes in the environment. Amsterdam, the Netherlands. *Elsevier.* pp. 39–69.

International Organization for Standardization 21528 (ISO 21528). 2004. Detection and enumeration by MPN with pre-enrichment. USA. Thermoscientific.com/microbiology. 1-51

Kar, M., Hoq, M.E., Islam, M.S.,Islam, M.M., Meghla, N.T., Suravi, and Kabir, M.H. 2020. Monitoring of proximate composition, heavy metal concentrations and pesticide residues in marine fish available in the coastal region of Bangladesh. Grassroots *J. Natural Resources,* 3(2): 30-41.

Kebede, Y. 2013. Groundwater in Ethiopia, Features, Numbers and Opportunities, *Springer Hydrogeology*. Heidelberg New York Dordrecht London, *ISBN*: 978-3-642-30390-6.

Keene, W.E., Mcanulty, JM., Hoesly, FC., Williams, LP., Hedberg, K., Oxman, GL., Barret, TJ, Pfaller, MA. And Fleming, DW. 1994. A swimming-associated outbreak of haemorrhagic colitis caused by Escherichia coli 0157:H7 and Shigella sonnei. N. Engl. *J. Med*. 331 579-584.

Kessler C, Manta V .1990. Specificity of restriction endonucleases and DNA modification methyl transferases a review (Edition 3). Gene 92: 1-248.

Kim, J.H.; Jung, S.; Oh, S.W. 2020. Combination of bacteria concentration and DNA concentration for rapid detection of *E. coli* O157: H7, *L. monocytogenes*, and *S. Typhimurium* without microbial enrichment. LWT 117, 108609.

Lalit, I., Brkti, G., and Dejen, Y. 2015. Magnitude of Hygienic Practices and its associated factors of food handlers working in selectedfood and drinking establishments in Mekele town Northern Ethiopia. *International Food Research Journal journal* 22(6)2650-2656. http://www. Ifrj.upm.edu.my

Lu, Z. and Breidt, F. 2015. *Escherichia coli* O157: H7. Bacteriophage +ª241 isolated from an industrial cucumber fermentation at high acidity and salinity. Frontiers in Microbiology, 6:67. Doi 10.3389

Mahendra, P. and Mahendra. R. 2016. *Escherichia coli* O 157: H7: An emerging bacterial zoonotic food borne pathogen of global significance. *International Journal of Interdisciplinary and Multidisciplinary Studies.* 4(1):1-4.

Manafi, M. and Kremsmeir, B. (2011).Comparative evaluation of different chromogenic/fluorogenic media for detecting *E. coli* O157: H7 in food. *International Journal of Food Microbiology* 71 257-262.

Manning SD .2010.*Deadly diseases and epidemics. Escherichia coli Infections*. 2nd edn. An imprint of Info base Publishing, 132 West 31st Street New York, NY 10001, USA, Pp: 8-43.

Manyi-Loh, C., Mamphweli, S., Meyer, E., and Okoh, A. 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules 23, 795. doi: 10.3390/molecules23040795(Acessed on March,26, 2022)

Mathusa, E.C., Chen, Y., Enache, E. and Hontz, L., 2010. Nevern-O157 Shiga toxin-producin *Escherichia coli* in foods. Journal of Food Protection 73, 1721–1736.

Maysoon, S.A. 2014. Isolation of bacteria from fish. Veterinary Medicine University of Bag dad, Iraq. *International Journal of Advanced Research*, 2: 274-279.

Melese Abate Reta, Mekonnin Tesfa Lemma, Ashete Adere Gemeda, Getasew Assefa Lemlem. 2021. Food handling practices and associated factors among food handlers working in public food and drink service establishments in Woldia town, Northeast Ethiopia. Pan African Medical Journal. https://www.panafrican-med-journal.com//content/article/40/128/full

Mersha, G., Asrat, D., Zewde, B.M., Kyule, M. 2010. Occurrence of *Escherichia coli* O157:H7 in faces, skin and carcasses from sheep and goats in Ethiopia. *Lett Appl Microbiol* 50(1): 71-6.

Minda Asfaw Geresu and Shimelis Regassa. 2021. *Escherichia coli* O157: H7 from Food of Animal Origin in Arsi: Occurrence at Catering Establishments and Antimicrobial Susceptibility Profile. *Hindawi the Scientific World Journal* https://doi.org/10.1155/2021/6631860

Muleta, S., Yohannes, F. and Rashid, S. 2006. Soil erosion assessment of Lake Haramaya catchment, Ethiopia. *LDD*, 17, 333–341

Müller, EE and Ehlers, MM. 2005. *Biolog identification of non-sorbitol fermenting bacteria isolated on E. coli O157 selective* CT-SMAC agar. Department of Medical Virology, PO Box 2034,

University of Pretoria/National Health Laboratory Services, Pretoria 0001, South Africa ISSN 0378-4738 = Water SA Vol. 31 No. 2 http://www.wrc.org.za

Nhung, N., Cuong, N., Campbell, J., Hoa, N., Bryant, J., Truc, V., Kiet, B., Jombart, T., Trung, N., Hien, V. 2015. High levels of antimicrobial resistance among *Escherichia coli* isolates from livestock farms and synanthropic rats and shrews in the Mekong Delta of Vietnam. *Applied and Environmental Microbiology*, 81(3), 812-820.

Nigatu Disassa, Berhanu Sibhat, Shimelis Mengistu, YimerMuktar, and Dinaol Belina. 2017. Prevalence and Antimicrobial Susceptibility Pattern of *E. coli*O157:H7 Isolated from Traditionally Marketed Raw Cow Milk in and around Asosa Town, Western Ethiopia. *Hindawi Veterinary Medicine International Volume*. https://doi.org/10.1155/2017/7581531

Niway Tadesse, Bheemalingeswara, K. and Mohammed Abdulaziz. 2010. Hydrogeological Investigation and Groundwater Potential Assessment in Haromaya Watershed, Eastern Ethiopia. *Momona Ethiopian Journal of Science*, 2(1): 26-48.

Oliveira, M., Ferreira, V., Magalhães, R. and Teixeira, P. 2018. Biocontrol strategies for Mediterranean-style fermented sausages. *Food Res. Int.* 103, 438–449.

Oliveira, M., Usall, J., Solsona, C., Alegre, I., Viñas, I. and Abadias, M. 2010. Effects of packaging type and storage temperature on the growth of foodborne pathogens on shredded 'Romaine' lettuce. *Food Microbiol.* 27, 375–380.

Park. J.Y., Lim. M.C., Park. K., Gyeongsik. O.K., Chang. H.J., Lee. N., Park. T.J., and Choi. SW. 2020. Detection of E. coli O157:H7 in Food Using Automated Immunomagnetic Separation Combined with Real-Time PCR. *Processes*. 8: 908

Posada-Izquierdo, G.D., Pérez-Rodríguez, F., López-Gálvez, F., Allende, A., Selma, M.V., Gil, M.I. and Zurera, G. 2013. Modelling growth of *Escherichia coli* O157:H7 in fresh-cut lettuce submitted to commercial process conditions: Chlorine washing and modified atmosphere packaging. *Food Microbiol*. 33: 131–138.

Puligundla, P. and Lim, S. 2022. Biocontrol Approaches against *Escherichia coli* O157:H7 in Foods. *Foods*. 11: 756. https://doi.org/ 10.3390/foods11050756 [Accessed on April 4, 2021]

Quinn, P.J., Carter, M., Markey, B., and Carter, G. 2002. Clinical Veterinary Microbiology, Elsevier Health Sciences, London, UK,

Rabia, A.R., Kimera, S. I. Wambura, P. N. Mdegela, R. H. Misinzo, G., Mbazi, E. ,Ngaya, R. and Mzula, A. 2017 .Knowledge, Attitude and Practices on Handling, Processing and Consumption of Marine Foods in Zanzibar, Tanzania Journal of Education, Society and Behavioural Science 22(1): 1-11 ISSN: 2278-0998

Radhika, R. 2018. A seasonal study on proximate composition of major food fishes of Cochin Back water, (CBW) of Kerala, India. *Int. J. Adv. Sci. Res. Manag.,* 3 (8): 229-236.

Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., Hebert, R. J., Olcott, E. S., Johnson, L. M., Hargrett, N. T., Blake, P. A., and Cohen, M. L. 1983. Hemorrhagic colitis associated with a rare Escherichia coli serotype. N. Engl. J. Med.308, 681–685.

Saldaña, G., Monfort, S., Condón, S., Raso, J. and Álvarez, I. 2012. Effect of temperature, pH and presence of nisin on inactivation of *Salmonella typhimurium* and *Escherichia coli* O157: H7 by pulsed electric fields. *Food Res*. *Int*. 45, 1080–1086.

Samuel Chane Teferi. 2020. A Review on Food Hygiene Knowledge, Practice and Food Safety in Ethiopia. *Scientific Journal of Food Science and Nutrition.* 2020; 6(1): 004-010.

Samuel, L., Marian, Apun, M.M., Kasing, Mauris, L. and Radu, S. 2011. Characterization of Escherichia coli isolated from cultured catfish by antibiotic resistance and RAPD analysis. *International Food Research Journal* 18(3): 971-976

Saxena, T., Kaushik, P. and Krishna, M. 2015. Prevalence of *E. coli* O157: H7 in water sources: An overview on associated diseases, outbreaks and detection methods, *Diagnostic Microbiology and Infectious Disease*. 82: 249–264.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L. and Griffin, P. M. 2011. Foodborne illness acquired in the United States Major pathogens. *Emerging Infectious Diseases.*17 (1): 7–15. doi:10.3201/eid1701.P11101

Scheutz F, Teel LD and Beutin L. 2012. Multicenter evaluation of asequence-based protocol for subtyping shiga toxins and standardizing Stx nomenclature. *Journal of clinical Microbiolog*. 50:2951–2963

Segni Bedasa, Daniel Shiferaw, Ashebr Abraha and Tesfanesh Moges. 2018. Occurrence and antimicrobial susceptibility profile of Escherichia coli O157:H7 from food of animal origin in Bishoftu town, Central Ethiopia. *International Journal of Food Contamination* 5:2-8. DOI 10.1186/s40550-018-0064-3

Setegn, S., Chowdary, V.M., Mal, B.C., Yohannes, F. and Kono, Y. 2011. Water Balance Study and Irrigation Strategies for Sustainable Management of a Tropical Ethiopian Lake: A Case Study of Lake Haramaya. *Water Resources Management,* 25: 2081-2107.

Sharma, H. and Mutharasan. 2013. R. Review of biosensors for foodborne pathogens and toxins. *Sensors Actuat B Chem*.183, 535–549.

Sheng, L., Wang, L. 2021. The microbial safety of fish and fish products: Recentadvances in understanding its significance, contamination sources, and control strategies. *Compr Rev Food Sci Food Saf*. 20:738–786.

Shimels Tesfaye, Misaw Kasye, Mersha Chane, Baseazinew Bogale and Zewudie Abebe. 2018. Preliminary Survey of Gram-Negative Bacterial Pathogens from Commonly Caught Fish Species in Lake Hayiq, *Ethiopia Fisheries and Aquaculture Journal*. 9(1):1-7. DOI: 10.4172/2150-3508.1000238

Silva, G.L., Anduro, G.G., Garza, Z.J. and Valle, A.F. 2009. *Food safety Issues and the microbiology of fish and shellfish*. Hoboken, N.J: John Wiley and Sons.

Soliman, M., Khalil, R., Saad, T, El-Gamal, M. and Gebril, A. 2010. Isolation and identification of *E. coli* from cultured freshwater fish. *Journal of Arabic and Aquatic Science* 5:8.

Tchaptchet, S., and Hansen, J. 2011. The Yin and Yang of host-commensal mutualism. *Gut Microbes* 2, 347–352.

Teka, W., Neverlkes, D., Getachew, Y., Mulachew, M. 2017. Microbiological quality of frozen raw and undercooked Nile tilapia (*Oreochromis niloticus*) fillets and food safety practices of fish handlers in Arbaminch town. *Ethiopia. Journal of veterinary medicine and animal Health*. http://www.academicjournals.org

Tesfaye G. and Wolf, M. 2014. The state of inland fisheries in Ethiopia: A synopsis with updated estimates of potential yield. *Ecohydrology and hydrobiology* 14: 200-219

Tesfaye, L.B., Tatek K. F., Kaleab. S. A., Firehiwot A. D., Samson G. G., Waktole G. S., Tigist Y. N. and Yosef. B. 2020. Microbiological and Public Health Status of Cooked Meat and Fish in Ethiopia. *The Open Microbiology Journal* 1874-2858

Thrusfield, M. 2005. *Veterinary Epidemiology*. London: Blackwell Science Ltd. (3):223-250.

Tizeta Bekele, Girma Zewde, Genene Tefera, Aklilu Feleke and Kaleab Zerom. 2014. *Escherichia coli* O157:H7 in Raw Meat in Addis Ababa, Ethiopia: Prevalence at an Abattoir and Retailers and Antimicrobial Susceptibility. *International Journal of Food Contamination* 1:4. Doi: 10.1186/s40550-014-0004-9 (Accesed, May 24, 2022)

Tuglo, L.T., Agordoh, P.D., Tekpor, D., Pan, Z., Gabriel Agbanyo, G. and Chu, M. 2021. Food safety knowledge, attitude, and hygiene practices of street-cooked food handlers in North Dayi District, Ghana. *Environmental Health and Preventive Medicine.* 25:54(2-13) https://doi.org/10.1186/s12199-021-00975-9

Tut, G. 2020. Assesment of Factors Affecting Fish Production and Marketing in Gambella Region, Ethiopia. *Hindawi the Scientific World Journal.* Volume 2020, Article ID 5260693, 8 pages https://doi.org/10.1155/2020/5260693 [Accessed on 8 February, 2021].

Tut, G., Wakjira, M., Tamire, G. 2019. Diversity, length-weight relationship and condition factor of fishes in Gilo River and its nearby wetlands in Akobo district, gambella region, Ethiopia, Journal *of FisheriesSciences*, vol. 13, Never. 1, pp. 019–028.

United State Environmental Protection Agency (USEPA). 2012. Water: Basic Information about Regulated Drinking Water Contaminants; Basic Information about E. coli O157:H7 in Drinking WaterAvailable:http://water.epa.gov/drink/contaminants/basicinformation/ecoli.cfm

World Fish Center. 2009. Fish Supply and Food Security for Africa. Malaysia, July, 2009.

Yagoub, S.O. 2009. Isolation of *Enterobacteriaceae* and *pseudomonas* spp. from raw fish sold in fish market in Khartoum state. *J. Bacteriol. Res* 1: 085-088.

Ye, L., Lu, Z., Li, X., Shi, L., Huang, Y. and Wang, H.H. 2013. Antibiotic resistant bacteria associated with retail aquaculture products from Guangzhou, China. *J. Food Prot.,* 76: 295-301.

Yilma, S., Busse. H., Desta. D.T. and Alemayehu. F.R. 2020. Fish Consumption, Dietary Diversity and Nutritional Status of Reproductive Age Women of Fishing and non-Fishing Households in Hawassa, Ethiopia: Comparative Cross Sectional Study. *Journal of Frontiers in Science.* 10(1): 7-13 DOI: 10.5923/j.fs.20201001.02

Yoder, S.F., Henning, W.R., Mills, E.W., Doores, S., Ostiguy, N. and Cutter, C.N. 2012. Investigation of chemical rinses suitable for very small meat plants to reduce pathogens on beef surfaces. *J. Food Protect.* 75, 14–21

Yokoyama, E., Hirai, S., Ishige, T. and Murakami, S. 2018. Application of whole genome sequence data in analyzing the molecular epidemiology of Shiga toxin-producing *Escherichia coli* O157:H7/H-, *International Journal of Food Microbiology*. 264: 39–45.

8. APPENDICES

Appendix 1: CHEMICAL, REAGENTS AND LABORATORY PROCEDURE FOR ISOLATION AND ANTIBIOTIC SUSCEPTIBILITY TEST

A. Composition and preparation of Medias used for transportation and isolation of *E. coli* O157:H7 and antimicrobial susceptibility test

1. Buffered peptone water (HIMEDIA, India)

Composition Gms/Litre

Proteose peptone 10.000, Sodium chloride 5.000, Disodium phosphate, anhydrous 3.500, Potassium hydrogen phosphate 1.500, Final pH (at 25°C) 7.2±0.2

Preparation: Suspend 20.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amounts into tubes or flasks or as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. If desired aseptically add rehydrated contents of one vial of EC O157:H7 Selective Supplement (FD247) to 1000 ml of medium for enrichment of *Escherichia coli* O157:H7.

2. **Nutrient Agar (OXOID CM3)**

Composition: beef extract 1.0 g, Yeast extract 2.0 g, Peptone 5.0 g, NaCl 5.0 g,Agar 15.0 g,

Preparation: suspended 28g in 1liter of distilled water. Boil to dissolve completely. Sterilize by autoclaving at 121 ^{O}C for 15 minutes.

3. MacConkey Agar MH081 (OXOID)

Composition: in g/l

peptone # 17.000, HMC peptone ## 3.000, Lactose monohydrate 10.000, Sodium chloride 5.000, Bile salts 1.500, Neutral red 0.030, Crystal violet 0.001, Agar 13.500, pH after sterilization (at 25°C) 7.1±0.2

Preparation: Suspend 49.53 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Boil for 1minute with constant stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Avoid overheating. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. The surface of the medium should be dry when inoculated.

4. Eosin Methylene Blue (EMB) (Oxoid, ® Hampshire, England)

Composition (gm /liter): Peptone 1 0 .00 g, Lactose 5. 0 0 g, Dipotassiummono hydrogen phosphate 2. 00 g, Mthylene blue 0. 06 g, Eosin Y 0.04 g, Agar 13.50 g,PH at 25^{O}C 7.1 ± 0.2

5. Sorbitol Macconkey Agar (Oxoid), Cefixime and Potassium Tellurite Supplement

Composition: grams per litre

Peptone 20.g, Sorbitol 10.g,Bile salts No.3 1.5 g,Sodium chloride 5.g, Neutral red 0.03 g,Crystal violet 0.001 g, Agar 15.0g,Cefixime and potassium tellurite Supplement: Potassium tellurite 2.5mg/L Cefixime 0.05mg/L

Preparation of Cefixime stock solution

Add 7.5 ml of sterile distilled water to the vial of Cefixime. Dissolve completely and gently vortex to ensure complete mixing. Then filter it using 0.02 µm pore sized polyether sulfone (PES) syringe filter paper suck up the filtered cifixime.

Preparation of Potassium Tellurite stock solution

Add 7.5 ml of sterile distilled water to the vial containing Potassium tellurite. Dissolve completely and gently vortex to ensure complete mixing. Then filter it 0.02 µm pore sized using polyethersulfone (PES) syringe filter paper suck up the filtered cifixime.

Preparation of CT-SMAC media

Preparation: suspend Sorbitol MacConkey Agar (51.5 grams / litre) in de-ionised water. Sterilise at 121°C for 15 minutes. Cool and aseptically add cefixime tellurite supplement as above, mix. Aseptically dispense into Petri dishes. Label dishes, wrap and label pack. After autoclaving, cool to 55°C Add 375 µl of the filtered cefixime stock solution (giving a final concentration of 0.05 mg/l). Add 375 µl of filtered potassium tellurite stock solution (giving a final concentration of 2.5 mg/l).Aseptically pour into petri-plates, cool and store at 4°C.

Principle CT-SMAC media

Cefixime inhibits *Proteus* spp. and tellurite inhibits non-O157 *E. coli* and other organisms, thus improving the selectivity of SMAC-CT for *E. coli* O157:H7. Bile salts and crystal violet, which inhibit gram-positive bacteria, especially enterococci and staphylococci, are also included. Differentiation of enteric microorganisms is achieved by the combination of sorbitol and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate sorbitol

McFarland turbidity Standards

Composition: 1.17% BaCl.H2O solution and 0.36N of 1% sulfuric acid (H2SO4)

Preparation: Add approximately 85 ml of 1% H2SO4 to 100ml of volumetric flask. Using a 0.5ml pipette add 0.5ml of 1.1% BaCl.H2O drop wise to the H2SO4 while constantly the flask. Bring to 100ml with 1% H2SO4. Place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible clumps. Dispense three to seven ml, cub tightly and seal with paraffin and keep at dark and room temperature.

B. Composition and preparation of Media used for Biochemical and Serological test

Tryptophan medium (OXOID)

Composition Gms/Litre

Proteose peptone 10.000, Sodium chloride 5.000, Disodium phosphate, anhydrous 3.500, Potassium hydrogen phosphate 1.500, Final pH (at 25°C) 7.2±0.2. Preparation: Suspend 20.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amounts into tubes or flasks or as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

MR-VP Medium (Glucose Phosphate Broth) M070 (HIMEDIA)

Composition (Gms / Litre): Buffered peptone 7.g, Dextrose 5.g, Dipotassium phosphate 5.g, Final pH (at 25°C) 6.9±0.2. Composition: Suspend 17 grams in 1000 ml of distilled water. Heat if necessary to dissolve the medium completely. Distribute in test tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Simmon citrate (OXOID)

Composition: Ingredients Gms / Litre, Magnesium sulphate 0.200, Ammonium dihydrogen phosphate 1.000, Dipotassium phosphate 1.000, Sodium citrate 2.000, Sodium chloride 5.000, Bromothymol blue 0.080, Agar 15.000. Final pH (at 25°C) 6.8±0.2. Prepparation: Suspend 24.28 grams in 1000 ml purified/ distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Precaution: Before using water, ensure pH of water is 6.5 to 7.0.Initial colour of the medium may deviate from expected colour, if the above precaution is ignored.

Triple Sugar Iron Agar (OXOID)

Composition. Per Liter: Beef Extract3.0 g, Yeast Extract 3.0 g, Pancreatic Digest of Casein15.0 g, Peptone 5.0 g, Dextrose 1.0 g, Lactose 10.0 g, Sucrose10.0 g, Ferrous Sulfate 0.2 g, Sodium Chloride 5.0 g, Sodium Thiosulfate 0.3 g, Agar12.0 g, Phenol Red 24.0 mg

Preparation: Suspend 65 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Dispense into tubes and autoclave at 121°C for 15 minutes. Cool in a slanted position so that deep butts are formed. Test samples of the finished product for performance using stable, typical control cultures.

2.5 Composition of *Escherchia coli* O157:H7 Latex kit

1. *Escherchia coli* O157 test latex (green cap) 4.0 ml: one dropper vial containing latex particle coated with E. coli O157 pooled rabbit anti body, suspended in solution with preservative.

2. *E. coli* H7 Test latex (blue cap) 4.0 ml: one dropper vial containing latex particle coated with *E. coli* H7 pooled rabbit anti body, suspended in solution with preservative.

3. *E. coli* control latex (neutral cap) 4.0 ml: one dropper vial containing latex particle coated with pooled rabbit normal rabbit globulin, suspended in buffered solution with 0.1% sodium azide

4. *E. coli* O157: H7 positive control (red cap) 4.0 ml: one dropper vial containing latex particle containing formalin-killed cell suspension of *E. coli* O157:H7 in buffered saline.

5. *E. coli* (not O157: H7) negative control (yellow cap) 3.0 ml: one dropper vial containing a formalin-killed cells suspension of non-toxigenic E. coli in buffered saline

6. Plastic string sticks (2 vials) 7. Disposable slides (35)

C. Principle of Biochemical and Serological Tests Used to Isolate *Escherchia 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 Methyl red and Voges proskeur test

Methyl Red and Voges- Proskauer test are among the two various tests used in the biochemical identification of bacterial species. MR - positive organisms continue to produce acids, resulting in a low pH (acidic) that overcomes the phosphate buffering system and maintains an acidic environment in the medium (pH 4.2 or less). MR-negative organisms further metabolize the initial fermentation products by decarboxylation to produce neutral acetyl methylcarbinol (acetoin), which results in decreased acidity in the medium and raises the pH towards neutrality (pH 6.0 or above)

2.3 Citrate Utilization Test principle

These media are used for the differentiation between Enterobacteriaceae and the members of aerogenes group on the basis of citrate utilization as sole carbon source. Initially the citrate

medium was developed by Koser containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating Escherichia coli and Enterobacter aerogenes

2.4 Sugar fermentation test procedures and principle

Preparation: Suspend 65 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Dispense into tubes and autoclave at 121°C for 15 minutes. Cool in a slanted position so that deep butts are formed. Test samples of the finished product for performance using stable, typical control cultures.

Procedure: stab the center of the TSI 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

Principle of Latex Kit for *Escherchia coli*

RIM ^{TM} E. coli O157:H7 Latex test includes three latex reagents. The particle in each reagent are coated with a different anti body: one against *E. coli* serotype O157, another against *E. coli* serotype H7, and the third with normal rabbit globulin, to serve as control latex. When test latex particles are mixed with fresh colonies of O157 and/ or H7 strain of *E. coli*, an immunochemical reaction occurs, resulting in agglutination. No agglutination indicates the test isolate is not E. coli O157:H7. The control latex reagent identifies non-specific agglutination.

D: Antibiotic sensitivity test procedure and decision criteria for *Escherchia 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:

1. Obtain culture broths of the test bacteria.
2. 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.
3. 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.
4. Replace the lid. Discard the swab. Label the plate. Allow the plates to dry for 2-5 minutes.
5. 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.
6. Repeat the alcohol-flame sterilization of the forceps and tap each disc gently onto the plate.
7. Replace the lid, and invert the plate. Complete the label at the bottom of plates and incubate at 37°C for 24hrs.
8. Record the results by measuring the diameters of the zone of inhibition (ZOI).
9. The data is recorded and interpreted using CLSI manual for the (CLSI, 2015). The interpretation criteria is summarized in CLIS guide line

Appendix 2 Different pictures of Fish sample, storage, processing practice, Colonial morphology and Biochemical profile of *Escherchia coli* O157

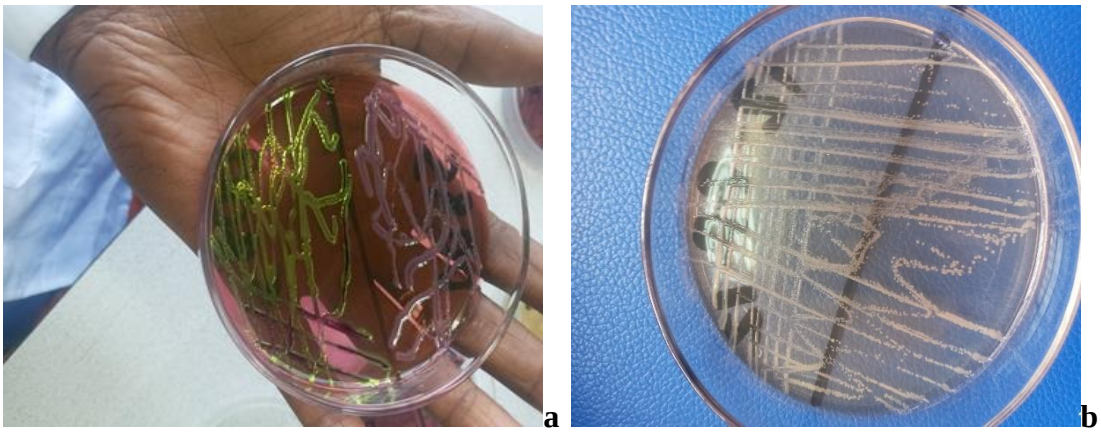
Figure 2: Types of Fish sample included in study

a. Retailed fish RTE; **b.** Frozen fish; **c.** Freshly caught fish sample

Figure 3: Stored Fish, filleting and gutting practice at Lake's environment

a. Frothen fish **b.** filleting and gutting practice at Lake's Environment.

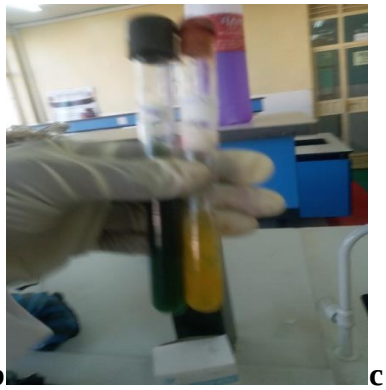
Figure 4: Colony characteristics *Escherchia coli* O157:H7



a. Golden metallic sheen on EMB. **b.** Sorbitol non fermenting colony on SMAC agar

Figure 5: Biochemical characteristics of *Escherchia coli*O157:H7


a

b

c

a. Indole positive, **b.** MR positive VP negative, **c.** Citrate negetive and TSI

Figure 6: Antimicrobial susceptibility test


a


b

Figure 7: Latex agglutination test and Kit for *Escherchia coli* O157:H7


a

b

a. *Escherchia coli* O157:H7 Latex kit. **b**. Latex agglutination test card

Appendix 3. QUESTIONARE AND OBSERVATIONAL ASSESMENT ON HYGIENIC PRACTICE ALONG FISH SUPPLY CHAIN

Dear Participant My name is ABAS MOHAMMED HASSEN and I am MSC Student in Veterinary public Health at HARAMAYA UNIVERSITY. I need your willingness to participate in my questionaire survey. It may take 20 minute

Are you willing to participate? Yes_____ No_____

A. Demographic information

Date: _____
Location: _____
Respondent's Code_____

Respondent type A= Fisherman B= Restaurant/retail worker C= Consumer

1. AGE A-Less than 20 B-20-40 C-Over 40

2 SEX A-Male B-Female

3.Level of education A-Illiterate B-Primary C-Secondary D-Collage &University

4. Experience A< 5 yearB- 6-15 year C- over 15 year

5. Training A- Trained B- trained

6. Job A- Fisher man B-Retail/restaurant worker C- Consumer

B. Questionaire on hygienic handling practice assessment along Supply chain

1. Do you inspect fish? A=Always B=Never C= some time

2. Use clean water and detergent A= Always B=Never C= some time

3. Store raw fishes in clean equipment with ice during transportation? A= B=Never C= some time

4. Proper hand wash and equipment during working A= Always B=Never C= some time

5. Proper fish handling and use refrigerator. A= Always B=Never C= some time

6. A void cross contamination with visceral organ while filleting and evisceration? A= Always B=Never C= some time

7. Clean surface and equipment cleaning using detergent. A= Always B=Never C= some time

8. Dispose inedible fish part and waste properly A= Always B=Never C= some time

9. Avoid waste material disposal in the lake. A= Always B=Never C= Some time

10. Protect fish from contamination; store old and new stock separately? A= Always B=Never C= some time

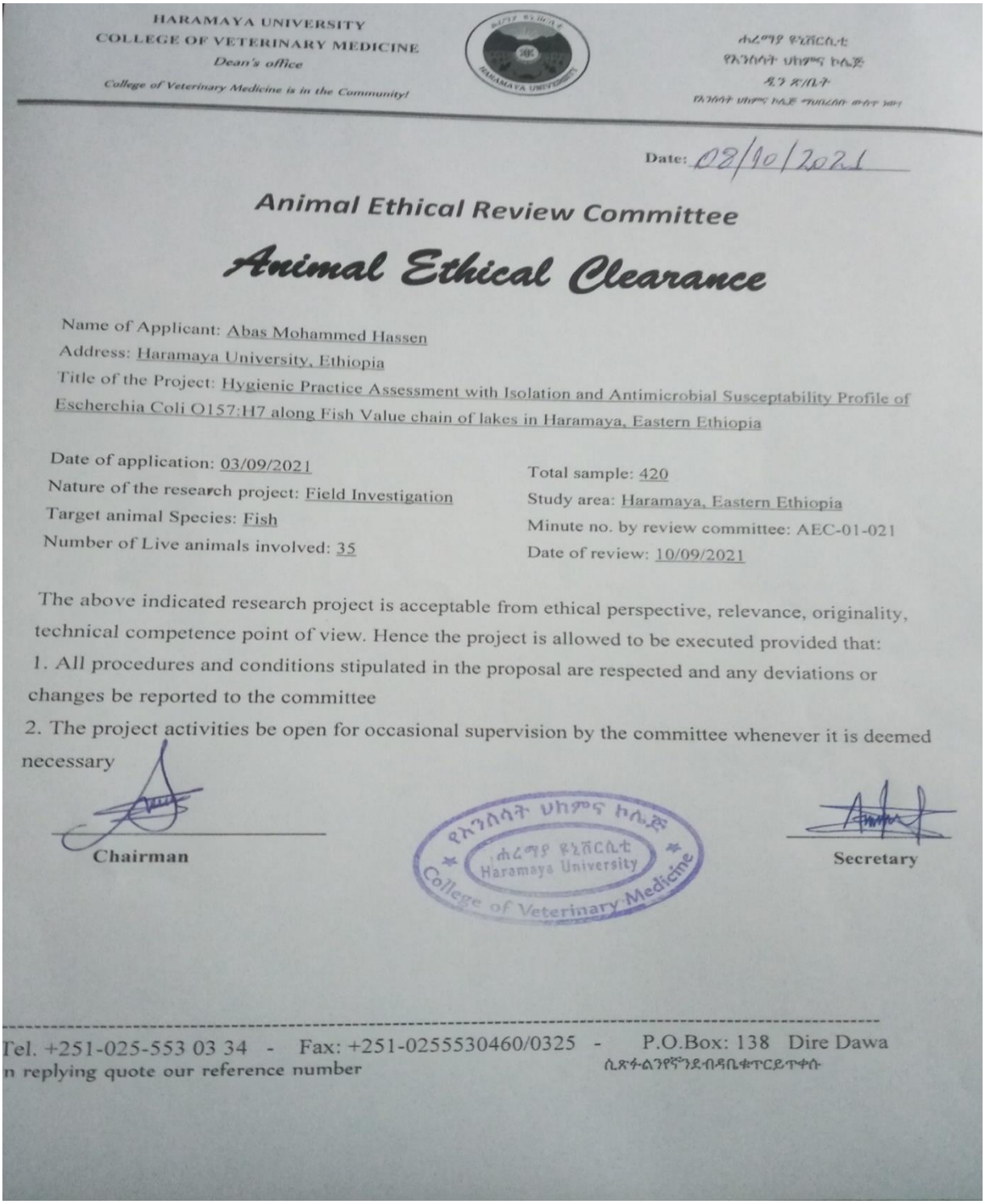
11. Appropriately cook fish with adequate heating A= Always B=Never C= some time

12. Avoid reheating fish left over? A= Always B=Never C= Sometime

C. Personal Hygienic Practice assessment along Supply chain

1. Regularly clean and Wear clean, protective clothing. Yes _____No_____
2. Use detergent to wash hands after going to the toilet. Yes _____No_____
3. Wash hands before and after handling fish. Yes _____No_____
4. Avoid Spitting; at work place and cover face during coughing / sneezing
5. Avoid finger nails to grow long. Yes_____ No_____
6. Avoid Wearing jewelry during work. Yes_____ No_____
7. Fish handlers have a medical certificate. Yes_____ No_____
8. Avoid handling fish when you are sick. Yes_____ No_____
9. Avoid Touch mouth, tongue, and Neverse during working. Yes_____ No_____
10. Training Yes_____ No_____

Appendix 4:Ethical clearance Approval for Involvement of Live Fish.



HARAMAYA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE
Dean's office
College of Veterinary Medicine is in the Community!

ሐረማያ ዩኒቨርሲቲ
የእንስሳት ህክምና ኮሌጅ
ዲን ጽ/ቤት
የእንስሳት ህክምና ኮሌጅ ማህበረሰብ ውስጥ ነው!

Date: 03/10/2021

Animal Ethical Review Committee

Animal Ethical Clearance

Name of Applicant: Abas Mohammed Hassen
Address: Haramaya University, Ethiopia
Title of the Project: Hygienic Practice Assessment with Isolation and Antimicrobial Susceptability Profile of Escherchia Coli O157:H7 along Fish Value chain of lakes in Haramaya, Eastern Ethiopia

Date of application: 03/09/2021
Nature of the research project: Field Investigation
Target animal Species: Fish
Number of Live animals involved: 35

Total sample: 420
Study area: Haramaya, Eastern Ethiopia
Minute no. by review committee: AEC-01-021
Date of review: 10/09/2021

The above indicated research project is acceptable from ethical perspective, relevance, originality, technical competence point of view. Hence the project is allowed to be executed provided that:
1. All procedures and conditions stipulated in the proposal are respected and any deviations or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee whenever it is deemed necessary

Chairman

የእንስሳት ህክምና ኮሌጅ ሐረማያ ዩኒቨርሲቲ Haramaya University College of Veterinary Medicine

Secretary

Tel. +251-025-553 03 34 - Fax: +251-0255530460/0325 - P.O.Box: 138 Dire Dawa
In replying quote our reference number
ሲጽፉልንየኛንደብዳቤቁጥርይጥቀሱ

Figure 8: Animal Ethical clearance certificate