OCCURRENCE, ANTIMICROBIAL SUSCEPTIBILITY AND EFFECT OF PHYSICO-CHEMICAL FACTORS ON POTENTIALLY PATHOGENIC AND ZOONOTIC BACTERIA ISOLATED FROM FARMED AND WILD *OREOCHROMIS SPECIES* IN TAITA-TAVETA COUNTY, KENYA

MERCY MATUMA HAMISI (BSc Applied Aquatic Science)

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR MASTERS OF SCIENCE IN VETERINARY PATHOLOGY, MICROBIOLOGY AND PARASITOLOGY (FISH SCIENCE OPTION)

DEPARTMENT OF VETERINARY PATHOLOGY, MICROBIOLOGY AND PARASITOLOGY

FACULTY OF VETERINARY MEDICINE
UNIVERSITY OF NAIROBI

NOVEMBER, 2024

DECLARATION

I declare that this is my original work and has not been submitted or presented for examination in any institution.

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MERCY MATUMA HAMISI, J56/41758/2022

This thesis has been submitted with our approval as the university supervisors.

1. Dr. Lucy Wanjiru Njagi (BVM, MSc, PhD)

Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi

Signature Date: 29th November 2024

2. Dr. Christine Minoo Mbindyo (BVM, MSc, PhD)

Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi

Signature Date: 29th November 2024

3. Prof. Phillip Njeru Nyaga (BVM, MSc, MPVM, PhD)

Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi

Signature Date: 29th November 2024

DECLARATION OF ORIGINALITY FORM

Name of student	Mercy Matuma Hamisi
Registration Number	J56/41758/2022
Faculty/ School/Institute	Veterinary Medicine
Department	Veterinary Pathology, Microbiology and Parasitology, University of Nairobi
Course Name	Master of Science in Veterinary Pathology, Microbiology and Parasitology **(**Fish Science option)
Title of the work	Occurrence, Antimicrobial Susceptibility and Effect of Physico-chemical Factors on Potentially Pathogenic and Zoonotic Bacteria in Farmed and Wild *Oreochromis Jipe* in Taita Taveta County, Kenya

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DEDICATION

This work is dedicated to the Almighty God, my parents; Mr Josphat Hamisi and Mrs Jackline Hamisi, my husband Mr. Lekanaya Sanin'go and my sons; Ethan Sanin'go and Leon Hamisi.

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LIST OF ABBREVIATIONS AND ACRONYMS

AHA	Aquatic Animal Health
AMR	Antimicrobial Resistance
APW	Alkaline Peptone Water
BMU	Beach Management Unit
CLSI	Clinical Laboratory Standards Institute
CO_2	Carbon Dioxide
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
GAP	Good Aquaculture Practices
GDP	Gross Domestic Product
IUCN	International Union for Conservation of Nature
KMFRI	Kenya Marine and Fisheries Research Institute
KWS	Kenya Wildlife Service
NACOSTI	National Commission of Science, Technology and Innovation
NTU	Nephelometric Turbidity Units
P	Phosphorus
pH	Potential of Hydrogen ion concentration
MALDI-TOF MS	Matrix Assisted Laser Desorption/Ionization –Time of Flight Mass Spectrometry
NORAD	Norwegian Agency for Development Corporation
NVI	Norwegian Veterinary Institute
TSIA	Triple Sugar Iron Agar

H_2S	Hydrogen Sulfide
WF	World Fish

ABSTRACT

Outbreaks of bacterial diseases in fish under intensive culture results in economic losses in aquaculture industry worldwide. In Kenya, fish diseases and antimicrobial resistance are among constraints for aquaculture sustainability. The objectives of this study were to determine physico-chemical factors, occurrence and antimicrobial susceptibility of potentially pathogenic and zoonotic bacteria in farmed and wild *Oreochromis species* in Taita-Taveta County, Kenya**.** One hundred and eleven apparently healthy fish consisting of 67 *Oreochromis jipe,* 34 *Oreochromis niloticus* and 10 hybrids; and 9 water samples were examined. Four organs from each fish were sampled, namely; skin and kidney swabs, gills and intestinal tissues. Conventional culture, biochemical and Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF) tests were done to identify bacterial isolates. The physico-chemical parameters of pond and lake-water were assessed "*in situ*" and further analyzed in the laboratory using the American Public Health Association's standard methods. Following Clinical Laboratory Standards Institute protocols, forty-five bacteria isolates were screened for resistance against six antibiotics using Kirby Bauer disc-diffusion method.

This study found that external organs of the fish had the highest prevalence of the bacterial isolates with the gills having 29.7 % (177/596) while the kidneys had the lowest with 11.8% ($p<0.05$). The source water had a lower prevalence of bacteria with 4.7%. Potential pathogenic and also zoonotic bacteria identified were *Bacillus cereus* (8%), *Aeromonas veronii* (8%), *Aeromonas hydrophilla* (5%), *Acinetobacter Johnsonii* (4%), and *Acinetobacter solii* (4%). All the bacteria were 100% (45/45) sensitive to Tetracycline and Gentamicin, 93.3%, 71.1% and 57.8% to Streptomycin, Co-trimoxazole and Ampicillin, respectively. A total of 42.2% of the bacteria showed multiple drug resistance against the antibiotics.

The physico-chemical parameters of the ponds and lake water were either within normal, above or below the recommended range. Temperatures ranged between (20.68 -25.38^{O}C) which is within the normal range of what is expected for tilapia farming. Wundanyi farm and lake Jipe were found to have the highest concentrations of dissolved oxygen, Wundanyi farms also had the highest concentration of Nitrates and Ammonia. The pH was alkaline (ranging between 8.2 and 10.9) and unsuitable for fish farming, with lowest value in Mwatate and highest in Wundanyi. Electrical conductivity was found to be highest in the lake compared to the farms. Mwatate farm had the highest concentration of phosphorous. Total suspended solids and turbidity levels were highest at Taveta farms. Sites with high Phosphorous, pH and NH_3 had high number of pathogenic and zoonotic bacterial isolates {*Aeromonas* spp (*A. hydrophila*, A. *veronii, A. hormaechei*); *Pseudomonas* (*P. fulva, P. putida, P. stutzeri*); *Bacillus cereus* and *Klebsiella pneumonia}.*

In conclusion, water, farmed and wild fish harbored potentially pathogenic and zoonotic bacteria which may cause diseases for fish and pose public health risks. In addition, some of the bacterial isolates showed high antimicrobial resistance to common antibiotics of human and animal health importance. Therefore, there is a need to educate fish farmers and other key value chain actors on proper control measures of fish bacterial infections, as well as water quality and antimicrobial resistance issues in Taita Taveta County

CHAPTER ONE: INTRODUCTION

1.1 Background information

Global fish production has been projected to be 179 million tonnes with aggregate worthy 401 billion US dollars in 2018. Of them, aquaculture accounts for 82 million tonnes worth USD $250 billion. In Africa, the main suppliers are Egypt and Nigeria at about 2.7%. Kenya was placed 4th largest producer of aquaculture in Africa, with a contribution of 0.2% in the period 2009-2013 (Obiero *et al.*, 2019) and 7th largest in 2020 with 0.9% contribution (FAO, 2024).

In the quest to improve aquaculture production in Kenya, the Government launched the Economic Stimulus Programme (ESP) which was a large-scale aquaculture support program during the period 2009-2013, aimed at supporting fish farmers with input supply, fish production, post-harvest management, and related activities (Maina, 2014). In this programme Six hundred and fifty (650) fishponds were constructed in Taita Taveta County, of these only 380 fish ponds are still actively productive while 270 fish ponds are dormant (Ministry of Fisheries and Livestock Development 2023). This is because fish farmers feel abandoned because of lack of sustainable technical support. In addition, some farmers have incurred huge losses after stocking their ponds with sub-standard fish fingerlings and the high cost of commercial fish feeds (Munguti *et al.*, 2014; Outa and Ogello, 2019) and fish diseases.

Taita-Taveta County is located on Kenya's coast. The county depends on both capture fisheries and aquaculture as the main source of fish (Ministry of Fisheries and Livestock Development 2023) . Capture fisheries are derived from lake Chala and lake Jipe. The main fish species of these two lakes include *Oreochromis hunter* of Lake Chala; and *Oreochromis niloticus, Clarias gariepinus* and *Oreochromis jipe* from Lake Jipe (Ministry of Fisheries and Livestock Development 2023) . The lake Jipe produces an average of 3-4 metric tonnes monthly of Tilapia and Clarias fish species , this is a key livelihood activity for the local communities surrounding the lake as well as serving as a source of fish for the nearby towns such as Taveta, Voi, Wundanyi and Mwatate.The industry is employing approximately 200 fishermen directly and supporting over 20,000 livelihoods indirectly in Kenya and Tanzania through fishing (Ministry of Fisheries and Livestock Development 2023). Hence, identifying the pathogens that limit aquaculture and wild fish productivity will enhance the livelihood of many communities.

In this county, the majority of fish farmers rear *Oreochromis niloticus* (90%) and *Clarias gariepinus* (10%) (Ministry of Fisheries and Livestock Development 2023). Most farmers use earthen ponds as the major units of culture, followed by liner ponds and 2% use concrete fishponds (Taita-Taveta, Fisheries Department annual report, 2021). The use of earthen ponds predisposes the fish to health challenges since they have been associated with heavy worm burdens and bacterial pathogens (Opiyo *et al.*, 2018). In 2019, Kenya Marine and Fisheries Research Institute (KMFRI) and County government of Taita-Taveta launched an initiative to restore the endangered *Oreochromis Jipe* and prevent its extinction (Shechonge *et al*., 2019). The reason for this diminished number is not clear (Mwachiro *et al.*, 2019). Could pathogen infections, especially bacterial ones be a reason? This needs to be investigated.

Oreochromis jipe is an endangered fish species that is threatened by extinction, according to (Shechonge *et al.*, 2019). This fish is mainly found in Lake Jipe and “Nyumba ya Mungu” in

Tanzania. It is on the Red List Assessment of Critically Endangered Species of the International Union for Conservation of Nature (IUCN) (Shechonge *et al.,* 2019). The introduction of *Oreochromis niloticus* into the lake was one of the reasons for its extinction which was examined against potential threat to biodiversity, actual stocks, and future potential fisheries yields (Bradbeer *et al.,* 2019). There are at least twelve (12) species of fish belonging to at least five families found in Lake Jipe. These include the endemic tilapia (*Oreochromis jipe*), *O. esculentus, Oreochromis niloticus, Clarias mozambicas* (mudfish) , *Astatotilapia bloyeti, Petersius tangensis* and *Rastineobola argentae* (sardine) *(* (Omweno *et al.,* 2023; Orina *et al.*, 2024). Unsustainable anthropogenic activities in lake Jipe ecosystem catchment have brought the species to endangered status while causing decline of some other fish species (Omweno *et al*., 2023). Despite this imminent danger, there have been no studies conducted to determine the health status of both wild and farmed fish establishments (Mwachiro *et al.*, 2019).

Environment pollution caused by globalization, intensification in fish farming and climate change and increased foreigh trade in aquatic foods have led to unsatable biosecurity in fish farms (Radhakrishnan *et al.,* 2023). According to the World Organization of Animal Health (WOAH) and Aquatic Animal Health Code, there is need for surveillance to be carried out with the following objectives (Thompson *et al.,* 2024):(1) Declaring the absence of a disease; (2) Establishing events that necessitate notification; (3) Regulating the incidence or prevalence of endemic diseases, including changes in incidence or prevalence, in order to: (i) Give information on disease control in Kenya; and (ii) Provide trading partners with relevant disease occurrence information for both qualitative and quantitative risk assessments (*National Aquatic Animal*

Health and Biosecurity Strategy – FAO project TCP/MIC/3603/C2 for The Federated States of Micronesia, 2020; World Health Organization. Regional Office for South-East Asia, 2022).

Most fish farmers are unfamiliar with proper health management practices, so they continue to suffer from continuous losses as a result of various pathogens, particularly bacteria, in their fish farms (Magouz *et al.*, 2024). Therefore, this study will provide essential information to fish farmers in Taita-Taveta County on potential bacterial pathogens likely to cause diseases in *Oreochromis* specis and possibly cross to humans, sensitize them on good fish farm husbandry to ensure sustainable aquaculture activities and production of healthy fish.

The importance of aquaculture is shifting, thus the need for more surveillance for fish diseases, not only during production, but also for its contribution to development of antimicrobial resistance (AMR) through misuse of antibiotic treatments and its zoonotic potential.This can occur after direct skin contact (when humans have injured skin) with the infected fish, culture water, or when the fish are consumed raw or when people are immunocompromised (Orabi, 2017; Haenen *et al.*, 2023) .

With an increasing demand for healthier foods consumption especially fish and fisheries products, zoonotic bacterial pathogens have become a major issue of concern worldwide. However; research on fish derived diseases are still inadequate (Ziarati *et al.*, 2022). Therefore, this study aims to find out the occurrence, antibiotic susceptibility and effect of physico-chemical parameters on potentially pathogenic and zoonotic bacteria isolated from farmed and wild *Oreochromis jipe* in Taita-Taveta County, Kenya.

1.2 Study objectives

1.2.1 General objective

To investigate effects of physico-chemical factors, occurrence and antimicrobial susceptibility profiles of potential pathogenic and zoonotic aerobic bacteria in farmed and wild *Oreochromis species* and source water in Taita-Taveta.

1.2.2 Specific objectives

1. Isolate and characterize aerobic bacteria from wild and farmed *Oreochromis jipe*, *Oreochromis niloticus*, their hydrid and source water in Taita-Taveta County, Kenya.
2. Establish the antimicrobial susceptibility profiles of the isolated bacteria in Taita-Taveta County.
3. Assess the effects of physico-chemical factors in the source pond and lake Jipe water in Taita-Taveta County.

1.3. Null Hypotheses

1. Farmed and wild *Orechromis* species and source water in Taita-Taveta County are not contaminated by bacteria and there is no antimicrobial resistance among these bacteria.

2. Wate quality does not affect bacterial load and diversity in wild and farmed *Oreochromis jipe* and *Oreochromis niloticus* in Taita-Taveta County, Kenya.

1.4 Justification

Aquaculture is considered as a promising sustainable venture in Taita Tavtea County. However, there is no baseline data or information on the occurrence of potential pathogenic and zoonotic bacteria pathogen in fish farms and lake Jipe which is a mojor source of fish brooders for most of the farmers in the County. Fish farmers also use organic manure in pond fertilization a practice that has not been verified for its responsibility in transmitting antibiotics in fish farms. Dispite many agricultural activities along the River Lumi which is the main inlet for lake Jipe, no studies have been carried out to determine the possibility of transferring antibiotic in the lakes during runoff. The water quality parameter for the Lake water and the fish farms have not been document. Threfore, this study aims to enhance biosecurity at farm management levels by identifying bacterial infections in *Oreochromis jipe* and other *Oreochromis species* of Taita-Taveta County.

Diseases in culture systems pose significant threat to economic and social development in many countries including Kenya around the world (Radhakrishnan *et al.*, 2023). A paradigm shift is required to address aquaculture biosecurity risks. When the pathogen has been identified and its host scope has been established, it is possible that it has already spread globally (including to wild populations) as a result of live animal migration with unknown health status, most commonly for aquaculture expansion (Scarfe and Palić, 2020).

In 2019, the Kenya Marine and Fisheries Research Institute (KEMRI) and the Taita-Taveta County government launched an initiative to restore *Oreochromis jipe*, which was on the verge of extinction (Shechonge *et al.*, 2019), nonetheless, it is one of the most preferred fish among the local community. The fish is mostly found in Lake Jipe and “Nyumba ya Mungu” in Tanzania, and it is listed on the International Union for Conservation of Nature's (IUCN) Red List of Critically Endangered Species (Shechonge *et al.*, 2019). Despite its current status and the need to farm it, no studies on the prevalence of bacterial infections or other pathogens have been conducted, indicating an urgent need for this study.

The project will adhere to the Sustainable Development Goals of Zero Hunger-End Hunger, attain Improved Nutrition through reduced malnutrition especially for women and childrens, and Promote Sustainable Aquaculture (Troell *et al.*, 2023).

CHAPTER TWO: LITERATURE REVIEW

2.1 Aquatic Farming

Aquatic animals can live in a variety of settings and incidences of disease-causing pathogens in aquaculture are mainly due to several factors that act individually or jointly in aquaculture systems (MacAulay *et al.*,2022). There are several human activities around aquatic ecosystems including; microbiological pollution of lakes, agriculture, urbanisation, cutting down of trees and rural settlements.These have resulted to advance effect to the lake water physical factors like dissolved gases, temperature and chemical factors which affects the survival and health of fish in aquatic bodies directly or indirectly (David *et al.*, 2009; Guerrero *et al.*, 2020).

Bacteria are among disease causative organisms that may initiate diseases or act as opportunistic invaders. Their primary entrance sites for infections in aquatic environments are the gills, epidermis, and digestive systems. These pathogens can easily proliferate and spread the infection throughout the body and fluids of fish (Irshath *et al* 2023). Bacterial diseases are characterized by reddish and inflamed parts of the body such as fins, scales, exophthalmos, dropsy and fin rot (Sanil, 2008; Wu *et al.*, 2021).

2.2 Factors that contribute to introduction of bacteria in aquatic systems

Disease predisposition is a result of an interaction between host susceptibility, pathogen virulence, and environmental factors. The environment is a complex of relationships with plants, soil and other animals living in water to form a food cycle of events which must be at equilibrium with each other for a healthy survival of the various biodiversity components. The three main factors which are necessary in the assessment of water quality are physical, biological, and chemical properties (Ikeogu *et al.*, 2010).

Aquatic ecosystem contamination is caused by increased human activities around the riparian region Agricultural activities such as overuse of pesticides and fertilizers and sewage from residential have found their way to water environment leading to the deterioration of the water quality and increased susceptibility of fish to infectious diseases (Bashir *et al.*, 2020).

Intensive tilapia fish farm systems are characterized by high nutrient concentrations due to feeding and high nitrogenous waste from the fish and high fish stocking per meter square, these conditions lead to favarouble conditions for bacterial growth , some of which are potentially pathogenic to fish and others having potential zoonosis which have led to global tilapia disease outbreaks, with bacterial infections causing mortalities and morbidities, threatening sustainable production (Haenen *et al.*, 2023).

The ability of a pathogen to cause disease in fish is determined by; virulence of the pathogen, exposure dose required to initiate an infection, contact time required to initiate infection, the ability of the pathogen to survive and multiply without a host present, and the route of transmission and therefore is crucial to determine the specific properties of the pathogens in assessing the risk of disease spread to wild and cultured fish (Aziz *et al.*, 2020).

2.3 Bacteria associated with wild and farmed *Oreochromis* species

Infectious disease threats must be addressed at the human-animal-environmental interface, as well as a robust surveillance system capable of supporting early detection and response. Most bacterial diseases in fish are caused by Gram negative organisms from the genera *Aeromonas*, *Citrobacter, Edwardsiella*, *Flavobacterium, Pseudomonas,* and *Vibrionaceae*, as well as acid fast *Mycobacterium* species (Declercq *et al.*, 2013; Wu *et al.*, 2021). The Gram-positive bacteria are mainly *Renibacterium* and *Streptococcus* species (Sani *et al.*, 2023). Do any of these occur in *O. jipe* and other *Oreochromis species*? This was investigated.

2.3.1 *Aeromonas* species

These are Gram-negative, rod shaped, facultative anaerobic bacteria and they are catalase and oxidase positive (Chen *et al.*, 2019). *Aeromonas* species are responsible for most natural diseases outbreaks in fish farms, the bacteria are normally isolated from the hematological organs of the fish and the most isolated species are; *A. hydrophila, A. Sobria*, *A. dhakensis, A. veronii*, and *A. jandaei* (Olga *et al.*, 2023). The fish infected by this bacterium normally shows signs of lethargy, and apathy, ulcerations, pale spots, and haemorrhages. Co-infections with *A. jandaei* and *A. veronii; Aeromonas hydrophila* and *Streptococcus agalactiae* occurs naturally in the aquatic environment and causes mass mortalities in fish farms. In intensive fish farming, diseases caused by *Aeromonas* often occur when water quality changes which is normally caused by improper farming practices and poor feed quality leading to rapid depletion of oxygen (Aziz *et al.*, 2020; Ahmed 2019; Chen *et al.*, 2019)

2.3.2 *Citrobacter* species

Citrobacter species belongs to the family *Citrobacteriaceae* of the *Enterobacteriaceae* family. Members of this genus are motile and grows well in blood agar. The colonies are normally smooth edged concave and colourless (Chen *et al.*, 2021). The diseased fish displays anorexia, lethargy, hemorrhage and darkened skin color externally. Internal organs also display ascites and hemorrhages.

2.3.3 *Edwardsiella* species

The genus *Edwardsiella* is Gram-negative, glucose fermentative, catalase positive and capable of producing hydrogen sulphide and indole (Kerie *et al*., 2019). The main species that are responsible for fish diseases are mainly of *E. tarda*, *E. hoshnae*, *E. ictaluri*, *E. piscicida* and *E. anguillarum* (Kerie *et al*., 2019). In fishes, *edwardsiella* is a major causative agent of enteric septicemia or putrefactive diseases (EPD), hepatomegaly, and recurrent multifocal nodulation of the spleen, head, and kidney (Ahmed, 2019). *Edwardsiella tarda* is considered as a serious pathogen of fish and of important public health concern because of its zoonotic potential and causes gastroenteritis and meningitis in humans (Olga *et al.*, 2023).

2.3.4 *Flavobacterium* species

Most members of this family are a non spore-forming , filamentous , Gram negative, motile , and are non-flagellated with rare exceptions, *Flavobacterium columnare* is a causative agent for columnaris disease, also called saddle back disease or bacterial warm water disease of fish which is responsible for devastating losses in aquaculture systems and wild fresh water fish (Declercq *et al.*, 2013; Loch and Faisal, 2015; Charo *et al.*, 2022).

Flavobacterium psychrophilum is mostly found in wild fish that are close to fish farms and may be introduced into the farms by the wild fish (Mulei *et al.*, 2021). *F. psychrophilum* in fishes can be recovered from several external and internal body organs including skin mucus, gills, brain, kidney, spleen and reproductive products of spawning adults (Starliper, 2011).

2.3.5 *Vibrio* species

Vibrios are Gram negative short rod, motile, facultative and oxidative bacteria. Species that usually cause fish mortalities are *Listonella anguillarum* , *Vibrio ordalii, V. salmonicida, V. harveyi, V. alginolyticus, Moritella viscosa* and *V. vulnificus* (Toranzo *et al.*, 2005). Fish that are severely infected exhibit serious disease symptoms which include skin ulcers, hemorrhage at the body's surface and fin bases, rotten fins and tails, and stomach distension. A build up of crimson ascitic fluid is seen in the peritoneal cavity of severely diseased fish, and the liver, kidneys, and spleen all appear pale with petechiae (Jun and Woo, 2003) **.**

2.3.6 *Streptococcus* species

Streptococcus is a Gram-positive bacterium. Species that are of economic importance in fish farming include *Streptococcus iniae, S. difficilis, S. agalactiae Lactococcus garvieae* and *Vagococcus salmoninarum* (Roque *et al.*, 2009). Clinical signs in fish associated with streptococcal infections include exophthalmia, ocular opacity, skin blackening, petechial hemorrhages, intensive mucous production at the pectoral, caudal fins and mouth (Roque *et al.*, 2009).

2.3.7 *Pseudomonas* species

Pseudomonas are Gram negative bacterias that are responsible for major outbreaksin aquaculture. The common species isolated from fish include; *Pseudomonas pulva, Pseudomonas* *fluorescence, Psudomonas putida, Pseudomonas aeruginosa, Pseudomonas alcaligenes, Pseudomonas stutzeri, and Pseudomonas alcaligenes.* Affected fish show exophthalmia, severe distended and congested stomach and anal opening, ascites, hemorrhages, fin rot, detached scales, skin and tail ulceration (Yousuuf *et al.,* 2020; Wamala *et al.,*2018).

2.4. Fish and aquatic - derived zoonoses

Fish diseases that are potentially zoonotic have caused considerable problems in the aquaculture industry in addition to the possibility of spreading these diseases to humans. With the world's growing population and increase in global trade and movement of fish and their by-products, the risk of aquatic contamination and development of aquatic-derived zoonoses in humans is increasing. Zoonotic bacterial agents are either Gram-positive or Gram negative; Gram-positive are; *Mycobacteriaceae, Streptococcaceae, Erysipelothricaceae* families and Gram-negative include; *Aeromonadaceae, Vibrionaceae, Pseudomondaceae, Enterobacteriaceae,* and *Hafniaceae* families. Humans get exposed to the pathogens mainly through consumption of improperly cooked or raw fish or fish products, aquaculture workers become infected when they get in contact with the super-bacteria and there is a threat of spreading these pathogens to their family and community as they automatically become carriers of these pathogens. This can be minimized by properly processing fish and fish product and ensuring proper biosecurity at farm level. The prevalence of zoonotic agent's changes with seasons and should be regularly monitored to evaluate the prevalence of pathogens in both wild and cultured fish populations (Swaimanathan *et al.*, 2021; Ziarati *et al.*, 2022; Preena *et al.*, 2022).

2.5 Challenges facing aquaculture farming

A major limitation to the sustainability and development of aquaculture is incidences of fish deaths. Mass mortalities are normally caused by diseases, including bacterial infection and influence of poor water quality but these two have not been carefully studied (Assefa and Abunna, 2018; Martinez *et al.*, 2023). Moreover, intensification in aquaculture brings about risks of diseases caused by bacteria, parasites, viruses and fungi.

Fish reared in aquaculture are often kept under crowded and stressful conditions (Leung *et al.*, 2019). Fish are susceptible to a wide variety of bacterial pathogens especially when they are subjected to stress due to poor water quality, overstocking and overfeeding (Kerie *et al.*, 2019) . Fish diseases can be prevented and managed by providing vaccinations and medications, improving the hygiene of existing fish hatcheries, increasing the number of hatcheries and enhancing bio-security measures leading to improved farming practices that will help in reducing mortality rates (Yassien, 2022).

2.6 Water quality and bacteria in fish

Water quality is the most compelling factor in fish farming, as it has a direct effect on fish feeding efficiency, fish feeds conversion ration, reproduction and health. Any significant deviation from such water quality can be considered a potential stressor (Mramba *et al.*, 2023). Toxic algae and high fish densities in aquaculture facilities put farmed fish at higher risk of exposure to water quality related stressors, increasing their susceptibility to disease when exposed to pathogens compared to wild fish, which have a wide area in which they can migrate to (St-Hilaire *et al.*, 2022) .

The water environment that is constantly in contact with fish must have an acceptable quality in terms of its biological, chemical and physical attributes for it to be tolerated by aquatic animals. Overcrowding leads to declining water quality, as crowded culture systems often have low dissolved oxygen levels, declining pH values, and high nitrates and ammonia levels that rise daily after feeding. In addition to lowering the fish's resistance to diseases, overcrowding also increases the chances of pathogens getting into contact with susceptible hosts and cannibalism which leads to skin injuries that are key entry points to opportunistic pathogens (Ikeogu *et al.*, 2010; Aly *et al.*, 2018) .

2.6.1. Specific water parameters that affect fish health

Aquatic life is heavily reliant on the physico-chemical and biological features of water, including dissolved oxygen, temperature, pH, turbidity, and ammonia which must be within the optimal range for their survival. Variations within the ranges must be gradual for fish to survive within their targeted ranges, since a sudden shift in the water quality parameters can put fish under a lot of stress, diminish their capability to fight off diseases, and occasionally even kill them (Mramba and Kahindi, 2023). It is possible that unfavorable environmental conditions will reduce organisms' strength to sustain an effective immunological response system, resulting in heightened susceptibility to various infections (Verma *et al.*, 2023). Organic pollution of water, followed by a reduction in dissolved oxygen content, creates an ambient condition for bacterial growth (Xia *et al.*, 2022).

Phosphorus (P) is the rate-limiting nutrient in the most freshwater ecosystems, while nirtogeneous wastes enter the aquatic system directly from excreted by fish or degradation of fish feeds. Ammonia in its unionized form (NH_3) and at a concentration of >0.1 mg L is highly harmful as it leads to gill damage, destruction of mucous-producing membranes, "sub-lethal" effects such as reduced growth, poor feed conversion, and reduced disease resistance at concentrations lower than lethal concentrations, osmoregulatory imbalance, and kidney failure (Verma *et al.*, 2023).

Alkalinity is measured by the amount of acid (hydrogen ion) water can absorb (buffer) before achieving a designated pH. Total alkalinity is expressed as milligrams per liter or parts per millions of calcium carbonate (mg/L or ppm $CaCO_3$). A total alkalinity of 20 mg/L or more is necessary for good pond productivity. The pH of well buffered water normally fluctuates between 6.5 and 9.0. Waters with low alkalinity, pH can reach dangerously low (CO_2 and carbonic acid from high respiration) or dangerously high (rapid photosynthesis). The release of carbonate converted from bicarbonate by plant life can cause pH to increase dramatically (above 9) during periods of rapid photosynthesis by dense phytoplankton (algal) blooms (Verma *et al.*, 2023) .

In highly stocked fish farms, carbon dioxide (CO_2) concentrations is usually high as a result of respiration, the free CO_2 released reacts with water, producing carbonic acid (H_2CO_3), and pH is lowered (Mendes *et al.*, 2019). Tabel 1 shows vital water quality parameters that limit aquaculture productivity.

Table 1: Tolerant, desirable and lethal ranges of some water quality parameters for *Oreochromis niloticus*.

S/N	**Water quality parameters**	**Water quality parameter characteristics**	
		Optimal	*Reference*
1	Temperature (Degrees Celsius)	22-30	Nivelle *et al.*, 2019
2	Dissolved oxygen (mg/L)	5-7	Mramba and Kahindi, 2023
3	Carbon dioxide (mg/L)	0.5	Ikeogu *et al*. 2010
4	Nitrates (mg/L)	0.0	Ikeogu *et al*. 2010
5	Unionized Ammonia (mg/L)	0.002	Ikeogu *et al*. 2010
6	Hydrogen sulphide (ppm)	0.00	Ikeogu *et al*. 2010
7	pH (H+)	4.6-6.5	Ikeogu *et al*. 2010
8	Hardness(ppm)	10-20	(Ikeogu *et al*. 2010)
9	Turbidity (NTU)	1000-20,000	(Ikeogu *et al*. 2010)

2.7 Antimicrobial resistance

The growth of fish in closed and controlled systems have led to conditions that favours growth of pathogenic bacteria (Schar *et al.*, 2020). This has necessitated the use of antibiotics in treatment of fish diseases, the antibiotics of which now have gained entry in aquatic environment, both marine and fresh water, and led to the wider theoretical risks associated with the development of antimicrobial resistance by fish pathogens (Thornber *et al.*, 2022). The appearance of acquired resistance in fish pathogens and other aquatic bacteria means that such resistant bacteria can serve as a reservoir of resistance genes from which can spread further and may eventually end up in human pathogens through conjugation.

Antibiotics must be used prudently in aquaculture under veterinary supervision for the safety of aquaculture commodities (Gazal *et al.*, 2020). The most commonly used antibiotics in terms of environmental and health risks in fish are oxytetracycline, ampicillin, tetracycline, co-trimoxazole, streptomycin, kanamycin, gentamicin (Wanja *et al.*, 2020). Considerable concerns ranging from antibiotic resistance, biodiversity changes, food safety and occupational health hazards to feed industry and farm workers have been raised *(Lulijwa et al.*, 2020).

Antimicrobial resistant bacteria in fish food pose a risk to public health because of the possibility of spreading antibiotic resistant genes. The human-derived bacterium *Escherichia coli*, contains certain strains which are harmful to fish, and to humans (Burridge *et al.*, 2010).

2.8. Matrix Assisted Laser Desorption/Ionization –Time of Flight technique

This techniques is used for accurate and rapid identification of both Gram negative and positive bacteria, which is based upon the detection of highly abundant proteins in a mass range between 2 and 20 (kilodalton) kDa by computing their mass (m) to charge (z), m/z values. A typical fingerprint is then generated for each microorganism which is used for comparison with the stored reference spectra and therefore providing identification for the sample (Singhal *et al* 2015).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Ethical clearance

Research approvals were granted by Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (REF: FVM BAUEC/2023/419; Appendix 1) and National Commission for Science, Technology (NACOSTI) (REF: 549523; Appendix 2). An informed verbal consent was also sought from fish farmers prior to field sampling

3.2 Study area and sites

The study was carried out in four sub counties of Taita Taveta county namely: Voi, Mwatate, Wundanyi and Taveta where Tilapia Jipe farming is practiced and in Lake Jipe. In lake Jipe, the study was carried outlong the shores (particularly at the inlet of River Lumi, Mkwajuni beach, Kenya Wildlife Service [KWS] station and at the outlet of River Ruvu) of Kenya.

Taita-Taveta County is a Kenyan county located approximately 140 kilometers northwest of Mombasa and 380 kilometers southeast of Nairobi. The county is bordered to the north by the counties of Makueni, Kitui, and Tana River, to the west by Kajiado, to the south by the Tanzanian border, and to the east by Kilifi and Kwale (Figure 1). The county has a tropical wet and dry or savanna climate. The county yearly temperature is 25.44ºC (77.79ºF) and it is 2.94% higher than Kenya's averages. Taita-Taveta typically receives about 144.51 millimeters (5.69 inches) of precipitation and has 158.92 rainy days annually. The area around Lake Jibe has two rainy seasons: the long rains between March and May and the short between November and December. The average rainfall during the entire year is up to 640mm. The driest month is usually February with 7mm and the wettest is May with up to 156mm. Temperatures range between 23^{0}C and 34^{0}C.

Some farmers in Mwatate, Voi and Wundanyi sub counties rear *Oreochromis jipe*. InTaveta Sub –county the same fish species is found both in Lake Jipe and aquaculture systems. The County has a population of 284,659 and covers 17,083.9 square kilometers (Kenya National Bureau of Statistics, 2019).

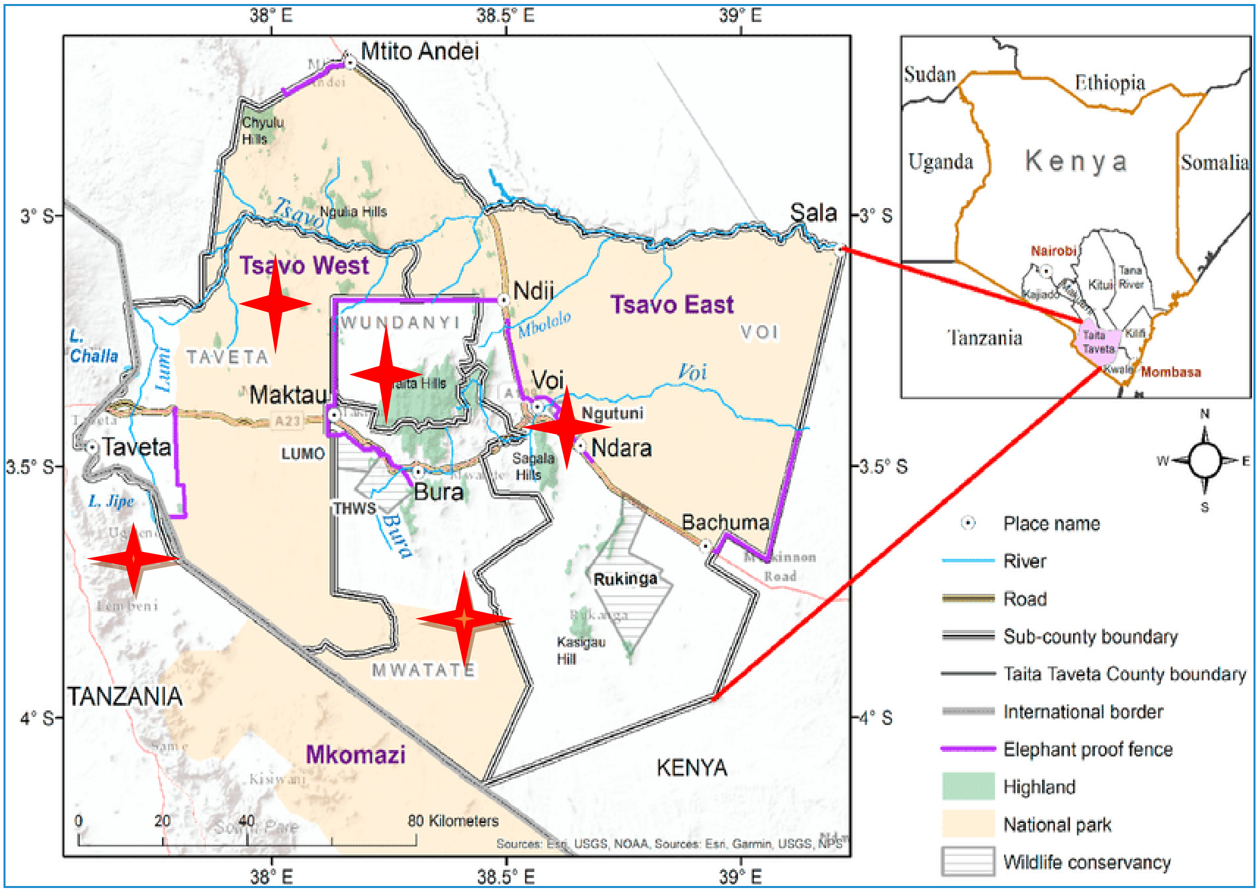


Figure 1: A map of Taita Taveta county showing the study sites **(red stars)** *(Wekesa et al., 2020)*.

Lake Jipe has an elevation of 705 meters above sea level and is located at 3.58330 latitude and 37.750 longitude. It is a small, shallow lake (28 square kilometers with a moderate depth of less than 3 meters) on the Kenya-Tanzania border, just to the east of the northern Pare Mountains in Mwanga district, in the Kilimanjaro region of Tanzania. It is 12 kilometers long and 2.5 kilometers wide, with Tanzania managing 12 square kilometers and Kenya owning 14 square kilometers.

Lake Jipe draws most of its water from Mt Kilimanjaro in Tanzania through river Lumi, which flows through Kenya. The river Muvulani, which flows from the Pare Mountains, is the other major inflow. Many semi-permanent streams, primarily from the Pare Mountains, forms the major inlet into Lake Jipe. The lake has one drainage, the river Ruvu, which is based in Tanzania and to the south of the main inflow, the river Lumi. Increased soil erosion has caused siltation and eutrophication in the lake, promoting the growth of invasive aquatic macrophyte such as *Prosopis juliflora* on land and *Typha domingensis* in water. The rainfall at the slopes of Mt. Kilimanjaro is often heavy and run-off that causes floods that are a menace to both people and infrastructure (Orina *et al.*, 2024).

Typha domingensis, also known as 'Gugu maji,' and Cyperus swamps cover 4045 hectares of the lake's surface area. This area is a breeding ground for fish such as *Oreochromis jipe* and is inhibited by several bird species. This fish thrives in the lake, benefiting the local fishing industry (Mwachiro *et al.,* 2019) .

Oreochromis niloticus is the preferred commercial aquaculture species accounting for up to 80% in the Kenya's total aquaculture production (Munguti *et al.*, 2022). Its regional widespread is largely attributed to a wide range of ecological and feeding adaptations, low food conversion ratio (FCR) and the ease of captive breeding (Lind *et al.*, 2019). Hence, most Kenyan aquaculture farmers prefer the semi-intensive production of *O. niloticus* because of its presumed moderate operational costs (Hinneh *et al*., 2022).

Lake Jipe has been the main source of fish in Taita Taveta region in Kenya and Mwanga District in Tanzania. Fishing is the key economic activity for communities living in the lake's catchment, making it their main source of livelihood (Figure 2). Since it is a freshwater lake, the communities also use it as a source of drinking water and for other domestic purposes. Increased population over the years has led to encroachment of the lake's riparian areas for crop farming and livestock grazing.

Kachero and Mkwajuni are the only Beach Management Unit (BMU) within Lake Jipe. The unit members comprise of fishermen and the lake users within the Lake. They are involved in conservation of the shoreline and maintaining security and cleaning of the lake beach.

Wildlife in the lake Jipe catchment area, including herbivores, predators, ungulates, reptiles, and other animals, have adapted to the local habitat. Lesser jacanas, purple gallinules, Madagascar squacco herons, black herons, African darters, and African skimmers are frequent waterbirds (Avifauna) (Mwachiro *et al.*, 2019). These have the potential to affect the lake's bacterial flora and fish. The fish include mudfish (*Clarias mosambicus*), endemic tilapia (*Oreochromis jipe*; *Oreochromis niloticus*), and sardine "omena" (*Rastineobola argentae*) (Orina *et al.*, 2024). Crocodiles, hippopotamuses, water monitors, otters, and other animals are also present. The lake is significant to the local fauna because it provides a constant source of water and year-round greenery.

Figure 2: A fisherman at lake Jipe Mkwajuni beach *(Mwachiro et al., 2019)*

3.3 Study design

A cross-sectional study comprising sampling of wild and farmed *Oreochromis niloticus*, *O.jipe* and their *hybrid* from L. jipe and farms within Taita-Taveta County and respective source water was carried out between April 2023 and May 2023. The fish were sampled from both the lake and purposively selected active fish farms (with at least one pond stocked with *Oreochromis jipe*) within Taita-Taveta County.

In addition, physico-chemical parameters of water were assessed *in-situ* and *ex-situ* (chemical analysis done at Government Chemist). Collected fish samples were subjected to gross examination and thereafter, organ samples were taken for conventional bacteriological isolation and identification. Following Clinical Laboratory Standards Institute (CLSI) protocols, the antimicrobial susceptibility profiles of the isolated bacteria against particular antibiotics were established by disc-diffusion procedures.

3.4 Study animals

The study animals were grow-out to table size (200g) tilapia jipe (*Oreochromis jipe*) which is locally known as "Asilia". The fish were sampled from both the lake and randomly selected active fish farms (with at least one pond stocked with tilapia jipe) within Taita-Taveta County. *Oreochromis jipe* is easily recognized by steep and nearly straight upper profile of the head, presence of 34 scales along the lateral line and some vertical stripes on the caudal fin (Hamerlynck *et al.*, 2008).

In total, three fish species were sampled; *O. Jipe* (Figure 3A) which had brownish to silver coloration, the fins were pink to orange and had a steep upper front of the head. The *hybrid* (Figure 3B) had brownish to silver colorations and blackish fins. *Oreochromis niloticus* (Figure 3C) appeared to be bronze to brownish gray color with a deep body and a compressed shape

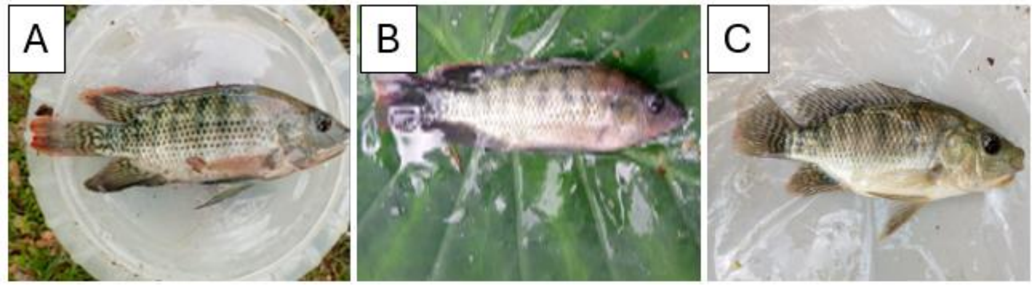


Figure 3: *Oreochromis jipe* (A), hybrid (B) and *Oreochromis niloticus* (C) sampled in the study

3.5 Sample size determination and sampling procedure

Five farms within Voi (n=1), Mwatate (n=1), Wundanyi (n=1) and Taveta (n=2) sub-counties in Taita-Taveta County were recruited. They were purposively selected based on availability of active fish farms rearing tilapia Jipe. Similarly, the 4 sites within the lake Jipe (River Lumi inlet, Kachero Beach, Mkwajuni Beach and Kenya Wildlife Service (KWS) area were purposively selected based on accessibility.

The sample size was calculated using the formula by Bartlett *et al*. (2001): where n=$\frac{z^2 p\,(1-p)}{d^2}$

Wherby n is the sample size, Z = Z statistic for a level of confidence (1.96 for 95%), 'P' = prevalence (assumed pathogen prevalence level of 92%) and d = precision, which is equal to 5% (0.05). This gave a sample size of 114 samples. However, one hundred and eleven (111) healthy-appearing fish consisting of 67 *Oreochromis jipe* (*O. Jipe*), 34 *Oreochromis niloticus* (*O. niloticus*) and 10 hybrids; and nine water samples (7 from farms and 2 from Lake Jipe) were taken. Four (4) organs (skin, gills, intestines and kidney) samples were taken from each fish (Table 2). This made a total of four hundred and fifty-three (453) samples that were obtained.

Table 2: Fish species and samples collected from different sites

Fish species	Sampling site	Number of fish samples	Number of water samples collected
Oreochromis jipe	Voi farm	10	2
Oreochromis jipe	Dembwa (Mwatate) farm	10	1
Oreochromis niloticus	Dembwa (Mwatate) farm	10	1

Hybrids (Oreochromis jipe and *Oreochromis niloticus)*	Dembwa (Mwatate) farm	10	0
Oreochromis jipe	Wundanyi farm	11	1
Oreochromis jipe	Taveta farm	20	1
Oreochromis niloticus	Taveta farm	5	1
Oreochromis jipe	Lake Jipe	16	1
Oreochromis niloticus	Lake Jipe	19	1
Total		111	9

3.6 Fish and water sampling for bacteriological analysis

The Beach Management Units (BMU) assisted the researcher in harvesting fish from the lake using seine nets and/or fish traps. Fish traps were set at river Lumi inlet, Kachero beach, Kenya wildlife Service station and river Ruvu outlet. Similarly, farmed fish samples were harvested from the ponds using seine nets with the help of local fish harvesters, upon getting consent to sample from fish farm owners. A total of 10-15 fish were collected per pond from the selected farms. Thereafter, surface water for bacteriological assay, were collected aseptically from each lake site and pond in a sterile screw-capped 20-ml universal bottle. The fish samples were transported live to the Taveta and Wundanyi Fisheries offices using oxygen polythene bags which were two-thirds full oxygenated by pumping oxygen from oxygen tanks into the bags. A temporary field laboratory was set up for necropsy and organ sampling within the premises of Fisheries offices.

3.6.1 Necropsy and organ sampling

Post-mortem examination was done as described by Roberts (2012). Prior surface decontamination of the working benches was done using 70% alcohol. The dissecting instruments were initially autoclaved at 121ºC for 15 minutes. For re-use, the instruments were immersed in 70% ethanol for 2 minutes followed by flame sterilization. The fish were humanely killed by giving a sharp blow on the cranium and pithed. An external examination of individual fish organs was then done to check for gross lesions such as wounds, hemorrhage, ulceration, and erosion. The specimen was later laid on a sterile tray.

A square inch of the skin's lateral surface was swabbed with sterile cotton swabs. A sterile aluminum metal plate with square inch fenestration was used as a guide. The operculum was removed to reveal the gills, after which a portion of the gills was excised. The ventral-lateral surface of the fish was decontaminated with 70% alcohol prior to opening the body cavity (Wanja *et al.*, 2020). The fish were then dissected to expose abdominal organs. A portion of the intestine and kidney swabs were aseptically removed. Organ samples (skin and kidney swabs, portions of gills and intestines) were placed separately in bijou bottles containing Stuart’s transport media, labeled, and put inside a cool box with ice. As soon as possible, all samples were transported to the bacteriology laboratory in the department of Veterinary Pathology, Microbiology, and Parasitology for bacterial culture, identification and characterization.

3.7 Sample processing and isolation of fish bacteria

All bacteria analysis was done at the bacteriology laboratory, department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi. Each fish's intestines and gills were aseptically pulverized in sterile physiological saline using a sterile pestle and mortar. The homogenate, as well as skin and kidney swabs were incubated overnight at 37°C in freshly prepared alkaline peptone water (APW; pH 8.4) (Dissasa *et al.*, 2022).

An inoculum was sub-cultured by streaking one by one colony aseptically onto plates containing blood agar and MacConkey agar. According to Sanders (2012) the plates were subsequently incubated aerobically at 37^0C. After 24-48 hours of incubation, during which time growth occurs, the plates were then examined, and the colony morphology on the plates was documented. To obtain pure colonies, distinct colonies were sub-cultured.

3.8 Identification of isolated bacteria

Following Austin and Austin (2016) and Bergeys Manual of Determinative Bacteriology (Krieg, 2010), the isolates were identified using colony morphology, Gram staining characteristics, and conventional biochemical tests as shown in (Appendices 3 and 4) and further bacterial identification was done at the International Livestock Research Institute (ILRI) Kabete, using Matrix Assisted Laser Desorption/Ionization –Time of Flight technique (MALDI-TOF MS).

3.8.1 Morphological characterization of isolates

The bacterial colonies on the culture medium were morphologically examined using their size, elevation, margin, surface, pigmentation, opacity and whether grown inside or on the surface of the medium, following criteria given by Austin (2019). The following biochemical tests were performed to identify the isolates: cytochrome oxidase spot test, catalase, methyl red test, indole production, Simmon’s citrate utilization, urease, Indole, Methyl red, Voges Proskauer and citrate test were done on separate tubes, Triple Sugar Iron Agar Test (TSIA) was also done by inoculating organisms by stabbing at the butt and then streaking on the slant surface. Acid and gas production from four sugars (glucose, sucrose, mannitol and lactose) tests were carried out, following standard procedure as described by Dura (2020).

3.8.2 Spore testing

Spore testing was done by preparing a smear on a microscope slide and heat fixing, then the slide was flooded with 5% aqueous solution of malachite green. Steaming was done gently for 30 seconds, and the slide was left to stain for 2-3 minutes, washing with tap water was done and afterwards diluted with safranin for 30 seconds, the slide was washed again with tap water, blot dried and examined using oil immersion objective (x1000).

3.9 Measurement of physicochemical parameters of water

Prior to sampling, different physio-chemical conditions were evaluated in sub-counties of Voi, Mwatate, Wundanyi and Taveta; and Lake Jipe in Taita-Taveta County shown in (figure 1) they included temperature, dissolved oxygen (DO), and pH which were measured *in-situ* before 0800 hours’ Kenyan time using a portable digital multi-probe meter (Hanna Instruments Inc., USA). In addition, 2L of pooled water sample was collected from each of the 4 farms/ponds and 4 sites within the lake and kept inside a cooler box with ice and transported to the Faculty of Agriculture, University of Nairobi for further chemical analysis namely; concentration of total ammonia, total alkalinity, nitrates, nitrites, carbonates, and total orthophosphate

3.10 Antibiotic susceptibility testing

A total of 45 (forty-five) bacteria isolates were screened for resistance against six (6) antibiotics (Appendix 6). The antibiotics were chosen based on their importance in fish farming and other of veterinary and medical importance. Bacteria screened for resistance belonged to various genera namely: *Klebsiella*, *Streptococcus*, and *Escherichia coli* were tested because of their high zoonotic potential and public health concerns while *Aeromonas* and *Bacillus* genus were tested due to their pathogenicity potential for fish. Members of the genus *Lycinibacillus*, *Acinetobacter*, *Staphylococcus*, *Kytococcus*, *Empedobacter and Enterobacter* were tested due to their emerging nature as potential fish pathogens. Commercially available antibiotic disks (Himedia®) and their concentration are shown in Table 3. *Stahylococcus aureus* (ATCC 29213), *E. coli* (ATCC 35218) and *Pseudocomona aeruginosa* ATCC were used as reference strains.

Table 3: Antibiotics used in susceptibility testing

Antibiotic	Symbol	Concentration (mg)
Ampicilin	AMP	10
Streptomycin	S	30
Tetracycline	TET	30
Gentamicyn	GEN	1
Co-trimazole	CTX	10
Oxacillin	OX	1

The antibiotic susceptibility testing for selected bacterial isolates was performed using Kirby-Bauer disc diffusion according to CLSI (2015). Briefly, suspension of the bacterial isolates and the reference strains were vortexed and inoculated on nutrient broth separately and incubated for 24 hours aerobically at 37°C. After overnight incubation, the bacterial suspension was vortexed and diluted to turbidity that was equivalent to that of 0.5 McFarland standard. The bacterial suspension was then spread onto the surface of the Mueller-Hinton agar (Oxoid®), to make uniform growth. Antibiotic discs were then automatically placed on the surface of the agar plate using a disk dispenser and incubated aerobically at 37°C for 16 hours. Inhibition zones for various isolates, were measured and interpreted as sensitive, intermediate, or resistant; according to Clinical Laboratory Standards Institute (CLSI, 2015; Humpries *et al.*, 2021). Multiple Antibiotic Resistances (MAR) indices of the study isolates against tested antibiotic was calculated using the formula by Shakir *et al.* (2021) as shown below.

$$indice = \frac{x}{yz}$$

Where; X is the total of antibiotic resistance case, Y is total of antibiotic used in the study and Z is the total of isolates tested.

3.11 Data management and analysis

The collected raw data was stored Microsoft Excel® 2013 and was used to calculate means, proportions and drawing of graphs and tables. The association of the prevalence of bacterial isolates and sampling sites was determined using Chi-square test of association at 95% confidence interval.

CHAPTER FOUR: RESULTS

4.1 Bacterial isolation and characterization

4.1.1. Overall bacterial isolation

A total of five hundred and ninety-six (596) bacteria were isolated from 444 (four hundred and forty-four) fish organs and 9 (nine) water samples. Out of these 568 (five hundred and sixty-eight) isolates were recovered from sampled fish organs and 28 (twenty-eight) from water samples (Figure 4). The isolates belonged to 23 genera and 53 species. The common genera identified include *Staphylococcus* species at 19.8% (118/596), *Bacillus* species at 17.8% (106/596), *Acinetobacterium* species 16.4% (98/596), *Aeromonas* species 15.6% (93/596) and *Pseudomona* species 5% (30/596) (Appendix 7).

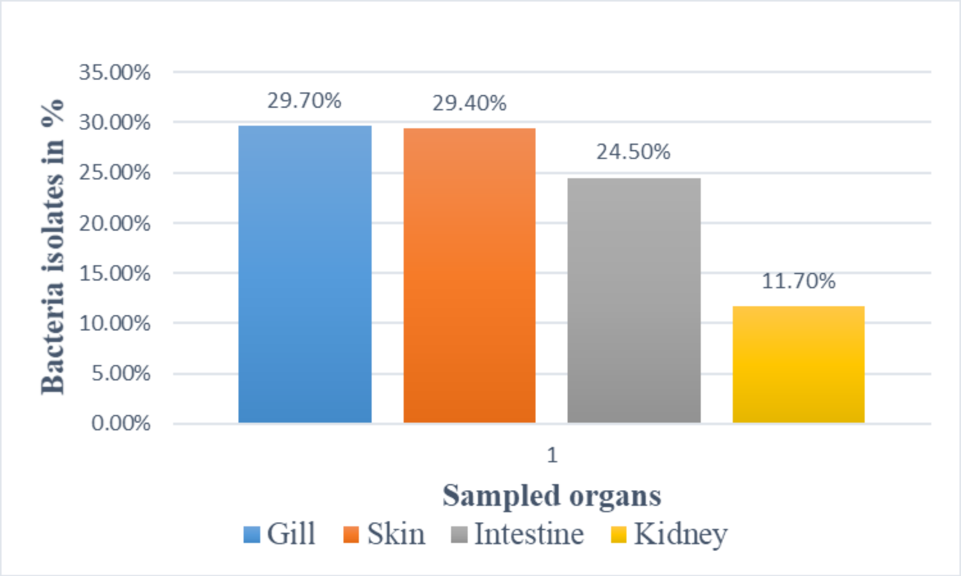


Figure 4: Percentage of bacterial isolates recovered from different fish organs

Key: % = Percentage

4.1.2. Identification of bacteria isolates

4.1.2.1. Characteristics of isolates based on colony morphology

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 (Figure 5; Appendix 3). *Aeromonas hydrophila* colonies were circular grey haemolytic colonies on sheep blood agar after incubation for 24 hours at 37^0C (Fig 5A)*; Pseudomonas fulva* colonies were non-haemolytic on sheep blood agar after incubation for 24 hours at 37^0C (Fig 5B); *Staphylococcus lentus* colonies on mannitol salt agar appearing as yellowish small round and convex (Fig 5C); *Streptococcus scuiri* appeared as round grey (β-hemolytic) colonies on blood agar after incubation for 24hrs at 37^0C (Fig 5D); *Klebsiella variicola* produced pink-reddish (prodigiosin) colonies on MacConkey Agar (Fig 5E); *Klebsiella pneumoniae* produced pink irregular shaped mucoid medium size colonies on MacConkey Agar after incubation for 24 hrs at 37^0C (Figure 5 F). *Bacillus cereus* on blood agar (Fig 5G); *Lysinibacillus fusiformis* was non-hemolytic, forming a characteristic swarming following spot inoculation in the center of 10% sheep blood agar plate (Fig 5H), *Micrococcus luteus colony* produced yellow pigmented colonies on Triple Sugar Agar (Fig 5I).

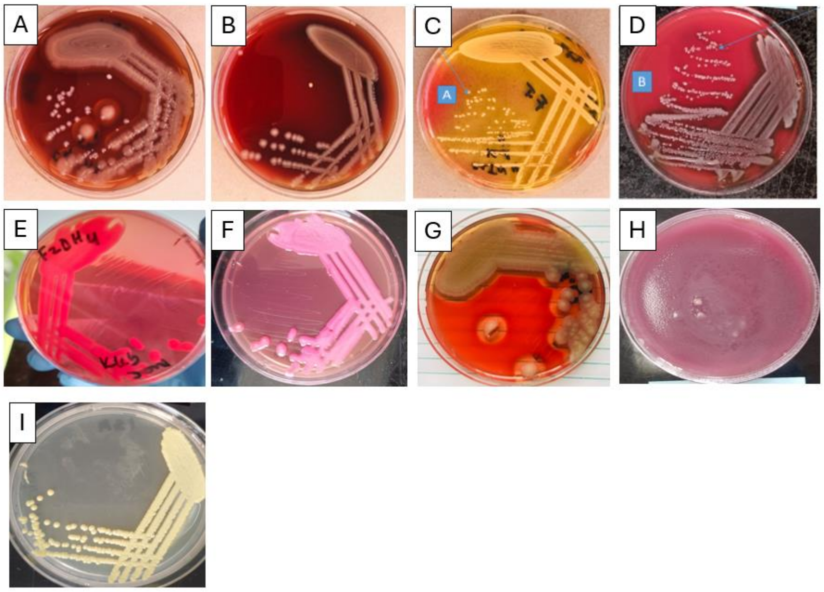


Figure 5: Colonial morphology of some bacterial isolates

Key A= *Aeromonas hydrophila, B=Pseudomonas fulva, C=Staphylococcus lentus, D= Streptococcus scuiri, E= Klebsiella variicola, F=Klebsiella pneumoniae G= Bacillus cereus H=Lysinibacillus fusiformis and I =Micrococcus luteus*

4.1.2.2. Gram staining and other phenotypic characteristics of the isolates

The Gram-negative bacteria isolates were 313 (52.6%) and the Gram-positive isolates were 283 (47.4%). Most of the isolates were Gram-negative short bacilli (Figure 6A), broad Gram-positive bacillus (Figure 6B) to coccobacilli (Figure 6C) and spore forming rods (Figure 6D).

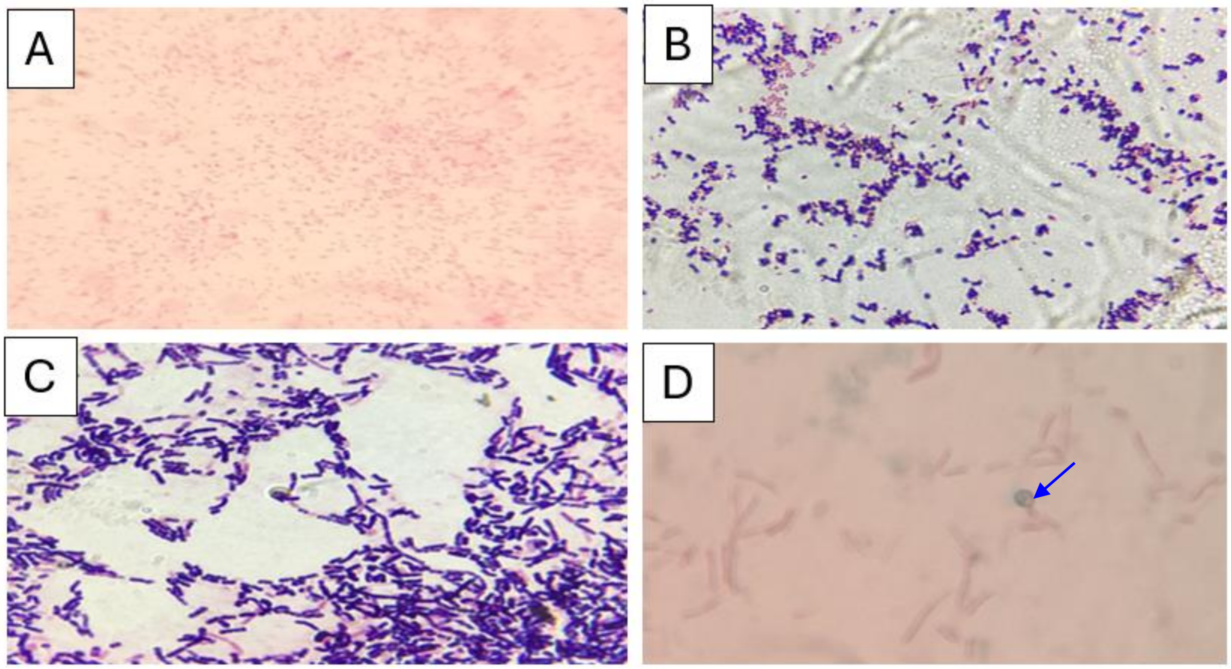


Figure 6: Gram staining characteristics of bacterial isolates

Key: (A) Gram-negative smear showing short bacillus of *Aeromonas spp;* (B) Gram-positive *Micrococcus leteus* cocci in tetrad arrangement*;* (C) *Bacillus cereus* Gram-positive rods in chain; and (D) *Lysinibacillus fusiformis green* spore (blue arrow) and red vegetative cells (x 100).

4.1.2.3 Biochemical characteristics of the bacteria isolated from fish and water samples

Figure 7 shows negative (Fig 7A) and positive (Fig 7B) reactions for the catalase test. Some of catalase producing organisms were *Staphylococcus lentus, Escherichia coli, Bacillus cereus* and *Mycobacterium oleivorans* (Figure 7A). Catalase negative bacteria included *Streptococcus sciuri* and *Enterobacteria cloacae* (Figure 7B) and the oxidase negative organisms were *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter brakii* (Figure 7C) and oxidase positive organisms included *Pseudomonas and Aeromonas* species (Figure 7D).

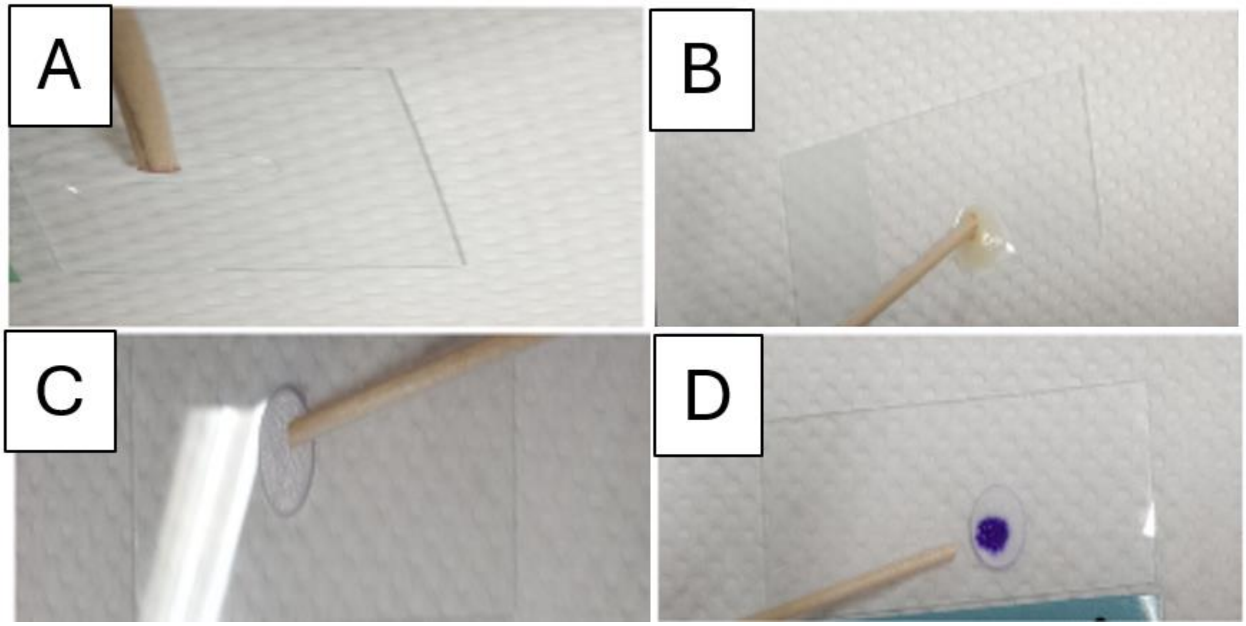


Figure 7: Catalase and oxidase test reactions of bacterial isolates

Key: (A) Negative *Enterobacteria cloacae* and (B) positive *Staphylococcus lentus* catalase test reaction; (C) negative *Klebsiella pneumoniae* and (D) positive *Aeromonas veronii on* oxidase test.

Thirty-seven percent of the tested bacteria isolates were positive on Simmon's citrate. They included *Escherichia coli*, *Enterobacter, Bacillus* and *Streptococcus species* (Figure 8A), while the rest were negative. Figure (8B) represents a citrate negative test for *Aeromonas hydrophila*. Sixteen percent of the isolates comprising *Pseudomonas* and *Klebsiella* species were urease posive (Figure 8C). The rest of the fish samples collected from Mwatate farm in Taita-Taveta County were urease negative (Figure 8D).

Forty-eight percent of the isolates comprising *Aeromonas*, *Citrobacter*, *Escherichia coli*, *Klebsiella and Enterobacter species* fermented glucose. Sixteen percent were lactose positive and included *Aeromonas*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Escherichia coli*. Forty-Seven percent of the organisims mainly; *Aeromonas, Pseudomonas*, *Klebsiella* and *Enterobacter* were sucrose positive, while 29% of the isolates comprising *Aeromonas*, *Citrobacter* and *Escherichia coli* tested positive for mannitol. Figure 8E shows carbohydrate fermentation positive test with gas production by *Escherchia coli*, while Figure 8F demonstrates positive results without gas by *Enterobacter cloacae* (Yellow color) and Figure 8G shows carbohydrate non-fermentative test result by *Micrococcus luteus* from fish collected from Taita-Taveta County. Three percent of bacteria mainly *Escherichia coli*, *Aeromonas* and *Streptococcus* were indole positive (Figure 8 H) while Figure 8I is a negative indole test of *Escherichia coli* from fish samples collected from Taita-Taveta County. A total of twenty -nine percent of the isolates comprising *Streptococcus*, *Citrobacter*, *Escherichia coli* were methyl red positive (Figure 8J) while Figure 8K shows negative methyl test by *Klebsiella pneumoniae* from fish samples collected from Taita-Taveta County (Appendix 4).

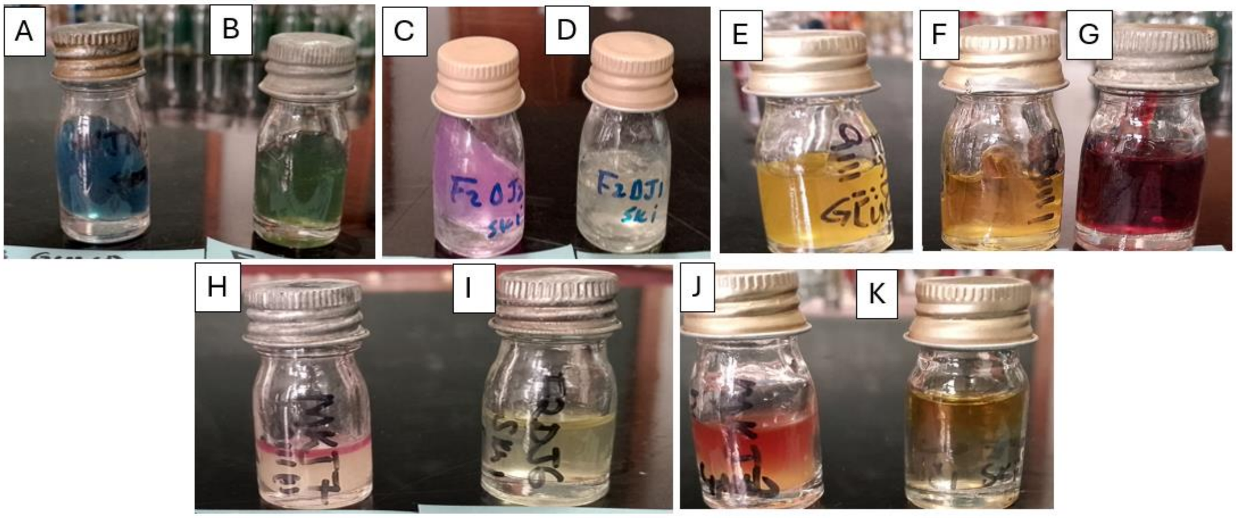


Figure 8: Biochemical reactions of the bacteria isolated from fish and water

Key: A=positive citrate, B=negative citrate, C=positive urease, D=negative urease, positive sugar fermentation test without gas, F= positive sugar fermentation test with gas, G=negative sugar fermentation test, H=positive indole, I=negative indole, J=positive methyl red and K= negative indole test.

Eighteen percent of the organisims namely *Citrobacter*, *Escherichia coli* and *Klebsiella* species were found to produce hydrogen sulphide (Figure 9; B, C and G) the rest were negative. Six percent of the tested isolates comprising *Aeromonas*, *Pseudomonas*, *Citrobacter*, *Escherichia coli* and *Entrobacter* were motile (Figure 9; B, C and G). Thirty-nine percent tested negative for sugar fermentation test and they included; *Acinetobacter* and *Psudomonas* species (Figure 9A). Thirty-three percent of the isolates comprising *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumonia* fermented lactose, glucose and sucrose (Figure 9 D and F) while eleven percent (11%) comprising C*itrobacter* species fermented glucose only (Figure 9 E).

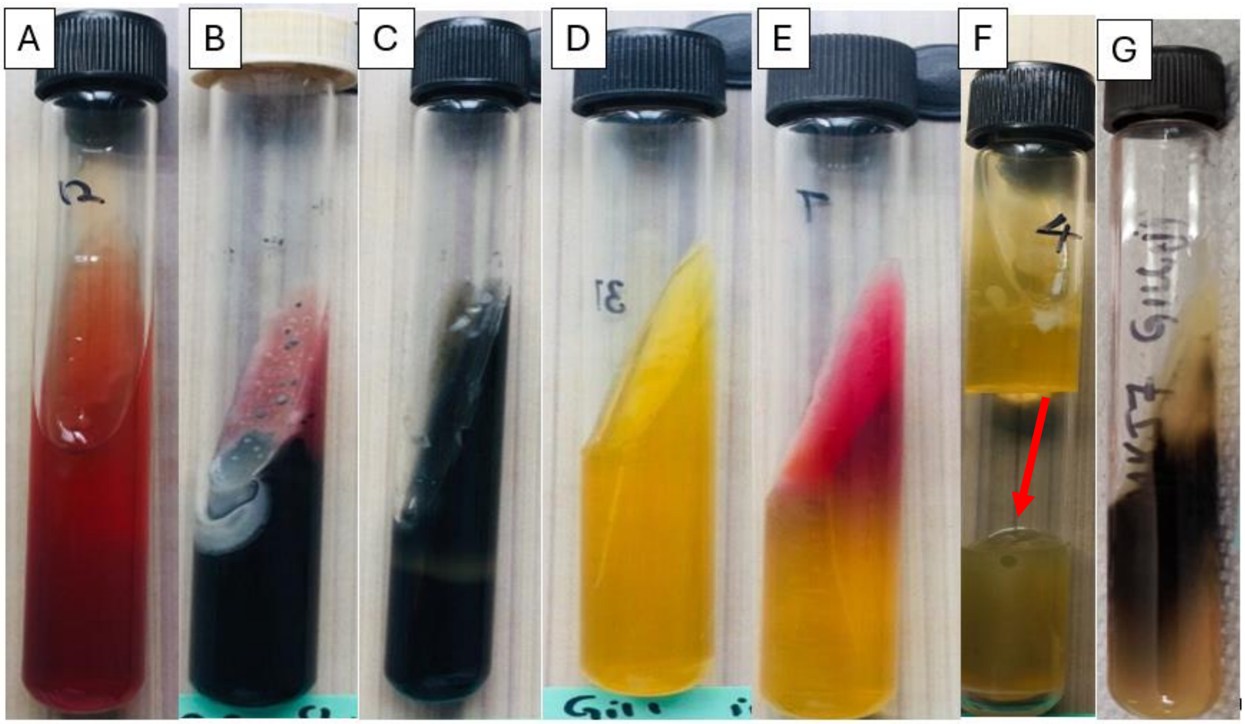


Figure 9: Reactions of some bacteria isolates in Triple sugar ion agar

Key: A, D and E= negative for hydrogen suphide gas production; B, C and G=positive for hydropgen sulpphide gas production (blackening); D and E= negative reaction and F=positive gas production; E and G = positive motility; A, B, C, D and G non-motile.

4.1.2.4 Further characterization of the bacterial isolates using MALDI-TOF

Empedobacter falseni had the highest score value of 2.52, *Acinetobacter soli* had 2.5, *Bacillus cereus* 2.4, *Escherichia coli* 2.33 and *Psudomonas putida* 2.25 (Appendix 5).

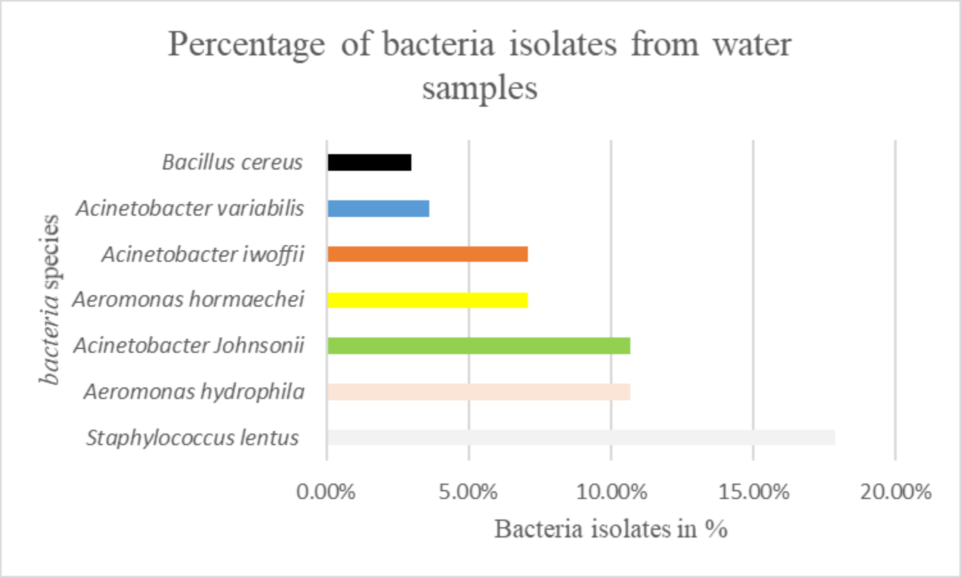
4.2 Potential pathogenic and zoonotic bacterial isolates from fish and water samples

Potential pathogenic and zoonotic bacteria isolated from fish organs and water samples include *Staphylococcus lentus* 12.9% (77/596), *Bacillus cereus* 8.1% (48/596), *Aeromonas veronii* 7.6%

(45/596), *Aeromonas hydrophila* 5.2% (31/596), *Acinetobacter johnsonii* 4.4 (26/596), *Acinetobacter iwoffii* 3.4 (20/596), *Escherichia coli* 3.2 (19/596), *Bacillus pumilus* 3.0% (18/596), *Pseuodomonas fulva* 2.9 (17/596), *Microbacterium oleivoran* 2.7 (16/596), *Klebsiella pneumoniae* 1.7% (10/596) and *Streptococcus scuiri* 1.7% (10/596) (Appendix 7).

4.2.1 Potential pathogenic and zoonotic of bacteria isolated from source water

Potential pathogenic and zoonotic bacteria isolated from water were; *Staphylococcus lentus 17.9%* (5/28), *Aeromonas hydrophilla 10.7 %* (3/28), *Aeromonas hormaechei 7.1%* (2/28), *Acinetobacter johnsonii* 10.7 % (3/28), *Acinetobacter iwoffii* 7.1% (2/28), *Acinetobacter variabilis* 3.6% (1/28) and *Bacillus cereus* 3.6% (1/28) (Figure 10; Appendix 7).



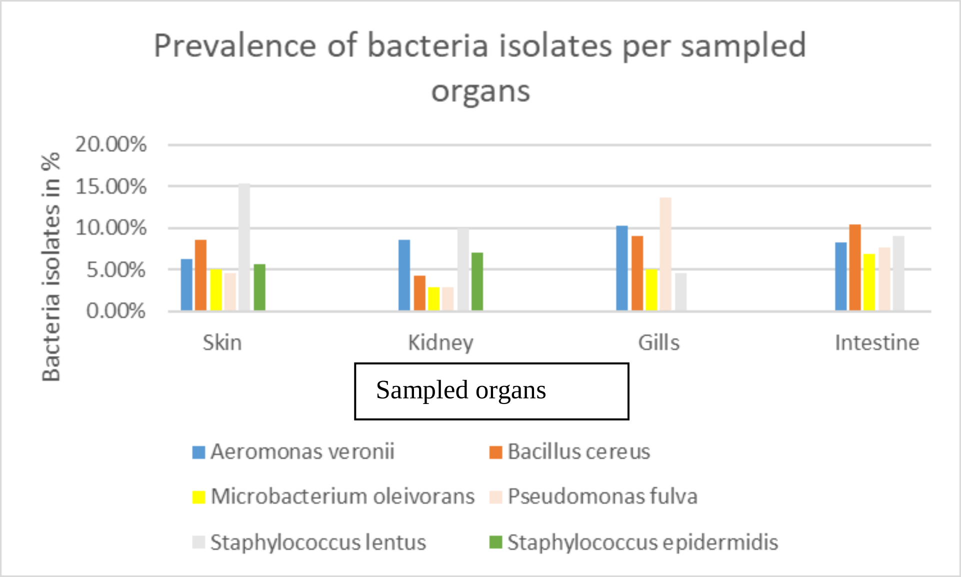
Key: %=Percentage

Figure 10: Prevalence of bacteria isolated from source water

4.2.2. Prevalence of bacteria isolated from fish organs

The following bacteria were isolated from the gills; *Aeromonas veronii* 10.2% (18/177), *Bacillus cereus* 9.0% (16/177), *Lysinibacillus fusiformis* 5.1% (9/177), *Staphylococcus lentus* 13.6% (24/177) and *Aeromonas hydrophila* 4.5% (8/177). The skin had the following bacterial isolates; *Staphylococcus lentus* 15.4% (27/175*), Bacillus cereus* 8.6% (15/175), *Aeromonas veronii* 6.3% (11/175), *Staphylococcus epidermidis* 5.7% (10/175), *Microbacterium oleivorans* 5.1% (9/175) and *Pseudomonas fulva* 4.6% (8/175). *Staphylococcus lentus* 8.9% (13/146), *Bacillus cereus* 10.3% (15/146) *Aeromonas veronii* 6.8% (10/146), *Aeromonas hydrophila* had 8.2% (12/146) and *Lysinibacillus fusiformis* 7.6% (11/146) were the most prevalent bacteria from the intestine.

Bacteria isolated from the kidney included *Staphylococcus lentus* 10% (7/70), *Acinetobacter johnsonii* 7.1% (5/70), *Aeromonas veronii* 8.6% (6/70), *Microbacterium oleivoran* 4.3% (3/70) *Escherichia coli* 2.9% (2/70) and *Klebsiella varricola* 2.9% (2/70) ($p<0.05$). The prevalence of the bacteria isolates recovered from different organs are summarized in (Figure 11; Appendix 7).

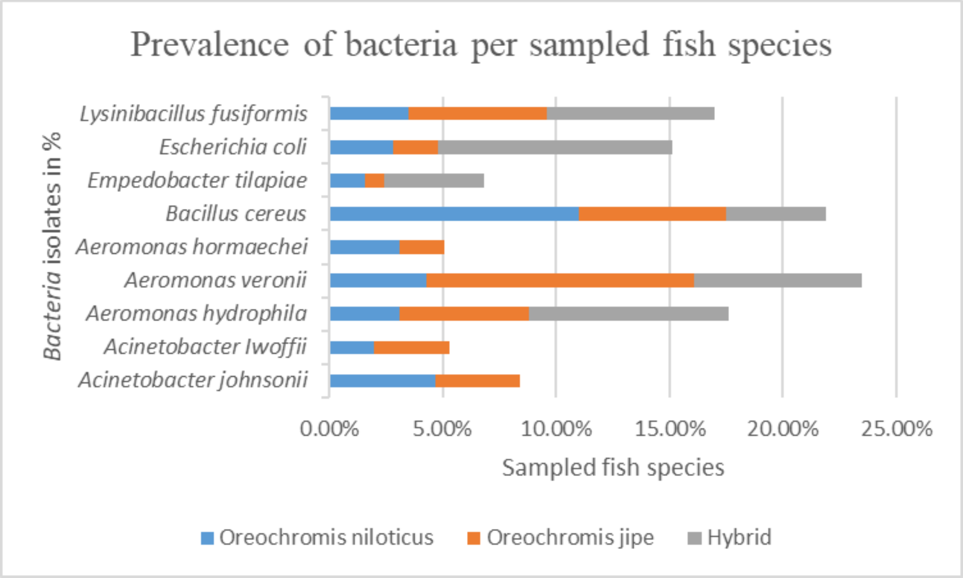


Key: % =Percentage

Figure 11: Prevalent of the most common bacterial isolates per organs sampled

4.2.2.1. Proportion of bacteria species isolated from different fish species

From the three sampled fish species 42.6% (254/596) of the bacteria isolates were recovered from *Oreochromis niloticus*. The most prevalent bacteria species from this fish were *Acinetobacter johnsonii* 4.7% (12/254), *Acinetobacter iwoffii* 2.0% (5/254), *Aeromonas hydrophila* 3.1% (8/254), *Aeromonas veronii* 4.3% (11/254), *Aeromonas hormaechei* 3.1 (8/254), *Bacillus cereus* 11.0% (28/254), *Empedobacter tilpiae* 1.6% (4/254), *Escherichia coli* 2.8% (7/254) and *Lysinibacillus fusiformis* 3.5% (9/254). *Oreochromis jipe* had 41.3% of the isolated bacteria species. The most prevalent bacteria were *Acinetobacter Johnsonii* 3.7% (9/246), *Acinetobacter iwoffii* 3.3% (8/246), *Aeromonas hydrophila* 5.7% (14/246), *Aeromonas veronii* 11.8% (29/246), *Bacillus cereus* 6.5%, *Empedobacter tilapiae* 0.8% (2/246), *Escherichia coli* 2.0% (5/246), *Lysinibacillus fusiformis* 6.1% (15/246) and *Aeromonas hormaechei* 2.0% (5/256). Total bacterial species isolated from the hybrid were sixty-eight (68). The most prevalent ones from the hybrid were *Aeromonas hydrophila* 8.8% (6/68), *Aeromonas veronii* 7.4% (5/68), *Bacillus cereus* 4.4% (3/68) and *Empedobacter tilapiae* 4.4% (3/68). There was a significant difference between the numbers of isolates recovered from the different fish species ($p<0.05$). A breakdown of the bacterial isolates by their frequency and prevalence per species of fish are shown on Figure 12 and Appendix 8.

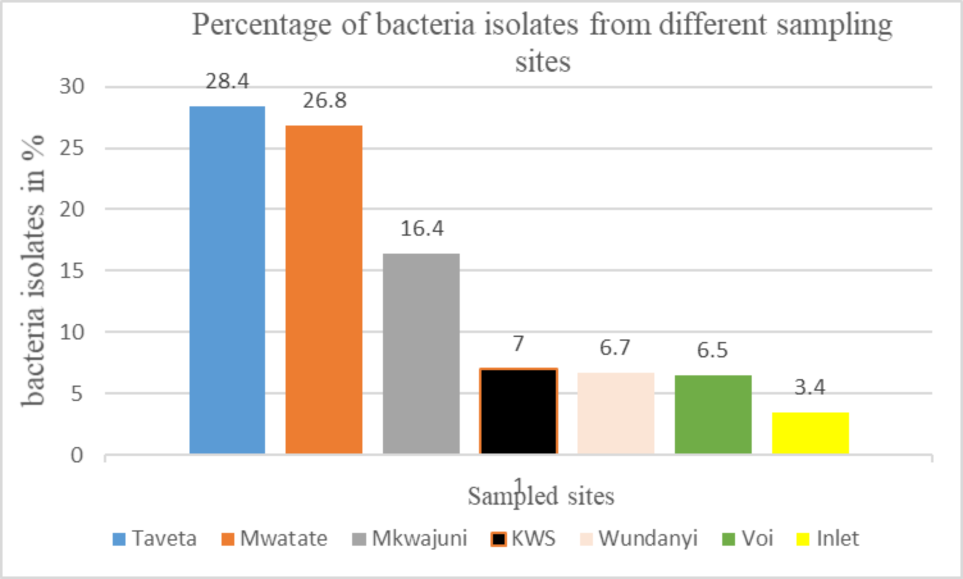


Key %=Percentage

Figure 12: Prevalence of bacterial isolates per sampled fish species

4.2.3. Prevalence of bacteria from different study sites

Prevalence of bacteria from fish samples per sampling site were as follows; 28.4% (169/596) of the total isolates were collected from Taveta, then Mwatate with 26.9% (160/596), Mkwajuni 16.4% (98/596), KWS 7.0% (42/596), Wundanyi 6.7% (40/596), Voi 6.5% (39/596) and the River Lumi Inlet had a 3.4 % (20/596) [$p<0.05$] (Figure 13; Appendix 9).



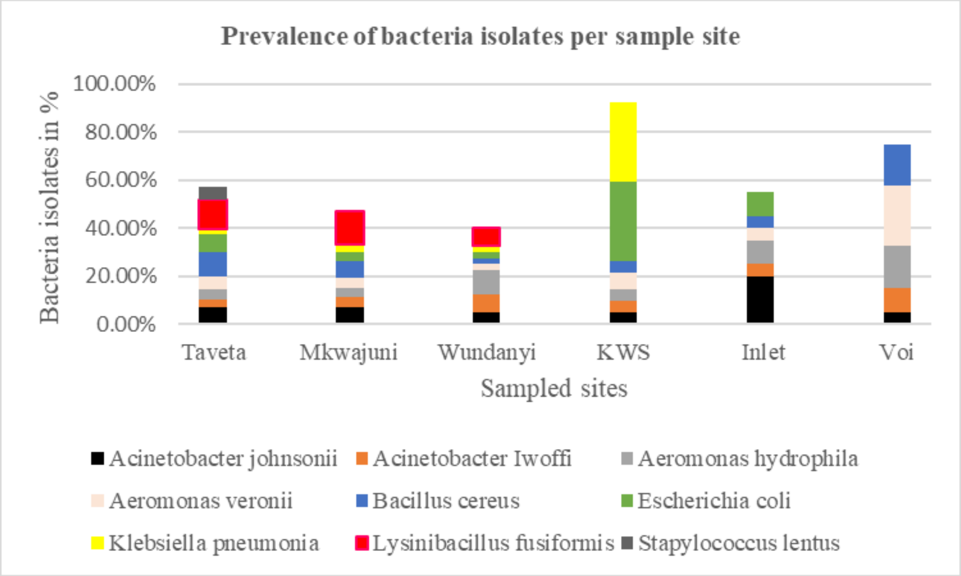
Key: KWS=Kenya Wildlife Service, %=Percentage

Figure 13: Percentage of total bacterial isolates from different sites

The most prevalent bacteria isolates from the River Lumi inlet were *Staphylococcus lentus* 10.0%, *Psudomonas stutzeri* 5.0%, and *Klebsiella pneumonia* 5.0% and from Mwatate, *Acinetobacter iwoffi* 5.0%, *Aeromonas hydrophila* 5.0%, and *Aeromonas veronii* 5.7 (Appendix 8). In Mkwajuni, *Acinetobacter johnsonii* 7.1%, *Aeromonas hormaechei* 3.3%, and *Aeromonas veronii* 4.0% were the most prevalent. From Voi, *Acinetobacter johnsonii* 5.0%, *Aeromonas hormaechei* 10.0%, and *Bacillus cereus* 17.5% were the most prevalent bacteria. While in

Wundanyi, *Acinetobacter iwoffii* 5.0%, *Aeromonas hormaechei* 7.0%, and *Aeromonas hydrophila* 10.0% were the most preavelent. In Taveta and the KWS the most prevalent bacteria were; *Aeromonas veronii* 4.8%%, *Aeromonas hydrophila* 4.8% and *Escherichia coli* 4.8% as the most prevalent (Figure 14; Appendix 8).

Bacteria isolates common in lake and aquaculture water were; *Staphylococcocus lentus, Bacilus cereus, Aeromonas hydrophila, lycinibacilus fusiformis, Acinetobacter johsonii* and *Priestia megaterium.*



Key: KWS=Kenya Wildlife Service,%=Percentage, Inlet = River lumi intake

Figure 14: Prevalence of different bacterial isolates from different sites

4.2.4. Prevalent of potentially zoonotic and pathogenic bacterial isolates

Possible zoonotic bacteria isolated were *Bacillus cereus*, *Aeromonas veronii*, *Aeromonas hydrophilla*, *Escherichia coli*, *Klebsiella pneumoniae* among others *(*Appendix 9*).* Some of potential fish pathogens included; *Bacillus cereus (*8%; 48/596), *Aeromonas veronii* (8%; 45/596), *Aeromonas hydrophila* (5%; 31/596), *Acinetobacter Johnsonii* (4%; 26/596), and *Escherichia coli* (3%; 19/596) (Table 4; Appendix 10).

Table 4: Potential pathogenic and zoonotic bacteria

Bacteria genera	**Bacteria species**	**Bacteria isolates**	**Percentage**	**Pathogenic**	**Zoonotic**	**References**
Bacillus species	*Bacillus cereus*	48	8.05	+	+	Mursyidah *et al.*, 2021
Aeromonas species	*Aeromonas veronii*	45	7.55	+	+	Ziarati *et al.*, 2022; Irshath *et al.*, 2023
	Aeromonas hydrophilla	31	5.2	+	+	Ziarati *et al.*, 2022; Basri *et al.*, 2020
	Aeromonas hormaechei	16	2.68	New	-	Not known
	Acinetobacter johnsonii	26	4.36	+	+	Cao *et al.*, 2018
	Acinetobacter solii	26	4.36	-	+	Not known
	Acinetobacter lwoffii	20	3.35	+	+	Arafa, 2021; Cao *et al.*, 2018
	Acinetobacter gyllenbergii	1	0.17	Emerging+	+	Not known
Escherichia coli	*Escherichia coli*	19	3.18	-	+	Mumbo *et al.*, 2023; Bedane *et al.*, 2024
Pseudomonas species	*Pseudomonas fulva*	17	2.85	-	+	Stark, 2022
	Pseudomonas putida	5	0.8	+	+	Ziarati *et al.*, 2022; Rahman *et al.*, 2024
Klebsiella species	*Klebsiella pneumoniae*	10	1.67	+	+	Ziarati *et al.*, 2022; Xu *et al.*, 2022
Streptococcis species	*Streptococcus sciuri*	10	1.68	-	-	Not known
Leclercia species	*Leclercia adecarboxylata*	7	1.17	-	+	Zayet *et al.*, 2021

Empedobacter species	*Enterobacter cloacae*	4	0.67	+	+	Hardi *et al.*, 2018; Aly; Rawash *et al.*, 2019
Enterobacter species	*Enterobacter bugandensis*	3	0.5	+	+ New	Hardi *et al.*, 2018

Key: += (pathogenic or zoonotic), normal=commensal bacteria, new=bacteria isolated for the first time

4.3 Antibiotic resistance of selected bacteria isolated from fish organs and source water

In this study, all the bacteria showed 100% (45/45) resistance to Oxacillin (OX), followed by ampicillin at 42.2% (19/45), co-trimoxazole (CTX) 28.9% (13/45) and Streptomycin (S) 6.7% (3/45). All the organisms were 100% (45/45) sensitive to tetracycline (TET) and gentamicin (GEN), 93.3% (42/45) sensitive to Streptomycin (S), 71.1% (32/45) sensitive to co-trimoxazole (CTX) and 57.8% (26/45) sensitive to ampicillin (AMP) (Figure 15; Appendix 10).

The antibiogram susceptibility of reference strains showed that they were 100% resistant to ampicillin (AMP) and Oxacillin (OX), 100% susceptible to Ciprofloxacin and sulpamethoxazole (SX), 66% resistant to streptomycin (S) and 33% resistant to tetracycline (TE).

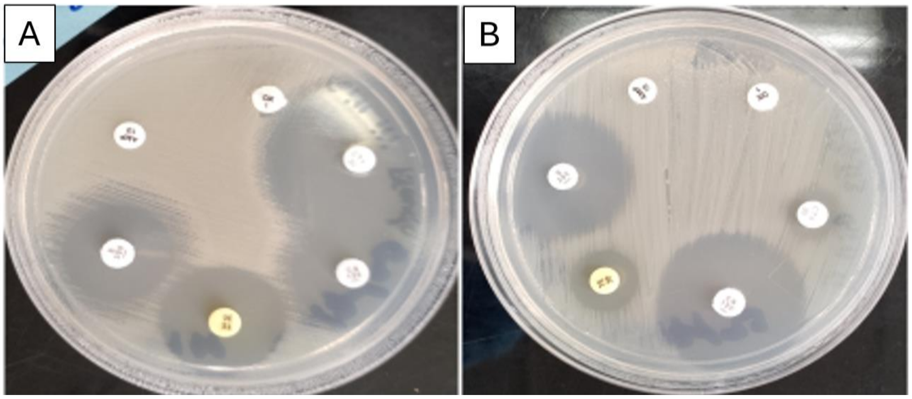


Figure 15: Antibiotic susceptibility patterns for selected bacteria

Actinobacter soli (A) and (B) *Staphylococcus lentus* showing sensitivity (clear zone around antibiotic disc) and resistance zones (confluent growth around the antibiotic disc) selected from the tested bacteria genera to illustrate Kirby-Bauer disc diffusion method.

Forty-two (42.2%; 19/45) bacteria showed Multiple Drug Resistance against the antibiotics tested, the highest was observed in *Acinetobacter iwofii* (0.5600), *Bacillus pumilus* (0.500), *Klebsbiella pneumoniae* (0.333) and *Streptococcus sciuri* (0.067). The least AMR was observed from *Staphylococcus lentus* at 0.042 and *Pseudomonas fulva* at 0.083 (Figure 16; Appendix 5). The multiple antibiotics resistance (MAR) index ranged from 0.042-0.56 with *Acinetobacter iwoffii* having the highest index value.

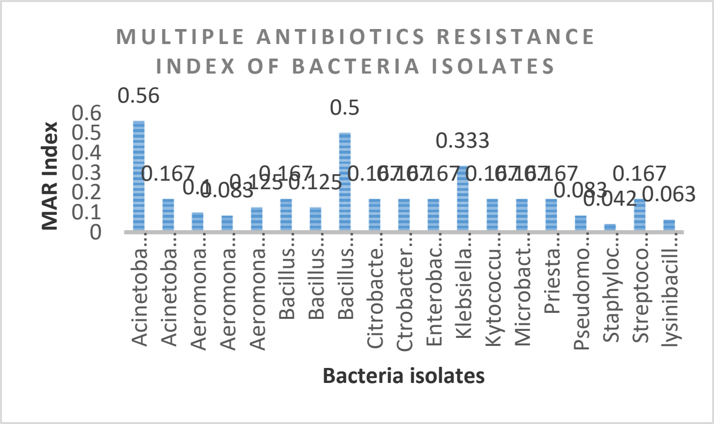


Figure 16: Multiple Antibiotics Resistance (MAR) index of bacterial isolates

4.4 Physicochemical parameters in the source pond and lake Jipe water

From the different sampling sites, temperatures ranged between (20.68 -25.38^{O}C) which is within the normal range of what is expected for tilapia farming apart from Wundanyi farm which was 20.68^{0}C. Wundanyi farm and lake Jipe were found to have the highest concentrations of dissolved oxygen, Wundanyi farms also had the highest concentration of Nitrates and Ammonia. The pH and electrical conductivity range was between 8.2 and 10.9, with lowest value in Mwatate and highest in Wundanyi pH. Electrical conductivity was found to be highest in the lake compared to the farms. Voi farm had the highest concentration of phosphorous. Total suspended solids and turbidity levels were highest at Taveta farms (Table 5). Other related findings are as shown in figure 17A (Voi farm) over fertilized fish pond; figures 17 B (Mwatate farm) and C (Taveta farm) are showing overgrowth with excess vegetation around the fish pond and figure 17 D (Wundanyi farm) shows bird droppings (black arrow) on fish pond liner.

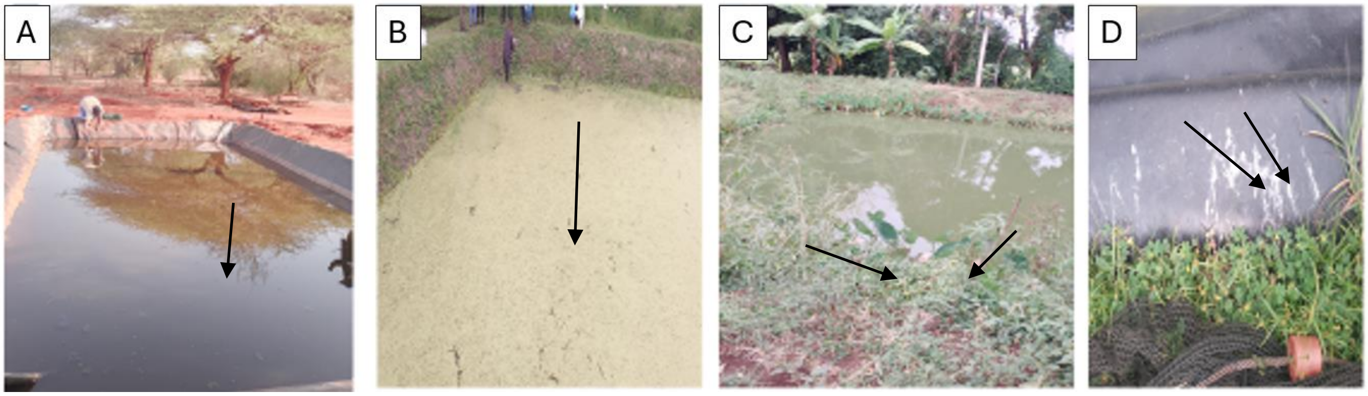


Figure 17: General observations of some sampled fish ponds that have effect on physicochemical factors

Table 5: Water quality factors in different locations

	Physiochemical factors								
Farms	**D.O (mg/L)**	**Temp (^{0}C)**	**P(mg/L)**	**EC (Ds/m)**	**Turbidity(mg/L)**	**TSS (mg/L)**	**NH3(aq) (mg/L)**	**N03 (mg/L)**	**pH(H^{+})**
Voi Farm	4.72±1.28	25.38±0.7	4.61±5.78	0.49±0	9.8±0	200±0	0.50±0	3.36±0	8.7
Mwatate Farm	5.18±1.01	22.15±0.13	7.8±0.53	0.26±0	13.2±0	240±0	0.841±0	7.01±0	8.2
Wundanyi	8.46±0.38	20.68±0.41	5.5±7.64	0.04±0	7.2±0	160±0	0.11±0	9.81±0	10.9
Taveta Farm 1	7.3±0.17	22.37±0.06	7.22±4.77	0.27±0	6.4±0	120±0	0.6±0	4.48±0	9.6
Taveta farm 2	3.85±0.49	21.98±0.33	5.51±4.66	0.29±0	45.2±0	640±0	0.36±0	5.6±0	8.3
Lake Jipe	6.55±2.62	23.0±0	4.91±5.63	0.6±0.1	21±7.92	340±28.28	0.56±3.97	4.62±0.59	10.6
Optimal levels	5-7	15-30	0.03-2	100-2,000	20-40	1000-40,000	0	0.1-0.4	6.5-8.5

Mean ± SD values for physiochemical factors of ponds water and lake water in different sub-counties in Taita-Tavata County.

DO: dissolved oxygen, P: phosphorus, EC: electrical conductivity, NO3: Nitrates, NH#: Ammonia; TSS: total suspended solids.

4.4.1. Effect of physico-chemical factors on bacteria recovered fom different sites

Bacterial isolates were high in Taveta farms followed by Mwatate and Mkwajuni (>70) with aquacultural farms having higher than lake (except at Mkwajuni beach). High phosphorous influenced isolation of many bacteria species (especially emerging ones) unlike low levels of phosphorous. Low dissolved oxygen seems to influence prevalence of emerging bacteria than high dissolved oxygen. High Phosphorous, pH and ammonia were associated with high number of pathogenic and zoonotic bacterial isolates in particular *Aeromonas* spp (*A. hydrophila, A. veronii, A. hormaechei*); *Pseudomonas* (*P. fulva, P. putida, P. stutzeri*); *Bacillus cereus* and *Klebsiella pneumoniae* in respective sites. Zoonotic *E. coli*, *Stretococcus scuiri* and *Microbacterium oleivorans* were isolated only in areas with high DO and low phosphorous levels in the lake. *Lactococcus graviae* was isolated in Wundanyi sites that had low Temperature, high ammonia and high pH (Table 6).

Table 6: Effects of physico-chemical water parameters on the species of bacteria recovered from different sites

Sampling sites	Occurrence of bacteria per site	Important physico-chemical parameters	Emerging bacteria isolated	Pathogenic and zoonotic bacteria
Voi	6.9% (39/568)	Low DO high NH_3	*Acinetobacter johnsonii, Aeromonas hormaechei* *Empedobacter tilapiae*	*Cytobacter kochi, Aeromonas hormaechei*
Mwatate	28.2% (160/568)	High NH_3 High phosphorus	*Lysinibacillus fusiformis* *Klebsiella pneumoniae*	*Aeromonas hormaechei, A. hydrophila, A. veronii, Bacillus cereus, Citrobacter brakii,*

				Klebsiella pneumoniae, Pseudomonas fulva, *P. putida, P. stutzeri.*
Wundanyi	7.0%(40/568)	Low Temp High NH3 Hgh pH High NO_3	*Cryseobacterium gambrini Bacillus thuringiensis* *Klebsiella varicola* *Priestia megaterium*	*Acinetobacter iwofii, Aeromonas veronii, Bacillus cereus, Lactococcus garvieae, Pseudomonas putida.*
Taveta Farms	29.8%(169/568)	High phosphorus High pH Low DO Very high TTS	*Psudomonas fulva* *leclercia adecarboxylata* *Empedobacter tilapiae Corynebacterium jeikem*	*Acinetobacter johnsonii, A. iwoffii, Aeromonas hormaechei, A. hydrophila, A. veronii, Klebsiella pneumoniae, Psudomonas fulva, P. stutzeri*
Lumi inlet	3.5%(20/568)	Low phosphorus Low DO	*Exiguobacterium indicum, Strenotrophomona maltophila*	*Klebsiella pneumoniae, Pseudomonas stutzeri*
KWS	7.4%(42/568)	High DO Low phosphorus	*Enterobacter cloacae,* *Kytococcus sedenatr*	*Acinetobacter johnsonii, Aeromonas hydrophila, A. veronii, Enterobacter cloacae, Escherichia coli, Psudomonas stutzeri, Stretococcus Scuiri*
Mkwajuni	17.3% (98/568)	High DO Low phosphorus	*Leclercia adecarboxylata, Microbacterium oleivorans*	*Leclercia adecarboxylata, Microbacterium oleivorans*

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussion

Fish diseases have caused serious challenges to aquaculture expansion and management. In addition, some pathogens associated with aquatic environment have been found to be zoonotic and of public health concern (Subasinge *et al*., 2023).In this study, there was more Gram negative bacteria isolates identified compared to Gram positive bacteria isolates which agreed with Dissasa *et al.* (2022) in a study on live, processed and water samples of Ethiopian rift valley lakes athough at different percentage. Wanja *et al.* (2020) also identified more Gram negative bacteria compared to the Gram positive bacteria. Similarly, Wanja *et al.* (2020) also identified four species that were common to the current study including; *Aeromonas hydrophilla, Klebsiella pneumoniae, Enterobacter cloacea* and *Escherichia coli*. His study however, differed from the current study in that the researchers identified 17 bacteria genera and 18 species while in the current study 23 genera and 53 bacteria species have been identified.Wamala *et al.* (2018), identified two important pathogens; *Aeromonas hydrophila* (43.8%) and *Pseudomonas stutzeri* that were common in this study. Wamala *et al*. (2018) study differed from the current study in reference to the number of bacteria genera and species identified and also on the species of fish that they sampled.

Bacillus cereus, Aeromonas veronii and *Aeromonas hydrophila* were the most predominant pathogenic bacteria isolated, the three leading bacteria were also found in pond water, confirming the important inter-relationship between bacteria prevalence of source water (Wanja *et al*., 2020; Charo *et al*., 2022). Santos *et al.* (2023) identified *Aeromonas veronii* as a highly pathogenic bacteria to *Oreochromis niloticus* juvenile fish, isolation of this pathogen from organs of fish and culture water in the current study is an indication of possible pathogenic presence of a pathogenic strain. Unlike Santos *et al*. (2023) study differed from the current study in that the researchers conducted an experimental study on the juvenile *Orechromis niloticus* fish while in this study examined both wild and cultured fish of different *Oreochroms* species were purposively sampled from farms and the Lake.The high number of isolated pathogenic bacteria can be attributed to insufficient biosecurity including; pond disinfection, stocking of ponds using fingerlings from authenticated sources and use of poor fish feeds as reported by Charo *et al.* (2022).

Aeromonas hydrophilla (31%) was the predominant isolate identified in the sample population, in consistent with the results of Charo *et al.*, (2022) who reported similar findings although with varying intensity unlike this study. Charo *et al.* (2022) isolated *flavobacterium species* which were not reported in this study. *Acinetobacter* species were also some of the most prevalent bacteria. The isolation of these bacteria in fish is a threat to achieving profitable aquaculture farming enterprise in Taita Taveta County.

Potential zoonotic bacteria isolated include *Bacillus cereus, Escherichia coli, Klebsiella, Lactococcus, Staphylococcus, Acinetobacter* and *Streptococcus,* these results were similar to those of Chitambo *et al*. (2023). However their findings differed with the current findings in that *Closridium, Aequorivita, Aerococcus, Dermatophilus, Enterococcus, Flavobacterium, Planococcus and Proteus Rhodococcus* were not isolated from the current study. Haenen *et al.* (2023) also positively identified zoonotic fish bacteria associated with aquaculture farms including, *Streptococcus, Aeromonas and Enterobacterium* which were common in the current study findings. This shows that fish farmers and fishermen of lake Jipe in Taita-Taveta County are at risk of contracting these diseases if proper biosecurity is not institutionalized. Incidences of zoonosis from fish bacteria pathogens can be reduced by consumption of well cooked fish products and ensuring proper biosecurity at farm level as reported by Ziarati *et al.* (2022).

The findings of important pathogenic bacteria such as *Aeromonas hydrophilla, Aeromonas veronii, Bacillus cereus, Escherichia coli, Lysinibacillus fusiformis, Priestia megaterium and Staphylococcus lentus* isolated from the intestines was in conformity with a study by Helmi *et al.*(2020) who reported similar results. Helmi *et al.* (2020) reported *Aeromonas hydrophila, Yersinia ruckeri and Staphylococcus epidermidis,* to be pathogenic to *Oreochromis niloticus,* however, from the current study *Klebsiella pneumoniae* and *Escherichia coli* were the most important zoonotic bacteria isolated.

There was an equal representation of bacteria isolated from fish and water samples confirming that aquatic systems where fish grows has an influence on the fish bacterial composition as repoted by El Bably *et al.* (2010). The isolation of different bacteria species from water samples were in partial agreement with those of Wanja *et al.* (2020).This study however, did not isolate *flavobacterium, citrobacter, enterobacterium* and *proteus* which were reported by Wanja *et al.* (2020). Wanja *et al.* (2020) did not isolate *Klebsiella* from the source water pond which was present in the current study. This can be attributed to the difference in fish pond management practices by farmers at the different sampled areas and also the variable climatic condition. Another study on bacteriological study of pond water for aquaculture by Ajayi and Okoh (2013) reported similar findings although at different percentage from the current study. This confirms that water used for purposes of rearing fish determines the composition of bacterial flora of fish, although most bacteria are naturally found in the aquatic system. Also Irshath *et al.* (2023) isolated *Aeromonas hydrophila* form water from culture systems, which was in conformity with this study finding, however the current study did not isolate *Vibrio, Edwardsiella tarda, Flavobacterium* and *Pseudomonas anguillarium* species.

Nine genera (9) including *Acinetobacter Courvalinii, Acinetobacter gyllenbergii, Acinetobacter schindleri, Cryseobacterium gambrini, Enterobacter tilapiae, Lactococcus garvieae, Pseudomonas stutzeri, Staphylococcus ureilyticus* and *Staphylococcus haemolyticus,* were isolated for the first time in *O. jipe*. Intensification in fish farming is characterized by high stocking density, anoxic condition, and increased human-fish interactions; this coupled with with the current trend of aquatic pollution and climate change may make normal bacteria flora more important fish pathogens as reported by Wamala *et al.* (2018). This therefore can be one of the reasons for high number of bacteria isolation from sampled sites in Taita-Taveta County.

Low bacteria isolation from the kidneys in this study is in agreement with the findings of Charo *et al.*(2022) although at variable percentage in their study, however, Charo *et al.*(2020) observed more bacteria isolates in intestines compared to the skin which was different from this study. *Wanja et al*. (2020) also reported similar results which was in agreement with the results from this study. High bacterial count on the fish skin may be due to contamination by normal aquatic bacteria flora as well as contamination of the fish during handling, example of bacteria isolated that are indication of contamination are *Pseudomonas* and *Streptococci* which were also reported by Shinkafi and Ukwaja (2011). Isolation of 12% of the bacteria from the kidney confirms the presence of bacteremia or septicaemia in the sampled fish. This is an indication of fish infection after suppression of fish’s internal defense mechanisms as reported by Pech *et al*. (2017).

Different bacteria commensals isolated from the current study were, *Staphylococcus epidermids, Bacilus pumilus, Staphylococcus ureilyticus* and *Peribacillus simple*. Although most *Enterobacteriaceae* are normal bacteria flora causing no harm to the host, but some such as *E. coli, Klebsiella penumoniae* and *Pseudomonas spp*. are capable of causing human diseases as reported by Pech *et al*. (2017). *Bacillus, Aeromonas and Enterococcus* are bacteria occurring naturally in fish guts and can be used as probiotics in aquaculture industry additives as reported by Cui *et al.* (2023) and Austin (2006).

In terms of bacteria diversity from farms and the Lake, 12 bacterial genera were common from aquaculture set up and also from the wild, this therefore shows fish from both wild and aquaculture set up can be affected by the same bacteria community, this results also agrees with those of Ajayi and Okoh (2013). Therefore, positive isolation of these micro-organisims proves lack of proper biosecurity measures and could potentially become harmful to both fish and fish handlers across the value chain.

In reference to bacteria distribution among species studied, 10 bacteria genera were common in the *Oreochromis niloticus*, *Hybrid* and *Oreochromis Jipe,* this shows that different fish species can be affected with the same bacterial pathogens while in the same environment, similar findings were also reported by Kerie (2019) and de Pádua *et al.* (2014). This therefore confirms that isolation of these bacterial species in the fish poses a great challenge to sustainable development of the aquaculture sector and also conservation of endemic fish species as they may cause mass fish mortality. The presence of members of family genus *Aeromonas, Bacillus, Enterobacter, flavobacter, Klebsiella, E.coli, Micrococcus, Pesesudomonas, Staphylococcus* and *Streptococcus* in Taita-Taveta posses a public health concern to people who are in a constant contact with fish and their by-products as reported by Olga *et al.* (2023); Boylan (2011); Ziarati *et al.* (2022) and Ahmed (2019) in other studies elsewhere

Aquatic environment has a lot of micro-organisms, some of which are potentially pathogenic to fishes and others opportunistic under favorable environmental conditions (Low Dissolved oxygen, high temperature, high organic material load, acidic or basic pH condition (Olga *et al.*, 2023). Dissolved oxygen (DO) is crucial water quality parameter in the lake ecosystem that can cause massive fish kills if depleted and it is normally used as an indicator of high organic load and biological activity (Verma *et al.*, 2023). Dissolved oxygen results showed the lowest averages at Taveta Farm two and at Voi Farm, this might be attributed to organic degradation by oxygen consuming micro-organisms and also respiration of plants that were grown inside the pond, which agrees with (Elvines *et al.,* 2023).

Temperature is another water quality parameter that affects the physiology, growth, reproduction and metabolism of aquatic flora and fauna. The optimum temperature range for normal development, reproduction and growth of tilapia is about 22-28°C, depending on the fish species (Ikeogu *et al.*, 2010a). The temperatures of the farms and lake were within the acceptable range apart from Wundanyi farm which was 20.68^{0}C lower than 22.0^{0}C, this might be because the farm is located in the wet areas of Taita-Taveta County.Current study foubd out that *Aeromonas hydrophila* was reported in almost all farms. Semwal *et al.* (2023) associated this to high temperatures where it is capable in surviving in the optimal temperature range for tilapia. However, it occurred in Wundanyi with suboptimal temperature for tilapia. May be due to other factors.

Ammonia concentration (NH3) was found to be above the recommended level of 0-0.025 (mg/L) in the farms and in the lake, this can be attributed to usage of organic manure for ponds fertilization, overfeeding and increased siltation , this was in agreement with research findings by Ikeogu *et al.* (2010) who attributed this to overfeeding and over fertilization of ponds using organic manure. According to a study by Verma *et al.* (2023), a rise in ammonia concentration is attributed to increased metabolism by fish and bacteria during decomposition of organic waste such as unconsumed fish feed, dead planktons as well as organic fertilizers. Lethal effects of ammonia are gill erosion, and destruction of mucosal glands "sub-lethal" effects such are reduced growth, poor feed conversion, and reduced disease resistance, osmoregulatory imbalance, and kidney failure. These findings of increased ammonia levels were in agreement with those of Chen *et al.*(2015) who reported that fish feed are the main entry point of nitrogenous protein waste in pond , only 30% of the feed given to fish in the pond is utilized for growth and weight gain while the remaining 70% enter the culture water as uneaten feeds and feces or excreted as ammonia. From this study high ammoiaum in farms were associated with *Aeromonas, Psudomonas, Bacillus* and *Klebsiella* species as reported by Chitambo *et al.* (2023).

The optimal nitrates concentration in aquaculture system is zero. In this study findings, the nitrates concentration was above normal for all farms and the lake. The highest was reported at Wundanyi farm with 9.81 and the lowest at Taveta farm one at (4.4mg/L). High nitrate concetration can be attributed to poor feeding and anoxic sediments contitions and low temperatures as explained by Lu *et al.* (2021) who also reported the high ammonia concentration in regions of low temperatures. One potential risk to aquatic organisms exposed to high conductivity in aquatic ecosystems is that fluctuations in levels of dissolved solids require organisms to exert energy in maintaining osmoregulatory balance (Armstead *et al*., 2016) .

Phosphorus is a crucial plant nutrient and the most limiting nutrient in aquatic systems and necessary plankton development, which is used as alternative source of feed for fish in aquatic systems. In water, it is in the form of phosphate, which is generally present in surface water as attached to living or dead particulate matter, and in the soil as insoluble $Ca_3\,(P04)_2$. In this study, the phosphorus concentration was above normal in all study sites. The highest levels were measured at Mwatate farm with a concentration of (7.8 mg/L) and the lowest at the lake Jipe (4.91 mg/L), this finding might be attributed to the fact~~s~~ that ponds at Mwatate farm did not have a frequent water flow in and out of the ponds while the lake ecosystem contained both the inlet and the outlets maintaing a balanced ecosystem.This current findings were also reported by Carpenter (2005) who recorded high concentration of phosphorus on the river outlet compared to the inlate, however Capenter (2005) study differed from the current one in that he also sampled fish from the rivers' ecosystem. High concentation of phosphorus was associated with isolation of *Pseudomonas* and *Bacillus* as reported by Timofeeva *et al.* (2022) although they did not associate *Aeromonas* and *Acinetobacter* species, to its high level in the environment.

The pH optimal range is 6.5-8.5. In this study, the pH was above the normal range in all the sampled sites confirming the basic nature of Taita Taveta soils and therefore need for liming of aquaculture ponds in the area. High pH in aquaculture systems increases stress in fishes and negatively affects their growth and reproduction as reported by Liu *et al.* (2023).

Antibiotic susceptibility patterns of *Acinetobacter soli, lysinibacillus*, *Staphylococcus lentus*, *Cytrobacter brakii*, *Escherichia coli*, *Aeromonas veronii*, *Klebsiella pneumoniae* and *Kytococcus* *sedentarius* spp showed high susceptibility to gentamicin and tetracycline in all the tested isolates, this means that gentamicin and tetracycline can be used for treatment of infections caused by these bacteria. In another study by Hamom *et al.* (2020), the reseachers reported high susceptibility of tetracycline to *Strepocococcus* and *Aeromonas* isolated from *Oreochromis niloticus*. Other studies in Kenya have found out that *Klebsiella, Escherichia coli, Citrobacter spp* are highly susceptible to tetracycline and gentamycin (Wanja *et al.*, 2020) . Another study by Wamala *et al.* (2018) who tested the susceptibility of oxacillin on *Aeromonas* spp found out that the bacteria was 100% resistant to the drug. However, 100% were found to be resistant to Oxacillin. In another study by Marijani (2022), the researchers reported high resistance of *E. coli* to gentamicin and tetracycline which was contrally to the current study findings. Multiple antibiotics resistance index (MAR) shows the degree of bacteria resistance in a given population. MAR index greater than 0.2 indicates that the bacteria strain is derived from an environment where different antibiotics are used. This study established that all the bacteria species tested except *Klebsiella pneumonia*, *Bacillus pumilus* and *Acinetobacter Iwoffii* showed an MAR ration of less than 0.2. Multiple antibiotic resistant bacteria might have found their way through water from these agriculture activities to the aquaculture ponds.

5.2. Conclusions

1. Farmed, wild fish and culture water harbor potentially pathogenic and zoonotic bacteria which may cause diseases in fish and pose public health risks.
2. *Aeromonas hormaechei* is being reported for the first time in fish, aquatic and capture environments in Kenya.

3. Aquaculture and the Lake Jipe were found to be potential reservoirs for human pathogens.
4. Some of the bacterial isolates showed high antimicrobial resistance to common antibiotics of human and animal health importance. Multi drug resistance was also observed in this study.
5. The physico-chemical parameters of the ponds and lake water were either above or below the recommended range. They also influenced the occurrence of various pathogenic and zoonotic bacteria.

5.3 Recommendations

- Fish and fish by-products for human consumption must be properly handled to prevent human infection by zoonotic bacterial fish pathogens.

- Seasonality study on occurrence of fish bacteria in fish farms and the lake is encouraged to understand the variation and correlation of the water quality and bacteria composition.

- Further research on pathogenicity of some bacteria isolated in this study is recommended.

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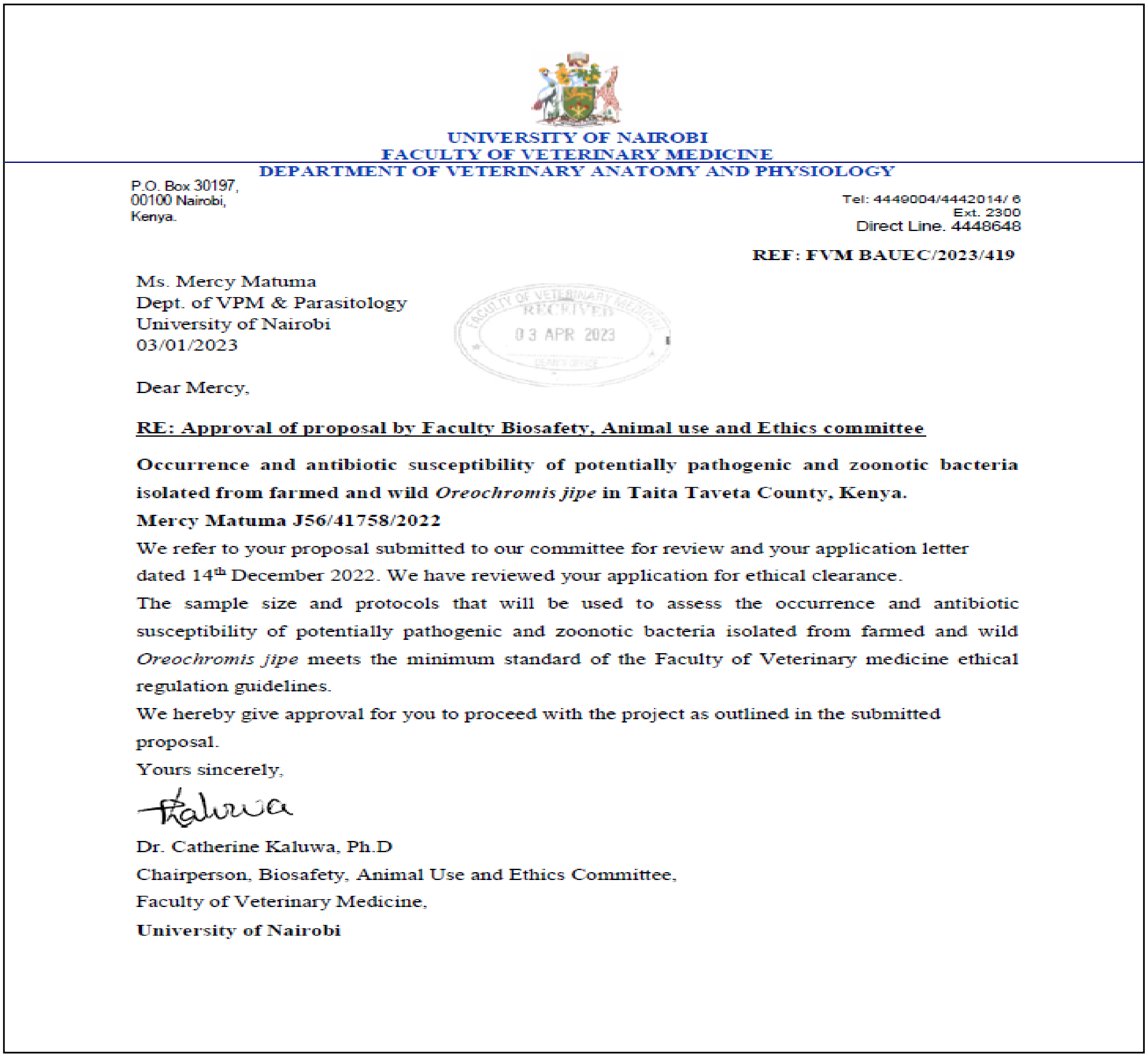
APPENDICES

Appendix 1: Biosafety, Animal use and Ethics committee certificate



UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2023/419

Ms. Mercy Matuma
Dept. of VPM & Parasitology
University of Nairobi
03/01/2023

RECEIVED
03 APR 2023

Dear Mercy,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

Occurrence and antibiotic susceptibility of potentially pathogenic and zoonotic bacteria isolated from farmed and wild *Oreochromis jipe* in Taita Taveta County, Kenya.

Mercy Matuma J56/41758/2022

We refer to your proposal submitted to our committee for review and your application letter dated 14th December 2022. We have reviewed your application for ethical clearance.

The sample size and protocols that will be used to assess the occurrence and antibiotic susceptibility of potentially pathogenic and zoonotic bacteria isolated from farmed and wild *Oreochromis jipe* meets the minimum standard of the Faculty of Veterinary medicine ethical regulation guidelines.

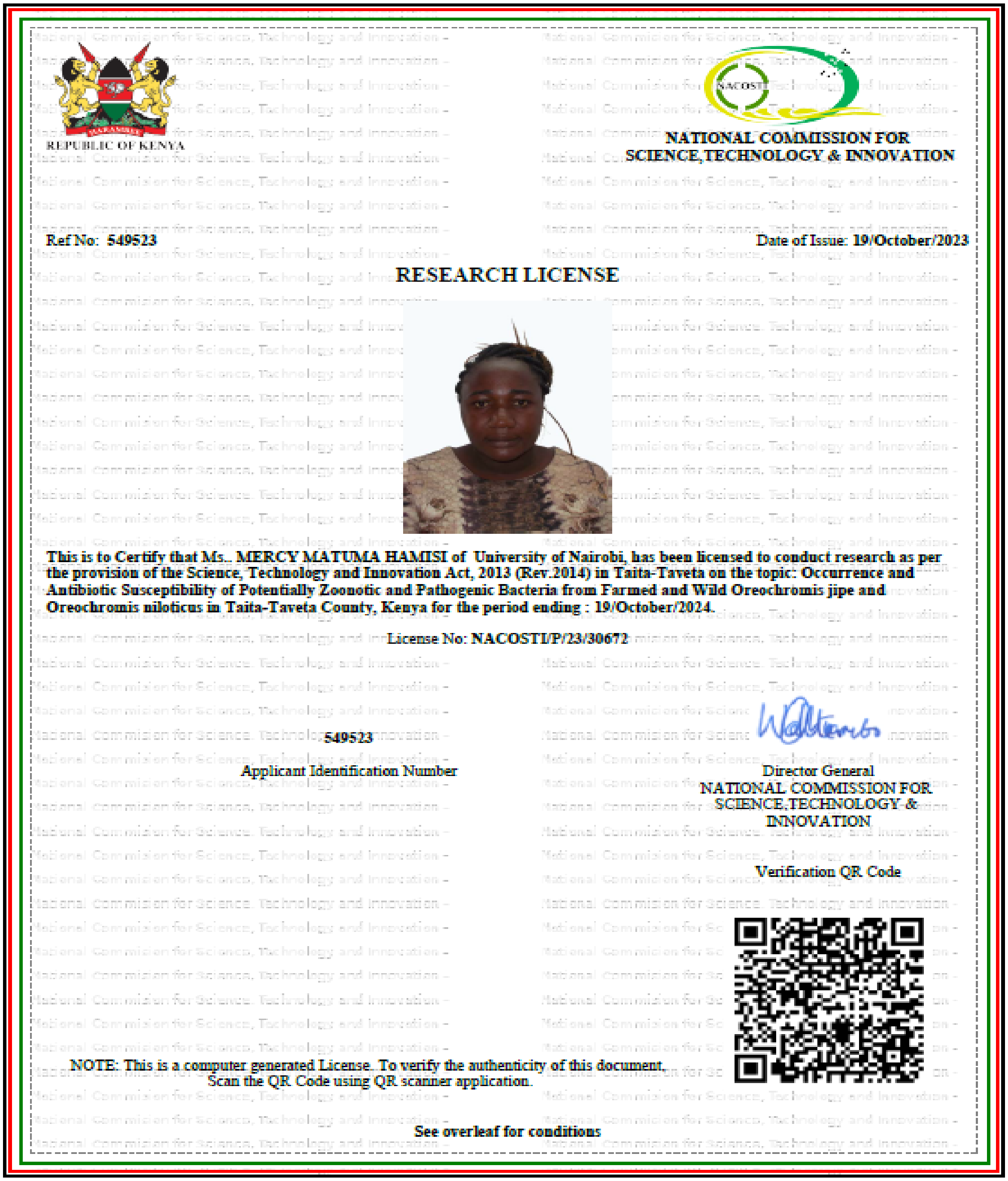
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix 2: National Commission of Science Technology and Innovation Research License



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Date of Issue: 19/October/2023

RESEARCH LICENSE

This is to Certify that Ms.. MERCY MATUMA HAMISI of University of Nairobi, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Taita-Taveta on the topic: Occurrence and Antibiotic Susceptibility of Potentially Zoonotic and Pathogenic Bacteria from Farmed and Wild Oreochromis jipe and Oreochromis niloticus in Taita-Taveta County, Kenya for the period ending : 19/October/2024.

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National Commission for Science, Technology and
Innovation(NACOSTI),
Off Waiyaki Way, Upper Kabete,
P. O. Box 30623 - 00100 Nairobi, KENYA
Telephone: 020 4007000, 0713788787, 0735404245
E-mail: dg@nacosti.go.ke
Website: www.nacosti.go.ke

Appendix 4: Phenotypic characteristics of bacterial isolates

Bacteria isolate	Gram stain	Catalase	Oxidase	Indole	MR	Citrate	H_2S	TSI	Glucose	Urease	Sucrose	Mannitol	BA	MAK	Other features
Aeromonas	-	+	+	+	-	+	-		+	-	+	+	H	LF	B-H
Pseudomonas	-	-	+	+	-	+	-		-	+	-	-	H	NLF	
Bacillus	+	+	-	-	-	-	-		-	-	-	-	H	NLF	Giant
Micrococcus	+	+	-	-	-	-	-		-	-	-	-	NH	LF	Pale yellow
Streptococcus	+	-	-	+	+	-	-		+	-	-	-	H	LF	Pin-point colonies
Staphylococcus															Pinpoint colonies
E. coli	-	+	-	+	+	-	+		+	-	-	+	NH	L.F	
Klebsiella	-	+	-	-	-	+	-		+	+	+	+	NH	LF	mucoid
Enterobacter	-	+	-	-	-	+	-		+	_	+	+	NH	LF	

Appendix 3: Conventional biochemical tests

Catalase test is using 3% H2O2 (hydrogen peroxide) that is placed on a clean microscope slide, using a sterile platinum wire loop, a loop-full of the bacteria colony was transferred on to the H202 on the microscope, observation of bubbles (effervescence), is a positive reaction. Catalase test detects the ability of a bacteria to produce catalase enzyme which breaks down hydrogen peroxide to water and oxygen. It is the oxygen gas produced that causes the effervescence.
Oxidase test detects the bacteria that produce cytochrome oxidase when it undergoes an electron transport chain. The filter paper used is soaked with the reagent tetramethyl -p phenylenediamine which develops dark blue-purple color when rubbed with an organism with oxidase enzyme giving a positive result after 10-30 seconds.
Indole test is carried out to detect the production of indole. 1 % peptone broth was put in a sterile bijou bottle. A bacterial inoculum was added using a sterile wire loop and incubated at 370C for 24 hours. 4-8 drops of Kovacs reagent were added and mixed. The observation was made for the development of cherry red color, black precipitation for H2s production indicating a bacteria spread for mobility.
Methyl Red Voges Proskauer (MR-VP) test is used to detect organism's ability to maintain stable acid or not as some organisms undergoes mixed acid fermentation. For this test GPPW broth was inoculated and incubated overnight at 37^{o}C. After incubation 4-6 drops of methyl red was added to the culture broth which turns red at low Ph which is a positive result and yellow at high ph as a negative result.

Citrate test is used to test for organisms that use citrate as sole source of carbon for metabolism, Simmons citrate agar is used with Bromothymol blue as a pH indicator which gives Prussian blue. For this test, bacteria organisms are inoculated in a zigzag mannter on the simmon slant surface at 37^{0}C for 24 hours.
Urease test is used to detect organisms that produce nitrate reductase enzymes which reduces nitrate to nitrite.4 (four) grams of urea base was suspended in 900ml of distilled water, boiled to completely dissolve the agar and then autoclaved at 121^{0}C and 15 Pounds Per Square inch (PSI) for 15 minutes and then left to cool .4ml of 40% urea was added to the cooled agar aseptically and mixed thoroughly before dispensing 4ml into sterile bijou bottles. The bottles were slopped during cooling until agar solidified. The slant urea agar was then inoculated with bacterium and then incubated overnight at 37^{0}C. A pink color indicated a positive reaction whereas yellow color indicated a negative reaction.
Carbohydrate fermentation test is used to detect bacteria that can produce acid only with gas from carbohydrates. Glucose, Sucrose, Lactose and Mannitol were used. Peptone water sugar media was used with 1% of respective sugar. Peptone red indicator was added into the media and autoclaved at 121^{0}C for 15 minutes. Durham tubes were put in each bijou bottle in an inverted position and then phenol peptone water sugar broth was added to cool. Bacterial inoculum was then added using a sterile wire loop and incubated for 18-24 hours at 37^{0}C. A change from red to yellow color of the phenol red peptone water sugar broth indicated acid production and any gas produced was trapped in the Durham tubes.
Sulphur-indole-motility test uses Sulphur indole motility (SIM) medium is used to detect motility as well as Hydrogen Sulphide and indole production. 23g SIM was dissolved in 1L of

dissolved water. The solution was boiled to completely ensure that all agar dissolved and left to cool before dispensing into sterile screw cap tubes. The medium was then autoclaved at 121^0C for 15 minutes. Pure culture of the bacteria was picked using a straight sterile inoculating needle. Inoculations was done by stabbing at the butt and then streaking on the slant surface and then incubated aerobically at room temperature for 36 hours. If the bacterium is motile, the medium will become turbid with diffuse growth. If the bacterial species is non motile, only the cragies tube will have visible bacterial growth. Hydrogen Sulphide, production was confirmed by blackening. Indole production was detected by addition of 4 drops of kovac’s where a red color indicates a positive reaction and yellow indicates a negative reaction.

Appendix 5: MALDI-TOF results of some characterized isolates

Strain ID	*Identification*	Score value
MJN3 Kd i	*Empedobacter falsenii*	2.52
MKJM4 Kid	*Acinetobacter soli*	2.5
F2DJ2 Kd	*Klebsiella variicola*	2.46
MJM Sk i White	*Acinetobacter soli*	2.46
MJN Kd	*Acinetobacter soli*	2.45
MJN8 Gill i	*Empedobacter falsenii*	2.43
F4J3 Kd ii	*Acinetobacter gyllenbergii*	2.42
F4J12 Gill ii White	*Acinetobacter johnsonii*	2.41
F4B4 kd	*Staphylococcus warneri*	2.4
MNJH kd i	*Priestia megaterium*	2.4
F4J3 int i	*Bacillus cereus*	2.4
F4J5 Gill i	*No Organism Identification*	2.39
F4J3 Kd i	*Acinetobacter soli*	2.39
F5J3 int i	*Micrococcus luteus*	2.37
KWSN3 Gill i	*Macrococcus caseolyticus*	2.37
F3JW2	*Acinetobacter johnsonii*	2.36
IJ2 sk	*Acinetobacter johnsonii*	2.35
FIV8 int i	*Pseudomonas stutzeri*	2.35
F4J8 kd i	*Acinetobacter johnsonii*	2.34
MJN I Kd i	*Brevibacillus invocatus*	2.34
F2DH7 int i	*Bacillus pumilus*	2.34
KWSN7 Gill i	*Macrococcus caseolyticus*	2.34
F2DH2 spreading	*Escherichia coli*	2.33
KWSNI Kd i	*Bacillus pumilus*	2.32
MKJ6 Kd i	*Escherichia coli*	2.31
F4J3 sk i	*Acinetobacter soli*	2.29
F5J3 sk i	*Acinetobacter johnsonii*	2.29
MJN5 sk i	*Acinetobacter soli*	2.29
KWSN3 Kd ii	*Acinetobacter modestus*	2.29
MJN 5 Gill ii	*No Organism Identification*	2.29
FIV 4 Gill i	*Bacillus cereus*	2.28
MJN3 Gill i	*No Organism Identification*	2.27

IJ2 sk ii	*Acinetobacter modestus*	2.27
KWSNI kd i	*Bacillus pumilus*	2.27
F2DJ2 Gill i	*Priestia megaterium*	2.27
F3J2 kd i	*Pantoea ananatis*	2.26
F4J15 gill ii Cream large	*Pseudomonas putida*	2.25
MJN 8 Gill i	*Empedobacter tilapiae*	2.25
F2DH7 sk ii	*Pseudomonas sp*	2.24
F2B7 Kd ii	*Staphylococcus equorum*	2.24
F5J3 Kd i	*Acinetobacter johnsonii*	2.24
F4J2 Kd ii	*Acinetobacter johnsonii*	2.24
MNJH kd iii	*Micrococcus luteus*	2.23
F5J10 int i	*Leclercia adecarboxylata*	2.23
F4J8 Gill i	*Acinetobacter johnsonii*	2.22
F5JWii	*Enterobacter bugandensis*	2.21
MNJH kd ii	*Staphylococcus epidermidis*	2.2
F3J2 ii kd	*Staphylococcus haemolyticus*	2.18
F2DH4 kd i	*Pseudomonas fulva*	2.18
FIVW v	*Bacillus cereus*	2.17
F4J3 Kd iii	*Staphylococcus ureilyticus*	2.16
FIV8 sk ii	*Priestia megaterium*	2.15
FIV8 Gill i	*Cytobacillus kochii*	2.15
MJN8 sk i	*Moraxella osloensis*	2.14
F3J7 Gill ii	*Chryseobacterium gambrini*	2.14
KWSN7 sk i	*Acinetobacter piscicola*	2.14
F5J3 int i	*Micrococcus luteus*	2.12
F2B9 kd	*Lysinibacillus fusiformis*	2.12
MJN 6 sk i	*Acinetobacter soli*	2.11
MKJM4 Kid I	*Acinetobacter soli*	2.1
FIVI sk ii	*Acinetobacter johnsonii*	2.08
F3J7 gill ii cream	*Chryseobacterium gambrini*	2.08
F3J7 gill ii yellow	*Chryseobacterium sp*	2.08
KWSN6 Spreading	*Acinetobacter soli*	2.06
F4J2 sk ii	*Acinetobacter johnsonii*	2.06
MKJ1 kd i	*Acinetobacter schindleri*	2.06
FKDHI Gill ii	*Peribacillus simplex*	2.05
F2DWI	*Metabacillus indicus*	2.04
F5J10 kd iii	*Acinetobacter courvalinii*	2.04
MJN8 sk i	*Bacillus pumilus*	2.02

MJN5 kd ii	*Acinetobacter johnsonii*	2.01
MKJK kd	*Comamonas aquatic*	1.99
F3J5 sk i	*Acinetobacter soli*	1.99
F3J4Kdi	*Micrococcus luteus*	1.99
FKDH1 Skiii	*Microbacterium oleivorans*	1.99
F2B6Kdi	*Pseudomonas stutzeri*	1.99
F2B1Kdi	*Micrococcus luteus*	1.98
F2DJ5Giii	*Lysinibacillus fusiformis*	1.97
F2DH2	*Lysinibacillus fusiformis*	1.96
F2DJ7 int	*Paenibacillus lautus*	1.96
F2B9	*Lysinibacillus fusiformis*	1.95
FIV2Gill ii	*Staphylococcus capitis*	1.94
MKJ2SK	*Escherichia coli*	1.93
F4J7G14 ii	*Acinetobacter johnsonii*	1.93
KWS96 int	*Bacillus pumilus*	1.9
F2DH2 Kdi	*Lysinibacillus pakistanensis*	1.89
FIVI0 int	*Lysinibacillus sp[2]*	1.86
MNJ2 All org	*Acinetobacter soli*	1.85
MNJ2 Kd	*Bacillus pumilus*	1.84
F2DH10	*Bacillus thuringiensis*	1.82
FIV6 gill iii	*Acinetobacter lwoffii*	1.81
F4JII Gill vi	*Acinetobacter lwoffii*	1.81
MJN3 gill i	*Exiguobacterium indicum*	1.8
F2DJI	*Bacillus pumilus*	1.79
F2DJ3 gill ii	*Acinetobacter variabilis*	1.77
KWSN9 SK ii	*Macrococcus caseolyticus*	1.75
F4J ii int	*Acinetobacter lwoffii*	1.74
F4JII int	*Exiguobacterium indicum*	1.74
F5JI int	*Peribacillus simplex*	1.74
50Tiv	*Psychrobacter pulmonis*	1.74
MJN2Ski	*Acinetobacter soli*	1.73
22Kii	*Bacillus pumilus*	1.72
48Tiv yellow	*Bacillus pumilus*	1.72
106Gill white	*Staphylococcus warneri*	1.71
106Gill yellow	*Macrococcus caseolyticus*	1.71
91Kii yellow	*Micrococcus endophyticus*	1.7
91Kii white	*Exiguobacterium mexicanum*	1.7

Appendix 6: Percentage (%) of antibiotics sensitive (S), resistant (R) and MAR index for selected bacteria

	Total isolates	**AMPICILLIN**		**COTRIMOXAZOLE**		**GENTAMICIN**		**TETRACYCLINE**		**STREPTOMYCIN**		**OXACILL**		**MAR**
Bacterial Species		**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	
Acinetobacter Iwoffii	3	3	0	3	0	3	0	3	0	3	0	0	3	0.56
Acinetobacter variabilis	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167
Escherichia coli	5	1	4	4	1	5	0	5	0	5	0	0	5	0.1
Aeromonas hydrophilla	4	2	2	4	0	4	0	4	0	4	0	0	4	0.083
Aeromonas veronii	4	0	4	3	1	4	0	4	0	4	0	0	4	0.125
Bacillus Subtilis	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167
Bacillus cereus	4	1	3	1	3	4	0	4	0	4	0	0	4	0.125
Bacillus pumilus	1	0	1	0	1	1	0	1	0	1	0	0	1	0.5
Citrobacter braakii	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167
Ctrobacter braakii	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167

Enterobacter cloacae	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167
Klebsiella pneumonia	1	0	1	1	0	1	0	1	0	1	0	0	1	0.333
Kytococcus sedentarius	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167
Microbacterium Oleivorans	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167
Priesta megaterium	1	1	0	1	0	1	0	1	0	1	0	0	1	0.167
Pseudomonas fulva	2	0	2	0	2	2	0	2	0	2	0	0	2	0.083
Staphylococcus lentus	4	3	1	3	1	4	0	4	0	4	0	0	4	0.042
Streptococcus sciuri	1	0	1	1	0	1	0	1	0	1	0	0	1	0.167
Lysinibacillus fusiformis	8	8	0	4	4	8	0	8	0	5	3	0	8	0.063
Total	45	26	19	32	13	45	0	45	0	42	3	0	45	
%		58%	42%	71%	29%	100%	0%	100%	0%	93%	7%	0%	100%	

Legend: Multiple Antibiotics Resistance Index of bacteria isolates

Key: AMP= Ampicillin, CTX=Cotrimoxazole, GEN= Gentamicin, TET= Terecycline, S= Streptomycin and OX= Oxacillin

Appendix 7: Prevalence of bacteria isolates in different fish organs and water samples

Characteristic	**Gill**, N = 177[1]	**Intestine**, N = 146[1]	**Kidney**, N = 70[1]	**Skin**, N = 175[1]	**Water**, N = 28[1]
Acinetobacter courvalinii	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Acinetobacter gyllenbergii	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Acinetobacter johnsonii	7 (27%)	4 (15%)	5 (19%)	7 (27%)	3 (12%)
Acinetobacter lwoffii	10 (50%)	1 (5.0%)	0 (0%)	7 (35%)	2 (10%)
Acinetobacter modesta	2 (25%)	2 (25%)	1 (13%)	3 (38%)	0 (0%)
Acinetobacter schindleri	1 (50%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)
Acinetobacter solii	7 (27%)	5 (19%)	7 (27%)	7 (27%)	0 (0%)
Acinetobacter variabilis	3 (23%)	2 (15%)	2 (15%)	5 (38%)	1 (7.7%)
Aeromonas hormaechei	7 (44%)	2 (13%)	0 (0%)	5 (31%)	2 (13%)
Aeromonas hydrophilla	8 (26%)	12 (39%)	1 (3.2%)	7 (23%)	3 (9.7%)
Aeromonas spp	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Aeromonas veronii	18 (40%)	10 (22%)	6 (13%)	11 (24%)	0 (0%)
Bacillus cereus	16 (33%)	15 (31%)	1 (2.1%)	15 (31%)	1 (2.1%)
Bacillus pumilus	4 (21%)	6 (32%)	3 (16%)	4 (21%)	2 (11%)
Bacillus subtilis	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Bacillus thuringiensis	0 (0%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)
Chryseobacterium gambrini	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Citrobacter brakii	0 (0%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)
Corynebacterium jeikeim	3 (43%)	1 (14%)	1 (14%)	2 (29%)	0 (0%)
Cytobacillus kochi	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Empedobacter falseni	0 (0%)	1 (50%)	0 (0%)	0 (0%)	1 (50%)
Empedobacter tilapiae	3 (33%)	1 (11%)	1 (11%)	4 (44%)	0 (0%)
Enterobacter bugandensis	0 (0%)	1 (33%)	0 (0%)	0 (0%)	2 (67%)
Enterobacter cloacae	1 (25%)	2 (50%)	1 (25%)	0 (0%)	0 (0%)
Enterobacter tilapiae	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Escherichia coli	6 (32%)	5 (26%)	2 (11%)	6 (32%)	0 (0%)

Exiguobacterium indicum	0 (0%)	1 (25%)	1 (25%)	2 (50%)	0 (0%)
Klebsiella pneumoniae	4 (40%)	4 (40%)	0 (0%)	2 (20%)	0 (0%)
Klebsiella variicola	1 (25%)	0 (0%)	2 (50%)	1 (25%)	0 (0%)
Kytococcus sedentarius	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Lactococcus garvieae	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Leclercia adecarboxylata	2 (29%)	1 (14%)	1 (14%)	3 (43%)	0 (0%)
Listeria monocytogenes	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Lysinibacillus fusiformis	9 (31%)	11 (38%)	5 (17%)	4 (14%)	0 (0%)
Macrococcus caseolyticus	7 (58%)	3 (25%)	0 (0%)	1 (8.3%)	1 (8.3%)
metabacillus indicus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Microbacterium oleivorans	2 (13%)	1 (6.3%)	3 (19%)	9 (56%)	1 (6.3%)
Micrococcus leteus	2 (25%)	2 (25%)	1 (13%)	1 (13%)	2 (25%)
Pantoea ananatia	0 (0%)	2 (40%)	1 (20%)	2 (40%)	0 (0%)
Peribacillus simplex	2 (40%)	1 (20%)	0 (0%)	2 (40%)	0 (0%)
Priestia megaterium	6 (27%)	10 (45%)	2 (9.1%)	3 (14%)	1 (4.5%)
Pseudomonas fulva	5 (29%)	3 (18%)	1 (5.9%)	8 (47%)	0 (0%)
Pseudomonas putida	1 (20%)	1 (20%)	2 (40%)	1 (20%)	0 (0%)
Pseudomonas stutzeri	2 (25%)	5 (63%)	1 (13%)	0 (0%)	0 (0%)
Staphylococcus ureilyticus	1 (10%)	3 (30%)	2 (20%)	4 (40%)	0 (0%)
Staphylococcus capitis	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Staphylococcus equorum	0 (0%)	2 (33%)	2 (33%)	2 (33%)	0 (0%)
Staphylococcus haemolyticus	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Staphylococcus kloosii	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Staphylococcus lentus	24 (32%)	13 (17%)	7 (9.2%)	27 (36%)	5 (6.6%)
Staphylococcus variabilis	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Staphylococcus warneri	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Stapylococclus epidermids	3 (14%)	7 (33%)	1 (4.8%)	10 (48%)	0 (0%)
Stenotrophomonas malltophilia	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Streptococcus sciuri	4 (40%)	3 (30%)	0 (0%)	3 (30%)	0 (0%)

Appendix 8: Proportion of bacteria species isolated from different fish species

Characteristic	**Hybrids** N = 68^1	***O. jipe*** N = 246^1	***O. niloticus*** N = 254^1	**Water** N = 28^1
Acinetobacter courvalinii	0 (0%)	2 (100%)	0 (0%)	0 (0%)
Acinetobacter gyllenbergii	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Acinetobacter johnsonii	2 (7.7%)	9 (34.8%)	12 (46%)	3 (12%)
Acinetobacter lwoffii	5 (25%)	8 (40%)	5 (25%)	2 (10%)
Acinetobacter modesta	0 (0%)	5 (63%)	3 (38%)	0 (0%)
Acinetobacter schindleri	0 (0%)	2 (100%)	0 (0%)	0 (0%)
Acinetobacter solii	0 (0%)	16(61.7%)	10 (38%)	0 (0%)
Acinetobacter variabilis	2 (15%)	5 (38%)	5 (38%)	1 (7.7%)
Aeromonas hormaechei	1 (6.3%)	5 (31%)	8 (50%)	2 (13%)
Aeromonas hydrophila	6 (19%)	14 (45%)	8 (26%)	3 (9.7%)
Aeromonas veronii	5 (11%)	29 (60%)	11 (24%)	0 (0%)
Bacillus cereus	3 (6.3%)	16 (33.3%)	28 (58%)	1 (2.1%)
Bacillus pumilus	3 (16%)	5 (26%)	9 (47%)	2 (11%)
Bacillus subtilis	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Bacillus thuringiensis	2 (100%)	0 (0%)	0 (0%)	0 (0%)
Chryseobacterium gambrini	0 (0%)	2 (100%)	0 (0%)	0 (0%)
Citrobacter brakii	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Corynebacterium jeikeim	0 (0%)	2 (29%)	5 (71%)	0 (0%)
Cytobacillus kochi	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Empedobacter falseni	0 (0%)	0 (0%)	1 (50%)	1 (50%)
Empedobacter tilapiae	3 (33%)	2 (22%)	4 (44%)	0 (0%)
Enterobacter bugandensis	1 (33%)	0 (0%)	0 (0%)	2 (67%)
Enterobacter cloacae	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Enterobacter tilapiae	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Escherichia coli	7 (37%)	5 (26%)	7 (37%)	0 (0%)

Exiguobacterium indicum	0 (0%)	3 (75%)	1 (25%)	0 (0%)
Klebsiella pneumoniae	2 (20%)	4 (40%)	4 (40%)	0 (0%)
Klebsiella variicola	1 (25%)	2 (50%)	1 (25%)	0 (0%)
Kytococcus sedentarius	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Lactococcus garvieae	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Leclercia adecarboxylata	1 (14%)	0 (0%)	6 (86%)	0 (0%)
Lysinibacillus fusiformis	5 (17%)	15 (52%)	9 (31%)	0 (0%)
Macrococcus caseolyticus	0 (0%)	7 (58%)	4 (33%)	1 (8.3%)
metabacillus indicus	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Microbacterium oleivorans	1 (6.3%)	5 (31%)	9 (56%)	1 (6.3%)
Micrococcus luteus	2 (25%)	3 (38%)	1 (13%)	2 (25%)
Pantoea ananatia	1 (20%)	3 (60%)	1 (20%)	0 (0%)
Peribacillus simplex	2 (40%)	0 (0%)	3 (60%)	0 (0%)
Priestia megaterium	1 (4.5%)	9 (41%)	11 (50%)	1 (4.5%)
Pseudomonas fulva	2 (12%)	9 (53%)	6 (35%)	0 (0%)
Pseudomonas putida	1 (20%)	4 (80%)	0 (0%)	0 (0%)
Pseudomonas stutzeri	0 (0%)	1 (13%)	7 (88%)	0 (0%)
Staphylococcus ureilyticus	0 (0%)	10 (100%)	0 (0%)	0 (0%)
Staphylococcus capitis	0 (0%)	0 (0%)	1 (100%)	0 (0%)

Appendix 9: Prevalence of bacterial isolates in different study sites

Characteristic	**Intake**, N = 20^1	**KWS**, N = 42^1	**Mkwajuni**, N = 99^1	**Mwatate**, N = 159^1	**Taveta**, N = 169^1	**Voi**, N = 39^1	**Water**, N = 28^1	**Wundanyi**, N = 40^1
Isolates								
Acinetobacter courvalinii	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)
Acinetobacter gyllenbergii	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Acinetobacter Johnsonii	0 (0%)	2 (7.7%)	7 (27%)	1 (3.8%)	11 (42%)	2 (7.7%)	3 (12%)	0 (0%)
Acinetobacter lwoffii	2 (10%)	0 (0%)	0 (0%)	8 (40%)	5 (25%)	1 (5.0%)	2 (10%)	2 (10%)
Acinetobacter modesta	1 (13%)	1 (13%)	0 (0%)	0 (0%)	5 (63%)	1 (13%)	0 (0%)	0 (0%)
Acinetobacter schindleri	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Acinetobacter solii	4 (15%)	1 (3.8%)	4 (15%)	1 (3.8%)	15 (58%)	0 (0%)	0 (0%)	1 (3.8%)
Acinetobacter variabilis	1 (7.7%)	0 (0%)	3 (23%)	6 (46%)	1 (7.7%)	0 (0%)	1 (7.7%)	1 (7.7%)
Aeromonas hormaechei	1 (6.3%)	0 (0%)	3 (19%)	2 (13%)	1 (6.3%)	4 (25%)	2 (13%)	3 (19%)
Aeromonas hydrophila	0 (0%)	2 (6.5%)	4 (13%)	8 (26%)	10 (32%)	0 (0%)	3 (9.7%)	4 (13%)

aeromonas spp	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Aeromonas veronii	0 (0%)	2 (4.4%)	16 (36%)	9 (20%)	17 (38%)	0 (0%)	0 (0%)	1 (2.2%)
Bacillus cereus	0 (0%)	1 (2.1%)	7 (15%)	17 (35%)	14 (29%)	7 (15%)	1 (2.1%)	1 (2.1%)
Bacillus pumilus	2 (11%)	2 (11%)	4 (21%)	3 (16%)	1 (5.3%)	5 (26%)	2 (11%)	0 (0%)
Bacillus subtilis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Bacillus thuringiensis	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Chryseobacterium gambrini	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)
Citrobacter brakii	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Corynebacterium jeikeim	0 (0%)	0 (0%)	1 (14%)	0 (0%)	6 (86%)	0 (0%)	0 (0%)	0 (0%)
Cytobacillus kochi	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Empedobacter falseni	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)
Empedobacter tilapiae	0 (0%)	0 (0%)	0 (0%)	3 (33%)	4 (44%)	2 (22%)	0 (0%)	0 (0%)

Enterobacter bugandensis	0 (0%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	0 (0%)	2 (67%)	0 (0%)
Enterobacter cloacae	0 (0%)	3 (75%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)
Enterobacter tilapiae	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Escherichia coli	0 (0%)	2 (11%)	4 (21%)	12 (63%)	0 (0%)	0 (0%)	0 (0%)	1 (5.3%)
Exiguobacterium indicum	1 (25%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (25%)
Klebsiella pneumonia	1 (10%)	0 (0%)	1 (10%)	4 (40%)	4 (40%)	0 (0%)	0 (0%)	0 (0%)
Klebsiella variicola	0 (0%)	1 (25%)	0 (0%)	3 (75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Kytococcus sedentarius	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Lactococcus garvieae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Leclercia adecarboxylata	0 (0%)	0 (0%)	3 (43%)	1 (14%)	3 (43%)	0 (0%)	0 (0%)	0 (0%)
Lysinibacillus fusiformis	0 (0%)	0 (0%)	3 (10%)	21 (72%)	4 (14%)	1 (3.4%)	0 (0%)	0 (0%)
Macrococcus caseolyticus	1 (8.3%)	1 (8.3%)	0 (0%)	0 (0%)	7 (58%)	2 (17%)	1 (8.3%)	0 (0%)

Metabacillus indicus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
Microbacterium oleivorans	1 (6.3%)	0 (0%)	6 (38%)	3 (19%)	5 (31%)	0 (0%)	1 (6.3%)	0 (0%)
Micrococcus leteus	0 (0%)	1 (13%)	0 (0%)	2 (25%)	1 (13%)	0 (0%)	2 (25%)	2 (25%)
Pantoea ananatia	0 (0%)	0 (0%)	0 (0%)	3 (60%)	1 (20%)	0 (0%)	0 (0%)	1 (20%)
Peribacillus simplex	0 (0%)	0 (0%)	0 (0%)	2 (40%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)
Priestia megaterium	0 (0%)	0 (0%)	5 (23%)	10 (45%)	3 (14%)	1 (4.5%)	1 (4.5%)	2 (9.1%)
Pseudomonas fulva	0 (0%)	0 (0%)	4 (24%)	3 (18%)	10 (59%)	0 (0%)	0 (0%)	0 (0%)
Pseudomonas putida	0 (0%)	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	3 (60%)
Pseudomonas stutzeri	1 (13%)	1 (13%)	0 (0%)	2 (25%)	2 (25%)	2 (25%)	0 (0%)	0 (0%)
Staphylococcus ureilyticus	0 (0%)	0 (0%)	0 (0%)	3 (30%)	7 (70%)	0 (0%)	0 (0%)	0 (0%)
Staphylococcus capitis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Staphylococcus equorum	0 (0%)	0 (0%)	0 (0%)	6 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Staphylococcus haemolyticus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Staphylococcus kloosii	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Staphylococcus lentus	2 (2.6%)	14 (18%)	16 (21%)	9 (12%)	13 (17%)	7 (9.2%)	5 (6.6%)	10 (13%)
Staphylococcus variabilis	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Staphylococcus warneri	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stapylococclus epidermids	1 (4.8%)	1 (4.8%)	4 (19%)	5 (24%)	9 (43%)	0 (0%)	0 (0%)	1 (4.8%)
Stenotrophoimonas maltophila	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Stenotrophomonas malltophilia	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Streptococcus sciuri	0 (0%)	4 (40%)	1 (10%)	3 (30%)	1 (10%)	0 (0%)	0 (0%)	1 (10%)

[1]n (%)

Appendix 10: Pathogenic and Zoonotic Bacteria Isolated

Bacteria genera	Bacteria species	Bacteria isolates	Percentage	Pathogenic	Zoonotic	References
Staphylococcus species	*Staphylococcus lentus*	77	12.9	+	-	(Rivera *et al.*, 2014)
	Staphylococcus equorum	6	0.1	Normal	-	Not known
	Staphylococcus capitis	1	0.17	-	-	Not known
	Staphylococcus haemolyticus	1	0.17	+	+	(‘Staphylococcus haemolyticus and Providencia vermicola Infections Occurring in Farmed Tilapia: Two Potentially Emerging Pathogens’, 2023),(Silago *et al.*, 2022)
	Staphylococcus kloosii	1	0.17	Normal	-	Not known
	Staphylococcus variabilis	1	0.17	Normal	-	Not Known
	Staphylococcus warneri	1	0.17	Normal	-	Not Known
	Stapylococclus epidermids	20	3.35	+/Normal	Normal	(Al-Harbi, 2023),(Arafa, 2021)
	Staphylococcus ureilyticus	10	1.67	Normal	-	Not Known
Bacillus species	*Bacillus cereus*	48	8.05	+	+	(Mursyidah *et al.*, 2021)
	Bacillus pumilus	18	3.02	Normal	Normal	(Mursyidah *et al.*, 2021), (An and Kj, 2017)
	Bacillus thuringiensis	2	0.33	Normal	Normal	(Wekesa *et al.*, 2020),(Mursyidah *et al.*, 2021)
	Bacillus subtilis	1	0.17	-	+	(Mursyidah *et*

						al., 2021)
	Cytobacillus kochi	1	0.17	-	-	Not known
	Peribacillus simplex	5	0.84	Normal	-	Not known
	Lysinibacillus fusiformis	29	4.86	-	-	(Zabidi *et al.*, 2021)
Aeromonas species	*Aeromonas veronii*	45	7.55	+	+	(Ziarati *et al.*, 2022),(Basri *et al.*, 2020),(Irshath *et al.*, 2023),(Arafa, 2021),(Youssuf *et al.*, 2020)
	Aeromonas hydrophilla	31	5.2	+	+	(Ziarati *et al.*, 2022),(Basri *et al.*, 2020)
	Aeromonas spp	1	0.17	+	-	(Ziarati *et al.*, 2022)
	Aeromonas hormaechei	16	2.68	New	-	Not known
Acinetobacter species	*Acinetobacter courvalinii*	2	0.33	-	-	Not known
	Acinetobacter schindleri	2	0.33	-	+	Not Known
	Acinetobacter modesta	8	1.34	Not	-	Not known
	Acinetobacter johnsonii	26	4.36	+	+	Cao *et al.*, 2018
	Acinetobacter solii	26	4.36	-	+	Not known
	Acinetobacter lwoffii	20	3.35	+	+	(Arafa, 2021),(Cao *et al.*, 2018)
	Acinetobacter variabilis	13	2.18	Normal	Normal	Not known
	Acinetobacter gyllenbergii	1	0.17	Emerging+	+	Not known
Escherichia coli	*Escherichia coli*	19	3.18	-	+	(Mumbo *et al.*, 2023),(Bedane *et al.*, 2024)
Pseudomonas species	*Pseudomonas fulva*	17	2.85	-	+	(Stark, 2022)
	Pseudomonas stutzeri	8	1.34	-	-	Not known

	Pseudomonas putida	5	0.8	+	+	(Ziarati *et al.*, 2022),(Rahman *et al.*, 2024)
Micrococcus species	*Micrococcus leteus*	8	1.34	Normal	Normal	(D. Wanja *et al.*, 2020),(An and Kj, 2017)
Microbacterium oleivoran	*Microcobacterium oleivoran*	16	2.68	New		Not Known
Macrococcus species	*Macrococcus caseolyticus*	10	2.01	-	-	(Li *et al.*, 2018)
Priestia species	*Priestia megaterium*	22	3.69	Plant pathogen	-	(Cui *et al.*, 2023)
Klebsiella species	*Klebsiella pneumonia*	10	1.67	+	+	(Ziarati *et al.*, 2022),(Xu *et al.*, 2022),
	Klebsiella variicola	4	0.67	+	+	(Ziarati *et al.*, 2022)
Streptococcis species	*Streptococcus sciuri*	10	1.68	-	-	Not known
Corynebacterium species	*Corynebacterium jeikeim*	7	1.17	-	-	Not known
	Microbacterium oleivorans	14	2.35	-	-	Not known
Leclercia species	*Leclercia adecarboxylata*	7	1.17	-	+	(Zayet *et al.*, 2021)
Pantoea species	*Pantoea ananatia*	5	0.84	-	-	Not known
Empedobacter species	*Enterobacter cloacae*	4	0.67	+	+	(Hardi *et al.*, 2018), (Aly, Nouh and Salem-Bekhet, 2012),(Rawash *et al.*, 2019)
	Empedobacter falseni *Empedobacter tilapiae*	2	0.33	+	+	(Martinez *et al.*, 2023)
Enterobacter species	*Enterobacter bugandensis*	3	0.5	+	+ New	(Hardi *et al.*, 2018)
Exiguobacterium species	*Exiguobacterium indicum*	4	0.67	-	-	Not reported fish pathogen
Chryseobacterium species	*Chryseobacterium gambrini*	2	0.33	-	-	Not known
Citrobacter species	*Citrobacter brakii*	2	0.33	+	+	(An and Kj, 2017),(Hardi *et al.*, 2018),

						(Youssuf *et al.*, 2020)
Kytococcus species	*Kytococcus sedentarius*	1	0.17	-	-	Not known
Lactococcus species	*Lactococcus garvieae*	1	0.17	+	+ Emerging	(Swaimanathan *et al.*, 2021); (Bwalya *et al.*, 2021); (Irshath *et al.*, 2023)
Stenotrophoimonas species	*Stenotrophoimonas maltophila*	1	0.17	+	+	(Abraham *et al.*, 2015)
metabacillus species	*metabacillus indicus*	1	0.17	-	-	Not known
		596		21+ , 13, normal, 4 emerging, I plant pathogen	30+ ,2 emerging	

Key: += (pathogenic or zoonotic), normal=commensal bacteria, new=bacteria isolated for the first time