PREVALENCE OF ZOONOTIC BACTERIAL PATHOGENS AND ASSOCIATED RISK FACTORS AMONG CAGE-CULTURED NILE TILAPIA (*Oreochromis niloticus*) IN MWANZA GULF OF LAKE VICTORIA, TANZANIA

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ABSTRACT

Aquaculture has the potential to improve global food and nutrition security, but climate change and poor practices can lead to chemical accumulation and zoonotic bacterial pathogens. This study aimed to identify the prevalence of zoonotic bacterial pathogens and associated risk factors in cage-cultured Nile tilapia in Mwanza Gulf, Lake Victoria, Tanzania. A total of 210 Nile tilapia (*Oreochromis niloticus*) samples were collected from cages distributed in three districts: Nyamagana (60), Ilemela (70) and Misungwi (80). Tissue samples from the gills, skin, kidney and liver were examined for zoonotic pathogenic bacterial infections. Bacterial isolation and identification were performed using standard conventional bacteriological methods. Additionally, a structured questionnaire was administered to 120 fish farmers to gather information on aquaculture practices, fish health status, and disease management. Water and sediment quality in cage-cultured and control sites were assessed using established physicochemical and sediment analysis procedures. Nine genera of bacteria were identified, including *Salmonella* spp. (12.5%), *Klebsiella* spp., *Pseudomonas* spp. (23.5%), *Lactococcus* spp. (16.9%), *Bacillus* spp. (14.7%), *Shigella* spp., *E. coli*, *Streptococcus* spp., and *Staphylococcus* spp. (16.7%). The gills had the highest bacterial prevalence (28.6%), followed by the liver (16.7%), skin (12.9%) and kidney (6.7%). Bacterial isolates were most prevalent in cages in Ilemela (44.1%), followed by Misungwi (34.6%) and Nyamagana (21.3%), and with significant differences noted ($p < 0.001$). Additionally, analysis showed that most farms had high stocking densities (> 8 fish/m², 44.4%) and reared Nile tilapia (*Oreochromis niloticus*) (67.7%). Few farmers regularly measured water quality (16.7%) or removed dead fish daily (20.8%). Key factors influencing total mortality included stocking density ($p = 0.013$), fish species ($p = 0.031$), dead fish disposal methods ($p = 0.023$), and predator bird control ($p = 0.016$). Water samples from both cage-cultured and control sites showed no significant differences in quality ($p > 0.05$). Farmed fish and their aquatic environments harbor potentially pathogenic and zoonotic bacteria, posing significant risks to public health and leading to considerable economic losses. Therefore, the implementation of optimal management practices, along with strict biosafety and biosecurity measures, is essential.

DECLARATION

I, Richard Samwel Komba, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my work and that it has neither been submitted nor is being concurrently submitted for degree award in any other institution

Richard Samwel Komba Date

The above declaration is confirmed by the followings:

Dr. Esther G. Kimaro Date

Prof. Mwita Chacha John Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance and approval by the Senate of the Nelson Mandela African Institution of Science and Technology the dissertation entitled *“Prevalence of zoonotic bacterial pathogens and associated risk factors among cage-cultured Nile tilapia (Oreochromis niloticus) in Mwanza Gulf of Lake Victoria, Tanzania.”* in partial fulfilment of the requirements for the award of the Degree of Master of Science in Health and Biomedical Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

I dedicate this work to my parents, Mr. and Mrs. Samwel (Staff and Nyitawa), for their unwavering love and support. And also, to my greatest inspirations, my daughters, Alyssa and Ayla Richard Samwel.

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LIST OF ABBREVIATIONS AND SYMBOLS

Bp	Base pair
CREATES	The Africa Centre for Research, Agricultural Advancement, Teaching, excellence, and sustainability in food and nutrition
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
FAO	Food and Agriculture Organisation
FDA	The USA Food and Drug Administration
FDGS	Focus Group Discussion
G	Gram
MCCA	Mac Conkey agar
Mg/L	Milligram per Liter
MSD	Medical Stores Department
MR-VP	Methyl Red and Voges- Proskauer broth
MWG	Mean Weight Gain
NFQCL	National Fish Quality Control Laboratory
NGO	Non-Government Organization
NTU	Nephelometric Turbidity Units
PCA	Principal Component Analysis
PPM	Part per millions
PVC	Polyvinyl Chloride
Rpm	Revolutions per minute
SADC	Southern African Development Community
SD	Standard Deviation
SSA	Salmonella shigella agar
TaFReTEC	Tanzania Fisheries and Aquaculture Research Technical and Ethical Committee
TBE	Tris-borate-EDTA
TCBS	Thiosulphate Citrate Bile Salts Sucrose
TSA	Trypticase Soy Agar
UN	United Nations
USA	United States of America

URT	United Republic of Tanzania
µL	Microliter
WHO	World Health Organisation
XLD	Xylose Lysine Deoxycholate Agar

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

An increase in food security is essential to accommodate the projected growth of the human population, expected to reach 2 billion more people by 2050 (Hall *et al.,* 2017; Grafton *et al.,* 2015). The United Nations Member States' 2030 Agenda emphasizes the value of fisheries and aquaculture in exploiting natural resources to contribute to food security and nutrition, with fish as an essential source of protein (Gephart *et al.,* 2020). Aquaculture, being the fastest-growing food-producing industry globally, offers millions of people opportunities for income, employment, and food security (Salayo, 2022). However, the rapid expansion of aquaculture has also led to increased fish mortality and morbidity due to disease outbreaks, causing significant economic losses estimated at $6 billion annually worldwide (Irshath *et al.,* 2023; Funge & Bennett, 2019). In Tanzania, significant government investments are directed toward promoting freshwater aquaculture, particularly through cage cultures, in lake-rich regions such as Kagera and Mwanza (Rukanda *et al.,* 2011; Shoko *et al.,* 2017; Berg *et al.,* 2021). These initiatives are designed to increase fish production, improve food security, and stimulate local economic growth (Muringai *et al.,* 2021; Orinda *et al.,* 2021)

However, the intensification of caged fish farming has also raised concerns about the prevalence of fish diseases and environmental contamination, which could jeopardize the aquaculture's viability. To ensure the continued success of cage-cultured fish farming, it is crucial to implement effective management and prevention strategies to control fish diseases and safeguard the sector's growth (Afewerki *et al.,* 2023; Ibengwe & Sobo, 2016).

Nile Tilapia aquaculture around Lake Victoria has suffered significant economic losses due to infections brought on by poor water quality, physical and chemical factors within cage-culture systems in Lake Victoria (Ngodhe, 2021; Munguti *et al.,* 2022). These infections can be attributed to various environmental stressors, including elevated nutrient levels leading to eutrophication and harmful algal blooms, fluctuations in water temperature and decreased dissolved oxygen levels (Wurtsbaugh *et al.,* 2019). Poor water quality can also result from the runoff of agricultural pesticides and fertilizers, industrial pollutants, and untreated sewage (Akhtar *et al.,* 2021). Additionally, the high stocking densities in cage-cultured systems can exacerbate the spread of diseases by facilitating close contact among fish, thereby increasing the likelihood of zoonotic pathogen transmission (Kyule-Muendo *et al.,* 2022).

Zoonotic bacterial infections in fish aquaculture are caused by bacteria which can be transmitted from fish to humans and lead to zoonotic diseases in humans. These bacteria can pose a great public health risk to both fish populations in aquaculture settings and to humans who come into contact with the infected fish or contaminated water (Haenen, 2023). Direct handling of contaminated fish, such as consuming uncooked or undercooked fish, and exposure to contaminated water, can lead to infections. Key zoonotic bacteria reported in the existing literature include *Vibrio* spp., *Mycobacterium* spp., *Streptococcus iniae*, *Salmonella* spp., *Listeria* spp., *Escherichia coli,* and *Aeromonas* spp. (Ziarati *et al.,* 2022). External stresses can worsen the development of clinical infection, leading to reduced output and occasionally high death rates (Al-Saedi *et al.,* 2024; Admasu & Wakjira, 2021; Ndashe *et al.,* 2023; Siamujompa *et al.,* 2023). Examples of these stressors include high stocking densities, poor diet, and poor water quality.

Water physical-chemical quality parameters in fish rearing can also directly affect feed efficiency, growth rate, fish health, infection outbreaks and survival (Wanja *et al.,* 2020). Aquatic life depends on the physical, chemical, and biological factors of water, which play a substantial role in the biology and physiology of fish (Menon, 2023). In Lake Victoria, the increasing incidence of fish deaths due to poor water quality has led many farmers to abandon aquaculture, emphasizing the need for comprehensive strategies to improve water quality and implement disease surveillance and control programs (Jennings *et al.,* 2016).

The lack of awareness regarding zoonotic bacterial infections in Nile tilapia, along with the factors that heighten the risk, is a pressing issue in the cage aquaculture industry of Lake Victoria, Mwanza, Tanzania (Ngodhe, 2021). This gap underscores the immediate need for extension services to address these concerns and pinpoint areas for improvement within the sector. The substandard management practices employed by fish farmers only serve to worsen the situation, potentially leading to decreased fish survival rates. Moreover, the insufficient knowledge surrounding the presence of zoonotic bacteria in tilapia cage farming and the corresponding risk factors, particularly those related to water quality, presents a major obstacle to the expansion and long-term viability of Lake Victoria Mwanza's cage aquaculture sector. This lack of understanding hampers the implementation of effective infection prevention and control measures, thereby endangering both fish populations and human health.

1.2 Statement of the problem

The rapid growth of aquaculture in Tanzania, particularly in caged fish farming in Lake Victoria, has been well documented. According to the University of Sydney, Report on Aquaculture Development in Tanzania (2025), the number of fish cages increased dramatically from around 100 in 2016 to nearly 1000 by 2023, which resulted in a substantial rise in fish production, from 4790 tonnes annually to 33 525 tonnes. This expansion is attributed to the concerted efforts by the Tanzanian government to promote aquaculture as a strategic means to improve both food security and livelihoods. In particular, initiatives such as the Wezesha Aqua Farms project, reported by Financial Sector Deepening Africa (2024), have played a significant role in supporting local communities in adopting cage farming methods. There is limited research on the prevalence of these infections and their impact on Nile tilapia in the Gulf of Lake Victoria. Studies have shown that these infections pose health risks in other African countries, and similar concerns may arise in Tanzania (Ng'wigulu, 2021; Njiru *et al.,* 2018; Mavindu *et al.,* 2024).

Understanding zoonotic fish infections is crucial for both fish health and human safety, as these bacteria can be transmitted from fish to humans. The infections not only affect fish health but also impact the productivity and sustainability of the aquaculture sector. Poor water quality and inadequate farming practices worsen the spread of these infections, leading to high mortality rates and reduced fish quality (Wanja *et al.,* 2020). Therefore, this study aimed to address this research gap by assessing the prevalence of zoonotic bacterial pathogens and their associated risk factors among cage-cultured Nile tilapia in the Mwanza Gulf of Lake Victoria, Tanzania. The goal is to provide insights to help develop effective management and intervention strategies to improve fish health, ensure food safety, and support the sustainable growth of the aquaculture industry in Tanzania.

1.3 Rationale of the study

Fish are important sources of food and income for many people in developing countries, such as Tanzania. To successfully farm Nile tilapia (*Oreochromis niloticus*) in fish cages at the Mwanza Gulf of Lake Victoria, it is crucial to determine the prevalence of zoonotic bacterial infections and the associated risk factors contributing to fish diseases. The findings of this study will help strengthen the surveillance, prevention, and control of fish diseases and infections. The results will be used to educate cage farmers about the variability of risk factors (physical and chemical parameters) and their effects on fish. This information will also enlighten stakeholders about the levels of physical and chemical contamination in fish cages and their effects on Nile tilapia. Also, this will seek to raise public awareness about the need for proper fish cage management to prevent zoonotic bacterial pathogens, benefiting both fish and humans. Empower farmers with the knowledge to reduce farming costs, minimize disease risks, prevent outbreaks, and lower fish mortality rates.

1.4 Research objectives

1.4.1 General objective

To assess the prevalence of zoonotic bacterial pathogens and associated risk factors in cage-cultured Nile tilapia (*Oreochromis niloticus*) in the Mwanza Gulf of Lake Victoria, Tanzania.

1.4.2 Specific objectives

(i) To determine the prevalence of zoonotic bacterial pathogens in Nile tilapia (*Oreochromis niloticus*) cage-cultured in the Mwanza Gulf of Lake Victoria, Tanzania.

(ii) To establish the risk factors contributing to the prevalence of zoonotic bacterial infection identified from Nile Tilapia (*Oreochromis niloticus*) cage-cultured in Mwanza Gulf of Lake Victoria, Tanzania.

1.5 Research questions

(i) What types of zoonotic bacterial pathogens are found in Nile tilapia (*Oreochromis niloticus*) of cage-cultured farming in the Mwanza Gulf of Lake Victoria, Tanzania?

(ii) What are the potential risk factors contributing to the prevalence of zoonotic bacterial infections from Cage-Cultured Nile tilapia (*Oreochromis niloticus*) in the Mwanza gulf of Lake Victoria, Tanzania?

1.6 Significance of the study

This study investigated the prevalence of zoonotic bacterial pathogens in cage-cultured Nile tilapia in the Mwanza Gulf of Lake Victoria, Tanzania. It aimed to identify specific bacterial pathogens in the fish and assess the risk factors contributing to their spread. The study considered environmental factors such as water quality parameters (temperature, pH, dissolved oxygen, etc.) and management practices employed by fish farmers, including feeding routines, stocking densities, and hygiene practices. By analyzing these factors, the study sought to provide insights into how bacterial infections occurred and spread in the aquaculture environment and to offer recommendations for improving fish farming practices to enhance fish health and ensure the safety of fish for human consumption.

1.7 Delineation of the study

This study aimed to assess the quality of aquaculture management practices and the presence of zoonotic bacterial pathogens in cage-cultured Nile tilapia in the Mwanza Gulf of Lake Victoria. The research involved isolating and identifying bacterial pathogens using conventional bacteriological techniques and biochemical tests. In addition, standard laboratory procedures were used to measure water quality parameters such as temperature, pH, and dissolved oxygen. To understand management practices and associated risk factors, local fish farmers were interviewed through questionnaires and focus group discussions. This comprehensive approach combined biological assessments, environmental analysis, and qualitative data to gain a thorough understanding of the factors affecting fish health in the region.

CHAPTER TWO

LITERATURE REVIEW

2.1 Growth of aquaculture and its socio-economic importance

Fish farming, also known as aquaculture, is a rapidly expanding industry globally, with a growth rate averaging about 8% annually over the past three decades (Naylor *et al.,* 2021). This growth has been driven by the increasing demand for fish as a source of food and protein, with the per capita supply of fish from aquaculture rising from 0.7 kg in 1970 to 7.8 kg in 2006 (FAO, 2020). The Asia-Pacific region dominates world aquaculture, accounting for 89% of production by quantity and 77% by value (FAO, 2020). However, aquaculture is also seeing significant growth in countries in sub-Saharan Africa, such as Nigeria, Egypt, Uganda and Morocco, which are driving the industry's 11.7% annual growth on the continent (Bjørndal & Tusvik, 2020).

In Tanzania, fish farming, especially cage-cultured aquaculture, has been on the rise, with an increasing number of farmers managing ponds and cages nationwide (Cai *et al.,* 2017). Despite promising production volumes of approximately 360 000 metric tons annually, which is equivalent to 1% of global fish production, aquaculture in Tanzania still faces constraints on its impact on national food security and economic growth, primarily due to the predominance of subsistence-level production among farmers (Cai *et al.,* 2017). In the aquaculture industry, species such as Nile tilapia, catfish, carp, oysters and freshwater shrimp are widely farmed for commercial purposes and as home-grown protein sources (Mapfumo, 2022). China is a leading producer of carp and oysters globally, while Norway and Chile are recognized as top producers of farmed salmon (Subasinghe, 2017). The Nile tilapia (*Oreochromis niloticus*) is a highly valued and popular species for aquaculture globally due to its ease of breeding and adaptability to different culture systems (Geletu & Zhao, 2023).

Continued deficits in fish production could have negative implications for economically disadvantaged groups, as fish is a crucial source of animal protein in various countries (Béné *et al.,* 2016). Aquaculture plays a vital role in rural socio-economic development by providing income, employment, and food security through local resource utilization and trade (Allison, 2011; Mulokozi *et al.,* 2020). However, the trend towards intensified production in aquaculture, similar to other agricultural sectors, could lead to increased disease challenges (Little *et al.,* 2018). Overall, fish farming and aquaculture present significant opportunities for sustainable food production and economic development (Little *et al.,* 2016), but careful management and disease control measures will be essential to ensure continued growth and success in the industry (Lieke *et al.,* 2020).

2.2 Zoonotic bacterial pathogens in fish

Aquatic environments, particularly those used in aquaculture, harbor diverse bacterial communities, some of which are pathogenic to fish and humans. Ampofo and Clerk (2010) reported that *Pseudomonas* spp. were the predominant bacterial species in various tissue organs of tilapia cultured in organic waste fertilizer ponds in Ghana. Additionally, *Salmonella* spp. was identified as a major contaminant in the gills, muscles, and skin, posing both fish health and food safety concerns. Similarly, Richard (2011) found that *Edwardsiella ictaluri*, a gram-negative bacterium, thrives in catfish and pond-bottom mud, indicating the persistence of pathogenic bacteria in aquaculture systems. Tilapia naturally harbor bacterial flora in their gastrointestinal tracts (Tiamiyu *et al.,* 2015), which can either contribute to gut health or act as opportunistic pathogens under stressful conditions. Richard (2011) isolated 19 bacterial genera from the gills and intestines of tilapia, with a predominance of gram-negative rods, reinforcing the significance of bacterial diversity in fish health.

Further studies have identified key bacterial species in different aquaculture systems. Huicab-Pech (2016) detected *Aeromonas hydrophila, Shewanella putrefaciens, Corynebacterium urealyticum, Escherichia coli* and *Vibrio cholerae* in the intestine of hybrid tilapia cultured in earthen ponds in Saudi Arabia. Additionally, *Plesiomonas shigelloides* was found in both gastrointestinal regions of *Oreochromis niloticus*, with a significantly higher concentration in the posterior gut compared to the anterior gut and stomach. Moreover, *Aeromonas hydrophila, Escherichia coli* and *Flavimonas oryzihabitans* were isolated from the stomach, while *Citrobacter freundii* and *Burkholderia cepacia* were confined to the posterior gut. These findings emphasize the role of the gut microbiota in maintaining fish health and the potential risks associated with pathogenic bacteria.

In addition, *Chromobacterium violaceum* was the dominant bacterium in the stomach and anterior gut of tilapia (Haenen *et al.,* 2023), while *Pseudomonas* and *Aeromonas* species were found to be the predominant genera in the gut microbiome of tilapia across different environments. Although some of these bacteria are naturally occurring, others are linked to fish diseases. Shoemaker *et al.* (2017) further reported that *Streptococcus iniae* exhibited varying prevalence in tilapia and hybrid striped bass, with a higher prevalence in fish at the grow-out stage, indicating that disease susceptibility may increase as fish mature. Apart from pathogenic bacteria, some beneficial bacteria, such as *Bacillus* spp. play an essential role in aquaculture. *Bacillus* spp. in the intestines of fish create a favorable environment for probiotics, promoting a balanced gut microflora essential for digestion and overall fish health (Nayak, 2021). The establishment of resident microflora in fish has also been suggested to contribute to overall gut health and disease resistance (Banerjee & Ray, 2017).

While various studies have extensively documented the occurrence of bacterial species in tilapia across different regions globally, there remains a critical need to assess the prevalence of these bacteria in specific aquaculture systems, particularly in East Africa and Tanzania. In East Africa, several studies have reported bacterial pathogens affecting farmed tilapia. For instance, Wamala *et al.* (2018), Wanja *et al. (*2020), and Mwainge *et al.* (2021) found that *Pseudomonas* spp. and *Aeromonas* spp. were the most commonly isolated bacteria in tilapia farms in Uganda. In addition, *Streptococcus agalactiae* and *Edwardsiella tarda* have been identified in farmed fish in Kenya and Uganda, leading to significant disease outbreaks and economic losses in aquaculture (Nantongo *et al.,* 2019; Njagi *et al.,* 2019).

In Tanzania, bacterial infections in aquaculture have been increasingly reported (Mzula *et al.,* 2021) documented the presence of *Aeromonas hydrophila, Vibrio spp., Salmonella spp.*, and *Escherichia coli* in tilapia farms in Lake Victoria, raising concerns about fish health and potential zoonotic risks. Additionally, *Streptococcus iniae* has been detected in farmed tilapia, particularly in cage culture systems, where high stocking densities and environmental stressors may contribute to bacterial proliferation (Hossain *et al.,* 2014). These findings highlight the urgent need for continuous monitoring, improved management strategies, and biosecurity measures to mitigate bacterial infections in Tanzania's growing aquaculture industry.

2.3 Impact of zoonotic bacterial pathogens on fish health and disease

Aquaculture advancements are hindered by zoonotic bacterial illnesses, which reduce the quantity and caliber of aquatic organisms produced (Tuševljak *et al.,* 2012). The prevalence of zoonotic bacterial infection in the feed provided to cultured animals can significantly impact the diversity of bacterial species found in the tissue organs of fish (Gauthier, 2015; Burgos Valverde, 2018). The composition of microflora in the fish gastrointestinal tract is heavily influenced by their diet, as fish ingest a significant number of bacteria from their food (Uma *et al.,* 2020). With the increase in prices of raw ingredients, farmers may need to source cheaper and alternative feed ingredients from longer distances (Craig *et al.,* 2017). This could result in prolonged transport times under suboptimal conditions of heat and humidity, as well as storing the ingredients in greater-than-normal quantities under suboptimal storage conditions, leading to spoilage and subsequent fungal and bacterial contamination.

Aeromonads and *Pseudomonads* are known to cause mass mortalities and reduced production in aquaculture. *Aeromonas* spp. (*Aeromonas hydrophila, Aeromonas sobria, and Aeromonas caviae*) and *Pseudomonas* spp. (*Pseudomonas fluorescens, Pseudomonas putida, and Pseudomonas aeruginosa*) have been associated with severe outbreaks in fish hatcheries, particularly *Oreochromis niloticus* (Duman *et al.,* 2024). *Aeromonas* spp. is a common causative agent of fish diseases globally, while *Streptococcus agalactiae* and *Streptococcus iniae* are primary causes of streptococcosis in farmed tilapia (Shoemaker *et al.,* 2017). Streptococcosis, on the other hand, is prevalent in areas where warm-water fish are cultured, while Aeromonas *salmonicida* subspecies *salmonicida* causes furunculosis, resulting in significant losses in salmonids and other fish species (Zaheen *et al.,* 2022). Opportunistic pathogens like *Aeromonas* sp. and *Pseudomonas* sp. commonly lead to bacterial diseases in freshwater fish in regions like India (Duman *et al.,* 2024).

On the other hand, certain bacteria, including *Bacillus* sp., *Vibrio* sp., *Lactobacillus* sp., and *Saccharomyces* sp., are considered probiotics, benefitting fish health by modulating microbial communities (Iwashita *et al.,* 2022). Moreover, *Bacillus* species, in particular, have shown promising effects on fish health, producing beneficial antibiotics, amino acids and enzymes (Kawser *et al.,* 2022). Probiotics such as *Bacillus* spp. have demonstrated water quality improvement by efficiently converting organic matter to CO_2 (Kawser *et al.,* 2022). Additionally, *Carnobacterium* species have been found to inhibit pathogens like *Aeromonas salmonicida and Vibrio anguillarum*, showcasing their potential as beneficial probiotics in aquaculture (Kumari, 2017). These findings highlight the importance of probiotics in aquaculture to combat bacterial infections and enhance the overall health and productivity of aquatic organisms.

2.4 Public health aspect of zoonotic bacterial pathogens in fish

Bacterial contamination in fish poses a significant health risk to humans, especially when the fish comes from farms with poor biosecurity measures. Opiyo *et al.* (2020) found that bacteria isolated from fish can potentially cause infections in humans, particularly during fish cleaning and evisceration, when hands and surfaces can become contaminated. These bacteria, including species like Vibrio spp., have been linked to food poisoning (Domitila, 2019). Studies have identified various bacteria in fish products that pose serious health risks, such as *Citrobacter, Enterobacter, Escherichia, Salmonella, Vibrio, Aeromonas* and *Klebsiella* (Ampofo & Clerk, 2010). These contaminants harm both fish and humans, as they can cause diseases and spread foodborne infections and intoxications (Gauthier, 2015). Transmission occurs when humans consume contaminated fish, leading to the ingestion of harmful bacteria or toxins that were present in the aquatic environment (Adedeji *et al.,* 2012). For example, *Aeromonas hydrophila* has been known to cause gastroenteritis, septicemia, and peritonitis in humans (Pessoa *et al.,* 2022).

Furthermore, bacterial contamination of feed ingredients or diets with pathogens like *Salmonella* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., *Pseudomonas* spp., and *Clostridia* spp. can pose significant health risks to both fish and humans (Swelum *et al.,* 2021). *Escherichia coli* outbreaks have been reported globally, leading to foodborne illnesses in various countries (Yang *et al.,* 2017). To address these concerns and prevent fish-borne bacterial food poisoning, the Hazard Analysis and Critical Control Points (HACCP) system has been developed to ensure proper sanitation practices in fish processing (De Oliveira *et al.,* 2016). Proper biosecurity measures, effective sanitation practices, and the implementation of systems like HACCP can help mitigate the risks associated with bacterial pathogens in fish products.

2.5 Risk factors for the prevalence of fish diseases in cage-cultured Nile tilapia (*Oreochromis niloticus*)

In an unpolluted pond environment, good water quality and good husbandry practices, there will be a natural balance between fish (host) and agent (bacteria). However, a reduction in either quality of pond environment (e.g. poor water quality and pollution) or poor hygienic practices or high stocking density will lead to disease outbreaks (Camus *et al*., 1998; Roberts, 2012; Austin & Austin, 2016). Variations in physico-chemical parameters of water (dissolved oxygen, pH, salinity, ammonia, temperature, etc.) and poor management practices (overfeeding, inadequate nutrition, overcrowding, etc.) can cause stress to the cultured fish and thus make them more susceptible to disease outbreaks (Boyd & Tucker, 2012; Zamri-Saad *et al*., 2014).

2.5.1 Impact of physico-chemical parameters on water quality and zoonotic bacterial pathogens prevalence

Effective water management in a fish-holding facility is essential for a successful fish culture

(Ani, 2021). Fluctuations in water quality parameters, such as elevated levels of ammonia or nitrite, can lead to diseases and significant fish losses (Soler *et al.,* 2021). Fish growth is negatively impacted when temperatures drop to 20°C, and susceptibility to diseases increases at temperatures below 12°C (Bruneaux *et al.,* 2017). The organic manuring of cage water can deplete dissolved oxygen, increase biological, physical, and chemical oxygen demand, and elevate carbon dioxide and ammonia levels, causing stress in cultured fish (Romano, 2020). Cascarano *et al.* (2021) highlighted the importance of seasonal variations in pH, temperature and dissolved oxygen levels in promoting pathogen multiplication and contributing to fish disease outbreaks, including those caused by zoonotic bacteria. Water quality parameters such as temperature, pH, alkalinity and dissolved oxygen levels significantly influence seasonal aggregations of fish diseases (Fahmy *et al.,* 2022).

Menon *et al.* (2023) emphasized that water quality parameters like temperature, pH and dissolved oxygen levels pose significant challenges to fish, especially in intensive farming conditions, leading to discomfort and high mortalities. Elevated carbon dioxide levels in water and decreased oxygen levels can result in respiratory acidosis and nephrocalcinosis (Vatsos & Angelidis, 2010). The population of bacteria in water is strongly correlated with water quality for farmed freshwater fish species (Ismail *et al.,* 2016). Dissolved oxygen concentration in water bodies fluctuates due to various factors, including pH, temperature, atmospheric pressure, and salinity (Ismail *et al.,* 2016). Stressful environmental conditions, particularly low dissolved oxygen levels, promote the proliferation of fish pathogens and increase the risk of disease outbreaks (Zorriehzahra *et al.,* 2020). Therefore, maintaining optimal water quality is crucial for the success of fish culture operations (Saraswathy *et al.,* 2015).

Different fish species can survive within a certain tolerable and desirable range of physicochemical parameters (Bhatnagar & Devi, 2013), as shown in Table 1.

Table 1: Acceptable and desirable limits of some Physical-chemical water quality parameters for fish

Water quality parameter	Tolerable limits	Desirable limits
Dissolved oxygen(mg/L)	1-5	5-7
Temperature(°C)	15-32	22-28
pH	6.5-8.5	4.6-6.5
Alkalinity(mg/L)	5-200	25-100
Nitrate (mg/L)	0-100	0.1-4.5
Nitrites (mg/L)	0.1-0.4	0.00
Ammonia(mg/L)	0-0.05	0-<0.025
Hardness(mg/L)	10-100	10- 20
Phosphates(mg/L)	0.03-2	0.01-3

Bhatnagar and Devi (2013)

2.5.2 Improper fish nutrition

Improper fish nutrition in the form of either underfeeding or feeding unbalanced diet or feeds of low-quality composition and digestibility leads to retarded growth and organ development as well as a weak immune system which predispose fish to diseases. In addition, feeding excess protein-based feeds leads to high ammonia (waste product) levels in the water, leading to poor water quality (Wanja, 2020).

2.5.3 Overstocking density

High cage-cultured stocking density means more fish waste and consequently, poor water quality. In addition, overstocking may result in atypical behavior, especially cannibalism (Mawundu, 2024), as a result of competition for feed and space. Skin wounds and injuries inflicted from cannibalism invite the entrance of disease-causing agents, including bacteria.

2.5.4 Biosecurity on fish-cage-cultured farms

Inadequate biosecurity measures (lack of proper disinfection for items entering the fish pond or inadequate sanitary disposal arrangements for dead fish) allow the build-up and transfer of potentially harmful micro-organisms (Pattabhiramaiah & Mallikarjunaiah, 2023). Quarantine and/or isolation of a new stock of fish before placement within aquaculture facilities aims to prevent the introduction of disease agents (Mocho, 2022). Traffic measures, including restricting access to the farm establishments, prevent the spread of pathogens onto and off the farm (Robertson, 2020).

2.6 Bacterial disease control in aquaculture

Strict adherence to the principle of biosecurity (isolation, transport control and sanitation) at the farm level is the most effective disease control strategy. Disease control strategies include improved husbandry practices and consideration of the use of genetically disease-resistant fish strains, when available (Assefa & Abunna, 2018). Others are the use of well-formulated feeds/feed supplements, vaccines, prophylactic health products (non-specific immune stimulants, probiotics, prebiotics, Phyto biotics), antimicrobial compounds, water disinfection, and restriction in the transfer of infected fish stock (Assefa & Abunna, 2018). In addition, good farming practices such as maintenance of good water quality, proper stocking density, as well as minimal stress during handling, are vital.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites and sampling stations

The study was conducted along the Mwanza Gulf of Lake Victoria, Tanzania, representing other caged fish farming regions in Lake Victoria (Fig. 1). Mwanza Gulf, one of the largest gulfs at the southern end of Lake Victoria, stretches 60 km southward, with an average width of 5 km and a surface area of about 500 km² (Witte &Van Densen, 1995). It is situated between 1–3° S and longitudes 31°35'–34°05' E, at an elevation of 1134 meters above sea level (Witte & Van Densen, 1995). This location was selected due to its extensive Nile Tilapia (*Oreochromis niloticus*) cage farming practices.

The selected farm sites (Fig. 1 and 2) consist of approximately 158 cages with dimensions of 5x5x2.5 meters, covering an area of around 3950 m² distributed unevenly in the three districts of Ilemela, Nyamagana and Misungwi. Out of these, only 50 cages were used. Nile tilapia was stocked at a density of 172 fish per cubic meter and manually fed three times daily (8 am, 12:30 pm and 5:30 pm) with formulated extruded floating feed. The quantity of feed used per day is approximately 1750 kg, and annual production ranges between 200 and 300 tons. The average water depth in the Gulf is 8 meters, and the fish-cage-cultured farms (Fig. 2) have been operational since 2015.

The study sites (Fig. 1 and 2) were spread across three districts, including Site 1, Ilemela coordinates (50.2199^{0}E, 97.36889^{0}N), (48.6550^{0}E, 97.13078^{0}N) and (48.6794^{0}E, 97.12963^{0}N). Site 2, Nyamagana at (15.7644^{0}E, 97.10625^{0}N) and (48.4520^{0}E, 97.10630^{0}N). Site 3, Misungwi covered (48.0579^{0}E, 97.11647^{0}N) and (50.1945^{0}E, 97.37355^{0}N) Site 4, at (48.6721^{0}E, 97.12943^{0}N) and (48.4520^{0}E, 97.10630^{0}N) was located 400 meters away to the south and used as a control site.

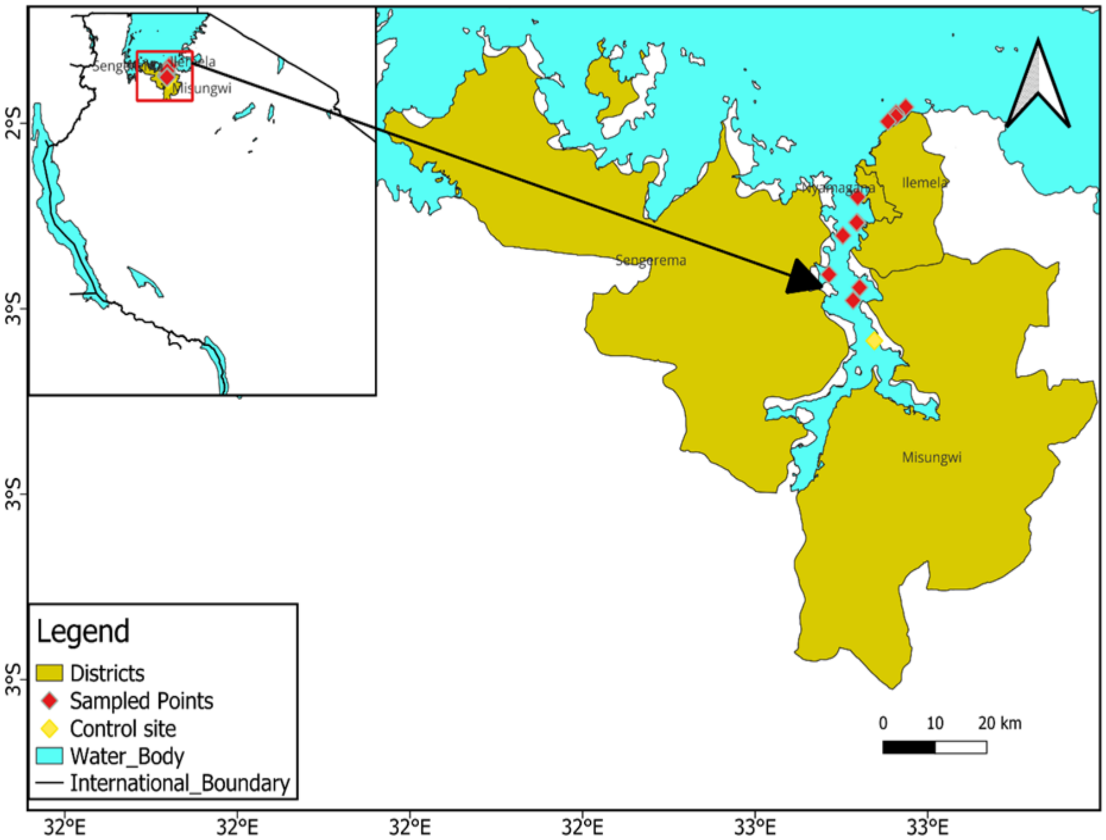


Figure 1: Map of the study sites showing the sampling points

Plate 1: The cages of Nile tilapia (Oreochromis niloticus) around the Mwanza Gulf of Lake Victoria with the sub-cages plots for grow-out, fingerlings and brood stocks

3.2 Study design

A simple random sampling technique was employed to select cage-culture farms from the gulfs located within three districts of the Mwanza region: Illemela, Nyamagana and Misungwi between March and October 2023. A total of 50 cages were randomly selected as sampling units. Cages were sampled 30 from Illemela, 10 from Nyamagana and 10 from Misungwi, along with control sites in the Mwanza Gulf, which were located in areas without fish farming activities, to analyze microbiological and physico-chemical parameters.

A total of 210 fish were collected from different cages. While fish from the same cage shared a common environment, each fish was treated as an individual sample for analysis. The collected fish were placed in separate plastic-covered containers and transported to the Microbiology Laboratory at the National Fish Quality Laboratory (NFQL)-Nyegezi in Mwanza for analysis within 8 hours. The Nile tilapia was humanely euthanized using a swift, stunning blow to the cranium prior to dissection, in accordance with ethical procedures for fish handling. Swab samples from the skin, gills, liver and kidneys were aseptically collected for bacteriological analysis. Before culturing, these swabs were temporarily placed in a nutrient broth to support bacterial growth. The physicochemical parameters of water were measured in the cage where sampling was done to supplement the questionnaire data. Bacteria were isolated from the sampled fish and identified by colony morphology, Gram staining and biochemical characteristics.

3.3 The study population

3.3.1 Inclusion criteria

The study included three sites from the Mwanza Gulf of Lake Victoria's fish farms. It was open to adult fish farmers or farm managers (over the age of eighteen) who could complete the questionnaire and were willing to participate.

3.3.2 Exclusion criteria

Fish farmers under the age of eighteen and those unable or unwilling to participate were excluded from the study. However, no participants under the age of eighteen were encountered during the data collection. Furthermore, farmers who declined to participate in the study or failed to consent were left out.

3.4 Sampling strategy and sample size determination

3.4.1 Qualitative study: Sampling procedure and sample size of fish farmers

A list of fish farmers in three different districts (Illemela, Nyamagana and Misungwi) was provided by the respective cage-cultured fisheries extension officers. The list of fish farmers formed the sampling frame. A purposive sampling was used to obtain active and available fish farmers and participant fish farmers who were drawn from the population of fish farmers in all three districts. The minimum sample size was guided by a formula based on statistical theory (Yamane, 1967), which assumes a 95% confidence interval and a maximum variability of *P*=0.05.

A total of 120 farmers were selected randomly with the help of the fisheries extension officers from all the three districts of Mwanza gulf of Lake Victoria as follow: Illemela (50), Misungwi (30) and Nyamagana (40). The number of farmers selected to participate in the study was guided by the number of fish farmers in the districts and the existing resources available for the study. Verbal consent was sought from the owners of the fish farms before sampling. The order of activities at the farm was as follows: First being measurement of water quality and water sampling for chemical analysis, followed by administration of questionnaires and observations, and lastly fish sampling.

3.4.2 Sampling procedure and sample size of fish (Nile tilapia) from cage-cultured

Simple random sampling was used to select active and available cage-cultured farms in the study area. The sample size was calculated using the formula given by Naing *et al.* (2006):

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

Whereby, n is the sample size, Z is the Z statistic for a level of confidence (1.96 for 95%), P is expected prevalence (assumed pathogen prevalence level of 50%) and d is the precision, which is equal to 5% (0.05). This gave a sample size of 384 samples. However, due to limitations in resources and time, a total of only 210 samples of Nile tilapia (*Oreochromis niloticus*) were collected from the three districts: Illemela 70) Nile tilapia samples), Nyamagana (60) Nile tilapia samples) and Misungwi (80) Nile tilapia samples).

3.5 Water sampling for physico-chemical analysis and collection and measurement of sediment samples

From March to October 2023, water quality measurements were conducted at cage and control sites. Samples were collected one meter below the water surface using pre-cleaned 1 L plastic bottles, which were rinsed with lake water prior to sampling and then sealed and labelled with detailed site information, time and date. These samples underwent analysis at the National Fish Quality Laboratory (NFQL) for various parameters including dissolved oxygen, ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen, electrical conductivity, total hardness, turbidity, alkalinity, chlorophyll-a, sulfate and phosphorus. Sampling and parameter measurements adhered to standard methods outlined by APHA/AWWA (2005), conducted between 9:00 am and 2:00 pm.

In-situ measurements of water temperature, electrical conductivity, dissolved oxygen, and pH were taken at 1m depth using a portable multi-parameter water quality probe (Model HQ40d) within both the fish cages and the surrounding areas (Fig. 3). Turbidity was measured using a HACH 2100P turbid meter. Laboratory analyses involved filtering samples through GF/C filter papers for dissolved nutrients analysis (NH_4-N, NO_2-N, NO_3-N), using Hach Lange DR 2800 Portable Spectrophotometer as per APHA (2005). Phosphates (PO_4-P) as total phosphorus (TP) was analyzed from the unfiltered portion using the ascorbic-acid method.

Plate 2: In situ water quality assessment

Sediment samples from both cage and control sites were collected using an Ekman bottom grab sampler, and stored in sealed plastic bags. Organic Carbon (OC) content was determined by grinding 5 g of dried sediment, adding dichromate and sulfuric acid, followed by titration with ferrous sulfate. Total Organic Matter (TOM) was assessed using the Losson Ignition method: samples were dried, heated and weighed to calculate TOM as a percentage of the initial dry weight after furnace treatment.

3.6 Questionnaire administration and researchers' observations

The study focused on Nile tilapia farming in the districts of Illemela, Nyamagana and Misungwi, specifically targeting cage tilapia farmers. A carefully designed, pretested and refined semi-structured questionnaire was used to interview 120 respondents from cage-cultured fish farms. The final version of the questionnaire incorporated feedback from a pretest, which involved interviews with two fish farmers and two researchers from the Tanzania Fish Research Institute (TAFIRI) in Mwanza. The questionnaire sought information on various aspects, including the farming system, water management methods, stocking details, death rates, awareness of clinical indicators, disease prevention measures and socio-economic characteristics of the respondents and owners.

It also explored management practices such as feeds, feeding, and seining practices, as well as the farmers' ability to detect or assess disease conditions and the challenges faced by aquaculture farms (Appendix 1). The GPS coordinates were recorded for each household to facilitate follow-up by researchers, and interviewers made observations on the fish, their environment and relevant farming practices. Farms were selected based on the willingness and accessibility of the farmers to provide detailed responses to the questionnaire.

3.7 Fish sampling and transport procedures for bacteriological analysis in cage-cultured tilapia and postmortem examination

After obtaining consent from fish farm owners, all fish were collected using a 1 × 1 m seine net following the procedures outlined by Mdegela *et al.* (2011). To ensure proportional sampling, 5–10 grow-out to market-size fish were captured from each selected cage-culture system.

3.7.1 Live fish collection for necropsy and pathogen isolation

Live Nile tilapia (*Oreochromis niloticus*) were collected directly from cage-culture systems and transported to the National Fish Quality Laboratory (NFQL) in Nyegezi for necropsy and bacteriological analysis. The fish were placed in sterile 18-liter buckets or polythene bags filled with source water and oxygen. The polythene bags were two-thirds filled with oxygen using an oxygen tank to maintain optimal fish survival conditions during transit. Transporting live fish allowed for the examination of fresh, uncontaminated tissues and facilitated accurate identification of clinical signs, external lesions, and internal pathological changes that may be associated with bacterial infections.

3.7.2 Collection of fish organ tissues from cage-cultured fish

Organ tissues (gills, liver, kidney and skin) were also excised from fish harvested directly from cage cultures. These organs were collected for bacteriological analysis when necropsy on live fish was not feasible due to field constraints or when immediate sampling was required. The excised tissues were carefully sealed in sterile polyethylene zip-lock bags and labeled with the farm initials and district (site), cage number (station) and sample number (e.g., ILEMELA-MAZE, CAGE I, C1). The samples were preserved at 4–5°C in iceboxes during transport to NFQL. This approach ensured that a broader range of samples could be analyzed across multiple locations even when live transport conditions were limited.

Upon arrival at the laboratory, the samples (skin, gills, liver and kidney) were brought out of refrigeration and left at room temperature (24–26°C) to equilibrate. The gill and liver tissues of each fish were aseptically pulverized separately in 4 ml of sterile physiological saline using a sterile pestle and mortar. A 0.5 ml portion of each homogenized gill and liver sample was transferred into 4.5 ml of alkaline peptone water (APW) (pH 8.4), achieving a 1:10 dilution. These were incubated aerobically at room temperature (24–26°C) for 2 days for pre-enrichment. Skin and kidney swabs were also transferred into 4.5 ml of APW and incubated under the same conditions. The use of APW as a non-selective enrichment medium facilitated the recovery of potentially stressed or low-abundance bacteria prior to further isolation and identification.

3.7.3 Postmortem examination

A postmortem examination was performed in accordance with standard protocols described by Noga (2010) and Roberts (2012). Prior to the procedure, all work surfaces were disinfected using 70% ethanol. Dissecting instruments were initially sterilized in an autoclave at 121ºC for 15 minutes. For reuse between samples, instruments were immersed in 70% ethanol for 2 minutes and flame-sterilized to maintain aseptic conditions. Nile tilapia (*Oreochromis niloticus*) was euthanized by applying physical trauma to the brain. External examination was carried out first to detect any visible lesions, injuries, or abnormalities, such as wounds. The fish were positioned laterally on a clean, disinfected bench.

A square inch section of the skin surface was swabbed with a sterile cotton swab, guided by a sterile aluminum plate to prevent contamination. Before internal examination, the ventrolateral body surface was disinfected using 70% ethanol. A midline incision was made with a sterile scalpel, beginning at the anterior margin of the anal opening and extending anteriorly toward the operculum. A second lateral incision extended from the anal opening along the abdominal wall up to the upper corner of the operculum. A third incision connected the ends of the previous two cuts at the operculum, allowing the removal of a skin and muscle flap and exposing the abdominal cavity.

Plate 3: Postmortem examination and organ sampling

3.8 Laboratory analysis

3.8.1 Fish tissue sampling

During transportation of fish organ samples from the sampling sites to the laboratory, the organ tissues (gills, skin, kidney and liver) were initially preserved in sterile peptone water. Peptone water helps maintain the viability of bacteria by keeping them alive without applying any selective pressure, making it suitable for short-term storage and transport of microbial samples. Once in the laboratory, the preserved tissues were aseptically transferred and cultured in Brain Heart Infusion (BHI) broths**,** as some bacterial species are fastidious in nature and require nutrient-rich media for optimal growth. Bijou bottles containing the BHI broths were labelled with numbers starting from one (1) for Nile tilapia (*Oreochromis niloticus)*, the organ to be excised (for example A/B/C/D = Organ type (e.g., A = gills, B = kidney, C = skin, D = liver), the initials of the farm name (for example TSHE, for TSHEBA), and the date of excision of tissue. This procedure was repeated for all fish samples from the sites and their respective stations. The fish to be worked on was first removed from the ice chest and placed in a clean and dry enamel bowl big enough for the purpose, and placed in the safety cabinet class II.

To obtain gill samples, the operculum was first carefully removed using aseptically sterilized scissors and forceps. After removal, the instruments were re-sterilised by dipping them in 70% alcohol and briefly flaming them with a Bunsen burner to maintain aseptic conditions. A portion of the gill was then gently excised using the sterilized instruments. The collected gill tissue was immediately placed into a sterile Bijou bottle containing Brain Heart Infusion (BHI) broth. Each bottle was properly labelled with the sample number, tissue type (A for gills), source (TSHE for TSHEBA), and the date of collection, following the standard protocol.

The kidney was the next tissue excised. Using aseptically sterilized scissors and forceps, a small incision was made just behind the urinogenital opening to access the internal organs. One blade of the scissors was inserted into the incision, and cutting continued along the ventral side, extending through one lateral side to the head region and then downward toward the pelvic fin. Care was taken not to puncture any internal organs during this process. The body wall was gently pulled back to fully expose the internal cavity. With the aid of sterile scissors and forceps, a tissue sample was carefully excised from the kidney and immediately placed into Brain Heart Infusion (BHI) broth contained in a sterile Bijou bottle. Each bottle was appropriately labelled to indicate the sample number, tissue type (k for kidney), source (TSHE for TSHEBA), and the date of collection, following the method described by Christopher and Bruno (2003). This procedure was repeated for all sampled fish from Site A (Stations I, II and III). The Bijou bottles containing the kidney tissue samples, along with a control, were incubated at 37°C for 24 hours.

The liver was then excised using sterilized scissors and forceps under aseptic conditions. After exposing the internal organs, the liver was located and carefully separated from the surrounding tissues. A section of liver tissue was gently cut and handled to avoid contamination. The excised liver sample was immediately placed into a sterile Bijou bottle containing Brain Heart Infusion (BHI) broth. The bottle was appropriately labelled with the sample number, tissue type (L for liver), source (TSHE for TSHEBA), and the date of collection, in line with the method described by Christopher and Bruno (2003).

For skin sampling, tilapia was prepared for swabbing by first selecting a defined square inch area on the skin surface. To standardize the swabbed area, a sterile, fenestrated aluminium metal plate was placed over the skin, guiding the swabbing process. The exposed area within the fenestration was then gently but thoroughly swabbed using a sterile cotton swab to collect surface bacteria. This technique helped ensure consistency across all samples by targeting the same surface area. The swabs were then immediately placed into sterile Brain Heart Infusion (BHI) broth in properly labelled Bijou bottles, indicating the sample number, tissue type (S for skin), source (TSHE for TSHEBA), and date of collection. All samples were incubated at 37°C for 24 hours alongside control samples.

3.8.2 Inoculation of bacterial suspension

Following incubation in Brain Heart Infusion (BHI) broth, bacterial suspensions were aseptically inoculated onto a range of selective and differential media to facilitate the isolation and identification of bacteria. These included MacConkey Agar (MA) for Gram-negative enteric bacteria, Blood Agar (BA) enriched with 5% sheep blood to support the growth of fastidious organisms and assess hemolytic activity, Xylose Lysine Deoxycholate (XLD) agar for the isolation of *Salmonella* and *Shigella* spp., Salmonella-Shigella (SS) agar for selective isolation of *Salmonella* spp., and Mannitol Salt Agar (MSA) for the selective growth of *Staphylococcus* spp. All inoculated plates were incubated at 37°C for 24–48 hours under aerobic conditions.

(i) Inoculation of bacterial suspension in BHI broths onto MacConkey (MA) and Blood Agar (BA) enriched with 5% sheep blood

MacConkey agar is a selective and an indicator medium which supports the growth of bacteria while inhibiting the growth of unwanted bacterial species, and Blood agar is an enrichment medium that supports the growth of fastidious bacteria. Plates were placed on the aseptically sterilized surface of the safety cabinet II (Plate D) so that the lids were on the bench and the agar was facing upwards (Christopher & Bruno, 2003). They were placed in this position to prevent and minimise the introduction of contamination. Care was taken not to plunge the loop into the agar during streaking (Christopher & Bruno, 2003).

Sterilized loops were used to pick a loopful of bacterial suspension from the BHI broth in the Bijou bottle labelled 1, A for gills, TSHE for TSHEBA and date of excision onto the surface (by making a well i.e. by passing the loop in an oval fashion on a small section of the portion to be streaked) of a sterile MA agar on the section with the same labelling. The first streaking was made from the well.

A second streak was made across the first streak. After this, the loop was re-sterilized aseptically with a Bunsen burner flame to red hot and cooled in air. Then a third streak was made across the second streak (Christopher & Bruno, 2003). Streaking was done to separate bacteria in the liquid cultures from each other. This procedure was repeated on the BA for the same 1 A. The same procedure was repeated on both MacConkey and Blood agar for the other tissue samples from 1 and the other tissue samples in BHI in the Bijou bottles from site A and site B, and Site C and their stations. Figure 5, after the inoculation, the agar plates with one sterile agar plate (control) each of the MacConkey and Blood agars were incubated for 24 hrs. at 37ºC.

(ii) Inoculation onto XLD agar

Xylose Lysine Deoxycholate (XLD) Agar, a selective medium designed for isolating and differentiating enteric Gram-negative bacteria, was used to support the growth of potential pathogens such as *Salmonella* spp., *Shigella* spp., and *Escherichia coli*. The XLD agar plates were aseptically placed inside a Biosafety Cabinet Class II (Plate D), ensuring that the lids were placed on the bench and the agar surfaces faced upward to avoid contamination. This setup minimizes external environmental contamination and ensures proper incubation conditions (Christopher & Bruno, 2003).

A sterile inoculating loop was used to transfer a loopful of bacterial suspension from the BHI broth contained in the Bijou bottle labelled "1, A for gills, TSHE for TSHEBA, and date of excision" onto the surface of the XLD agar. The streaking was performed in a systematic manner to achieve isolated bacterial colonies: First, a small well was made using the loop by streaking the loop in an oval fashion, then a first streak was drawn from the well. A second streak was made perpendicularly across the first streak to further separate bacterial growth, followed by a third streak after the loop was re-sterilized with the Bunsen burner flame.

The loop was allowed to cool before proceeding to avoid killing the bacterial culture (Christopher & Bruno, 2003). This streaking technique is critical for separating colonies and obtaining pure cultures. The same procedure was repeated for other tissue samples from Site A and Site B and Site C, as well as their respective stations. Additionally, control plates were prepared for comparison, with one sterile XLD agar plate for each control. After inoculation, the XLD agar plates were incubated at 37°C for 24 hours to allow the bacterial growth. During incubation, bacteria that ferment xylose or lactose will produce yellow colonies, while those that do not ferment these sugars will produce red colonies.

(iii) Inoculation onto SS Agar

Salmonella-Shigella (SS) Agar is a selective and differential medium specifically designed to isolate *Salmonella* spp. and *Shigella* spp., as well as other enteric pathogens. The medium contains bile salts to inhibit the growth of Gram-positive bacteria and lactose to differentiate between lactose fermenters and non-fermenters. The SS agar plates were placed inside a Biosafety Cabinet Class II (Plate D), ensuring that the lids were positioned on the bench while the agar surfaces faced upwards, minimizing contamination during handling (Christopher & Bruno, 2003).

A sterile inoculating loop was used to pick a loopful of bacterial suspension from the Brain Heart Infusion (BHI) broth in the Bijou bottle labelled "1, A for gills, TSHE for TSHEBA, and date of excision," which had been previously prepared. This suspension was streaked onto the surface of the SS agar plate in a specific manner to ensure proper separation of bacterial colonies. A small well was first made by streaking the loop in an oval pattern, then a first streak was made from the well. A second streak was made perpendicular to the first one, followed by a third streak after re-sterilizing the loop in a Bunsen burner flame. The loop was cooled in air before each streaking to ensure that it didn't kill the bacterial cells.

The same inoculation procedure was repeated for tissue samples from Site A, Site B, Site C and their respective stations. Additionally, control plates were prepared, with one sterile SS agar plate for the control. After inoculation, the SS agar plates were incubated at 37°C for 24 hours. During the incubation period, the *Salmonella* species, which do not ferment lactose, would produce colorless colonies, while lactose fermenters would produce pink colonies.

(iv) Inoculation onto mannitol salt agar (MSA)

Mannitol Salt Agar (MSA) is a selective and differential medium primarily used to isolate *Staphylococcus* species, especially *Staphylococcus aureus*, which is known for its ability to ferment mannitol. The MSA contains a high concentration of salt (7.5% NaCl), which inhibits the growth of most bacteria except for *Staphylococcus* species, which can tolerate high salt concentrations. Additionally, MSA contains mannitol, a sugar alcohol that can be fermented by some *Staphylococcus* species. When mannitol is fermented, the medium turns yellow due to the production of acidic byproducts. Non-fermenters, such as *Staphylococcus epidermidis*, produce pink or red colonies, as they do not acidify the medium.

To begin the inoculation process, the MSA plates were placed inside a Biosafety Cabinet Class II (Plate D), ensuring that the lids were placed on the bench and the agar surfaces faced upward. This orientation was carefully maintained to minimize the possibility of contamination during the process (Christopher & Bruno, 2003).

A sterile inoculating loop was used to pick a loopful of bacterial suspension from the Brain Heart Infusion (BHI) broth in the Bijou bottle labelled with "1, A for gills, TSHE for TSHEBA, and date of excision." This bacterial suspension was then streaked onto the surface of the MSA plate using the streak plate method. The loop was passed through the suspension to ensure even distribution, starting with a well in an oval shape. The first streak was drawn from the well, and a second streak was made perpendicular to the first.

The loop was re-sterilized by flaming it with a Bunsen burner until it was red hot, and after cooling in air, a third streak was made across the second streak. This process helped separate individual bacterial colonies. The same procedure was applied to inoculate MSA plates for other tissue samples from Sites A, B, C and their respective stations, ensuring proper labelling. Control plates were also prepared, including one sterile MSA plate for the control. After inoculation, the MSA plates were incubated at 37°C for 24 hours.

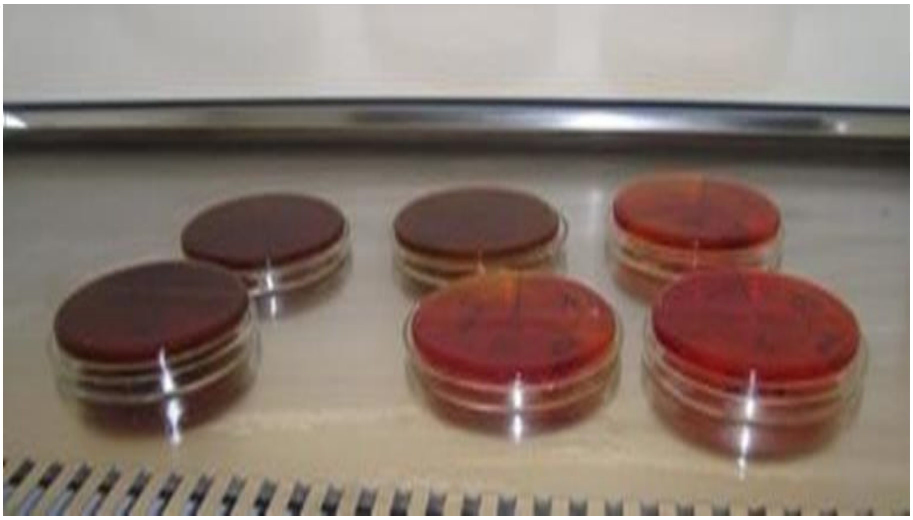


Plate 4: MacConkey and blood agar on the floor of the safety cabinet

(v) Bacterial identification

Physical characteristics of colonies

Bacterial colonies (Plate E) were examined for key morphological features such as colony shape, surface texture, elevation, edge or margin, color and general growth characteristics. Cultures exhibiting similar appearances were grouped together for subsequent testing.

On Blood Agar (BA), colonies were assessed for the presence or absence of hemolysis. Hemolysis patterns (complete, partial, or none) served as important indicators in differentiating types of bacteria based on their ability to lyse red blood cells. On MacConkey Agar (MA**)**, colonies were distinguished by their ability or inability to ferment lactose, indicated by changes in colony coloration. Colonies that fermented lactose appeared colored, while non-fermenters remained colorless or pale. On Xylose Lysine Deoxycholate (XLD) Agar, differentiation was based on colony color and the presence or absence of black centers, which indicate the production of hydrogen sulfide. Colonies could appear red, yellow, or red with black centers depending on their biochemical activities.

On Salmonella-Shigella (SS) Agar, colony appearance varied with the ability to ferment lactose and produce hydrogen sulfide. Colonies appeared colorless, colorless with black centers, or pink/red depending on their characteristics. On Mannitol Salt Agar (MSA), colonies were evaluated based on mannitol fermentation and salt tolerance. Fermenting colonies caused a color change in the medium around them, while non-fermenters maintained the original color of the medium.

Identification by staining

- **Preparation of smear on sterile microscope slides and inoculation of the same colonies into peptone**

In order to distinguish gram-negative bacteria from gram-positive bacteria, Prestone and Morrell’s modified staining technique was used. Some bacteria can display variable responses to stain or have extra waxy content in their cell wall, which makes staining difficult, but with Prestone and Morrell’s modified staining technique, it is easier. The sterile microscope slide was first labelled with the label on the section on which the colony is to be picked for staining. The surface of the sterile slide was divided into two sections with a non-greasy pencil.

Aseptically sterilized loop was used to pick a loopful of sterile water onto the centre of the section on the slide. The inoculation loop was sterilized aseptically again with a Bunsen burner flame to red hot and cooled in air, after which it was used to pick a bacterial colony from the same colony inoculated into the peptone water onto the sterile water on the slide. The colony was spread evenly on the slide while mixing it with the sterile water. The smear was allowed to air-dry for a few minutes.

With the aid of forceps, the slide was passed through the flame for a couple of seconds to make sure the bacteria were affixed to the slide to render the vegetative bacteria permeable to the stain and ensure that the material is firmly fixed to the slide (Quinn *et al.*, 1994). The same colony that was picked for staining was also picked with a sterilized loop by touching it to the surface of a colony and then inoculated into peptone water in labelled Bijou bottles. This was incubated at 37°C for 24 hrs. The above procedures were repeated for all the other colonies on the different plates.

- **Staining of bacterial colonies**

The next step was to take smears on the slides through Prestone and Morrell's modified staining technique to determine the gram-negative and gram-positive bacteria reaction.

(a) The fixed smears were placed on staining racks over a sink and covered with 0.5% Ammonium Oxalate-Crystal Violet at the section where the smear was fixed. This was allowed to sit and act for 30 seconds.

(b) After this, the Ammonium Oxalate-Crystal Violet was poured off, and the slide was washed with Lugol's iodine while placing the slide in a tilted position. The Lugol's iodine was then used to cover the slide, which had been placed back in the horizontal position and allowed to act for 30 seconds.

(c) The Lugol's iodine was then poured off and washed with iodine acetone while tilting the slide. The slide was then covered with iodine and acetone for 30 seconds. After which, the slide was washed thoroughly with water for a few seconds.

(d) The fixed bacterial colony on the slide was counterstained with 10% dilute Cabrol Fuchsin for 30 seconds.

(e) This was washed with water and air dried. Staining of the smears was done to distinguish gram-negative from gram-positive bacterial colonies. This procedure was repeated for all the other slides.

New frosted-edged slides were used for each staining. They were washed both with a detergent to rid them of any grease, rinsed in distilled water and stored in 70% alcohol. Sterile water was used to make the smear on the slide. Care was taken not to overheat the smear on the slide during the process of drying and affixing bacterial cells on the slide because overheating denatures the bacterial cells

Examination of slides under the microscope

The slides were examined under the microscope. The section with the affixed bacteria was covered with a drop of immersion oil (Plate F), and then the slide was placed under the objective lens with magnification x100. The observed morphological characteristics of the cells were recorded.

- **Inoculation of bacterial suspensions onto sterile MA and BA**

In order to ensure that pure colonies are used, bacterial suspensions in the incubated peptone water were inoculated onto MAC and BA. The various labels given to the various Bijou bottles were then transferred onto the side facing upward on MA and BA. The sides facing upward were divided into three separate sections for each agar. An aseptically sterilized inoculation loop was then used to pick a loopful of bacteria from the peptone and then transferred onto the MA agar by making a well at the section with the same label as that on the Bijou bottle.

After this, the inoculation loop was used to touch the surface of the well, and the first streaks were made from the well. A second streak was made across the first streak. After the second streak had been made, the loop was used to make a third streak across the second streak aseptically. The same procedure was repeated for the other cultures in incubated peptone on both MA and BA. The inoculated plates and a sterile agar plate (control) were then incubated for 24 hrs. at 37°C.

Biochemical tests

- **Inoculation of sugars**

Since some bacterial species are not able to ferment all sugars, this test was done to determine which sugars the bacteria are able to ferment. Isolated colonies from pure cultures were inoculated aseptically into sterile peptone water in an equivalent number of Bijou bottles. These were incubated for about 4 hrs at 37°Cin an incubator. A sterilized Pasteur pipette was used to aseptically dispense about 50 µl of the bacterial suspension in the Bijou bottles containing eleven (11) different types of sugars (Galactose, D-Glucose, D-Sorbitol, I (meso) Inositol, L-Arabinose, L-Rhamnose, Salicin, Dulcitol, Raffinose, Dextrose and Fructose) which had been sterilized by tindalization. This was done to determine which sugars the bacteria are able to ferment (Plates G & H). These were incubated at 37°C for 24 hrs.

- **Indole test and reaction**

With the aid of a sterile pipette, a few drops of Kovac solution were put into the inocula in the peptone water for the Indole tests. The observed changes (colour) were recorded (red colour on the surface is an indication of a positive reaction, while colourless means a negative reaction).

- **Catalase test**

A loopful of H_2O_2 was placed on a slide, after which the loop was used to pick a bacterial colony from BA onto the H_2O_2. This was done to determine bacterial species that have the ability to break down H_2O_2 to water and oxygen. New sterile frosted slides were used for each tissue during the Catalase examinations.

- **Oxidase test**

Two drops of the reagent (*N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride*) were added to an isolated colony on a BA agar plate. This was examined for blue colour within 10 seconds**.** The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme.

- **Urease and simmon citrate test**

With the aid of a loop, a suspension of bacteria was streaked onto Urea and Simmons citrate. The inoculated Simmon citrates were incubated at 37°C for 4 days. The urea was incubated overnight at 37°C. Urease test was done to determine the ability of bacteria to split urea through the production of the enzyme urease.

- **Sulphur indole motility test (S.I.M)**

A sterilized rod was used to pick bacteria suspensions and then stabbed into SIM. This was done to determine whether the bacteria could utilize Sulphur, indole and is motile. The stabbed media were incubated overnight at 37°C.

3.8.3 General safety and quality assurance

Hands were first washed with soap and water before and after the laboratory work was started (Christopher & Bruno, 2003). Virkon was used to clean and disinfect the floor and inside of the safety cabinet II before and after work. Virkon was put in a discarded jar for used glassware and pipettes. The floor of the safety cabinet was sterilized aseptically with 70% alcohol before and after each use (Christopher & Bruno, 2003). New sterile gloves were worn before handling the fish sample and throughout the research. These were done to reduce the introduction of cross-contamination to cultures. The caps of the Bijou bottles containing the Brain Heart Infusion broths, peptone water and the sugars were not placed on the floor of the safety cabinet II. This was done to prevent introducing contamination.

Microscope slides, agar plates, Bijou bottles and all glassware used were all sterilized by subjecting them to high temperature and pressure in an autoclave. Inoculation loops used for the streaking were aseptically sterilized to red hot with a Bunsen burner flame before and after each use. They were cooled in air before they were used to pick colonies and bacterial suspensions. This was done to reduce the introduction of contamination and ensure effective growth.

Control media (sterile sugars, BA, MA, Sulphur Indole Motility, Urease and Simmon citrate of the same batch) were incubated alongside those that had been inoculated. This was done to ensure that the media being used had no contamination and that the incubator was also sterile. The necks of the Bijou bottles containing the various cultures were passed through the flame about three times after the caps were removed with the index finger of the palm holding the inoculation loop or the scissors (Christopher & Bruno, 2003). Time of exposure for sterile media, cultures or bacteria to air was reduced by working quickly. New Pastor Pipettes were aseptically sterilized with a benzene flame before they were used to inoculate all the sugars for each culture from the peptone water.

3.9 Data analysis

To classify bacteria to the genus and species levels, we used references including Quinn *et al.* (1994), Inglis *et al.*, (1994) and Bergey's Manual (Buchanan & Gibson, 1974). Bacterial species and their frequencies from three sites were plotted, and statistical analysis was done using SPSS version 26.0, with Pearson correlation, to find associations at a p-value of 0.05 or less. Physical and chemical parameters were analyzed using Jamovi version 2.3.28.0, with descriptive statistics and Student’s t-tests for significance at a 5% probability value. Questionnaire data on risk factors were entered into Excel and exported to SPSS version 22.0 for further analysis. Descriptive statistics were presented in tables, and logistic regression analysis with odds ratios was used to examine associations between risk factors and outcomes, with standardized beta coefficients and p-values indicating significance.

3.10 Ethical approval

The Tanzania Fisheries and Aquaculture Research Technical and Ethical Committee (TaFReTEC) reviewed and approved the research protocol in Dar es Salaam before the study commenced- TAFIRI/HQ/RES.CLEARANCE/ot.il/6 (APPENDIX A). Consent was obtained from fish farmers or farm managers before administering the questionnaire and collecting samples at each cage-cultured farm (Appendix 2).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Prevalence of zoonotic pathogenic bacteria at cage-cultured Nile tilapia (*Oreochromis niloticus*) in Mwanza Gulf, Lake Victoria

(i) Fish samples and their biodata

Plate 5: Photos of Nile tilapia (Oreochromis niloticus) for the isolation of bacteria

The mean weights of the sampled fish were 167.3±13 g, the mean total lengths of fish were 19.7±0.6 cm and the mean standard length of fish was 16.7±0.6 cm (Table 2).

Table 2: Mean ± SEM Values of Weight and Lengths of Sampled Tilapia

Fish Type (District)	Weight (g) Mean ±SEM (Range)	Standard Length (cm) Mean ± SEM (Range)	Total Length (cm) Mean ± SEM (Range)
Tilapia (Illemela)	167.33 ± 12.96 (15–657)	19.73 ± 0.60 (8–42)	16.71 ± 0.56 (7–29)
Tilapia (Misungwi)	348.66 ± 29.99 (68–1020)	36.87 ± 1.11 (23–57)	32.00 ± 0.97 (20–50)
Tilapia (Nyamagana)	39.33 ± 6.05 (4–140)	13.97 ± 0.63 (8–20)	9.78 ± 0.52 (5–15)

(i) Bacteria isolation and characterization

Bacterial isolates

A total of 136 bacterial isolates were recovered from fish organs, belonging to nine genera. Of these, 64.8% (n = 88) of the isolates were successfully characterized and identified based on the criteria used, while 35.2% (n = 48) could not be identified. The isolates belonged to five families, namely: *Enterobacteriaceae, Pseudomonadaceae, Bacillaceae, Streptococcaceae*, and *Staphylococcaceae.* The majority of the isolates (70) were Gram-negative bacteria, while 66 were Gram-positive (Table 3).

Table 3: The bacterial isolates from fish in Mwanza Gulf, Lake Victoria

Family	Bacterial isolates	Frequency (%)
Enterobacteriaceae	*Klebsiella* spp.	6(2.9)
	Salmonella spp.	17(8.1)
	E. coli	7(3.3)
	Shigella spp.	8(3.8)
Pseudomonadaceae	*Pseudomonas* spp.	32(15.2)
Bacillaceae	*Bacillus* spp.	20(9.5)
Staphylococcaceae	*Staphylococcus* spp.	19(9.0)
Streptococcaceae	*Streptococcus* spp.	4(1.9)
	Lactococcus spp.	23(11.0)

Colony morphology characteristics of the isolates

Colony morphology of the selected isolates was recorded with respect to their form/shape, pigmentation, size, margin, surface and visual characteristics, lactose fermentation and hemolytic activity (Table 4).

Bacillus formed dry, large, amoeboid-like colonies with a rough texture and distinct hemolysis on Blood Agar (BA). On MacConkey Agar (MA), mucoid, pink colonies were observed, indicating lactose fermentation, typically associated with enteric bacteria. These colonies developed well at room temperature (24–26°C). On Xylose Lysine Deoxycholate (XLD) Agar, colonies exhibited varying colours, ranging from yellow to red, with or without black centres, suggesting differences in xylose fermentation, lysine decarboxylation and hydrogen sulfide production. *Salmonella* was cultured on various selective and differential media to aid its identification. On Xylose Lysine Deoxycholate (XLD) Agar, *Salmonella* produced red colonies with black centres due to hydrogen sulfide (H_2S) production, which reacts with ferric ammonium citrate in the medium.

On Salmonella-Shigella (SS) Agar, it formed medium-sized, colourless colonies with distinctive black centres, also due to H_2S production. On Mannitol Salt Agar (MSA)**,** no growth was observed, as the high salt concentration in the medium inhibits the growth of non-halotolerant organisms like *Salmonella*. *Streptococcus* suspect strains produced purple pinpoint colonies on sodium aside crystal violet blood agar. *Staphylococcus* species typically form smooth, round, golden or white colonies on nutrient agar. On blood agar, they often produce large, creamy, or yellow colonies. *Pseudomonas* species typically form smooth, round colonies with a distinct green or blue-green colour due to the production of pyocyanin and pyoverdine pigments. On blood agar, they generally produce non-hemolytic colonies.

On MacConkey agar, *Pseudomonas* species may appear as pale colonies, as they do not ferment lactose. *Escherichia coli* was tested on a range of selective and differential media. On MacConkey Agar, *Escherichia. coli* produced characteristic pink colonies due to lactose fermentation, which led to a drop in pH of the medium. On Blood Agar, *Escherichia coli* colonies were generally non-hemolytic, exhibiting either alpha or gamma hemolysis depending on the strain. On XLD Agar, *Escherichia coli* formed yellow colonies as a result of xylose fermentation. On Salmonella-Shigella (SS) Agar, *Escherichia coli* typically produced pink to red colonies, indicating lactose fermentation, and did not form black centres, distinguishing it from hydrogen sulfide-producing organisms.

Shigella species typically form small, non-lactose fermenting and colourless or pale colonies on MacConkey agar, as they do not ferment lactose. On Salmonella-Shigella (SS) agar, *Shigella* species produce pale colonies without black centres, distinguishing them from *Salmonella*, which produces colonies with black centres due to hydrogen sulfide production. On blood agar, *Shigella* usually forms non-hemolytic, smooth colonies. On blood agar, *Lactococcus* species often produce small, pinpoint colonies that may be alpha-hemolytic (partial hemolysis, producing a greenish discoloration around the colonies), though some strains are non-hemolytic. They do not ferment lactose on MacConkey agar, so their colonies would remain colourless or pale.

Table 4: Phenotypic characteristics of bacteria isolated determined by colony morphology

Bacterial Isolate	**Lactose Fermentation**	**Hemolysis on Sheep Blood Agar**	**Other Features**
Bacillus spp.	Negative	Hemolytic	Dry, rough colonies on Nutrient Agar; no growth on MacConkey; poor/no growth on XLD, SS, MSA.
Pseudomonas spp.	Negative	Hemolytic	Blue-green colonies on MacConkey; non-lactose fermenter on XLD and SS; no growth on MSA; grows well on Nutrient Agar.
Escherichia coli	Positive	Non-hemolytic to β-hemolytic	Pink colonies on MacConkey (lactose fermenter); yellow colonies on XLD; colorless on SS; no growth on MSA; smooth colonies on Nutrient Agar.
Salmonella spp.	Negative	Non-hemolytic	Red colonies with black centers on XLD and SS; colorless on MacConkey; no growth on MSA; smooth colonies on Nutrient Agar.
Klebsiella spp.	Positive	Non-hemolytic	Large mucoid pink colonies on MacConkey; yellow on XLD; colorless or pale on SS; no growth on MSA; smooth mucoid colonies on Nutrient Agar.
Lactococcus spp.	Negative	α or non-hemolytic	Grows on Nutrient Agar and MSA (some species); no growth on MacConkey, XLD, or SS.
Staphylococcus spp.	Positive	Hemolytic (mostly β)	Yellow colonies on MSA (mannitol fermentation by *S. aureus*); no growth on MacConkey, XLD, or SS; golden/yellow on Nutrient Agar.
Streptococcus spp.	Negative	α, β, or γ hemolysis	No growth on MacConkey, XLD, SS, or MSA; small translucent colonies on Nutrient Agar.
Shigella spp.	Negative	Non-hemolytic	Pale colonies on MacConkey; red colonies without black centers on XLD; colorless on SS; no growth on MSA; grows on Nutrient Agar.

Phenotypic and Gram staining characteristics of isolates

Preliminary identification of isolates was based on Gram staining, catalase and oxidase, according to Bergey's Manual of Determinative Bacteriology (Fig. 7). Members of the family *Enterobacteriaceae, Pseudomonadaceae*, *Bacillaceae*, *Streptococcaceae*, *Staphylococcaceae* and *Lactococcaceae* showed positive catalase reaction. *Pseudomonadaceae*, *Streptococcaceae* and *Staphylococcaceae* showed positive oxidase reaction. The majority of the isolates were Gram-negative short bacilli to coccobacilli, including members of the family *Enterobacteriaceae* and non-*Enterobacteriaceae* bacteria such as *Pseudomonadaceae*, *Bacillaceae*, *Streptococcaceae* and *Staphylococcaceae.* Few isolates were Gram-positive.

Bacillus spp. produced medium-sized Gram-positive bacilli. *Streptococcus* spp. formed Gram-positive cocci in short chains.

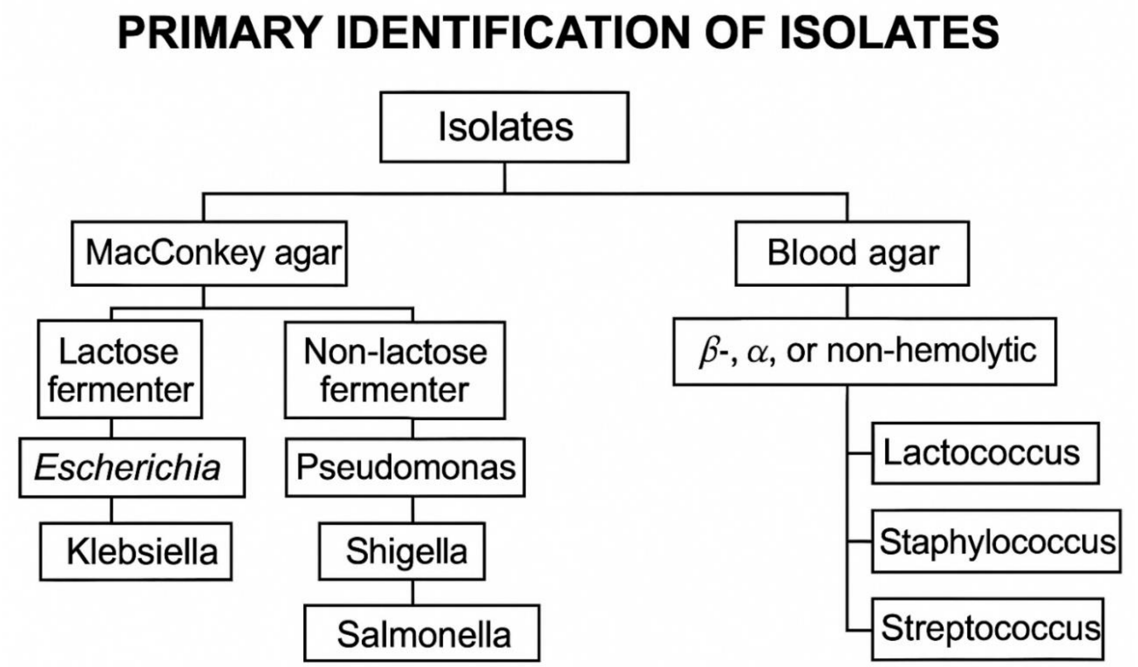


Figure 2: **Flowchart of primary identification of bacterial isolates (Modified from Bergey's manual of determinative bacteriology)**

Biochemical characteristics of isolates

Biochemical characteristics (indole production, methyl red test, citrate, urease, Sulphur indole-motility (SIM) test, gas and acid formation from glucose, sucrose, mannitol and arabinose) of the isolates were as given in Table 5. These were run and interpreted as given under the methodology.

Thirty-three per cent (33%, n=3) of the isolated genera (*Streptococcus, Salmonella* and *Escherichia*) were found to be producing indole from peptone water, while the other genera were indole negative. Twenty-two (22%, n=2) of the isolated genera (*Escherichia* and *Salmonella*) were found to be methyl red positive, while the other isolates were negative. Twenty-two per cent (22%, n=2) of the genera isolated were found to utilize Simmons' citrate (*Pseudomonas* and *Salmonella*), while the rest were negative.

Twenty-two per cent (22%, n=2) of the genera isolated were found to produce hydrogen sulphide (*Escherichia* and *Salmonella*) while the rest were negative. Thirty-three per cent (33%, n=3) of the genera isolated were motile (*Pseudomonas*, *Escherichia*, *Salmonella*) while the rest were non-motile. Thirty-three per cent (33%, n=3) of the genera isolated were found to degrade urea (*Streptococcus*, *Pseudomonas,* and *Klebsiella*) while the rest were negative.

Forty-four per cent (44%, n=4) of the genera isolated were found to ferment glucose (*Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*) while the rest were glucose non-fermenting.

Thirty-three per cent (33%; n=3) of the genera isolated were found to ferment sucrose (*Pseudomonas*, *Klebsiella*, *Enterobacter*) while the rest were sucrose non-fermenting. Forty-four per cent (44%; n=4) of the genera isolated were found to ferment mannitol (*Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter)* while the rest were non-fermenting. Forty-four (44%; n=4) of the genera isolated were found to ferment arabinose (*Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*) while the rest were non-fermenting.

Table 5: Biochemical Characteristics of Selected Bacterial Isolates Determined by Conventional Methods

Bacterial Isolate	Indole	MR	Citrate	H_2S	Motility	Urease	Glucose	Sucrose	Mannitol	Arabinose
Bacillus spp.	–	–	–	–	–	–	–	–	–	–
Streptococcus spp.	+	+	–	–	–	+	+	+	–	–
Pseudomonas spp.	–	–	+	–	+	+	–	–/+	–	–
Staphylococcus spp.	–	–	–	–	–	+	+	+	+	+
Salmonella spp.	–	+	+	+	+	–	+	–	+	+
Klebsiella spp.	–	–	+	–	–	+	+	+	+	+
Escherichia coli	+	+	–	+	+	–	+	–	+	+
Shigella spp.	+	+	–	–	–	–	+	–	+	+
Lactococcus spp.	–	+	–	–	–	–	+	–	+	–

–= Negative reaction += Positive reaction

(ii) Prevalence of bacteria per sample

Table 6 gives the prevalence of bacterial flora from the different samples of fish organs (skin, gills, kidney and liver). *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Klebsiella* spp., *Staphylococcus* spp., *Shigella* spp., *Lactococcus* spp. and *Escherichia* spp. were recovered in varying magnitudes in the skin, gills, liver and kidney samples of cage-cultured fish from Illemela, Nyamagana and Misungwi.

Table 6: Distribution of bacteria in the gills, skin, liver and Kidney of fish cage-cultured from Illemela, Nyamagana and Misungwi (N =210)

Isolated bacteria	Gills (%)	Skin (%)	Kidney (%)	Liver (%)	Total(%)
Staphylococcus sp.	10 (4.8)	3 (1.4)	0 (0)	6 (2.9)	19 (9.0)
Streptococcus sp.	3 (1.4)	1 (0.5)	0 (0)	0 (0)	4 (1.9)
Pseudomonas sp.	15 (7.1)	6 (2.9)	3 (4)	8 (3.8)	32 (15.2)
Escherichia sp.	5 (2.4)	2 (1.0)	0 (0)	0 (0)	7 (3.3)
Shigella sp.	0 (0)	3 (1.4)	0 (0)	5 (2.4)	8 (3.8)
Salmonella sp.	3 (1.4)	5 (2.4)	6 (2.9)	3 (1.4)	17 (8.1)
Klebsiella sp.	4 (1.9)	2 (1.0)	0 (0)	0 (0)	6 (2.9)
Lactococcus sp.	6 (2.9)	2 (1.0)	5 (2.4)	10 (4.8)	23 (11.0)
Bacillus sp.	14 (6.7)	3 (1.4)	0 (0)	3 (1.4)	20 (9.5)
Total	**60 (28.6)**	**27 (12.9)**	**14(6.7)**	**35 (16.7)**	**136 (64.8)**

The gills had the highest number of bacterial isolates (28.6%; 60/210), followed by the skin (12.9%; 27/210), kidney (6.7%; 14/210) and liver (16.7%; 35/210), with a statistically significant difference of a P-value of 0.0002118154 ($P < 0.001$). This suggests that the gills might act as a key site for bacterial colonization in cage-cultured fish (Fig. 8).

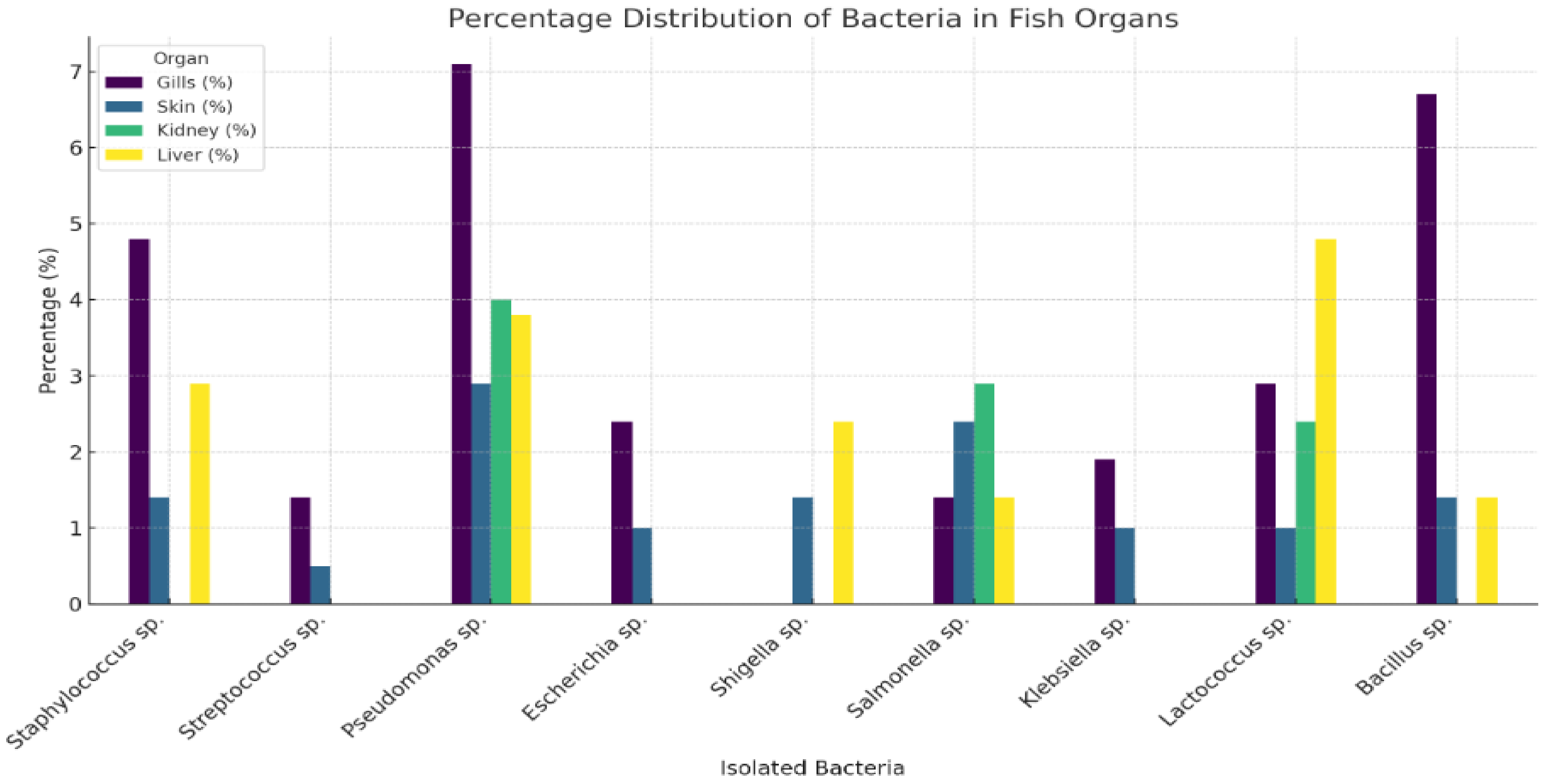


Figure 3: **Percentage distribution of bacterial isolates in different organs**

(iii) Prevalence of bacterial pathogens from the three sites of cage-cultured

Table 7, presents the types of bacteria isolated from Illemela, Nyamagana and Misungwi districts. Among the bacteria identified, *Bacillus spp.*, *Pseudomonas spp.*, *Salmonella spp.*, *Klebsiella spp.*, *Staphylococcus spp.*, *Shigella spp.*, *Lactococcus spp.* and *Escherichia spp.* were found across all three districts. *Pseudomonas spp.* (23.5%; 32/136), *Lactococcus spp.* (16.9%; 23/136), *Bacillus spp.* (14.7%; 20/136), *Staphylococcus spp.* (14.0%; 19/136) and *Salmonella spp.* (12.5%; 17/136) were the most prevalent bacterial isolates overall. Illemela recorded the highest number of total isolates (44.1%; 60/136), followed by Misungwi (34.6%; 47/136) and Nyamagana (21.3%; 29/136).

Table 7: Number (percentage) of bacteria isolated from cage-cultured fish samples from Illemela, Nyamagana and Misungwi

Isolatedbacteria	**Illemela *N*= 70**	**Nyamagana *N*= 60**	**Misungwi *N*= 80**	**Total *N*= 210**
Staphylococcus spp.	10 (14.3)	3 (5)	6 (7.5)	19 (4.2)
Streptococcus spp.	2 (2.9)	0 (0)	2 (2.5)	4 (1.9)
Pseudomonas spp.	13 (18.6)	9 (15)	10 (12.5)	32 (5.7)
Escherichia spp.	3 (4.3)	1 (1.7)	3 (3.8)	7 (3.3)
Shigella spp.	4 (5.7)	2 (3.3)	2 (2.5)	8 (3.8)
Salmonella spp.	8 (11.4)	3 (5)	6 (7.5)	17 (8.1)
Klebsiella spp.	2 (2.9)	2 (3.3)	2 (2.5)	6 (2.9)
Lactococcus spp.	11(15.7)	3 (5)	9 (11.5)	23 (11.1)
Bacillus spp.	7(10)	6 (10)	7 (8.8)	20 (9.5)
Total	**60 (85.7)**	**29 (48.3)**	**47 (58.8)**	**136 (64.8)**

Figure 9 illustrates the comparative prevalence of bacterial isolates across the three districts. Statistical analysis revealed no significant differences in bacterial prevalence between Illemela and Nyamagana or between Illemela and Misungwi (p = 0.988; $p > 0.05$). However, *Salmonella spp.* demonstrated a relatively higher prevalence in Misungwi compared to the other sites.

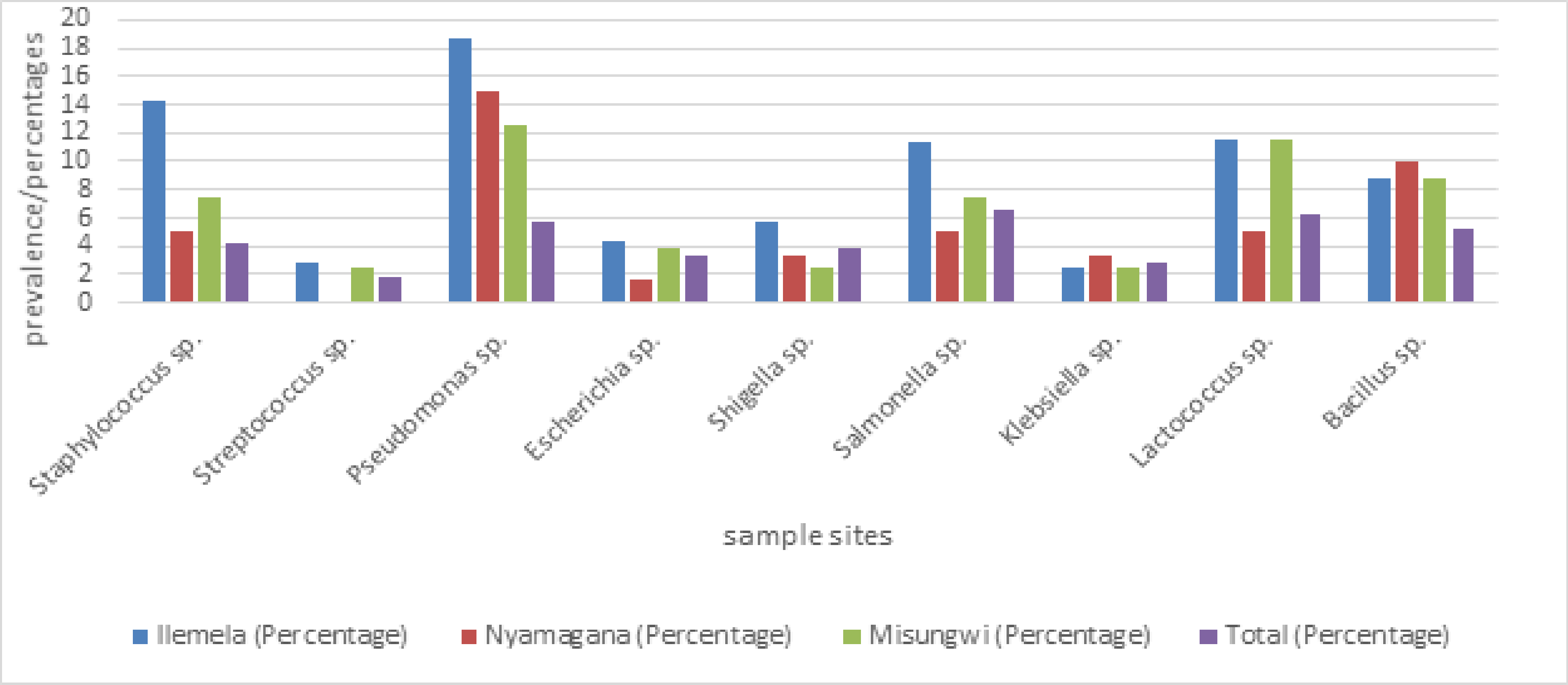


Figure 4: **Prevalence of bacteria at cage-cultured sites in the three districts**

(iv) Pearson correlation coefficient of bacterial prevalence (%) at cage-cultured sites (Ilemela, Misungwi and Nyamagana)

The Pearson correlation analysis revealed strong positive linear relationships in bacterial prevalence among the cage-cultured sites of Ilemela, Misungwi and Nyamagana, with correlation coefficients ranging from 0.971 to 0.987, all statistically significant at the 0.01 level. Biologically, this indicates that the presence and distribution of bacterial pathogens across these locations are closely related. Such high correlation values suggest that similar environmental conditions or aquaculture practices, such as high stocking densities, poor water quality and inadequate biosecurity measures, are likely influencing bacterial prevalence at all three sites. This implies that bacterial challenges in these districts are not isolated incidents but may reflect broader, shared risk factors associated with cage culture systems in the Mwanza Gulf (Table 8).

Table 8: Pearson correlation coefficient of bacterial prevalence (%) per cage-cultured Sites

Correlations	**Ilemela (Percentage)**	**Misungwi (Percentage)**	**Nyamagana (Percentage)**
	(Percentage)	(Percentage)	(Percentage)
Ilemela (Percentage)	1	0.987**	0.971**
Misungwi (Percentage)	0.987**	1	0.977**
Nyamagana (Percentage)	0.971**	0.977**	1

****Correlation is significant at the level of 0.01 (2-tailed)**

4.1.2 Water quality parameters

(i) Physical-chemical parameters

Cage-cultured water quality was assessed in 50 active cage-cultured farms and 10 non-cage-cultured control sites due to time (8:00–10:00 am) and resource (one water test kit) constraints. The mean ± standard deviation (SD) values for physicochemical parameters of water samples taken from different locations (Illemela, Misungwi and Nyamagana) are presented in Appendix 3. The overall mean ± SD values for the various water quality parameters were as follows: Dissolved oxygen (DO) was 6.70 ± 0.88 mg/L, water temperature was 28.68 ± 1.37°C, pH was 6.75 ± 0.44, conductivity was 54.07 ± 2.85 µS/cm, turbidity was 0.65 ± 0.27 NTU, total hardness was 27.36 ± 3.07 mg/L, alkalinity was 63.94 ± 10.39 mg/L, chlorophyll-a was 1.87 ± 0.50 mg/m^3, and total phosphorus was 0.80 ± 0.008 mg/L. Water temperature ranged from 20.5 to 31.7°C, falling within the recommended limits. Dissolved oxygen, essential for the survival of cage-cultured fish, ranged from 0.13 to 13.4 mg/L. Nitrite, which is highly toxic to fish, ranged from 0 to 3.06 mg/L (Table 9).

Among the 50 cage-cultured fish samples from Ilemela, Misungwi, and Nyamagana, elevated levels of nitrite, phosphate and ammonia were primarily recorded in samples from Nyamagana, indicating a higher likelihood of water quality stress at that site. Specifically, Nyamagana showed the highest nitrate concentration (0.15 mg/L), nitrite levels (0.04 mg/L), ammonia concentration (0.45 mg/L), and phosphate concentration (1.41 mg/L), which exceeded recommended thresholds, indicating potential nutrient accumulation and water quality stress. In contrast, Ilemela and Misungwi generally remained within safe limits for nitrate (Ilemela: 0.09 mg/L, Misungwi: 0.058 mg/L), nitrite (Ilemela: 0.02 mg/L, Misungwi: 0.023 mg/L), and phosphate (Ilemela: 0.15 mg/L, Misungwi: 0.79 mg/L).

The control site consistently showed values within the recommended limits for all measured parameters, with nitrate (0.08 mg/L), nitrite (0.03 mg/L), ammonia (0.24 mg/L), phosphate (0.80 mg/L), and sulphate (2.69 mg/L). While all sites met water quality standards for nitrate, nitrite, and phosphate, Nyamagana consistently exhibited the highest values across all three parameters, highlighting its proximity to exceeding safe limits and suggesting a relatively higher risk of water quality deterioration. Sulphate levels were highest at Nyamagana (2.83 mg/L) and lowest at the control site (2.69 mg/L) (Table 10).

(ii) Sediment quality of water samples collected from the gulf of lake victoria

The mean monthly concentrations of Total Organic Carbon (TOC) recorded at the cage farmed and control sites ranged from 0.22 - 4.27% and 0.35 - 4.56 % (Fig. 4) with overall mean values of 4.17% at Illemela, 3.12% at Misungwi, 0.20% at Nyamagana and 4.56% at the control site, respectively. The concentration at the control site was, however, higher than that at the cage-farmed site. The TOC values for both sites were generally low, and the difference in mean values was not significant ($p > 0.05$). The Total Organic Matter (TOM) content at the control site was marginally higher than at the cage-farmed site. Mean values for the farmed and control sites were 2.54% for Ilemela, 1.89% for Misungwi, 2.31% for Nyamagana and 3.92 % for the control site, respectively (Fig. 10). Total Nitrogen concentrations at the farmed and control sites were 0.02% at Illemela, 0.01% at Misungwi, 0.02% at Nyamagana, and 0.39% at the Control site, respectively.

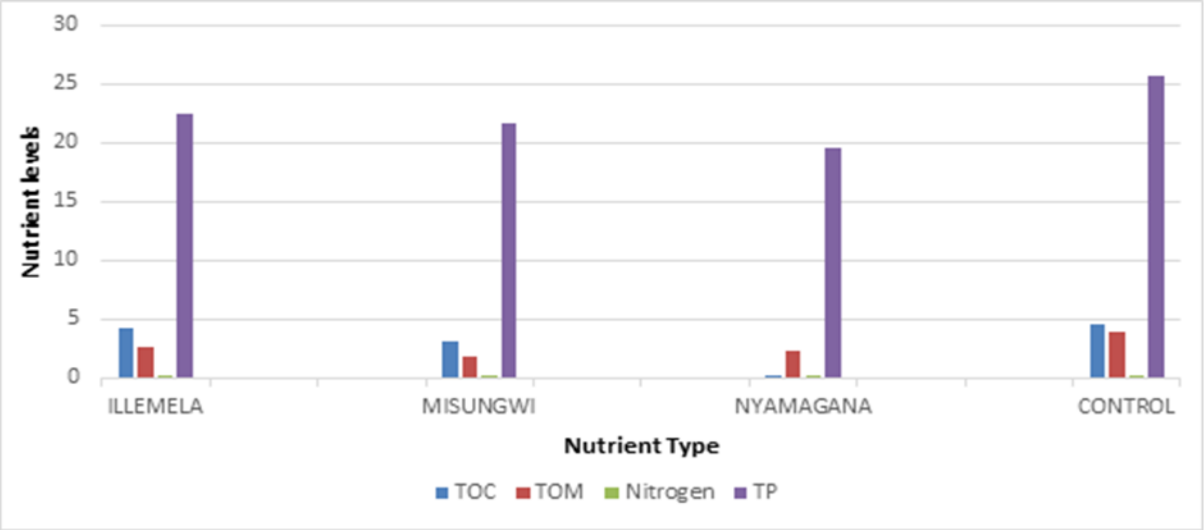


Figure 5: **Mean levels of nutrients in the sediment at the cage-farmed and control sites**

TOC: Total Organic Carbon TON: Total Organic Matter TP: Total Phosphorus

(iii) Comparison of nutrient levels over time in Mwanza gulf

Water quality parameters in Mwanza Gulf have changed notably from 2004 to 2024, with recent data under cage culture conditions showing elevated nutrient levels. In 2024, nitrate reached 150 µg/L, nitrite 40 µg/L, ammonia 450 µg/L, and total phosphorus 1410 µg/L—significantly higher than in previous years. Dissolved oxygen was 6.38 mg/L, pH 6.69, temperature 29.1°C, conductivity 67.97 µS/cm, and turbidity 1.63 NTU.

In earlier years, nutrient concentrations were lower, and water quality was generally better. For example, in 2004, nitrate was 28.5 µg/L, ammonia 141.4 µg/L, and phosphorus 96.9 µg/L, with higher dissolved oxygen and lower turbidity. These changes suggest increasing eutrophication and declining water quality, especially in areas with cage aquaculture (Table 11).

Table 9: Physical parameters recorded from the cages cultured Nile Tilapia (Oreochromis Niloticus) along Mwanza Gulf of Lake Victoria, from March 2023 to September 2023

Cage Site Parameters	Site 1 Control (10)	Site2 Cage-cultured (30)	Site3 Cage-cultured (10)	Site4 Cage-cultured (10)
Dissolved oxygen(mg/l)	5.78±0.85	6.70±0.88	6.22±0.71	6.38±0.6
pH	6.69±0.27	6.75±0.44	7.21±0.39	6.79±0.58
Conductivity (µs⁄cm)	67.97±0.46*	54.07±2.85	59.89±5.48	62.33±1.92
Turbidity (NTU)	1.63±0.78	0.65±0.27	1.01±0.85	1.47±0.173
Total Hardness(mg/l)	31.08±7.81*	27.36±3.07	28.95±4.58	22.20±1.56
Alkalinity(mg/l)	45.89±5.24	63.94±10.39	64.06±11.04	58.39±12.20
Chlorophyll- a(mg/m^3)	2.178±0.45*	1.87±0.50	1.96±0.45	2.04±0.191
Temperature(°C)	29.10±1.00	28.68±1.37	27.97±1.84	28.13±1.24

(Mean ± SD); Site 1; control site (non-cage-cultured), site 2; Ilemela, Site 3; misungwi, site 4; Nyamagana

Table 10: Chemical parameters at the cages cultured Nile Tilapia (Oreochromis Niloticus) along Mwanza Gulf of Lake Victoria, from March 2023 to September 2023

Cage site Parameters	Sites 1; Control (10)	Site2; Cage-cultured (30)	Site 3; Cage- cultured (10)	Site 4; cage-cultured (10)
Nitrate(mg/l)	0.15±0.042	0.09±0.039	0.058±0.095	0.08±0.005
Nitrite(mg/l)	0.04±0.004	0.02±0.009	0.023±0.008	0.03±0.004
Ammonia(mg/l)	0.45±0.947*	0.26±0.057	0.25±0.043	0.24±0.041
Phosphate(mg/l)	1.41±0.394*	0.15±0.059	0.79±0.409	0.80±0.008
Sulphate(mg/l)	2.83±0.318*	2.84±0.119	2.80±0.173	2.69±0.009

(Mean ± SD), Site 1; control site (non-cage-cultured), Site 2; Ilemela, Site 3; misungwi, site 4; Nyamagana

Table 11: Comparison of physico chemical parameters in Mwanza Gulf across time 2004–2024

	DO (mg/l)	pH	EC (µs⁄cm)	Turbidity (NTU)	T (°C)	NO_{3}+ (µg/l)	NO_{2}+ (µg/l)	HNO_{4}+ (µg/l)	TP (µg/l)
2024_Caged	6.38±0.6	6.69±0.2	67.97±0.4	1.63±0.7	29.10±1.0	150±42	40±4	450±94.7	1410±39.4
2021 (2000_2016)	6.67	9.06	113.2	6	24.9	28			
2012 (2005_2008)	7.1±0.6	8.6±0.6		2.5±0.1	25.5±1.1	110.6±32.8			103.5±38.0
2004	7.4±0.1		95.8±0.3	1.7±0.1	25.2±0.1	28.5±4.5	5.1±0.7	141.4±43.7	96.9±1.8

Makaka *et al.* (2022), Ngupula *et al.* (2012) and **Kishe (2004)**

DO-Dissolved Oxygen, **EC**-Electrical conductivity, **T**-Temperature, **NO_{3}+**-Nitrate, NO_{2}**+-Nitrite**, **HNO_{4}+**-Ammonia, **TP**-Total phosphorus

4.1.3 Risk factors contribute to the prevalence of zoonotic pathogenic bacteria

(i) Demographic characteristics of the respondents

Interviews were completed with 120 randomly selected fish farmers during the study. In the survey, certain variables were selected to determine characteristics of the majority of the fish farmers in the study areas. The social demographic attributes of fish cage-cultured owners were the individual's age, gender, level of education, occupation, reasons for keeping fish, prior training in aquaculture, and number of years in fish farming. Results on the socio-demographic attributes of fish farmers are given in Table 12. Of these, 69.2% were male, mostly aged between 36 and 55 years. Half had around 10 years of fish farming experience. The farms were distributed as follows: The 41.7% in Illemela, 33.3% in Nyamagana, and 25.0% in Misungwi. Additionally, 34.2% of the farmers used these practices as a supplementary income source.

Table 12: Demographic Characteristics of Respondents

Demographic Information	Category	Number (%) of Respondents
Sex	Male	83 (69.2)
	Female	37 (30.8)
Districts	Illemela	50 (41.7)
	Nyamagana	40 (33.3)
	Misungwi	30 (25.0)
Age	20 – 35	13 (10.8)
	36 – 45	50 (41.7)
	46 – 55	50 (41.7)
	56 – 65	7 (5.8)
Education Level	Primary	40 (33.3)
	Secondary	40 (33.3)
	University	9 (7.5)
	None	31 (25.8)
Cage Farming Experience (years)	Up to 10	60 (50.0)
	11 – 20	49 (40.8)
	21 & above	11 (9.2)
Training on Nile Tilapia Farming	Yes	70 (58.3)
	No	50 (41.7)
Is Nile Tilapia Farming the Sole Source of Income?	Yes	41 (34.2)
	No	59 (65.8)

(ii) The characteristics of cage-cultured farm production include the type and size of cages, the stocking density and the species of fish being raised

In the study region, farmers cultivated three tilapia species, with Nile tilapia (*Oreochromis* *niloticus*) being the most prevalent. Most farmers (73.3%; 88/120) practiced monoculture. Cage sizes were categorized as small (<300 m²), medium (301–500 m²), large (501–600 m²), and extra-large (>600 m²). The majority (47.5%; 57/120) used large cages, while 5% (6/120) used medium-sized ones. Many farmers (38.3%; 46/120) overstocked their cages with more than 8 fish per square meter. Half of the farms used externally sourced cages, and hatcheries were the main source of fingerlings (87.5%; 105/120). Regarding feeding, 35% (42/120) used formulated feed with carbohydrates, proteins, fats, and vitamins, while 55% (66/120) relied on market-sourced feed containing carbohydrates and fats (Table 13).

Table 13: Characteristics of Cage Farming, Including the Type and Size of Cages, Stocking Density, and the Species of Fish

Variable	Frequency	Per cent (%)	95% CI
Cage system Type			
Floating system	66	55	39–67
Fixed system	42	35	23–50
Other systems	12	10	2–19
Size of Cages (m²)			
<300	37	30.8	18–44
301 to 500	6	5	0–13
501 to 600	57	47.5	33–61
>600	20	16.7	6–27
Stocking Density			
<4 fish/m²	19	15.8	6–27
4 to 8 fish/m²	55	45.8	31–59
>8 fish/m²	46	38.3	25–52
Fish Species			
Nile tilapia (*Oreochromis niloticus*)	81	67.5	54–80
Three spot tilapias (*Oreochromis andersonii*)	34	28.3	16–41
Greenhead tilapia (*Oreochromis macrochir*)	5	4.2	0–10
Farming Techniques			
Monoculture	88	73.3	61–85
Polyculture	32	26.7	14–39
Sources of Fingerlings			
Wild environment	15	12.5	3–21
Hatcheries	105	87.5	79–97
Other	0	0	-
Did you build the fish cage?			
Yes	97	80.8	62–86
No, it was from another farmer	23	19.2	15–40

(iii) Water quality monitoring and feeding trends

Water quality monitoring was insufficient, with only 25.8% (31/120) of farmers conducting tests for parameters such as dissolved oxygen and pH among those who did monitor; 75% (24/120) conducted tests just once a month (Table 14).

Table 14: Water-Quality-Monitoring and Feeding Trends

Variable	Frequency	Percent (%)	95% CI
Water quality monitoring conducted			
No	89	68.5	62–87
Yes	31	25.8	13–37
Frequency of Water Quality Monitoring			
Monthly	24	75	65–89
Weekly	5	15.6	5–26
Daily	3	9.4	0–15
Feeding frequency			
Once / day	81	67.5	54–80
Twice-thrice/day	34	28.3	16–41
Never	5	4.2	0–10
Feed composition			
Carbohydrate + Fat sources	66	55	39–67
Carbohydrate + Protein + Fat + Vitamin sources	42	35	23–50
Neither	17	14.2	04–24

n = 120, * n = 32, CI, confidence interval

(iv) Fish disease management and mortality trends

Disease and mortality trends among cage farmers

mainly during the early growth stage (3–20g**.** Most mortality events were short-lasting (less than 5 days), and over 60% reported low mortality rates (below 5%) per production cycle (Table 15A).

Table 15: Disease and Mortality Trends Reported by Cage Farmers (n = 114)

Variable	Category	Frequency	Per cent (%)	95% CI
Did you experience fish disease mortality?	High	105	87.5%	79–97
	Normal	15	12.5%	3–21
Growth Stage with Highest Mortality	3–20 g	77	67.5%	45–73
	21–50 g	16	14.0%	3–21
	51–150 g	16	14.0%	3–21
	251 g and above	5	4.4%	0–10
Duration of Mortality Episodes (days)	Less than 5	35	71.4%	59–84
	5–10	6	12.2%	3–21
	More than 10	2	4.1%	0–10
Mortality Rate per Production Cycle (%)	Less than 5%	69	60.5%	39–67
	5–10%	12	10.5%	2–19
	More than 10%	33	28.9%	12–37

n=114, CI, confidence interval

Clinical signs and treatments

The most commonly reported clinical sign of disease, observed by 35.1% (40/120) of farmers, was fish gasping for air near the water surface. Other frequently observed signs included cotton wool-like skin lesions, red skin, fin rot or erosion, lethargy, eye opacity (exophthalmia), pale and eroded gill filaments, necrosis, and general abnormal behavior (Fig. 11). Notably, 18.4% (21/120) of the farmers could not identify any clinical signs during episodes of high mortality. In response to disease outbreaks, salt (sodium chloride) was the most commonly used treatment, applied by 42.1% (48/120) of respondents, whereas 43.9% (50/120) reported not using any treatment at all (Table 15B).

Plate 6: Clinical signs of Disease in cage-cultured Nile tilapia (*Oreochromis niloticus*)

Table 16: Clinical Signs and Treatments Used During Disease Outbreaks

Variable	Category	Frequency	Percent (%)	95% CI
Clinical Signs Observed	Gasping at surface	40	35.1%	18–44
	Cotton wool on skin	16	14.0%	3–21
	Reddish discoloration	8	7.0%	0–13
	Fin rot/erosion	8	7.0%	0–13
	Swimming in circles	5	4.4%	0–10
	Other signs (lethargy, corneal opacity)	16	14.0%	3–21
	I don't know	21	18.4%	6–27
Medications Used	None	50	43.9%	25–52
	Salt	48	42.1%	23–50
	Potassium permanganate	11	9.6%	0–16
	Lime	5	4.4%	0–10

n=114, CI, confidence interval

(v) Biosecurity management

In cage farming, key biosecurity measures are widely practiced to ensure the health and safety of the fish. A large proportion of respondents (69.2%) restrict entry to the area used for fish production, and the same percentage have some form of pest control in their cages. Additionally, 50.8% maintain a regular cleaning schedule for instruments and equipment. Predator management techniques are common, with 30.8% using bird nets and another 30.8% chasing predators away. When it comes to handling dead fish, 39.5% leave them in the water, while 32.5% bury them. Though less common, 12.5% of respondents wear safety equipment when fishing, indicating attention to personal protection (Table 16).

Table 17: Biosecurity Measures in Cage Farming: Practices and Trends in Fish Production

Variable	Frequency	Per cent (%)	95% CI
Limit Entry to the Area Used for Fish Production			
Yes	83	69.2	57–83
No	37	30.8	18–45
Maintain a Cleaning Schedule for Instruments and Equipment			
Yes	61	50.8	36–64
No	59	49.2	34–64
For Equipment Cleaning, Use Disinfectants			
Chlorine	47	39.2	25–53
None	39	32.5	21–45
Quaternary Ammonium Chloride	34	28.3	16–42
Do the Cages Have Any Kind of Pest Control System?			
Yes	83	69.2	56–83
No	37	30.8	18–45
Manage Predator Birds and Other Predators (Techniques)			
Use bird nets	37	30.8	18–45
Chase them away	37	30.8	18–45
Use scarecrows	24	20	9–32
Use fireworks	12	10	2–19
Use no method	10	8.3	0–16
How to Get Rid of Dead Fish			
Leave the dead fish in the water	45	39.5	20–48
Bury the fish	37	32.5	16–42
Dispose of the fish in uncovered pits	19	16.7	4–24
Incinerate the fish	14	12.3	2–19
Wearing Safety Equipment When Fishing			
Yes	15	12.5	3–20
No	105	87.5	78–97

n = 120, CI, confidence interval

(vi) Diagnostic and extension services for fish health

In the event of disease outbreaks, (78.1%; 90/114) of farmers said they received competent technical assistance through phone consultations or on-site inspections (Table 17). The majority (67.6%; 71/105) asked aquaculturists for advice; in this survey, aquaculturists were defined as people who held degrees or certifications related to fisheries and aquaculture.

Table 18: Diagnostic and Extension Services for Fish Health

Variable	Frequency	Per cent (%)	95% CI
Seek Expert Assistance When Disease and Mortality Occurs			
Yes	90	78.1	56–82
No	24	21.1	8–29
Experts Offering Services			
Aquaculturists	71	67.6	50–77
The source hatchery	22	21.0	6–27
Training institution personnel	6	5.7	0–10
Veterinarian	6	5.7	0–10

n = 114, * n = 105, CI, confidence interval

(vii) Risk factors associated with the prevalence of zoonotic pathogenic bacteria infections on cage-cultured (*Oreochromis niloticus*) farms

Multivariate analysis of cage size, fish species, predator control, and disposal practices associated with zoonotic pathogen prevalence in Nile tilapia cage culture

The multivariate analysis reveals several factors associated with the prevalence of zoonotic pathogenic diseases on cage-cultured Nile tilapia farms, including cage size, fish species, techniques for disposing of dead fish, and strategies for managing predatory birds. Specifically, cages smaller than 300 m^2 were significant ($p = 0.013$), with a 39% increase in the number of farmers in this category for every unit increase in the cumulative death rate. Farmers raising Nile tilapia experienced a 30% rise in the cumulative death rate for every unit increase ($p = 0.031$). The practice of burying deceased fish resulted in a 46% reduction in the cumulative death rate for every unit increase ($p = 0.023$). Preventing predatory bird attacks by using scarecrows ($p = 0.016$) and physically chasing birds away ($p = 0.003$) was associated with increases in the cumulative death rate by 34% and 45%, respectively (Table 18).

Table 19: Multivariate Analysis of Factors Associated with Zoonotic Disease Prevalence in Nile Tilapia Cage Farms

Variable	Beta Coefficient	Odds Ratio	Standard Error	p-value	R²
Cage Size (m²)					
<300	0.33	1.39	1.45	0.013	0.09
301 to 500	-0.01	0.99	2.59	0.961	
501 to 600	0.24	1.27	1.29	0.057	
>600	-0.09	0.91	2.29	0.46	
Species					
Nile tilapia	0.26	1.30	1.31	0.031	0.04
Three spot tilapias	0.06	1.06	1.56	0.639	
Greenhead tilapia	0.04	1.04	2.97	0.761	
Dead Fish Disposal Methods					
Bury the fish	-0.62	0.54	2.92	0.023	0.14
Dispose of the fish in uncovered pits	-0.31	0.73	3.38	0.197	
Leave the dead fish in the water	-0.45	0.64	3.09	0.099	
Incinerate the fish	-0.18	0.84	3.25	0.358	
Control of Predator Birds					
Use scarecrows	0.29	1.34	1.32	0.016	0.25
Use bird nets	-0.16	0.85	1.71	0.202	
Chase them away	0.37	1.45	1.35	0.003	
Use no method	0.11	1.12	2.17	0.375	
Use fireworks	-0.14	0.87	2.17	0.249	

Multivariate analysis of feeding practices, fingerling sources, and stocking density in relation to the duration of high-mortality disease episodes in Nile tilapia cage farming

Factors independently associated with the duration of mortality episodes included the feed, the source of fingerlings, and stocking density. Feeding compositions like Carbohydrate + Fat sources ($p = 0.031$), Carbohydrate + Protein + Fat + Vitamin sources ($p = 0.031$), and neither ($p = 0.045$) were found to increase the duration of zoonotic bacterial infections and mortality episodes by 1.18, 0.78, and 1.11 units, respectively. Using hatcheries as the source of fingerlings ($p = 0.023$) was associated with an increase in duration by 0.26 units. Moreover, farmers who stocked fish at rates between 5 to 8 fish/m² ($p = 0.027$) experienced a duration increase of 0.24 units in mortality episodes (Table 11).

Table 20: Multivariate Analysis of Factors Associated with the Duration (Days) of High-Disease Mortality Episodes

Variable	Beta Coefficient	Odds Ratio	Standard Error	*p*-value	R²
Feeding Composition					0.12
Carbohydrate + Fat sources	1.18	3.25	3.33	0.031	
Carbohydrate + Protein + Fat + Vitamins	0.78	2.18	2.97	0.031	
Neither	1.11	3.03	3.18	0.045	
Source of Fingerling					0.06
Others	0.00	1.00	0.63	0.992	
Wild environment	0.16	1.17	0.63	0.138	
Hatcheries	0.26	1.30	0.89	0.023	
Stocking Density (Fish/m²)					0.02
<4	0.11	1.12	0.86	0.335	
4 to 8	0.24	1.27	0.62	0.027	
>8	0.09	1.09	0.68	0.426	

4.2 Discussion

Aquaculture plays a crucial role in supporting food and nutritional security in Tanzania and globally. However, the expansion of cage culture systems, particularly in natural water bodies like the Mwanza Gulf of Lake Victoria, has raised concerns about the emergence of zoonotic bacterial pathogens. These pathogens can significantly impact both fish health and public safety. This study was conducted to determine the prevalence of zoonotic bacterial pathogens in cage-cultured Nile tilapia (*Oreochromis niloticus*) and to identify factors contributing to their prevalence. The findings of this study were therefore focused on three key areas: Risk factors related to farming practices**,** water quality assessment through physico-chemical parameters, and identification of zoonotic pathogenic bacterial isolates in cage-cultured Nile tilapia (*Oreochromis niloticus*).

4.2.1 Factors contributing to zoonotic disease prevalence in cage-cultured *Nile tilapia*

Cage-cultured farming in Mwanza gulf of Lake Victoria was an activity of men as reported elsewhere in Illemela, Nyamagana and Misungwi (Ngwili *et al*., 2015), probably because most households are headed by men who controls economic activities. However, women play vital role in cage-cultured management, as they stay at home, therefore, keener in managing their cage-cultured as compared to men who are away most of the time (Shitote *et al*., 2013). Contrary to present findings, Ngwili *et al*. (2015) reported that fish farming was practiced by a large proportion of farmers below fifty years of age in Kiambu and Machakos from Kenya, unlike in this study where fish farming was undertaken by persons above 50 years. Low uptake of cage-cultured farming among the youth group (below 35 years), in this study, may be due to lack of important production factors such startup capital, technical knowledge, ownership or leasing of cages and related equipment and capital compared to farmers above 50 years who are retirees with some resources from savings and pension.

Aquaculture Farming in Tanzania was practiced mainly by people with formal education. The findings are in agreement with those of Maina *et al.* (2014) who investigated socio-economic and gender factors influencing fish farming in the Mwea Irrigation scheme (Kirinyaga County). Market demand for aquatic protein, as well as a lack of formal employment opportunities in the country, might have pushed the unemployed population to pursue aquaculture for income generation purposes.

Fish cage-cultured farmers in the Mwanza gulf of Lake Victoria indicated farming as their main source of daily bread. These findings are in agreement with those of Omasaki *et al.* (2013), who reported that in Western Kenya, the majority of fish farmers practised mixed (crop-livestock) farming for subsistence and income, a confirmation that agriculture is the main activity in rural areas. The majority of farmers (58.3%) in Mwanza Gulf of Lake Victoria had attended some training in aquaculture farming. This is contrary to a study in western Kenya by Omasaki *et al.* (2013), where only 33.5% of farmers in Kakamega had attended a course on fish farming, followed by Siaya (29.6%) and Kisii (11.8%). Training in aquaculture is critical to fish farming.

In this study, aquaculture was an old agricultural activity (More than 10 years) among the farmers, unlike observation by Maina *et al.* (2015); they reported that 90.9% had been in fish farming for less than three years. A probable explanation for this finding is due to the impacts of the Fish Farming Enterprise and Productivity program started countrywide during the 2008-09 financial year. Under this program, the government gave funds to farmers for the establishment of fish cages and also supplied other inputs such as fingerlings and feeds. Results of this study have given a good indication that farmers who were funded by the program are still practising cage-cultured fish farming; most of the fish farmers have been in the practice for many years, thus translating to more experience in fish farming. In addition, experienced fish farmers may have influenced new farmers to take up fish farming as an income-generating activity, giving an explanation for the 11% who had been practicing fish farming for less than 1 year.

Cage-cultured Farmers obtained their first fingerling stock mainly from a supplier's hatchery, as wild; this was occasioned by an economic stimulus programme when the suppliers supplied inputs such as fingerlings and feeds, as was reported by Ngwili *et al.* (2015). However, Governments and private hatcheries were preferred as sources of restocking fingerlings. Advice from other farmers, as well as individual assessment of performance/quality of first stock fingerlings from either the government or major private hatchery, may have influenced the choice of source of fingerlings for restocking.

Nile Tilapia (*Oreochromis niloticus*) were the commonly dominated reared species. The findings are in agreement with those of a previous study done by Maina et al. (2015) on farmed fish value chain analysis in Kirinyaga County. Nile Tilapia was the dominant species kept by the farmers under the monoculture system in the study area.

Cage-cultured treatments between crops aim at: Oxidising wastes; eradicating predators, pathogens, and vectors of pathogens; improving soil pH; increasing availability of planktons before restocking; removing or redistributing wastes (Suresh *et al.,* 2006). Cage removal or relocation activities, sometimes accompanied by localised sediment disturbance, were observed in some farms, and removal of wastes was found to be a common practice in cage-cultured in Mwanza gulf of Lake Victoria; however, the majority of farmers did not treat their cages before the next crop. The few who treated their cage relied on the natural dry-out method and liming.

A minority of farmers practising cage culture fertilise their fish cages with livestock manure as a source of organic nutrients. This may be attributed to the fact that many of them engage in mixed farming, raising livestock near water bodies, which makes it feasible to apply manure directly into the area surrounding the cages. In addition, animal manure is inexpensive compared to chemical fertilisers and therefore can help cut down the cost of investment, with respect to buying inorganic fertilizers.

Knowledge of fish diseases was found to be low among fish farmers in the Mwanza gulf of Lake Victoria, as only a few could tell when fish were sick. This echoes a similar finding by Faruk *et al.* (2008), who found low knowledge on basic fish health management among farmers in Bangladesh. Some farms had experienced disease incidences in the last year;

however, the exact causes of the deaths were not understood.

Several factors were identified as being associated with a higher likelihood of zoonotic disease prevalence in cage-cultured Nile tilapia systems. smaller cage sizes (<300 m²), high stocking densities, use of scarecrows, and manually chasing away predator birds contributed to increased risk. Other contributing factors included feeding fish with poorly formulated diets, sharing fishing nets and gear, and flooding of fish cages, all of which may facilitate the transmission of fish diseases.

Cage sizes smaller than 300 m² were significantly associated with a higher prevalence of zoonotic diseases in cage-cultured Nile tilapia, with an odds ratio of 1.39 ($p = 0.013$). Approximately 30.8% of the farmers surveyed reported using cages within this size range. Previous studies have shown that small cage sizes, particularly when coupled with high stocking densities, negatively impact fish health and growth. For instance, a study conducted in Lake Kuriftu, Ethiopia, demonstrated that Nile tilapia stocked at lower densities had better growth performance and survival rates compared to those at higher densities (Gizaw *et al.,* 2021). Similarly, research in Lake Albert, Uganda, reported higher body weights and survival rates in fish reared under lower stocking densities (Tumwesigye *et al.,* 2020). These findings are consistent with the present study's observation that smaller cage sizes are a risk factor for increased disease prevalence in farmed Nile tilapia.

High stocking densities were identified as a significant contributor to the increased prevalence of zoonotic diseases in cage-cultured Nile tilapia. In this study, 45.8% of farmers reported stocking densities ranging from 4–8 fish/m², a range significantly associated with prolonged disease outbreaks (odds ratio = 1.27, $p = 0.027$). Overcrowding under such conditions often leads to heightened competition for space and resources, increased stress levels, and weakened immune responses in fish. These stressors not only impair fish health but also reduce dissolved oxygen levels and deteriorate water quality, creating favorable conditions for the proliferation and transmission of pathogenic microorganisms (Ellis *et al.,* 2002; Wedemeyer, 1996).

Several studies across Africa have similarly demonstrated that lower stocking densities are linked with improved growth performance, higher survival rates and reduced disease incidence in Nile tilapia. For example, Gizaw *et al.* (2021) in Lake Kuriftu, Ethiopia, found that lower densities enhanced fish survival and growth, while Tumwesigye *et al.* (2020)

reported comparable findings in Lake Albert, Uganda. These results collectively emphasize the importance of maintaining optimal stocking densities to ensure fish welfare and minimize disease risks in cage aquaculture systems.

The use of scarecrows as a bird deterrent was significantly associated with a higher prevalence of zoonotic diseases in cage-cultured Nile tilapia. In this study, 30.8% of farmers employed scarecrows for predator control, and this method was linked to an increased risk of disease outbreaks (odds ratio = 1.34, $p = 0.016$). Although scarecrows offer a traditional and low-cost approach to managing avian predators, their effectiveness diminishes over time as birds become habituated to static decoys (Blackwell *et al.,* 2002).

Moreover, piscivorous birds, such as cormorants and herons, are not only capable of consuming juvenile and adult fish but may also serve as mechanical vectors of pathogenic bacteria, including Salmonella spp., thereby facilitating the horizontal transmission of infectious agents across cages and farms (Gibbs *et al.,* 2005; Friend *et al.,* 2001). The frequent interaction between birds and cages, when scarecrows fail, may significantly elevate the risk of pathogen introduction. This highlights the need for more robust, integrated bird management strategies, such as the installation of overhead bird netting, reflective tapes, or physical exclusion barriers, which have shown greater success in preventing avian contact and pathogen transmission in aquaculture settings (Glahn *et al.,* 2000; White *et al.,* 2008).

Manually chasing away predator birds was another practice significantly associated with an increased risk of zoonotic disease prevalence in cage-cultured Nile tilapia. Approximately 30.8% of farmers reported using this method, and it was found to increase the likelihood of disease outbreaks (odds ratio = 1.45, $p = 0.003$). While this approach may provide short-term relief, it is labor-intensive and ineffective as a long-term solution for bird predation control. Predator birds, such as herons, cormorants, and kingfishers, are known to act as mechanical vectors of fish pathogens, including Streptococcus spp., by transferring bacteria from infected environments to healthy fish stocks (Friend *et al.,* 2001; Hubálek, 2004; FAO, 2020).

Moreover, repeated human efforts to chase birds can lead to habituation, where birds gradually become less responsive to deterrent actions (Blackwell *et al.,* 2002; Cook *et al.,* 2008). This behavioral adaptation undermines the effectiveness of manual deterrence, allowing predator birds to continue interacting with fish cages and increasing the risk of disease spread. Research in aquaculture regions in Southeast Asia and Africa has confirmed the growing challenge of bird-borne diseases in cage systems (Ali *et al.,* 2016; Omasaki *et al.,* 2022). Studies have shown that more permanent and passive deterrent measures, such as bird netting, floating cover structures, motion-activated scare devices, or integrated pest management, are significantly more effective in reducing bird presence and minimising pathogen transmission in aquaculture systems (Glahn *et al.,* 2000; White *et al.,* 2008; Koelle *et al.,* 2020; Uddin *et al.,* 2021).

Feeding fish with poorly formulated diets was identified as a significant risk factor for increased zoonotic disease prevalence in cage-cultured Nile tilapia. Approximately 14.2% of farmers reported using diets lacking essential nutrients, such as proteins, fats, carbohydrates, and vitamins, which was significantly associated with a higher likelihood of disease outbreaks (odds ratio = 3.03, $p = 0.045$). Nutritionally imbalanced diets can compromise the immune function of fish, increasing their vulnerability to infections and disease (Kiron, 2012; Dawood & Koshio, 2016). Inadequate nutrition not only weakens immune responses but also results in poor growth, low survival rates, and higher susceptibility to stress and pathogen exposure in confined cage systems (El-Sayed, 2006; Abdel-Tawwab *et al.,* 2020). Previous research highlights that a balanced diet enriched with essential nutrients is crucial for maintaining fish health and enhancing resistance to diseases. For example, dietary supplementation with immune stimulants, vitamins (like C and E), and essential fatty acids has been shown to improve disease resistance in Nile tilapia (Ngugi *et al.,* 2007; Harikrishnan *et al.,* 2011).

Sharing fishing nets and other gear was identified as a significant factor contributing to the prevalence of zoonotic diseases in cage-cultured Nile tilapia. Although specific percentages for this practice were not provided, the analysis revealed an odds ratio of 3.03 ($p = 0.045$), indicating a higher risk of disease outbreaks associated with gear sharing. This practice promotes the transfer of pathogens—including bacteria, viruses, and parasites between farms, as contaminated nets and equipment can act as fomites, facilitating cross-contamination (Murray & Peeler, 2005; Subasinghe *et al.,* 2001). Several studies have underscored the role of shared equipment in disease transmission within aquaculture systems and stressed the importance of biosecurity protocols such as disinfection and the use of dedicated tools for each production unit (Bondad-Reantaso *et al.,* 2005; Rico *et al.,* 2013). Regular sanitation of gear and the implementation of strict hygiene practices can significantly reduce the risk of spreading infectious agents and protect fish health in cage aquaculture operations.

4.2.2 Physico-chemical parameters on water quality assessment

Recently, there has been an increase in caged fish farming in different gulfs within the Lake Victoria basin. Most of these gulfs are regarded as refugia and breeding grounds for fish (Ogello *et al.,* 2018; Mwamburi *et al.,* 2020). Nevertheless, the number of cages for fish farming is increasing tremendously in Tanzania (450), Kenya (3379), and Uganda (1827), totaling 5656 cages. However, according to satellite imagery, there are about 8024 cages installed in the whole of Lake Victoria: Tanzania (46), Kenya (6169), and Uganda (1809). This study investigated variations in physical and chemical factors between cage-cultured and non-cage-cultured areas along the Mwanza Gulf of Lake Victoria and compared the current levels of nutrients to those reported in the literature before the introduction of cages in the lake. Cage fish farming is characterized by the artificial feeding of fish in order to attain the market size within a short period of time. Consequently, extra feed, fish waste, and debris that are removed from the cage nets are deposited underneath the cages and thereby causing a rise in nutrient levels in the cage farming sites.

This study observed that all the cage-cultured sites had slightly acidic conditions, potentially attributed to the decomposition of waste from the cage fish farming activities (Njiru *et al.,* 2014). Additionally, the extraction of dissolved oxygen (DO) during fish respiration and the release of carbon dioxide by zooplankton may contribute to lowering pH levels. The elevated acidity levels noted in this research could also be linked to chemical additives used for improved production, high stocking densities, or insufficient buffering capacity within the farms, as indicated by pH measurements. These findings align with similar observations made in Nigeria by Adebami *et al.* (2020).

Electrical conductivity in cage-cultured sites was also recorded as high. Electrical conductivity is an indicator of mineralization in a water sample and is the numerical expression of an aqueous solution's ability to convey electrical current, and further lists all of the dissolved ions present in the solution (Etim *et al.,* 2013). In addition to Electrical conductivity (EC), turbidity was also recorded as high in cage-cultured sites, marking the presence of suspended and dissolved organic and inorganic elements, as well as plankton and other microbes. Increased turbidity usually limits light penetration, which hinders the photosynthesis of phytoplankton and other aquatic vegetation (Nunes *et al.,* 2022). High turbidity damages fish gills, clogs filters, and lowers fish productivity (Adeogun, 2012).

The levels of phosphates were elevated in Nile tilapia raised in cage culture, indicating potential pollution at these sites. Increased phosphate levels can hinder digestion in both humans and animals. While phosphorus-phosphate is typically not harmful at low concentrations, it can become problematic at higher levels (Magrí *et al.,* 2020). Soluble organic phosphorus is released during the decomposition of organic fertilizers under aerobic conditions (Gigliotti *et al.,* 2002).

The high values of ammonia obtained from cage-cultured Nile tilapia could be due to the high rate of feeding and fish densities; hence, the excess feed decomposes and pollutes the cage farm water (Ng'wigulu, 2021). Ammonia is introduced through dead phytoplankton, uneaten feeds, and dead and decaying organic matter. It is also attributed to the addition of manure to fertilize the cages or through the process of nitrogen fixation by algae and water plants (Suresh *et al.,* 2023).

A low concentration of DO in water causes suffocation in fish, while its supersaturation may result in the gas bubble disease, leading to the mass mortality of fish in both cases. The small variation could be caused by the site's high fish density (80 fish/m^3) in comparison to the control site. This might cause the fish to engage in greater metabolic activities like respiration and excretion. The biological oxygen requirement along the cages is increased by the microbial digestion of fish waste and excrement (Adebami *et al.,* 2020). The cage structures' potential for obstructing water current flows may also result in minimal water exchange, which would explain why the cage-cultured farmed site's DO concentrations are lower.

The increase in physical-chemical parameters observed in this study, as compared to those reported by previous studies (Miruka *et al.,* 2021), is a consequence of direct input loading (feeds, fertilisers/manure, additives and therapeutics) to facilitate fast fast-growing of fish for market purposes. For instance, in the study area, daily inputs of fish feeds amount to 1750 Kg daily, equivalent to 638.75 tons per annum to produce about 300 tons of fish. Given an apparent ratio of fish to wastes of 1:4.26 (Osei *et al.,* 2019; Hasan & Halwart, 2009), 70.4 tons are deposited at the current cage fish farm per annum as feed wastage and thus pollutants. Given 158 cages in this site receiving 638.75tones of feed per annum, the

12 086 cages already installed and operational in the lake (Musinguzi *et al.,* 2019; FAO, 2020) are receiving 48 879.45 tons of feed per annum to produce nearly 3000 tons of fish and thus an estimated 704.2 tons of feed wastes pollutants loaded into the lake per annum. This is only fish feeds, let alone other inputs such to include fertilizers/manures, feed additives, and chemotherapeutics applied for various reasons.

Katunzi and Zheng (2010) and Katunzi *et al.* (2006) stipulated that the gulfs in Lake Victoria are utilised as refugia for threatened species of fish as well as breeding grounds for most of the fish in the lake. If, however, the rate of pollutant loading in these gulfs remains at the projected level (this study), the fate of most species of fish in Lake Victoria will be at stake, putting the fishery of Lake Victoria in danger of collapsing in the near future. Henceforth, the riparian governments around the lake are argued to take measures that will ensure sustainable cage fish farming without jeopardising the environmental quality of Lake Victoria, with the consequential collapse of the existing fishery.

4.2.3 Pathogenic bacterial isolates

In cage-cultured Nile tilapia (*Oreochromis niloticus*) in the Mwanza Gulf of Lake Victoria, the presence of pathogenic bacteria poses a significant threat to fish health and human safety, often resulting in severe disease outbreaks. This study aimed to assess the prevalence of zoonotic pathogenic bacteria such as *Staphylococcus, Streptococcus, Pseudomonas, Escherichia, Shigella, Salmonella, Klebsiella, Lactococcus,* and *Bacillus* among tilapia from three districts in Mwanza.

The research noted that *Escherichia spp.* isolates appeared as Gram-negative, short, plump rods arranged singly, in pairs, or short chains (Cyuzuzo *et al.,* 2023). The incidence of *Escherichia spp.* varied among the surveyed districts: Illemela had 4.3% of samples positive for *Escherichia spp.* Nyamagana had 1.7%, and Misungwi had 3.8%, (Table 8*). Escherichia spp.,* a bacterium commonly found in the intestines of warm-blooded animals, including fish, indicates contamination of water sources with faecal matter (Guillen & Wrast, 2010). Elevated levels of *Escherichia spp* in aquatic environments pose health risks to both aquatic organisms and humans (Ribeiro *et al.,* 2016). The observed prevalence of *Escherichia spp.* in this study suggests its presence in samples of cage-cultured fish from these districts, although at relatively low levels compared to some other bacterial species. Possible sources of these bacteria in the waters of the Mwanza Gulf include proximity to surrounding villages, human excreta, domestic animal faeces, agricultural runoff, wastewater discharge, storm water runoff, and sewage leaks from ageing infrastructure.

Among the bacterial isolates identified in cage-cultured Nile tilapia, *Pseudomonas* spp. was the most prevalent, a finding that is consistent with previous studies conducted in both freshwater and marine environments. Duman *et al.* (2021) and Amin *et al.* (2022) also reported *Pseudomonas* spp. as a dominant pathogen in tilapia, while Ezzat *et al.* (2014) confirmed its presence in marine fish, demonstrating its widespread occurrence in aquatic ecosystems. The elevated prevalence of *Pseudomonas* in the current study may be linked to multiple on-farm risk factors. These include small cage sizes, high stocking densities, poor feeding practices involving improperly formulated diets, and inadequate biosecurity measures such as the sharing of fishing gear and the use of ineffective predator control methods like scarecrows and manual bird chasing.

Environmental factors, such as the discharge of untreated wastewater into the lake, flooding events, and excess aquatic vegetation, further contribute to deteriorating water quality, creating ideal conditions for bacterial proliferation. As noted by Hassan *et al.* (2015) and Soliman and Yacout (2016), such stressors compromise fish immunity and increase vulnerability to opportunistic infections like *Pseudomonas*. In addition, poor water quality characterized by low dissolved oxygen, high organic load, and nutrient pollution has been shown to favour the survival and spread of *Pseudomonas* in aquaculture systems (Khalil *et al.,* 2020; Yılmaz & Ergün, 2018). This underscores the need for comprehensive disease prevention strategies, including proper cage management, improved nutrition, and effective waste and water quality management, to minimise bacterial infections and safeguard fish health.

The high prevalence of *Staphylococcus spp*. observed in Illemela (54.2%) and Misungwi (43.7%) compared to Nyamagana (26.6%) may be attributed to a combination of anthropogenic influences and aquaculture practices that compromise water quality and biosecurity. *Staphylococcus* spp., particularly *Staphylococcus* species, are opportunistic pathogens introduced through untreated sewage, livestock runoff, and direct human contact, especially in regions practicing mixed farming or located near urban wastewater outlets (Austin & Austin, 2016; Saad & Atallah, 2014; Marijani *et al.,* 2022). Poor hygiene practices, such as the sharing of nets and gear without disinfection, coupled with environmental stressors like overcrowding and small cage sizes (<300 m²), increase fish susceptibility by weakening immunity and enhancing pathogen transmission (FAO, 2021; Menon *et al.,* 2023). These conditions are exacerbated by organic waste accumulation from uneaten feed and faeces, which create nutrient-rich environments ideal for bacterial growth (Ali *et al.,* 2020).

Furthermore, unfavourable water quality parameters, such as low dissolved oxygen, high turbidity, and elevated temperature, commonly observed in poorly managed cages, support the persistence of *Staphylococcus* spp. (Karthikeyan *et al.,* 2019). Wastewater discharge and stormwater runoff transporting faecal matter and contaminants further contribute to the spread of these bacteria in cage-culture systems (Othman, 2023; Okeke *et al.,* 2020), highlighting the urgent need for enhanced water management and farm biosecurity protocols.

The examination of bacterial prevalence highlights the presence of *Klebsiella* spp. in Tanzanian aquatic environments, with the highest occurrence recorded in Nyamagana (3.3%), followed by Illemela (2.9%) and Misungwi (2.5%). Though less dominant than other bacterial pathogens, *Klebsiella* spp. are significant due to their ability to cause ulcerative lesions in farmed fish, which can lead to secondary infections and increased mortality (Dubey *et al.,* 2016). Their detection in multiple regions suggests widespread contamination, likely linked to anthropogenic pollution such as untreated wastewater, poor sanitation, and runoff from livestock and agricultural activities. Marijani (2022) reported that *Klebsiella* spp. isolated from retail fish and shrimp in Tanzania demonstrated high proteinase activity, a virulence factor that may contribute to tissue degradation and poor fish health. These findings emphasize the need for regular microbial monitoring and improved water management practices to mitigate the risk posed by such opportunistic pathogens in aquaculture systems.

The prevalence of *Bacillus* spp. and *Lactococcus* spp. in cage-cultured fish across Illemela, Nyamagana, and Misungwi districts indicates potential microbial risks influenced by both environmental and management-related factors. In Illemela, *Bacillus* and *Lactococcus* spp. were detected in 10% and 15.7% of samples, respectively; in Nyamagana, both appeared at 10% and 5%; and in Misungwi, 8.8% and 11.5%, respectively. These bacteria, though generally recognized for their roles in nutrient cycling and organic matter decomposition (Richard, 2011), may act as opportunistic pathogens under certain stressors such as poor water quality, high stocking densities, and inadequate biosecurity measures (Loka, 2023;

Austin &Austin, 2016). Factors such as elevated water temperatures, low dissolved oxygen, and increased organic load can favour the proliferation of these bacteria, compromising fish immunity and facilitating infections (Zhou *et al.,* 2020; FAO, 2022).

Additionally, the use of poorly formulated feeds and overstocking contribute to stress-induced dysbiosis, enabling opportunistic bacteria like *Bacillus* and *Lactococcus* to shift from commensals to pathogens (Defoirdt *et al.,* 2011). Wastewater discharge and sediment accumulation in cage areas also alter the microbial dynamics, creating anaerobic conditions favourable to pathogen persistence (Marijani *et al.,* 2022). These findings underscore the importance of integrating proper water quality management, responsible feeding, and routine microbial monitoring to minimize the risk of bacterial infections in cage aquaculture systems.

The prevalence of *Streptococcus* spp. among cage-cultured Nile tilapia was relatively low compared to other bacterial isolates, with 2.9% detected in Illemela, 2.5% in Misungwi, and no presence in Nyamagana. Despite this low occurrence, *Streptococcus* spp. remains a significant pathogen in aquaculture, known to cause streptococcosis: A bacterial disease associated with high mortality and economic losses in tilapia farming (Mishra *et al.,* 2018; Sherif & Abul, 2022). Its presence, even at low levels, may be influenced by suboptimal environmental and management conditions. This study identified contributing factors such as high stocking densities, low dissolved oxygen, elevated water temperature, and accumulation of organic waste and vegetation, which can stress fish and compromise their immune response, increasing susceptibility to bacterial infections.

Similar findings have been reported by Amal and Abdelrahman (2015), who linked the emergence of streptococcosis to overcrowding and water quality deterioration. Poor water quality, particularly high levels of ammonia and nitrite, can favour the growth of *Streptococcus* spp. (Austin & Austin, 2012). Additionally, Othman (2023) and Menon *et al.* (2023) highlight those environmental stressors, including stormwater runoff and waste discharge into fish farming areas, may introduce and propagate pathogenic bacteria such as *Streptococcus* spp., posing risks not only to fish but also to public health. Therefore, while the prevalence is currently low, the conditions observed in these farming systems could facilitate future outbreaks if not properly managed.

Salmonella spp. was detected in 8.1% of the total fish samples collected, with Illemela showing the highest prevalence (11.4%), followed by Misungwi (7.5%) and Nyamagana (5.0%), Appendix 5. This is concerning due to *Salmonella*'s zoonotic potential, posing significant public health risks, particularly through the consumption of contaminated fish and improper handling practices (Hamilton *et al.,* 2018; Iyer *et al.,* 2013). Several risk factors identified in this study, such as improper waste disposal, sharing of fishing gear, and cage overcrowding, likely contribute to the presence and spread of *Salmonella* in cage-cultured systems (Marijani *et al.,* 2022; Noga, 2010).

Moreover, deterioration of water quality parameters, including low dissolved oxygen levels, elevated ammonia, and increased water temperatures, creates stress-inducing conditions for fish, making them more susceptible to infections (Boyd & Tucker, 1998; FAO, 2020). Runoff from agricultural fields and discharge of domestic wastewater, especially during heavy rains, introduce pathogens and organic matter into aquatic systems, further facilitating bacterial growth (Othman, 2023; Niyonzima *et al.,* 2015). Inadequate cage management practices and a lack of biosecurity amplify these risks. Therefore, improved monitoring, stricter hygiene measures, and regulation of water quality are essential to reduce *Salmonella* contamination and ensure both fish welfare and public health (Hatha *et al.,* 2005; Austin & Austin, 2016).

The prevalence of *Shigella* spp. in cage-cultured fish samples varied across districts, with the highest occurrence in Illemela (5.7%), followed by Nyamagana (3.3%) and Misungwi (2.5%), giving an overall prevalence of 3.8% (Table 5). These findings indicate potential microbial contamination linked to environmental and management practices. *Shigella* spp. are known human pathogens capable of causing shigellosis, a waterborne gastrointestinal disease primarily transmitted through consumption of contaminated food or water (Igbinosa *et al.,* 2012; Elbashir *et al.,* 2018).

The introduction of *Shigella* into aquaculture systems can occur through poor water quality, often worsened by the use of untreated livestock manure, inadequate waste management, and runoff from nearby settlements or farms (Yeboah *et al.,* 2021; Tadesse & Alemayehu, 2020). High organic loads, especially during rainy seasons, may lead to eutrophication, promoting the survival and proliferation of enteric bacteria like *Shigella* (Okeke *et al.,* 2020). Additionally, farming practices such as overcrowded cages, sharing of fishing gear, and insufficient disinfection protocols may contribute to increased bacterial presence and transmission (Mdegela *et al.,* 2021; Njiru *et al.,* 2019). These findings stress the importance of strict hygiene, regular water quality monitoring, and biosecurity measures to safeguard public health and enhance food safety in aquaculture.

Concerning the rate of bacterial isolates recovery from various organs, our investigation demonstrated that the prevalence of total bacterial isolates was (28.6%) in the gills so it is the most predominant site for isolation of bacterial pathogens that leads to respiratory difficulties, immune suppression, tissue damage, stress and increased susceptibility to secondary infections (Table 5). Followed by the livers (16.7%), then the skin (12.9%), and finally the kidney (6.7%) This result nearly agreed with (Mahmoud *et al.,* 2016) who concluded that bacterial infections affect the hematopoietic system mainly gills, liver, kidney and skin.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study examined the prevalence of zoonotic bacterial pathogens and associated risk factors in cage-cultured Nile tilapia (*Oreochromis niloticus*) in the Mwanza Gulf of Lake Victoria, Tanzania, revealing significant levels of zoonotic bacterial isolates.

Predominant bacteria in the Mwanza Gulf included *Pseudomonas*, *Lactococcus*, *Staphylococcus*, *Salmonella*, *Bacillus*, *Shigella*, *Escherichia*, *Streptococcus* and *Klebsiella*, with the highest prevalence recorded in Illemela district. Environmental factors such as wastewater discharge, stormwater runoff, and agricultural runoff were implicated in the proliferation of these pathogens. Although these environmental sources were not directly tested in this study, their influence was inferred based on the observed bacterial profiles and is supported by findings from previous studies.

The study identified key risk factors such as inadequate biosecurity practices, cage size, high stocking density, tilapia species, and methods for controlling predatory birds. It also highlighted moderate to low biosecurity and fish health management practices among farms, including infrequent water quality monitoring, high fish stocking densities, improper disposal of dead fish, and indiscriminate disease treatment with salt, lime, or potassium permanganate. Positively, most respondents had access to fish health technical services and could identify clinical signs of disease.

Overall, this study highlights the significant environmental impacts of cage fish farming in the Mwanza Gulf, showing how physical-chemical parameters of water quality differ between cage-cultured and non-cultured areas. Changes in water quality are primarily due to wastewater discharge, excessive feeding, and high fish densities, leading to increased nutrient loading and pollution. The findings suggest that current cage aquaculture practices are detrimental to both aquatic ecosystems and the overall health of Lake Victoria.

The study underscores the need for enhanced biosecurity measures, regular monitoring, and targeted interventions to manage bacterial infections, ultimately improving fish health and boosting aquaculture productivity.

5.2 Recommendations

The findings of this study highlight the urgent need for comprehensive fish farm management strategies to reduce the risk of bacterial infections in cage-cultured Nile tilapia.

(i) Enhanced monitoring and surveillance, especially in high-prevalence areas, are vital for early detection and timely response to disease outbreaks.

(ii) It is recommended to implement organ-specific diagnostic approaches and targeted treatment protocols to improve disease management efficiency.

(iii) Improving water quality through routine assessment and management, minimizing environmental stressors such as overcrowding and poor nutrition, and reinforcing biosecurity measures are essential steps toward preventing the spread of zoonotic pathogens.

(iv) In addition, ongoing research, farmer training, and the development of strong regulatory frameworks will play a critical role in promoting best aquaculture practices.

(v) By adopting these strategies, stakeholders—including farmers, extension officers, and policymakers—can work collaboratively to reduce the impact of bacterial diseases and enhance the sustainability, productivity, and health of cage aquaculture systems in the region.

REFERENCES

Abd-El-Hack, M. E., El-Saadony, M. T., Ellakany, H. F., Elbestawy, A. R., Abaza, S. S., Geneedy, A. M., Shafi, M. E., Khafaga, A. F., Elnesr, S. S., Alagawany, M., & El-Tarabily, K. A. (2022). Inhibition of microbial pathogens in farmed fish. *Marine Pollution Bulletin*, 183, 114003.

Abdallah, E. S. H., Metwally, W. G. M., Abdel-Rahman, M. A. M., Albano, M., & Mahmoud, M. M. (2024). *Streptococcus agalactiae* infection in Nile tilapia (*Oreochromis niloticus*): A review. *Biology, 13*(11), 914. https://www.mdpi.com/2079-7737/13/11/914

Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A. E., & Shalaby, A. M. E. (2020). Growth, feed utilization, and health performance of Nile tilapia (*Oreochromis niloticus*) fed diets with different protein and energy levels. *Aquaculture Research, 51*(4), 1433–1443. https://doi.org/10.1111/are.14497

Abu-Elala, N. M., Abd-Elsalam, R. M., & Younis, N. A. (2020). Streptococcosis, lactococcosis and enterococcosis are potential threats facing cultured Nile tilapia (*Oreochromis niloticus*) production. *Aquaculture Research, 51*(10), 4183–4195.

Action, S. I. (2020). *The State of World Fisheries and Aquaculture 2020: Sustainability in Action*. Food and Agriculture Organization of the United Nations. https:// www. fao. org/ documents/ card/en/c/ca9229en

Adebami, G. E., Fasiku, S. A., Solomon, O. D., & Babalola, B. A. (2020). Physicochemical and microbial evaluations of different fish ponds' wastewaters and the antibiotic profiles of isolated bacteria. *Ethiopian Journal of Environmental Studies and Management, 13*(4), 509–521. https://doi.org/10.4314/ejesm.v13i4.11

Adedeji, O. B., Okerentugba, P. O., Innocent-Adiele, H. C., Okonko, I. O., Ojeniyi, S. O., Adejoro, S. A., & Mohamed, S. A. (2012). Benefits, public health hazards and risks associated with fish consumption. *New York Science Journal, 5*(9), 33–61. http://www.sciencepub.net/newyork

Adeogun, A. O. (2012). Impact of industrial effluent on water quality and gill pathology of *Clarias gariepinus* from Alaro Stream, Ibadan, Southwest, Nigeria. *European Journal of Scientific Research, 76*(1), 83–94.

Admasu, F., & Wakjira, M. (2021). Pathology of epizootic-infectious diseases of fishes in aquaculture. *Biomedical Journal of Scientific & Technical Research, 40*(2), 31984–31995. https://doi.org/10.26717/BJSTR.2021.40.006413

Afewerki, S., Asche, F., Misund, B., Thorvaldsen, T., & Tveteras, R. (2023). Innovation in the Norwegian aquaculture industry. *Reviews in Aquaculture, 15*(2), 759–771. https://doi.org/10.1111/raq.12755

Akhtar, N., Syakir Ishak, M. I., Bhawani, S. A., & Umar, K. (2021). Various natural and anthropogenic factors responsible for water quality degradation: A review. *Water, 13*(19), 2660. https://doi.org/10.3390/w13192660

Ali, S. E., Abdel-Rahman, A. M., Shalaby, A. M., Soliman, W. S., Mahmoud, S. A., & Omar, E. A. (2016). Assessment of bird predation in freshwater fish farms and its impact on fish mortality. *Aquaculture Reports, 3*, 19–24. https://doi.org/10.1016/j.aqrep.2016.01.003

Allison, E. H. (2011). *Aquaculture, Fisheries, Poverty and Food Security*. WorldFish Centre. https://www.worldfishcenter.org/publication/aquaculture-fisheries-poverty-and-food-security

Al-Saedi, M. S., Naimi, S., & Al-Sharify, Z. T. (2024). The environmental impact of pollutants and heavy materials on the water quality in the Tigris River. *International Journal of Aquatic Science, 15*(1), 120–134. https://doi.org/10.18280/mmep.110202

Amin, H. F., Carne, A., & Bekhit, A. E. D. A. (2022). *Fish Roe Products: Middle East Perspective*. https://shop.elsevier.com/books/fish-roe/aladin-bekhit/978-0-12-819893-3

Ampofo, J. A., & Clerk, G. C. (2010). Diversity of bacterial contaminants in tissues of fish cultured in organic waste-fertilised ponds: Health implications. *The Open Fish Science Journal, 3*(1), 142–146. https://doi.org/10.2174/1874401X01003010142

Ani, J. S. (2021). *Effects of Stocking Density on Growth, Water Quality and Economic Performance of Monosex Nile tilapia (Oreochromis niloticus) Reared in an Aquaponics System* (Master's thesis, University of Eldoret). University of Eldoret Repository. https://erepository.uoeld.ac.ke/handle/123456789/1045

Assefa, A., & Abunna, F. (2018). Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary Medicine International, 2018*, 5432497. https:// doi. org/ 10. 1155/ 2018/ 5432497

Ayuti, S. R., Pratiwi, Z. A., Admi, M., Darniati, D., Isa, M., & Herrialfian, H. (2022). Isolation and identification of gram-negative bacteria on cattle farms indicated by mastitis. *The International Journal of Tropical Veterinary and Biomedical Research, 7*(2), 40–46. https://doi.org/10.21157/ijtvbr.v7i2.30735

Banerjee, G., & Ray, A. K. (2017). Bacterial symbiosis in the fish gut and its role in health and metabolism. *Symbiosis, 72,* 1–11. https://doi.org/10.1007/s13199-016-0451-1

Barcellos, L. J. G., Nicolaiewsky, S., De Souza, S. M. G., & Lulher, F. (1999). The effects of stocking density and social interaction on acute stress response in Nile tilapia (*Oreochromis niloticus*) fingerlings. *Aquaculture Research, 30*(11–12), 887–892. https://doi.org/10.1046/j.1365-2109.1999.00410.x

Basak, S., & Das, S. (2022). *Pseudomonas Aeruginosa, the Superbug*. Cambridge Scholars Publishing. https://scholar.google.com/

Bassetti, M., Peghin, M., Trecarichi, E. M., Carnelutti, A., Righi, E., Del Giacomo, P., & Tumbarello, M. (2017). Characteristics of *Staphylococcus aureus* bacteraemia and predictors of early and late mortality. *PLoS One, 12*(2), e0170236.

Béné, C., Arthur, R., Norbury, H., Allison, E. H., Beveridge, M., Bush, S., & Williams, M. (2016). Contribution of fisheries and aquaculture to food security and poverty reduction: Assessing the current evidence. *World Development, 79*, 177–196.

Berg, H., Mulokozi, D., & Udikas, L. (2021). A GIS assessment of the suitability of tilapia and *Clarias* pond farming in Tanzania. *ISPRS International Journal of Geo-Information, 10*(5), 354. https://doi.org/10.3390/ijgi10050354

Bjørndal, T., & Tusvik, A. (2020). *Economic Analysis of the Contributions of Capture Fisheries and Aquaculture to Future Food Security*. Centre for Applied Research at NHH. https://scholar.google.com

Blackwell, B. F., Seamans, T. W., Schulz, J. H., & Dolbeer, R. A. (2002). Habituation of birds to deterrent stimuli: Implications for aviation safety and aquaculture. *Wildlife Society Bulletin*, 30(2), 366–375.

Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., & Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinary Parasitology, 132*(3–4), 249–272.

Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2017). Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology, 31*(1), 216–226.

Burgos-Valverde, F. A. (2018). *Effect of Aquaculture Practices on Fish Microbial Communities (Master's thesis)*. Auburn University. https:// etd. auburn. edu/handle/10415/6345

Cai, J., Quagrainie, K., & Hishamunda, N. (2017). *Social and Economic Performance of Tilapia Farming in Africa*. FAO Fisheries and Aquaculture Circular No. 1130. Food and Agriculture Organization of the United Nations. https://scholar.google.com/

Cascarano, M. C., Stavrakidis-Zachou, O., Mladineo, I., Thompson, K. D., Papandroulakis, N., & Katharios, P. (2021). Mediterranean aquaculture in a changing climate: Temperature effects on pathogens and diseases of three farmed fish species. *Pathogens, 10*(9), 1205. https://doi.org/10.3390/pathogens10091205

Chatreman, N., Seecharran, D., & Ansari, A. A. (2020). Prevalence and distribution of pathogenic bacteria found in fish and fishery products: A review. *Journal of Fisheries and Life Sciences, 5*(2), 53–65.

Christopher, K., & Bruno, E. (2003). *Identification of Bacterial Species. In Tested Studies for Laboratory Teaching (Vol. 24, pp. 103–130). Association for Biology Laboratory Education.* https://scholar.google.com

Craig, S. R., Helfrich, L. A., Kuhn, D., & Schwarz, M. H. (2017). *Understanding Fish Nutrition, Feeds, and Feeding*. Virginia Cooperative Extension. https:// scholar. google. com

Cunningham, F. L., Jack, S. W., Hardin, D., & Wills, R. W. (2012). Pond-level risk factors associated with columnaris disease on Mississippi commercial catfish farms. *Journal of Aquatic Animal Health, 24*(3), 178–184.

Cyuzuzo, E., Amosun, E. A., Byukusenge, M., & Musanayire, V. (2023). Antimicrobial resistance profiling of *Escherichia coli* isolated from chickens in Northern Province of Rwanda. *African Journal of Biomedical Research, 26*(1), 151–154.

Dawood, M. A. O., & Koshio, S. (2016). Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture, 454*, 243–251.

De Oliveira, C. A. F., Da Cruz, A. G., Tavolaro, P., & Corassin, C. H. (2016). Food safety: Good manufacturing practices (GMP), sanitation standard operating procedures (SSOP), hazard analysis, and critical control point (HACCP). In J. Barros-Velázquez (Ed.), *Antimicrobial Food Packaging* (pp. 129–139). Academic Press. https:// scholar. google. com

Dickson, O. W. K. (2016). *Characterization of Salmonella and Other Gram-Negative Bacterial Pathogens Obtained from Stool and Blood at Cape Coast Teaching Hospital (Master's Thesis).* Kwame Nkrumah University of Science and Technology. https://ir.knust.edu.gh/handle/123456789/9187

Domitila, K. M. (2019). *Profiles, Diversity and Antibiotic Response Patterns of Bacterial Isolates from Fish and Processed Fish Products Retailed in Kirinyaga County, Kenya* (Master's thesis). Kenyatta University. https://irlibrary.ku.ac.ke

Donbæk, C., Mørk, A., & Ravlo, I. (2019). *Early Mortality in Tilapia Fingerlings on Lake Kariba in Zambia*. Norwegian University of Life Sciences. https://scholar.google.com/

Dubey, S., Saini, P., & Singh, P. (2016). *Prevalence and Antibiotic Susceptibility of Enterobacter.* https://scholar.google.com

Food and Agriculture Organization of the United Nations. 2021). *Nile tilapia*. https://scholar.google.com

Friend, M., & Franson, J. C. (1999). *Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds* (Information and Technology Report 1999–0001). U.S. Geological Survey. https://pubs.usgs.gov/publication/itr19990001

Funge-Smith, S., & Bennett, A. (2019). A fresh look at inland fisheries and their role in food security and livelihoods. *Fish and Fisheries, 20*(6), 1176–1195.

Gauthier, D. T. (2015). Bacterial zoonoses of fishes: A review and appraisal of evidence for linkages between fish and human infections. *The Veterinary Journal, 203*(1), 27–35. https://doi.org/10.1016/j.tvjl.2014.10.028

Geletu, T. T., & Zhao, J. (2023). Genetic resources of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in its native range and aquaculture. *Hydrobiologia, 850*(10), 2425–2445. https://doi.org/10.1007/s10750-022-04989-4

Gephart, J. A., Golden, C. D., Asche, F., Belton, B., Brugère, C., Froehlich, H. E., & Allison, E. H. (2020). Scenarios for global aquaculture and its role in human nutrition. *Reviews in Fisheries Science & Aquaculture, 29*(1), 122–138.

Gigliotti, G., Kaiser, K., Guggenberger, G., & Haumaier, L. (2002). Differences in the chemical composition of dissolved organic matter from waste material of different sources. *Biology and Fertility of Soils, 36*(5), 321–329. https://doi.org/10.1007/s00374-002-0551-8

Glahn, J. F., Dorr, B. S., Tobin, M. E., Harrel, J. B., & Preusser, K. J. (2000). Evaluation of low profile barriers to exclude cormorants from catfish ponds. *North American Journal of Aquaculture*, 62(3), 300–307.

Grafton, R. Q., Daugbjerg, C., & Qureshi, M. E. (2015). Towards food security by 2050. *Food Security, 7*, 179–183. https://doi.org/10.1007/s12571-015-0445-x

Haddock, N. L. (2023). *Bacteriophage as Diagnostic and Pathogenic Factors in Human Disease (Doctoral Dissertation, Stanford University*). https://scholar.google.com

Haenen, O. L., Dong, H. T., Hoai, T. D., Crumlish, M., Karunasagar, I., Barkham, T., & Bondad-Reantaso, M. G. (2023). Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. *Reviews in Aquaculture*, 15(1), 154–185.

Hall, C., Dawson, T. P., Macdiarmid, J. I., Matthews, R. B., & Smith, P. (2017). The impact of population growth and climate change on food security in Africa: Looking ahead to 2050. *International Journal of Agricultural Sustainability, 15*(2), 124–135.

Hamilton, K. A., Chen, A., Johnson, E. D. G., Gitter, A., Kozak, S., Niquice, C., Birkner, N., & Gurian, P. L. (2018). Salmonella risks due to consumption of aquaculture produced shrimp. *Microbial Risk Analysis, 9*, 22–32.

Harikrishnan, R., Balasundaram, C., & Heo, M. S. (2011). Diet enriched with vitamin C and E enhances the immune response in Nile tilapia against *Aeromonas hydrophila* infection. *Fish and Shellfish Immunology, 31*(3), 430–438.

Hasan, M., Faruk, M., Anka, I., & Azad, M. (2014). Investigation on fish health and diseases in rural pond aquaculture in three districts of Bangladesh. *Journal of Bangladesh Agricultural University, 11*, 377–384. https://doi.org/10.3329/jbau.v11i2.19936

Heckman, T. I. (2021). *Understanding and Controlling the Widespread Piscine Pathogen Streptococcus iniae* (*Doctoral dissertation, University of California, Davis).* https://www.proquest.com/openview

Henriksson, P. J. G., Troell, M., Banks, L. K., Belton, B., Beveridge, M. C. M., Klinger, D. H., Pelletier, N., Phillips, M., & Tran, N. (2021). Interventions for improving the productivity and environmental performance of global aquaculture for future food security. *One Earth*, 4(9), 1220–1232. https://doi.org/10.1016/j.oneear.2021.08.009

Hossain, M. F., Hasan, S. Z., Zaoti, Z. F., Hasan, M. F., Acharjee, U. K., Islam, M. A., & Sikdar, B. (2017). Isolation and characterization of *Pseudomonas syringae* pv. *lachrymans* from angular leaf spot disease of cucumber (*Cucumis sativus* L.) and evaluation of its antibiotic sensitivity. *Journal of Pharmacognosy and Phytochemistry, 6*(6), 233–238.

Hossain, M. M. M., Ehsan, A., Rahman, M. A., Haq, M., & Chowdhury, M. B. R. (2014). Transmission and pathology of *Streptococcus iniae* in monosex Nile tilapia (*Oreochromis niloticus*) in aquaculture of Bangladesh. *Journal of Fisheries, 2*(1), 90–99.

Hubálek, Z. (2004). An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of Wildlife Diseases, 40*(4), 639–659.

Huicab-Pech, Z. G., Landeros-Sánchez, C., Castañeda-Chávez, M. R., Lango-Reynoso, F., López-Collado, C. J., & Platas Rosado, D. E. (2016). Current state of bacteria pathogenicity and their relationship with host and environment in tilapia (*Oreochromis niloticus*). https://scholar.google.com/

Hulme, P. E. (2020). One Biosecurity: A unified concept to integrate human, animal, plant and environmental health. *Emerging Topics in Life Sciences, 4*(5), 539–549.

Ibengwe, L., & Sobo, F. (2016). *The Value of Tanzania Fisheries and Aquaculture: Assessment of the Contribution of the Sector to Gross Domestic Product. In Freshwater, Fish and the Future: Proceedings of the Global Cross-Sectoral Conference* (pp. 131–145). Food and Agriculture Organization of the United Nations. https://scholar.google.com

Irshath, A. A., Rajan, A. P., Vimal, S., Prabhakaran, V. S., & Ganesan, R. (2023). Bacterial pathogenesis in various fish diseases: Recent advances and specific challenges in vaccine development. *Vaccines, 11*(2), 470.

Islam, M. J., Mahbub-E-Elahi, A. T. M., Ahmed, T., & Hasan, M. K. (2016). Isolation and identification of *Salmonella* spp. from broiler and their antibiogram study in Sylhet, Bangladesh. *Journal of Applied Biology and Biotechnology, 4*(3), 46–51.

Ismail, N. I. A., Amal, M. N. A., Shohaimi, S., Saad, M. Z., & Abdullah, S. Z. (2016). Associations of water quality and bacteria presence in cage-cultured red hybrid tilapia, *Oreochromis niloticus* and *O. mossambicus*. *Aquaculture Reports,* 4, 57–65.

Iwashita, M. K. P., Addo, S., & Terhune, J. S. (2022). *Use of Pre- and Probiotics in Finfish Aquaculture. In Feed and Feeding Practices in Aquaculture (PP. 269–289).* Woodhead Publishing. https://doi.org/10.1016/B978-0-12-823190-0.00011-9

Jama, M. A., Mohammed, O. H., Guled, A. A., Khaled, M., & Hossain, M. R. A. (2020). Isolation and identification of pathogenic bacteria in pond water and fish bodies and evaluation of their antibiotic susceptibility. *Journal of Fisheries and Aquaculture Research, 5*(1), 54–60.

Jennings, S., Stentiford, G. D., Leocadio, A. M., Jeffery, K. R., Metcalfe, J. D., Katsiadaki, I., & Verner-Jeffreys, D. W. (2016). Aquatic food security: Insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment. *Fish and Fisheries, 17*(4), 893–938. https://doi.org/10.1111/faf.12152

Kabuusu, R. M., Aire, A. T., Stroup, D. F., Macpherson, C. N. L., & Ferguson, H. W. (2018). Production-level risk factors for syncytial hepatitis in farmed tilapia (*Oreochromis niloticus* L.). *Journal of Fish Diseases, 41*, 61–66. https://doi.org/10.1111/jfd.12782

Karim, M. R., Giasuddin, M., Samad, M. A., Mahmud, M. S., Islam, M. R., Rahman, M. H., & Yousuf, M. A. (2017). Prevalence of *Salmonella* spp. in poultry and poultry products in Dhaka, Bangladesh. *International Journal of Animal Biology*, *3*(4), 18–22.

Katunzi, E. F. B., Van Densen, W. L. T., Wanik, J. H., & Witte, F. (2006). Spatial and seasonal patterns in the feeding habits of juvenile *Lates niloticus* (L.) in the Mwanza Gulf of Lake Victoria. *Hydrobiologia*, *568*(1), 121–133.

Katunzi, T. M., & Zheng, Q. (2010). Tanzanian SMEs' perceptions towards the adoption of supply chain management (SCM) strategy. *International Journal of Business and Management*, *5*(10), 42–50. https://doi.org/10.5539/ijbm.v5n10p42

Kavitha, M., Raja, M., & Perumal, P. (2018). Evaluation of the probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). *Aquaculture Reports*, *11*, 59–69.

Kawser, A. R., Islam, T., Alam, M. S., Rahman, M. M., & Salam, M. A. (2022). Mechanisms of the beneficial effects of probiotic *Bacillus* spp. in aquaculture. In S. Ghosh, D. Sen, & S. Das (Eds.), *Bacilli in Agrobiotechnology: Plant Stress*

Tolerance, Bioremediation and Bioprospecting (pp. 453–486). Springer International Publishing. https:// scholar. google. com

Kiron, V. (2012). Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology*, *173*(1–2), 111–133.

Koelle, K., Rodger, H., Munro, L., & Baily, J. (2020). Integrated management of disease risks in cage aquaculture systems. *Aquaculture International,* 28(2), 321–336.

Kumari, K. M. (2017). Role of probiotics as bio-controlling agents in aquaculture: A review. *International Journal of Fisheries and Aquatic Studies*, *6*(8), 17–21.

Fish Cage Farm. (2023). Fish cage farming practices in Ghana. *Ghana Journal of Science*, 60(1), 1–16.

Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., & Steinberg, C. E. (2020). Sustainable aquaculture requires environmentally friendly treatment strategies for fish diseases. *Reviews in Aquaculture*, *12*(2), 943–965. https://doi.org/10.1111/raq.12359

Little, D. C., Newton, R. W., & Beveridge, M. C. M. (2016). Aquaculture: A rapidly growing and significant source of sustainable food? Status, transitions, and potential. *Proceedings of the Nutrition Society*, *75*(3), 274–286.

Little, D. C., Young, J. A., Zhang, W., Newton, R. W., Al Mamun, A., & Murray, F. J. (2018). Sustainable intensification of aquaculture value chains between Asia and Europe: A framework for understanding impacts and challenges. *Aquaculture*, *493*, 338–354. https://doi.org/10.1016/j.aquaculture.2017.12.033

Logan, N. A., & De Vos, P. (2015). *Bacillus*. In *Bergey's Manual of Systematics of Archaea and Bacteria (pp. 1–163)*. Wiley. https://doi.org/10.1002/9781118960608.gbm00905

Loka, J. (2023). *Environment Management and Probiotics in Mariculture*. https:// scholar. google. com

Magrí, A., Carreras-Sempere, M., Biel, C., & Colprim, J. (2020). Recovery of phosphorus from wastewater profiting from biological nitrogen treatment: Upstream, concomitant or downstream precipitation alternatives. *Agronomy*, *10*(7), 1039.

Mahboub, H. H., & Shaheen, A. A. (2021). Mycological and histopathological identification of potential fish pathogens in Nile tilapia. *Aquaculture*, *530*, 735849.

Mahmoud, M. A., Abdelsalam, M., Mahdy, O. A., El Miniawy, H. M., Ahmed, Z. A., Osman, A. H., & Ewiss, M. Z. (2016). Infectious bacterial pathogens, parasites and pathological correlations of sewage pollution as an important threat to farmed fishes in Egypt. *Environmental Pollution, 219*, 939–948.

Maldonado-Simán, E., González-Ariceaga, C. C., Rodríguez-de Lara, R., & Fallas-López, M. (2018). Potential hazards and biosecurity aspects associated on food safety. In *Food Control and Biosecurity* (pp. 25–61). Academic Press. https://doi.org/10.1016/B978-0-12-814264-6.00002-7

Mapfumo, B. (2022). *Regional Review on Status and Trends in Aquaculture Development in Sub-Saharan Africa – 2020*. FAO Fisheries and Aquaculture Circular No. C1237. https://www.fao.org/documents/card/en/c/cc0611en

Marijani, E. (2022). Prevalence and antimicrobial resistance of bacteria isolated from marine and freshwater fish in Tanzania. *International Journal of Microbiology*, *2022*, 4652326. https://doi.org/10.1155/2022/4652326

Mawundu, S. (2024). *Ecological Carrying Capacity and Growth Performance of Nile tilapia (Oreochromis niloticus) in Cage Aquaculture within Kadimo Bay, Lake Victoria, Kenya* (Doctoral dissertation, University of Eldoret). https://scholar.google.com

Mdegela, R. H., Omary, A. N., Mathew, C., & Nonga, H. E. (2011). Effect of pond management on prevalence of intestinal parasites in Nile tilapia (*Oreochromis niloticus*) under small-scale fish farming systems in Morogoro, Tanzania. *Livestock Research for Rural Development*, *23*(6), 127.

Menon, S. V., Kumar, A., Middha, S. K., Paital, B., Mathur, S., Johnson, R., & Asthana, M. (2023). Water physicochemical factors and oxidative stress physiology in fish: A review. *Frontiers in Environmental Science*, 11, 1240813.

Miruka, J. B., Getabu, A., Sitoki, L., James, O., Mwamburi, J., George, O., & Odoli, C. (2021). Water quality, phytoplankton composition and microcystin concentrations in

Kisumu Bay (Kenya) of Lake Victoria after a prolonged water hyacinth infestation period. *Lakes & Reservoirs: Research & Management,* 26(4), e12380.

Mishra, A., Nam, G. H., Gim, J. A., Lee, H. E., Jo, A., & Kim, H. S. (2018). Current challenges of Streptococcus infection and effective molecular, cellular, and environmental control methods in aquaculture. *Molecules and Cells*, 41(6), 495–505.

Mjoun, K., Rosentrater, K., & Brown, M. L. (2010). *Tilapia: Environmental Biology and Nutritional Requirements (P. 8)*. South Dakota State University. https:// scholar. google. com

Mosha, S. S., & Daudi, J. M. (2020). A review of aquaculture production in Tanzania: Recent status, challenges and opportunities, and its impact in poverty alleviation. *Journal of Agriculture and Veterinary*, 3(8), 107–115.

Mousum, T. (2021). *Isolation and Identification of Escherichia coli and Salmonella from Drinking Water in and Around the SAU campus (Doctoral Dissertation, Sher-e-Bangla Agricultural University, Dhaka*). https://www.researchgate.net

Müller, W., Bocklisch, H., Schüler, G., Hotzel, H., Neubauer, H., & Otto, P. (2007). Detection of Francisella tularensis subsp. holarctica in a European brown hare (Lepus europaeus) in Thuringia, Germany. *Veterinary Microbiology*, 123(1–3), 225–229.

Mulokozi, D. P., Mmanda, F. P., Onyango, P., Lundh, T., Tamatamah, R., & Berg, H. (2020). Rural aquaculture: Assessment of its contribution to household income and farmers' perception in selected districts, Tanzania. *Aquaculture Economics & Management*, 24(4), 387–405. https://doi.org/10.1080/13657305.2020.1753731

Muringai, R. T., Mafongoya, P., Lottering, R. T., Mugandani, R., & Naidoo, D. (2021). Unlocking the potential of fish to improve food and nutrition security in sub-Saharan Africa. *Sustainability,* 14(1), 318. https://doi.org/10.3390/su14010318

Murray, A. G., & Peeler, E. J. (2005). A framework for understanding the potential for emerging diseases in aquaculture. *Preventive Veterinary Medicine*, 67(2–3), 223–235. https://doi.org/10.1016/j.prevetmed.2004.10.012

Musinguzi, L., Lugya, J., Rwezawula, P., Kamya, A., Nuwahereza, C., Halafo, J., & Ogutu-Ohwayo, R. (2019). The extent of cage aquaculture, adherence to best practices, and reflections for sustainable aquaculture on African inland waters. *Journal of Great Lakes Research*, 45(6), 1340–1347.

Mwainge, V. M., Ogwai, C., Aura, C. M., Mutie, A., Ombwa, V., Nyaboke, H., & Nyaundi, J. (2021). An overview of fish disease and parasite occurrence in the cage culture of Oreochromis niloticus: A case study in Lake Victoria, Kenya. *Aquatic Ecosystem Health & Management*, 24(1), 43–55. https://doi.org/10.14321/aehm.024.01.05

Mwamburi, J., Basweti, G., Owili, M., Babu, J., & Wawiye, P. (2020). Spatio-temporal trends of nutrients and physicochemical parameters on lake ecosystem and fisheries before the onset of cage farming and re-opening of the Mbita passage in the Nyanza Gulf of Lake Victoria. *Lakes and Reservoirs: Science, Policy and Management for Sustainable Use*, 25(3), 292–313. https://doi.org/10.1111/lre.12329

Mzula, A., Wambura, P. N., Mdegela, R. H., & Shirima, G. M. (2021). Present status of aquaculture and the challenge of bacterial diseases in freshwater farmed fish in Tanzania: A call for sustainable strategies. *Aquaculture and Fisheries*, 6(3), 247–253. https://doi.org/10.1016/j.aaf.2020.06.002

Naing, L., Winn, T. B. N. R., & Rusli, B. N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences*, 1, 9–14. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=7b6f8ebbe6dc68c7fba40cfb1673be4db5eec85e

Namonje-Kapembwa, T., & Samboko, P. (2017). *Assessing the Profitability of Small-Scale aquaculture Fish Production in Zambia. Indaba Agricultural Policy Research Institute (IAPRI).* https://pdf.usaid.gov

Nantongo, M., Mkupasi, E. M., Byarugaba, D. K., Wamala, S. P., Mdegela, R. H., & Walakira, J. K. (2019). Molecular characterization and antibiotic susceptibility of Edwardsiella tarda isolated from farmed Nile tilapia and African catfish from Wakiso, Uganda. Uganda *Journal of Agricultural Sciences,* 19(1), 51–64. https://doi.org/10.4314/ujas.v19i1.5

Nawar, S., Rashid, M. T., Ahmed, A., Hossain, M. M., & Afzal, A. B. (2021). A study of prevalence and pathogenic activity of bacteria in the air of Dhaka City and their antimicrobial resistance pattern. *American Journal of Molecular Biology,* 11(2), 51–62. https://doi.org/10.4236/ajmb.2021.112004

Nayak, S. K. (2021). Multifaceted applications of probiotic Bacillus species in aquaculture with special reference to Bacillus subtilis. *Reviews in Aquaculture,* 13(2), 862–906. https://doi.org/10.1111/raq.12501

Naylor, R., Fang, S., & Fanzo, J. (2023). A global view of aquaculture policy. *Food Policy*, 116, 102422. https://doi.org/10.1016/j.foodpol.2023.102422

Ndashe, K., Hang'ombe, B. M., Changula, K., Yabe, J., Samutela, M. T., Songe, M. M., Mubita, C., Phiri, M., Mataa, 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), 49. https://doi.org/10.3390/fishes8010049

Ng'wigulu, L. K. (2021). *Cage fish farming in Lake Victoria, Tanzania: A recently rising food production area and its effects on the environment and community (Master's thesis, World Maritime University)*. World Maritime University Dissertations. https://commons.wmu.se/all_dissertations/808

Niyonzima, E., Ongol, M. P., Kimonyo, A., Tumwebaze, R., & Byaruhanga, Y. B. (2021). Microbial diversity of Rwandan fermented milk products revealed by culture-dependent and culture-independent approaches. *International Dairy Journal*, 112, 104846.

Njagi, I. G. (2019). *Microbial Contamination of Nile Tilapia (Oreochromis Niloticus) And African Catfish (Clarias Gariepinus) Fingerlings Reared in Hatchery Tanks at Sagana, Jambo, and Mwea Fish Farms (Doctoral Dissertation, Kenyatta University*). https://scholar.google.com

Njiru, J., van der Knaap, M., Kundu, R., & Nyamweya, C. (2018). Lake Victoria fisheries: Outlook and management. *Lakes & Reservoirs: Research & Management*, *23*(2), 152–162. https://doi.org/10.1111/lre.12226

Njiru, M., van der Knaap, M., Taabu-Munyaho, A., Nyamweya, C. S., Kayanda, R. J., & Marshall, B. E. (2014). Management of Lake Victoria fishery: Are we looking for easy solutions? *Aquatic Ecosystem Health & Management*, *17*(1), 70–79.

Nunes, P., Roland, F., Amado, A. M., Resende, N. da S., & Cardoso, S. J. (2022). Responses of phytoplanktonic chlorophyll *a* composition to inorganic turbidity caused by mine tailings. *Frontiers in Environmental Science*, *9*, 605838.

Nyamweya, C. (2017). *Ecological modelling of Lake Victoria: Threats to Aquatic Biodiversity and Possible Management Strategies in Lake Victoria* [Doctoral Dissertation, University of Iceland]. https://opinvisindi.is/handle/20.500.11815/185

Odhiambo, E., Angienda, P. O., Okoth, P., & Onyango, D. (2020). Stocking density induced stress on plasma cortisol and whole blood glucose concentration in Nile tilapia fish (*Oreochromis niloticus*) of Lake Victoria, Kenya. *International Journal of Zoology*, *2020*, 9395268. https://doi.org/10.1155/2020/9395268

Ogello, E. O., Kembenya, E. M., & Muthoni, C. (2018). *State of Cage Culture in Lake Victoria, Kenya*. Kenya Marine and Fisheries Research Institute (KMFRI). https://scholar.google.com

Omasaki, S. K., Munguti, J., Karisa, H. C., & Charo-Karisa, H. (2022). Zoonotic bacterial risks in East African aquaculture: A review. *African Journal of Aquatic Science*, *47*(2), 115–124. https://doi.org/10.2989/16085914.2022.2043123

Opiyo, M. A., Mwainge, V. M., Musa, S., Kyule-Muendo, D., Hinzano, S., Wainaina, M., Magondu, E., Werimo, K., & Ombwa, V. (2020). Fish disease management and biosecurity systems. In J. Munguti, K. Obiero, P. Orina, D. Mirera, D. Kyule-Muendo, J. Mwaluma, M. Opiyo, S. Musa, J. Ochiewo, J. Njiru, E. Ogello, & A. Hagiwara (Eds.), *State of Aquaculture in Kenya 2020: Towards Nutrition-Sensitive Fish Food Systems* (pp. 97–126). Kenya Marine and Fisheries Research Institute (KMFRI). https://scholar.google.com

Orinda, M., Okuto, E., & Abwao, M. (2021). Cage fish culture in the Lake Victoria region: Adoption determinants, challenges and opportunities. *International Journal of Fisheries and Aquaculture*, *13*(2), 45–55. https://doi.org/10.5897/IJFA2020.0798

Osei, L. K., Asmah, R., Aikins, S., & Karikari, A. Y. (2019). Effects of fish cage culture on water and sediment quality in the gorge area of Lake Volta in Ghana: A case study. *International Journal of Fisheries and Aquatic Studies*, *7*(3), 36–42. https://doi.org/10.22271/fish.2019.v7.i3a.6928

Othman, C. O. (2023). *Bioaccumulation of Heavy Metals and their Resistance Genes in E. Coli Isolated from Carp Fish Cultured in Dukan Lake* (Doctoral Dissertation, University of Sulaimani, College of Veterinary Medicine). https://scholar.google.com/

Otieno, O. (2022). *Nile tilapia (Oreochromis niloticus Linnaeus, 1758) Culture in Kenya: Emerging Production Technologies and Socio-Economic Impacts on Local Communities*. https://scholar.google.com

Pang, J., Liu, Z. Y., Hao, M., Zhang, Y. F., & Qi, Q. S. (2017). An isolated cellulolytic *Escherichia coli* from bovine rumen produces ethanol and hydrogen from corn straw. *Biotechnology for Biofuels, 10*, 1–10. https://doi.org/10.1186/s13068-017-0926-2

Parija, S. C. (2023). *Textbook of Microbiology and Immunology* (Vol. 1579). Springer. https://link.springer.com/book/10.1007/978-981-19-3315-8

Pattabhiramaiah, M., & Mallikarjunaiah, S. (2023). *Foodborne Pathogens and Food Safety Regulations. In Food Microbial and Molecular Biology* (pp. 179–212). Apple Academic Press. https://www.taylorfrancis.com/chapters/edit/10.1201/9781003300601-9

Pessoa, R. B. G., Oliveira, W. F. D., Correia, M. T. D. S., Fontes, A., & Coelho, L. C. B. B. (2022). *Aeromonas* and human health disorders: Clinical approaches. *Frontiers in Microbiology, 13*, 868890. https://doi.org/10.3389/fmicb.2022.868890

Plumb, J. A., & Hanson, L. A. (2010). *Health Maintenance and Principal Microbial Diseases of Cultured Fishes*. John Wiley & Sons. https://books.google.co.tz/books

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., El Zowalaty, M. E., Rahman, A., Alam, M., Noreddin, A., & Ashour, H. M. (2020). Zoonotic diseases: Etiology, impact, and control. *Microorganisms, 8*(9), 1405.

Rakocy, J. E., Valerio, C., & Michael, N. (2009). *Oreochromis niloticus*. Cultured Aquatic Species Fact Sheets. FAO. https://scholar.google.com/

Rasool, U., Ahmad, A., Badroo, G. A., Mudasir, M., Fayaz, S., & Mustafa, R. (2017). Isolation and identification of *Bacillus cereus* from fish and their handlers from

Jammu, India. *International Journal of Current Microbiology and Applied Sciences, 6*, 441–447. https://d1wqtxts1xzle7.cloudfront.net/108318965

Ribeiro, L. F., Barbosa, M. M. C., de Rezende Pinto, F., Guariz, C. S. L., Maluta, R. P., Rossi, J. R., Silva, A. G. M., & Do Amaral, L. A. (2016). Shiga toxigenic and enteropathogenic Escherichia coli in water and fish from pay-to-fish ponds. *Letters in Applied Microbiology*, 62(3), 216–220. https://doi.org/10.1111/lam.12541

Richard, T. (2011). *Occurrence of Bacterial Infection in Two Commonly Cultured Fish Species on Two Fish Farms in Southern Ghana* (Doctoral Dissertation, University of Ghana, Legon). https://www.researchgate.net/profile/Richard-Takyi/publication/360747538

Rico, A., Phu, T. M., Satapornvanit, K., Min, J., Shahabuddin, A. M., Henriksson, P. J. G., Murray, F. J., Little, D. C., & Van den Brink, P. J. (2013). Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture*, 412–413, 231–243.

Robertson, I. D. (2020). Disease control, prevention and on-farm biosecurity: The role of veterinary epidemiology. *Engineering, 6*(1), 20–25.

Romano, N., & Sinha, A. K. (2020). *Husbandry of Aquatic Animals in Closed Aquaculture Systems. In Aquaculture Health Management (pp. 17–73).* Academic Press. https://www.sciencedirect.com/science/article/abs/pii/B9780128133590000026

Ismail, N. M. M., Ghoneim, E., El-Baz, F., & Hafez, M. (2023). Impacts of climate change on water quality using GIS technique in water courses at Egyptian Delta governorates. *Egyptian Journal of Aquatic Biology & Fisheries,* 27(6), 387–409.

Sagwe, Orina. (2019, June). *Kenya Marine and Fisheries Research Institute*. https://www.researchgate.net

Saraswathy, R., Muralidhar, M., Sundaray, J. K., Lalitha, N., & Kumararaja, P. (2015). Water *Quality Management in Fish Hatcheries and Grow-Out Systems. In Advances in Marine and Brackishwater Aquaculture* (pp. 217–225). https://scholar.google.com

Sarker, M. S. (2018). *Antimicrobial Resistance and Beta-Lactamase Genes Detection in E. coli, Salmonella spp. and Vibrio spp. From Food Animals in Southeast Asia* (Master's thesis, Chiang Mai University, Thailand). https://www.researchgate.net/profile/Md-Sarker-31/publication/333995536

Schenone, N. F., Vackova, L., & Cirelli, A. F. (2011*).* Fish-farming Water Quality and Environmental Concerns in Argentina: A Regional Approach. *Aquaculture International, 19*, 855–863. https://doi.org/10.1007/s10499-010-9395-y

Sherif, A. H., & AbuLeila, R. H. (2022). Prevalence of some pathogenic bacteria in caged Nile tilapia (Oreochromis niloticus) and their possible treatment. *Jordan Journal of Biological Sciences*, 15(2), 239–247. https://doi.org/10.54319/jjbs/150211

Shoko, A. P., Mgaya, Y. D., Limbu, S. M., & Hoza, R. B. (2017). *Aquaculture and Fisheries Extension. In Lake Victoria Fisheries Resources: Research and Management in Tanzania* (pp. 137–160). https://link.springer.com/chapter/10.1007/978-3-319-69656-0_7

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.

Singh, A., Amod, A., Pandey, P., Bose, P., Pingali, M. S., Shivalkar, S., Varadwaj, P. K., Sahoo, A. K., & Samanta, S. K. (2022). Bacterial biofilm infections, their resistance to antibiotic therapy and current treatment strategies. *Biomedical Materials*, 17(2), 022003.

Soler, P., Faria, M., Barata, C., García-Galea, E., Lorente, B., & Vinyoles, D. (2021). Improving water quality does not guarantee fish health: Effects of ammonia pollution on the behaviour of wild-caught pre-exposed fish. *PLOS ONE, 16*(8), e0243404. https

Uddin, M. N., Islam, M. S., Hossain, M. A., & Sultana, S. (2021). Management practices to reduce avian predation in tilapia farming in Bangladesh. *Aquaculture Reports*, 19, 100592. https://doi.org/10.1016/j.aqrep.2020.100592

Uddin, N., & Al-Harbi, A. H. (2012). Bacterial flora of polycultured common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*). *International Aquatic Research, 4*, 1–9. https://doi.org/10.1186/2008-6970-4-7

Uma, A., Subash, P., & Abraham, T. J. (2020). Importance of gut microbiota in fish: A review. *Indian Journal of Animal Health, 59*, 181–194.https://www.researchgate.net/profile/Tj-Abraham/publication/348069534

Van der Heijden, P. G. M., Shoko, A. P., Van Duijn, A. P., Rurangwa, E., & Bolman, B. (2018). *Review and analysis of small-scale aquaculture production in East Africa: Part 3. Tanzania*. Wageningen University & Research.https://doi.org/10.18174/459741

Vásquez-García, A., Silva, T. D. S., Almeida-Queiroz, S. R. D., Godoy, S. H., Fernandes, A. M., Sousa, R. L., &Franzolin, R. (2017). Species identification and antimicrobial susceptibility profile of bacteria causing subclinical mastitis in buffalo. *Pesquisa Veterinária Brasileira, 37*, 447–452. https://doi.org/10.1590/s0100-736x2017000500007

Vatsos, I. N., & Angelidis, P. (2010). Water quality and fish diseases. *Journal of the Hellenic Veterinary Medical Society, 61*(1), 40–48.

Wamala, S. P., Mugimba, K. K., Mutoloki, S., Evensen, Ø., Mdegela, R., Byarugaba, D. K., & Sørum, H. (2018). Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda. *Fisheries and Aquatic Sciences, 21*, 1–10.

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*, 6839354. https://doi.org/10.1155/2020/6839354

Wato, T., Amare, M., Bonga, E., Demand, B. B. O., & Coalition, B. B. R. (2020). The agricultural water pollution and its minimization strategies: A review. *Journal of Resources Development and Management, 64*, 10–22.

White, D. H., et al. (2008). Use of visual and sound deterrents to reduce bird predation in aquaculture. *Applied Animal Behaviour Science, 114*(3–4), 288–294.

Witte, F., & van Densen, W. L. (1995). *Fish Stocks and Fisheries of Lake Victoria: A Handbook for Field Observations*. Samara Publishing Limited. https://scholar.google.com

Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater-to-marine continuum. *Wiley Interdisciplinary Reviews: Water, 6*(5), e1373. https://doi.org/10.1002/wat2.1373

Yang, S. C., Lin, C. H., Aljuffali, I. A., & Fang, J. Y. (2017). Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. *Archives of Microbiology, 199*, 811–825. https://doi.org/10.1007/s00203-017-1355-1

Zaheen, Z., War, A. F., Ali, S., Yatoo, A. M., Ali, M. N., Ahmad, S. B., Dar, S. A., & Paray, B. A. (2022). Common bacterial infections affecting freshwater fish fauna and the impact of pollution and water quality characteristics on bacterial pathogenicity. In B. A. Paray (Ed*.), Bacterial Fish Diseases (pp. 133–154).* Academic Press.

Ziarati, M., Zorriehzahra, M. J., Hassantabar, F., Mehrabi, Z., Dhawan, M., Sharun, K., Kumar, A., & Shamsi, S. (2022). Zoonotic diseases of fish and their prevention and control. *Veterinary Quarterly*, 42(1), 95–118.

Zorriehzahra, M. J., Yazdanpanah-Goharrizi, L., Rokhbakhsh-Zamin, F., Kazemipour, N., &Kheirkhah, B. (2020). Isolation, biochemical, and molecular detection of *Aeromonas hydrophila* from cultured *Oncorhynchus mykiss*. *Iranian Journal of Fisheries Sciences, 19*(5), 2422–2436. http://jifro.ir/article-1-4637-en.html

APPENDICES

Appendix 1: Questionnaire for risk factors assessment of the prevalence of zoonotic pathogenic bacteria infection in cage-cultured Nile Tilapia (Oreochromis niloticus) at Mwanza Gulf of Lake Victoria, Tanzania

The Nelson Mandela African Institution of Science and Technology (NM-AIST)

CREATS-FNS-PROJECT
General information
Fish farmer information
Name of Respondent Date of Interview / /
Region and Village Name of farmer group/enterprise
Age: years. ☐
Sex: male…. Female ……….☐
Farm information
What size of the farm? m^2
Type of rearing system: Cages/ others
Age of the system: years;
Year operation started: ;

General Cage description and use of water
How many cages do you own? cages
Is Nile tilapia (*Oreochromis niloticus*) farming your sole source of income?
How big are the respective cages (m^2)? A: B: C:
Please describe the location of your cage. A: B: C: (1 = residential area, 2 = paddy field area, 3 = upland field area, 4 = other(s).
What are the respective cages used for? A: B: C: (1 = grow-out, 2 = nursery, 3 = other(s):)
Was the fish cage cultured and constructed for tilapia fish production? Yes…. No….
If no, what was the original purpose of the cage?
When did you build the respective cage (year)? A: B: C:
Do you get training on Nile tilapia production?
Do you dry out the cage nets before stocking? A: B: C: (1 = yes, always, 2 = yes, often, 3 = yes, sometimes, 4 = never, 5 = other(s); other(s):

Fish stock
When did you stock the respective cage? A: / B: / C: /

(Month and year)

Where do you get fingerings for restocking your cages? kg^{-1})

Source offish

How were the fish transported from the place of purchase to your farm?

Do you also stock non-fish aquatic products (e.g., shrimps, snails, etc.) in your cage? Yes ☐ No ☐ If yes, what?

Do you know the stocking density of your cage?

How long do you harvest the fish?

What harvesting gear do you use?

Are there any instances of harvested fish not getting customers?

Feed management

Which ingredients do you use to make your fish feed?

How often do you feed per day?

(1=once, 2 =twice, 3=seldom, 4 =never)

Is there a difference in feeding practices between the different ponds? Yes ☐ No ☐ If yes, which?

Manure management

Do you use manure in your cages? Yes ☐ No, ☐ if yes, which cages?

What kinds of manure do you usually use? / /

(1= cattle, 2 = pig, 3 = chicken, 4 = green manure, 5 = humus, 6 = other(s))

Please estimate the amount and frequency of manure application. A: / B: / C: / (e.g. kg/ twice a week)

In which form do you usually supply manure? (1 = fresh, 2 = dried, 3 = processed, 4 = Other(s);)

Fish diseases

Have you ever had to cope with fish diseases in their respective cage? A: B:-----C: (1 = often, 2 = sometimes, 3 = seldom, 4 = never)

Do you know anything about fish diseases? Yes------No------

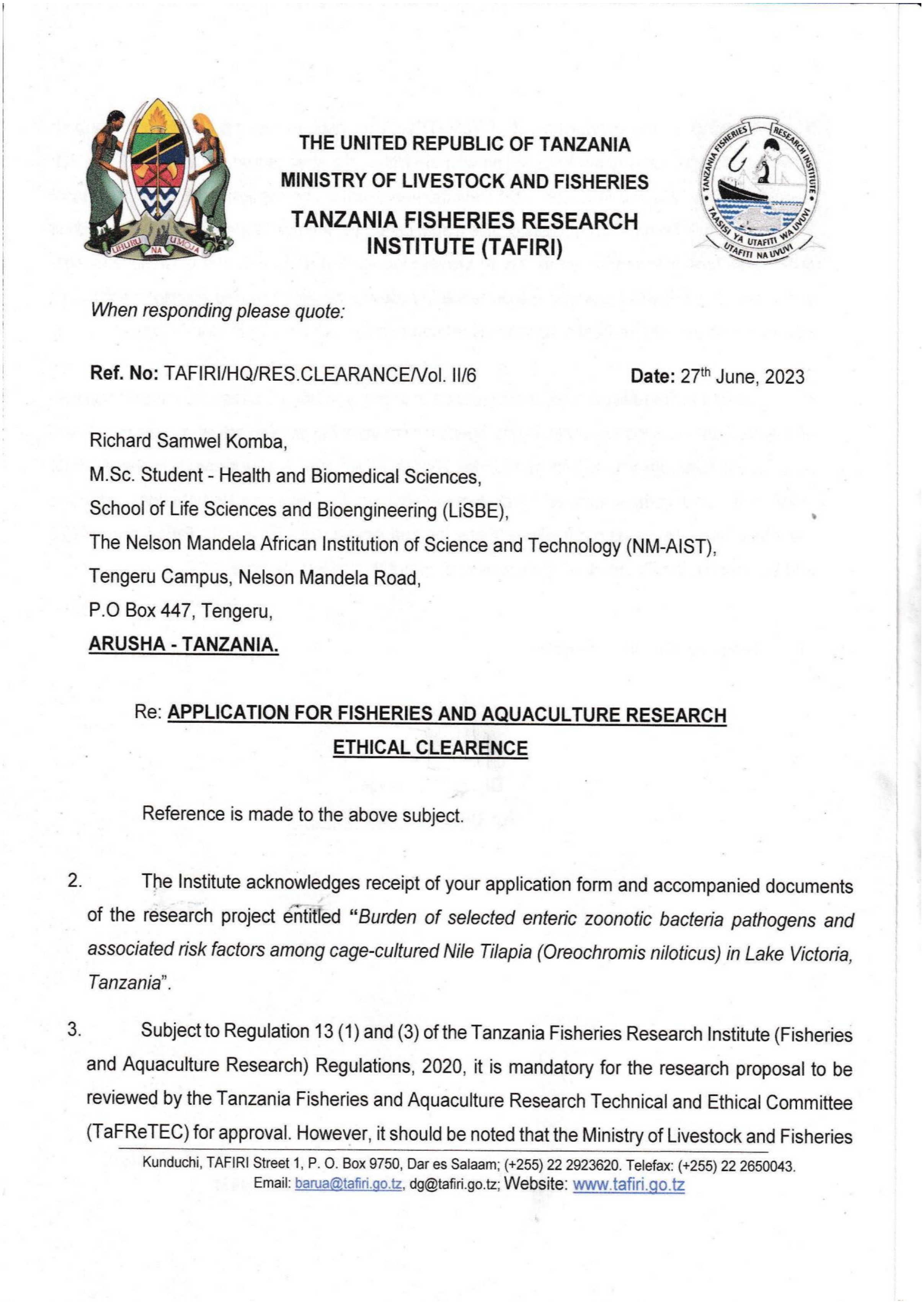
Do you know the name of the disease? Please describe the symptoms:

Since which year have you faced problems with this fish disease? Do you know the disease prevention and control?

How many fish have died? . What was the approximate weight of the dead fish (g)?

Do you practice pest control in fish cages?

Appendix 2: Ethical clearance certificate



THE UNITED REPUBLIC OF TANZANIA
MINISTRY OF LIVESTOCK AND FISHERIES
TANZANIA FISHERIES RESEARCH INSTITUTE (TAFIRI)

When responding please quote:

Ref. No: TAFIRI/HQ/RES.CLEARANCE/Vol. II/6 **Date:** 27th June, 2023

Richard Samwel Komba,
M.Sc. Student - Health and Biomedical Sciences,
School of Life Sciences and Bioengineering (LiSBE),
The Nelson Mandela African Institution of Science and Technology (NM-AIST),
Tengeru Campus, Nelson Mandela Road,
P.O Box 447, Tengeru,
ARUSHA - TANZANIA.

Re: **APPLICATION FOR FISHERIES AND AQUACULTURE RESEARCH ETHICAL CLEARENCE**

Reference is made to the above subject.

2. The Institute acknowledges receipt of your application form and accompanied documents of the research project entitled "*Burden of selected enteric zoonotic bacteria pathogens and associated risk factors among cage-cultured Nile Tilapia (Oreochromis niloticus) in Lake Victoria, Tanzania*".

3. Subject to Regulation 13 (1) and (3) of the Tanzania Fisheries Research Institute (Fisheries and Aquaculture Research) Regulations, 2020, it is mandatory for the research proposal to be reviewed by the Tanzania Fisheries and Aquaculture Research Technical and Ethical Committee (TaFReTEC) for approval. However, it should be noted that the Ministry of Livestock and Fisheries

Kunduchi, TAFIRI Street 1, P. O. Box 9750, Dar es Salaam; (+255) 22 2923620. Telefax: (+255) 22 2650043.
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is now working on the establishment of TaReTEC. The Institute has reviewed your research proposal on behalf, and found to be in line with the National Fisheries and Aquaculture Research Agenda (2021 – 2025) and Sustainable Development Goals. The implementation of this project will increase fish farmers' awareness of Nile tilapia diseases, the risk factors associated with their occurrence, and intervention measures to prevent the spread and treatment of these diseases. The research work will moreover, will enhance knowledge on handling and management of fish diseases and overall fish health specifically related to cage fish farming in Lake Victoria.

4. Subject to Regulation 13(5), I am pleased to inform you that your research project "*Burden of selected enteric zoonotic bacteria pathogens and associated risk factors among cage-cultured Nile Tilapia (Oreochromis niloticus) in Lake Victoria, Tanzania*" has approved. However, your study shall also comply with all other requirements of the research undertaking under the Tanzania Fisheries Research Institute (Fisheries and Aquaculture Research) Regulations, 2020 and your Institution Research Regulations throughout the project life time.

5. Thank you for your cooperation.

Dr. A. P. Mwijage

Ag. DIRECTOR GENERAL

RESEARCH OUTPUTS

(i) Publications

Komba, R. S., Kimaro, E. G., & Mwita, C. J. (2024). The Burden of Bacteria Pathogens Associated with Cage-cultured Nile Tilapia (*Oreochromis niloticus*) along the Mwanza Gulf of Lake Victoria, Tanzania. *Uttar Pradesh Journal of Zoology*, *45*(22), 174-184.

(ii) Poster presentation