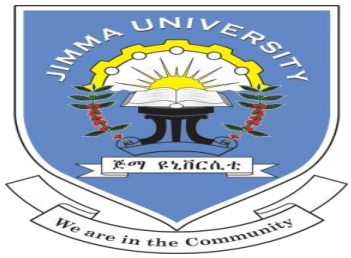

College of Natural Sciences

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Department of Biology

Microbiological Quality and Safety of Fish Fillet Collected from Gilgel Gibe and Jimma City Fish fillet selling shops, Southwest Ethiopia

By: Asfaw Beyene

A Thesis Submitted to Department of Biology, College of Natural Sciences, Jimma University in partial fulfilment of the requirement for the Degree of Master of Science in Biology(Applied Microbiology)

October 2021

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Candidate

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Signature _____

Date _____

The research has been done under supervision of

Name _____

Signature _____

Date _____

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List of Acronyms

AMB	Aerobic Mesophilic Bacteria
ANOVA	Analysis of Variance
CV	coefficient of variation
MR	Methyl red
MSA	Mannitol Salt Agar
O/F	Oxidation Fermentation
PCA	Plate Count Agar
PDA	Potato dextrose agar
SIM	Sulphide Indole Motility
TSCBSA	Thiosulfate Citrate Bile Salt agar
VP	Voges-Proskauer
VRBA	Violet Red Bile Agar
VRBGA	Violet Red Bile Glucose Agar
XLD	Xylose lysine Deoxy cholate

Abstract

Fish fillets (muscles) are sterile, however, they can be cross-contaminated during handling and storage. The present study aims to assess the microbial quality and safety of fish fillets sold in Gilgel Gibe and Jimma city shops. Across sectional study design was used. A total of 30 fish fillets were collected from both sites and analysed for microbial load and detection of pathogens. The analysis of microbial load, detection of pathogens, and antibiotic resistance patterns of pathogens were conducted based on the standard procedures. Moreover, the risk associated with fish fillets was also assessed using a semi-structured interview. The findings showed that the count of AMB 8.28 ± 0.09 log CFU/g followed by staphylococci 6.86 ± 0.17 Log CFU/g was higher in fish fillets collected from Jimma city shops. Similarly, the AMB and staphylococci counts were also greater than others in the Gilgel Gibe site. The mean microbial counts in both sites showed a significant difference at P <0.05 with % CV < 10%. A total of 312 bacterial isolates were obtained, characterized, and grouped into seven genera and one family. Generally, Gram-positive bacteria greater in count than the Gram-negative bacteria. Among the isolates Staphylococcus spp. Were the predominant in both sites which accounted for 32.37%, followed by Enterococcus spp. 20.19% and Bacillus spp. 18.91%. Overall, 43.33% of fish fillets were positive for S.aureus and Listeria spp 23.33. while Vibrio spp was the lowest prevalent 16.67%. On the other hand, S.aureus were highly resistant to Penicillin G 100%, followed by Ampicillin 92.31%, and tetracycline 84.62% while Listeria spp was also highly resistant to Penicillin G 100% followed by clindamycin, erythromycin, and vancomycin 85.71%, each). Moreover, Salmonella spp were highly resistant to ampicillin whereas Vibrio spp were highly resistant to vancomycin. Lack of washing hands before work, lack of using gloves during work, lack of disinfecting working surfaces and tools using disinfectants, sellers direct contact of paper currency, lack of wearing a mask during work, use open toilet around vending area, storing and processing fish fillet in open filed, lack of training on the safety and quality, and transporting long distances for selling fish fillet are the associated risk factors. In general, the analysed fish fillets were contaminated by pathogens and had a high load of microbes. Hence, proper handling and storing can enhance the quality and safety of fish fillet that benefits both producers and consumers.

Key words; Fillets, Fish, Microbial quality, Microbial safety

1. Introduction

1.1. Background of the study

Fishes are classified as any of the cold-blooded aquatic vertebrates of the superclass Pisces typically showing gills, fins, and a streamlined body. There are about 22,000 species of fish that began evolving around 480 million years ago (Pal and Mahendra, 2015). Fisheries are one of the important and renewable natural resource bases for many developing countries, and the livelihood of many rural communities thatrely on the fishery sector reduces poverty and it could be considered as a potential strategy because it helps to diversify the household income directly and indirectly (Olale and Henson, 2013). In the developing world, about 116 million people are benefited from the fishery sector and about 90% of them are working in the small-scale fisheries sector (Hebano and Wake, 2020). Historically, Africa's fisheries output is dominated by capture fisheries and the total amount of fish produced from aquaculture is grown from time to time over the past decade (Adeleke *et al*., 2021).

In Ethiopia, fish production depends on the inland waters for the supply of fish as a cheap source of animal protein which indirectly contributes by providing revenue for purchasing food for deficient areas (Dawit *et al*., 2013). The country has different geological formations and climatic conditions is endowed with considerable water resources and wetland ecosystems, including river basins, major lakes, many swamps, floodplains, and man-made reservoirs. The fish supply in most cases comes from the major lakes and some reservoirs such as Hawassa, Tana, Chamo, Ziway, Koka, Abaya, Gilgel Gibe, and rivers in the country. The benefits gained from the development of fisheries are significant. From local to global levels, fisheries play an important role in the food supply, income generation, employment creation, and nutrition security (Hebano and Wake, 2020).Fish is an important part of a healthy diet due to its high-quality protein, other essential nutrients, and omega 3-fatty acids, and its low-fat content as compared to other fillets (Rhea, 2009). In a world where nearly 30 percent of humanity suffers from malnutrition and more than 70% of the planet is covered by water, aquatic food especially fish forms an essential part of human nutrition and play a crucial role within the nutrition of the poor as a reasonable and far needed source of top-quality animal protein and essential amino acids, omega-3 fatty acids, vitamins, minerals, and

trace elements which maintain a healthy life (Mohanty, 2020). Fish fillet is the product that constitutes an important food commodity in the international trade due to its ever-increasing consumer demand due to 60% of the world supply of protein, and 60% of the developing world derives more than 30% of their animal protein from fish, and low cholesterol level and presence of essential amino acids (Emikpe *et al.*, 2011).

Fish fillets are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues (WHO, 2007). However, contamination often occurs from human and animal sources, and thus, fish can be involved in the transmission of pathogenic microorganisms and toxins (Pal, 2012).Microbial contamination on environmental surfaces may be transferred to the food products directly through surface contact or by vectors such as personnel, pests, air movements, or cleaning regimes, careless handling of landed fish, its stowing, and cutting (Sheng and Wang, 2020).

It is well accepted that microorganisms are commonly present on fish surfaces, such as skin and gills, as well as inside of the fish in areas such as the digestive tract and internal organs, for example, the kidney, liver, and spleen. However, fish and fish products, especially raw or undercooked products, have been involved in outbreaks associated with bacterial pathogens, bio toxins, histamine, viruses, and/or parasites (Galaviz-Silva*et al.*, 2009). Fish have been identified as reservoirs of bacterial pathogens linked to human diseases causing microbes including *Mycobacterium spp., Streptococcus spp, Vibrio spp,* pathogenic *Escherichia coli, Aeromonas spp., Salmonella spp., Staphylococcus aureus, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens,* and *Campylobacter jejuni*. (Novotny*et al.*, 2004). Furthermore, concerns about the contamination and proliferation of human pathogens in fish and fish products, the growth of certain spoilage, and pathogens (Biji *et al.*, 2016). Thus, improperly handled and processed fish fillet consumption may cause diseases due to intoxication (Adebayo-Tayo *et al.*, 2012a). In addition, the presence of antibiotic-resistant genes in these microorganisms has caused concerns about spreading antibiotic resistance in the environment and to human beings (Brunton *et al.*, 2019; Preena *et al.*, 2020). In addition to human pathogens, bacteria are considered the primary cause of fish spoilage (Rippen & Skonberg, 2012). Some pathogens isolated from fish fillet such as *Mycobacterium spp* are completely insusceptible to isoniazid, rifampicin, clofazimine, streptomycin, and

erythromycin (Guz *et al*. 2013). Sing *et al*. (2016) recovered multiple drug-resistant zoonotic pathogens, Salmonella spp with higher multiple antibiotic resistance from African catfishes belonging to different fish farms, which highlighted the exposure of cultured catfishes to several antibiotics. There are also several non-pathogens and beneficial bacteria associated with commensal bacteria in fishes that are also found to possess antimicrobial resistance genes, which acquire from pathogenic microorganisms (Duran and Marshall, 2005).

Foodborne illnesses have become a significant global health problem due to the increased consumption of foods contaminated with pathogenic microorganisms or their toxins that causes approximately 24,484 illnesses, 5677 hospitalizations, and 122 deaths in the United States in 2017 with an increase in a 96% increase compared to 2014-2016 (CDC, 2017). Among foods, fresh fish fillets are the ones considered highly perishable, and both fresh fish and processing environments are potential sources of different foodborne pathogens like the outbreaks of *Listeria monocytogenes* with refrigerated fish fillet (Jami *et al*., 2014).

The economic losses due to spoilage and health problems call to need for control of the quality of the kinds of seafood to avoid high microbial contamination, which may lead to antibiotics resistance which increases the rate of seafood borne illnesses, in turn, increasing an urgent means of assuring the quality of seafood (Adebayo-Tayo *et al*., 2012a). Many pathogenic and spoilage bacteria can attach to food contact surfaces, and remain viable even after cleaning and disinfection (Pal, 2010). Thus, the food processing industry is difficult to provide safe, wholesome, and acceptable food to the consumer. Control of microorganisms exerted through a high level of hygiene and efficient cleaning, and disinfection practices during the processing and preservation procedures are essential to meet this goal. Even though people may get the tremendous advantage of the fish fillet to generate an income for jobless people and nutritionally rich food, insufficient value chain control, fish marketing infrastructure, and environmental factor leads to cross-contamination.

Food consumers in developing countries including Ethiopia suffer from food-borne bacterial illnesses especially from those of *Salmonella spp., Shigella spp. Staphylococcus aureus* and *Bacillus cereus*. Food-borne diseases result from ingestion of a wide variety of foods contaminated with pathogenic microorganisms, microbial toxins, or chemicals (Bean.N.H.,et al, 1990), found that over 90 percent of confirmed food-borne human illness cases and deaths

reported to the Centres for Disease Control and Prevention (CDC) are attributed to bacteria. Bacteria are commonly found in soil, water, plants and animals (including humans). People can also be exposed to some bacteria through inhalation, contaminated drinking water and contact with infected pets, farm animals and humans. In recent year the report of (Garedew, et al, 2015) indicated that the overall prevalence of *L. monocytogenes* and other *Listeria species* from foods of animal origin isolated from cake, raw meat, ice cream, minced beef, fish, unpasteurized milk and pizza in Gonder Were 6.25%. In these specific sites, fish fillets are handled carelessly and it may cause serious risks. Moreover, there was no scientific research has been done regarding fish fillets and associated risk factors like wearing proper cloths, hair cover, using gloves during working knowledge of microbial contamination of fish fillet and the like were risk factors to be assessed. To this effect, the present study aims to assess the microbial quality and safety of the fish fillet sold in Gilgel Gibe and Jimma city shops.

1.2.Statement of the problems

The problem is stated to find a solution for rapidly growing world's population which, is expected to reach 9.8 billion by 2050, and feed this population, food production must increase by 60% worldwide (FAO, 2014). However, food production from the agricultural sector fails to meet such high demand due to climatic effects. Thus, climate-smart aquaculture is vital to increase food production. It is one of the primary sources of cheap animal protein for the rapidly growing human population. Aquaculture production has increased from 61.8 million tons in 2011 to 80 million tons in 2016 (FAO, 2018). Thus the need for quality fish production to meet population need is of vital questionable concern of this study.

Increasing fish and fish products such as fillet-free from any contamination help to compensate for the scarcity of foods. Despite increased global demand for fish and fish-based products, challenges such as contamination and environmental factors play a vital role (Yu *et al*., 2018). Fishers obtain an income by selling their product to street vendors, restaurants, bars, and/ selling for themselves. However, illnesses due to microbes in fish and fish products make microbial quality to be the most important aspect of food safety and may pose a serious

threat to human health (Riener *et al*., 2010). Fish fillets can be classified as high-risk food due to poor sanitation practices and handling of food, insufficient food safety laws, weak regulatory systems, and lack of education for food-handlers on food-borne infections is important for developing countries like Ethiopia (Hailemichael & Gutema, 2021).Risk factor assessment to evaluate fish fillets due to contamination through production systems, improper handling and storage condition is basically considered. Fish fillet is one of the most perishable food products during handling, storage and has a short lifetime even at refrigeration temperature (Lauzon *et al.,* 2010). The most prevalent cause of food spoilage is microbial growth and residence in the food, which results in numerous undesirable metabolites being produced in the food that cause unwanted flavours and odours (Gram and Dalgaard, 2002).Fish and fish products are often associated with human disease, especially when raw or undercooked fish and fish products are consumed.

The presence of different bacteria species including human pathogenic bacteria in fish can be linked to direct contact with a contaminated water environment and ingestion of bacteria from sediments or contaminated feed. Thus, bacteria detected in fish reflect the condition and safety of aquatic environments **(**Novoslavski *et al*., 2016**).** Gilgel Gibe fishing site is one the best site that provides fish from Sekoru district to Omo Nada district, Kersa district, and Jimma city. Even though they produce a large fish fillets to these districts issue related with quality and safety is questioned because they transport long distance, and producers handle improperly. Thus, assessing the microbial quality and safety of the fish fillet is inevitable.

As indicated above food born illnesses due to eating contaminated fish fillets and other food items are the world's serious problem which is the basic concern of the researcher.

1.3. Objectives of the study

1.3.1. General Objective

- To assess microbiological quality and safety of Fish fillet collected from Gilgel Gibe and Jimma city fish fillet selling shops, southwest Ethiopia

1.3.2. Specific Objectives

- To assess the microbiological load of fish fillets
- To isolate the different bacterial groups from fish fillet samples.
- To detect the prevalence of pathogenic microbes associated with fish fillets.
- To evaluate the antibiotic susceptibility patterns of the pathogens.
- To identify the risk factors associated with fish fillets collected from both sites.

1.4.Significance of the study

This study is very significant in the way that it provides somebody with valuable information about the microbiological quality and safety of Fish fillets sold in Gilgel Gibe and Jimma city for further studies. The study also provides awareness about physical features that indicate spoilage in the fish fillet. It could also be used by concerned bodies to re-visit the existing fishery management practice and its processing in fishing grounds forthe production of microbiologically safe and qualified fishery products and to take the corrective measures to control the likely occurrence of foodborne diseases. The finding could also enrich the available literature on the microbiology of Ethiopian foods to be used for setting microbiological standards.

2. Literature Review

2.1. Fish Nutritional Value

Fishes are classified as any of the cold-blooded aquatic vertebrates of the typically showing gills, fins, and a streamlined body. In addition, ‘fish’ also refers to the flesh of such animals used as food. There are about 22,000 species of fish that began evolving around 480 million years ago (Pal and Mahendra, 2015). Fish and seafood products constitute an important food commodity in international trade due to their ever-increasing consumer demand. Fish contributes about 60% of the world's supply of protein, and 60% of the developing world derives more than 30% of their animal protein from fish (Emikpe *et al*., 2011). Fish allows for protein improved nutrition in that it has a high biological value in terms of high protein retention in the body, low cholesterol level, and presence of essential amino acids (Emikpe *et al*., 2011). There are more kinds of fishes than all other kinds of water and land vertebrates put together, and fish differ so greatly in shape, colour, and sizes (Adebayo-Tayo *et al*2012b).

Figure 1. Morphology of Nile tilapia

2.2, Fish Production and marketing in Ethiopia

Ethiopia is a country with an area of 1,127,127 km^2, water bodies cover approximately 7400 km^2 (Cheffo *et al*., 2015). In Ethiopia, water bodies are home to diverse aquatic life including more than 180 fish species of which about 40 are endemic (Birhanu, 2015). Currently, the annual exploitable fish potential of Ethiopia is 51,481 tons of which only 24% is exploited right now (Hirpo, 2017). According to FAO (2008), fish farming has become an alternative to agriculture that depends on seasonal rainfall like Ethiopia. This indicated that fishery resources in Ethiopia have a vital role in poverty reduction, food insecurity, and employment creation, yet the sector is not given due attention to any degree (Lemma, 2012).

In the Oromia region, fishery shares 18.5% of the country's total fish production of which Gilgel Gibe river has a 0.8% share (Assefa, 2013). Gilgel Gibe is the source explained that the river has huge fish potential and is well known in *Barbus* intermediary, *Oreochromis niloticus*, and *Labeo spp* .*Barbus* fish species. According to Lemma (2012), although there is some form of fisheries practiced in most freshwater bodies in Ethiopia, commercial fishery is concentrated at Lakes Tana, Chamo, Ziway, Abaya, Koka, Langano, Hawassa, and Turkana. Similarly, Hirpo (2017) underscored that 40% and 50.2% of fish supply to the major urban centres in Ethiopia are captured from the Rift Valley lakes and Lake Tana. This indicates that farm households who live near the Gilgel Gibe River do not participate in fish production and marketing though fish marketing has been given prior attention by the government of Ethiopia (Hirpo, 2017).

Almost all the fish consumed in Ethiopia are collected from the wild using artisanal methods. The current total fish production potential of the country is estimated to be around 51,481 tons annually for the main water bodies, of which only around 38,400 were exploited very recently (FAO, 2014). According to Lemma(2012),the major fish supply to the major cities and towns in Ethiopia are captured from the Rift Valley lakes (40%) and Lake Tana (50.2%) in the north, and the remaining percentage going to riverine fisheries. For instance, the rivers and floodplains in Gambella Region are estimated to have annual fish yield potentials of 15,000 to 17,000 tons while the rivers and floodplains in Benishangul Gumuz Region are estimated to have potentials of 2,400 tons per year (Alayu, 2012). Fish plays a vital role in domestic trade as well as in import and export market. The Ethiopian cross-border fishtrade is

currently not properly documented. The country imports significant amounts of fish from neighbouring countries though some of these imports end up being exported to Sudan through the porous border with neighboring South Sudan. The per capita fish supply is around 200 g, significantly below the mean 2.6 kg per capita per year for the East African sub region (FAO, 2015). Although most fish traders do not have access to basic cold chains with ice and insulated containers, a few basic fish handling and preservation institutions which are equipped with electricity and freshwater supplies are available in the Ethiopian fisheries. By such a ways many people are engaged in this sector as source of income. As a result of the general shortage of basic cold chains, fresh fish storage usually lasts only up to two days. Consequently, fish marketers concentrate their trade during religious fasting periods when there is more demand (Ann *et al.*, 2013).

2.3 . Microbial contamination of fish

Fish are very tolerant to most of the environmental factors, stressful conditions can affect them adversely and make them more susceptible to diseases. Stress can result from nutritional differences especially due to vitamin and protein imbalances, environmental quality culture conditions, physicochemical and biological interference for example polluted water sources, crowding of fish, contaminated feeds, transportation, and organic enrichment can cause sufferingof many different infections; such as bacterial, fungal and other infections (Mwajuma *et al.*, 2010).

Fish spoilage is a complex process, in which physical, chemical, and microbiological mechanisms are implicated (Adebayo-Tayo *et al*., 2012b). Many spoilage-producing bacteria (*Aeromonas, Alcaligenes, Bacillus, Enterobacter, Enterococcus, Escherichia coli, Listeria, Pseudomonas, Shewanella*) and fungi (*Aspergillum ,Candida, Cryptococcus, Rhodotorula*) are isolated from fresh and spoiled fish and other seafood (Pal, 2012). Degradation of lipids in fatty fish produces rancid odours. In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine, the compound responsible for fishy off odours. Iron is a limiting nutrient in fish and this favors the growth of bacteria such as pseudomonads that produce siderophores that bind iron.

Spoilage is the result of a series of changes brought about in the dead fish mainly due to enzymatic and bacterial action starts as soon as a fish is caught and dies(Pal, 2012). In areas where the temperature is high, fish spoils within 15-20 hours depending on the species, and the method captures an extremely perishable commodity due to its high water content (Pal and Mahendra, 2015). Spoilage is defined as a change in fish or fish products that renders it less acceptable, unacceptable, or unsafe for human consumption Fish undergoing spoilage has one or more of the following signs, discolorations, slime formation, changes in texture, off-odours, off-flavours, and gas production(Pal, 2012). Microbial growth is the first mechanism deteriorating fish, being the spoilage factor that most affects the quality of fresh or lightly preserved fish. Initially, the fish muscles are sterile, but after death, they are contaminated by the microbial population present at the fish skin (Comi, 2017).

The high water activity, low acidity (pH > 6), and high amount of nonprotein nitrogenous compounds typical of fish result in the fast growth of microorganisms, leading to undesirable changes in appearance, texture, flavor, and odor, reducing its quality. Spoilage created by microorganisms generates volatile amines, biogenic amines, organic acids, sulphides, alcohols, aldehydes, and ketones, which have unpleasant and unacceptable off-flavours (Sperber & Doyle, 2010). The main compounds formed during microbiological spoilage are depended on the microorganisms (Table 1). Biogenic amines, such as histamine, cadaverine, tyramine, and putrescine, are produced by the decarboxylation of specific free amino acids by microorganisms during storage and are used to monitor fish safety and quality (Silbande *et al.*, 2018).

Table 1. Spoilage compounds produced by microorganisms during the storage of fresh fish (Gram *et al*., 2000)

Spoilage Microbes	Spoilage Compound(s) Produced
Pseudomonas spp.	CH3SH, $(CH3)_2S$, ketones, esters, aldehydes, NH_3, and hypoxanthine
Vibrionaceae	Trimethylamine and H2S
Enterobacteriaceae	Trimethylamine, H2S, ketones, esters, aldehydes, NH_3, hypoxanthine, and acids
Lactic acid bacteria	H2S, ketones, esters, aldehydes, NH_3, and acids
Yeast	Ketones, esters, aldehydes, NH_3, and acids
Aerobic spoilers	NH_3, acetic, butyric, and propionic acids
Anaerobic rods	Ketones, esters, aldehydes, and NH_3

2.3.1. Risk factors associated with fish fillet

2.3.2. Fish handling and processing

Fish product handling, processing, and Marketing in Ethiopia follow still traditional methodologies due to a lack of expertise in the area and undeveloped technology in the Field. Regarding Fresh fish handling and distribution in Ethiopia, most private traders do not use even basic cold chains because of lack of ice as well as in the appropriate amount of ice for the given catch (Deng, 2020). Good trade practices indicate that retailers should only keep one day stock of cooled Fish or Fish products such as Fillets, beheaded, and gutted fish. In formal Fishermen on Lake Chamo have to walk daily 10 km to Arbaminch,Carrying Fish in Sacks on their heads, in order to bring to traditional markets as there is no road access to Lack shore. In the case of Lake, Tana women gut and skin the Fish and remove the gills and tie about 2kg in a bundle by threading reed through the eyes of the fish. Then they sell these products either to hotel owners or direct to consumers in the street (Doda, 2008). The additional processing of Fish includes Sun-drying when Fish is filleted made in strips that are hanging on a string and allowed to stay for two to three days and after it is partially dried

stored in poor condition using available material like sacks. This leads to significant deterioration of these products including physical losses(FAO, 2006).

2.3.3.Environmental Factors

This refers to the assessment of the physical, chemical, or biological parameters that affect fish production. There are environmental factors such as water quality and nutrients that affect the growth of fish and the quality of fish fillets. Water quality is the most limiting factor in fingerlings production is water quality (Ibrahim*et al*., 2010). It is hard for any farm to predict, manage and understand the quality of water in its systems. The quality of water affects directly the growth rates, efficiency of feeds, feeds conversion, and growth. Temperature affects the development of fungal contaminations in fish. When temperatures are low outbreaks due to disease occur, but high temperatures are also implicated to cause outbreaks (Mwaura, 2005). Water quality and its frequent checking (temperature, dissolved oxygen content, pH, and salinity) are crucial in detecting issues in a fishery (Adebayo *et al*., 2012).

Moreover, the physicochemical conditions and the interactions between the microorganisms impose a selection of the organisms capable of growing under such as the environmental factors that affect fish fillet. The initial microflora of the fish is dependent on different factors, such as the environment where the fish lives, the fishing season, water temperature, the method of capture, the handling on the ship, or the technological and sale process, but, regardless of the variety of microflora present in the fresh fish and the diverse parameters used for preserving, the species growing are consistent in the different products. The different microbial species that can develop on fish produce the off-odours and off-flavours, named specific spoilage organisms due to environmental factors (Leisner and Gram, 2014).

2.3.4. Slaughter, Storage, and Packing

Fish fillet preparation during slaughter, storage and packing is very important. Appropriate processing should enable maximal use of raw material and thus contribute to increased economic profitability. Thus, the process results in practically no unused waste material. However, achievement of this goal essentially requires that mechanization be introduced into

processing, albeit on a small scale. Persons serving in food processing industries may be sources of microbial inoculation, food poisoning, food intoxication, and food spoilage. Several organisms including Staphylococcus aureus have been isolated from the hands of employees working in food establishments (Pal, 2012; Pal and Mahendra, 2015).Hence, it is important to mention that any person with purulent skin lesions or having respiratory infections should not be allowed to work in the food industry (Pal and Mahendra, 2015).

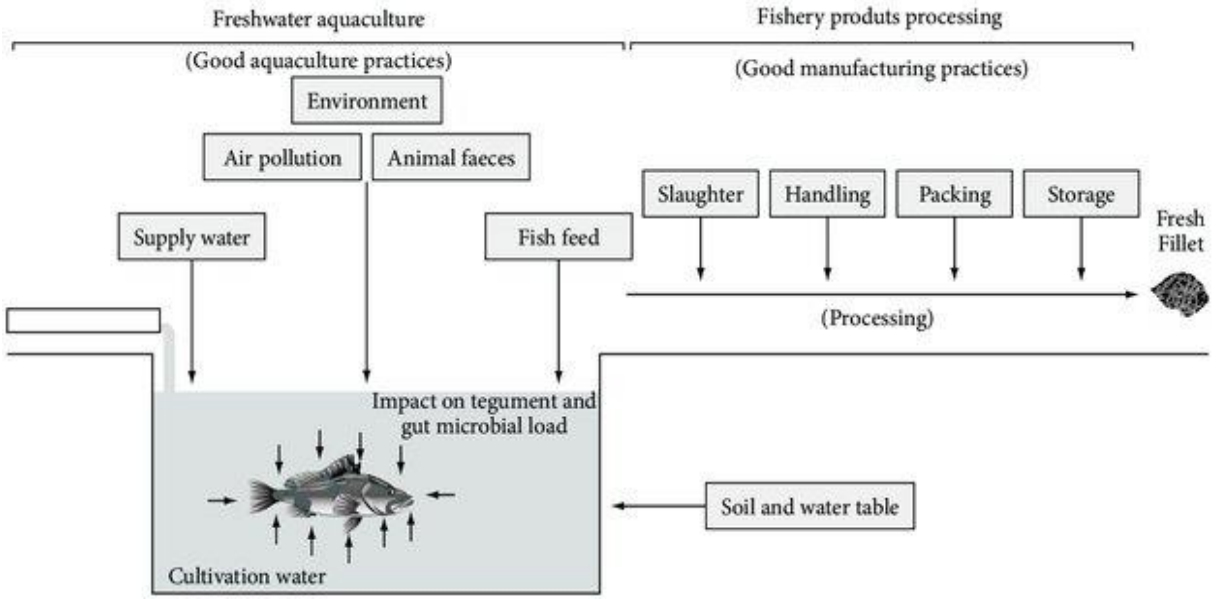

Figure 2. Risk factors associated microbial quality of fresh fillet (Boari*et al.*, 2008)

2.4. Some pathogens Related to Fish

2.4.1. Vibrio spp

Vibrio spp is Gram-negative bacteria that are rod-shaped and curved, halophilic, facultative anaerobic, non-spore-forming, motile with polar flagella and sheath, and oxidase positive. Their natural habitat is in estuarine and coastal areas, where they are found free-living in water, sediments, plankton, and nearly all flora and fauna found in coastal environments (Scharer *et al*., 2011). The major human pathogenic Vibrio species are *V. parahaemolyticus*, *V. vunificus*, and *V. cholerae* (Parveen and Tamplin, 2013). They constitute a significant human health hazard causing outbreaks and sporadic foodborne illnesses associated with the consumption of raw or undercooked contaminated seafood (Scallan *et al*., 2011). The presence of predisposing factors, such as the use of antacid medications, absence of gastric hydrochloric acid (achlorhydria), and partial or complete gastrectomy, increase the risk of illness (Nishibuchi & DePaola, 2005).

2.4.2. Salmonella spp

Salmonella Salmonellae are Gram-negative, small, rod-shaped, facultative anaerobic bacteria, usually motile with peritrichous flagella and are catalase positive, oxidase negative, and produce gas from glucose. Salmonella is a non-lactose fermenter. Salmonella produces enterotoxins and causes inflammatory reactions and diarrhoea. Symptoms often start 12 to 72 h after the ingestion of contaminated food. The onset of acute symptoms may last for 1 to 2 days or more depending on the individual host variation, ingested dose, and strains (Jay *et al*., 2005). Salmonella infections from the consumption of seafood products are most commonly associated with raw, undercooked, and/or poorly cooked finfish (NACMCF, 2008). Salmonella cross-contamination of seafood may take place during processing and storage, however, this can be prevented by good manufacturing practices hazard analysis critical control point (Iwamoto *et al*., 2010).The risk of seafood-borne salmonellosis can be reduced by using specific control measures focused on: monitoring harvest water pollution levels; employing best management hygienic production practices; establishing biosecurity measures in production areas; and ensuring appropriate cooking temperatures, appropriate storage temperatures, and prevention of cross-contamination during harvesting, handling, and processing (FDA, 2011).

2.4.3. Listeria spp

Listeria monocytogenes Listeria monocytogenes is a Gram-positive, rod-shaped non-acid fast, non-spore-forming, catalase-positive bacterium that ferments glucose-producing lactic acid. The bacterium is ubiquitous in soil, water, animal excreta, and plants. The ability of the bacterium to accommodate a vast range of temperatures (1- 45 ºC), and pH (4.1- 9.6), form biofilms and remain viable for long periods promote its broad distribution, colonization, and adaptation to various environments (Jay *et al*., 2005). Listeriosis caused by *Listeria spp*. is recognized as a foodborne disease including seafood of increasing public health and food safety concerns since early 1981. Ingestion is the main route of transmission to humans; mainly through the consumption of ready-to-eat foods (Miya *et al*., 2010). Research indicated that 93% of raw seafood is contaminated with less than 1 CFU/serving. However, raw and processed crab fillets the foremost incriminated seafood that transmits Listeria. Approximately 7.2% of raw seafood contains 1 to 3 CFU/serving, 1.2% contain 3 to 6 CFU/serving (NACMCF, 2008).

Different *Listeria spp* were isolated from ready-to-eat minced tuna and fish roe products in Japan (Miya *et al*., 2010). Little information is available on the prevalence and sources of *Listeria spp* in ready-to-eat seafood products which passively affects the seafood industry. *L. monocytogenes* isolated from popular seafood products sampled at the retail market and processing facilities were found to be resistant to penicillin, ampicillin, tetracycline, and vancomycin (Fallah*et al.*, 2013).

2.4.4. Staphylococcus aureus

S. aureus is a Gram-positive coccus and catalase-positive chemotrophic bacterium. It produces Staphylococcus food poisoning (food intoxication syndrome), although it is not considered a problem with raw seafood (NACMCF, 2008). Staphylococcus aureus enterotoxins are responsible for the pathogenicity and virulence of the bacterium (Pinchuk*et al*., 2010). S. aureus is not found in the normal microflora of fish. Their presence or absence in a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment (Huss *et al*., 2003). The handling of fish products during the process involves a risk of contamination by pathogenic bacteria like *S. aureus*, causing foodborne human intoxication. This bacterium is salt-tolerant and therefore can contaminate all cured preparations such as cold-smoked fish,

and fish-based preserves. It was also evidenced that *S. aureus* in fish can survive at a wide range of temperatures (Mariapan *et al*., 2010).

2.5. Antibiotic Susceptibility of pathogens

Knowledge about the antibiotic susceptibility of bacteria is vital for the proper management of the diseases they cause. Worldwide, the use of antibiotics in aquaculture and the potential transmission of resistant bacteria between terrestrial and aquatic environments have been reported (Cabello 2006). The common mechanism of this transmission is via transfer of resistance plasmids with fish bacteria acting as intermediate vectors (Sørum 2006). However, several studies have focused on the transmission of antimicrobial resistance (AMR) between humans and terrestrial food animals, with less attention to the aquatic ecosystem including fish. This has left inadequate information available about the antimicrobial drug susceptibility of the aquatic ecosystem, a very important aspect in the epidemiology of AMR (Biyela*et al*. 2004).

The high levels of antibiotic misuse and antibiotic resistance in the human and livestock populations have been reported (Byarugaba *et al*. 2011). Though the available information suggests minimal use of antibiotics in aquaculture, the use of animal wastes to fertilize ponds and the close interaction between humans, livestock and the aquatic ecosystem indicates a possible transmission of AMR to the aquatic environment. The widespread occurrence of naturally resistant bacteria in the aquatic environment and soil could also contribute to the passage of antibiotic resistance genes to fish bacteria (Cantas*et al*. 2013).

All the bacterial isolates showed multiple resistance to various drugs tested except for *Salmonella enteritidis*, and *Escherichia coli*. However, all bacterial isolates were susceptible to gentamicin, and this is in agreement with the findings of Gufe *et al*. (2019) reported that the resistance levels to kanamycin, streptomycin, chloramphenicol, and tetracycline were relatively low. Indeed, co-trimoxazole had the highest levels of resistance. On the other hand, Newaj-Fyzul *et al*.(2008) reported a relatively low resistance of 6.4%. Resistance against ampicillin was markedly high (65%); it is comparable to studies by Karimi (2015)

2.6.Disease outbreaks of fish

The disease outbreaks in aquaculture are the result of a complex network of interactions on aquatic systems between the produced organism, several environmental and zoo technical

aspects, and possible pathogenic agents that present a series of unique challenges in aquatic organism's health (Peeler & Taylor, 2011). Infectious pathologies in a farmed fish approach like epidemiological studies on main areas of aquatic animal health as transboundary and emerging aquatic animal diseases, animal health surveillance, and biosecurity program development should be performed. These are crucial to disease prevalence monitorisation, early detection of emerging exotic and new diseases, and quality management improvement of aquaculture operations (Oidtmann *et al.*, 2014). Nevertheless, to obtain proper epidemiological models, animal health surveillance and biosecurity programs must integrate environmental information and information from different areas like pathogenesis, disease diagnosis, disease resistance, physiological response to pathogens, pathogen characterization, host immune system responses characterization, disease biomarkers, and organism response to disease treatment

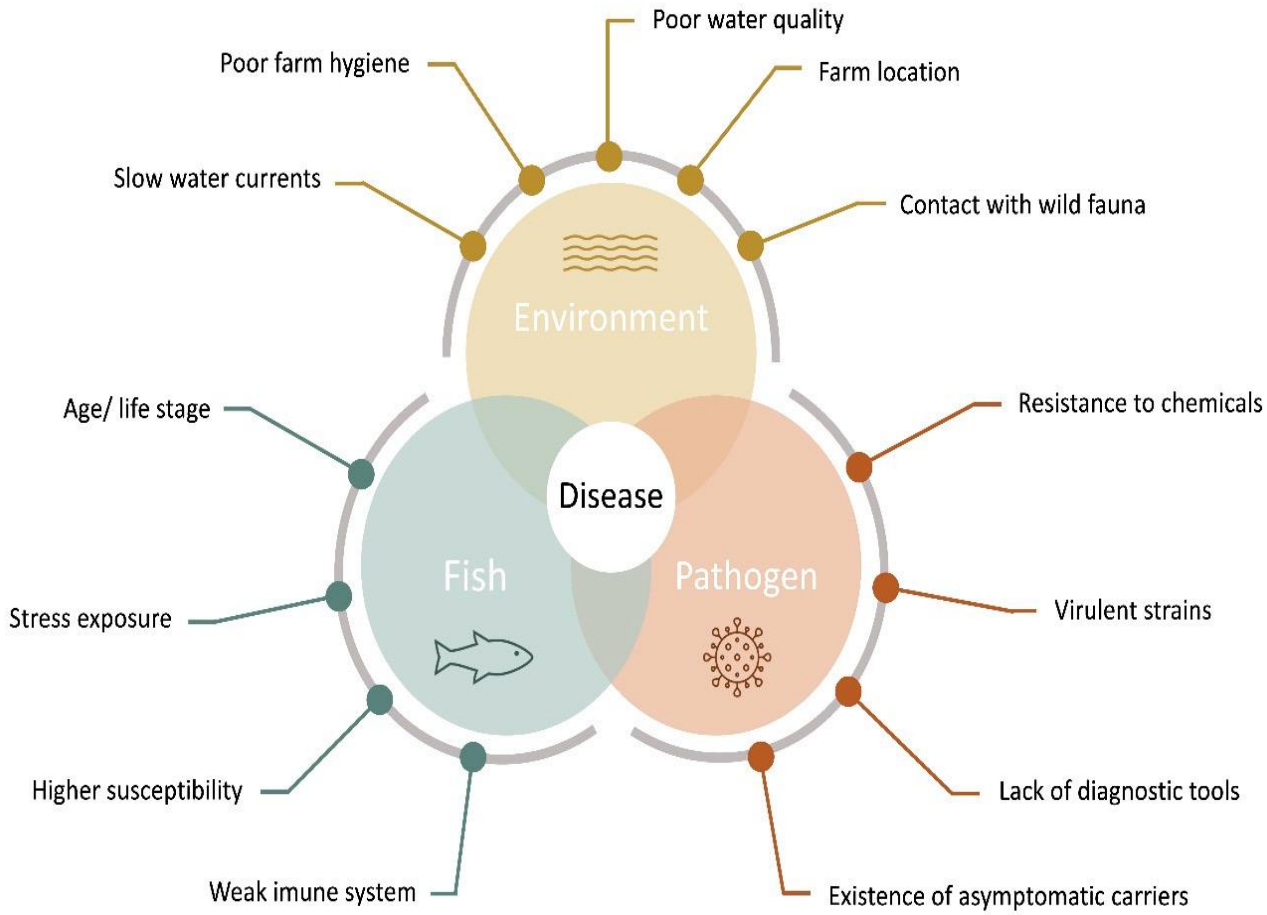

Figure 3. Aquaculture disease diagram and host-pathogen interactions intervening in fish disease outbreaks (Freitas *et al.*, 2020; Sitjà-Bobadilla & Oidtmann, 2017).

3. Materials and Methods

3.1. Description of the study area

The study was conducted in Gilgel Gibe reservoir and Jimma city shops. Gilgel gibe dam I reservoir is located in Oromia regional state, Jimma Zone about 260 km southwest of Addis Ababa and 60 km northeast of Jimma City. It is enclosed with four districts namely Sekoru, Omo Nada, Kersa, and Tiro Afeta; with the area coverage of 62 square kilometres and astronomically it is found within 7º3' to 8º3' and 36º7'to 37º6' with an average altitude of 1,650 m. above sea level, mean annual rainfall ranges between 1300 mm and 1800 mm and it is the reservoir of Gilgel Gibe Hydroelectric dam I (Bahiru, 2010). With-in these four districts, there are Eight beneficiaries of rural kebele's, and about 12 small-scale fish producer cooperatives were organized from these kebeles and engaged in fish production and marketing. The main fish types it contains are the *Barbes spp* and Nile Tilapia(*Oreochomis niloticus*) (Wakjira, 2013).

Jimma city is the largest city in southwestern Ethiopia located in the Jimma zone of the Oromiya region. This city has a latitude and longitude of $7^0$40'N, $36^0$50'E/7.667^{0}N,36.833^{0}E. It was found at an altitude of the area ranges from 1300m to 2100m. The mean annual rainfall of the area is between 1800 mm to 2300 mm with maximum rainfall between June and September. The annual mean temperature of the area is between 15℃ and 22℃ (Alemu, 2011). Jimma city has sold several types food, plant and animal origin food. From animal origin food milk, cattle fillet, sheep and goat fillets and fish fillet are common.

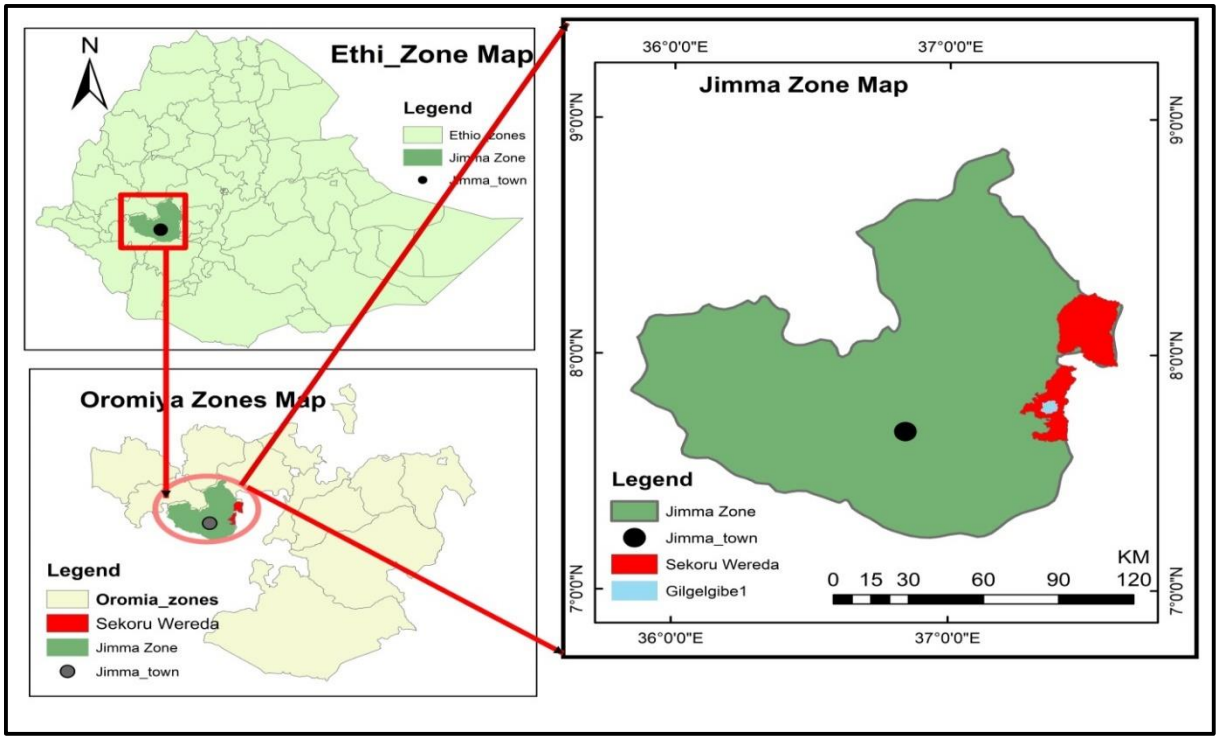

Figure 4. Map of the study area

3.2. Study Period

The duration of this study was between January and June 2018.

3.3. Risk Factor Associated with Fish fillets

Risk factors associated with fish fillets were assessed using interviews and onsite observation. Accordingly, a semi-structured interview was prepared to collect information on socio-demography, personal hygiene, fishing materials, storage materials, and some basic knowledge on microbes associated with fish fillets (Appendix 1).

3.4. Study design, sample size, and Technique

A cross-sectional study design was used. The total fish fillet sellers sites were 15 (10 in Gilgel Gibe Site and 5 in Jimma city). From each site, two round samples were taken. Then, the total sample size was 30 . A purposive sample technique was employed during sample collection.

3.5. Sample collection

A total of 30 fish fillets from Gilgel Gibe and Jimma city fish fillet selling shops were collected. About 250 g of Fish fillets were purchased and wrapped with sterile aluminium foil

and placed in an icebox. Then, all fillet samples were transported to Research and Postgraduate Laboratory, Department of Biology, College of Natural Sciences, Jimma University for microbial analysis. The sample preparation was started within one to five hours of collection while fillet samples were kept in the refrigerator at 4 ^{o}C until microbial analysis was conducted.

3.6. Sample preparation

The collected fish fillets were analysed for microbial quality and safety by crushing them into small pieces using a sterile pestle and mortar. Briefly, 25 g of the pieces fish fillets were taken and mixed with 225ml of peptone water and homogenized using a shaker at 150 rpm for about 2 minutes. Then 1ml of the homogenate was serially diluted in 9ml peptone water up to 10^{-6} serial dilution. Finally, 0.1 ml of appropriate dilution wastaken from 10^{-4} and 10^{-5} dilutions and transferred to different pre-sterilized surface of dry agar plates. The colonies were counted from a plate containing microbial colonies between 30 and 300. The counted colonies were expressed in colony-forming units per gram (CFU/g) and later converted to log CFU/g.

3.7. Microbial Enumeration

3.7.1. Aerobic Mesophilic Bacteria Count

From appropriate serial dilutions, 0.1ml aliquot was spread-plated on the pre-dried surface of Plate count Agar (Oxoid) plates. Colonies were counted after the culture media were incubated at 32 ℃ for 24 hours.

3.7.2. Counts of Staphylococci

From appropriate dilutions, 0.1 m1 aliquot was spread-plated on Mannitol Salt Agar (MSA) and incubated at 32 ℃ for 48 hours. Then golden yellow colour surrounded by red colour was counted as staphylococci.

3.7.3. Counts of Enterobacteriaceae

From appropriate dilutions, 0.1 ml appropriate dilution was spread-plated on pre-dried surfaces of Violet Red Bile Glucose Agar (VRBGA) plates. The culture plates were

incubated at 32 ℃ for 24 hours and after which pink to red-purple colonies with or without haloes of bile precipitation were enumerated as members of Enterobacteriaceae.

3.7.4. Counts of Coliforms

From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-dried surfaces of Violet Red Bile Agar (VRBA) plates. The culture plates were incubated at 30- 32℃ for 24 hours after which purplish-red colonies surrounded by a reddish zone of precipitated bile were counted as coliforms.

3.7.5. Counts of Yeasts and Molds

From appropriate dilutions, 0.1 ml appropriate dilution was spread-plated on pre-dried surfaces of Potato dextrose agar (PDA) supplemented with 0.1g Chloramphenicol. The culture plates were incubated at 30℃ for three to five days. Smooth (non-hairy) colonies without an extension at the periphery (margin) were counted as yeasts

3.8. Microbial Analysis

After the enumeration of aerobic AMB, about 10 to 15 colonies were picked randomly from countable plates and inoculated into 5 ml Nutrient Broth and incubated at 32°C overnight for purification. After cultures were purified by repeated sub-culturing, further characterization was done using morphological and biochemical tests various bacterial groups were grouped into genus or family level based on Jon's classification attached to appendix 3 (John, 2012)

3.9. Characterization of the isolates

3.9.1. Cell morphology

To assess the cell morphology gram staining, motility and endospore tests were employed.

3.9.1.1. Gram Staining

A smear of pure isolates was prepared on a clean slide and allowed to air-dry and heat-fix. The heat-fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water for 3 seconds. Then, the slide was flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing, the smear was decolorized with 96% of ethanol for 20 seconds and washed slide gently under tap water for 3 seconds. Thereafter, the smear was counterstained by safranin and dried with absorbent paper. Finally, the air-dried smear was observed under the oil immersion objective. After the Gram staining, Gram-negative

bacteria were stained pink/red and gram-positive bacteria were stained blue/purple (Gram, 1884).

3.9.1.2. Motility Test

A purified broth culture of bacteria was taken by a sterile needle and stabbed straight vertically into a test tube containing motility medium (Tryptose (10g/ L), NaCl (5g/ L), and Agar (5g/ L) final pH adjusted to 7.2 ± 0.2) to the bottom of the tube and incubated aerobically at 32°C for 24 h. A positive motility test was indicated by a turbid area diffusing away from the line of inoculation and a negative test was indicated by growth along the inoculation line only with no further (Shields and Cathcart, 2012).

3.9.1.3. Endospore Staining

A smear of isolates was prepared on a clean glass slide and allowed to air-dry followed by heat fixing and placed on wire gauze at the top steaming, then flooded with 0.5% (w/v) malachite green solution and steam for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin, and stay for 30 seconds. The slide was washed with tap water and air-dried, then observed under the oil immersion objective (×1000) pink colour for vegetative cell and green colour confirmed for the presence of endospore (Schaeffer and Fulton, 1933).

3.9.2. Biochemical tests for isolate

3.9.2.1. KOH test

Two drops of 3 % KOH solution were placed on a clean microscopic slide. A colony was aseptically picked from MRS agar using an inculcating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass when the KOH solution becomes viscous, the thread of slime followed the loop for 0.5 to 2 cm or more (Gram-negative bacteria) and no slime, the watery suspension did not follow the loop (Gram-positive bacteria) (Gregerson, 1978).

3.9.2.2. Cytochrome oxidase test

This test will be conducted following the method outlined by (Kovacs, 1956). A young colony (overnight culture) will be rubbed on Whatman filter paper No 1 and flooding with oxidase reagent, appearance of blue colour on the colonies within 30 seconds to 2 minutes

will be indicated a positive reaction. Any very weak or no colour change that occurred after 2 minutes were taken as negative.

3.9.2.3. Oxidation Fermentation (O/F) test

This test is used to assess the ability of the isolate to utilize glucose and to determine the metabolic way (i.e. fermentation or oxidation). Ingredients (g/l): Peptone, 2 g; yeast extract, 1 g; NaCl, 5 g; K2HPO4, 0.2 g; glucose,10 g; bromothymol blue, 0.08 g; agar, 2.5 g; distilled water, 1000 ml; pH, 7.10. Accordingly, test tubes containing 15 ml of freshly prepared medium for the O/F test were autoclaved and immediately cooled under tap water to avoid the dissolution of oxygen in the medium. Then, the broth cultures were inoculated into the medium by stabbing with a sterile straight wire to the bottom. An organism with oxidative metabolism displayed yellow in the upper half of the tube and green in the lower half. An organism with fermentative metabolism displayed yellow in both halves of the tube. Acid formation and growth regions were interpreted after 2 to 5 days of incubation at 32°C (Hugh and Leifson, 1953).

3.9.2.4. Catalase test

A catalase test was carried out after young colonies flooded with a 3% solution of H_2O_2. The formation of bubbles indicated the presence of catalase and no bubbles for the absence of catalase enzyme (MacFaddin, 1980).

3.10. Detection of Pathogens

3.10.1. *Staphylococcus aureus*

After counting staphylococci, golden-yellow colonies on MSA plates were aseptically picked and transferred into 5 ml nutrient broth and incubated at 37ºC for 24 hrs for further purification. Then, a loop full of culture from the nutrient broth was streaked on nutrient agar supplemented with 0.75% NaCl and again incubated at 37ºC for 24 hrs. Finally, the distinct colonies were characterized using the established microbiological methods. Gram-positive cocci with a clustered arrangement under the microscope were subjected to preliminary biochemical tests (oxidase, catalase, and coagulase tests) (Acco *et al*., 2003).

Coagulase test

Coagulase test was done using slide test procedures (Chees brough, 2006). In the slide test, a colony of the purified isolates was emulsified in a drop of distilled water on two ends of a

clean glass slide to make thick suspensions. One was labeled as test and the other was as control. A loop full of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for coagulase-positive organisms.

3.10.2. Detection of *Listeria spp*

The fish fillet samples were tested for the presence of *Listeria spp*. using the selective enrichment and isolation protocol of McClain and Lee (1988). Twenty-five grams of each fillet sample were taken aseptically, blended for 2 min in 225 ml *Listeria* enrichment broth, and incubated at 37°C for 24 h. A 1 ml sample of this primary enrichment was transferred to a 9 ml Listeria secondary enrichment broth (Frazer broth) and incubated at 37°C for 24 h. Then, a loopful of broth from secondary enrichment was streaked onto Listeria selective agar and incubated at 37°C for 48 h. The plates having a colony with black colonies with the black sunken centre were taken as presumptive of *Listeria spp* and further purified by sub culturing in Tryptone Soya Agar. All isolates were subjected to standard biochemical tests including Gram staining, catalase test, motility, carbohydrate fermentation (acid production from glucose, sucrose, mannitol, and xylose), and nitrate reduction, hydrolysis of esculin, MR/VP test, and β-haemolytic activity (Aygun and Pehlivanlar 2006). Gram-positive rods, aerobic and facultatively anaerobic, non-spore-forming, catalase-positive (although there are reports of catalase-negative Listeria), oxidase-negative, fermentative in sugars, and producing acid without gas. Most strains are motile at 28 C and non-motile at 37 C was taken as Listeria spp

Carbohydrate fermentation of *Listeria spp*

The ability of microorganisms to ferment certain sugars was performed by inoculating 1 ml of overnight culture to 10 ml of Phenol Red Broth containing 2% of glucose, sucrose, mannitol, and xylose separately inserted with Durham tube and incubated for 24 hours at 37°C. Finally, the change of color from red to yellow indicated the production of acid (fermentation only), bubble formation (CO_2) in Durham tubes indicated the positive result for gas production. No color change showed no fermentation.

βhaemolysis Activity

The *Listeria spp* β haemolysis activities were conducted by streaking of overnight cultures onto the blood gar supplementing with 5% (v/v) sheep blood and incubated at 37°C for 48 h. Then, lyses zones or clear zone around colonies were considered β-haemolytic activity.

3.10.3. Detection of *Salmonella spp.*

For the detection of *Salmonella spp,* a 25 g fish fillet was mixed with 225 ml of BPW and incubated at 37°C for 24hrs. Then 1ml pre-enrichment broth culture was added to 10ml of Rappaport-Vassiliadis broth (Oxoid) and again incubated at 37°C for 24 hrs. Thereafter, a loopful of suspension from a tube was streaked onto Xylose lysine Deoxycholate (XLD) (Oxoid). The presumptive *Salmonella* colonies (black colony surrounded by red color) were picked off, transferred to 5 ml nutrient broth (Oxoid), incubated at 37°C for 24 hrs, then streaked onto Nutrient Agar (Oxoid) for purity, and incubated at 37 °C for 24 hrs. The presumptive *Salmonella* spp. were characterized by the below standard biochemical tests

Triple Sugar Iron Test

The butt was stabbed and the slant was streaked and incubated at 37°C for 24hrs to detect fermentation of glucose, sucrose, and lactose as well as production of H_2S. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H_2S was considered as presumptive for Salmonella spp.

Test for deamination of lysine or decarboxylase lysine

Test for deamination of lysine or decarboxylase lysine was done using Lysine Iron Agar (LIA). The butt of LIA was stabbed and the slant was streaked and incubated at 37°C for 24hrs. Then, the production of an alkaline reaction (purple colour) throughout the medium indicated the delaminating of lysine, positive for *Salmonella spp*.

Urease test

The slant was streaked and the tube was incubated at 37°C for 24 hrs to assess the hydrolysis of urea. No color change was considered as negative and thus presumptive for *Salmonella spp*. Urease-producing organisms hydrolyse urea to form ammonia and the medium may change to purple-red. Salmonella did not produce the enzyme urease and the colour of the urea slant was unchanged.

Citrate utilization

The slant of Simmons Citrate Agar was streaked and the tube was incubated at 37^{o}C for 24hrs to determine citrate utilization as a sole source of carbon. The presence of growth and colour change from green to blue was considered as presumptive for *Salmonella spp*.

Sulphide Indole Motility (SIM) test

The SIM medium was stabbed to the bottom and incubated at 37^{o}C for 24 hrs for the determination of H_2S production, indole production, and motility. Production of indole was investigated by adding Kovac's reagent (HCl, 250ml, amyl alcohol, 750ml, and Para dimethylamine-benzaldehyde 50g/l) to growth in this culture medium. The non-utilization of indole and absence of deep red colour at the surface of agar was considered as presumptive for *Salmonella spp*.

Methyl red Voges –Proskauer reagent

This medium was stabbed to the bottom and incubated at 37°C for 24 hrs for the determination of acid production for methyl red and acetoin production in Voges – Proskauer. Methyl red was added to the MR tube. A red colour indicates a positive result (glucose can be converted into acidic end products such as lactate, acetate, and formate). A yellow colour indicates a negative result; glucose is converted into neutral end products. For Voges – Proskauer first alpha-napthol and then potassium hydroxide was added to the VP tube. The culture was allowed to sit for about 15 minutes for colour development to occur. If acetoin was produced then the culture turns a red colour (positive result); if acetoin was not produced then the culture appears yellowish to copper in colour (a negative result). The non-production of red color as a result of the absence of deep red colour in the medium was considered as presumptive for *Salmonella spp*.

3.10.4. Detection of *Vibrio spp*

Determination of the Vibrio spp. 25g of fish fillet was homogenized in 225 Buffered peptone water. Then, 0.1 ml of aliquot was spread plated on Thio Sulphate Citrate Bile Salt agar (TCBS) agar plates after serial dilution were inoculated by spreading 0.1 ml of the serial dilutions and incubated at 37°C for 48 h. TCBS plates were examined for the presence of either yellow or green colour with round colonies were taken as presumptive for *Vibro spp*.

For purification, a single colony from each grown type of Vibrio spp suspect colonies was streaked onto TCBS agar and incubated overnight at 37℃. This process was repeatedly performed until pure consistent colonies. Biochemical tests including Oxidase, Triple Sugar Iron (TSI), Sulphur reduction – Indole – Motility (SIM), Methyl Red (MR), and Voges-Proskauer (VP).

3.10.5. Antimicrobial Susceptibility of Pathogens

The antimicrobial susceptibility test was done by agar disc diffusion assay as described by the Clinical and Laboratory Standards Institute (CLSI, 2020).A standardized suspension of the bacterial isolates was prepared and the turbidity of the inoculum was matched with the turbidity standard 0.5 McFarland (concentration equivalents to a cell density of about 10^7-10^8 CFU/g) (Bauer *et al*., 1966).Briefly, a pure culture of the colony was taken from nutrient agar and transferred into a test tube containing 5ml of a sterile saline solution and mixed gently to make a homogenous suspension equal with the turbidity to 0.5 McFarland. Then, a bacterial suspension was swabbed by using a cotton swab on the pre-dried surface of Muller Hinton agar. Antibiotic discs were then placed on the cultured agar surface using clean and sterile forceps. The plates were incubated aerobically at 37 ℃ for 24 h. Antimicrobials used for susceptibility testing and their concentrations (µg) are Ampicillin (AM), Chloramphenicol (C), Ciprofloxacin (CIP), Clindamycin (CN), Erythromycin (E), Kanamycin (K), Gentamycin (G), Nalidixic acid (NA), Penicillin G (P), Streptomycin (S), Tetracycline (TE) and Vancomycin (VA). Finally, the diameters of the zone of inhibition around the discs were measured to the nearest mm using a digital electronic calliper and the isolates were classified as susceptible, intermediate, and resistant to the drugs tested according to the interpretation standards of CLSI (2020).

3.10.6. Data Analysis

The percentage of coefficient of variation (% CV) was calculated for significant variation in counts within the fillet samples analysed. The mean and standard deviation of each site and the significant difference between sites were analysed using SPSS software version 20. Then, the values were compared using one-way ANOVA and the significance of differences were considered at a 95% confidence interval ($P < 0.05$).

4. Results

4.1.Socio-demographic characteristics of Fish sellers

The socio-demographic characteristics of the fish sellers revealed that 100% of fish fillet sellers from Gilgel Gibe were males. Unlike the Gilgel Gibe site, the fish fillet sellers from Jimma city were40% females and 60% males. Educationally, 45% of Gilgel Gibe sellers were unable to read and write while 70% of sellers from Jimma city attended secondary education. Majorities of Gilgel Gibe (60%) and Jimma city (70%) had 3 to 5 years of experience in selling the fish fillets (Table 2).

Table 2. Socio-demographic characteristics of fish fillet sellers

Parameters	Gilgel Gibe (N=20)		Jimma City (N=10)	
	Frequency	%	Frequency	%
Sex				
Male	20	100	6	60
Female	-	-	4	40
Age				
<20	5	25	-	-
20- 30	13	65	6	60
31-40	2	10	4	40
Educational status				
Uneducated	9	45	-	-
Primary	7	35	3	30
Secondary	4	20	7	70
Experience selling fish fillet				
1-2 years	3	15	2	20
3-5 years	12	60	7	70
>5 years	5	25	1	10

4.2.General sanitation of Fish fillet sellers

The general sanitation of fish fillet sellers were observed that they didn't wash hands before work, didn't use gloves during work, didn't get training on the safety of fish fillets from the Gilgel Gibe site (Table 3). On the other hand, 100% of Jimma city sellers transported long distances and didn't disinfect using the disinfectants. In both sites, there was dust in selling sites and the sellers didn't use basic methods of cross-contamination (Table 3).

Table 3. General sanitation of fish fillet sellers from Gilgel Gibe and Jimma City shops

S.N	Parameters	Fish fillet from GG (N=20)		Fish fillet from Jimma City (N=10)	
		Yes	No	Yes	No
		Fre. (%)	Fre. (%)	Fre. (%)	Fre. (%)
1	Neatness of fishing net	Observed(no)	Observed(no)	-	_
2	Washing hands before work	Observed	observed	observed	observed
3	Using gloves during work	Observed	observed	-	observed
4	Proper handling of instruments	2 (10)	18(90)	4 (40)	6 (60)
5	Cleaning the surfaces and tools with soap and water	10 (50)	10(50)	10 (100)	-
6	Disinfecting working surfaces and tools using disinfectants	-	20(100)	-	10 (100)
7	Selling using direct contact of paper currency	20 (100)	-	10 (100)	-
8	Wear a mask during work	-	20(100)	-	10(100)
9	Hair cover during work	-	20(100)	4 (40)	6(60)
10	Wash hands after touching fish fillet	4 (20)	16(80)	6 (60)	4(40)
12	Use open toilet around vending area	20 (100)	-	-	10(100)
13	Use refrigerator until sell	6 (30)	14(70)	8 (80)	2(20)
14	Store more than 4h in container	8 (40)	12(60)	-	10(100)
15	Storing in open filed until collection	Observed	observed	-	observed
16	Dust available on the vending site	Observed	-	observed	observed
17	Obtaining training on the safety of fish fillet	-	20(100)	-	10 (100)
18	Having information about food and waterborne disease	11 (55)	9(45)	10 (100)	10 (100)
19	Water of fishing area is pure	observed	observed	-	_
20	Transporting long distances for selling	-	20(100)	10 (100)	-
21	Sterilizing the transport container	-	20(100)	-	10(100)

Where: Fre, Frequency

4.3. Microbial Load

The count of AMB was higher (8.28 ± 0.09log CFU/g)in fish fillets collected from Jimma city shops. Similarly, the counts of staphylococci were also higher(6.86 ± 0.17 Log CFU/g)in Jimma city shops. In comparison, the highest mean microbial counts were observed from fish fillets of Jimma city shops. The microbial counts of AMB from both sites showed a significant difference (P< 0.05) and the % CV was< 10 % in all counts (Table 4).

Table 4. Microbial mean counts (log CFU/g ± SD) of fish fillet

Microbes	Gilgel Gibe Site		Jimma City Site		P-value
	mean Log CFU/g ± SD	% CV	mean Log CFU/g ± SD	% CV	
AMB	7.19 ± 0.12	1.67	8.28 ± 0.09	1.09	0.000
Staphylococci	6.52 .± 0.37	5.67	6.86 ± 0.17	2.49	0.003
Enterobacteriaceae	5.73 .± 0.17	2.97	5.9 ± 0.1	1.69	0.003
Coliform	5.32 .± 0.18	3.38	5.12 ± 0.09	1.76	0.001
Yeast and Mould	4.55 .± 0.06	1.32	4.63 ± 0.1	2.16	0.014

Where: AMB= Aerobic Mesophilic Counts SD= Standard Deviation

4.4. Microbial Analysis

From the total of 30 fillet samples analysed, a total of 312 bacterial isolates were obtained, characterized, and classified into the following groups(Appendix 5). Generally, Gram-positive bacteria 276(88.46%) dominated the Gram-negative bacteria 36(11.54 %) (Figure 5). Among the isolates *Staphylococcus* spp. were the dominant in both sites which accounted for 101 (32.37%), followed by *Enterococcus spp*. 63 (20.19%) and *Bacillus spp*. 59(18.91%). However, *Salmonella spp* 8(2.56%) was the lowest as compared to all of the microbes.

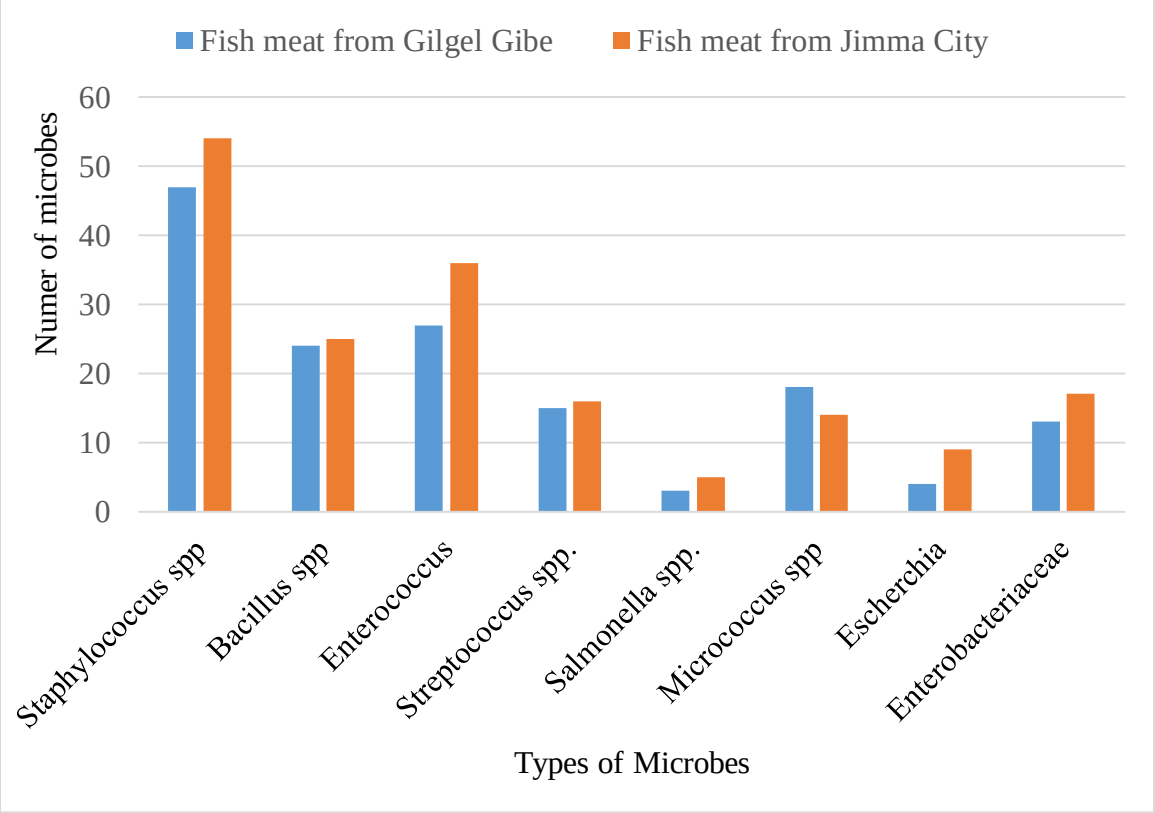

Figure 5. Frequency distribution of dominant bacteria in fish fillet

4.5.Prevalence of *S. aureus, Salmonella spp, Vibrio spp and Listeria Spp*

The presence of pathogens in fish fillets showed the overall 43.33%of samples were positive for *S.aureus* followed by *Listeria spp*(23.33%). However, *Vibrio spp* was the lowest prevalent (16.67%). In comparison, pathogens were less prevalent in fish fillets collected from Gilgel Gibe than Jimma city (Table 5).

Table 5. Prevalence of *S. aureus, Salmonella spp ,Listeria spp* and *Vibrio spp* from fish fillet

Pathogens	Fish fillet from Gilgel Gibe (n=20)	Fish fillet from Jimma City (n=10)	Total (n=30)
	Frequency (%)	Frequency (%)	Frequency (%)
S.aureus	8 (40)	5 (50)	13 (43.33)
Listeria spp	4 (20)	3 (30)	7 (23.33)
Salmonella spp	3 (15)	3 (30)	6 (20)
Vibrio spp	4 (20)	1 (10)	5 (16.67)

4.6.Antibiotic susceptibility of pathogens

A total of 12 antibiotics were applied to assess the resistance and susceptibility of the isolates. Accordingly, all isolates were classified as resistant, intermediate, and susceptible. However, for seek of interpretation the intermediate isolates were classified as resistant (Appendix 6). Briefly, the isolates of *S.aureus* were highly resistant to Penicillin G (100%), followed by Ampicillin (92.31%), and tetracycline (84.62%).*Listeria spp was* also highly resistant to Penicillin G (100%) followed by clindamycin, erythromycin, and vancomycin (85.71%, each). Moreover, Gram-negative bacteria such as *Salmonella spp* were highly resistant (100%) to ampicillin whereas Vibrio *spp* were highly resistant (100%) to vancomycin. On the other hand, S.aureus (84.62%), *Listeria spp* (71.43%), *Salmonella spp* (83.33%), and *Vibrio spp* (80%) were susceptible to ciprofloxacin(Table 6).

Table 6. Antimicrobial susceptibility patterns of *Salmonella spp, Listeria spp, Salmonella spp,* and *Vibrio spp*

Antibiotics	Disc Potency	*S.aureus*(n=13)		*Listeria spp*(n=7)		*Salmonella spp*(n=6)		*Vibrio spp*(n=5)	
	(µg/mL)	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Ampicillin (AM)	10	12 (92.31%)	1(7.69%)	5 (71.43%)	2(28.57%)	6 (100%)	-	4 (80%)	1(20%)
Chloramphenicol (C)	30	5(38.46%)	8 (61.54%)	3(42.86%)	4 (57.14%)	3 (50%)	3(50%)	3 (60%)	2(40%)
Ciprofloxacin (CIP)	5	2 (15.38%)	11(84.62%)	2 (28.57%)	5(71.43%)	1 (16.67%)	5(83.33%)	1(20%)	4(80%)
Clindamycin (CN)	2	9 (69.23%)	4(30.77%)	6 (85.71%)	1(14.29%)	5 (83.33%)	1(16.67%)	4 (80%)	1(20%)
Erythromycin (E)	15	10 (76.92%)	3(23.08%)	6 (85.71%)	1(14.29%)	ND	ND	ND	ND
Kanamycin (K)	30	6 (46.15%)	7(53.85%)	2 (28.57%)	5(71.43%)	2 (33.33%)	4(66.67%)	1 (20%)	4(80%)
Gentamycin (G)	10	3 (23.08%)	10(76.92%)	3 (42.86%)	4(57.14%)	2 (33.33%)	4(66.67%)	2(40%)	3 (%)60
Nalidixic acid(NA)	30	ND	ND	ND	ND	5 (83.33%)	1(16.67%)	3(60%)	2(40%)
Penicillin G(P)	10	13 (100%)	-	7 (100%)	-	ND	ND	ND	ND
Streptomycin(S)	10	5 (38.46%)	8(61.54%)	2 (28.57%)	5(71.43%)	3 (50%)	3(50%)	2(40%)	3 (60%)
Tetracycline(TE)	30	11 (84.62%)	2(15.38%)	7 (100%)	-	4 (66.67%)	2(33.33%)	4 (80%)	1(20%)
Vancomycin(VA)	5	8 (61.54%)	5(38.46%)	6 (85.71%)	1(14.29%)	3 (50%)	3(50%)	5 (100%)	-

Where, ND= Not Done

4.7.Multidrug Resistance (MDR) Pattern of the selected Pathogens

The MDR pattern of all pathogens showed the isolates were resistant to three, four, five, six, seven, and eight antibiotics (Table 6). Among these isolates, *S.aureus*, *Listeria spp*, *and Vibrio spp* were dominant by being resistance to seven antibiotics which accounted for 53.84, 42.86, and 40% respectively. However, *Salmonella spp* were dominating (50%) by being resistance of six antibiotics (Table 7).

Table 7. MDR of S.aureus, Listeria spp, Salmonella spp. and Vibrio spp

Pathogens	No. of Antibiotic resistance	Antimicrobial resistance pattern	No of isolates	Total
S.aureus	Three	C,P,E	1 (7.69%)	1 (7.69%)
	Five	AM,C,CN,P,S	1 (7.69%)	2 (15.38%)
		AM,E,P,TE,VA	1 (7.69%)	
	Six	AM,C,CN,E,G,P	1 (7.69%)	3 (23.08)
		AM,C,CA,E,G,P	1 (7.69%)	
		AM,CN,E,P,TE,VA	1 (7.69%)	
	Seven	AM,C,CN,E,K,P,TE	1 (7.69%)	7 (53.84%)
		AM,CN,E,K,P,S,TE	3 (23.08)	
		CN,E,G,P,S,TE,VA	1 (7.69%)	
		AM,CN,K,G,P,S,TE	1 (7.69%)	
		AM,C,CIP,E,P,TE,VA	1 (7.69%)	
Listeria Spp	Six	C,CN,G,P,TE,VA	1 (14.29)	2 (28.57)
		AM,CIP,CN,E,P,TE	1 (14.29)	
	Seven	C,E,K,P,S,TE,VA	1 (14.29)	3(42.86)
		AM,CN,E,G,P,TE,VA	2 (28.57)	
	Eight	AM,CIP,CN,E,P,S,TE,VA	1 (14.29)	2 (28.57)
		AM,C,CN,E,K,P,TE,VA	1 (14.29)	
Salmonella Spp	Five	AM,C,CN,NA,TE	2 (33.33)	3(50)
		AM,CIP,K,S,VA	1 (16.67)	
	Six	AM,C,CN,K,NA,S	1 (16.67)	2(33.33)
		AM,CN,G,NA,TE,VA	1 (16.67)	
	Seven	AM,CN,G,NA,S,TE,VA	1 (16.67)	1 (16.67)
Vibrio spp	Four	AMP,CIP,S,VA	1 (20)	1 (20)
	Five	CN,NA,S,TE,VA	1 (20)	1 (20)
	Six	AM,C,CN,K,TE,VA	1 (20)	1 (20)
	Seven	AM,C,CN,G,S,TE,VA	1 (20)	2(40)
		AM,C,CN,G,NA,TE,VA	1 (20)	

Where: AM =Ampicillin, C= Chloramphenicol, CIP= Ciprofloxacin, CN= Clindamycin, E= Erythromycin, K= Kanamycin, G =Gentamycin, A= Nalidixic acid, P=Penicillin G, S= Streptomycin, TE= Tetracycline, VA=Vancomycin.

5. Discussion

In the current study, the count of AMB in fish fillets from both sites exceeds 7 log CFU/g with a significant difference ($P<0.005$) between Gilgel Gibe and Jimma city shops sites. In comparison, the fish fillets obtained from Jimma city shopping had higher counts of AMB than the Gilgel Gibe site. In line with the present study, Pao*et al*. (2008) reported a high aerobic mesophilic count of bacterial count has been reported from different fresh fillet samples count ranging from 3.84 to 8.23 log CFU/g. Eizenberga *et al*., (2015) also reported a total viable count in fish fillets ranging up to 7.57 log CFU/g. The AMB count could be due to the lack of the neatness of fishing net, store place (stored in an open field and dirty container), long-distance transportation (for Jimma city shops), and personnel hygiene.

Results from the survey and observation showed that the vending sites had dust,and the sellers didn't obtain training on the safety of fish fillets. On the other hand, 100% of Jimma city sellers responded as transport fish with long-distance and didn't disinfect the working place using the disinfectants. The sellers' hygiene, dust, and litter from the vending site lead to the proliferation of large populations of aerobic mesophilic microorganisms (Sudershan, *et al*., 2009).The result of microbial aerobic load present study falls in unacceptable condition based on the International Commission for Microbiological Specifications for Foods (ICMSF, 1998) which suggests acceptance of samples that contain a total viable count below 7 log CFU/g. This indicates all samples from Gilgel gibe and Jimma city shops were recorded > 7 log CFU/g TVC, which is unacceptable. A high load of total bacterial count could be an indicator of a possible risk of spoilage and/or safety that could further affect the health of consumers since all the different bacteria have varying effects (Gram *et al*., 2000). This could be due to improper handling practices along the supply chains of the fish.

In the present study, the count of staphylococci was higher than 6 log CFU/g. The higher count of staphylococci in the present study could be due to the cross-contamination of hand to fillet or money or paper currency to fillet, lack of using a mask (prevent the sneezing). The result of the interview showed that 100% of the fillet sellers from both sites didn't use a mask which prevents the cross-contamination of droplets, 100% exchange paper currency with bare hands without using gloves, and presence of dust in selling sites. Mensah (2002), reports the use of bare hands to increase the contamination of staphylococci that exist in air, dust, sewage, water, and food or on food equipment and environmental surfaces. *S.aureus* can be

found in the nose (50%), on hands (5-30%), in the hair, eyes, and throat of healthy persons (Hammad, 2004).

In the current study, the count of Enterobacteriaceae and coliform was found greater than 5 log CFU/g. In lower to the present study (Hailemichael and Gutema, 2020) reported the count of coliform from fish fillet samples from shops of Addis Ababa showed relatively lower TCC than fish samples from supermarkets. Coliforms count of samples from supermarkets was in a range between 2.45 ± 0.03 to 5.00 ± 0.00 CFU/g. The presence of Enterobacteriaceae and coliforms in fish fillets could be due to environmental contamination such as poor sanitation, washing water, faecal contamination, and relative humidity during the handling and storage of the fish. The results of the interview and observation showed that 100% the Gilgel Gibe sites used open toilet that exist around vending area.A study by Ahimed and Sirag, (2016) reveals that the presence of enteric microorganisms is due to faecal contamination resulting from improper hand washing after the use of the toilet. According to Otokunefor *et al*. (2020),the presence of the Gram-negative bacteria which are a member of the coliforms indicates the possibility of the presence of faecal contamination on the public conveniences.

In the present study, the yeast and mold count was greater than 4 log CFU/g. In the lower to the present Centeno and Rodriguez (2005) reported for frozen fish fillet mold and yeast counts were 3.28 log CFU / g in samples of fish. The presence of yeast and mold in fish fillets could be due to cross-contamination from the river or the pond or the serving utensils, the poor management of fish ponds increases the chances of fungal infection in fishes. Moreover, certain other conditions in the pond increase the possibility of fungal infection including poor pond management, injured fish or fish having other diseases, or large amounts of decomposing organic matter in a pond (Eli *et al.*, 2011).The presence of fungi and yeasts in food can be an indicator of contamination derived from inadequate hygienic and sanitary conditions and the ability to resist adverse environmental conditions (Centeno and Rodriguez, 2005; Machado *et al*., 2015)

In general, the microbial load of fish fillets was very high in the present study > 4 log CFU/g. The higher microbial load could be due to personal hygiene, cross-contamination during and after fishing from soil, hands, and equipment during transportation,and water quality from which fish lives. In fish, the microbial load depends, to a large extent, on the microbiological contamination of the aquatic environments, as well as the hygienic-sanitary level during the

handling in cultivation, harvest, processing, storage, and transportation practices (Fuertes Vicente *et al*., 2014).

In the present study *S.aureus, Salmonella spp, Listeria spp*, and *Vibrio spp* were isolated. *S. aureus* was the dominant followed by *Listeria spp*. Similarly, scholars obtained these pathogens from aquaculture and fishing activities are widely distributed and provided with food, which has been related to outbreaks and cases of diseases; therefore, fish are considered potential carriers and transmitters of pathogens to humans, being the most relevant *Escherichia coli, Staphylococcus aureus, Salmonella spp., Listeria spp.,* and *Vibrio spp.,* (Vázquez-Sánchez *et al*., 2018) The presence of *Salmonella spp* is because of poor hand washing and contact with infected pets are some of the contamination routes (Munck *et al*., 2020). *Listeria spp* could be introduced into fish products during the processing of raw fillet and fillet products, an important source of contamination at processing facilities, resulting in subsequent contamination of products (Miettinen and Wirtanen 2005). Evisceration and scalding of the fish before marketing can contribute to the introduction of *Listeria spp* into the surroundings, leading to cross-contamination of fish, utensils, personnel, and environment (Papadopoulos *et al*., 2010).

In the current study, 43.33% of fish fillet was contaminated by S.aureus. In lower to this, Saito *et al*. (2011) reported *S. aureus* in the retail fish samples was19.6%. Hygienic practices in fish-processing facilities have recently improved, with some facilities adopting measures such as the use of ozone water and ultraviolet sterilizers. Pathogenic and spoilage microorganisms can be introduced into fish and fish products at any point throughout the production and supply chain. Studies have demonstrated several bacterial species encountered in different fish, which are potentially pathogenic under certain conditions as reported for *Pseudomonas spp, vibrio spp, E.coli,* and *S.aureus*(Emikpe *et al*., 2011).

In the current study, S. aureus was found to be resistant to ampicillin, clindamycin, erythromycin, penicillin G, tetracycline, and vancomycin. Similarly, Albuquerque *et al*. (2007) reported a multidrug resistance of *S. aureus* isolated from fish was resistant to ampicillin, penicillin, and erythromycin. On the other hand, *Listeria spp* was 100% resistant to Penicillin G & tetracycline, 85.71% to erythromycin, and 71.43 % resistant to ampicillin. However, they were susceptible to chloramphenicol, ciprofloxacin, kanamycin, gentamycin, and streptomycin. In lower to this, Rodas-Sua´rez *et al*. (2006) reported *L. monocytogenes* isolated from fish water were resistant to ampicillin (60.3%), erythromycin (30.9%),

penicillin (57.4%), and tetracycline (16.7%). Pagadala *et al*. (2012) also reported resistance among the tested *L. monocytogenes* to erythromycin, and tetracycline, and were susceptible to gentamicin, trimethoprim, and kanamycin.

In the present study, majorities of the isolates of *Salmonella spp* were resistant to ampicillin, clindamycin, nalidixic acid, and tetracycline. Similarly, Zhao *et al*. (2006) reported Salmonella spp from seafood were resistant to tetracycline, ampicillin, chloramphenicol, nalidixic acid. On the other hand, majorities of the isolates of *Vibrio spp* were resistant to ampicillin, erythromycin, vancomycin, and streptomycin. Similarly, Amalina *et al*. (2019) reported as majorities of the *Vibrio spp* isolated from groupers (fish of any genera) ranged from 57- 100% resistance to ampicillin, erythromycin, vancomycin, and streptomycin. The resistance of the *Vibrio spp* to these antibiotics could be due to resistance genes located in plasmid and chromosomes and the increasing use of these antibiotics in aquaculture. Letchumanan *et al*. (2015) reported that the resistance level of pathogenic Vibrio spp. toward antibiotics used in aquaculture was increasing every year and some antibiotics are ineffective in controlling bacterial pathogens. Moreover, bacteria are overly exposed to antibiotics, they tend to acquire antimicrobial resistance genes, either via horizontal gene transfer or vertical gene transfer (Serrano, 2005).

6. Conclusion

- The overall mean microbial load of fish fillets of Gilgel Gibe and Jimma city shops were greater than 4 log CFU/g with the AMB count the highest recorded (> 7 log CFU/g) in both sites.

- Among the microbes distributed in fish fillets, Staphylococci spp was the highest prevalent followed by Enterococcus and *Bacillus sp*.

- Pathogens in fish fillets showed the overall 43.33% of samples were positive for *S.aureus*, 23.33% for *Listeria spp*,20% for *Salmonella spp*, and 16.67% for *Vibrio spp*. In comparison, the overall pathogens isolated from Gilgel Gibe were less prevalent in fish fillets sold in Jimma city shops.
- Gram-positive pathogens (*S.aureus* and *Listeria spp)* were resistant to Penicillin G, ampicillin, erythromycin, tetracycline, vancomycin, and clindamycin. Moreover, Gram-negative pathogens (*Salmonella* and *Vibrio spp)* were resistant to ampicillin, clindamycin, nalidixic acid, tetracycline, and vancomycin. However, all pathogens were relatively susceptible to kanamycin, gentamycin, streptomycin, and ciprofloxacin.
- The high microbial load, and presence of pathogens have significantly increases the loss of consumer acceptability, the shelf life of the fish fillet, and an indicator for having health impact.

- Lack of washing hands before work, direct contact of paper currency, use open toilet around vending area, storing and processing fish fillet in open filed, lack of training on the quality and safety, and transporting long distances for selling fish fillet are the risk associated factors with it.

7. Recommendations

- The higher microbial load and the presence of pathogens should call for cleaning or treating the reservoir with proper disinfectants, or further need of appropriate heat treatment before consumption.

- The contamination of pathogens could be originated from sellers' hygiene, materials used for storage, water sources, and improper management of the fish fillets. Hence, sanitary assessments in both sites are advisable.

- For disease caused by the isolated pathogens better to use ciprofloxacin, gentamycin, kanamycin, and streptomycin antibiotics.
- The high health risks of pathogens need due attention by responsible authorities like Jimma zone Agricultural sectors, the zonal and Jimma city Health Bureau for further remedies

- Disease like listeriosis was reported around Gonder, in this study therefore the responsible health offices should take preventive measures in Gonder and other parts of the country.

- Proper processing, storage, and good handling procedures of fish fillet better to be employed.

- The microbial and physicochemical properties of Gilgel Gibe water should be studied to identify the exact source of contamination

- Identification of the isolates were done using morphological and biochemical tests. Thus, better to use the molecular approach to overcome this drawback.

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This interview was designed to obtain the general sanitation and knowledgeof fish fillet sellers towards fillet. This interview was prepared only for research purposes and cannot be used for another purpose.Your genuine response is very critical to get the necessary information and is crucial for this study. Thank you in advance for your cooperation.

Appendix 1. Socio-demographic characteristics of the fillet sellers

Parameters	Fish fillet from GG (N=20)		Fish fillet from Jimma City (N=10)	
	Frequency	%	Frequency	%
Sex			-	-
Male				
Female				
Age				
<20				
20- 30				
31-40				
Educational status				
Uneducated				
Primary				
Secondary				
Experience selling fish fillet				
1-2 years				
3-5 years				
>5 years				

Appendix 2. General sanitation of fish fillet producers

S. N	Parameters	Fish fillet from GG (N=20)		Fish fillet from Jimma City (N=10)	
		Yes	No	Yes	No
		Fre. (%)	Fre. (%)	Fre. (%)	Fre. (%)
1	Fishing net is neat				
2	Washing hands before work				
3	Using gloves during work				
4	Proper handling of instruments				
5	Cleaning the surfaces and tools with soap and water				
6	Disinfecting working surfaces and tools using disinfectants				
7	Selling using direct contact of paper currency				
8	Wear a mask during work				
9	Hair cover during work				
10	Wash hands after touching raw fillet				
12	use open toilet around the vending area				
13	Use refrigerator until sell				
14	Store more than 4h in container				
15	Storing in open filed until collection				
16	Dust available on the vending site				
17	Obtaining training on the safety of fish fillet				
18	Having information about food and waterborne disease				
19	Having information about food and waterborne disease				
20	Transporting long distances for selling				
21	Sterilizing the transport container				

Appendix 3. John's bacterial identification (John's, 2012)

Gram reaction (young culture)	+	+	+	+	+	-	+	+	+	+	–	–	–	–	–
Shape	CCl	CCl	CCh	CT	Rod	rod	IR	rod	rod	rod	rod	rod	rod	rod	Rod
Aerobic growth	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Anaerobic growth	–	+	+	+	+	–	–	+	+	–	–	–	+	+	+
Endospore	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
Motility	–	–	–	–	–	-	–	+/-	+/-	+/ -	+/-	+/ -	–	+	+
Catalase reaction	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+
Oxidase reaction	+	–	–	–	–	–	–	–	+/-	+/ -	+	+	–	–	+
OF Test	–	+	+	+	+	–	–	+/-	+	–	+	–	+	+	+
Micrococcus	X														
Staphylococcus		X													
Streptococcus			X												
Lactococcus			X												
Enterococcus			X												
Leuconostoc			X												
Pediococcus			X	X											
Aerococcus				X											
Lactobacillus					X										
Acinetobacter						X									
Arthrobacter							X								
Clostridium								X							
Bacillus									X	X					
Alcaligenes											X				
Pseudomonas												X			
Klebsiella													X		
Shigella													X		
Salmonella														X	
Escherichia														X	
Other enteric genera														X	
Aeromonas															X

Where; CCl= Coccus (clusters), CCh =Coccus (chains), CT= Coccus (tetrad), IR= Irregular Rod

Appendix 4. One way ANOVA result among a different microbial group

		Sum of Squares	df	Mean Square	F	Sig.
AMB	Between Groups	8.911	1	8.911	740.037	.000
	Within Groups	.337	28	.012		
	Total	9.248	29			
Staphylococci	Between Groups	.847	1	.847	10.217	.003
	Within Groups	2.320	28	.083		
	Total	3.167	29			
Enterobacteriaceae	Between Groups	.213	1	.213	10.553	.003
	Within Groups	.566	28	.020		
	Total	.779	29			
Coliform	Between Groups	.304	1	.304	15.030	.001
	Within Groups	.566	28	.020		
	Total	.870	29			
Yeast and mould	Between Groups	.048	1	.048	6.925	.014
	Within Groups	.194	28	.007		
	Total	.242	29			

Appendix 5. Frequency distribution of bacteria

Microbial groups	Fish fillet from Gilgel Gibe		Fish fillet from Jimma City	
	Frequency	Percentage	Frequency	Percentage
Staphylococcus spp	47	31.13	54	30.68
Bacillus spp	24	15.89	25	14.20
Enterococcus	27	17.88	36	20.45
Streptococcus spp.	15	9.93	16	9.09
Salmonella spp.	3	1.99	5	2.84
Micrococcus spp	18	11.92	14	7.95
Escherichia	4	2.65	9	5.11
Enterobacteriaceae	13	8.61	17	9.66

Appendix 6. Antibiotic susceptibility pattern of Pathogens

Antibiotics	*S.aureus*			*Listeria spp*			*Salmonella spp*			*Vibrio spp*		
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Ampicillin	10(76.92%)	2(15.38%)	1(7.69%)	4(57.14%)	1(14.29%)	2(28.57%)	5(83.33%)	1(16.67%)	-	3(60%)	1(20%)	1(20%)
Chloramphenicol	3(23.08%)	2(15.38%)	8(61.54%)	1(14.29%)	2(28.57%)	3(57.14%)	3(50%)	-	3(50%)	1(20%)	2(40%)	2(40%)
Ciprofloxacin	-	2(15.38%)	11(84.62%)	1(14.29%)	1(14.29%)	5(71.43%)	-	1(16.67%)	5(83.33%)	-	1(20%)	4(80%)
Clindamycin	8(61.54%)	1(7.69%)	4(30.77%)	4(57.14%)	2(28.57%)	1(14.29%)	4(66.67%)	1(16.67%)	1(16.67%)	3(60%)	1(20%)	1(20%)
Erythromycin	7(53.8%)	3(23.08%)	3(23.08%)	6(85.71%)	-	1(14.29%)	ND	ND	ND	ND	ND	ND
Kanamycin	4(30.77%)	2(15.38%)	7(53.85%)	1(14.29%)	1(14.29%)	5(71.43%)	1(16.67%)	1(16.67%)	4(66.67%)	1(20%)	-	4(80%)
Gentamycin	3(23.08%)	-	10(76.92%)	1(14.29%)	2(28.57%)	4(57.14%)	1(16.67%)	1(16.67%)	4(66.67%)	1(20%)	1(20%)	3(60%)
Nalidixic acid	ND	ND	ND	ND	ND	ND	4(66.67%)	1(16.67%)	1(16.67%)	3(60%)	-	2(40%)
Penicillin	13(100%)	-	-	7(100%)	-	-	ND	-	ND	ND	ND	ND
Streptomycin	3(23.08%)	2(15.38%)	8(61.54%)	1(14.29%)	1(14.29%)	5(71.43%)	1(16.67%)	2(33.33%)	3(50%)	1(20%)	1(20%)	3(60%)
Tetracycline	10 (76.92 %)	1(7.69%)	2(15.38%)	6(85.71%)	1(14.29%)	-	3(50%)	1(16.67%)	2(33.33%)	1(20%)	3(60%)	1(20%)
Vancomycin	6 (46.15%)	2(15.38%)	5(38.46%)	3(42.86%)	2(28.57%)	1(14.29%)	-	-	3(50%)	3(60%)	2(40%)	-

Where ND= **Not** done

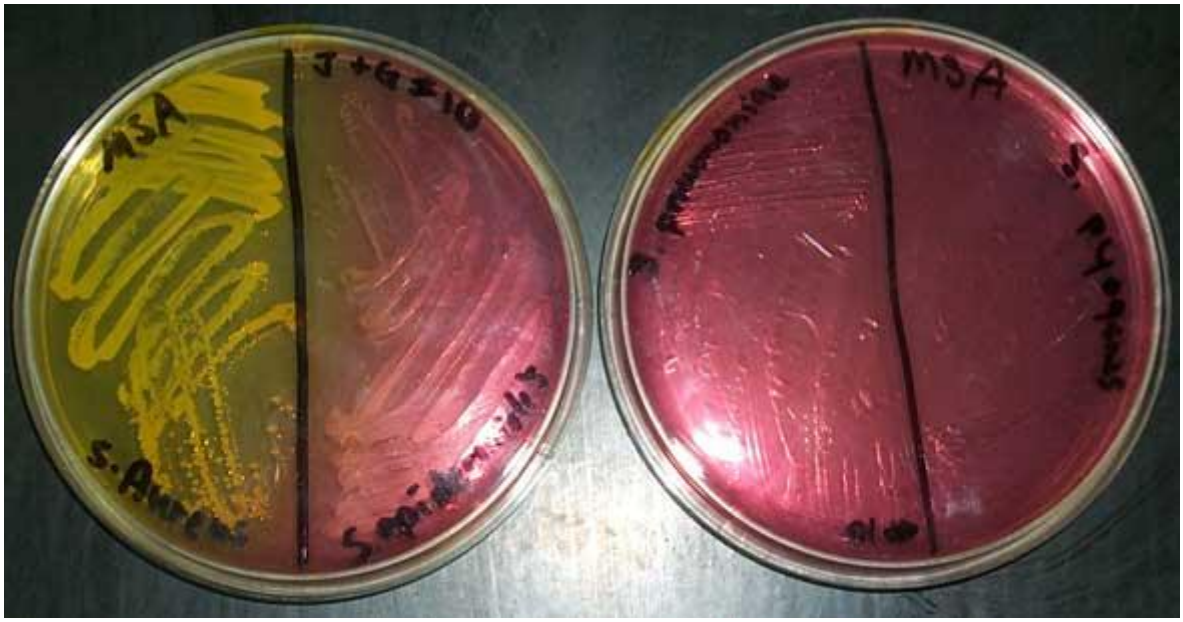
Appendix 7. Some images of the study

Fish storage and fishing

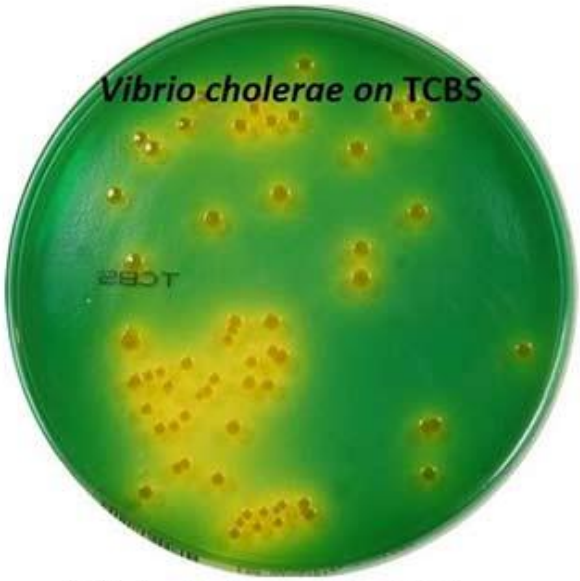
Fish fillet processing by the small scale fisher men

Fish fillet processing and storing in the field

Appendix. 8. Detected pathogens



Colony morphology of S. aureus on the left and S. epidimis on the right from MSA



Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar