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BY

MIKIAS JUFAR KEDIDA

ASSESSMENT OF BACTERIOLOGICAL QUALITY OF FISH AND FISH PRODUCTS AND KNOWLEDGE, ATTITUDE AND PRACTICE OF FISH HANDLERS IN BATU AND KOKA, OROMIA, ETHIOPIA

JUNE, 2023

BISHOFTU, ETHIOPIA

ASSESSMENT OF BACTERIOLOGICAL QUALITY OF FISH AND FISH PRODUCTS AND KNOWLEDGE ATTITUDE AND PRACTICE OF FISH HANDLERS IN BATU AND KOKA, OROMIA, ETHIOPIA

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

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STATEMENT OF THE AUTHOR

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ABBREVIATIONS

APHA	American Public Health Association
BPW	Buffered Peptone Water
CFS	Center for Food Safety Organization
CFU	Colony Forming Unit
EMB	Eosine Methylene Blue
FAO	Food and Agricultural Organization
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis of Critical Control Points
ISO	International Organization for Standardization
KAP	Knowledge, Attitude and Practice
LIA	Lysine Iron Agar
PCA	Plate Count Agar
SIM	Sulfide Indole Motility
SSOP	Sanitation Standard Operating Procedure
TCC	Total Coliform Count
TPC	Total plate Count
TSI	Triple Sugar Iron
TVBC	Total Viable Bacterial Count
XLD	Xylose-Lysine Deoxycholate

ABSTRACT

Fish and fishery product contribute to 17% of the global animal protein intake, but its contamination can cause food borne infections and constitute a risk to the public health. Across sectional study design was conducted comprising of field survey and laboratory analysis of fish samples from December 2022 to June 2023 in Koka and Batu towns. The aim of the study was to determine the bacterial load, detect common food borne pathogenic bacteria as well as to assess food safety knowledge, attitude and hygienic practices of fish and fish products. A total of 130 fish products were collected randomly from cooked (n= 20), frozen (n= 15), and fresh fish fillets (n= 65) as well as swab samples from hands, knife and filleting table (n=30). All collected samples were transported and analyzed at Aklilu Lemma Institute of Pathobiology Medical Microbiology laboratory of Addis Ababa University. Salmonella, *E. coli*, and *S. aureus* were detected by selective media and biochemical tests. Total viable bacterial count was assessed by using Plate Count Agar, whereas total Coliform count was determined by Violet Red Bile agar. The data were analyzed using STATA version 14 and descriptive statistics, Chi squared and one way ANOVA were employed to generate required information. The overall prevalence of *E. coli, S. aureus* and Salmonella were 32.31%, 16.9% and 3.1% respectively. The mean of TPC from cooked, frozen and fresh fillets were 4.54, 5.91 and 7.55 log cfu/g respectively. The mean of TCC from cooked, frozen and fresh fillet were 3.67, 5.02 and 6.1 logcfu/g respectively. The mean of TPC and TCC showed above center for food safety standard level. The survey study revealed that 23.75%, 38.75%, and 51.25% of respondents had poor knowledge, negative attitudes, and poor hygienic practices of fish and fish products respectively.Similarly62.5% and 68.75% of participants had negative attitudes regarding consumption of raw and inadequately cooked fish, implying the potential health risk to consumers. Generally, the study showed detection of pathogenic bacteria and unacceptable bacterial load with unhygienic handling and processing of fish. Education and awareness creation regarding hygienic practice and risk of food borne disease is essential.

Keywords: *Bacterial load, E. coli, Fish product, KAP, Salmonella, S. aureus*

1. INTRODUCTION

Fish and fish product contribute to around 17% of the global animal protein intake and worldwide expansion of human population has resulted in increases the demand for fish production (Scallan *et al*., 2011). Aquatic food sources provide a significant contribution to the global food supply, thereby advancing human nutrition, health, and wellbeing in light of the fact that over 70% of the planet is encompassed by water. Despite the low per capita consumption of fish in developing countries, it provides 180 kilocalories per day in few countries with a developed fish preference (Arfatahery *et al.*, 2015). Fish is nutritionally rich food item, possessing excellent nutritive properties due to its abundance in proteins, vitamins, and unsaturated fatty acids Omega 3 as well as highly perishable. It is imperative to uphold appropriate hygienic measures during catching, handling, processing, vending and consuming perishable goods (Khalifa *et al.*, 2014 and Sorsa *et al.*, 2019). Ethiopia has number of beautiful lakes, reservoirs and small water bodies that distributed throughout the countries which makes comfortable for fish production (Tesfaye *et al*., 2014).

Food safety is essential in order to improve the community health. In spite of considerable advancements in food safety knowledge and techniques, the act of ingested contaminated food continues to be a leading cause for food borne diseases. The handling procedures for fish that includes catching, filleting, and processing for human consumption may present potential safety risks (Issue *et al.*, 2015).The contamination of fish from domestic, industrial, and agricultural wastes in their natural habitat has emerged as a crucial issue. Aquatic organisms inclusive of fish are susceptible to numerous environmental hazards (Evans *et al*., 2009).Depending on the patient's interaction with fish and the surrounding environment, their eating habits, and the status of their immune system, human infections caused by pathogens transmitted from fish or the aquatic environment are relatively frequent. The presence of pathogenic bacteria that impact both fish and human populations can be observed in fish samples, despite the absence of any visible indication of disease (Wodajo, 2020).

According to annual reports, the USA exclusively has documented 48 million cases of illnesses caused by consumption of contaminated food, resulting in 128000 hospitalizations and 3000 fatalities. The results from the analysis indicate that fish accounted for the highest proportion (17%) of food borne outbreaks (Scallan *et al.*, 2011).Bacteria are the primary causative agents for food borne infections that develop from fish. Several bacterial pathogens cause infections and fatalities in both fish and humans. Unhygienic handling practices lead to contamination of fish ready for human consumption with potentially food borne pathogens such as Salmonella species, *Escherichia coli*, and *Staphylococcus aureus*; especially if fish is consumed raw or undercooked; it poses a significant public health risk (Lowry and Smith, 2007 and Austin *et al.*, 2005).

According to reports of Mitiku *et al*. (2022), the consumption of fish products are associated with high prevalence of food borne diseases in Ethiopia. This can be due to the poor handling practices and inadequate sanitation conditions in food preparation, lack of stringent food safety regulations and effective regulatory frameworks. Additionally, lack of proper training for food handlers contributes to the widespread prevalence of food borne infections. This improper food safety and quality practice in developing countries like Ethiopia provoke fish spoilage and contamination (Fratamico *et al*., 2005).

The knowledge, attitude, and practice (KAP) approach is frequently used to identify potential issues with food safety along the entire value chain. Knowledge of food safety is important as it can help to prevent the spread of food borne illnesses (Jianu and Goleț, 2014). In general, the food handler and consumer attitudes and practices influence their degree of food safety perception (Zanin *et al*., 2017). Their attitude has an impact on their actions and practices related to food safety since their optimistic mentality suggests that they recognize the importance of food safety and want to ensure that everyone can consume food safely (Kwol *et al*., 2020).There is limited research conducted in relation to assessment of food safety knowledge, practices and attitudes of food handlers it suggests a safety issue that has to be established. The processors involved in final product

preparation, processing, and packaging for onward sale to consumers are a crucial part of any food value chain (Raspor, 2008; Delia, 2005, and Cheraghi *et al*., 2014).

The bacteriological quality of fish is a critical aspect of public health that direct related with spoilage and the potential for food borne illnesses. It is necessary to ensure that the presence of potential microorganisms remains within acceptable thresholds that align with the product's shelf-life standards. Study on assessment of the bacteriological load of fish and isolation of most common food-borne bacterial pathogens from fish products was not well addressed in Ethiopia which is a critical for understanding of the possible prevalence of microorganisms of public health concern. Furthermore, such evaluation offers into the hygienic quality of fish, temperature abuse and hygiene practice during processing and handling.

The identification of food borne pathogens alone could not provide required data for policy development on risk factors such as unhygienic fish handling practices. Fishing practice in Ethiopia is important to ensure food security which need to have basic information on knowledge, attitude and practices (KAP) of the fish stake holders and hence research need to be done on the prevailing KAP on fish foods. To reduce food borne outbreaks, it is very important to have a clear understanding of the interaction of the prevailing KAP of food handlers and consumers.

General Objective

- To evaluate bacterial quality of fish products and assess the knowledge, attitude and hygienic practice towards food safety of fish products in Batu and Koka, Oromia, Ethiopia.

Specific Objectives

- To determine bacterial load of fish products in Koka and Batu.
- To detect the most pathogenic bacteria including *Escherichia coli*, salmonella species and *staphylococcus aureus* in the study sites.
- To assess the current status of food safety knowledge, attitude and practice of fish handlers.

2. LITERATURE REVIEW

2.1. Bacterial quality of fish and fish products

The microbial flora existing inhuman and animal including fish comprises of microorganisms that habituate either externally or internally within various region of the body. The host and the microorganisms in the natural flora may have symbiotic relationships (Pal, 2010). The enteric bacteria, which constitute the indigenous micro biota of the intestinal tract, are involved in the biosynthesis of vitamin K along with certain B complex vitamins. In commensalism, microbes are neither helpful nor unfavorable to their host, as is the case with the broad group of microbial flora that resides on the skin, as well as the mucous membranes of the upper respiratory system, intestines, and vagina (Pal *et al.*, 2016).

Fish constitute a significant component of dietary intake in developing nation attributable to its enriched protein contents as well as its nutritional value. It is valuable source of animal protein that is widely consumed by populations across various countries.Fish salting or brining, drying, or smoking are the traditional procedures for enhancing and storing fish (Couliarha *et al*., 2004). However, it spoils fast, especially in hot temperatures and tropical places where cold preservation techniques are generally lacking (Zambuchini *et al*., 2008). According to Pal *et al*. (2016), annually, roughly 8 million tons of fish, constituting approximately 25-30% of the global catch intended for human consumption**,** undergo preservation methods such as drying, salting, smoking, or a combination of these techniques.

The concept of fish quality is complicated, encompassing variety of factors that are relevant to consumers. These factors include safety, nutritional value, accessibility, ease of preparation, integrity, freshness as well as physical attributes such as species, size and product type (Abbas *et al.*, 2008).

Organoleptic techniques are commonly employed to evaluate the extent of freshness of fish by analyzing attributes such as the overall texture of flesh, scent, tint, eyes and gill condition (Clucas, 2003). Total plate count and total coliform counts serve as crucial for determining the microbial load of fish. The total number of microbial flora present on fish or fish products undergoes fluctuations over time. The determination of viable bacterial count in fish samples is achieved through the culture of bacteria species utilizing proficient bacteriological media, which facilitates the recovery of a maximal quantity of bacterial cells from fish tissue(FAO and WHO, 2012).

The Enterobacteriaceae count is regarded as an indicator of fish quality, as interconnected with factors such as ice storage, washing techniques, and removal of internal organs. It has been considered to use the monitoring of these bacteria as a fish quality indicator (Zambuchini *et al.,* 2008).

The enumeration of viable microorganisms does not necessarily serve as definitive indicator of the suitability of fish consumption. Fish with low bacterial count may harbor pathogens that could be more than hazardous if ingested (Negash *et al*., 2017). Therefore, qualitative analysis is conducted in order to assess the existence of pathogenic bacteria. Numerous kinds of selective media are employed in discernment of pathological microorganisms of aquatic organisms. The three types of media commonly employed in detection of *Escherichia coli*, Salmonella, and *Staphylococcus aureus* are Eosine Methyline Blue (EMB), Xylose Lysine Deoxycholate (XLD) agar, and Mannitol salt agar (MSA) respectively (WHO, 2020).

Effective management of risks in the fish processing chain is based upon scientific understanding of microbiological hazards together with comprehensive knowledge of primary production, processing, manufacturing technologies as well as proper handling techniques to be applied throughout the various stages of food preparation and storage, transport, retail and catering (Reilly, 2006; Galaviz-silva and Ez-anduro, 2009).

2.2. Risk factors associated with the occurrences of bacterial load in fish

The origin, quantity, and quality of the diet affect the quantity and variety of microorganisms in fish. It is expected the fish that eat more food (referred to as heavy feeders) have a higher level of bacteria than fish that don't feed (Nickelson *et al.*, 2001).Fish deterioration is caused by a number of reasons, including the geographic location of the catch, the season, and the techniques used for harvesting, handling, and storage (Novotny *et al.,* 2004). Fish may be predisposed to additional contamination through various stages of handling, including onboard, at docking areas, and upon presentation at market places, where they are susceptible to exposure and contamination by human pathogens transmitted via avian and insect sources (Adams and Moss, 2008).

The study carried out by Harwood *et al.*(2004), revealed that the primary source of microbial contamination in fish was attributed to inadequate adherence to sanitary protocols in the handling of the product during transportation from its marine environment to the commercial marketplace as well as during the point of sale. Additionally, these results have shown that the initial micro flora on fish's surface is directly tied to the aquatic environment, whereas the micro flora in the fish's gastrointestinal tract is related to its diet and overall health (Aliyu *et al*., 2018).

Upon capture, it is common practice to store fish in ice or chilled seawater prior to their transportation to shore (Adams and Moss, 2008). Consequently, it is imperative that a hygienic cooling agent is employed in the preservation of fish. Failure to do so may result in the accumulation of microorganisms and hastened spoilage of stored fish, which could compromise its quality and safety (Iwu *et al*., 2017).

Temperature and time are crucial variables that must be taken into consideration during the storage and handling of fish, as they play a significant role in the proliferation of microorganisms (Gelman *et al*., 2005). The authentic storage temperature of a product is contingent not only upon the temperature to which it is ultimately exposed within the

market container, but rather its complete temperature trajectory throughout its storage and handling processes (Nai *et al.*, 2007).

The presence of microorganisms in fishery products that have been isolated might be attributed to inadequate handling, transportation, and storage methods. Enhancement of the bacteriological quality can be attained through the implementation of hygienic handling methods throughout the supply chain, with particular emphasis on transportation, and by ensuring a continuous cold chain from the initial point of capture to the final point of consumption (Gauthier, 2015).The maintenance of the cold chain in the Ethiopian fish supply chain is inefficient, which can be attributed to a variety of obstacles, including inadequate access to essential resources such as ice and refrigerated transportation (Hailemichael and Gutema, 2021).

2.3.Major food borne bacterial infections associated with fish and fish products

Bacterial gastrointestinal infections persist as a significant public health burden across various regions of the world, encompassing even high-income nations with extensive surveillance and control efforts. These infections are responsible for causing morbidity, mortality, as well as causing considerable economic implications (Bienfang *et al*., 2011).

The ingestion of food contaminated with pathogenic microorganisms can lead to the manifestation of food borne illnesses. In particular, individuals who have been afflicted with diarrheal disease as a result of such food borne illnesses could have implications on their growth, physical development, and health care systems (Ali *et al*., 2020).

In developing nations, food borne illnesses significantly impact the children, with fatal cases being a frequent occurrence. The ingestion of fish has been identified as a potential cause of various diseases stemming from pathogenic infection or toxin exposure. Several microbial species have been identified as potentially harmful to humans, resulting in serious and sometimes fatal illnesses (Beyari *et al.*, 2021).

2.3.1. Escherichia coli

There are more than 250 known bacterial species that have been identified as causative agents of foodborne illnesses in humans. Among this, *Escherichia coli* (*E. coli*) are recognized as a prominent pathogen that has been implicated in numerous outbreaks of food poisoning on a global scale (Charles *et al.*, 2021).*It is* Gram negative, rod shaped, facultative anaerobic bacteria that commonly reside in the gastrointestinal tracts of various animal species. Although, the majority of *E. coli* strains do not pose a threat to human health, certain serotypes have been known to cause food borne illness and subsequent product recalls due to their presence in food products. The beneficial strains, which are normal gut flora, produce vitamin K for their hosts and function to prevent pathogenic bacteria from colonizing the intestines (Barguigua *et al*., 2011).

The *E. coli* bacteria have been frequently found in the intestines of humans, animals, and fish. Most *E. coli* that naturally reside in the colon and small intestine are not dangerous. These non-pathogenic bacteria can, however, cause disease if they enter other organs after leaving the colon. Enterotoxigenic *E. coli* is one of the pathogenic strains of *E. coli* that may produce and secrete toxins to cause diarrhea and even cause fish food to deteriorate (Iwu *et al.*, 2017 and Hiko *et al*., 2018).

E. coli contamination in fish based meals most commonly occurs during the handling and production processing of the fish. The presence of these bacteria in fish samples obtained from different nations across the globes has been reported. According to Mostafa *et al*. (2016) and Chandraval and Chandan (2016), the prevalence of *E. coli* in Egypt and India were recorded as 4.50% and 65%, respectively. The presence of *E. coli* in fish samples from Ethiopia has been found to range from 2.4% to 53.30%, as indicated in Table 1.

Table 1: The Prevalence of *E. coli* from fish samples of different parts of Ethiopia

Study area	Site of sampling	Prevalence of *E. coli*	References
Lake Tana	Intestine	2.4%	Anwar *and* Yimer, 2012
Lake Abaya and Chamo	Raw fillet	42.5%	Wondesen *et al*., 2017
Lake Tinike	Kidney, Oral cavity	20%	Hiko *et al.*, 2018
Lake Adelle	Liver, Gill, Heart, Intestine	53.3%	Hiko *et al.*, 2018
Lake Hayke	Skin, Gill, Intestine	25%	Tesfaye *et al.*, 2018
Lake Hawassa	Skin, Muscle	23.3%	Tilahun and Engidawerk, 2020
Lake Hayik and Tekezze	Muscle, swab	1.45%	Assefa *et al*., 2019
Lake Tana	Raw and cooked fillet	36%	Yohans, 2021
Lake Tana	Raw and cooked fillet, hand swab, knife swab, and table swab	21.21%	Mitiku *et al.*, 2022

2.3.2. Salmonella species

The bacterium Salmonella is classified under the Enterobacteriaceae family and is known to be a frequently encountered waterborne pathogen. This pathogen may probably present within the tissues of normal fish. The outbreak of disease accompanied by mortality in fishes is a significant consequence of injury or environmental stress (Li *et al*., 2019). Environmental stresses like high temperature and insufficient water quality are significant factors influencing the onset and severity of Enterobacteriaceae infections in fish population. Salmonella species are widely distributed and have been found in a variety of living things, including humans (Khosravani *et al.*, 2017).

The Salmonella species is a highly pathogenic bacterium that typically does not naturally occur in marine water. Its presence is often attributed to improper handling and unhygienic conditions. Fish have the potential to act as a carrier for various species of salmonella, with such contamination being attributed to terrestrial sources. These microorganisms are recognized as highly pathogenic and are deemed unsuitable for consumption in food products. Moreover, their persistent presence has been observed in tropical waters contaminated by their introduction (Chatreman *et al*., 2020).

Typhoid fever, which is caused by *Salmonella typhi*, has been identified as one of the most lethal strains of bacteria. It has been observed to manifest in several low-income countries that have been significantly impacted by inadequate hygienic conditions, particularly due to the consumption of contaminated food and water(Chatopadhyay, 2000 and Raufu *et al*., 2014).

Fish species, geographic regions, sampling methods (fish farms vs. retail stores), sampling components (skin vs. intestine), sources (imported vs. domestic), and fish product types (raw vs. cooked) all affect the prevalence of Salmonella contamination in fish and fish products. According to reports of Busani *et al.* (2005) and Nguyen *et al.* (2016), contamination levels were varies from 0.3% in Italy to 36.6% in Vietnam. In a recent finding across China, 2,328 retail freshwater fish were collected, and Salmonella was present at a level of 3.5% (Li *et al.*,2019).Raufu *et al*. (2014) identified 11.5% prevalence of Salmonella from retail catfish and catfish collected from fish farms in Nigeria.

Salmonella was detected at a prevalence of 7.5% in raw fillet from the Abaya and Chamo Lakes and a prevalence of 30% in kidney and oral cavity samples of fish from the Lake Tinike (Hiko *et al.,* 2018). There haven't been any cases of human salmonellosis associated to the consumption of fish or fish products in Ethiopia. However, using unclean filleting procedures, holding fish by hand, and consuming raw or just slightly cooked fish meals could expose people to the illness (Sorsa *et al.,* 2019).

2.3.3. Staphylococcus aureus

Staphylococcus aureus (S. aureus) is one of the leading bacteria causing food-borne diseases in humans globally. It has been reported as *S. aureus* is the third most common cause of food borne illness within the European Union caused by fish and its products(Naas *et al.,* 2019).*S. aureus* is a Gram positive bacterium that can cause a wide range of illnesses, from mild skin infections to fatal ones like pneumonia and endocarditis. Inadequate cooking, contaminated water, or raw materials used in food preparation are a few factors that could contribute to outbreaks (Baumgartner, 2014).

Fish do not typically have *S. aureus* in their usual microbiota. Fish products may or may not contain food-borne *S. aureus* depending on the harvest environment, hygienic conditions, and practices related to equipment in the processing environment. This microorganism has the potential to infect all cured food products as it displays salt tolerant properties, including cold smoked fish and preserved fish based items. Moreover, *S. aureus* is capable of withstanding elevated temperatures in fish and fish products (Huss *et al.,* 2003 and Boss *et al.,* 2016).

The detection of *S. aureus*, a pathogenic bacterium with significant public health implications, within frozen fish products suggest that improper handling by processor may have occurred, resulting in contamination of the processed frozen fish products from the outset. Poor handling and unsanitary practices can lead to contamination of ready to eat foods, posing a serious risk to the health of consumers (Okonko *et al.,* 2008).

The detection of methicillin resistance *S. aureus* in food producing animals, including fish, is increasingly becoming a matter of concern. Staphylococcus is therefore considered crucial for the fish food chain. Fish can become contaminated when people handling food have *S. aureus* on their skin or mucous membranes (Kumar and Rupam, 2018). However, *S. aureus* enterotoxins, which are present in fish and fish products, cause humans to develop gastroenteritis (Novotny *et al.,* 2004 and Vazquez-Sanchez *et al*., 2012).The consumption of raw fish is practiced in several countries, including

Ethiopia it pose significant health risks to the individual involved, potentially leading to the transmission and spread of disease (Obaidat *et al.*,2015).

2.4.Source and route of transmission of bacterial fish borne infections

The bacterial infection interaction among humans and aquatic species can be challenging, given the diverse modes of disease transmission, combined with the observation that numerous food borne pathogens do not provoke disease manifestation in aquatic organisms (Yukgehnaish *et al*., 2020).

Healthy fish possess the capacity to disseminate infections among individuals as asymptomatic carriers. Moreover, commensal microorganisms that typically present minimal challenges for aquatic organisms have the potential leading to the emergence of zoonotic pathogens capable of impacting human health. Another challenge with identifying pathogens in fish is that many clinical indications of the sickness in aquatic species vary from those that appear in infected humans (Jami *et al*., 2014).

2.4.1. Route of transmission

Humans may contract zoonotic infections through ingesting contaminated fish or water, direct topical contact through stings, bites, spine/pincer injuries, or penetrating wounds on the handler. Fisherman or fish processing workers that regularly exposed to fish, fish products, or their surrounding environment are susceptible to risks that might affect human health (Wodajo, 2020).

People with compromised immune systems may exhibit increased susceptibility. The phenomenon of seasonal variability and dietary preferences, specifically the consumption of raw fish, exhibit a consistent association with risk factors pertinent to seafood related illnesses in the human population (Ranjbar *et al*., 2019).

2.4.2. Source of contamination in fish

Waterborne infections persist as a prominent global health concern, from those infection bacteria are commonly found in fish and related products during the packaging process. About 80% of all illnesses are linked to use of water of poor microbiological quality (Adedeje, 2012).Seafood products obtained from contaminated water or those that have been inadequately preserved or after being harvested, are recognized for their significant contribution to infections caused by pathogenic bacteria (Wafaa *et al.,* 2011).

Food processors and handlers have the potential to serve as source of bacterial induced food poisoning, food intoxication, and food spoilage. As a result, they may hinder rather than aid efforts towards public health by posing a risk to the well being of individuals, as well as causing significant financial losses (Bankole *et al.,* 2009).

It is crucial to implement sanitary procedures throughout its manufacture and sales as well as for stores selling raw or pre-processed foods to have a reliable source of power supply. The presence of index of water quality and indicators of fecal contamination such as E. coli and Salmonella species are as a result of possible contamination during sales or unhygienic handling of fish right from the processing plants and this might have adverse influence the consumers' health (Okonko et al., 2008).

2.5.Food safety and hygiene of fish and fish products

Food safety is fundamental component that plays vital role in promoting and maintaining the overall wellbeing and health status of individuals. Every year, one in 10 people fall ill and 33 million healthy life years are lost due to the consumption of unsafe foods. Children under five years of age are particularly vulnerable, accounting for almost 40% of all foodborne diseases and 30% of total deaths related to unsafe food annually (WHO, 2015).

According to Grace (2016) and Hofmann *et al*. (2017) findings, low and middle income nations bear the majority of the public health burden associated with food borne illnesses, with sub Saharan Africa having the greatest per capita burden across all age groups. Foods that are most frequently recognized are those with an animal source, such as fish and fresh veggies, both of which are abundant with nutrients. Inadequate handling practices and substandard hygienic measures employed by vendor facilitate the proliferation of pathogens in food, which can result in detrimental health effects for consumers (Basha *et al.,* 2019).

The implementation of food safety standards measures, like personnel protection equipment and training throughout the food production chain, including processes related to food production, preparation, processing, labeling, hygiene, additives, residue, and policies relevant to food biotechnology that is important (FAO, 2006).Several researchers examined the microbial quality of different street food items, particularly fish, across African nations. These studies have demonstrated that street vended food is unsuitable for human consumption due to the detection of microbes known to cause food borne infections, as reported in the works of Tafida *et al.* (2013), Ndahi *et al*. (2013), Tamba *et al*. (2016), and Akabanda *et al*. (2017).

In recent decades, there has been a global rise in the consumption of fish and fishery products. This trend can be attributed to a shift towards fish protein, which is lower in cholesterol content, compared to animal protein (Wim *et al.,* 2007). The increasing global demand for aquatic products across developed and developing nations has spurred the necessity of preserving the current per capita consumption of such products for the future. The assurance of high quality and safe fish and fishery products as a significant source of protein has a global concern (Huss *et al.,* 2003).

2.5.1. The ways to maintain food safety hygiene

Good manufacturing practices (GMP): Exclusion, removing unwanted and foreign matter, inhibiting, and destroying undesired microorganisms are the four pillars of GMP.

The building and its surroundings, staff members, cleaning and sanitization procedures, equipment and utensils, processes and controls, and storage and distribution are the components that make up GMP. The GMP program analyzes and controls these factors with the goal of producing high quality foods. GMPs are one method for preventing food-borne illnesses (Cruz *et al*., 2006).

Sanitation standard operating procedure (SSOP):SSOP are documented procedures created and used in a facility in order to prevent product adulteration or direct contamination. In order to keep utensils and equipment clean and free of pathogenic bacteria and little deteriorating microbiota, and to avoid contaminating foods that come into touch with these utensils and equipment, SSOP include a full explanation of the precise tasks required (Cunha *et al*., 2015).

Good hygiene practices (GHP): The hygiene of foods is described as the processes and circumstances required to regulate risks and guarantee that that a food item is fit for consumption by people, considering its final use GHP are frequently referred to as the prerequisite measure upon which other food safety and quality management systems are constructed. Staff personal hygiene and training are among the extensive list of steps they provide(Food Standards Agency, 2009).

Hazard Analysis of Critical Control Points (HACCP):A structured set of operations known as HACCP is used to manage food production in order to assure food safety and prevent changes in foodstuffs. The system is based on the application of control procedures at specific manufacturing stages where there is a higher likelihood of encountering health risks. GMP and SSOP are the prerequisite programs for HACCP implementation in food industries. These programs cover a variety of food industry topics, including physical structure and maintenance, water supply, personal hygiene, pest control, sanitization techniques and equipment, instrument calibration, and quality control of raw materials and ingredients (Kamboj *et al*., 2020).

2.6.Food safety knowledge attitude and hygienic practice of food handler

The Knowledge, Attitudes, and Practice (KAP) framework is used to carry out representative research on particular groups, and it focuses on examining what people know and believe about a subject as well as the actions that go along with those beliefs (WHO, 2008).Due to the fact that experienced handlers frequently act and behave in ways that indicate to their knowledge level, knowledge level might be a crucial factor in determining the relationship between attitudes and actions (Zanin *et al*., 2017). Attitude is used to describe a complex mental state including opinions, emotions, values, and dispositions to act in certain ways (Sharif and Al-Malki, 2010). In essence, it refers to the routine actions that food handlers take to ensure food safety. In order to ensure that food is safe for everyone, a positive attitude will therefore suggest a disposition that tends to act out the understanding of food safety (Akabanda *et al*., 2017).

The safety of food is the main topic of several KAP research on fish (Trondsen *et al*., 2004, Ahmad *et al*., 2016, and Ali *et al*., 2020), which also assess the needs of consumers for particular types of fish and estimate fish consumption rates. Although, several studies have reported the occurrence of common fish borne pathogens in Ethiopia such as: Tesfaye *et al*. (2018), Hiko *et al*. (2018), and Wendesen *et al*., (2017) showed *Edwardsiella tarda*, Salmonella, *E. coli*, *S. aureus*, Aeromonas and Vibrio species are among the bacterial pathogens found in fish and fish products in the country. Food safety KAP of fish and fish products not well studied in which it needs special attention in order to control and prevent food borne infection and intoxication.

2.7. Public health importance of fish borne bacterial infection

The occurrence of food borne diseases has increased on a global scale. Annually the United States of America reports 48 million instances of food borne diseases, resulting in 128,000 admissions to hospitalsand3,000 recorded deaths. According to the results of the study, fish emerged as the predominant food category, accounting for a significantly large proportion (17%) of the total number of food borne outbreaks (Scallan *et al.,* 2011).

Food borne infections represent a crucial role of public health concern globally. Annually, numerous individuals suffer from illnesses arising from the consumption of animal origin food that is contaminated (Cantas and Suer,2014).Fish disease may result in financial losses due to death and healthcare expenses, a delay in or loss of the opportunity to sell the fish, and the transfer of zoonotic diseases to consumers and fish handlers. Moreover, apart from the potential transmission of diseases from fish to humans fish themselves are prone to numerous illnesses and have the ability to transfer food borne microbial infections and toxicities (Emikpe *et al.,* 2011).

Consuming raw or undercooked fish involves greatest risks that may be naturally contaminated with food-borne diseases found in the aquatic environment. The risk is considerably high if the food is incorrectly handled during preparation, when microbes may quickly multiply in favorable conditions (Adedeje *et al.,* 2012).Fish are susceptible to bacterial contamination and may serve as a vehicle of known bacterial pathogens,includingSalmonella and Vibriospecies.They are the most common bacterial causative agents in food poisoning resulting from the consumption of fish (Wafaa *et al*., 2011).

2.8.Control and prevention of fish borne infection

The management of fish diseases is fairly complicated because the system in which fish are cultured depends on the condition of the natural environment. Environmental factors greatly influence the health of fish and the majority of illnesses affecting fish are brought on by deterioration in the aquatic environment (Ali *et al*., 2020).The implementation of multidisciplinary approaches, encompassing the characteristics of highly pathogenic microorganisms in fish, the biological aspects of fish, and an enhanced comprehension of environmental factors facilitate the utilization of suitable measures for the prevention and management of disease which impede fish production (Toranzo *et al.,* 2005 and Shemsi, 2019).

Educating the public concerning microorganisms and the potential hazards associated with the consumption of raw or undercooked fish is importance as microbial agents present in fish may exacerbate public health issue. The regular monitoring and implementation of quality control measures are imperative for the consumption of fish(Bibi *et al.*, 2015). The expeditious and efficacious regulation of disease, coupled with the delivery of requisite intelligence, enables the prevention and treatment of aquatic zoonotic pathogens (Ibrahim and Sheshi, 2014). It is imperative to don disposable gloves and refrain from coming into direct contact with fish mucous. One of the most effective measures is the regular practice of hand hygiene, particularly following contact with aquatic environments or fish (William *et al*., 2022). Additionally, it's imperative to wait until consuming or eating before washing your hands. Transmission of zoonotic diseases can occur through direct or indirect contact with insects, vectors, contamination from inanimate objects, ingestion, and inhalation (Boylan, 2011).

Aquaculture systems exhibit a wide range of dimensions and configurations, stretching from modest residential aquariums to expansive hectares-wide bodies of water. Despite the variation in size and design, these systems uniformly entail water that is abundant in nutrients and fosters the proliferation of bacteria (Lehel *et al*., 2021). As a result, several studies on the chemical disinfection of contaminated habitats have been done. In order to effectively prevent fish zoonoses, contact duration, adequate and safe handling of disinfectants and accurate disinfectant dosage should all be addressed. Remarkably, desiccation or the process of drying has also been established as a potent disinfection method for the control of zoonotic bacterial pathogens (Ziarati *et al.*, 2022).

The ingestion of raw or insufficiently cooked fish and fish products as well as exposure to contaminated water/ fish, represent the most significant risk elements for bacterial zoonoses transmitted through fish (Zorriehzahra and Talebi, 2021).It is desirable to avoid contamination of the natural environment to reduce infections because fishing is mostly dependent on the natural environment in developing nations like Ethiopia (Sorsa*et al.*, 2019).

3. MATERIAL AND METHODS

3.1. Description of the Study Area

The study was conducted in Koka and Batu towns of East Shewa zone of Oromia region, Ethiopia. East Shewa zone has a total population of 1,356,342 within an area of 8,370.90 square kilometers with a population density of 162.03. While the majorities (74.9%) of the population are rural dwellers practicing mixed crop-livestock farmers or other, the remaining proportions are urban inhabitants (25.1%) or few pastoralists (0.05%). The majority of the fish populations in Ethiopia are situated within the rift valley lakes, including naturally occurring water bodies Lake Ziway (Dambel) in Batu town and artificially constructed Lake Koka near Koka town. Lake Koka, is found at latitude 8^{o}24’12.66’’N and longitude 39o05’31.50’’E, and was dammed hydroelectric reservoir constructed in the river Awash. The Koka reservoir is a crucial supply of fish for riparian societies and small-scale fishermen (Tesfaye and Wolf, 2014).

Lake Ziway, situated at latitude 7°58'18.37’’N and longitude 38°50'29.88’’E, is a shallow Lake in the Ethiopian Rift Valley, located approximately 40 km and 120 km south of Lake Koka and Addis Ababa, respectively. The prominent fish species in Batu, including tilapia, carp and catfish, exhibit most demand and high exploitation. Both Lake Koka and Lake Ziway are situated in an area with a climate that varies between semi-arid to sub-humid. The annual precipitation and temperature of the area range, respectively, from 600 mm and 25^{o}C in the semi-arid parts to 1200 mm and 15^{o}C in the humid areas (Willen, 2011). The annual fish yield is estimated to be approximately 542 tons. The common carp (*Cyprinus carpio*), catfish (*Clarias gariepinus*), tilapia (*Oreochromis niloticus*), and barbs (*Labeobarbus intermedius*) are the existing species that have commercial value and produce considerable annual catches (Abera and Tibesso, 2021).

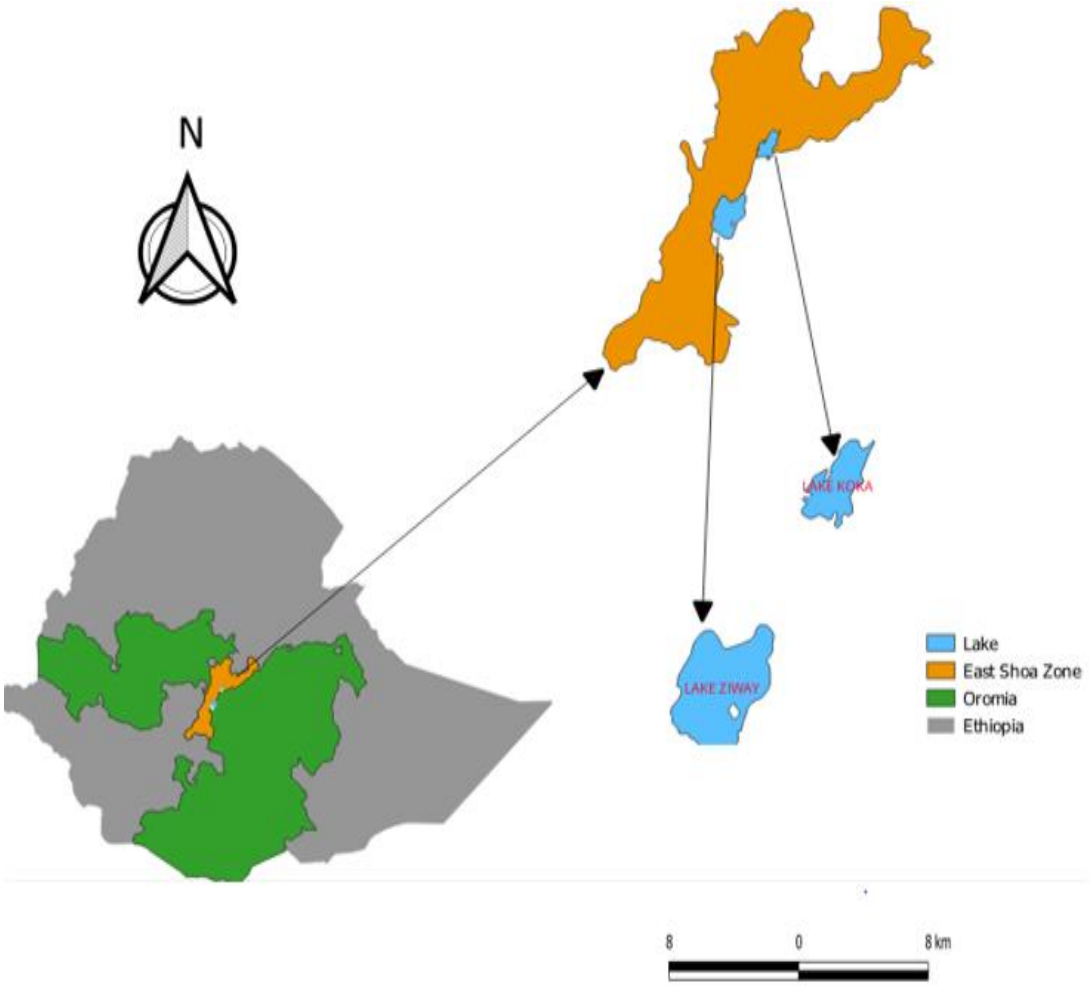

Figure 1: Map of the Lakes where fish product was harvested (Source: GIS Software).

3.2. Study design, Sample size and sampling techniques

A cross sectional study design was conducted from December 2022 to June 2023, comprising of both survey and laboratory analysis. Fresh, frozen, cooked fillet samples were purchased from purposively selected fishermen at landing sites, retailers at shop, restaurants found in Koka and Batu towns and also swab samples were collected from hands of fish handlers and filleting equipment including knife and filleting board. Questionnaire survey on the current status of food safety knowledge, attitude and practice among fish handlers, retailer and consumers were also performed. The study areas were selected purposively based on practices of fish and fish products.

Due to limitations of resources a total of 130 samples (65fresh fish fillet, 15 frozen fillet, 20cooked fillet, 15 hand swab, 8 knife swab, 7 table swabs) were collected from fisherman at landing sites, retailer and restaurant in Koka and Batu towns for laboratory analysis. The sample size required for a qualitative survey study was estimated to be a minimum of 50 participants according to the recommendation by Dworkin (2012). Survey data were collected from a total of 80 selected respondents, comprising 15 fishermen, 19 food workers, 13 fish retailers and 34consumerswere interviewed and hygienic status of each place observed from the study areas.

3.3. Sample collection handling and transportation

A total collection of 130 samples (65 fresh fillet, 15 frozen fillet, and 20 cooked fillets as well as 15 hand swab, eight knife swab and seven table swab) were collected from Batu and Koka. A total of 13 samples were taken during each trip (8 raw fillet, 2 cooked, and three swab samples) in order to evaluate bacterial quality. The Sterile plastic bags were used for collection of sample to avoid the sample taken from contamination. The inner surface of the bag was used to touch the sample only. The swabbed sample was inserted into transport media. All samples were appropriately identified by specifying their sample type, sampling location, date of collection, and assigned an identification code. All samples were then transported to the Aklilu Lemma Institute of Patho-biology medical microbiology laboratory, Addis Ababa University, Ethiopia, using icebox containing ice packs to ensure optimal conditions for microbiological analysis. Upon arrival, the samples were immediately processed within a period of 24hr of collection.

3.4. Bacterial analysis

3.4.1. Sampling for Laboratory Analysis

One hundred fish fillet samples were used through aseptic condition, representing different fillet types including cooked (20 samples), frozen (15 samples), and fresh (65 samples) for bacterial load evaluation. Twenty five gram of each sample was introduced

into 225ml of sterile buffered peptone water (BPW) and subsequently homogenized with the aid of laboratory blender (Stomacher 400) at high speed for two minutes. In order to perform analysis of both total bacterial count and total coliform count, a volume of 1milliliter from a homogenized sample was aseptically transferred into a test tube containing 9 milliliters of sterile BPW, which was appropriately labeled as 10^{-1}. 1ml of the initial suspension was subsequently transferred into subsequent sterile BPW for the purpose of generating further dilutions to attain desired level of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. The bacterial colony count was determined using the spread plate method (Wise, 2006 and Nur *et al*., 2020).

For the analysis of Salmonella species, *E. coli*, and *S. aureus* a homogenized suspension containing 25g of sample in 225ml of BPW and swab samples that transferred from transport media to nutrient broth were subjected to incubation at 37^{o}C for a period of24hours, after which further bacterial analysis was carried out. A total of one hundred thirty distinct samples, comprising both swab samples and fish fillet samples, were utilized for purpose of detecting thus three most common food borne bacteria. The laboratory procedures were processed in accordance with the prescribed codex (FAO, 2009 and WHO, 2015).

3.4.2. Preparation and sterilization of media and laboratory reagents

The media and reagents used in this study were prepared and subjected to appropriate sterilization process, as per the instructions provided by the respective manufacturers (Appendix 4). The sterility control plates for each media and diluents were generated through an incubation process lasting for a required time.

3.4.3. Total viable bacterial count

The use of Plate Count Agar (PCA) or the standard plate methodology was employed to evaluate the total bacterial load of fish. The Total Viable Bacterial Count was determined through the utilization of the spread plate technique, which is a commonly employed

methodology for the microbial evaluation of food products, including fish. This technique enables the isolation and identification of a diverse range of microbial flora on PCA medium (Acharjee *et al*., 2014, and Nur *et al*., 2020).

A volume of 0.1mL from each selected dilution suspension was aseptically dispensed onto the center of individual, labeled, and sterilized plates containing PCA agar. The samples were uniformly distributed onto the agar surface by means of a sterile glass spreader, while simultaneously rotating the Petri dish at an angle of 45^{o} in a careful manner. The Petri dish was subjected to incubation at a temperature of 37°C for 24-48hours (Appendix 1).Upon completion of colony counting, the obtained values were subjected to multiplication by the corresponding dilution factor, with the ultimate goal of determining the concentration of colony forming units per gram of the original sample.

3.4.4. Total coliform count

The utilization Violet red bile agar (VRBA) was employed in order to establish the presence of total Coliform count (TCC) as per the accepted protocols (Wehr *et al.,* 2004). A desired dilution series suspension of 0.1ml was aseptically spread over the center of designated, sterile VRBA plates using sterile glass spreaders. The plates were subjected to incubation at a temperature of 37^{o}C for 18 to 24hour (Appendix 2).Upon assessing the colonies present; they were subsequently multiplied by the corresponding dilution factor in order to calculate the quantity of colony forming units per gram in the initial specimen.

Total bacterial count (CFU/g) for both total viable bacterial count and total coliform count were calculated as:

$$TBC\ (CFU/g) = N/V*D$$

Whereas, TBC= total bacterial count, CFU/g= colony forming unit per gram, N= total number of colonies on plate, V= volume of suspension, D= dilution factor.

3.4.5.Isolation of E. coli

Twenty five gram of raw or cooked fish samples was homogenized by laboratory blender (Stomacher 400) in a sterile plastic bag containing 225 ml of BPW for 2 minutes at high speed. The swab samples were transferred from carry Blair medium into nutrient broth and incubated at 37^{o}C for 24hr. The homogenized sample and swab suspension from nutrient broth was inoculated into the Eosine Methylene Blue medium (EMB) and subjected to incubator at temperature of 37^{o}C for duration of 24hr (Gupta *et al.,* 2013 and Alexander *et al*., 2010). The suspected colonies on EMB agar showed a green metallic sheen appearance and were sub cultured on nutrient broth incubated for 24 hr, then inoculated on EMB to get a pure colony and were incubated at 37°C for 24 hr. The pure colonies have been preserved for further identification after being sub cultured on nutrient agar slant.

3.4.6.Occurrences of Salmonella

A twenty five gram portion of raw and cooked fillet was placed in sterile plastic bag with 225 ml of BPW and homogenized with stomacher for two minutes at higher speed and incubated for 24 hr at 37°C for pre-enrichment broth media. The swab samples were transferred from transport medium into nutrient broth and incubated at 37^{o}C for 24hr. Thus, 1 ml suspension from pre-enrichment broth was inoculated into tube containing 10 ml Rappaport Vassiliadis broth and it was incubated at 42^{o}C for 24 hr. A loop full of (approximately 10 µl) broth inoculums was transferred and streaked onto the surface of XLD agar. The plates were subjected to incubation at temperature of 37°C for 24 hr (Mooijman *et al.,* 2019).The pure colony with the black center on the XLD was sub cultured into nutrient broth and inoculated to nutrient agar slant to preserve for an additional identification process.

3.4.7. Detection of *Staphylococcus aureus*

The detection and identification of *S. aureus* in fish samples was conducted through a standardized process whereby 25 grams of both raw and cooked fillets were aseptically placed in sterile plastic bag containing 225ml of BPW. The homogenization of the samples was then performed using stomacher blender. The swab samples were transferred from transport medium into nutrient broth and incubated at 37^{o}C for 24hr. A loop full homogenized sample was streaked onto the surface plate of MSA media, followed by incubation for 24-48 hours at 37°C (Ramesh and Souissi, (2018). The fermentation of mannitol produced pigmented colonies with a yellow halo around them, which was a positive result.

3.4.8. Summary of biochemical tests

The different biochemical tests were performed for further confirmation after cultured on their selective media of the indicator bacteria. Salmonella suspected colony having black center of culture test was biochemically performed by inoculating into Urea agar and those Urease negative tests were further confirmed by Triple sugar iron agar, Simmons' citrate agar, lysine iron agar, and SIM (Sulfide Indole Motility) medium, and then incubated at 37°C for 24 hours. According to ISO-6579-1, (2017), the outcome was determined. Confirmatory biochemical tests including Indole test, Simon citrate test, Triple Sugar Iron agar (TSI) test, and Urease test, were subsequently performed for E. coli identification according to ISO 6887-3, (2017) guidelines. Suspected positive samples of *S. aureus* on Mannitol salt agar were identified using biochemical tests including the Catalase and coagulase tests as well as Gram staining reactions, in which Staphylococci had been expected to be present in smears that showed gram-positive, spherical cells grouped in irregular clusters (Appendix 3).

3.5. Questionnaire survey and observation for KAP analysis

The knowledge, attitude, and hygienic practice (KAP) of fish and fish products were studied among fish handler, processor and consumer in order to determine food safety awareness, belief and practice of participants. The format of the drafted questionnaire was based on numerous published questionnaires (Al-Kandari *et al.,* 2019, Grema *et al.,* 2019; Ansari-Lari *et al.,* 2010; Eltholth *et al.,* 2014).

Direct observation was employed to assess the hygienic status and practices including proper handling and processing of fish products and quality of filleting area by the fish handlers at landing site in Koka and Batu lakes place where filleting takes place, fish handlers at kitchen of different restaurant found in Koka and Batu towns. Fish handlers, retailers, and consumers were interviewed by using detailed and well-structured questionnaire in order to obtain relevant information on food safety practices and fish handling.

The questionnaires were translated to local languages (Afan Oromo and Amharic) prior to interview administration. Information about the individual, such as educational background and fish handlers' training in hygienic handling of fish food and procedures, was gathered. Informed consent was obtained from fish handler after a brief explanation about the benefits of the study. The respondent's confidentiality was maintained by ensuring that his or her responses wouldn't be made public, would remain anonymous, and not have his or her name on them questionnaire or be kept in any other records.

In order to assess KAP of fish handlers the levels of individual elements of knowledge were assessed as good or poor, attitude as positive or negative and practices as good or bad. A total of 18 KAP questions were grouped into three sub-topics; eight questions measure knowledge of food safety of fish and fish products, five questions focused on attitudes, and 5 questions assessed hygienic practice towards handling, processing and consuming fish products. When respondents were asked about their knowledge, attitudes and practices related to fish and its products, their responses were mostly framed as

“yes/no/don’t know”. Responses to the knowledge, attitude and practice questions were mainly dichotomized into “correct vs. incorrect.

A value of 1 or 0 was assigned to each question, meaning that a '1' value was provided for a valid response and a '0' value for an unsure or incorrect response. Thus, a given participant would receive a cumulative score ranging from 0 to 8 points for the knowledge, 0-5 points for attitude and practice questions. Using the original Bloom's cut-off point, the aggregated KAP score was then divided into three categories: good for scores between 80% and 100%, moderate for scores between 60% and 79%, and poor for scores below 60% of correct responses.

3.6. Ethicalclearance

The study was approved by Addis Ababa University College of Veterinary Medicine and Agriculture ethical review committee (Ref No. VM/ERC/02/01/15/2023). All study participants provided written informed consent, and to protect their businesses and identities, privacy information is kept confidential.

3.7. Data quality control

The collection and processing of samples were conducted utilizing aseptic techniques. Each specimen was appropriately labeled. The bacterial colony count, culture, and biochemical test were conducted under the guidance and supervision of skilled laboratory personnel. The media utilized in this experiment were prepared in accordance with the procedures outlined by the manufacturer. The sterility of the prepared media was assessed through an incubation process at a temperature of 37°C (APHA, 2005).The management of the questionnaire and observational checklists adhered to the standard guidelines of the Codex Alimentarius Commission of Food and Agriculture Organization (FAO, 2009).

3.8. Data analysis

The data obtained from the participant questionnaire and laboratory findings were documented in Microsoft Excel and subsequently analyzed by Stata software, version 14. The TPC and TCC values were log transformed prior to statistical analysis to enhance the symmetry of the frequency distribution. In this study, an analysis of variance (ANOVA) was used to compare the means of TPC and TCC in samples of cooked, frozen, and fresh fillet obtained from different types of fish. The Chi-square test was used to determine statistical association detected bacteria and to compare the proportions of observations among the different categories as well as for food safety KAP of fish and fish products. The present study employed descriptive statistics including: percentage, frequency, minimum, maximum, and mean, to analyze TPC, TCC and detect the presence *E. coli*, *Staphylococcus aureus*, and Salmonella species as well used the data collected through a questionnaire-based survey. Throughout all of the analyses, the confidence level maintained at 95%, with p-value assumed that less than 5%.

4. RESULTS

4.1. Colony counts of bacteria

Bacterial growth represents the primary cause for the deterioration of fish and poses a significant risk to public health and hence the determination of total bacterial count and total Coliform count serve as general index for evaluating fish quality. The present findings of the bacteriological load analysis from 15 frozen, 20 cooked and 65 fresh fillet samples demonstrated that the mean TPC of the fresh fillet recorded 7.55 (log cfu/g), with the samplerevealingthe highest count ranging from 5.95 to 9.99 log cfu/g. The cooked fillet exhibited the lowest overall counts of viable bacteria, falling within the range of 2.09-6.82log cfu/g (Table 2). Whereas the frozen fish sample's TPC was found to have a mean value of 5.91log cfu/g. The mean value of TCC (log cfu/g) of examined types of fish samples:cooked fillet, frozen and fresh raw fillet were 3.67, 5.02, 6.1 with minimum-maximum of 1.53-5.5, 3.52-6.39 and 4.04-7.5 (log cfu/g) respectively.The mean of TPC showed that there was statistically significant difference between types of sample (F=74.8, degree of freedom=2, 94, p=0.0000).

Table 2: Mean log of total plate count and total Coliform count based on type of fish Samples.

Measure	Sample type	Minimum count (log cfu/g)	Maximum count (log cfu/g)	Mean (log cfu/g)	P value /F statistics (degree of freedom)
TPC	Cooked fish	2.09	6.82	4.54	0.0000/ 74.8 (2, 94)
	Frozen fillet	4.38	7.62	5.91	
	Fresh fillet	5.95	9.99	7.55	
TCC	Cooked fish	1.53	5.5	3.67	0.0000/52.09 (2, 94)
	Frozen fillet	3.52	6.39	5.02	
	Fresh fillet	4.04	7.5	6.1	

As indicated on Table 3 based on source of sample the mean value, the range from minimum to maximum counts (log cfu/g) were 5.82, 3.65-7.5 for fishermen, 5.94, 2.93-6.9 for fish retailer and 3.62, 1.53-5.5 for restaurants respectively. Based on the source of fish sample the mean value forrestaurant, retailer and fishermen were 4.54, 7.05 and 7.36log cfu/g respectively. The mean of TPC showed that there was statistically significant difference between source of sample (F=36.95, degree of freedom=2, 94 p=0.0000).

Table 3: Mean Log of total plate count and total Coliform count based on source of fish sample.

Measure	Source of samples	Number of observation	Minimum count (log cfu/g)	Maximum count (log cfu/g)	Mean (log cfu/g)	P value /F statistics (degree of freedom)
TPC	Restaurant	20	2.09	6.82	4.54	0.0000/36.95 (2, 94)
	Fish retailer	38	3.47	8.99	7.05	
	Fishermen	42	4.47	8.5	7.36	
TCC	Restaurant	20	1.53	5.5	3.62	0.0000/ 33.45 (2,94)
	Fish retailer	38	2.92	6.9	5.94	
	Fishermen	42	3.65	7.5	5.82	

4.2. Detection of indicator bacteria

Frequency data for the three most common food borne bacteria*(E. coli*, Salmonella, *Staphylococcus aureus*) studied from a total of 130 (7 table swab, 8 knife swab, 15 hand swab, 15 frozen fillet, 20 cooked fillet and 65 fresh fillet) samples collected during study period are summarized below.

4.2.1. Occurrence of E. coli

From a total of 130 samples, 42 (32.31%) were found positive for *E. coli.* The prevalence of *E. coli* was 32.8% and 31.7% from Batu and Koka, respectively. The highest prevalence of *E. coli* was found in Tilapia species whereas no *E. coli* contamination was encountered in Barbus fish species. From Retailer, 20/49(40.8%) isolates were positive which is prevalent than fisherman and restaurant. The distribution of *E. coli* among species, study sites, source of samples and sample types were listed in Table 4.

Table 4: Prevalence of *E. coli* based on fish species, sample types, study site and source of samples

Category	Number of tested sample	*E. coli* positive in number (%)	Chi square (P value)
Fish species			
Tilapia	26	10 (38.5%)	4.25 (0.235)
Catfish	25	8 (32%)	
Carps	15	2 (13.3%)	
Barbus	3	0	
Study site			
Batu	67	22 (32.8%)	0.0176
Koka	63	20 (31.7%)	(0.894)
Sample type			
Cooked fillet	20	5 (25%)	
Fresh fillet	65	18 (27.7%)	
Frozen fillet	15	8 (53.3%)	7.97 (0.158)
Table swab	7	4 (57.1%)	
Hand swab	15	6 (40%)	
Knife swab	8	1 (12.5%)	
Source of sample			
Fisherman	52	12 (23%)	
Retailer	49	20 (40.8%)	3.71 (0.156)
Restaurant	29	10 (34.5%)	
Overall prevalence	**130**	42 (32.31%)	

4.2.2. Detection of Salmonella

From a total of 130 samples taken, 4 (3.1%) samples were positive for salmonella species.Salmonella was found in fresh fillet 3 out of 65 samples (4.61%) and 1 out of 7 (14.3%) samples of filleting board/table. However, cooked fillets, frozen fillets, hand swab, and knife swabs were showed negative result. With regard to source of the fish samples, the prevalence of 3.85% from fishermen and 4.1% from retailer was recorded. No Salmonella spp was detected from 29 samples collected from restaurants. Table 5 shows the occurrences of Salmonella from different study sites, fish spp, source of samples and type of samples in detail.

Table 5: Prevalence of Salmonella speciesbased on fish species, sample types, study lakes and source of samples

Category	Number of Tested Sample	Salmonella Positive Number (%)	Chi square (p value)
Fish species			
Tilapia	26	1(3.85)	
Catfish	25	1 (4)	0.3533
Carps	15	1(6.67)	(0.950)
Barbus	3	0	
Study site			
Batu	67	2 (3.17)	0.0039
Koka	63	2 (2.98)	(0.950)
Sample type			
Cooked fillet	20	0	
Fresh fillet	65	3 (4.47)	5.3061
Frozen fillet	15	0	(0.380)
Table swab	7	1 (14.3)	
Hand swab	15	0	
Knife swab	8	0	
Source of sample			
Fisherman	52	2	
Retailer	49	2	1.1897
Restaurant	29	0	(0.5520)
Overall prevalent	**130**	4 (3.1%)	

4.2.3 Detection of Staphylococcus aureus

S. aureus was detected from 130 different fish samples with a prevalence of 16.9%. The frequency of positive samples of 7, 11 and 4 were observed from fishermen, fish retailer and restaurant respectively. As indicated on Table 6, prevalence of 19.4% and 14.3% of *S. aureus* were collected from Batu and Koka respectively. Based on fish species, 36% of Catfish, 11.6% of Tilapia and 26.7% of Carps species were positive of pathogenic bacteria. However, no positive result from sampled Barbus species was detected.

Table 6: The Prevalence of *Staphylococcus aureus* based on fish species, sample types, study site and source of samples

Category	Number of tested sample	*S. aureus*positive in number (%)	Chi square (P value)
Fish species			
Tilapia	26	3 (11.6)	5.2925 (0.152)
Catfish	25	9 (36)	
Carps	15	4 (26.7)	
Barbus	3	0	
Study site			
Batu	67	13 (19.4)	0.6048 (0.437)
Koka	63	9 (14.3)	
Sample type			
Cooked fillet	20	1 (5)	8.7379 (0.12)
Fresh fillet	65	16 (24.6)	
Frozen fillet	15	0	
Table swab	7	2 (28.6)	
Hand swab	15	2 (13.3)	
Knife swab	8	1 (12.5)	
Source of sample			
Fisherman	52	7 (13.4)	1.7095 (0.425)
Retailer	49	11 (22.4)	
Restaurant	29	4 (13.8)	
Overall prevalence	**130**	22 (16.9%)	

4.3. Results for KAP analysis

4.3.1. Demographic characteristics of respondents

Among a total of 80respondents participated in assessment of knowledge, attitude, and hygienic practice of handling, processing and consuming fish products 61.25% were male. As indicated in (Table 7**)** 46.25% of participants were range in age of 20-30 year and only 15% of respondents were above 40 years. Based on educational status the percentage of respondents of no formal education, diploma, high school, degree and above, and elementary school were 13.75%, 15%, 18.75%, 25%, 27.5% respectively. Among the respondents 41.25% were fish consumer and 23.75% were food worker.

Table 7: Socio-demographic characteristics of respondent from Koka and Batu towns

Characteristics		Frequency	Percent
Gender	Male	49	61.25
	Female	31	38.75
Age (Years)	< 20	15	18.75
	20-30	37	46.25
	30-40	16	20
>40		12	15
Educational Status			
Informal		11	13.75
	Elementary	22	27.5
High school		15	18.75
	Diploma	12	15
	Degree and above	20	25
Role of Respondents			
	Fishermen	15	18.75
	Food Worker	19	23.75
	Retailer	13	16.25
	Consumer	33	41.25

4.3.2. Food safety knowledge of respondents

Assessment of the food safety knowledge in the study area indicated (Table 8) that most of respondents (83.75%) had good knowledge that hand washing can reduce risk of fish product contamination. About 62.5% and 67.5% of respondents had good knowledge of using protective equipments (glove, hair coat) and proper cleaning and handling of fish products can reduce risk of contamination respectively. Among respondents 78.75% know refrigerator is the safest fish storage material. Only 33.75% of participant takes food safety training and 50% of respondents know diarrheic person can contaminate the fish products.

Table 8: Food safety knowledge of respondents

Statements	Respondents Responses Yes	
	Frequency	Percent
Hand washing reduce risk of fish contaminations	67	81.7
Protective equipments reduce risk of contaminations	5062.5	
Proper cleaning and handling reduce contamination	5467.5	
Diarrheic person can contaminate fish products	4050	
Wound on skin can infect fish products	37 46.25	
Do get food safety training?	27 33.75	
Contaminated fish can transmit disease	44 55	
Refrigerator is safest fish storage	63 78.75	

4.3.3. Food safety attitude of respondents

Most of respondents (86.25%) have positive attitude towards the occurrence of human diseases when fish stored at room temperature is used for consumption and most of them (83.75%) agreed that fish stored at room temperature can be contaminated. As indicated in Table 9**,** 56.25%, 62.5% and 68.75% negative attitude with the statements of raw and cooked should be stored separately, eating raw fish can increase risk to human health and eating undercooked fish product can increase human health risks respectively.

Table 9: Assessment of food safety attitude of fish and fish products

Statements	Number of Respondents Agree (%)
Raw and cooked fish should be stored separately	35 (43.75%)
Eating raw fish increases the risk to human health	30 (37.5%)
Eating undercooked fish increases the risk to human health	25 (31.25%)
Fish kept at room temperature can be contaminated	67 (83.75%)
Fish stored at room temperature when consumed can cause disease	69 (86.25%)

4.3.4. Hygienic practice of respondents towards handling processing and consuming fish products

As showed in (Table 10), 95% of respondents eat fish and 67.5% of them also consume undercooked fish. However, only 33.75% of participants preferred consuming raw fish as well as only 48.75% respondents clean transportation materials, vehicles with clean water after use.

Table10: Assessment of hygienic practice of fish and fish products

Statements	Number of Respondents yes (%)
Do you eat fish	76 (95%)
Do you eat undercooked fish	54 (67.5%)
Do you clean transportation materials, Vehicleswith pure water after use?	39 (48.75%)
Do you prefer consuming raw fish	27 (33.75%)
Do you store leftover fish at room temperature for sale the next day?	10 (12.5%)

4.3.5. Overall knowledge

From all interviewed participants 42.5% have good knowledge, 33.75% have moderate, and 23.75% have poor knowledge on food safety practice of handling, processing and consuming fish and fish products. As indicated in the following Table 11**,** 44.9% of male respondents have good knowledge whereas 22.5% of them have poor knowledge. Furthermore 32.2%, 41.9% of female respondents have moderateand good knowledge regarding safety of fish and its products respectively.

Based on educational status, respondents with degree and above have moderate and good knowledge on fish and fish products at percents of 35 and 40 respectively. Only 13.3% of respondents at high school level have poor knowledge.From participated interviewer 40% of fishermen, 26.3% of food workers, 18.2% of consumers, and 15.4% of fish product retailers have poor knowledge towards fish safety.

Table 11: Overall knowledge of respondents

Characteristics		Overall Knowledge (%)		
		Poor	Moderate	Good
Gender	Male	22.45	34.7	42.86
	Female	25.8	32.25	41.93
Age	<20 year	26.7	26.7	46.7
	20-30	21.6	43.2	35.1
	30-40	31.25	25	43.75
>40 year		16.7	25	58.3
Educational Status	No Formal Educ.	27.2	36.4	36.4
	Elementary	27.27	31.8	40.9
	High school	13.3	33.3	53.3
	Diploma	25	33.3	41.7
	Degree and Above	25	35	40
Role of Respondents	Fishermen	40	13.3	46.7
Food Worker		26.3	26.3	47.4
	Retailer	15.4	46.1	38.5
Consumer		18.2	42.4	39.4

4.3.6. Overall attitudes

Generally 38.75% of respondents have positive attitudes and 22.5% have moderate attitude towards food safety of fish products. As showed in the Table 12**,** there was statistically significant association between respondents based on their roles (p= 0.017). Among the respondents, 45.45% of consumers and 57.9% of food workers have positive attitudes towards fish food safety. However, 66.7% of fishermen and 61.5% of fish retailer have negative attitudes towards handling, processing and consuming fish products.

Only 8.3% and 26.7% of respondents have positive attitude to food safety of fish products with more than 40 years old and less than 20 years respectively. About 45.2% of female respondents and 34.7% of male respondents have negative attitudes towards handling, processing and consuming fish products.

Table 12: Overall attitudes of respondents

Characteristics		Overall Attitude			P Value (Chi square)
		Good	Moderate	Poor	
Gender	Male	22	10	17	0.365 (2.01)
Female		9	8	14	
Age	<20 year	4	3	8	0.126 (9.96)
	20-30	10	8	10	
	30-40	7	4	5	
>40 year		1	3	8	
Educational Status	No Formal Educ.	2	7	2	0.08 (13.77)
Elementary		8	4	10	
High school		7	2	6	
Diploma		5	1	6	
	Degree and Above	9	4	7	
Role of Respondents	Fishermen	2	3	10	0.017 (15.5)
	Food Worker	11	6	2	
	Retailer	3	2	8	
	Consumer	15	7	11	

4.3.7. Overall practice

From 80 interviewed participants, about 51.25% have poor fish products handling practices. But, only 13.75% of respondents have good practices to the safety of fish products regarding to handling, processing and consumption habits. As showed in the Table 13, 48.9% of male respondents and 54.8% of female respondents were found poor towards practices to food safety. Based on age of respondents less than 20 year and those ages of more than 40 years had poor practice, whereas between age of 20 and 40 relatively good practices. Furthermore 60.6% of interviewed fish consumers and 69.2% of fish product retailer were found experiencing bad hygienic practices.

Table13: Overall Practice of Respondents

Characteristics		Overall Practice (%)		
		Poor	Moderate	Good
Gender	Male	48.9	40.8	10.3
	Female	54.8	25.8	19.4
Age	<20 year	46.7	40	13.3
	20-30	62.2	29.7	8.1
	30-40	50	31.25	18.75
>40 year		25	50	25
Educational Status	No Formal Educ.	45.4	54.6	
	Elementary	40.9	40.9	18.2
	High school	53.3	33.3	13.4
	Diploma	66.7	8.3	25
	Degree and Above	55	35	10
Role of Respondents	Fishermen	33.3	33.3	33.3
	Food Worker	36.8	47.4	15.8
	Retailer	69.2	23.1	7.7
	Consumer	60.6	33.3	6.1

4.3.8. Observations of hygienic status during handling, processing and consuming fish and fish products

The hygienic status of landing sites (the place where caught fish were filleted and sold to vendor), fish retailer house, and food worker were assessed. Hand washing with soap before and after handling fish products was not observed except in food worker at restaurants. Use of personal protective equipment such as glove and hairnets was observed in very few food workers by researcher. However, all observed fish retailer house used refrigerator in order kept to fish products in hygienic ways.

From this observation, it is found that all the fishermen did not handle and process under hygienic conditions at landing sites of Lake Ziway and Koka. The activity of filleting were performed without basic processing facilities such as clean processing area, clean water, equipment washing and disinfection facility, hand washing facility, and product handling facility. All fish was processed with a single knife and the same cutting board, with infrequent unclean water washing and no disinfection in between (Appendix 7). The consumption of raw fillet and undercooked (Asalebleb) were common in both study sites.

5. DISCUSSIONS

5.1. Bacterial load and indicator bacteria

Fish products are essential diets of consumers, because they are an affordable source of protein for a large portion of the global population (Edris *et al.,* 2017).However, pollution and human activity cause the environment to become contaminated. Foods may become contaminated as a result of pathogenic bacteria from humans, animals, water, or land that are found in freshwater or saltwater (Nnenna and Blessing, 2022).

Fish quality, shelf life, cross contamination, and bacterial population in the sample can be determined by the total viable bacterial count on fish and fish products (Deribe *et al.,* 2020). The bacterial load of fresh fish fillet, cooked fillet, and frozen fillet was assessed in this study. A total bacterial load of 2.00–6.00 log cfu/g is considered as normal for freshly caught fish and fish products, and most consumer safety guidelines specify that a total bacterial load of 6.00 log cfu/g is acceptable (CFS, 2014). Almost all TPC mean value of fresh fillets inthe current study was above acceptable level.

The mean of frozen raw fillet (5.91 log cfu/g) was higher than mean of cooked fish (4.54 log cfu/g), but lower than mean of fresh filleted fish samples (7.55log cfu/g). This may occur as a result of heating fish fillets, which lowers the load of bacteria even it can eliminate them at high temperature. It depends on the types processed fillet including under cooked (Asa lebleb, Asa Dulet) and properly cooked (Asa Kotelet, Asa Tibs/Wet) (Wendesen *et al*., 2017).The present research reveals that the mean of frozen fillet was lower than fresh fillet because the refrigerator or low temperature suppresses bacterial development.

The mean value of present study (5.91log cfu/g) was found to be higher than the result of Dhanapal *et al.*(2012) who found $4.9x10^4$ (4.69 log cfu/g) in frozen raw fillet samples, but lower microbial load compared with the results of Salaudeen *et al*. (2010), who reported $4.63x10^6$cfu/g (6.66 log cfu/g) in frozen raw. As compared to the present

finding, the lower mean of cooked fillet was reported in Arbaminch with mean of $4.92x10^3$ cfu/g (3.69 log cfu/g) (Wendesen *et al.*, 2017) and higher bacterial load of cooked fillet was recorded from Lake Tana (Mitiku *et al.*, 2022) with mean of $8.4x10^4$ cfu/g (4.9 log cfu/g). This variation may be brought about by improper fish preparation techniques and different types of cooked fish (either undercooked or adequately cooked).

The TPC of fresh fillet of the present finding (5.95log cfu/g to 8.99 log cfu/g) was higher than findings of Pao *et al*. (2008), who reported a total viable count ranging from 3.84 to 8.23 log cfu/g and findings of Eizenberga *et al*. (2015), who recorded a total viable count ranging up to 7.57 log cfu/g. But, reports of Hailemichael and Gutema (2021) from Supermarket of Addis Ababa indicated that the TPC of fish samples with range of 6 log cfu/g to 9.08 log cfu/g were higher than current study. A significant increase of bacterial load could be a sign of a possible spoilage and/or safety risks that can have a negative impact on the health of customers (Pao *et al*., 2008).

With regard to the source of sample the current finding revealed that a statistically significant difference between the mean of TPC of fishermen, fish retailer and restaurants was observed. The fish sampled from restaurant was ready to eat (undercooked/properly cooked), in which bacterial load could be lower. Fresh fillet samples, which were all purchased from fishermen, were expected to have a greater bacterial burden. Furthermore, bacterial contamination of the fillet could be due to poor water quality, a single filleting knife, a filleting board, and unhygienic handling conditions at the landing site. Because of the fluctuating electric power, the degradation of the fishing product may occur due to the increasing bacterial load of frozen fillet (Dhanapal *et al*., 2012).

TCC are a frequently used bacterial indicator of the hygienic quality of fish products. Coliform bacteria are ubiquitous and abundantly present in animal excrement as well as the soil, vegetation, and aquatic environments. They are simple to culture, and their presences suggest the possibility of other pathogenic organisms with fecal origin being present (Ebenezer *et al.,* 2018). The total coliform count of fresh fillet (6.1log cfu/g) was higher than the result of Mitiku *et al.*, (2022), who reported the mean result of raw fish

sample was 4.71log cfu/g. The majority of the samples were found to have coliforms, which could be due to environmental contamination from contaminated washing water, improper handling of temperature during handling and storing the fish.

The findings of the current study showed that 32.3% of 130 different types of fish samples tested positive for E. coli. It was higher than the finding of Mitiku *et al.*(2022),who isolated with prevalence of 21.21% from different fish samples in Lake Tana and its surrounding districts, findings of Assefa *et al*. (2019), who detected prevalence of 1.45% from different fish sample in Hayik and Tekeze Lakes as well as higher than the result of Tilahun and Engdawerk, (2020), who reported the prevalence of 23.3% that detected from different fish samples in Hawassa, Ethiopia. However, the prevalence detected in the current study was lower than that found in previous studies by Yohans (2021), who isolated from cooked and raw fillet from Lake Tana with a prevalence of 36% and Kumar *et al.* (2005), who isolated the Prevalence of 47% in tropical seafood.

In the present finding fresh fillet samples of fish had a higher *E. coli* prevalence than cooked fish samples. This could be due to the heat effect during processing. The findings of Gupta *et al.* (2013), who isolate 48.95% *E. coli* in raw fish samples and 12.96% *E. coli* in ready-to-eat fish product samples, support this work. Similar findings were observed by other researchers on the frequency of bacteria isolated from heat-treated fish and other ready-to-eat food items (Shaltout, 2017 and Omnaz *et al.*, 2020).

The current study revealed that 53.3% of frozen raw fillet samples were positive for *E. coli*. This result is higher than the result of Wendesen *et al.* (2017) who reported that the prevalence of 42.5% *E. coli* from frozen raw fish in Arbaminch, Ethiopia. Based on fish species higher prevalence of 38.5% from Tilapia was detected than Catfish with 32% positive for *E. coli*. This study agree with findings of Mitiku *et al.*(2022);Sujatha *et al*. (2011); Hanson et al. (2008),thus findings were reported higher presence of *E. coli* from Tilapia species. However, according to Tilahun and Engdawerk (2020), there was no difference in the prevalence of *E. coli* in species of fish, which is contrary to this finding.

This disagreement could be due to variations in sample sizes, the environment of the study location, or sampling techniques.

The prevalence of Salmonella spp that was collected from 130 different fish samples during the study period was3.1%.The presence of Salmonella in this study was found to be lower than the report in Brazil with a prevalence of 4% (Ferreira *et al*., 2014), Nigeria with a prevalence of 11.5% (Raufu *et al*., 2014), Saudi Arabia with a prevalence of 26.6-64% (Elhadi, 2014), as well as Ethiopia with a prevalence of 6.82% at Lake Tana, 7.5% at Lake Chamo, and 30% at Lake Tinike(Mitiku *et al.*, 2022and Hiko *et al.*, 2018).

The prevalence of salmonella of present study was higher than the finding recorded in Spain (2.5%) (Martinez *et al.,* 2004) and in Brazil (2.8%)(Duarte *et al.,* 2010).These variations in the occurrences of Salmonella species could be due to difference in hygienic practice, environmental factors, sample size, type of fish samples taken (Gawish *et al*., 2021).

The detection of Salmonella in different fish samples could be due to unhygienic filleting of fish, bacterial contamination from gut of fish by contaminating handler's hand, filleting knife or filleting board (Fernandes *et al*., 2018). Additionally, unsanitary conditions may exist during fish harvesting, food processing, and fish storage, and they may be caused by human or animal excrement (FDA, 2021).

The current study showed that the prevalence of *S. aureus* was 16.9%.In Ethiopia; Mitiku *et al.* (2022) reported the lower prevalence (4.8%) of *Staphylococcus aureus* than the current findings. The higher occurrences of thus bacteria were reported from fish samples according to the findings of Obiadat *et al.*(2015), Hammad *et al.*(2012) and Muhammed *et al.*(2015), with prevalences of 47.2%, 87%, and 31.8% in Jordan, Japan and Egypt respectively.

Staphylococcus aureus infections in fish may result from cross-contamination and improper handling of fish and fish products (Da Silva *et al.*, 2020). The heat-resistant *S. aureus* enterotoxins pose a risk to the public's health. Poor hygiene practices during food processing are strongly associated with *S. aureus* enterotoxin infection. Inadequate cooking, contaminated water, or raw materials used in food preparation are a few factors that may contribute to infections(Naas *et al.*, 2019). The occurrences of *S. aureus* from all types of sample of the present studywere 5%, 24.6%, 13.3%, 25.6% and 12.5% from cooked fillet, fresh fillet, hand swab, filleting board and filleting knife swab respectively. The higher prevalence of bacteria from fresh fillet could be due to improper handling of filleted fish. The presence of *S. aureus* from cooked sample was lower than fresh fillet this may be due to effect of heat during food processing of fish fillet and sample size analyzed for cooked fish was less.

Staphylococcus aureus was found in 28.5%, 13.3%, and 12.5% of the samples from the filleting table, hand swab, and filleting knife, respectively. Additionally, a hand swab of a fish handler's, a filleting table, and a filleting knife, respectively, revealed 40%, 57.1%, and 12.5% of *E. coli* positives. As well as the current study showed 13.3% Salmonella positive were from filleting boards. The detection of those bacteria from hands of handler’s and filleting equipment could be due to contamination of human caused sewage pollution, animal waste, or inadequate sanitary conditions during fish handling, processing and preparation of food (Penakalapati *et al*., 2017).

5.2. KAP of handling processing and consumption of fish product

In addition to detect the prevalence of Salmonella, *E. coli, S. aureus* and determine the load of bacteria, in this study we assessed KAP of handling, processing and consumption of fish and fish product using a combination of questionnaire survey and direct observation to measure the hygienic condition. Training of fish handlers, fishermen, fish retailer and food workers about personal hygiene and hygienic handling of fish holds the potential to guarantee the delivery of safe fish products to the consumer. However, the majority of participants (66.25%) had no formal training of food safety practices of fish

and fish products. This result was similar with the previous result of Mekonnen *et al.*, (2012) who obtained 61.5% of meat handlers not to take food safety training.

Although most of fish retailer and fishermen of the current study attention to continue fish handling for the sake of business/economic reasons with less concern to public health requirement, the several studies have showed that infected individual is at risk of transmitting pathogens and contamination of fish (Rane, 2011 and Alimi, 2016). According to the findings of Adam and Moss (2008) and WHO (2006), number of food handlers lacked awareness regarding the critical role of basic temperature control measure in controlling microbial growth in food and the associated risks of microbial hazards. However, 78.75% of respondents of the present study had good awareness about storage of fish products in which they preferred refrigerators were safest storage equipments.

Potential causes of the contamination of natural environments include the exposure of water bodies to tainted water and open feces by nearby people and animals. The use of contaminated water for fillet was observed in current study that contradicts the codex Alimentarius Commission. The codex general principle of food hygiene declares the control of water quality to minimize the risk of potential biological, chemical, and physical hazards (FAO and WHO, 2021). Similar to our study, Huss *et al*. (2003) reported unsanitary processing or inadequate water quality are the main causes of contamination of fish products.

Similar to study by Bedane *et al*. (2022), our finding revealed that fishermen engaged in fish processing at unsanitary landing place nearby lake may have contributed for contamination of the fish products. In addition to unhygienic landing site, the utilization of single knife and filleting board for processing all varieties of fish, without undertaking any sanitary procedures for equipment, manifests hazardous practices that results the possibilities of contamination of fish during the production process. However the current findings were contrary to FAO advices, which says that improper post-harvest handling and processing techniques compromise consumer safety (FAO, 2009).

The present study showed that 23.75% of respondents had poor knowledge on food safety of fish and fish products and 38.75% of participants had negative attitude toward handling, processing and consumption of fish products as well as 51.25% of interviewer poor hygienic practice of fish products. Similar to our finding Grema *et al*. (2018) and Walker *et al*. (2003) reported that having good knowledge of food safety is insufficient for positive sanitary attitude and good practice among fish handlers and consumer. But, most of study indicated the importance of food safety knowledge reporting that good knowledge mostly translates into positive behavior and practices leading to safe food production and handling/ strong positive correlation between knowledge, attitude and practices/ (Sani and Siow, 2014; Al-Shabib *et al*., 2016; Hashanuzzaman., 2020).

The present study showed among participants including fishermen, fish retailer, food workers and fish consumers only 37.5% and 31.25% believed consuming raw and undercooked fish increases risks to human health respectively. However, several findings revealed that consumption of raw, undercooked or cooked fish could result in food borne disease or food poisonings due to contamination and cross contamination of fish and fish products (Austin *et al*., 2005; FAO, 2009; Kalimuddin *et al*., 2017 and Mitiku *et al*., 2022).

Unhygienic handling and processing practices of fish products reported in this study may not be attributed to insufficient regulation of food safety. Consequently, a prompt and imperative intervention is required to develop and implement a national plan for fish quality and safety, thereby safeguarding the safety of the general public. Promoting the consumption of properly cooked fish is an effective mitigation strategy in addressing the risks associated with fish borne zoonotic diseases. Such proactive measures support the prevention of potential health hazards inflicted by contaminated fish products.

Limitation of the study

The molecular confirmation for identification of bacteria was not implemented due to lack of resources.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed that the total viable count and total coliform count obtained from cooked, frozen and fresh fish fillet samples exceeds the standard levels recommended by center for food safety. The pathogenic bacteria such as Salmonella species, *Escherichia coli*, and *Staphylococcus aureus* were detected from swab sample as well as both raw and cooked fillet that could be great risks to public health. High bacterial load and pathogenic bacteria was detected in frozen and cooked fillets that could be due to cross contamination, in adequate cooking, lack of cold chain storage, and fluctuation of electric power which is suitable for bacterial proliferation that degrade fish products. Thefindings showed that 23.75%, 38.75%, and 51.25% of interviewer had poor knowledge, negative attitudes, poor food safety practices of fish and fish products respectively. The majority of fish handlers and processors had not participated in a formal food safety training program. Unhygienic practices includes washing fish products by tainted water and use poor quality filleting board and single knife without disinfection or infrequent cleaning was observed and this leads to contamination of fish products. The habit of consuming raw and improper cooked fillet was very common. The study showed poor KAP high bacterial load and presence of food borne pathogens that have great risks to public health.

Therefore, based on the above conclusion, the following recommendations are forwarded:

- It is essential to give training for fish handlers and processors on the hygienic and sanitary handling of food products.
- Awareness regarding to risks of consuming raw and improperly cooked fish products and about food borne disease should be given.
- The hygienic status of filleting equipment's and fish landing area in Lake Koka and Lake Ziway should be kept clean.
- Cold chain equipment with uninterrupted power supply should be implemented in fish product retailer.

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8. APPENDICES

Appendix 1: Procedures of Total Viable Bacterial Counts

Twenty five grams of raw or cooked fish samples was homogenized by laboratory blender (Stomacher 400) in a sterile plastic bag containing 225 ml of buffered peptone water. A series of 6 test tubes for each homogenate fish sample containing 9 ml of sterile peptone water was prepared. By using sterile pipette, 1ml of sample in the first tube was added and labeled as 10^{-1}, the dilutions was mixed using a vortex mixer. From the first tube, 1ml of the sample was taken and transferred to second tube. This was labeled as 10^{-2}. The procedure was repeated with all the remaining tubes labeling them until 10^{-6}. 0.1 ml from the appropriate desired dilution series was putted onto the center of the surface of separate, marked, sterilized PCA agar plate. The L-shaped circular glass spreader (hockey stick) was dipped into alcohol. The glass spreader was flamed over a Bunsen burner. The sample was evenly spread over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at an angle of 45^{o} at the same time. The plate was incubated at 37°C for 24-48 hours.

Appendix 2: Procedures of Total Coliform Bacterial Counts

25 g of raw or cooked fish samples was homogenized by laboratory blender (Stomacher 400) in a sterile plastic bag containing 225 ml of buffered peptone water. A series of 4 test tubes for each homogenate fish sample containing 9 ml of sterile peptone water was prepared. By using sterile pipette, 1ml of sample in the first tube was added and labeled as 10^{-1}, the dilutions was mixed using a vortex mixer. From the first tube, 1ml of the sample was taken and transferred to second tube. This was labeled as 10^{-2}. The procedure was repeated with all the remaining tubes labeling them until 10^{-5}. 0.1 ml from the appropriate desired dilution series was putted onto the center of the surface of separate, marked, sterilized VRBA plate. The sample was evenly spread over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at an angle of 45^{o} at the same time. The plate was incubated at 37°C for 18-24 hours.

Appendix 3: Principles and Procedures of Biochemical test

Indole test: Indole test ascertain the capacity of an organism to enzymatically cleave indole from the amino acid tryptophan via tryptophanase activity (Kumar and Rupam, 2018). The occurrences of E. coli was determined by formation of pink to red or (cherry red ring) within seconds subsequent to the addition of Kovac's reagent to the top layer of the medium. Procedures were:

- A small amount of a pure culture was inoculated into the tube of tryptone broth
- Incubated at 37°C for 24 to 48 hr.
- After incubation, few drops of Kovac's reagent were added to the tube.

Simmon's Citrate Test: This test was performed in order to differentiate bacteria based on the capacity to utilize citrate as primary source of carbon or not. Salmonella species were positive for citrate, whereas *E. coli* positive were negative for citrate utilization test. The following procedures were performed for citrate test:

- A sterile wire loop was used to inoculate the isolates into the slants and incubated at 37˚C for 24 h after which it was examined for color change.
- A change in color of the medium to bright blue color gives a positive citrate utilization test while green coloration is considered negative.

Urease Test: The current study employed the Urease test in order to assess the ability of microorganisms to decompose urea through the catalytic activity of the enzyme Urease. It was performed as to differentiate Urease positive Proteus spp from Salmonella and Urease positive klebsiella from E. coli. Procedures were:

- Salmonella positive from XLD agar and *E. coli* positive from EMB were inoculated on the urea slant media by means of loop of inoculation.
- Non-inoculated tube was kept as control.
- The tubes were incubated at 37°C for 24 hours and the reaction was observed.
- The inoculated tubes were observed to see if pink colour has developed or not.

- Development of pink colour indicator a positive test and no colour change shows a negative test. Both tested bacteria (Salmonella species and *E. coli*) were Urease negative that helps us to differentiate them from Urease Positive bacteria including Proteus spp. and Klebsiella spp.

Triple Sugar Iron Agar (TSI): TSI test was utilized to recognize the microorganisms based on the capacity to ferment the carbohydrates. The identification of *E. coli* samples was confirmed when acid Slant / acid butt with gas and the absence of H2S production were observed in results. The utilization of TSI medium enabled the observation of the alkaline slant/acid butt with gas production as well as the generation of hydrogen sulfide which was indicative of the presence of Salmonella species in a test suspected of Salmonella. The procedures were as follows:

- *E. coli* and Salmonella suspected samples were sub cultured on nutrient broth and inoculated at 37^{o}C for 24hr.
- The suspension was inoculated into the labeled TSI slant media by means of stab and streak inoculation.
- Non inoculated tube was kept as control, both tubes were incubated at 37°C for 24 hours and the reaction was observed.

Lysine Iron Agar (LIA) Slant Test: The utilization of LIA slant tests utilized to differentiate bacterial strains that hold the potential to decarboxylate lysine and/or generate hydrogen sulfide from those that lack such attributes. Four of the suspected Salmonella suspensions displayed blackening at the apex of the butt, along with purple slant.

Procedures:

- The Sterile LIA slant medium was inoculated by Salmonella suspected enrichment from nutrient broth twice stabbing through the center of the medium to the bottom of the tube and slant was streaked.
- The tube capped tightly and incubated at 37°C for 18 to 24 hours.
- At 24 and 48 hours for growth and color changes in tube butt and slant and for blackening at the apex of slant were examined.

SIM (Sulfur Indole Motility) Agar Test: The ability of an organism to reduce sulfur, produce indole, and exhibit motility through agar is assessed through the utilization of SIM. The SIM test was employed in current study for the purpose of differentiating discerning Salmonella from other bacterial species like Shigella. The procedure of SIM was:

- Each of sterile SIM medium was labeled clearly.
- Using a sterile inoculating needle, the center of a well isolated colony was touched.
- Aseptically a depth of 3/4 in the middle of the corresponding tube was stabbed once. The needle was withdrawn and sterilized it by Bunsen burner.
- The remaining tubes were inoculated aseptically in the same way and incubated aerobically at 37°Cfor 24 hours.
- The motility and formation of black precipitate were observed. Motile organisms spread out into the medium from the site of inoculation. Non motile organisms remain at the site of inoculation.

Catalase Test: The present study employed the catalase test as a means to distinguish between bacteria that exhibit catalase enzyme activity, in this case the Staphylococci, and non catalase producing such as Streptococci. The procedure for catalase test was:

- Few drops of 3% hydrogen peroxide were poured into a pure colony of Staphylococcus suspected bacteria on nutrient agar.
- The production of the bubble indicates a positive catalase test.

Coagulase Test: The coagulase was used for detection and identification of Staphylococcus aureus, a bacterium that exhibit the ability to secret the enzyme coagulase.

Procedure for coagulase test was:

- A drop of distilled water was placed on each end of a clean grease-free slide.
- A colony of the test organism was emulsified in each of the drops to make two thick suspensions and mixed gently.
- Rabbit Plasma was added to one of the suspensions.

The clumping of the organism after 10 seconds indicates a positive result.

Gram Staining

Principle: Some bacteria are able to retain the primary stain after being stained with the primary stain Crystal Violet and fixed by the mordant, while other bacteria are discolored by alcohol. Gram positive bacteria have low levels of lipid and a thick covering of peptidoglycan, a protein-sugar complex. The cell's thick cell wall becomes dehydrated and shrinks as a result of decolorization, which seals the cell wall's pores and keeps the stain from leaving.

The procedure of Gram staining was:

- Fixation of Staphylococcus suspected samples to the surface of the microscope slide by using methanol.
- Application of the primary stain (crystal violet). Crystal violet is a dark blue to purple dye. It stains all cells blue/purple.
- Application of mordant: The iodine solution (mordant) is added to form a crystal violet iodine complex, all cells continue to appear
- The decolorization step distinguishes gram-positive from gram-negative cells. The gram-negative bacteria appear colorless, and gram-positive bacteria remain blue.
- Application of counterstain (safranin): The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue.

Appendix 4: Procedures of Media and Laboratory Reagents used for biochemical tests, colony counting and isolating bacteria

1. Plate Count Agar

PCA (plate count agar) /standard methods agar were prepared according to manufacturer (Himedia-M091).

- Suspend 23.5g of PCA in 100ml distilled water.

- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 121^{o}C for 15 minutes.
- Cool to 45-50^{o}C. Mix well and pour into sterile petriplate

2. Violet Red Bile Agar (VRBA) (Oxoid)
    - Suspend 38.5g VRBA in 1 liter of distilled water.
    - Bring to the boil to dissolve completely.
    - No further sterilization was necessary. Cool to 50^{o}C and mix well before pouring into sterile petri plates.
3. Buffered Peptone Water (HIMEDIA/M614-500G)
    - Suspend 20g in 1000ml distilled water
    - Dissolve well using magnetic stirrer
    - Dispense in 9ml for serial dilution and dispense in sterile bottle for further sample preparation
    - Sterilize by autoclaving at 121^{o}C for 15 minutes.
    - Pour 225ml into sterile Plastic bag for sample preparation.
4. Rappaport Vassiliadis (RV) Enrichment Broth (OXOID/CM0669)
    - Weigh 30g RV and add 1litre distilled water
    - Heat gently until completely dissolved
    - Dispense 10ml volumes into test tubes
    - Sterilize by autoclaving 115^{o}C for 15 minutes. This medium is very hydroscopic and must be protected from moisture.
5. Nutrient Broth (OXOID/CM001)
    - Add 13g to 1litre distilled water
    - Mix well and distribute 3-4ml into test tubes.
    - Sterilize by autoclaving at 121^{o}C for 15 minutes
6. Xylose-Lysine Deoxycholate Agar (XLD)(HIMEDIA/M031-500G)
    - Suspend 56.68g in 1000ml distilled water.
    - Heat with frequent agitation until the medium boil.
    - Don’t autoclave / overheat
    - Transfer immediately to a water bath at 45-50^{o}C.
    - Mix well and pour into sterile petri plate

7. Nutrient Agar (OXOID/CM003)
    - Suspend 28g in 1litre of distilled water.
    - Bring to boil to dissolve completely
    - Sterilize by autoclaving at 121^{o}C for 15 minutes.
8. Mannitol Salt Agar (OXOID/CM0085)
    - Suspend 111g in 1litre of distilled water and bring to boil to dissolve completely.
    - Sterilize by autoclaving at 121^{o}C to 15 minutes, mix well before pouring.
9. EMB Agar (HIMEDIA/M317-500G
    - Suspend 35.96g in 1000ml distilled water. Mix until suspension is uniform.
    - Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Avoid overheating.
    - Cool to 45-50^{o}C and shake the medium in order to oxidize the methylene blue (to restore its blue color) and to suspend the flocculent precipitate.
    - Mix well and pour into sterile petri plates.
10. Transport media (Carry-Blair Medium)(BIO-MARKLaboratory/B017)
    - Formula: Disodium phosphate, sodium thioplyccllate, sodium chloride and Agar
    - Suspend 12.6g in 991ml distilled water and boil to dissolve the medium completely.
    - Cool to 50^{O}C and aseptically add 9ml of 1% aqueous calcium chloride solution.
    - Distribute in7ml amounts in screw capped tubes. Steam for 15minutes. Cool and tighten the caps.
11. Triple Sugar Iron Agar (HIMEDIA/M021-500G)
    - Suspend64.52g in 1000ml distilled water
    - Heat to boiling to dissolve the medium completely
    - Mix well and distribute into test tubes.
    - Sterilize by autoclaving at 115^{o}C for 15 minutes.
    - Allow the medium to set in sloped form with a butt about 2.54cm.

12. Simmons Citrate Agar (HIMEDIA/M099-500G)
    - Suspend 24.28g in 1000ml distilled water and heat to boiling to dissolve the medium completely.
    - Dispense as desired in tubes or flasks.
    - Sterilize by autoclaving at 121^{o}C for 15 minutes. Allow the medium to set in sloped form.
13. Lysine Iron Agar (Accumix^{R})
    - A medium for differentiation of enteric organisms especially Salmonella species, based on their ability to decarboxylate or deaminate lysine and production of hydrogen sulphide.
    - Suspend 34.56g of the powder in 1000ml distilled water. Mix thoroughly.
    - Boil with frequent agitation to dissolve the powder completely. Don't overheat.
    - Sterilize by autoclaving at 121^{o}C for 15minutes. Prepared appearance: purple colored, slightly opalescent gel forms in tubes.
14. SIM medium
    - Suspend 30g of the powder in 1litre of distilled water.
    - Mix well thoroughly, heat with frequent agitation and boil for 1 minute.
    - Suspend into test tubes and capped well.
    - Autoclave at 121^{o} C for 15 minutes.
15. Tryptone Broth
    - Ingredient: Tryptone 10g and Sodium chloride 5g.
    - Dissolve the ingredients in 1litre of sterile water.
    - Dispense 4ml per tube and autoclave at 121^{o}C for 15 minutes. Store the tubes in the refrigerator at 4 to 10^{o}C.
16. Urea Agar Base
    - Suspend 29g of the powder in 100ml of purified water. Mix thoroughly.
    - Suspend 15g of Agar in 900ml of distilled water. Autoclave at 121^{o}C for 15minutes. Cool to 50^{o}C and add 100ml of urea agar base. Mix thoroughly and dispense aseptically in sterile tubes.
    - Cool the medium in tubes in slanted position so that deep butts are formed.

17. Kovac's Reagents for Indoles (SIGMA-ALDRICH/60983-100ML)
    - Composition: n-butanol, HCL, 4-(dimethylamino) benzaldehyde.
    - Description: In the presence of oxygen, some bacteria, like *E. coli*are able to split tryptophan into indole and alpha amino propionic acid. This reagents was used for detecting the indole and identify the indole positive and indole negative microorganisms.
18. Rabbit Plasma

    Rabbit plasma is preferable, as it gives better clotting, is free from inhibitors and is safe. When bound coagulase is present on the bacterial cells, then presence of plasma can cause the bacterial cells to clump.
19. Hydrogen Peroxide

    3% hydrogen peroxide is the reagents used to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.

Appendix 5: Questionnaire survey

A. Demographic and operational characteristics of respondent from Koka and Batu town.

1. Gender: a. Men b. Women

2. Educational level a. no formal education b. elementary school c. high school d. diploma e. degree and above

3.Role of respondent a. fishermen b. food worker c. retailer d. consumer

4. Age a. <20 b. 20-30 c. 30-40 d. > 41

B. Assessment of food safety knowledge of respondent towards to handling, processing and consumption of fish

1. Washing hands before work reduces the risk of fish contamination a. true b. false c. I don't know

2. Using protective equipment during work reduces the risk of food contamination a. yes b. no c. I don't know

3. Proper cleaning and handling of contact surfaces reduces the risk of food contamination a. yes b. no c. I don't know.

4. If you have diarrhea, it is necessary to stay away from handling fish for consumption/business a. true b. false c. I don't know

5. If you have skin wound on hands, it is not necessary to cover it when handling fish a. true b. false c. I don't know

6. Food safety training a. yes b. no

7. Contaminated fish can transmit disease a. yes b. no c. I don't know

8. The refrigerator is the safest fish storage facility a. yes b. no c. I don't know

C. Food safety attitude of respondenttowards handling, processing and consumption of fish

1. Raw and cooked fish should be stored separately to reduce risk of food contamination a. yes b. no c. I don't know

2. Eating raw fish increases the risk to human healtha. true b. false c. I don't know

3. Eating undercooked fish increases the risk to human health a. true b. false c. I don't know

4. Fish kept at room temperature can be contaminated a. true b. false c. I don't know

5. Fish kept at room temperature can cause disease a. true b. false c. I don't know

D. Food safetypracticeof respondent towards handling, processing and consumption of fish

1. Do you eat fisha. yes b. no

2. Do you eat undercooked fish? a. yes b. no

3. Do you clean transportation materials, vehicles with clean water after use? a. always b. sometimes c. never

5. Do you prefer consuming raw fish? a. yes b. no

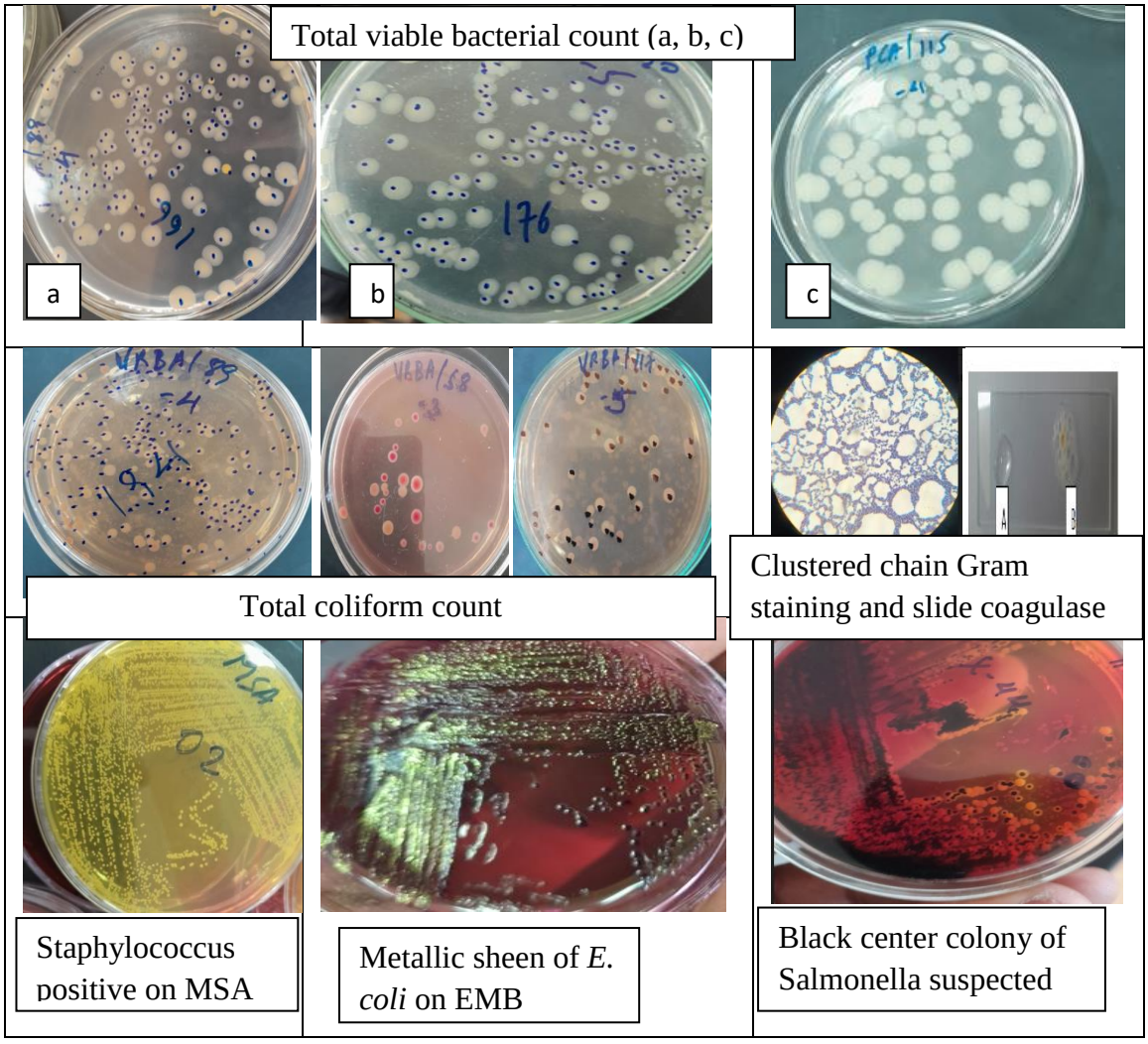
6. Do you store left over fish at room temperature for sale the next day?a. always b. sometimes c . never

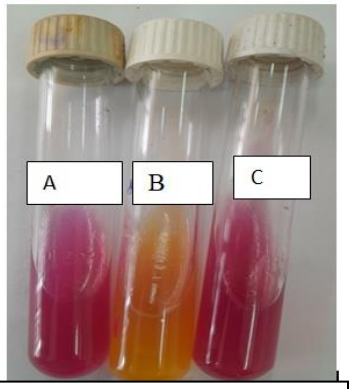
Appendix 6: Standard level of bacterial colony by Center for Food Safety organization.

Level	Mean Value (LOG cfu/g)
Satisfactory	< 1.3
Intermediate	1.3-2
Unacceptable	>2

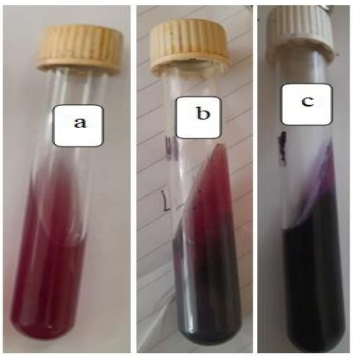
Appendix 7: Picture that shows hygienic status of landing area and Fish species in the Study Area

Appendix 8: Picture of Bacteria Colonies, Cultured bacteria on selective media and Biochemical tests

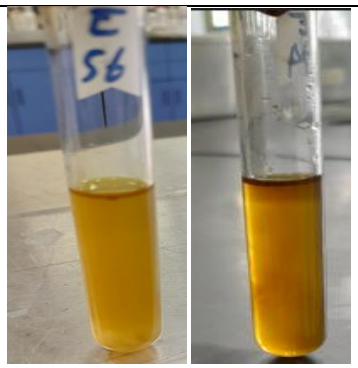




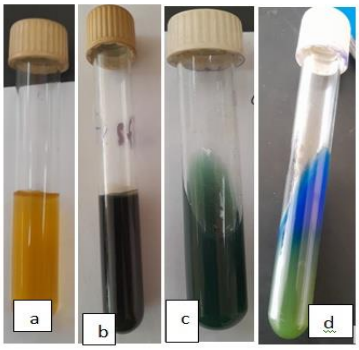
Urease test (A and C= positive, B= negative)



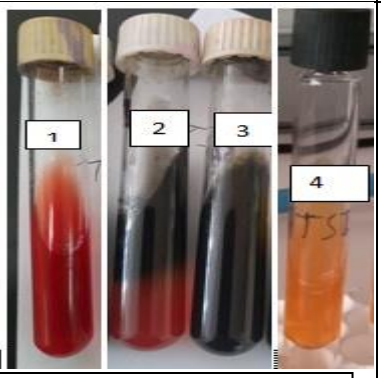
LIA test (a= negative control, b and c= positive results



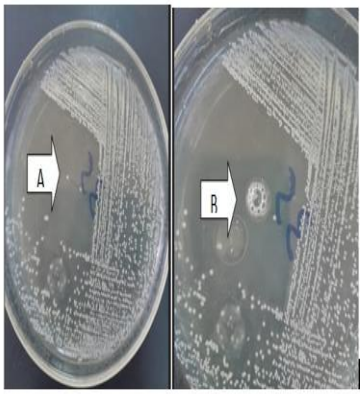
Positive Indole test of *E.coli*



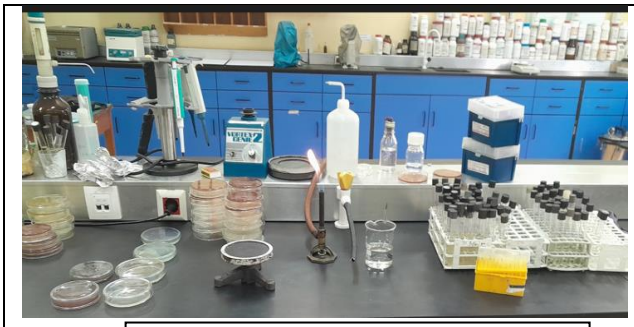
SIM= a (negative), b (positive), Citrate= c(negative), b



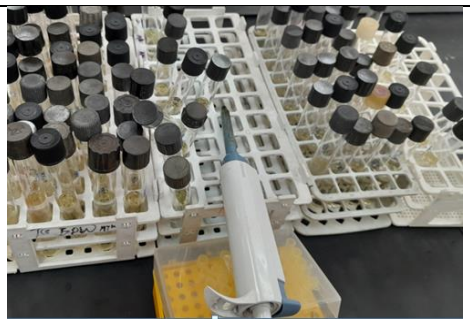
TSI 1 (negative), 2 and 3 (positive for Salmonella), 4 (positive for *E. coli*)



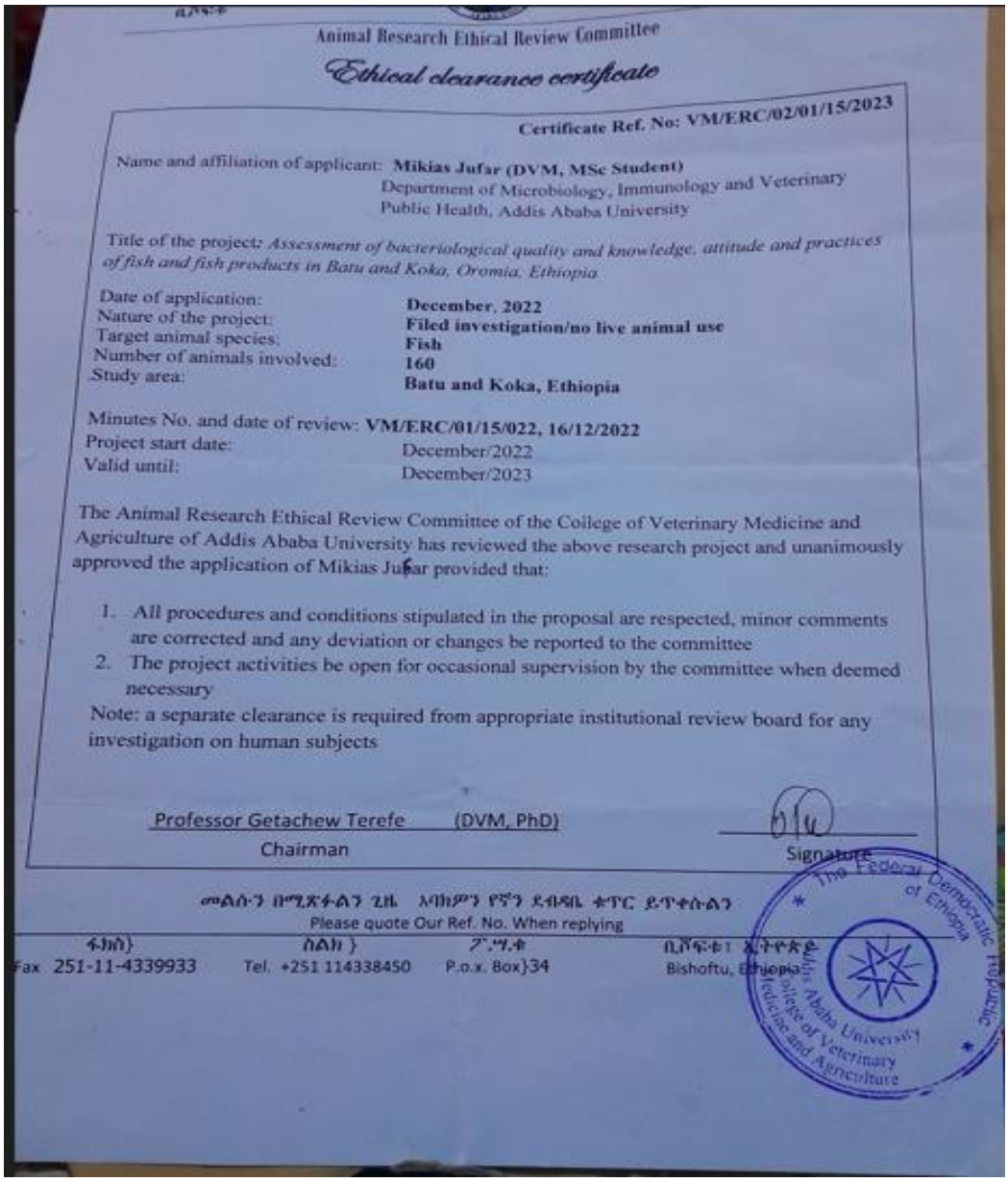
Bubble formation of Catalase reaction



Spreading suspension on plate



BPW of serial dilution



Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/02/01/15/2023

Name and affiliation of applicant: **Mikias Jufar (DVM, MSc Student)**
Department of Microbiology, Immunology and Veterinary Public Health, Addis Ababa University

Title of the project: *Assessment of bacteriological quality and knowledge, attitude and practices of fish and fish products in Batu and Koka, Oromia, Ethiopia*

Date of application:	**December, 2022**
Nature of the project:	**Filed investigation/no live animal use**
Target animal species:	**Fish**
Number of animals involved:	**160**
Study area:	**Batu and Koka, Ethiopia**

Minutes No. and date of review: **VM/ERC/01/15/022, 16/12/2022**

Project start date:	December/2022
Valid until:	December/2023

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Mikias Jufar provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when deemed necessary

Note: a separate clearance is required from appropriate institutional review board for any investigation on human subjects

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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

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Please quote Our Ref. No. When replying

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