BACTERIAL CONTAMINATION LEVELS IN FRESH FISH FILLETS SOLD IN LUSAKA DISTRICT, ZAMBIA

BY

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A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SAFETY AND RISK ANALYSIS

THE UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE

DECLARATION

I, **Malifa Mwendelema**, declare that this dissertation is an authentic piece of my own work. It adheres to the guidelines outlined for MSc dissertations at the University of Zambia. I confirm that it has not been presented for the purpose of obtaining a degree from this University or any other university.

Signature: ……. Date: ……...

CERTIFICATE OF APPROVAL

The dissertation submitted by Malifa Mwendelema has been reviewed and approved by the University of Zambia, satisfying the partial requirements for the conferment of the Master of Science in Food Safety and Risk Analysis.

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ABSTRACT

Zambia has seen rapid growth in aquaculture, with fish increasingly becoming a source of emerging bacterial zoonotic diseases. This cross-sectional study assessed the levels of bacterial contamination in 132 fresh fish fillets, consisting of 69 hake and 63 tilapia sold in Lusaka District, Zambia, addressing the research gap in local fish contamination and antimicrobial resistance. The isolates were identified using their morphological characteristics and conventional biochemical tests. The antibiotic susceptibility of selected bacteria was determined by the Kirby–Bauer disc diffusion method. Total viable count (TVC) and faecal coliform presence revealed that 31% of the samples exceeded the TVC limit, and 45% exhibited faecal coliforms. While hake fillets had no faecal contamination, 93% of tilapia fillets were contaminated, with only four samples testing negative for faecal coliforms. *Escherichia coli* was the predominant bacterium (53.8%), followed by *Klebsiella pneumoniae* (46.2%), and other species such as *Vibrio parahaemolyticus* and *Staphylococcus aureus*. Antimicrobial susceptibility testing (AST) revealed chloramphenicol's broad-spectrum efficacy against most bacteria, while penicillin resistance was noted in *Staphylococcus* and *Serratia* species. Ciprofloxacin and doxycycline were mostly effective, though one *E. coli* strain showed resistance. The high levels of contamination, especially in tilapia fillets, pose significant health risks, particularly to vulnerable populations such as children, the elderly, and immunocompromised individuals. While the exact quantity required for negative health effects varies based on bacterial load and individual susceptibility, ingestion of contaminated fish could lead to gastrointestinal illness or more severe outcomes. The study highlights the need for better farming practices, improved food safety standards, and stricter regulatory enforcement to mitigate microbial contamination in fish products. Public awareness on proper fish handling and cooking is also crucial to minimize health risks.

DEDICATION

With immense gratitude and love, I dedicate this dissertation to the two extraordinary individuals who have shaped my world - my mother, Mrs Ketty Mwape, and my father, Mr Lee Mwendelema. Your boundless sacrifices, enduring love, and unwavering belief in my potential have been the foundation of my academic journey. This achievement is a tribute to the values and strength you have instilled in me.

To the rest of my family, especially my sister, Tukiya and brother, Lee, your constant belief in my abilities and your encouragement during challenging times have been a source of strength. This work reflects the values and resilience instilled in me through our shared experiences.

To my gorgeous friends, you have been the bright stars illuminating the academic sky, making the journey not only intellectually enriching but also joyous and memorable. Your camaraderie and understanding have been invaluable.

This dissertation is dedicated to each of you - my family and friends - for being the pillars of my life. Your presence has infused my academic pursuit with meaning, and I am profoundly thankful for the love and support that has sustained me throughout this journey.

ACKNOWLEDGEMENTS

I would like to begin by expressing my deepest gratitude to God for providing me with the incredible opportunity to successfully undertake this academic journey. His guidance, strength, and boundless blessings have been my constant companions, and I am profoundly thankful for the wisdom and perseverance bestowed upon me.

I extend my sincere appreciation to my supervisor, Professor Benard Hang'ombe, whose unwavering support, valuable insights, and encouragement have been instrumental in shaping this thesis. His dedication to excellence and scholarly guidance have been invaluable throughout this research process.

I extend my heartfelt thanks to ACEIDHA (Africa Centre of Excellency for Infectious Disease of Human and Animals), our generous sponsors, for their financial support, which has made this research possible. Their commitment to fostering education and research is commendable, and I am grateful for the opportunities their sponsorship has provided.

Lastly, I want to acknowledge the exceptional contributions of the laboratory technicians whose expertise and commitment to excellence greatly contributed to the success of this research. Their assistance in conducting experiments and handling laboratory procedures has been invaluable. I also extend my heartfelt appreciation to my classmates. Their support, discussions, and encouragement have made this academic journey more fulfilling. This thesis is a culmination of collective efforts, and I am grateful for the support and encouragement from all who have played a role in this academic achievement.

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LIST OF ABBREVIATIONS

AMP	Ampicillin
AMR	Antimicrobial Resistance
AMC	Amoxiclav
AMX	Amoxicillin
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic-resistant genes
CDC	Centres for Disease Control and Prevention
CFUs	Colony Forming Units
CI	Confidence Interval
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standard Institute
COT	Cotrimoxazole
CTX	Cefotaxime
DO	Doxycycline
DNA	Deoxyribonucleic acid
ERES	Excellence in Research Ethics & Science Converge
FAO	Food and Agriculture Organization
FZDO	Fish Zoonotic Disease Outbreaks
GMP	Good Agricultural Practices
LCMS	Liquid Chromatography-Mass Spectrometry
LAMP	Loop-Mediated Isothermal Amplification
MDR	Multi Drug Resistance
MFL	Ministry of Fisheries and Livestock
MOH	Ministry of Health.
NHRA	National Health Research Authority
NGS	Next-Generation Sequencing
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
SADC HT	Southern African Development Community
SIM	Sulphide Indole Motility

SPP	Species
STEC	Shiga Toxin *Escherichia coli*
TCBS	Thiosulfate-Citrate-Bile Salts-Sucrose Agar
TVC	Total viable Count
UN	United Nations
UNZA	University of Zambia
WHO	World Health Organisation
ZABS	Zambia Bureau of Standards
ZADA	Zambia Aquaculture Development Association
ZEMA	Zambia Environmental Management Agency

CHAPTER ONE

INTRODUCTION

1.1 Background

In recent years, the consumption of fish has become an integral part of the diet for millions of people worldwide, owing to its nutritional value and the growing awareness of the health benefits associated with it (Siamujompa et al., 2023). Fish and fish products currently constitute the most traded food products globally (FAO/WHO, 2020). Zambia has recorded significant growth in the aquaculture sub-sector, recording 400% growth in the past decade however, the safety of fresh fish, particularly in regions with limited resources, remains a critical concern (Siamujompa et al., 2023). As the demand for fresh fish continues to rise, so does the need for a thorough understanding of the microbial safety of these aquatic products. Bacterial contamination poses significant risks to public health, causing foodborne illnesses and economic losses within the food industry (Ndashe et al., 2023).

Fresh fish fillets are a highly perishable retail food item, and their bacteriological quality is a concern to the food industry and consumers (Sheng et al., 2021). During the filleting of fish as practiced in filleting plants or shops, it is impossible to avoid contamination of the initially virtually sterile fish flesh (Møretrø et al., 2016). Subsequently, this primary contamination will proliferate, depending on storage conditions. However, post-harvest handling, processing, and storage of fish lead to food contamination losses and waste (Novoslavskij et al., 2016). Therefore, contamination occurs at all stages in the fish supply chain from capture to consumer (Akande et al., 2010).

Fish and fish products, especially raw or undercooked products, have been involved in outbreaks associated with bacterial pathogens, biotoxins, histamine, viruses, and parasites (Sheng et al., 2021). According to estimates, there are ten to twelve million tons of fish lost annually in the world, or about 10% of the entire catch from aquaculture and capture fish (FAO, 2018). Fish have been identified as reservoirs of bacterial pathogens linked to human diseases caused by species such as *Mycobacterium* spp*., Streptococcus aureus,*

Photobacterium damselae, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, Erysipelothrix rhusiopathiae, pathogenic Escherichia coli, Aeromonas spp*., Salmonella* spp*., Staphylococcus aureus, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens, Campylobacter jejuni, Delftia acidovorans, Edwardsiella tarda, Legionella pneumophila,* and *Plesiomonas shigelloides* (Novotny et al., 2004).

Concerns have been raised regarding the spread of antibiotic-resistant genes (ARGs) in the environment. The presence of these genes in bacteria from contaminated fish poses a threat to the environment and human health, as it can lead to the emergence and spread of antimicrobial resistance (AMR) among different organisms (Brunton et al., 2019). The use of antibiotics in fish farming is a significant concern, as it can result in antibiotic residues in the aquatic environment, affecting aquatic animals, plants, and terrestrial creatures that consume the water. A review by Kimera et al. (2023) highlights the widespread antimicrobial resistance in aquaculture across Africa, with bacteria exhibiting high levels of multidrug resistance to commonly used antibiotics such as tetracycline, ampicillin, cotrimoxazole, gentamicin, and amoxicillin. The study further reports that tetracycline residues were found in fish at a prevalence of up to 56.7%, raising concerns about potential human exposure through consumption. Despite limited data on antimicrobial use in fish farming across the continent, there is a growing need for surveillance and regulation to mitigate the burden of AMR on public health (Kimera et al., 2023).

Fresh fish is one of the widely consumed sources of proteins in Zambia, especially in Lusaka district, where it is sold in various markets and restaurants (Songe et al., 2012). However, fish deserves more attention in food policy because it has a special role in food security, nutrition, and sustainability. Compared to other agricultural systems, fish has unique nutritional benefits and higher production efficiency (Valero et al., 2016). To improve the quality and safety of fish for both local and global markets, it is essential to maintain high standards of primary and post-harvest operations in the fisheries industry (Akande et al., 2010)Many factors can cause fish disease in intensive production facilities, such as poor husbandry practices and inadequate biosecurity systems (Maulu et al., 2020).

Studying the prevalence, ecology, concentration, and dynamics of pathogenic and spoilage microorganisms throughout the fish production chain can help develop and apply new intervention strategies.

This study aims to determine the levels and types of bacteria present in fresh fish fillets sold in Lusaka District, Zambia. The study will identify the bacterial species in the fish fillets and assess their prevalence. Additionally, it will evaluate the antibiotic susceptibility profiles of the isolated bacteria, providing insights into potential risks to public health and food safety.

The findings of this study are crucial for public health, the local economy, and food safety regulations. By identifying the levels of bacterial contamination and their resistance to antibiotics, the study will provide valuable data for policymakers, food safety experts, and industry stakeholders to improve safety practices in the fish supply chain in Lusaka District, Zambia.

1.2 Problem Statement

Despite the importance of fresh fish consumption in the diet of the Lusaka District community, concerns persist regarding the bacterial contamination levels in locally sold fish fillets. The unique environmental and market conditions in Lusaka District create an essential backdrop for examining the bacterial quality of fresh fish fillets, providing valuable insights into potential health hazards and informing strategies for improved food safety practices. Fresh fish fillets are highly perishable retail food items, and their bacteriological quality is a concern to the food industry and consumers (Sheng et al., 2021). Pathogenic and spoilage microorganisms can be introduced into fish products in both preharvest and postharvest stages (Ibrahim et al., 2020). These microorganisms can lead to foodborne illnesses, including gastroenteritis and food poisoning, with significant health impacts. In sub-Saharan Africa, foodborne diseases are responsible for an estimated 91 million cases of illness and 137,000 deaths annually (World Health Organization, 2015).

The rate of spoilage and the associated disposal costs of contaminated fish fillets also represent a significant economic burden. According to studies, the postharvest losses of fish in sub-Saharan Africa can reach as high as 30-40%, with poor handling and storage conditions being key contributors to spoilage (Odeyemi et al., 2020). Improper storage temperature, pH, water availability, or moisture, along with indigenous microflora such as bacteria and fungi, processing operations (Odeyemi et al., 2020), transportation (Hammond et al., 2015), and food handlers, all influence the extent of spoilage. Furthermore, the use of antibiotics in fish farming, if not managed prudently, can contribute to the development of antibiotic-resistant bacteria, which poses a significant risk to both fish and human health. It is estimated that antibiotic resistance causes at least 700,000 deaths globally each year, and this number could rise to 10 million by 2050 if no action is taken (O'Neill, 2014).

Limited research has been conducted to assess the extent of contamination and identify predominant bacteria in fish fillets, hindering efforts to implement targeted fish safety measures (Onjong et al., 2018). Therefore, there is a critical need to investigate the bacterial quality of fresh fish fillets, focusing on tilapia and hake, and assess the susceptibility of identified bacteria to commonly used antibiotics. Addressing these gaps in knowledge is essential for safeguarding public health, preventing the spread of antibiotic resistance, reducing spoilage and disposal costs, and ensuring the safety of fish products in the local market.

1.3 Justification

Fish products, especially raw or undercooked products, have been involved in outbreaks associated with bacterial pathogens (Galaviz-Silva et al., 2009). Fish have been identified as reservoirs of bacterial pathogens linked to human diseases including *Mycobacterium* spp*., Vibrio cholera,* pathogenic *Escherichia coli, Salmonella* spp*., Staphylococcus aureus, Listeria monocytogenes* and *Clostridium botulinum* (Novotny et al., 2004b). In addition to human pathogens, bacteria are considered the primary cause of fish spoilage. Pathogenic and spoilage microorganisms can be introduced into fish and fish products at any point throughout the production and supply chain (Rippen et al., 2012). Therefore, understanding the extent of contamination and identifying the predominant bacteria can

help pinpoint areas for improvement in the fish supply chain, leading to better food safety practices and reduced health risks for consumers. Additionally, assessing the antimicrobial susceptibility of the identified bacteria provides valuable information for healthcare professionals in selecting appropriate treatment options for potential infections caused by consuming contaminated fish. Overall, investigating the microbial quality of fresh fish fillets is essential for promoting food safety, protecting public health, and enhancing the overall well-being of the community. In Zambia, the Lusaka District, a region known for its vibrant fish market and growing urban population, represents a focal point for investigating the levels of bacterial contamination in fresh fish fillets. The findings of this study have the potential to inform policy changes, improve regulatory frameworks, and strengthen food safety measures in the fish industry at both local and national levels.

1.4 Research Questions

1. What are the levels of bacterial contamination, and which bacteria are present in fresh fish fillets sold in Lusaka District, Zambia?
2. What is the antibiotic susceptibility profile of the bacteria isolated from fresh fish fillets?

1.5 Objectives

1.5.1 General Objective

To determine the levels, characteristics, and antibiotic susceptibility of bacteria associated with fresh fish fillets sold in Lusaka District, Zambia.

1.5.2 Specific Objectives

1. To determine the levels of bacterial contamination and identify the bacteria present in fresh fish fillets sold in Lusaka District.
2. To determine the antibiotic susceptibility profiles of the bacteria isolated from fresh fish fillets.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of fish contamination and its significance

Globally, fish is a critical source of nutrition, providing approximately 20% of the average per capita animal protein intake for about 3 billion people (Chitambo et al., 2023). Over the years, the demand for fish has surged significantly. Between 1961 and 2018, global fish production rose sharply to 179 million tons, with a market value of $401 billion (FAO/WHO, 2020). During the same period, per capita fish consumption increased from 9.0 kg to 20.5 kg (FAO/WHO, 2020). This growth has been driven by a combination of factors, including the rising demand for high-quality protein, declining wild fish stocks, and advancements in aquaculture technology. Notably, aquaculture accounted for 46% of global fish production and contributed 62% of total revenue in 2018 (U.S. Food and Drug, 2001). Projections indicate that global aquaculture production will double by 2050, but this expansion poses challenges to fish product safety (Ibrahim et al., 2020).

Regionally, fish contamination remains a significant concern in Africa, where the risk of foodborne illnesses linked to fish is high. The Centres for Disease Control and Prevention (CDC) reported that between 1998 and 2018, fish accounted for 937 foodborne outbreaks globally, resulting in 5,011 illnesses, 364 hospitalizations, and four deaths (CDC, 2018). Fish and fish products are consistently implicated in 6% to 8% of all confirmed foodborne-illness outbreaks, a higher rate than chicken and beef, which accounted for 3.6% and 1.9%, respectively, between 2011 and 2017 (CDC, 2018). Despite the belief that the muscles of healthy fish are sterile (Austin, 2006), these statistics highlight the persistent challenges in maintaining fish safety and underscore the need for robust control measures.

In Zambia, fish plays an essential role in dietary nutrition, contributing up to 55% of the population's animal protein intake (FAO, 2022). With the expansion of fish farming practices, Zambia has seen significant growth in aquaculture. However, this growth raises

concerns about food safety and the potential for fish borne zoonotic disease outbreaks (FZDOs) among consumers and workers (FAO/WHO, 2020).

2.2 Types of Bacteria associated with fish fillets

The fish industry and public health face a significant challenge from bacterial contamination of fish and fish products. Cultured tropical freshwater fish are mainly affected by bacterial diseases, which often cause mass mortality in fish. A study reported that the following common fish pathogens were found at these prevalence rates on lake Kariba, Siavonga, Zambia: *Aeromonas* spp. (13%), *Pseudomonas* spp. (10.3%), *Micrococcus* spp. (9.7%), *Klebsiella* spp. (8.7%), *Lactococcus* spp. (7.3%), *Streptococcus* spp. (7.0%), and *Acinetobacter* spp. (7.0%) (Siamujompa et al., 2023). Another study in Lusaka and the Southern Province of Zambia detected the following bacteria in fish with zoonotic potential at these farm prevalence rates: *Aeromonas* (13.2%), *Bacillus* (2.1%), *Clostridium* (2.1%), *Escherichia coli* (0.7%), *Klebsiella* (6.9%), *Lactococcus* (2.1%), *Listeria* (0.7%), *Staphylococcus* (18.1%), and *Streptococcus* (0.7%) (Chitambo et al., 2023). The prevalence of bacterial contamination in fish and fish products depends on various factors, such as the country, the fish species, the geographic location, the sampling stage (fish farm vs. retail stores), the sampling part (skin vs. intestine), the source (imported vs. domestic), and the fish product type (raw vs. ready to eat) (Sheng et al., 2021).The next section will discuss in detail the occurrences of *Salmonella* spp., *L. monocytogenes, Escherichia coli.,* and *A. hydrophila* in fish and fish products, as well as their resistance to antimicrobials.

2.2.1 *Salmonella* spp

Salmonella is a Gram-negative bacterial genus in the Enterobacteriaceae family that comprises the species *S. enterica* and *S. bongor. S. enterica* is further divided into six subspecies (enterica, salamae, arizonae, diarizonae, houtenae, and indica) with over 2,600 serotypes (Modarressi et al., 2010). *Salmonella* can contaminate fish and fish products through poor hygiene practices during handling, and other suboptimal fish breeding, processing, or storage methods (Amagliani et al., 2012)

Salmonella with antibiotic resistance is classified as a "Serious Threat Level pathogen" (CDC, 2019). However, detection of *Salmonella* spp. in seafood is essential as it is accountable for most of the foodborne illnesses or gastroenteritis characterized by diarrhoea, abdominal cramp, vomiting, nausea, and fever. According to Centres for Disease Control and Prevention, *Salmonella* is the primary cause of bacterial foodborne infections causing approximately 1.4 million nontyphoidal cases, 15,000 hospitalizations, and 400 deaths in the USA annually (Sanjee et al., 2016). Regrettably, *Salmonella* strains isolated from fish and fish products have exhibited resistance to a wide range of antibiotics including ampicillin, tetracycline, and streptomycin. Some *Salmonella* isolates have multidrug resistance (MDR), with resistance to at least one antibiotic in three or more drug classes (CDC, 2018). MDR is a greater concern as it can augment bacterial virulence and result in more severe diseases in patients (Brunton et al., 2019).

2.2.2 *Listeria monocytogenes*

A gram-positive bacterial species, *Listeria monocytogenes,* causes high mortality (around 16%) in infected individuals (Papadopoulos et al., 2010). *L. monocytogenes* is widely distributed in the environment and can be found in various habitats, such as soil, silage, animal faeces, fresh and marine water, and sediments. *Listeria* spp. is frequently detected in water sources that are contaminated or rich in organic matter, for example, rivers and coastal areas (Jami et al., 2014). One of the factors that enable *Listeria* spp. to persist in the environment is their capacity to grow and reproduce at very low temperatures. *L. monocytogenes* showed the longest survival time in water and sewage at 4°C, with a maximum duration of 120 to 141 days (Budzińska et al., 2012). Hence, it is essential to implement effective sanitation and control measures in fish processing plants to minimize the risk of *L. monocytogenes* contamination in fish and fish products.

The presence of antibiotic-resistant *L. monocytogenes* isolates further complicates fish safety. In India, isolates from fish and fish products were often resistant to antibiotics such as ampicillin, clindamycin, erythromycin, and tetracycline (Basha et al., 2019). Additionally, Chen et al., (2010) reported that 71% and 69% of *L. monocytogenes* isolates from domestic catfish fillets and their processing environments were resistant to

cefotaxime and clindamycin, respectively. Furthermore, 75% of *L. monocytogenes* isolates from imported fish and other seafood were found to be resistant to nitrofurantoin (Wang et al., 2011). These antibiotic-resistant strains highlight the need for rigorous microbial safety measures in the fish industry.

2.2.3 *Vibrio cholerae*

The World Health Organization reports that Zambia has faced repeated cholera epidemics since 1977, with the latest one in 2017-2018, causing 5,935 infections and 114 fatalities (WHO, 2011). Cholera has been prevalent in some regions of the country, such as Lusaka province, which has registered the most cases. Lusaka province had 4,464 infections and 73 fatalities (CFR 1.63%) (MoH, 2018) during the 2009 epidemic. This was succeeded by another epidemic in 2010-2011, with 173 infections in Lusaka district. The Cholera epidemic was also announced in October 2017 and impacted more than 3,534 individuals with more than 77 fatalities (MoH, 2018). The cholera epidemic interfered with many economic activities in the country, particularly in the impacted areas (Malata et al., 2021).

Cholera, a waterborne disease, often spreads concurrently as a foodborne disease. Fish accumulate bacteria as they consume contaminated food in the environment. Eating contaminated fresh fish has been associated with zoonotic disease transmission in humans, as fresh fish contain various spoilage bacteria and pathogens, including *V. cholerae*, which causes cholera in humans. (Kobayashi et al., 2010) conducted a study to verify whether cholera can be transmitted by food, especially fresh fish, and thus infect humans. The results indicated that vibrio agar was contaminated with *Vibrio* spp. Another study evaluated the risk of exposure to *V. cholerae* species through consumption of fresh fish in Lusaka province of Zambia. The results showed that the risk of contracting cholera through consumption of contaminated fresh fish is generally low for both restaurant and household exposure pathways. The low risk in this study could be explained by the preparation methods of fresh fish and meat in Zambia, which involve boiling and frying for long periods (Malata et al., 2021). However, the consumption of fresh fish, which is not well done, poses risk to consumers, especially those eating from restaurants. Equally, cross-contamination with other ready-to-eat products handled in the same kitchen with raw fish may increase the risk of exposure to *V. cholerae*. Despite intensive efforts, its

ecology and transmission via contaminated fish remains unclear (Novoslavskij et al., 2016).

Recent research on diseased marine fish in South China has highlighted the escalating issue of antimicrobial resistance in *Vibrio* species (Deng et al., 2020). Among 70 isolates, 64.3% were resistant to more than three antibiotics, with a multi-antibiotic resistance index ranging from 0.00 to 0.60. Over 50% of the strains exhibited resistance to vancomycin, amoxicillin, midecamycin, and furazolidone, while all were sensitive to florfenicol, norfloxacin, and ciprofloxacin. Notably, the study found higher resistance in Hainan compared to Guangdong, which was attributed to warmer temperatures and excessive antibiotic use (Deng et al., 2020).

These findings add to the growing body of evidence demonstrating the significant contribution of excessive antibiotic use to the development and spread of antibiotic resistance among *Vibrio* species. Strains of *Vibrio* have developed an enhanced ability to resist antibiotics that were previously effective (CDC., 2018). This resistance complicates the clinical treatment of *Vibrio* infections and is linked to the imprudent use of antibiotics in human medicine, agriculture, and animal husbandry (Loo et al., 2020). As a result, it jeopardizes food security, threatens public health, and hinders economic growth. Food can serve as a vehicle for antibiotic-resistant *Vibrio*, with resistant genes transmitted to humans through the ingestion of contaminated food. The food chain may become contaminated with resistant strains during the harvesting or processing of animals (Loo et al., 2020). Exposure to these resistant bacteria can lead to longer and more severe infections, potentially increasing mortality due to treatment failures caused by antimicrobial resistance (WHO, 2017).

The growing challenge of antibiotic resistance in aquaculture, underscored by these findings, necessitates improved ecological regulation and antibiotic management.

2.2.4 *Escherichia coli*

Escherichia coli represents a bacterial species capable of contaminating fish, thereby inducing foodborne illnesses. Certain *E. coli* strains can generate toxins responsible for

precipitating diarrheal and severe intestinal infections (Songe et al., 2017). Typically present in the faeces of both humans and animals*, E. coli* infiltrates the food chain through suboptimal hygiene practices. This bacterium commonly resides in the gastrointestinal tracts of humans and warm-blooded animals (Songe et al., 2017). The emergence of antibiotic-resistant strains of *E. coli* poses challenges in treating diarrheal diseases. *E. coli* serves as a pertinent indicator of antimicrobial resistance within faecal bacteria due to its widespread presence and facile acquisition of resistance (Yohans et al., 2022).

Research indicates that *E. coli* shows increased resistance to older antibiotics used in both human and veterinary medicine (Sivaraman et al., 2020). Ideally, the presence of *E. coli* in food should be minimal, with acceptable levels being less than 20 colony-forming units per gram (cfu/g). In fish and other consumables, levels between 20 and 100 cfu/g are considered borderline, while levels above 100 cfu/g are unacceptable and signal contamination (Yohans et al., 2022). The use of antimicrobial agents to treat *E. coli* infections has led to the rise of antibiotic-resistant bacteria, making resistance genes a significant and growing concern in modern medicine. *E. coli's* resistance to antimicrobials is causing serious issues for healthcare systems globally (Yohans et al., 2022). Therefore, monitoring bacterial loads, identifying antibiotic resistance patterns, and evaluating hygienic practices in the fish industry are essential for understanding public health risks related to fish and fish products. Assessing both the bacterial load and antibiotic resistance of *E. coli*, along with examining hygiene practices in fish production, provides valuable insights into public health risks. These evaluations guide the development of effective management strategies to reduce foodborne diseases associated with fish consumption(Songe et al., 2017).

2.2.5 *Klebsiella* spp

Klebsiella pneumoniae is a bacterium that can cause serious infections in humans and animals. It can also contaminate fish and fish products, posing a risk to food safety and public health. One of the ways that *Klebsiella pneumoniae* can contaminate fresh fish is through the water in which the fish are cultured or harvested. According to a study by (Chitambo et al., 2023), *Klebsiella pneumoniae* was isolated from the internal organs of farmed Nile tilapia (*Oreochromis niloticus*) and the water samples from their habitat in

Zambia. The authors suggested that the bacteria could have originated from the surrounding environment, such as the soil, faecal matter, or runoff water. The bacteria could also have been introduced by human activities, such as handling, processing, or transporting the fish (Chitambo et al., 2023).

Another way that *Klebsiella pneumoniae* can contaminate fresh fish is through the food that the fish consume. According to a report by Mohan et al., (2016), *Klebsiella pneumoniae* was detected in the fish feed used in some aquaculture farms in Zambia. The report stated that the bacteria could have been present in the raw materials or the manufacturing process of the fish feed. The bacteria could then be transferred to the fish through ingestion, and subsequently to the consumers through consumption.

Klebsiella pneumoniae can cause various diseases in fish, such as haemorrhages, ulcers, abscesses, and redness of the skin. These symptoms can reduce the quality and marketability of the fish, as well as the profitability of the aquaculture industry. Moreover, *Klebsiella pneumoniae* can also cause zoonotic infections in humans, such as pneumonia, urinary tract infections, septicemia, and meningitis (Mohan et al., 2016). These infections can be serious and life-threatening, especially for people with weakened immune systems or underlying conditions (Mohan et al., 2016). Therefore, it is important to prevent and control the contamination of *Klebsiella pneumoniae* in fresh fish in Zambia.

Klebsiella spp. isolated from fish have raised significant concerns due to their potential to harbor and transmit antibiotic resistance. These bacteria, commonly found in aquatic environments, can contaminate fish during handling and processing (Mohan et al., 2016). A study in Tanzania focusing on the prevalence and antimicrobial resistance of bacteria in retail fish and shrimp found *Klebsiella* spp. to be notably prevalent. Out of 92 fish and 20 shrimp samples, *Klebsiella* spp. was identified in 28% of the samples, making it the second most common bacteria. Contamination rates were 26% in fish from open-air markets and 33% in those from supermarkets. *Klebsiella* isolates from these samples exhibited significant resistance to multiple antibiotics, including commonly used ones like ampicillin, tetracycline, and ciprofloxacin (Marijani, 2022). This resistance poses a public health risk, particularly for individuals with compromised immune systems, as it

can be transferred to humans through the consumption of contaminated fish or contact with aquatic environments. The presence of antibiotic-resistant *Klebsiella* spp. in fish highlights the need for stringent monitoring and control measures in aquaculture and fisheries to mitigate the spread of resistant pathogens and ensure food safety (Thongka et al., 2019).

2.3 Effects of Bacterial Contamination of fish on Human Health

The impact of microbial contamination on human health is a significant concern, particularly in the context of fish consumption. This issue not only jeopardizes the well-being of fish but also poses risks to human health (Wanja et al., 2020). Harmful microorganisms that can affect both fish and humans are present in various natural and man-made aquatic environments, including lakes, rivers, ponds, and fish farms. These microorganisms may originate from freshwater habitats or result from water pollution (Novoslavskij et al., 2016).

Consuming fish contaminated with these microorganisms can lead to a range of diseases and health complications, with the specific outcome dependent on the type and quantity of microorganisms ingested. Illnesses such as gastroenteritis, listeriosis, yersiniosis, salmonellosis, and botulism may arise, each with its severity based on the extent of exposure (Novoslavskij et al., 2016).

The repercussions of such diseases can be particularly grave, especially for vulnerable populations, including pregnant women, infants, the elderly, and individuals with compromised immune systems. In some cases, these diseases can be fatal, underscoring the importance of addressing and mitigating microbial contamination in fish to safeguard public health (Sudheesh et al., 2012).

2.4 Microbial contamination sources

Bacterial contamination of fish and fish products is a serious issue that affects the quality and safety of seafood. Bacteria can contaminate fish through various sources, such as the water environment, the harvesting and processing techniques, and the unhygienic practices. Some of the bacteria can cause spoilage of fish, resulting in undesirable sensory

properties and biochemical changes (Sheng et al., 2021). Some of the bacteria can cause human diseases, such as food poisoning, infections, and allergies. Some of the bacteria can also produce toxins, such as histamine, that can have harmful effects on human health (Onjong et al., 2018).

2.4.1 Water

Previously, it has been recorded that water plays a role in shaping the microbiome of fish. Unfortunately, water acts as a storage site for various pollutants across multiple layers (State, 2010). Initially, examinations of water samples from the environments where fish reside or are bred have uncovered the existence of pathogens harmful to humans, microorganisms causing spoilage, and antimicrobial resistant bacteria (ARB). The identified ARB include a range of bacteria such as *Salmonella* spp*., Aeromonas* spp*., Acinetobacter* spp*., Pseudomonas* spp*., Bacillus* spp*., Pseudoalteromonas* spp*., Proteus* spp., and others (Fattal et al., 1992). For example, *Salmonella* has been found not just in seafood but also in freshwater and freshwater fish. This can be linked to contamination of the water source and inadequate hygiene during the capture, handling, and transportation of fish. Studies have detected *Salmonella* in freshwater sources, including stream water and groundwater, with the potential for transmission to ponds (Budiati et al., 2013). The improper disposal of human and animal waste, along with insufficient sanitation, can contribute to *Salmonella* contaminating water sources (Amagliani et al., 2012). *Vibrios* are also abundant in aquatic surroundings and have been seen on the skin, gills, and digestive tracts of fish or shellfish. The concentration of *V. vulnificus* and *V. parahaemolyticus* was noted to be higher in fish intestines compared to water and sediment samples (Kobayashi et al., 2010). Other factors such as water salinity and temperature can impact the prevalence of *Vibrio* spp. in fish and aquatic environments.

2.4.2 Hygiene Practices during Processing

Considerable emphasis has been placed on maintaining hygienic practices in handling fish from the moment of capture to ensure high quality and prolonged storage. Processing, however, poses a significant risk of contamination at processing facilities. Microbes can infiltrate fish products during the processing of raw fish (Shikongo et al., 2011). The act

of evisceration and scalding fish before marketing, for instance, can introduce *L. monocytogenes* into the surroundings, leading to cross-contamination of fish, utensils, personnel, and the environment (Papadopoulos et al., 2010). Poor personal hygiene among fish processing workers can introduce harmful microorganisms to the fish fillets (Møretrø et al., 2016). Adequate training and adherence to hygiene protocols are essential to minimize contamination.

A study conducted on African catfish by Sing, (2016) from four farms and four wet markets in Malaysia revealed that the prevalence of *Salmonella* in the 14 retail fish surveyed (28.6%) was higher than that in the 16 fish collected directly from the farms (12.5%). This underscores the fact that *Salmonella* is introduced into fish and fish products during postharvest processing (Sing, 2016). Additionally, another study conducted by Duffes, (1999) demonstrated that *L. monocytogenes* could be transferred from the flesh to cut surfaces, equipment, and tables during salmon filleting, creating a potential source of contamination.

2.4.3 Fish Feed

The quality and composition of the feed, the environmental conditions, and the fish species are some of the factors that determine how fish feed can affect the microbial contamination in fish (Novoslavskij et al., 2016). The number of microorganisms detected can also vary depending on the season, the part of the fish's digestive tract, and the type of feeding. For instance, detritus eaters have more bacteria in their digestive tract than filter-feeding water. Moreover, some fish feed can increase the risk of pathogens such as *Salmonella* in fish (Modarressi et al., 2010). This was observed in fish that were fed chicken eggs and chicken offal, as well as in fish feed from Norway that contained fish meal and fish oil.

Additionally, fish feed can change the natural microbiota of fish, which are the helpful microorganisms that assist fish in digestion, immunity, and health. For example, some fish feed may have antibiotics or other additives that can disturb the microbiota and make fish more prone to infections (de Bruijn et al., 2018). Therefore, it is important to monitor

and control the microbiological status of fish feed, as well as the water and sediment quality, to ensure the safety and health of fish and humans.

2.5 Factors influencing Bacterial contamination in Fish fillets

Microbial contamination in fresh fish fillets is influenced by a combination of intrinsic and extrinsic factors. Understanding these factors is crucial for implementing effective control measures and maintaining the safety and quality of fresh fish products (Sing, 2016). The following are factors that influence microbial contamination in fresh fish fillets:

2.5.1 Temperature

Temperature is a critical factor affecting microbial contamination. A proper and consistent cold chain is essential from harvest to sale. Temperature fluctuations or insufficient refrigeration during storage and transport can enhance bacterial proliferation and contamination risk. However, some bacteria like *Listeria* spp. can survive and multiply at very low temperatures. Budzińska et al., (2012) found that *L. monocytogenes* had the longest survival time in water and sewage at 4 °C, ranging from 120 to 141 days. It can also withstand freezing temperature and even grow in fish at refrigerator temperatures (Novoslavskij et al., 2016b).

Yersinia spp can grow in both aerobic and anaerobic conditions, with the best temperature at 29 °C, and the range from 4 to 42°C (Novoslavskij et al., 2016b). *Y. enterocolitica's* ability to live and multiply at very low temperatures is of special interest. This microorganism adapts to cold by using various factors that keep the cell functions during and after the cold shock. The cold adaptation process involves increasing the levels of specific cold shock proteins and their genes, fatty acid composition and compatible solutes that balance the osmotic pressure in the cells (Rakin et al., 2006). Therefore, raw products can be a key factor for the risk of wider contamination with *L. monocytogenes, Y. enterocolitica* and many other microorganisms especially if products are eaten without heating them first.

2.5.2 Biofilms

Biofilms are an important factor for the survival of pathogens. They are bacterial populations enclosed by an extracellular polymeric matrix, attached to each other and/or to surfaces. Biofilms protect bacteria like *Listeria* spp. from environmental stresses and make them less sensitive to antibiotics and disinfectants than free-floating bacteria (Costeron et al., 1995). *Salmonella* and *Y. enterocolitica* can form biofilms that increase their survival in water environments and help them resist desiccation and antimicrobial treatments (Brunton et al., 2019). Bacterial cells in biofilms are more resistant to antimicrobials than the same cells without biofilms This is because the extracellular polymeric substance (EPS) matrix of biofilms acts as a physical barrier, hindering the penetration of antimicrobial agents. This matrix also traps and neutralizes antimicrobials, reducing their efficacy (Sing, 2016).

2.5.3 Aquatic Microbial Environment

Various factors, such as environmental conditions, can influence the occurrence of harmful microorganisms in fish and fish products (Choongo et al., 2009). These microorganisms can be classified into two main categories: those that naturally live in freshwater habitats and those that are linked to water pollution. Some examples of bacteria that belong to both categories are *Vibrio* spp.*, Listeria monocytogenes, Salmonella serovars, Clostridium botulinum,* and *Yersinia* spp (Novoslavskij et al., 2016). These bacteria can come from different sources, such as sewage, wild animals, livestock, and feed. They can spread widely in aquatic environments and cause serious diseases in humans, such as listeriosis, botulism, and *V. vulnificus* infection. Therefore, understanding and addressing these factors through comprehensive quality control measures and adherence to hygiene practices at each stage of the supply chain are crucial for minimizing microbial contamination in fresh fish fillets and ensuring the safety of the food supply.

2.6 Systems of Detecting Bacteria in Fish and Fish Products

Detecting microorganisms in fish and fish products is crucial to ensure food safety and prevent the spread of foodborne illnesses. Various methods and systems are employed for microbial detection in these products. Here are some common approaches:

2.6.1 Culture-based methods

Culture-based methods for isolating and identifying microorganisms from fish samples are pivotal in microbiology due to their reliability in detecting specific pathogens and spoilage organisms (Watanabe et al., 1973). They offer direct observation of microbial growth, facilitating quantification and detailed characterization through biochemical and serological assays. These methods are cost-effective, accessible, and provide essential data on microbial ecology, aiding in food safety assessments and targeted interventions. Despite advancements in molecular techniques, culture-based methods remain crucial for their established protocols, reproducibility, and comprehensive microbial insights.(Novoslavskij et al., 2016).

2.6.2 Molecular Methods

Molecular methods refer to a set of techniques and technologies used to study biological molecules, particularly DNA and RNA, at the molecular level. These methods allow researchers to analyse, manipulate, and understand the structure, function, and interactions of these molecules within living organisms. Some common molecular methods include:

a. Polymerase Chain Reaction (PCR): PCR is a molecular biology technique that amplifies specific DNA sequences, enabling the detection of targeted microorganisms through DNA analysis (Austin, 2019).

b. Real-Time PCR (qPCR): This method allows for the real-time monitoring of the PCR process, providing quantitative data on the initial amount of DNA present (Modarress et al., 2010).

c. Loop-Mediated Isothermal Amplification (LAMP): LAMP is an isothermal DNA amplification technique that can be used for rapid and specific detection of microorganisms (Austin, 2019).

2.6.3 Next-Generation Sequencing (NGS)

Metagenomic Sequencing: NGS technologies can be used for metagenomic analysis, providing a comprehensive view of the microbial community in a sample, including potential pathogens (Austin, 2019).

2.6.4 Biochemical Tests

Biochemical tests are essential for identifying bacteria based on their metabolic activities and enzymatic properties. These tests typically include observations of colony morphology, such as shape, colour, pigmentation, and haemolytic activity, followed by Gram staining for initial grouping. Specific tests like sugar fermentation in phenol red broth, sulphur reduction, indole production, and motility using SIM (Sulphide Indole Motility) media are commonly employed (Chitambo et al.,2023).

It is important to note that the choice of method depends on factors such as the specific microorganisms of concern, the required sensitivity, the speed of analysis, and available resources. Additionally, regulatory standards may dictate the use of specific methods for monitoring and ensuring the safety of fish and fish products (Tavares et al., 2021). This research used the culture-based method for the isolation and identification of microorganisms from fish samples using selective media, biochemical tests, and sensitivity media.

2.7 Antibiotic Susceptibility Testing and Antibiotics in Fish Health

Antibiotic susceptibility testing (AST) plays a vital role in ensuring the appropriate use of antibiotics in aquaculture, particularly for treating bacterial infections in fish. AST helps determine which antibiotics are effective against specific bacterial strains, guiding the choice of treatment. This not only ensures better outcomes but also prevents the misuse of antibiotics, which could contribute to the development of antimicrobial resistance (AMR) (Ferri et al., 2022). The World Health Organization (WHO) has

highlighted that improper antibiotic usage, particularly in food-producing animals such as fish, can accelerate AMR, reducing the efficacy of antibiotics over time and complicating the treatment of infections in both humans and animals (WHO, 2019). Therefore, conducting AST in aquaculture farms is essential to combat AMR and preserve the effectiveness of antibiotics.

In aquaculture, antibiotics like oxytetracycline, florfenicol, and chloramphenicol are commonly used to treat bacterial diseases in fish. However, some antibiotics, such as chloramphenicol, are banned in the European Union due to concerns over potential residues in fish products, which could pose a risk to human health (European Medicines Agency, 2020). These antibiotics are administered through various routes, including feed, water immersion, or injections, depending on the specific requirements of the farm and the type of infection. However, improper administration, such as using antibiotics that are not approved for use in food-producing animals, can exacerbate the problem of AMR by increasing the likelihood of resistant strains developing (Ferri et al., 2022).

The global issue of antibiotic resistance is compounded by the widespread use of antibiotics in aquaculture, where fish farming often involves high stocking densities. This creates an environment conducive to bacterial outbreaks, leading to increased antibiotic use. Unfortunately, the overuse and misuse of antibiotics can lead to the emergence of antibiotic-resistant bacteria (ARB) (Wamala et al., 2018). These resistant strains can transfer their resistance genes to humans through the consumption of contaminated fish, posing significant health risks. Moreover, the aquatic environment itself, including wastewater from fish farms, can act as a reservoir for resistant bacteria and genes, further promoting the spread of AMR. This underscores the importance of adopting stringent regulations and best practices in aquaculture to mitigate the environmental and public health impacts of AMR (Ferri et al., 2022).

2.8 Prevention and Control of Microorganisms in fish and fish products

To prevent or reduce the risk of microbial contamination of fish, a comprehensive approach involving various measures can be implemented:

a. Monitoring and controlling water and feed quality: Regular monitoring and strict control over the quality of water and feed used in aquaculture are essential (Mantovani, 2015). Ensuring that these inputs are free from contaminants helps maintain the health of the fish and reduces the risk of microbial contamination.

b. Implementing good hygiene and husbandry practices: Throughout the entire supply chain, from harvesting to distribution, strict adherence to good hygiene practices is crucial (Wanja et al., 2020). This includes maintaining cleanliness during handling, transportation, processing, and distribution of fish and fish products. Proper sanitation measures should also be in place to minimize contamination risks.

c. Applying adequate preservation methods: Employing suitable preservation methods such as refrigeration, freezing, salting, smoking, drying, or canning can effectively inhibit or eliminate microbial growth (Lokuruka, 2016). These methods help maintain the freshness and safety of fish products during storage and transportation.

d. Educating consumers: Consumers should be educated about proper handling, preparation, and consumption of fish and fish products (Hoffman et al., 2003). This includes guidance on washing, cooking to appropriate temperatures, and avoiding cross-contamination with other foods.

e. Following recommendations and regulations: It's crucial to adhere to the recommendations and regulations set forth by public health authorities regarding the safety and quality of fish and fish products (U.S. Food and Drug, 2001). Compliance with these standards helps ensure that fish reaching the market are safe for consumption.
Additional recommendations for consumers include:

i. Choosing fish from clean and safe sources: Selecting fish from reputable sources known for maintaining high standards of cleanliness and safety is advisable (Møretrø et al., 2016).

ii. Avoiding consumption of raw or undercooked fish: Cooking fish thoroughly reduces the risk of microbial contamination and foodborne illnesses (Austin, 2006).

iii. Cooking fish to proper internal temperature: Ensuring that fish reaches an internal temperature of at least 145°F (63°C) or until the flesh is opaque and flakes easily with a fork helps kill harmful bacteria (Al-Reza et al., 2015).

iv. Prompt refrigeration or freezing: Storing fish in the refrigerator or freezer immediately after purchase or catch helps maintain its freshness and prevents microbial growth (Sanjee et al., 2016).

v. Discarding spoiled fish: Any fish exhibiting signs of spoilage, such as a bad odor or unusual appearance, should be discarded to avoid the risk of foodborne illness (Odeyemi et al., 2020).

vi. Practicing proper hygiene: Washing hands and utensils thoroughly before and after handling fish helps prevent cross-contamination and ensures food safety (Hanyinza et al., 2020).

2.9 Regulatory Guidelines and Standards for fish products

Regulations have been instituted to offer directives for good farming and processing practices within the fish industry (Haambiya et al., 2014.). Achieving global harmonization of laws, regulations, and manufacturing/storage/packaging practices is paramount to guaranteeing the microbial safety of fish and fish products. The following governmental and private agencies are tasked with the development and enforcement of national standards and quality control measures in Zambia. Their pivotal role encompasses promoting consumer protection, facilitating trade, and upholding the quality and safety standards of products in the Zambian market.

a. Zambia Bureau of Standards (ZABS): ZABS is a statutory body mandated to develop, promote, and enforce national standards across various sectors, including

fisheries. It plays a crucial role in establishing quality control measures and certification schemes for fish products to safeguard consumer health and promote trade.

b. Ministry of Fisheries and Livestock (MFL): The MFL is the primary government agency responsible for overseeing the fisheries sector in Zambia. It formulates policies, regulations, and guidelines to ensure the sustainable management of fisheries resources and the safety of fish products.

c. Zambia Environmental Management Agency (ZEMA): ZEMA may be involved in regulating aspects related to environmental impact assessments, which could be relevant to the fishing industry.

d. Zambia Aquaculture Development Association (ZADA): This association may play a role in promoting and regulating aquaculture practices in Zambia, and their guidelines may impact the production of fish products.

These regulators work in collaboration to establish and enforce regulations, standards, and quality control measures to ensure the safety and quality of fish and fish products in the Zambian market.

This literature review underscores the complex nature of bacterial contamination in fresh fish fillets, emphasizing its public health significance. The review highlights various contamination sources, including water quality, handling practices, and processing environments, all of which contribute to the microbial load in fish products. Several pathogenic bacteria, such as *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli*, have been identified as potential risks to consumers, reinforcing the need for stringent control measures.

Despite extensive research on fish contamination, key gaps remain. Notably, there is limited data on the bacterial contamination of fresh fish fillets in Zambia, particularly in high-end retail outlets in Lusaka. Additionally, the reviewed literature primarily focuses on broad strategies for controlling bacterial contamination but does not address specific

interventions tailored to fresh fish fillets sold in the Zambian market. The regulatory framework for fish safety has been discussed extensively, yet there is limited information on its enforcement and effectiveness within local retail chains.

Given these gaps, this study aims to assess the bacterial contamination of fresh fish fillets sold in Lusaka's retail outlets, with a focus on determining microbial load, bacterial species present, and their antibiotic susceptibility patterns. The findings will contribute to evidence-based recommendations for improving food safety in the fish supply chain. Understanding the extent of contamination will inform policymakers, retailers, and consumers about potential risks and necessary interventions to enhance fish quality and safety in Zambia.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

In the examination of microbial contamination levels in fish fillets obtained from retail outlets in Lusaka District, a cross-sectional study design was applied. Stratified random sampling was employed for the sampling technique. The identification focused on three main stores recognized for supplying fish fillets, each with outlets distributed across six different locations within Lusaka District: Longacres, Woodlands, East Park, Levy Mall, Twin Palm Mall, and Lewanika Mall. Fish fillets were then randomly selected from each study stratum. Lusaka was selected for its central role as the capital city, with a wide range of retail outlets and a high demand for fish products, providing a representative sample for the study. This study was conducted between June and July 2023, aiming to assess the extent of bacterial contamination in 132 fresh fish fillet samples purchased from Lusaka district.

3.2 Study Area

The comprehensive investigation into microbial fish contamination was executed within the confines of Lusaka district (Figure 3.1), a pivotal location situated in Lusaka Province, Zambia. Precisely defined by geographical coordinates 15.38 degrees South latitude and 28.32 degrees East longitude, Lusaka district emerges as a strategic focal point for this study on microbial contamination in fish.

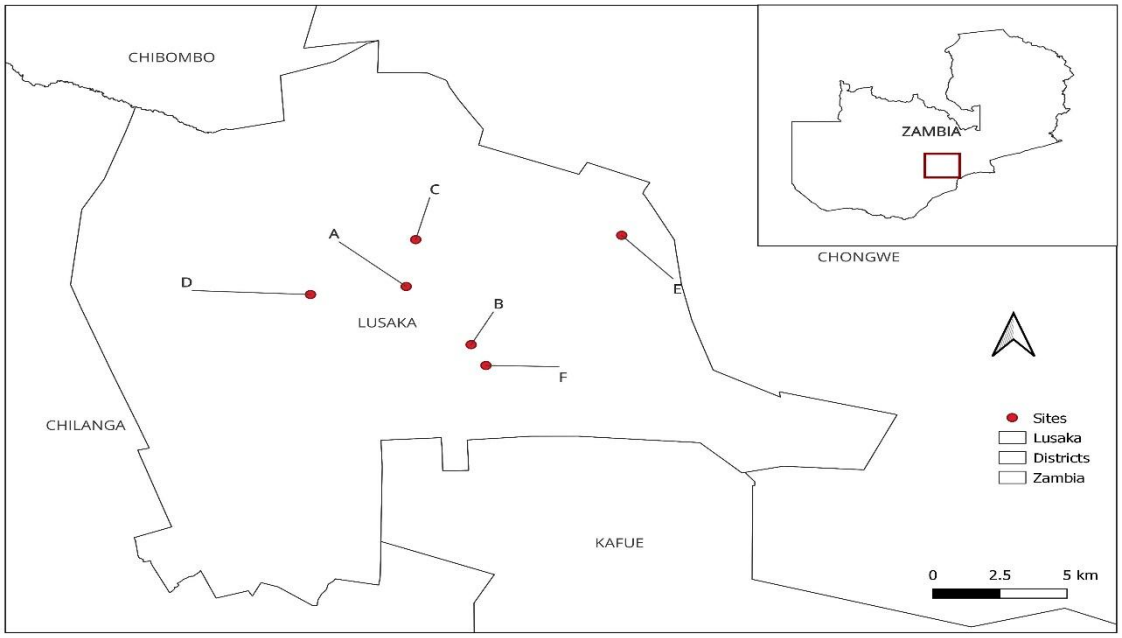


Figure 3.1: Map of the study area.

3.3 Sample size calculation

The Lusaka district has approximately 200 retail outlets that sell fresh fish fillets (Ziba et al, 2017). To ensure a representative sample for presentation, a 5% absolute precision was employed at a 95% confidence interval. The calculation involved key parameters:

- Z: Critical value of the normal distribution at the required confidence level
- P: Sample proportion
- d: Absolute precision
- n = Population size

The formula used for sample size calculation is given by:

$$n = \frac{Z^2 . P . (1-P)}{d^2}$$

$$n = \frac{(1.96)2 \ (.50) \ (.50)}{(0.05)^2}$$

$$n = \frac{0.9604}{0.0025}$$

$$n = 384$$

Therefore, the adjusted finite sample calculation is applied to determine the sample size:

$$N = \frac{n.x}{(x + n - 1)}$$

$$= \frac{384 * 200}{200 + 384 - 1}$$

$$= \frac{76800}{583}$$

= 132 total number of fresh fish fillets samples

Regarding the number of samples collected from each store/outlet, a total of 132 fish fillet samples were collected, with 22 samples taken from each of the six locations (Longacres, Woodlands, East Park, Levy Mall, Twin Palm Mall, and Lewanika Mall). Of these, 69 samples were hake, and 63 samples were tilapia, ensuring a balanced representation of both species.

For the inclusion and exclusion criteria, the following were considered:

Inclusion Criteria: Only fresh fish fillets obtained from retail outlets within Lusaka District were included in the study. The fillets were purchased within the study period (June to July 2023) to ensure freshness. Fish fillets were selected randomly from each outlet.

Exclusion Criteria: Fish fillets that were past their expiry date or improperly labelled were excluded from the study. Additionally, fillets that appeared visibly spoiled or had an unusual odour were not considered.

3.4 Sample Collection

This research focused on three prominent retail outlets in Lusaka District, known for their supply of high-quality produce. A strategic sampling approach was used, with the study covering six diverse locations within the district to ensure a comprehensive representation of microbial contamination levels. The locations included Longacres, Woodlands, East Park, Levy Mall, Twin Palm Mall, and Lewanika Mall. Equal quantities of fresh fish

fillets were collected from each of these selected retail outlets (figure 3.2a & 3.2b). To avoid cross-contamination, samples were immediately placed in polythene plastic bags and stored in a cooler box with ice to preserve their original microbial content.

The samples were then transported to the University of Zambia Veterinary Medicine Microbiology Laboratory (figure 3.3a & 3.3b). Every precaution was taken during transport to ensure that the microbial integrity of the samples was maintained. The cooler box was kept sealed, and the samples were handled carefully to avoid any contamination or degradation before reaching the laboratory for analysis.

Figure 3.2a and b: Selecting fresh fish fillets from retail outlets

Figure 3.3a: Arrival with Fresh Fish samples in Cooler box at the UNZA Veterinary Laboratory and Figure 3.3b: Preparation of Agar for Culturing Fresh Fish Fillets Respectively.

3.5 Bacterial Identification

Upon arrival at the laboratory, the fish fillet samples underwent a rigorous microbiological analysis. Techniques involved culturing for bacterial growth, staining, and other microbiological methods were employed to identify and quantify microbial contaminants. The laboratory setting ensured controlled conditions for accurate and reliable results as described below:

3.5.1 Bacteria Culture

Sterile test tubes were individually labelled with dilution factors (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). A sterile container containing 1g of fish and 9 mL of normal saline produced a 1:10 dilution. This process was repeated, transferring 1 mL of the diluted fish sample to another container with 9 mL of sterile normal saline for each subsequent dilution, reaching a final dilution of 10^{-5}. Colony count agar plates and MacConkey agar were labelled with corresponding dilution factors. Using a sterile pipette, 0.1 mL of each dilution was evenly spread onto separate agar plates using a sterile spreader rod. The plates were allowed to dry before inoculation and were then incubated upside down at 37 °C to facilitate general microbial growth in the fish sample.

3.5.2 Colony Counting

After 18-24 hours of incubation, the plates were observed for microbial growth. Colonies on plates with counts ranging from 1 to 300 were counted, providing a reliable estimate. The colony counts for each dilution were recorded. The CFUs per gram of the original sample were calculated by multiplying the number of colonies by the dilution factor using the formula:

$$\text{CFUs/gram} = (\text{Number of colonies}) \times (\text{Dilution factor}).$$

3.5.3 Gram Staining

At the bacteriology laboratory, pure cultures were obtained through subculturing procedures on MacConkey, Nutrient agar, and Blood agar. The isolated bacterial strains underwent a second round of incubation at room temperature (20°C to 25°C) for 24 hours to ensure uncontaminated cultures (MacFaddin et al., 1976).

The initial step in bacterial identification involved Gram staining to classify the isolates into Gram-positive and Gram-negative groups based on their cell wall structure. A smear of the bacterial culture was prepared on a clean glass slide, heat-fixed, and sequentially stained with crystal violet, iodine, decolorized with ethanol, and counterstained with safranin. Observations under the microscope revealed the Gram reaction (purple for Gram-positive and pink for Gram-negative) and cell morphology, such as cocci or bacilli (Chitambo et al.,2023). Representative isolates from each morphological group were selected for further analysis.

3.5.4 Biochemical Characterization

To characterize the isolates further, a series of conventional biochemical tests were conducted, starting with the examination of colony morphology. Attributes such as shape, colour, pigmentation, haemolytic activity, size, edges, and elevation were documented, and isolates were grouped accordingly.

Sugar Fermentation Test: A loopful of bacteria was aseptically inoculated into phenol red broth containing 1% sugar (e.g., glucose, lactose, sucrose) and incubated at 37°C for 24 hours. The change in the broth's colour indicated acid production, signifying sugar fermentation.

Sulphur Reduction, Indole Production, and Motility (SIM) Test: Sulphide indole motility (SIM) media was used to detect three characteristics. After inoculation with a loopful of bacteria and incubation at 37°C for 24 hours, the following were observed:
Sulfur Reduction: Black precipitate formation indicated hydrogen sulfide production.
Indole Production: Kovac’s reagent was added to the media; a red layer on top confirmed the presence of indole.

Other Biochemical Tests: Additional tests included the catalase test (to detect the presence of the enzyme catalase by adding hydrogen peroxide), the oxidase test (to identify cytochrome c oxidase activity), and citrate utilization tests (to assess the ability of bacteria to use citrate as the sole carbon source) (Siamujompa et al.,2023).

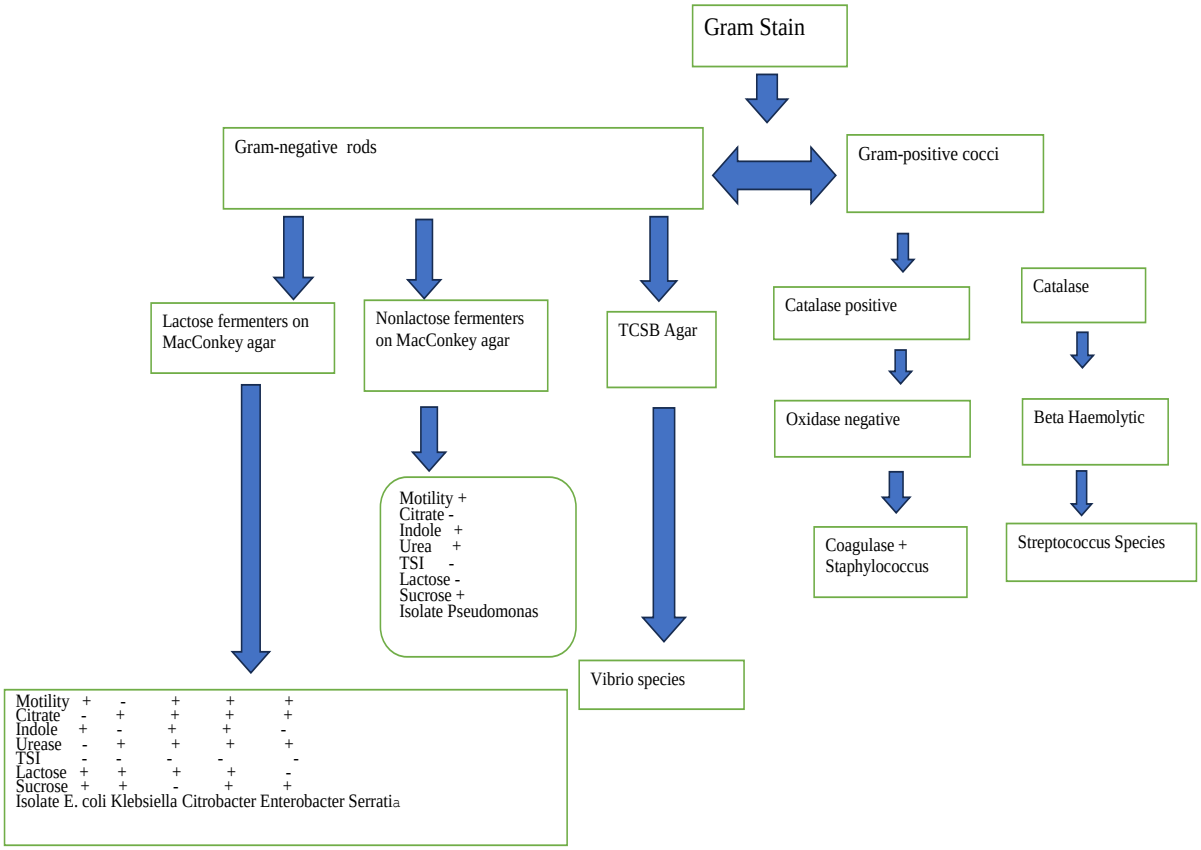


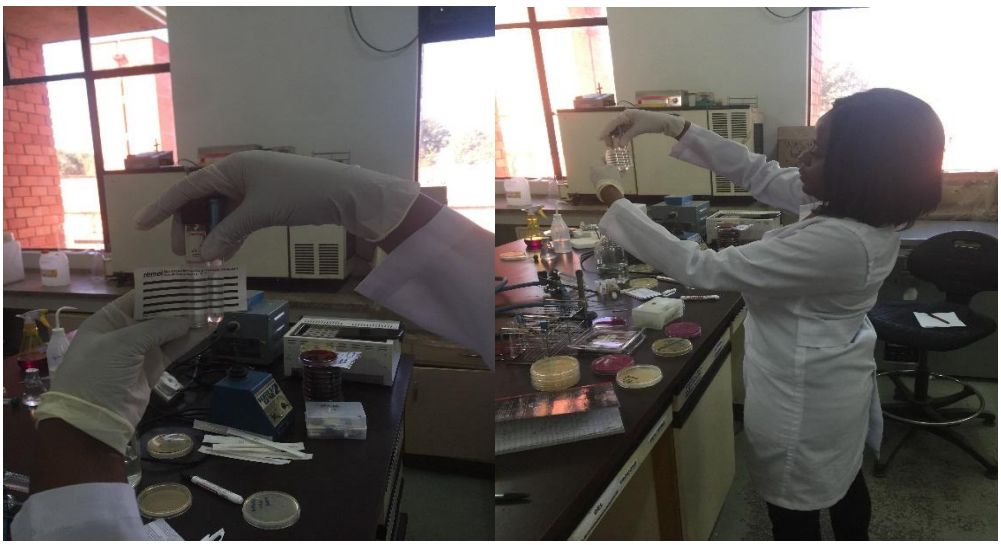
Figure 3.4: Flow diagram of the gram staining and some biochemical tests used to identify different bacterial isolates.

3.6 Determination of the Antibiotic Susceptibility of the Isolates

The Kirby-Bauer disc diffusion method was employed to assess the antibacterial sensitivity of bacterial isolates (figure 3.5a & b). The antimicrobial agents utilized, included Penicillin-G (P 10µg), Amoxiclav (AMC 30 µg), and Amoxicillin (AMX 10µg), chosen for their availability and efficacy against Gram-positive bacteria. Cefotaxime (CTX 30 µg), Ciprofloxacin (CIP 5 µg), and Chloramphenicol (30 µg) were selected due to their known effectiveness against Gram-negative bacteria, while Tetracycline (TE 30 µg), Doxycycline (DO 30 µg), and Co-trimoxazole (COT 25 µg) were chosen for their broad spectrum of activity. Mueller-Hinton agar was prepared according to the manufacturer's instructions, and the organisms were cultured on nutrient agar. Bacterial

colonies were then streaked onto Mueller-Hinton agar plates, and antibiotic discs were placed equidistantly on the agar surface. Only five antibiotic discs were placed per plate to ensure result clarity. The plates were incubated upside down at room temperature for 24 hours, and the diameter of the clear zones around each disc was measured in millimetres. Results were recorded and categorized as susceptible, intermediate, or resistant based on established criteria.

The results were recorded and categorized as susceptible, intermediate, or resistant based on the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2023). These guidelines provided standardized interpretative criteria for zone diameters, ensuring consistency and reliability in the classification of antibiotic susceptibility.



Figures3.5a and 3.5b: Comparison of turbidity of one or two colonies to a 0.5 McFarland turbidity standard in 4 mL of 0.9% sodium chloride solution (normal saline).

3.7 Quality Assurance and Control Measures

To ensure the accuracy and reliability of the results, stringent quality assurance protocols were implemented throughout the study. These measures were designed to minimize contamination, maintain consistency, and validate findings.

1. Calibration of Laboratory Equipment

Laboratory equipment, including incubators, autoclaves, and pipettes, was calibrated before analysis to ensure optimal performance. Calibration was performed using

standardized protocols, with thermometers verifying the accuracy of incubation temperatures and balances checked for precise weighing. This step minimized measurement errors and ensured reproducibility.

2. Sample Handling and Transportation

To maintain sample integrity, fresh fish fillets were collected in sterile polythene bags and immediately placed in a cooler box containing ice packs. This method helped preserve the microbial load by maintaining a controlled temperature during transportation to the laboratory, preventing bacterial proliferation or degradation before analysis.

3. Use of Positive and Negative Controls

Each batch of tests included positive and negative controls to validate results. Positive controls confirmed the effectiveness of culture media in supporting bacterial growth, while negative controls ensured no contamination occurred during testing. This approach enhanced result reliability.

4. Replicate Testing

To enhance result accuracy, tests were performed in replicates. Multiple samples from the same batch were analyzed to assess consistency, reducing the likelihood of false positives or negatives. The reproducibility of findings across replicates strengthened the reliability of the study.

5. Reference Bacterial Strains

Bacterial identification was confirmed by comparing isolates with reference strains. This step ensured that bacteria were correctly classified, minimizing the risk of misidentification. The use of standard bacterial strains further validated the identification process.

These quality assurance measures were crucial in ensuring the validity of the findings, particularly given the study's focus on assessing bacterial contamination in fresh fish fillets sold in Lusaka’s retail outlets.

3.7 Data Analysis

Data storage, prevalence computation, and analysis of levels of microbial contamination in relation to the type of fish were conducted using Microsoft Excel 2013. For the antibiotic susceptibility testing (AST) data, zones of inhibition were measured in millimeters and categorized as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines.

3.8 Ethical Considerations

This study was conducted following ethical guidelines, with approval granted by Excellency in Research and Science (ERES) and the National Health Research Authority (NHRA). The ethical clearance number assigned to the study is Ref.No.2023-Mar-008.

As this was a blind study, explicit permission from the retail outlets was not obtained prior to sample collection. However, ethical approval was granted, and all samples were purchased from the selected retail outlets. Strict confidentiality measures were implemented, with retail outlets assigned unique codes to replace identifiable information. The collected data was securely entered and stored in encrypted files, accessible only to the principal investigator, to ensure the anonymity of the stores and participants.

CHAPTER FOUR

RESULTS

4.1 Samples Collected

This study was conducted between June and July 2023, aiming to assess the extent of bacterial contamination in 132 fresh fish fillet samples purchased from Lusaka district. Among these samples, 89 were found to contain bacteria, while 43 tested negatives across all dilutions. The analysis unveiled a diverse spectrum of bacterial species present in fish sourced from different retail outlets. Notably, multiple types of bacteria were frequently found coexisting within individual samples. It's important to highlight that 69 of these samples were hake fish, which were imported, while 63 were locally sourced tilapia. The sourcing information for the fish was confirmed through direct inquiries with the vendors and by reading the packaging labels, which indicated the origin of the fish.

4.2 Bacterial Count

The findings from the microbial contamination analysis of fresh fish chilled fillets revealed that bacterial levels, particularly the total viable count and the presence of *E. coli*, were high, indicating significant contamination (figure 4.1). Among the 132 samples analysed, it was observed that a significant portion exceeded the acceptable standard of 10^{-5} for total viable count (Table 4.1).

Table 4.1: Microbiological limits for fresh fish and chilled fish according to SADC HT 82: 2023 standards

S/N	Micro-organisms	Max. limits	Method of test
1	*Salmonella* per 25 g	Absent	ISO 6579
2	*E. coli* per gram	Absent	ISO 7251
3	*Staphylococcus aureus* cfu per gram	10^{-2}	ISO 6888
4	*Vibrio* spp per gram	Absent	ISO 21872
5	Total viable count per gram	10^{-5}	ISO 4833
6	*Listeria monocytogenes*	Absent	ISO 11290

Of the 69 hake samples that passed the total viable count criterion by exhibiting a mean count of less than 7 in the first dilution, all showed negative results for Enterobacteriaceae (Table 4.2). This suggests that while these samples met one aspect of the standard, they still harboured other potential contaminants. Notably, among the remaining 63 samples, which were specifically of tilapia fillet, only 4 recorded zero Enterobacteriaceae counts. The majority of these samples tested positive for the presence of *E. coli* and other bacterial species, indicating a higher risk of contamination associated with this particular fish type.

Table 4.2: Total viable Count and Enterobacteriaceae Means of Fresh Fish Fillets

Sample	N	Total bacterial count CFU/gram Mean at each dilution					Faecal contamination means				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Tilapia	63	238.3	199.5	155.5	99.5	44.6	169.5	121.3	88.8	35.0	11.6
Hake	69	7.01	0.21	0	0	0	0	0	0	0	0

Furthermore, only 19 tilapia samples showed zero counts in the 5th last dilutions on the total bacterial count.

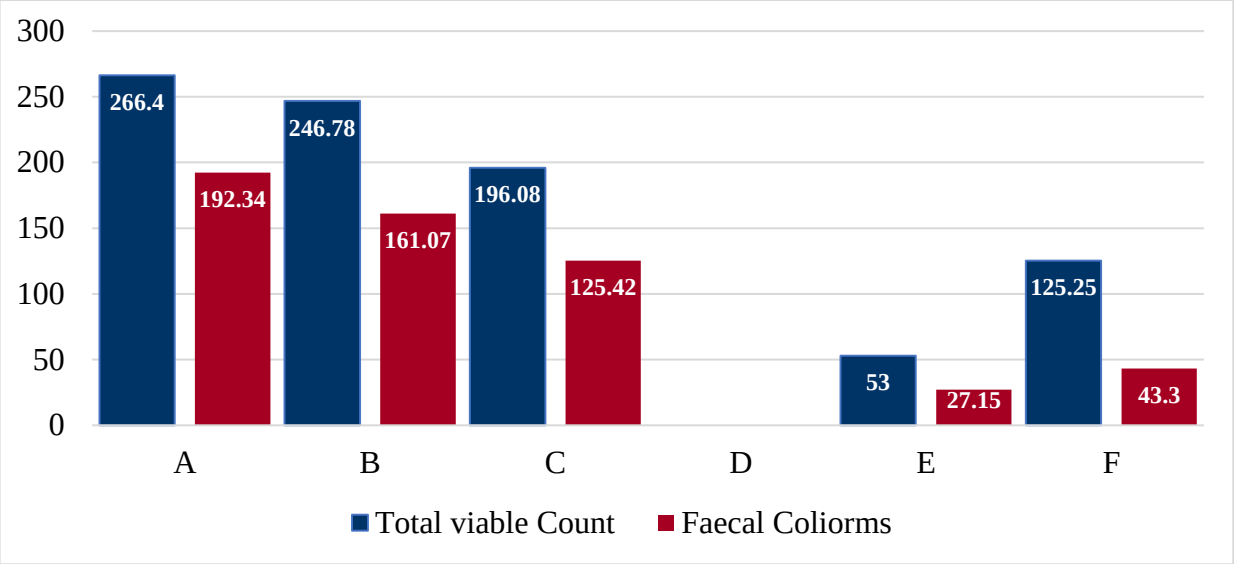


Figure 4.1: Mean levels of faecal and Total viable Counts in Tilapia fish across different locations.

The data in figure 4.1 reveals that location A exhibits the highest levels of both faecal counts and TVC, indicating substantial microbial contamination. In contrast, Location E reports the lowest levels.

Notably, Location D is excluded from this comparison as it did not have any tilapia fillets available for testing. The absence of data from Location D is marked clearly in the figure to avoid any misinterpretation.

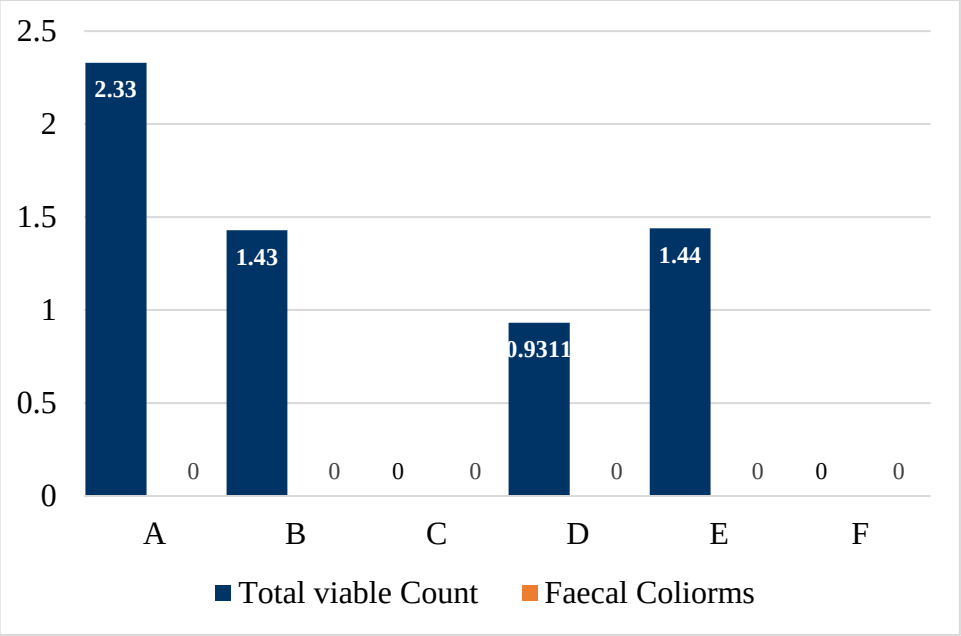


Figure 4.2: Mean levels of Faecal coliform and Total Viable count in Hake Fresh Fillets across different location

The analysis of total and faecal coliform counts per location for hake fillets revealed that location A exhibited the highest contamination levels, while locations C and D showed the least contamination levels (figure 4.2). Notably, there was zero faecal contamination detected across all six locations for hake fish. Additionally, the total viable count remained within acceptable limits.

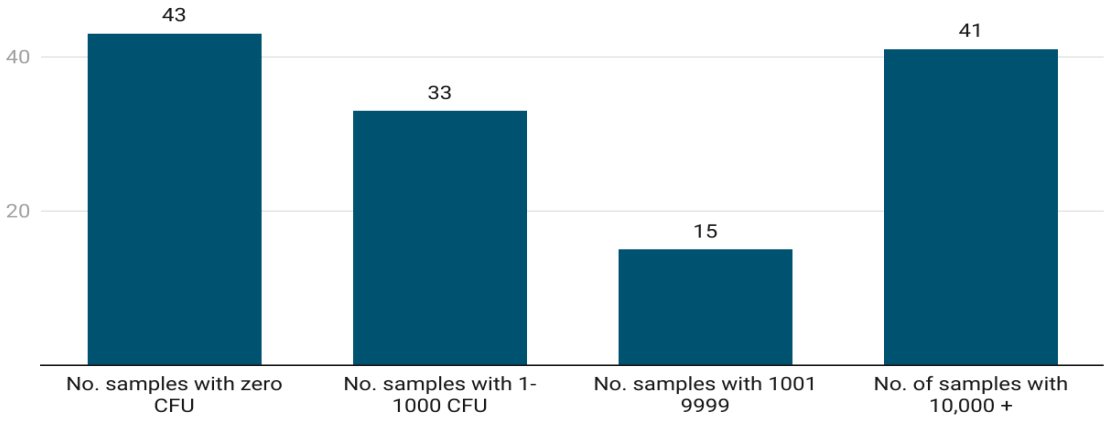


Figure 4.3: Average number of CFUs in collected samples for Total Viable Count

Among the 132 samples analysed for total viable count, 43 samples recorded zero colony-forming units (CFU). The highest CFU counts exceeding 10,000, were observed in 41 samples (figure 4.3).

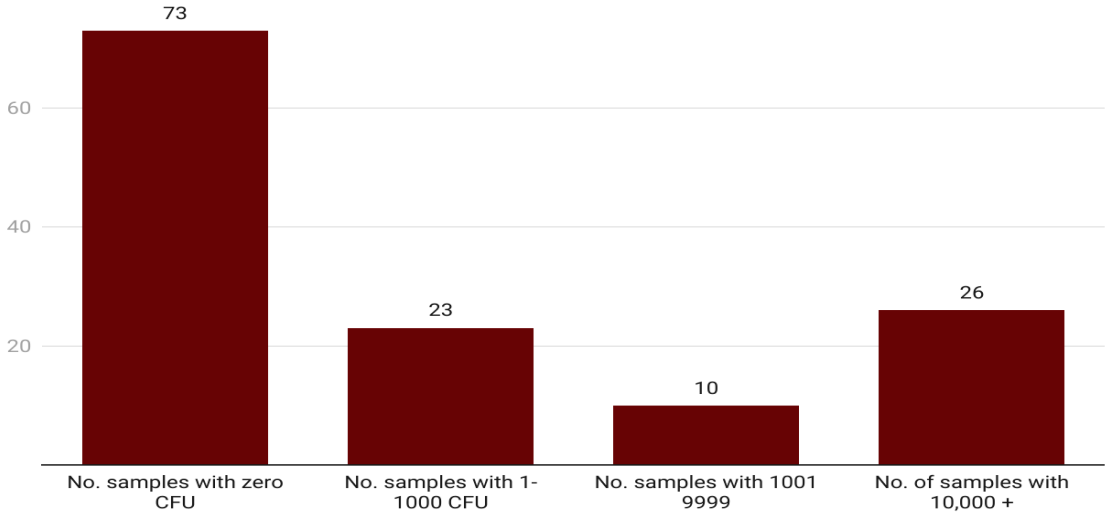
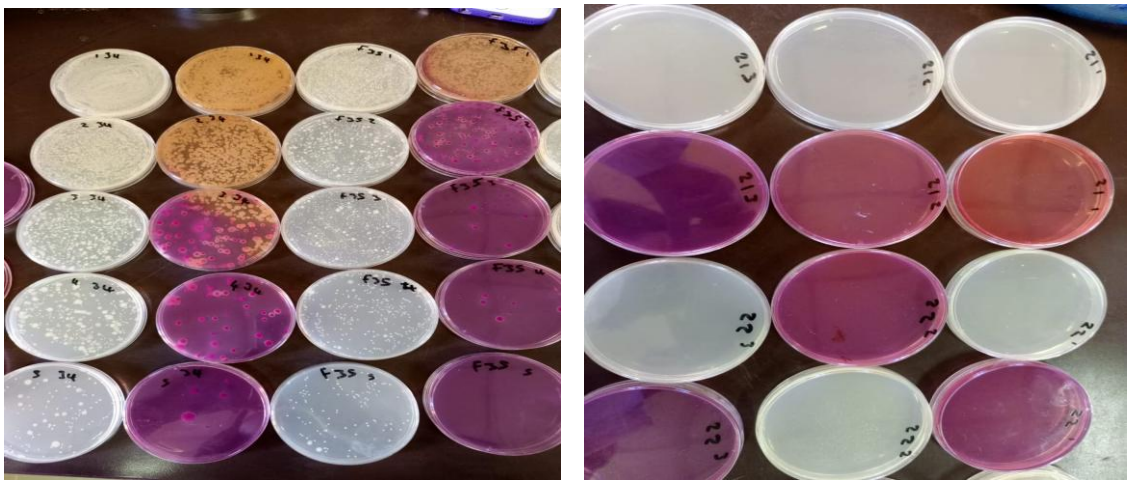


Figure 4.4: Average number of CFUs in collected samples for faecal coliform counts

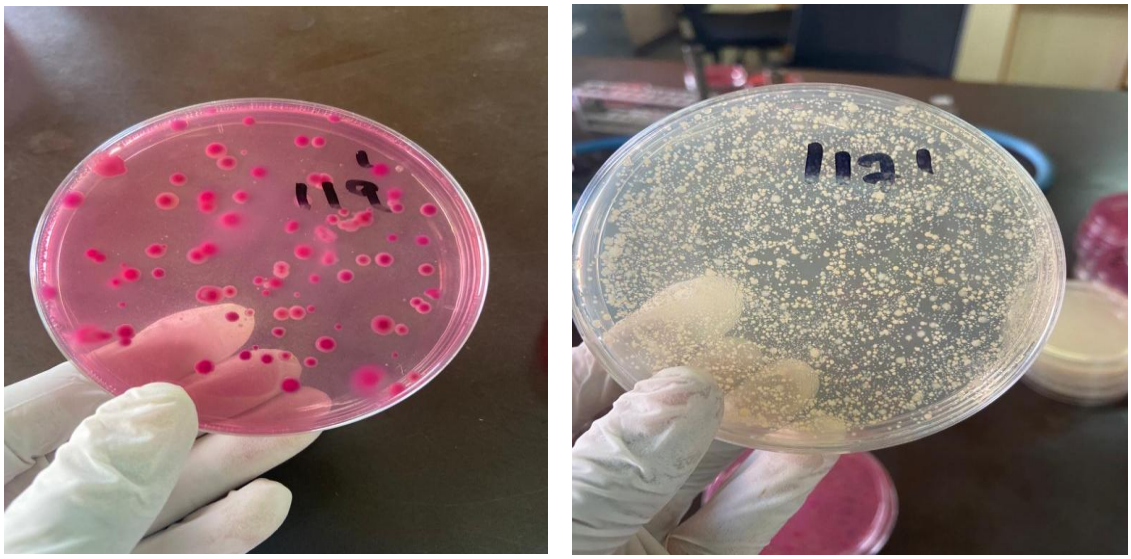
Figure 4.4 illustrates the average number of CFUs in collected samples for faecal contamination. Among the samples, 73 recorded zero faecal CFU, indicating no faecal contamination. In contrast, 26 samples exhibited high contamination levels, recording more than 10,000 CFUs.



(a) (b)

Figure 4.5a shows the Colony Forming Units (CFUs) from Tilapia fish samples, which exhibit fermentation with pink colonies on MacConkey agar, indicating lactose fermentation. Additionally, visible growth is observed on the Colony Count Agar. In

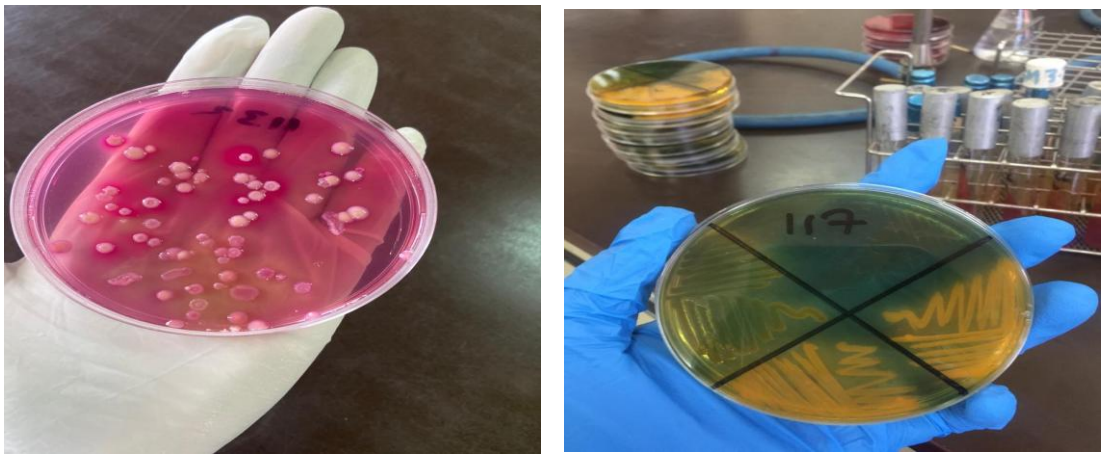
contrast, Figure 4.5b displays the absence of bacterial growth from Hake fish samples on both MacConkey agar and Colony Count Agar, suggesting no bacterial presence or growth.



(a) (b)

Figure 4.6a and 4.6b: Colony Forming Units (CFU) on MacConkey (on the left) and plate count agar (on the right) following a 22-hour incubation period.

The figures above provide important insights into the bacterial colonies isolated throughout the study. Figure 4.5a and 4.5b depict the Colony Forming Units (CFU) on MacConkey media and Colony Count Agar, respectively. In Figure 4.6a, the MacConkey agar shows clear differentiation of lactose-fermenting colonies, appearing as pink or red, which is typical of gram-negative bacteria such as *Escherichia coli*, whereas Figure 4.6b presents the overall bacterial load as quantified on Colony Count Agar.



(a) (b)

Figure 4.7(a) Colony Forming Units (CFU) cultivated on MacConkey media, a selective medium primarily for gram-negative species, 4.8(b) indicates *Vibrio* species growing on TCBS Agar, as non-fermenters and fermenters.

In Figure 4.7, the focus remains on the CFUs cultivated exclusively on MacConkey media, providing a selective environment for gram-negative bacteria. The distinct separation of colonies in this figure reflects the effectiveness of the selective medium in isolating potential pathogens from the fish fillet samples, with a significant number of *E. coli* colonies observed. Figure 4.8 shows the growth of *Vibrio* species on TCBS Agar, which is a differential medium. Non-fermenters and fermenters are clearly differentiated in this figure, with fermenting species producing yellow colonies due to the fermentation of sucrose, while non-fermenters remain green. This figure provides key information for identifying and distinguishing *Vibrio* species, which are of particular concern due to their potential pathogenicity in fish.

4.3 Bacteria Isolated and Identified

Based on figure 4.9, the most prevalent bacterial species in the sample is *Escherichia coli*, constituting 53.8% of the total bacteria identified. Following *E. coli*, *Klebsiella pneumoniae* is the next most prevalent, making up 46.2% of the sample. *Vibrio parahaemolyticus* and *Staphylococcus aureus* both contributed to a significant portion, with 9.10% and 7.60% respectively. *Klebsiella oxytoca* followed closely behind, constituting 6.80% of the bacteria. *Vibrio spp* accounted for 7.60%, while *Enterobacter*

had a higher percentage at 15.2%. *Pseudomonas* accounted for 2.30%, while *Serratia* and *Streptococcus* spp each made up 1.50% and 3.00% of the sample, respectively. *Citrobacter freundii* and unidentified bacteria both contributed to 3.00% and 14.4% of the total bacteria identified, as shown in figure 4.9.

Table 4.3: Shows the colony morphology of the suspected bacteria.

Blood Agar	MacConkey Agar	Suspected bacteria
Yellow round raised smooth shiny colonies	Tiny pink colonies	*Staphylococcus* species
Grey round flat haemolytic with coliform smell	Bright pink round colonies	*E. coli*
Grey round raised with coliform smell	Pink round colonies	*Coliforms*
Blue-green flat, round, haemolytic fruity smell	Pale round raised colonies	*Pseudomonas* species
Large grey mucoid colonies	Pale-pink colonies	*Klebsiella/Enterobacter* species
Large grey mucoid colonies	Pink round colonies	*Klebsiella/Enterobacter* species

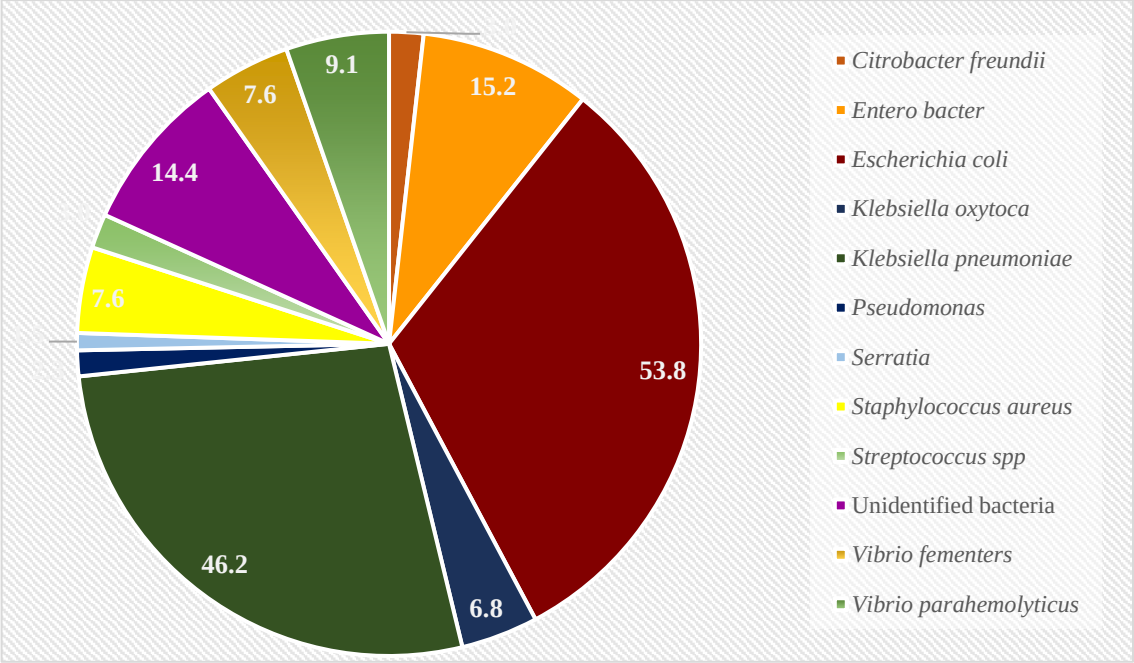
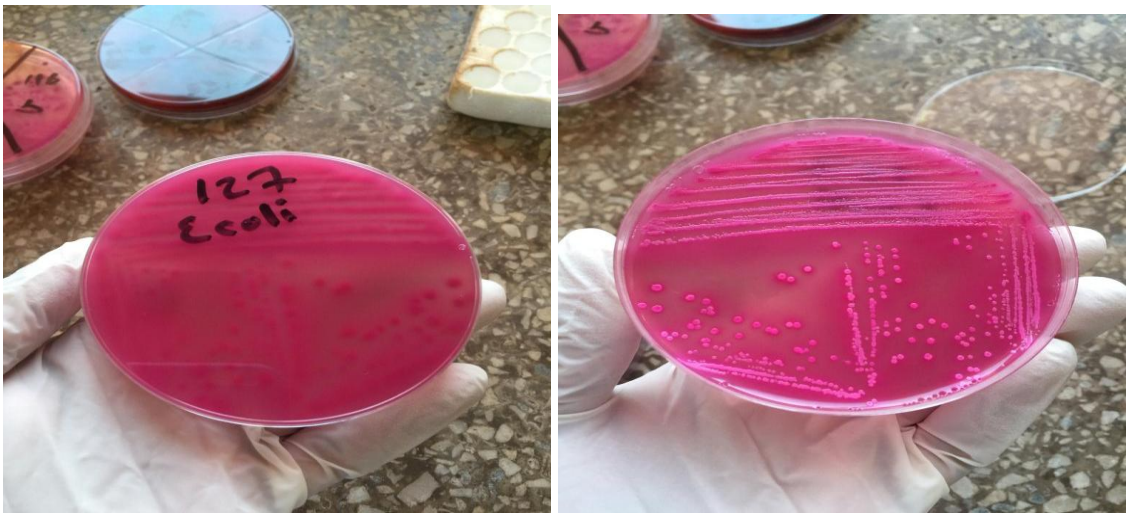


Figure 4.9: Prevalence of isolated bacteria from fresh fish fillet



(a) (b)

Figure 4.10a and 4.10b: Cultivation of *E. coli* on MacConkey culture media following an 18-hour incubation period, showing lactose fermentation as indicated by pink colonies.

(a) (b)

Figure 4.11a and 4.11b: Growth of *Klebsiella* species on MacConkey agar after an 18-hour incubation period, exhibiting characteristic mucoid, lactose-fermenting pink colonies.

(a) (b)

Figure 4.12a and 4.12b: Biochemical test results after 18-24 hours of incubation, including Triple Sugar Iron (TSI), Citrate, Indole, Urease, and Oxidase tests.

Figures 4.10a and 4.10b present the cultivation of *Escherichia coli* on MacConkey culture media following an 18-hour incubation period. In these figures, the lactose-fermenting *E. coli* colonies are visibly pink, characteristic of their ability to ferment lactose. The rapid growth and distinct colony morphology seen in both figures confirm the presence of this common gram-negative bacterium. Similarly, Figures 4.11a and 4.11b depict the cultivation of *Klebsiella* species on MacConkey media after an 18-hour incubation. The colonies appear mucoid and pink, indicating that *Klebsiella* is also a lactose fermenter,

and the appearance of its characteristic shiny, moist colonies helps in distinguishing it from other enteric bacteria.

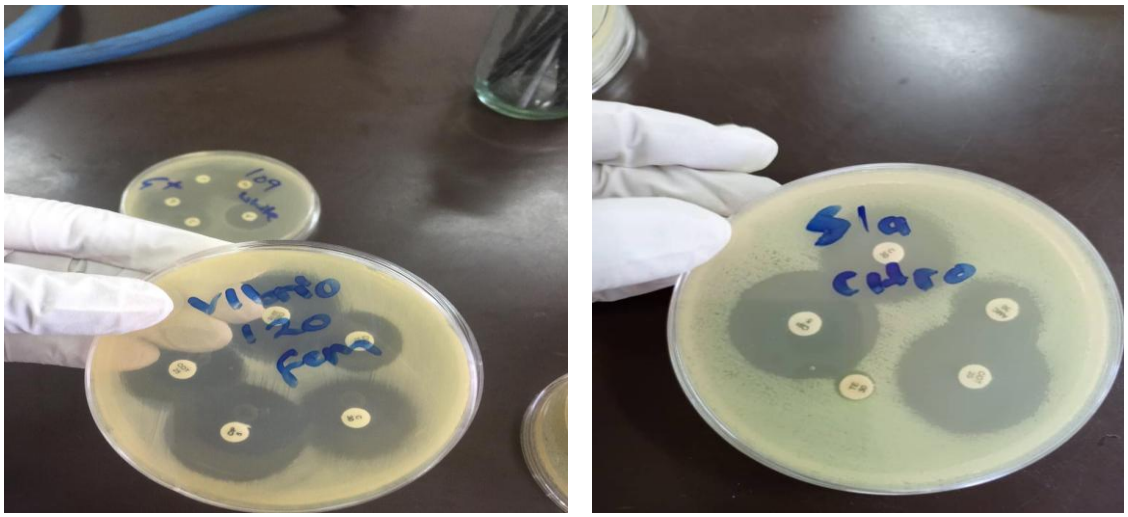
Figures 4.12a and 4.12b illustrate the biochemical test results obtained after 18–24 hours of incubation. These tests are essential for differentiating bacterial species based on their metabolic activities. The Triple Sugar Iron (TSI) test indicated the ability of the isolates to ferment glucose, lactose, and/or sucrose, as well as their potential for gas and hydrogen sulphide (H_2S) production. The citrate utilization test determined whether the bacteria could use citrate as a sole carbon source, while the Sulphide-Indole-Motility (SIM) test assessed hydrogen sulphide production, indole formation, and bacterial motility. The indole test confirmed the ability of some isolates to produce indole from tryptophan metabolism, and the urease test evaluated the ability of bacteria to hydrolyse urea into ammonia, indicating urease enzyme activity. These biochemical characteristics provide important insights into the metabolic capabilities of the isolated bacteria.

4.4 Antibiotic Susceptibility

Following the antimicrobial susceptibility test, it was observed that chloramphenicol, a broad-spectrum antibiotic, was effective against a wide range of bacterial species, including *Vibrio*, *Klebsiella*, *E. coli*, *Streptococcus*, *Staphylococcus*, *Serratia*, and *Pseudomonas*. In contrast, penicillin, which is narrower spectrum, showed resistance against *Staphylococcus* and *Serratia* species. Ciprofloxacin, another broad-spectrum antibiotic, was effective against all tested bacteria, except for a specific strain of *E. coli* (1b), as shown in figure 14a. Cotrimoxazole exhibited broad-spectrum activity against most bacteria, except for *Pseudomonas* and *E. coli* (1b), which were resistant. Doxycycline, also a broad-spectrum antibiotic, was effective against all bacteria except for *E. coli* (1b), which displayed intermediate resistance. Amoxicillin, which has a broad-spectrum profile, demonstrated activity against most bacteria, except for *Citrobacter*, which showed intermediate resistance, and *Vibrio spp.*, which were resistant (see figures 4.13a & b). Lastly, Dexamethasone, though not an antibiotic but a steroid, showed activity against all bacteria except for a specific strain of *Citrobacter* (51a).

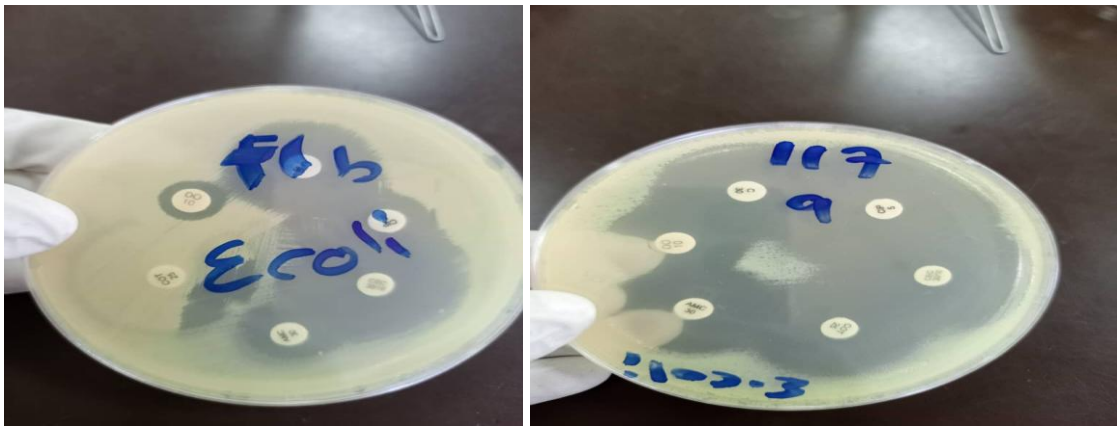
In determining antibiotic susceptibility, zone diameter breakpoints are used to classify bacteria as susceptible, intermediate, or resistant. These breakpoints, based on established

guidelines such as those from the Clinical and Laboratory Standards Institute (CLSI), are essential for accurately interpreting the test results and determining the appropriate antibiotic treatment.



(a) (b)

Figures 4.13a and 4.13b: *Vibrio* fermenters and *Citrobacter*, respectively, on Muller Hinton Agar following a 24-hour inoculation period.



(a) (b)

Figures 4.14a and 4.14b: *E. coli* (1b) and *E. coli* (117a), respectively, on Muller Hinton Agar following a 24-hour inoculation period.

Chloramphenicol demonstrates broad-spectrum activity, showing effectiveness against a wide range of bacterial species, making it a potentially valuable option for treating various

bacterial infections. On the other hand, the resistance observed in certain bacteria to penicillin highlights the need for responsible antimicrobial use to prevent resistance. The varying susceptibility patterns of bacteria to ciprofloxacin, clotrimazole, doxycycline, and amoxicillin underscore the importance of selecting antibiotics based on the specific susceptibility profiles of the bacteria (see figures 4.14a & b).

Table 4.4: Antibiotic susceptibility patterns of bacterial isolated from tilapia fillets

Bacteria	Amox	Doxy	Cotrim	Cipro	Pen	Chlor	CEC	Cefotax	Te
Vibrio non fem 113	R	S	S	S	-	S	-	-	-
Vibrio non fem	R	S	S	S	-	-	-	-	S
Citro 51a	I	-	S	S	-	S	-	-	R
E. coli 1b	S	I	R	R	-	S	S	-	-
Klebsiella 121	S	S	S	S	-	S	-	40	-
E. coli 117b	S	S	S	S	-	S	S	-	-
Citro 34a	S	-	S	S	-	S	-	-	S
Streptococcus spp	S	-	S	S	-	S	-	-	S

Staphylococcus aureus	S	-	S	S	R	S	-	-	-
Klebsiella 61b	S	S	S	S	-	S	S	-	-
E. coli 117a	S	S	S	S	-	S	S	-	-
Serratia	S	-	S	S	R	S	-	-	-
Pseudomonas 53c	-	-	R	S	-	-	-	-	S
Vibrio fem 120	-	S	S	S	-	S	S	-	-
Vibrio fem 121	-	S	S	S	-	S	-	S	-

Table 4.5: Antibiotic susceptibility patterns of bacterial isolated from hake fillets

Bacteria	Amox	Doxy	Cotrim	Cipro	Pen	Chlor	CEC	Cefotax	Te
Staphylococcus aureus	S	S	S	S	S	S	S	S	S

According to Clinical and Laboratory Standards Institute (CLSI) criteria: S = susceptible; R = resistance; I= intermediate.

CHAPTER FIVE

DISCUSSION

5.1 Bacteria Counts in Fresh Fish Fillets

The present investigation examines the microbial quality of fish fillets in retail markets within Lusaka District, Zambia, with a particular focus on Nile tilapia and hake. Drawing upon established standards, such as those outlined by the Southern African Development Community (SADC HT 82: 2023) in Table 4.2 which sets the maximum permissible microbiological limit for Total Viable Count (TVC) at 10^{-5} CFU/g, our findings reveal a noteworthy disparity between prescribed limits and observed bacterial counts. Our study indicates a significant proportion of fish samples surpassing this threshold, with 53.8% exhibiting counts above 10^{-5} CFU/g for TVC, and 45% displaying faecal coliform contamination.

Several factors could contribute to the observed exceedance of bacterial counts in the fish samples. Inadequate handling and hygiene practices, such as lack of proper handwashing, use of contaminated equipment, cross-contamination between raw and processed fish, inadequate refrigeration, and exposure to unsanitary conditions during processing, storage, and transportation, may have led to bacterial contamination (Al-Sheraa, 2018). Improper refrigeration and storage conditions, such as incorrect temperatures and extended storage periods before sale, are likely contributors. The initial quality and source of fish, including potential exposure to polluted waters or contamination at the point of capture, play a significant role (Møretrø et al., 2016b). Additionally, cross-contamination from other food products, contaminated surfaces, utensils, or hands could exacerbate the issue (Chou et al., 2006). Finally, gaps in regulatory enforcement and monitoring of food safety standards might contribute to the observed discrepancies in microbial quality (Rathebe et al., 2015).

These findings are inconsistent with previous research by Onjong et al., (2018) and Sarkar, (2020), who reported lower bacterial counts in fresh Nile tilapia fillets. Such discrepancies underscore the pressing food safety concerns associated with Nile tilapia

consumption, particularly given its popularity among Zambians. Notably, our study contrasts with findings from Al-Sheraa, (2018) in Saudi Arabia, wherein locally cultured fish met acceptability standards more consistently than imported varieties. This contrast can be attributed to several factors: coliforms are indicator organisms signifying contamination of a product by faecal matter. The presence of high bacteria and coliform in the fish samples could reflect poor initial fish quality pre-freezing (Nig et al., 2017). Additionally, the kind and number of microorganisms found on frozen fish depend on the source of the fish, additional contamination introduced on the fishing boat, freezing temperature during storage, severity of the freezing process with respect to lethality to microorganisms, and contamination by handlers and market sellers (Aboagye et al., 2020).

Moreover, the contrasting microbial profiles observed between hake and tilapia fillets highlight potential differences in processing techniques, environmental conditions, and handling practices along the supply chain. Hake, being a saltwater fish, is often subject to advanced processing methods and stringent hygiene protocols, which contribute to its lower microbial counts and negligible faecal contamination compared to locally produced tilapia (Shikongo et al., 2011). Additionally, the saltwater environment of hake may inherently support a lower microbial load than the freshwater environment of tilapia (Antunes et al., 2019). These disparities may stem from variations in regulatory standards, water quality, and handling practices.

Furthermore, insights from Mumbo et al., (2023) emphasize the multifaceted nature of contamination risks, implicating various stages of processing, handling, and storage. Inadequate cleaning and disinfection procedures during industrial processing, as highlighted by Møretrø et al., (2016), contribute to microbial contamination, exacerbated by factors such as equipment hygiene and introduction of water and nutrients.

Considering these findings, proactive measures are imperative to enhance the microbial safety of fresh fish products in local markets. Interventions targeting improved processing standards, sanitation practices, and adherence to hygiene protocols throughout the supply chain are paramount. By addressing these underlying factors, stakeholders can mitigate the risks associated with microbial contamination and safeguard public health.

5.2 Bacteria Isolation and Identification from Fresh Fish Fillets

The Microbiological Specifications for Fresh and Frozen Chilled Fish, as outlined by the Southern African Development Community (SADC HT 82: 2023) in Table 4.1, prescribe stringent standards, aiming to ensure the absence of pathogens such as *Vibrio* spp. and *E. coli* in fish products. Regrettably, our study identified these pathogens, alongside other potentially pathogenic bacteria including *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii, Pseudomonas* spp., *Serratia* spp., *Staphylococcus aureus*, and *Streptococcus* spp., in apparently healthy Nile tilapia (*Oreochromis niloticus*) fresh fish fillets sold in reputable retail outlets (figure 4.9).

This study highlights the critical need for establishing baseline data and implementing early warning systems to manage zoonotic diseases in fish. In this context, an early warning system refers to a proactive monitoring framework that identifies potential hazards within the food supply chain at an early stage, facilitating timely interventions to prevent the spread of contaminants (Faour et al., 2020). Such a system would involve routine microbial testing of fish products, surveillance of critical control points throughout the supply chain, and rapid communication channels to notify relevant authorities and stakeholders about any detected hazards. By leveraging data from regular inspections, microbiological assessments, and environmental monitoring, these systems can predict and respond to contamination risks, thereby mitigating the likelihood of foodborne outbreaks and enhancing overall food safety (Pigłowski, 2019). The identification of ten different bacterial genera in seemingly healthy fish accentuates the potential risks associated with fish consumption. Comparisons with prior studies further illuminate the prevalence and diversity of bacteria in fish. For instance, Chitambo et al., (2023) reported twenty-seven bacterial genera in eels, including *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Citrobacter* spp., and *Pseudomonas* spp., mirroring several findings in our study. Similarly, Siamujompa et al., (2023) documented ten bacterial genera in diseased Nile tilapia, including *Streptococcus* spp., *Pseudomonas* spp., and *Klebsiella* spp., aligning with our observations.

Discrepancies in the prevalence rates of isolated bacteria, as depicted in Figure 4.9, underscore diverse contamination sources, including sewage effluents, human handling,

and industrial and agricultural wastes. Figure 4.9 presents a pie chart illustrating the prevalence of bacteria isolated from fresh fish fillets, highlighting the dominance of *E. coli* and the presence of other coliforms such as *Klebsiella pneumoniae*. This indicates a high level of microbial contamination in the fish samples, which poses risks to consumer health. Notably, our study's finding of *E. coli* dominance aligns with results from Zimbabwe, where *E. coli* and *S. aureus* were prevalent (Gufe et al., 2019). The presence of *E. coli* and other coliforms like *Klebsiella* in food clearly indicates environmental and faecal contamination, stemming from either human or animal sources, as well as poor handling practices (Gufe et al., 2019).

The presence of enteric bacteria like *E. coli, Klebsiella* spp., and *Citrobacter* spp., alongside *S. aureus* and *Pseudomonas* spp., suggests multifaceted pollution sources, including sewage effluents, human activities, and industrial and agricultural waste discharge (Sheng et al., 2021). Haemolytic *E. coli* detection raises concerns regarding diarrheal illnesses, particularly in vulnerable populations. The presence of coliforms in fish products signals environmental and faecal contamination, emphasizing the importance of proper handling practices.

Although similar results were recorded with Al-Sheraa, (2018), the presence of the coliform group of bacteria, including *Citrobacter, Enterobacter, Escherichia*, and *Klebsiella*, was observed in fish products. It is noteworthy that *Salmonella, Listeria*, and *Shigella* genera were absent, contrasting with previous studies conducted by Al-Sheraa, (2018). The presence of *S. aureus* suggests contamination from human and animal waste, either at the source or during handling (Sheng et al., 2021).

Opportunistic bacteria like *Streptococcus* spp. thrive under poor husbandry conditions, predisposing fish to diseases (Bwalya et al., 2020). Additionally, *Pseudomonas aeruginosa*, *Citrobacter* spp., and *Klebsiella* spp., linked to fish disease outbreaks, underscore the risks associated with consumption (Sheng et al., 2021). The isolation of these bacteria from apparently healthy fish underscores the necessity of stringent processing standards to mitigate contamination risks.

Previous research by Al-Sheraa, (2018). suggests these pathogens could constitute part of fish intestinal microflora. Environmental contamination pathways, including air, water, and hygiene deficiencies, raise significant food safety concerns, highlighting the need for robust food safety management systems along the fish value chain. Implementing periodic monitoring programs, spanning fish capture to processing, is essential for enhancing performance and mitigating microbial contamination risks.

5.3 Antimicrobial Susceptibility and Resistance Testing

The Antibiotic Resistance results obtained from fresh fish fillets sold at supermarkets in Lusaka District reveal significant patterns that warrant careful consideration. Firstly, the susceptibility of all tested bacteria to Chloramphenicol suggests its efficacy in combating a wide array of bacterial strains commonly found in fish. This is an encouraging finding, indicating Chloramphenicol as a viable option for controlling bacterial contamination in fish products. Similarly, the susceptibility of most bacteria to Ciprofloxacin is promising, although the resistance exhibited by *E. coli 1b* as indicated in figure 4.14a raises concerns regarding the potential dissemination of Ciprofloxacin-resistant strains in the food chain, posing risks to public health.

Doxycycline 100 µg was effective against all bacteria tested, consistent with the findings by Siamujompa et al., (2023), except for *E. coli* 1b, which displayed intermediate susceptibility. The results from the antibiotic susceptibility tests conducted in this study indicate that all bacterial isolates examined were sensitive to doxycycline. Consequently, doxycycline emerges as a prime candidate for the treatment of bacterial infections, given its broad efficacy against the tested strains.

However, the resistance observed against Clotrimazole, particularly in *E. coli* 1b and *Pseudomonas* spp., underscores the growing challenge of antibiotic resistance in commonly used antibiotics (Sivaraman et al., 2020).

Furthermore, the resistance of *Vibrio* species to amoxicillin raises alarms, given the pathogenic potential of some *Vibrio* strains, which can cause severe foodborne illnesses (Novoslavskij et al., 2016). This highlights the necessity for enhanced surveillance and

control measures to prevent the spread of antibiotic-resistant *Vibrio* strains through contaminated food sources (Kobayashi et al., 2010).

The intermediate resistance displayed by *Citrobacter* to amoxicillin warrants further investigation into the underlying mechanisms and implications for public health (Ibrahim et al., 2020). Understanding the factors contributing to intermediate resistance can aid in devising strategies to mitigate the spread of antibiotic resistance in both clinical and environmental settings (Cabello et al., 2013).

Moreover, the resistance of *Staphylococcus* and *Serratia* to penicillin aligns with global trends of rising antibiotic resistance among common bacterial pathogens (Gufe et al., 2019). This emphasizes the critical need for judicious antibiotic stewardship practices and the development of alternative treatment strategies to combat emerging antibiotic resistance(Gufe et al., 2019).

Overall, these findings underscore the complex interplay between antibiotic usage, bacterial resistance, and food safety. Addressing this issue necessitates a multidisciplinary approach involving collaboration among public health authorities, veterinarians, food producers, and policymakers. Efforts should focus on promoting responsible antibiotic use, implementing robust surveillance programs, and raising awareness among stakeholders about the implications of antibiotic resistance for food safety and public health.

5.4 Bacteria of Public Health Significance

In concurrence with prior research, *E. coli* emerges as a significant pathogen implicated in diarrheal illnesses, particularly prevalent in developing regions (Ishii et al., 2008). The infectious dose for *E. coli*, estimated to range from 10-100 colony-forming units (CFUs), underscores its potential as a foodborne pathogen transmitted through contaminated Nile tilapia. The presence of *E. coli* may signify the co-occurrence of other pathogenic agents in fish, amplifying health risks, even in the absence of direct isolation (Mumbo et al., 2023).

Klebsiella pneumoniae, widely distributed in both natural and mammalian mucosal environments, including fish, poses notable risks to food safety due to its histamine-producing capacity. Histamine accumulation in seafood, associated with high intake levels, can precipitate seafood poisoning (Mohan et al., 2016). Our study identifies *Klebsiella pneumoniae* in fresh tilapia fillets, substantiating concerns regarding its pathogenic potential. While existing literature acknowledges *Klebsiella's* role in seafood contamination, particularly in histamine production, studies directly attributing it as a pathogen in marine environments remain limited. Moreover, *Klebsiella's* status as an opportunistic human pathogen underscores the importance of vigilance in food safety protocols, especially in mitigating risks for hospitalized or immunocompromised individuals (Mohan et al., 2016).

Staphylococcus spp., *Pseudomonas* spp., and pathogenic *Vibrio* species constitute major foodborne pathogens associated with contaminated fish consumption. The escalating trend of antimicrobial resistance in these pathogens poses a formidable global health challenge, necessitating continuous surveillance along the food chain to combat foodborne illnesses effectively (Mumbo et al., 2023).

Our findings align with a study conducted in Nairobi, Kenya (Mumbo et al., 2023), which assessed the prevalence and antimicrobial resistance patterns of bacterial foodborne pathogens in fresh Nile tilapia from retail markets. Although bacterial species prevalence varied, the study revealed concerning rates for *S. aureus, P. aeruginosa, Vibrio* spp., and *V. parahaemolyticus*, underscoring the persistence of foodborne pathogens in fish products.

The identification of *Streptococcus*-like bacteria in tilapia fillets echoes findings by (Bwalya et al., 2020), who detected similar strains in the fish farming environment of Lake Kariba, Zambia. *Streptococcus*-like bacteria emerge as significant disease agents in farmed fish, corroborated by observations of streptococcosis clinical manifestations, including erratic swimming and haemorrhages, as documented by (Siamujompa et al., 2023) and reflected in our study. These findings collectively emphasize the multifaceted

microbial landscape of fish products, highlighting the imperative of robust surveillance and mitigation strategies to ensure food safety and public health.

5.5 Factors Leading to Contamination of Fish Fillets

Consuming contaminated fish fillets poses significant public health risks, especially if the fillets are improperly handled, cooked, or disposed of. The following scenarios could outline potential risks associated with interacting or associating with such fish products:

a. Improperly Cooked Fillets: If fish fillets are not cooked to the appropriate internal temperature, bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Vibrio parahaemolyticus* may survive and cause foodborne illnesses (Novoslavskij et al., 2016a) The recommended internal cooking temperature for fish to ensure safety is 63°C (145°F) for at least 15 seconds. However, designing effective thermal processes for fish products can be challenging. Typical thermal processing involves temperatures in the range of 60–95°C for 10 to 30 minutes (Rosnes et al., 2011). This process can be difficult as it must balance between inactivating target microorganisms and avoiding undesirable changes to the product's quality, especially in the lipid and protein fractions (Rosnes et al., 2011). This risk is particularly high with tilapia fillets, given the high contamination rates observed

b. Mishandling During Preparation**:** Improper cleaning and handling of fish fillets can result in the persistence and spread of harmful bacteria. Even when fillets are contaminated, inadequate cleaning of surfaces, utensils, and hands can perpetuate bacterial presence. For example, bacteria such as *Staphylococcus aureus* and *Pseudomonas spp.* can survive on kitchen surfaces and equipment if they are not thoroughly cleaned and disinfected (Møretrø et al., 2016).

Disinfectants commonly used in kitchens, such as bleach, alcohol, and quaternary ammonium compounds, may not always effectively eliminate bacteria. Factors such as insufficient concentrations, improper usage, or the presence of bacterial biofilms can reduce their efficacy. Bleach requires proper dilution and contact time to be effective (Al-Dabbagh et al., 2015), alcohol may not be effective against all pathogens (CDC, 2019), and quaternary ammonium compounds can lose effectiveness in the presence of organic

matter. Bacterial biofilms, which are clusters of bacteria protected by a protective matrix, can also shield bacteria from disinfectants, making them more difficult to eliminate (Al-Dabbagh et al., 2015).

c. Injury During Cooking: Improper handling of fish fillets, such as cutting oneself during preparation, can lead to bacterial contamination of wounds. For example, *Staphylococcus aureus*, which was identified in our study, is a common pathogen found in contaminated fish fillets. This bacterium can cause localized or systemic infections if it meets open wounds (Sheng et al., 2021). Similarly, *E. coli*, another bacterium isolated from the fish samples, is known to cause severe infections and gastrointestinal illnesses if introduced to wounds or ingested (Gufe et al., 2019). Additionally, *Klebsiella pneumoniae*, also identified in our study, can cause infections and is known for its ability to produce toxins that can lead to severe health complications (Mohan et al., 2016).

d. Incorrect Disposal of Water Used to Clean Fillets: Water used to clean contaminated fish fillets may carry pathogenic bacteria. If this water is not disposed of correctly, it can contaminate the environment, including water sources, further spreading harmful bacteria and increasing the risk of waterborne diseases (Choongo et al., 2009).

e. Incorrect Disposal of Water Used to Clean Fillets: Improper disposal of water used to clean contaminated fish fillets can lead to environmental contamination and spread of bacteria. For instance, *E. coli* and *Klebsiella pneumoniae* were among the bacteria isolated in our study. *E. coli* thrives at temperatures between 37°C and 44°C and can multiply rapidly, with a doubling time of approximately 20 minutes under optimal conditions (Gufe et al., 2019). *Klebsiella pneumoniae* also grows well at similar temperatures, with a doubling time of around 30 minutes (Mohan et al., 2016). At these temperatures, bacteria can rapidly increase in number, potentially reaching levels sufficient to cause disease if the contaminated water is not disposed of properly. For example, if *E. coli* reaches a concentration of 10^5 CFU/mL in improperly disposed water, it could pose significant health risks if it contaminates water sources or food.

f. Inadequate Storage Conditions: If fish fillets are stored at incorrect temperatures, bacterial growth can be exacerbated, leading to an increased risk of foodborne illness upon consumption. For example, *E. coli* and *Klebsiella pneumoniae*, which were identified in our study, can proliferate rapidly if fish is not stored at appropriate temperatures. *E. coli*, known for its role in causing gastrointestinal illnesses, can multiply quickly in improperly stored fish, increasing the risk of infection (Gufe et al., 2019). Similarly, *Klebsiella pneumoniae*, which was also found in the samples, can thrive in suboptimal storage conditions and contribute to severe infections (Mohan et al., 2016). These findings underscore the importance of maintaining proper refrigeration to inhibit bacterial growth and ensure food safety.

5.6 Study Limitations

1. Limited Geographic Scope – The study focused on fresh fish fillets sold in Lusaka's retail outlets, which may not be representative of contamination levels in other regions of Zambia. The findings may not fully capture variations in fish handling, processing, and storage conditions in different locations.
2. Sample Size Constraints – Due to logistical and financial limitations, the number of fish fillets sampled may not have been large enough to provide a comprehensive assessment of contamination trends across all retail outlets. A larger sample size would have strengthened the study's generalizability.
3. Temporal Limitations – The study was conducted over a specific period, meaning seasonal variations in bacterial contamination could not be assessed. Factors such as changes in temperature, supply chains, and demand fluctuations may influence bacterial load over time.
4. Methodological Constraints – The study relied on culture-based and biochemical methods for bacterial detection, which, while effective, may have limitations in identifying all potential pathogens. The absence of molecular techniques such as PCR or whole genome sequencing restricted the ability to achieve precise bacterial identification and detect non-culturable or low-abundance pathogens. This may have led to an underestimation of the true contamination levels in fresh fish fillets.

5. Lack of Comparative Data – Since there is limited existing research on bacterial contamination in fresh fish fillets in Zambia, especially in high-end retail outlets, benchmarking results against local studies was challenging. This made it difficult to contextualize findings within the Zambian food safety landscape.
6. Antimicrobial Resistance Analysis Limitations – The study assessed antimicrobial susceptibility patterns, but due to constraints in testing a broader range of antibiotics, the full spectrum of resistance among bacterial isolates may not have been fully explored.
7. Scope of Assessment – The study focused solely on the end product, limiting the ability to determine critical points in the food production chain where contamination occurs. As a result, it was not possible to identify specific stages for targeted interventions to mitigate bacterial contamination. A more comprehensive approach, including an assessment of handling practices, processing environments, and supply chain dynamics, would be necessary to develop effective control measures.

Despite these limitations, our study provides valuable insights into the bacteriological safety of fresh fish fillets in Lusaka's retail market. The findings contribute to a growing body of knowledge on foodborne pathogens in Zambia and highlight areas for further research and policy improvement.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1.The research findings indicate significant bacterial contamination of fresh fish fillets sold in the Lusaka district, Zambia, particularly in tilapia.

2. The contamination is characterized by high levels of faecal coliforms and the predominance of pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae*, and *Vibrio parahaemolyticus.*

3.The antimicrobial susceptibility testing revealed varying degrees of resistance among the identified bacteria, with notable resistance observed in *E. coli* and *Klebsiella pneumoniae* to multiple antibiotics.

6.2 Recommendation

1.0 Addressing microbial contamination in fish products requires a multidisciplinary approach, encompassing enhanced processing standards, rigorous hygiene practices, and continuous monitoring of antimicrobial resistance patterns. Recommending further studies to examine the entire fish value chain from farm to fork is vital for identifying contamination sources and implementing targeted interventions at each stage of production and distribution, ultimately ensuring food safety and public health.

2.0 Implement a robust monitoring and surveillance system for microbial contamination in fresh fish fillets at both retail outlets and processing facilities across Lusaka District. This system should involve regular sampling and testing for bacterial pathogens to ensure compliance with food safety standards.

3.0 Encourage adoption of Good Aquaculture Practices (GAP) among fish farmers to minimize contamination risks at the production level. This may include proper pond management, water quality monitoring, and responsible use of antibiotics and other inputs.

4.0 Implement and adhere to GMP guidelines in all processing facilities involved in handling fish products, transportation, and retailing, to minimize the risk of microbial contamination. This includes maintaining cleanliness and sanitation standards, proper equipment maintenance, and employee training on hygiene practices.

5.0 For retail outlets, it is imperative to prioritize temperature monitoring by installing digital thermometers in refrigerators and display cases to maintain proper storage conditions for fish products. Additionally, emphasizing hygiene practices among staff, such as regular handwashing, sanitization of equipment and surfaces, and wearing protective gear, is crucial for preventing cross-contamination.

6.0 Future studies should investigate the impact of different fish farming practices on bacterial contamination, including examining the role of feed quality, pond sanitation, and the use of veterinary drugs. Additionally, research into consumer awareness and practices related to handling and cooking fish could provide valuable insights into preventing contamination at the household level.

REFERENCES

Aboagye, E., Tano-Debrah, K., & Kunadu, A. P.-H. (2020). Microbial quality of fish along the tilapia, African catfish, and Sardinella artisanal value chains in Kpong and James Town, Ghana. *International Journal of Bonorowo Wetlands*, 10(1). https://doi.org/10.13057/bonorowo/w100101

Akande, G., & Diei-Ouadi, Y. (2010). Post-harvest losses in small-scale fisheries: Case studies in five Sub-Saharan African countries. *FAO Fisheries and Aquaculture Circular*, (11–13). FAO.

Al-Sheraa, A. S. (2018). Microbial Quality of three Imported Fresh Locally Produced Marine Fishes in Al-Faw City, Basrah, Iraq. https://doi.org/10.4172/2155

Amagliani, G., Brandi, G., & Schiavano, G. (2012). Incidence and role of *Salmonella* in seafood safety. *Food Res Int*, 45, 780–788.

Antunes-Rohling, A., Artaiz, Á., Calero, S., Halaihel, N., Guillén, S., Raso, J., Álvarez, I., & Cebrián, G. (2019). Modelling microbial growth in modified-atmosphere-packed hake (Merluccius merluccius) fillets stored at different temperatures. *Food Research International*, 122, 506–516. https://doi.org/10.1016/j.foodres.2019.05.018

Austin, B. (2006). The bacterial microflora of fish. *The Scientific World Journal,* 6, 931–945.

Austin, B. (2019) Methods for the diagnosis of bacterial fish diseases. *Marine Life Science and Technology*, 1(1), 41–49. Springer. https://doi.org/10.1007/s42995-019-00002-5

Brunton, L. A., Desbois, A. P., Garza, M., Wieland, B., Mohan, C. V., Häsler, B., Tam, C. C., Le, P. N. T., Phuong, N. T., Van, P. T., Nguyen-Viet, H., Eltholth, M. M., Pham, D. K., Duc, P. P., Linh, N. T., Rich, K. M., Mateus, A. L. P., Hoque, M. A., Ahad, A. and Guitian, J. (2019) Identifying hotspots for antibiotic resistance emergence and selection, and elucidating pathways to human exposure: Application of a systems-thinking approach to aquaculture systems. *Science of the Total Environment*, 687, 1344–1356. https://doi.org/10.1016/j.scitotenv.2019.06.134

Budiati, T., Rusul, G., Wan-Abdullah, W. N., Arip, Y. M., Ahmad, R., & Thong, K. L. (2013). Prevalence, antibiotic resistance and plasmid profiling of Salmonella in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. *Aquaculture*. 372, 127–132.

Budzińska, K., Wroński, G., & Szejniuk, B. (2012). Survival time of bacteria *Listeria monocytogenes* in water environment and sewage. *Pol J Environ Stud*, 1, 31–37.

Bwalya, P., Simukoko, C., Hang'ombe, B. M., Støre, S. C., Støre, P., Gamil, A. A. A., Evensen, Ø. and Mutoloki, S. (2020) Characterization of streptococcus-like bacteria from diseased *Oreochromis niloticus* farmed on Lake Kariba in Zambia. *Aquaculture*, 523, 735185. https://doi.org/10.1016/j.aquaculture.2020.735185

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A., & Buschmann, A. H. (2013). Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology*, 15(7), 1917–1942. https://doi.org/10.1111/1462-2920.12134

CDC. (2018). *National Outbreak Reporting System (NORS).*

Chitambo, B., Munyeme, M., & Hang'ombe, B. (2023). Identifying Bacteria with Public Health Significance from Farmed Nile Tilapia (*Oreochromis niloticus*), Zambia. *International Journal of Microbiology*, *2023*. https://doi.org/10.1155/2023/6650378

Choongo, K., Hang'ombe, B., Samui, K. L., Syachaba, M., Phiri, H., Maguswi, C., Muyangaali, K., Bwalya, G., & Mataa, L. (2009). Environmental and climatic factors associated with epizootic ulcerative syndrome (EUS) in fish from the Zambezi floodplains, Zambia. *Bulletin of Environmental Contamination and Toxicology*, 83(4), 474–478. https://doi.org/10.1007/s00128-009-9799-0

Chou, C. H., Silva, J. L., & C. Wang. (2006). Prevalence and typing of *Listeria monocytogenes* in raw catfish fillets. *J. Food Prot*, 69, 815–819.

de Bruijn, I., Liu, Y., Wiegertjes, G.F. & Raaijmakers, J.M., 2018. Exploring fish microbial communities to mitigate emerging diseases in aquaculture. *FEMS Microbiology Ecology*, 94(1). https://doi.org/10.1093/femsec/fix161

Deng, Y., Xu, L., Chen, H., Liu, S., Guo, Z., Cheng, C., Ma, H., & Feng, J. (2020). Prevalence, virulence genes, and antimicrobial resistance of *Vibrio* species isolated from diseased marine fish in South China. *Scientific Reports*, 10(1). https://doi.org/10.1038/s41598-020-71288-0

Duffes F. (1999). Improving the control of *Listeria monocytogenes* in cold smoked salmon. *Trends Food Sci Technol*, 10, 211–216.

FAO/WHO. (2020). Code of Practice for Fish and Fishery Products. https://doi.org/https://doi.org/10.4060/cb0658en

Fattal, B., Dotan, A., & Tchorsh, Y. (1992). Rates of experimental microbiological contamination of fish exposed to polluted water. *Water Research*, 26(12), 1621–1627. https://doi.org/10.1016/0043-1354(92)90161-V

Food and Agriculture Organization of the United Nations. (2018). The state of world fisheries and aquaculture. *Agriculture Organization of the United Nations. Fisheries Department.*

Gufe, C., Canaan Hodobo, T., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., Jongi, G., Makaya, P. V., & Machakwa, J. (2019). Antimicrobial Profiling of Bacteria Isolated from Fish Sold at Informal Market in Mufakose, Zimbabwe. *International Journal of Microbiology*, *2019*. https://doi.org/10.1155/2019/8759636

Haambiya, L., Kaunda, E., Kambewa, D., & Muyangali, K. (n.d.). *Local-scale governance: a review of the Zambian approach to fisheries management*.

Hammond, S. T., Brown, J. H., Burger, J. R., Flanagan, T. P., Fristoe, T. S., Mercado-Silva, N., Nekola, J. C., & Okie, J. G. (2015). Food Spoilage, Storage, and Transport: Implications for a Sustainable Future. *BioScience*, *65*(8), 758–768. https://doi.org/10.1093/biosci/biv081

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

Ishii, S. and Sadowsky, M. J. (2008) *Escherichia coli* in the environment: implications for water quality and human health. *Microbes and Environments*, 23(2), pp. 101–108. https://doi.org/10.1264/jsme2.23.101

MacFaddin, J. F. (1976) *Biochemical Tests for Identification of Medical Bacteria*. Philadelphia: Williams and Wilkins.

Jami, M., Ghanbari, M., Zunabovic, M., Domig, K. J., & Kneifel, W. (2014). *Listeria monocytogenes* in aquatic food products-*A review. Comprehensive Reviews in Food Science and Food Safety*. *13*(5), 798– 813.

Kobayashi, T., Mudenda, Hang'ombe, B. and Yamamoto, H. (2010) The survival of *Vibrio cholerae* in the natural environment of Zambia: Fish, the suspicious gateway of cholera outbreak? *Journal of International Health*, 25(1), 33.

Loo, K.Y., Letchumanan, V., Law, J.W.F., Pusparajah, P., Goh, B.H., Ab Mutalib, N.S., He, Y.W. & Lee, L.H., 2020. Incidence of antibiotic resistance in *Vibrio* spp. *Reviews in Aquaculture*, 12(4), pp. 2590–2608. https://doi.org/10.1111/raq.12460

Malata, M., Bwalya Muma, J., Siamate, J. S., Bumbangi, F. N., Hang'ombe, B. M., & Mumba, C. (2021). Quantitative Exposure Assessment to *Vibrio cholerae* through Consumption of Fresh Fish in Lusaka Province of Zambia. *University of Zambia Journal of Agricultural and Biomedical Sciences*, 5(2), 37–55. https://doi.org/10.53974/unza.jabs.5.2.667

Marijani, E. (2022). Prevalence and Antimicrobial Resistance of Bacteria Isolated from Marine and Freshwater Fish in Tanzania. *International Journal of Microbiology*, *2022*. https://doi.org/10.1155/2022/4652326

Maulu, S., Hasimuna, O. J., Monde, C., & Mweemba, M. (2020). An assessment of post-harvest fish losses and preservation practices in Siavonga district, Southern Zambia. *Fisheries and Aquatic Sciences*, 23(1). https://doi.org/10.1186/s41240-020-00170-x

Modarressi, S., & Lin Thong, K. (2010). Isolation and molecular sub typing of *Salmonella* Enterica from chicken, beef and street foods in Malaysia. *Scientific Research and Essays*, *5*(18), 2713–2720. http://www.academicjournals.org/SRE

Ministry of Health (MoH) (2018) Situational Report No. 122. Outbreak name: Cholera. Investigation start date: 4th October 2017, 14 October. pp. 1–8.

Mohan, G., Sanjeevi, P., Gopi, M., Thankappanpillai Ajith Kumar, T., Prakash, S. and Mohan Gopi, C. (2016) Opportunistic pathogen *Klebsiella pneumoniae* isolated from Maldive's clown fish *Amphiprion nigripes* with hemorrhages at Agatti Island, Lakshadweep archipelago. *International Journal of Fisheries and Aquatic Studies*, 4(3), 464–467. https://www.researchgate.net/publication/304132682

Møretrø, T., Moen, B., Heir, E., Hansen, A. and Langsrud, S. (2016) Contamination of salmon fillets and processing plants with spoilage bacteria. *International Journal of Food Microbiology*, 237, 98–108. https://doi.org/10.1016/j.ijfoodmicro.2016.08.016

Mumbo, M. T., Nyaboga, E. N., Kinyua, J. K., Muge, E. K., Mathenge, S. G. K., Rotich, H., Muriira, G., Njiraini, B., & Njiru, J. M. (2023). Antimicrobial resistance profiles of *salmonella* spp. and *escherichia coli* isolated from fresh nile tilapia (*oreochromis niloticus*) fish marketed for human consumption. *BMC Microbiology*, *23*(1). https://doi.org/10.1186/s12866-023-03049-8

Ndashe, K., Hang'ombe, B. M., Changula, K., Yabe, J., Samutela, M. T., Songe, M. M., Kefi, A. S., Njobvu Chilufya, L., & Sukkel, M. (2023). An Assessment of the Risk Factors Associated with Disease Outbreaks across Tilapia Farms in Central and Southern Zambia. *Fishes*, *8*(1). https://doi.org/10.3390/fishes8010049

Nig, J. and Anim, M. (2017) Microbial load of some imported frozen fish species in Lagos, Nigeria. *Nigerian Journal of Animal Production*, 44(3), 152–160.

Novoslavskij, A., Terentjeva, M., Eizenberga, I., Valciņa, O., Bartkevičs, V. and Bērziņš, A. (2016) Major foodborne pathogens in fish and fish products: a review. *Annals of Microbiology*, 66(1), 1–15. Springer Verlag. https://doi.org/10.1007/s13213-015-1102-5

Novotny, L., Dvorska, L., Lorencova, A., Beran, V. and Pavlik, I. (2004) Fish: A potential source of bacterial pathogens for human beings. *Veterinarni Medicina*, 49(9), 343–358. https://doi.org/10.17221/5715-1

Odeyemi, O. A., Alegbeleye, O., Strateva, M., & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, *19*(2), 311–331. https://doi.org/10.1111/1541-4337.12526

Onjong, H. A., Ngayo, M. O., Mwaniki, M., Wambui, J., & Njage, P. M. K. (2018). Microbiological safety of fresh tilapia (*Oreochromis niloticus*) from kenyan fresh water fish value chains. *Journal of Food Protection*, *81*(12), 1973–1981. https://doi.org/10.4315/0362-028X.JFP-18-078

Papadopoulos, T., Abrahim, A., Sergelidis, T., Kirkoudis, I., & Bitchava, K. (2010). Prevalence of Listeria spp. in freshwater fish (Oncorhynchus mykiss and Carassius gibelio) and the environment of fish markets in Northern Greece. *J Hell Vet Med Soc*, *61*, 15–22.

Rakin, A., Garzetti, D., Bouabe, H., & Sprague, L. D. (2006). *Yersinia enterocolitica*. In *Molecular Medical Microbiology, Three-Volume Set*. Elsevier Ltd. https://doi.org/10.1016/B978-0-12-397169-2.00073-1

Rathebe, J. M., & Consultant, S. (2015). *The implementation of SPS Measures to facilitate safe trade Selected Practices and Experiences in Malawi, South Africa and Zambia*.

Rippen, T. E., & Skonberg, D. (2012). Handling of fresh fish. In *The Seafood Industry: Species, Products, Processing, and Safety* (pp. 249–260)

Sanjee, S. Al, & Karim, M. E. (2016). Microbiological quality assessment of frozen fish and fish processing materials from Bangladesh. *International Journal of Food Science*, *2016*. https://doi.org/10.1155/2016/8605689

Sheng, L., & Wang, L. (2021). The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies.

Comprehensive Reviews in Food Science and Food Safety, *20*(1), 738–786. https://doi.org/10.1111/1541-4337.12671

Shikongo-Nambabi, Shoolongela, A., & Schneider, M. (2011). Control of Bacterial Contamination during Marine Fish Processing. *Journal of Biology and Life Science*, 3(1), 1–17. https://doi.org/10.5296/jbls.v3i1.1033

Siamujompa, M., Ndashe, K., Zulu, F. C., Chitala, C., Songe, M. M., Changula, K., Moonga, L., Kabwali, E. S., Reichley, S., & Hang'ombe, B. M. (2023). An Investigation of Bacterial Pathogens Associated with Diseased Nile Tilapia in Small-Scale Cage Culture Farms on Lake Kariba, Siavonga, Zambia. *Fishes*, 8(9), 452. https://doi.org/10.3390/fishes8090452

Sigurgisladottir, S., Sigurdardottir, M. S., Ingvarsdottir, H., Torrissen, O. J. and Hafsteinsson, H. (2014) Microstructure and texture of fresh and smoked Atlantic salmon (*Salmo salar* L.) fillets from fish reared and slaughtered under different conditions. *Aquaculture Research*, 32, 1–10. https://doi.org/10.1111/j.1365-2109.2001.00503.x

Sing, K. S., Khan, M. Z. I., Daud, H. H. M. and Aziz, A. R. (2016) Prevalence of *Salmonella* spp. in African catfish (*Clarias gariepinus*) obtained from farms and wet markets in Kelantan, Malaysia, and their antibiotic resistance. *Sains Malaysiana*, 45(11), 1597–1602.

Sivaraman, G. K., Sudha, S., Muneeb, K. H., Shome, B., Holmes, M., & Cole, J. (2020). Molecular assessment of antimicrobial resistance and virulence in multi drug resistant ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* from food fishes, Assam, India. *Microbial Pathogenesis*, *149*. https://doi.org/10.1016/j.micpath.2020.104581

Songe, M. M., Hang'ombe, B. M., Knight-Jones, T. J. D., & Grace, D. (2017). Antimicrobial resistant enteropathogenic *escherichia coli* and *salmonella* spp. In houseflies infesting fish in food markets in Zambia. *International Journal of Environmental Research and Public Health*, 14(1). https://doi.org/10.3390/ijerph14010021

Songe, M. M., Hang'ombe, M. B., Phiri, H., Mwase, M., Choongo, K., Van der Waal, B., Kanchanakhan, S., Reantaso, M. B., & Subasinghe, R. P. (2012). Field observations of fish species susceptible to epizootic ulcerative syndrome in the Zambezi River basin in Sesheke District of Zambia. *Tropical Animal Health and Production*, 44(1), 179–183. https://doi.org/10.1007/s11250-011-9906-1

State, E. (2010). Water pollution: A review of microbial quality and health concerns of water, sediment and fish in the aquatic ecosystem. 9(January), 423–427.

Sudheesh, P. S., Al-Ghabshi, A., Al-Mazrooei, N., & Al-Habsi, S. (2012). Comparative Pathogenomics of Bacteria Causing Infectious Diseases in Fish. *International Journal of Evolutionary Biology*, *2012*, 1–16. https://doi.org/10.1155/2012/457264

Tavares, J., Martins, A., Fidalgo, L.G., Lima, V., Amaral, R.A., Pinto, C.A., Silva, A.M. & Saraiva, J.A., 2021. Fresh fish degradation and advances in preservation using physical emerging technologies. *Foods*, 10(4), p. 780. https://doi.org/10.3390/foods10040780.

Thongkao, K., & Sudjaroen, Y. (2019). Beta-lactamase and integrin-associated antibiotic resistance genes of Klebsiella pneumoniae isolated from Tilapia fishes (*Oreochromis niloticus*). *Journal of Applied Pharmaceutical Science*, 9(1), 125–130. https://doi.org/10.7324/JAPS.2019.90117

U.S. Food and Drug, A. (2001). *Fish and fisheries products hazards and controls guidance*. FDA & EPA Safety Levels in Regulations and Guidance.

Valero, A., Rodríguez, M.-Y., Posada-Izquierdo, G. D., Pérez-Rodríguez, F., Carrasco, E., & García-Gimeno, R. M. (2016). Risk Factors Influencing Microbial Contamination in Food Service Centers. In Significance, Prevention and Control of Food Related Diseases. *InTech*. https://doi.org/10.5772/63029

Wanja, D. W., Mbuthia, P. G., Waruiru, R. M., Mwadime, J. M., Bebora, L. C., Nyaga, P. N., & Ngowi, H. A. (2020). Fish Husbandry Practices and Water Quality in Central Kenya: Potential Risk Factors for Fish Mortality and Infectious Diseases. *Veterinary Medicine International*, *2020*. https://doi.org/10.1155/2020/6839354

Watanabe, K. & Ulstrup, G. (1973). Bacterial counts and quality of iced fish retailed at a Lusaka market, Zambia. *Journal of Applied Bacteriology*, 36.

World Health Organisation (WHO) (2011) *Zambia Global Task Force on Cholera Control, Cholera Country Profile.*Yohans, H., Mitiku, B. A. & Tassew, H. (2022) Levels of *Escherichia coli* as bio-indicator of contamination of fish food and antibiotic resistance pattern along the value chain in Northwest Ethiopia. *Veterinary Medicine: Research and Reports*, 13, pp. 299–311. https://doi.org/10.2147/vmrr.s373738

APPENDICES

APPENDIX 1: ETHICAL CLEARANCE