



**UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES**

**MULTIDRUG-RESISTANT BACTERIA IN AQUACULTURE SYSTEMS IN ACCRA,
GHANA**

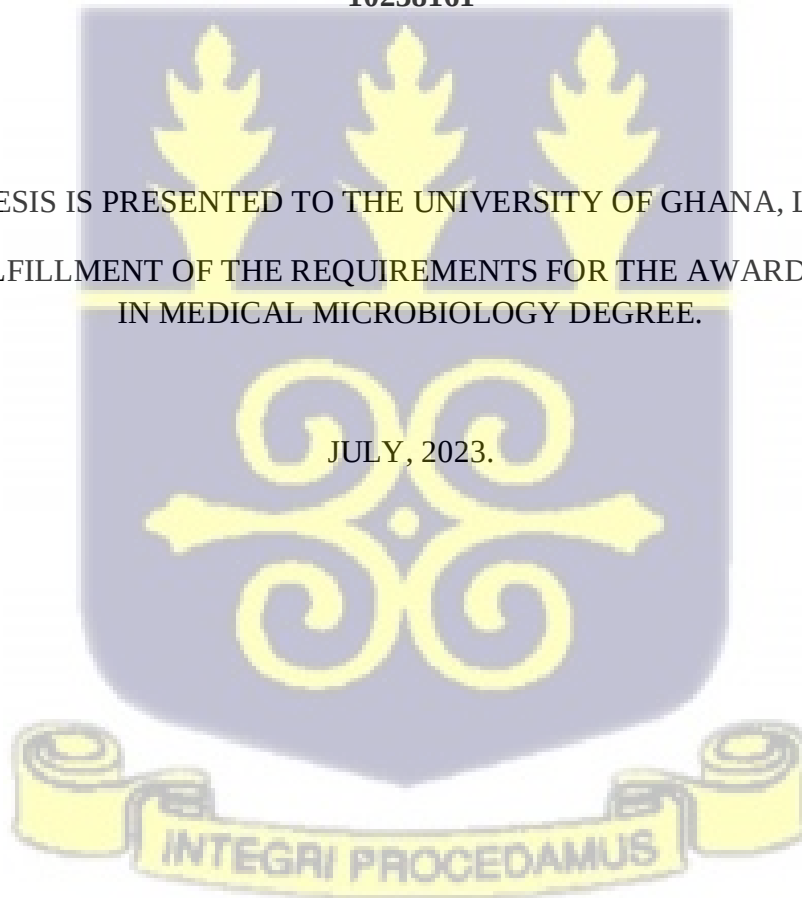
BY

FRANCES N. N. NATHAN-MENSAH

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THIS THESIS IS PRESENTED TO THE UNIVERSITY OF GHANA, LEGON, IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF AN MPhil
IN MEDICAL MICROBIOLOGY DEGREE.

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DECLARATION

I, Frances N. N. Nathan-Mensah, declare that the work presented in this thesis is a product of my independent research conducted at the Department of Medical Microbiology, University of Ghana Medical School (UGMS). This research was performed under the guidance of Professor Nicholas T. K. D. Dayie and Professor Eric Sampane-Donkor (Department of Medical Microbiology, UGMS). I affirm that this thesis has not been submitted for any degree or examination at any other university.

FRANCES N. N. NATHAN-MENSAH
(STUDENT)



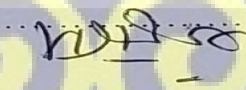
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.....12th June,2025

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CERTIFIED BY:

PROF. NICHOLAS T.K.D DAYIE
(MAIN SUPERVISOR)



SIGNATURE

....12th June, 2025

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PROF. ERIC SAMPANE-DONKOR
(CO-SUPERVISOR)



SIGNATURE

24th June, 2025

DATE

DEDICATION

This research is dedicated to my family, N. N. Mensah and Esther N. T. Nathan for always believing in me and bringing me this far.



ACKNOWLEDGEMENT

I am deeply grateful to the only true GOD for His guidance, wisdom, protection, and the priceless gift of life.

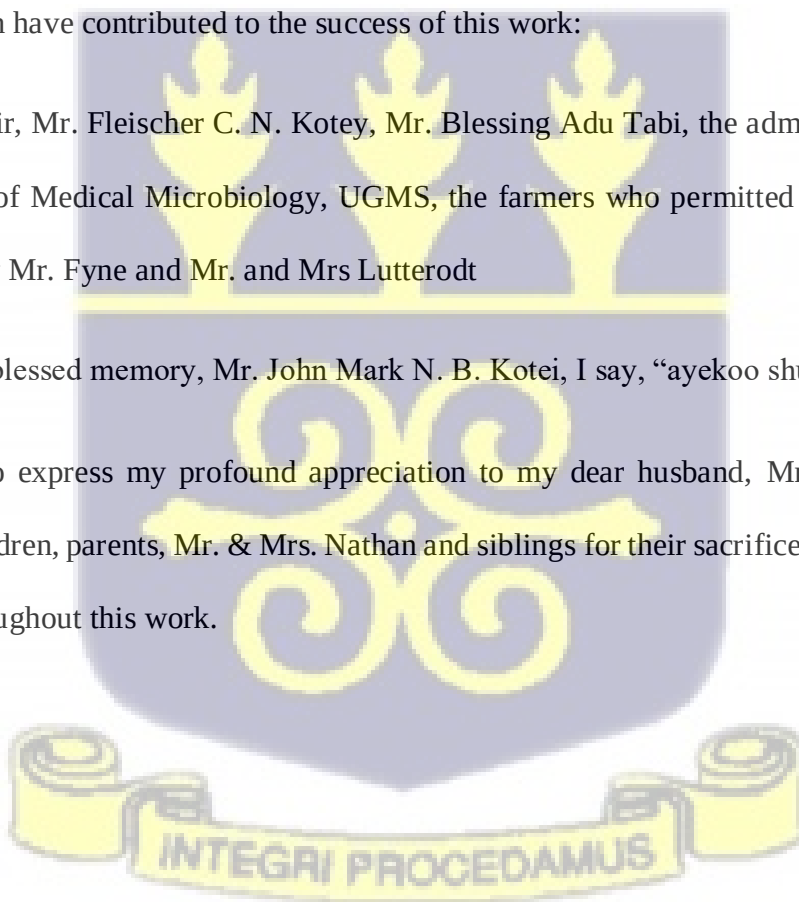
I wish to express my deepest gratitude to my supervisors, Prof. Nicholas T. K. D. Dayie and Prof. Eric Sampene-Donkor of the Department of Medical Microbiology, UGMS, for their valuable recommendations, insightful criticisms, and patience, which have been instrumental in bringing this work to a successful completion.

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To my uncle of blessed memory, Mr. John Mark N. B. Kotei, I say, “ayekoo shulo papa”.

Lastly, I wish to express my profound appreciation to my dear husband, Mr. Nicholas Nixon Mensah, my children, parents, Mr. & Mrs. Nathan and siblings for their sacrifices, encouragement, and support throughout this work.



ABSTRACT

Background: Antimicrobial resistance (AMR) is an existential global health threat, associated with high mortality, morbidity, and economic impacts. For remedial efforts to be effective, AMR surveillance needs to encompass both human and animal health. However, most AMR surveillance studies, particularly those conducted in sub-Saharan Africa, have usually focused on humans, and rarely on animals. The few that have been conducted on animals have usually focused on poultry, etc., with very little attention given to the aquaculture sector.

Aim: To evaluate AMR in bacteria pathogens harboured in marketed reared fish sold in Accra and the pond water from which they originated.

Methodology: The study was cross-sectional, involving 160 inland fish marketed to consumers and the corresponding pond water from which the fish were reared. The samples were bacteriologically cultured, and their total viable and coliform counts determined, following standard procedures. The bacterial isolates were identified using MALDI-TOF, and the AMR pattern determination and ESBL screening done via the Kirby-Bauer method. Further, the sequence types, serotypes, and resistance genes of the ESBL isolates were determined via whole genome sequencing.

Results: The overall total viable count was 12.4×10^5 cfu/ml (18.25×10^5 cfu/ml in the fish and 6.55×10^5 cfu/ml in the pond water). The overall total coliform count was 0.31×10^5 cfu/ml (0.35×10^5 cfu/ml in the fish and 0.27×10^5 cfu/ml in the pond water). In total, 39 different types of bacteria, totalling 391, were isolated from the fish and ponds – 38 different in the fish (totaling 201) and 34 different in the pond water, totaling 190. As a composite, the top five bacteria were

Escherichia coli (17%), *Aeromonas veronii* (11%), *Citrobacter freundii* (8%), *Bacillus cereus* (5%), and *Klebsiella pneumoniae* (5%). In the fish, the top five bacteria were *Escherichia coli* (15.4%), *Aeromonas veronii* (12.4%), *Citrobacter freundii* (9.5%), *Edwardsiella tarda* (5.0%), and *Klebsiella pneumoniae* (4.5%). In the pond water, the top five bacteria were *Escherichia coli* (19.5%), *Aeromonas veronii* (8.9%), *Bacillus cereus* (5.8%), *Citrobacter freundii* (5.8%), and *Proteus mirabilis* (5.3%). The AMR rates, respectively for the fish, pond water, and overall, were cefotaxime (29%, 35%, and 32%), gentamicin (1%, 0%, and 1%), ciprofloxacin (4% each), chloramphenicol (19% each), tetracycline (42%, 31%, and 37%), meropenem (0% each), and ertapenem (0% each). The bacterial MDR rates were 13.6% ($n = 53$) overall, 13.4% ($n = 27$) for the fish, and 13.7% ($n = 26$) for the pond water. The bacterial ESBL rates were 1.3% ($n = 5$) overall, 1.0% ($n = 2$) for the fish, and 1.6% ($n = 3$) for the pond water. The sequence types of the ESBL isolates were ST4684 (80%, $n = 4$) and ST2005 (20%, $n = 1$), and the serotypes were H34:09 (80%, $n = 4$) and H7 (20%, $n = 1$), and the AMR genes were *blaCTX-M-15*, *fosA7*, and *qnrS1*.

Conclusion: The fish and the pond water had high microbial contamination, mainly accounted for by *Escherichia coli* and *Aeromonas veronii*. The AMR, MDR, and ESBL rates were low to moderate. Moreover, the main sequence type and serotype of the ESBL isolates were ST4684 and H34:09, respectively, and the AMR genes were *blaCTX-M-15*, *fosA7*, and *qnrS1*.

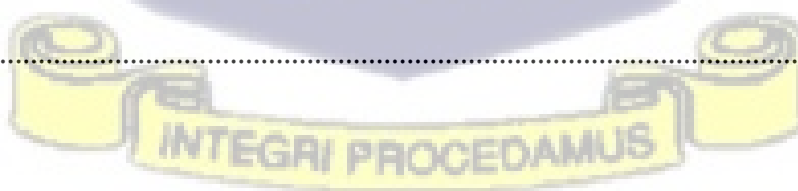


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LIST OF ABBREVIATIONS

AMC – Amoxicillin–clavulanate

AMP – Ampicillin

AMR – Antimicrobial Resistance

CC – Coliform count

CEFP – Cefepime

CEFT – Ceftriaxone

CEFU – Cefuroxime

CFTZ – Ceftazidime

CFU – Colony Forming Units

CIP – Ciprofloxacin

CLSI – Clinical Laboratory Standard Institute

ERT – Ertapenem

ESBL – Extended-Spectrum Beta-Lactamase

GDP – Gross Domestic Products

HEV – Hepatitis E Virus

LAB – Lactic Acid Bacteria

MALDI TOF – Matrix-Assisted Laser Desorption/Ionization Time of Flight

MEM – Meropenem

MPN – Most Probable Number

STEC – Shiga toxin-producing *E. coli*

TMS– Trimethoprim-sulphamethoxazole



CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Globally, antimicrobial resistance (AMR) is increasing in prevalence in a wide range of microorganisms, which is a substantial threat to global health (Yewale, 2014; WHO, 2015; Nadimpalli, 2018). It puts in peril the effective treatment and management of illnesses in patients, consequently leading to an increase in morbidity and mortality in humans. Drug-resistant microbes have a variety of reservoirs, including humans, animals, food, and the environment, and they often develop and spread in response to anthropogenic activities (World Organization for Animal Health, 2018; WHO, 2021; WHO, 2021). Key anthropogenic drivers of AMR include non-compliance with prescribed antimicrobial treatments, self-medication, and the misuse of broad-spectrum antibiotics. These practices lead to the inappropriate consumption of antimicrobials, characterized by overuse, underuse, or misuse, further exacerbated by similar issues in animal husbandry and fish farming (Organización Mundial de la Salud, 2001; Wall *et al.*, 2016; Rousham *et al.*, 2018; Schar *et al.*, 2020). Environmental factors also play a significant role, as the release of antimicrobials into natural settings fosters the selection and spread of resistance (von Wintersdorff *et al.*, 2016). The interactions among these drivers facilitate the continuous exchange of AMR determinants between humans, animals, and the environment, creating a cycle that amplifies the challenge of controlling AMR (Rousham *et al.*, 2018). In response to the AMR crisis, the United Nations promotes the One Health framework (WHO, 2018). This holistic approach acknowledges that “human and animal health are interdependent and bound to the health of the ecosystems in which they exist” (World Organization for Animal Health, 2021).

Pathogens responsible for infections thrive across a myriad of ecosystems, ranging from aquatic zones and hospitals to forests, animals, and even humans. The coexistence of antimicrobial resistance (AMR) agents, pathogens, and genes alongside non-AMR counterparts propels the spread of AMR. Additionally, genetic traits, environmental factors, and the presence of antimicrobial agents can enable pathogens to develop sophisticated resistance mechanisms to outwit the pharmacodynamics and potency of antimicrobials, thus, ensuring their survival in their host. The phenomenon of multidrug resistance (MDR) exacerbates healthcare challenges by increasing morbidity and mortality rates, prolonging disease treatment times, and inflating the cost of healthcare (WHO, 2017).

In Ghana, various industries, including healthcare, agriculture, veterinary medicine, and particularly aquaculture, face significant challenges due to antimicrobial resistance (AMR). The expansion of fish farming in Ghana and other developing nations has been rapid in recent years (Assefa & Abunna, 2018; Okocha *et al.*, 2018), significantly boosting exports over the past two decades (Assefa & Abunna, 2018). Farming of fish was introduced to Ghana in the 1950s during the colonial era aimed at improving nutrition and community livelihoods (Janananda *et al.*, 2023). The foundation of Ghana's aquaculture industry is the farming of two main fish varieties: African catfish (*Clarias gariepinus*) and the Nile tilapia (*Oreochromis niloticus*). The sector witnessed a dramatic production increase, soaring from less than 11,000 tons in 2010 to over 57,000 tons by 2017. Approximately 75% of Ghana's aquaculture output comes from private commercial entities, which are primarily responsible for the growth of this sector within the local fisheries industry. (Amenyogbe *et al.*, 2018; Shellfishers & Project, 2022).

Despite the promising growth and potential of fish farms in Ghana, concerns about unhygienic conditions and the existence of multidrug-resistant bacteria in fishery products and waters cast a

shadow over the industry (Agoba *et al.*, 2017; John *et al.*, 2017). These circumstances present significant health hazards, particularly considering the strong consumer preference for tilapia and catfish as primary animal protein sources (Amenyogbe *et al.*, 2018). The consumption or handling of contaminated, improperly cooked fishery products can lead to infections that are challenging to treat, further exacerbating healthcare burdens through prolonged hospital stays, increased mortality risks, and elevated medical expenses (Dadgostar, 2019).

Aquaculture, supplying over half of global seafood for human consumption, is the world's fastest-growing food production sector (Troell *et al.*, 2014; FAO, 2016). It is an ideal candidate for surveillance of AMR using the One Health approach, for several reasons. The use of antimicrobials in intensive fish farming serves not only therapeutic and preventive roles but also promotes growth, a practice that has long-term implications for AMR development (Cabello, 2006; Cabello *et al.*, 2013). The consequential release of antimicrobial-resistant organisms and residues into the environment through feed and waste contaminates soil and water, posing a risk to the entire aquaculture ecosystem and beyond (Wall *et al.*, 2016). Moreover, the unique conditions within aquaculture facilitate rapid genetic exchange (including AMR determinants) and recombination among microbes (Watts *et al.*, 2017). This accelerates the spread of resistance across different species and into the environment, affecting wildlife, human health, and ecosystem stability (Cabello, 2006; Wall *et al.*, 2016).

Situated in western sub-Saharan Africa, a region with the highest reported AMR burden globally, Ghana's aquaculture industry represents a critical junction for AMR dissemination (Murray *et al.*, 2022). The discovery of multidrug-resistant bacteria in fish farms across the Ashanti, Central, and Greater Accra regions underscores the critical need for targeted AMR surveillance within the aquaculture sector (Agoba *et al.*, 2017; Assefa & Abunna, 2018).

1.2 Problem statement

Antimicrobial resistance was implicated in the deaths of 4.95 million individuals in 2019, qualifying it as a global health concern (Murray *et al.*, 2022). Without effective AMR countermeasures by 2050, projections indicate 10 million annual deaths and a 2-3.5% GDP decline, amounting to a staggering US\$100 trillion loss (O'Neill, 2014). Sub-Saharan Africa, which is disproportionately burdened with AMR, is expected to experience a relatively higher impact, with a GDP reduction of US\$2895 billion, equivalent to 20% of the region's total economic output (O'Neill, 2014; Murray *et al.*, 2022).

Due to the extensive use of antimicrobials, aquaculture is a major driver of antimicrobial resistance. Approximately 70-80% of these drugs are released into aquatic environments, as fish cannot effectively metabolize them and predominantly eliminate them through their waste (Burridge *et al.*, 2010; Romero *et al.*, 2012). Additionally, antimicrobials incorporated into fish feed can persist in aquatic ecosystems for prolonged durations, rapidly disseminating through water bodies and imposing selective pressures that foster the emergence of AMR (O'Neill, 2015). To help tackle the antimicrobial resistance menace, it is important to continuously conduct surveillance, targeting interphases that hold high relevance regarding the selection and transmission of AMR organisms among wildlife, humans, and the environment, such as aquaculture ecosystems, using the One Health approach.

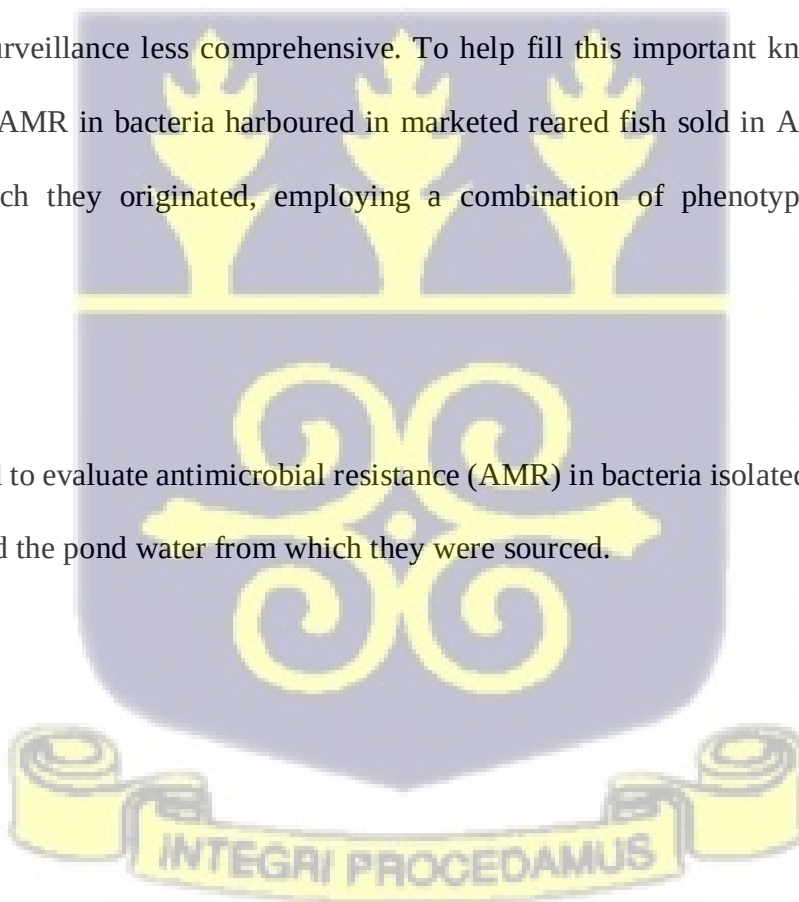
1.3 Justification

For antimicrobial stewardship to be effective, AMR surveillance efforts need to encompass the health of humans, animals, and the environment. Yet, the scope of AMR surveillance studies, particularly those conducted in sub-Saharan Africa, have mainly centered on humans and with

limited focus on animals and the environment (Eibach *et al.*, 2018; Adzitey *et al.*, 2019; Donkor *et al.*, 2019; Appiah *et al.*, 2020). This oversight extends to the aquaculture sector, which has received scant attention compared to other areas of animal agriculture, such as poultry farming (Adzitey *et al.*, 2019; Yafetto *et al.*, 2019; Adzitey *et al.*, 2020; Kunadu *et al.*, 2020). In Ghana, the few reported AMR surveillance efforts in the aquaculture sector have mainly been on phenotypic approaches to characterize antibiotic resistance (Agoba *et al.*, 2017; Assefa & Abunna, 2018). However, there is a general dearth of information on the genotypic characteristics of antibiotic resistance within Ghanaian aquaculture environments. Additionally, there is a significant lack of data on the overall patterns and trends of AMR in the aquaculture environment, making data on AMR surveillance less comprehensive. To help fill this important knowledge gap, this study evaluated AMR in bacteria harboured in marketed reared fish sold in Accra and the pond water from which they originated, employing a combination of phenotypic and genotypic techniques.

1.4 Aim

This study aimed to evaluate antimicrobial resistance (AMR) in bacteria isolated from farmed fish sold in Accra and the pond water from which they were sourced.



1.5 Specific objectives

The specific objectives were:

- To determine the microbial loads (total viable and coliform counts) in the fish and the pond water
- To determine the distribution of bacteria harboured in the fish and the pond water
- To determine the AMR patterns, as well as ESBL phenotypes, genotypes, and sequence types, among the bacteria isolated from the fish and the pond water



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aquaculture in Ghana

The abundance of aquatic resources in Ghana, including rivers, lakes, and vast coastal areas, have significantly bolstered fish farming, making it a lucrative and sustainable complement to capture fisheries (Amenyogbe *et al.*, 2018a). The inception of aquaculture in Ghana dates back to around 1953, during the era of the British colonial regime, marking the beginning of a journey towards enhancing the nation's fish production (Kwadwo Frimpong & Adwani, 2015). Recognizing the potential of aquaculture to support and augment traditional fishing methods, the Government of Ghana, in 1957, rolled out a policy to construct fishponds at most irrigation sites in Ghana (NDPC, 2017). This strategic move was aimed at increasing aquacultural outputs, thereby elevating the levels of fish available for consumption, and reinforcing the capture fisheries sector. Through these efforts, aquaculture in Ghana has evolved into a vital venture, significantly contributing to the country's food security and economic development.

2.2 Importance of Ghana's aquaculture industry

In 2004, the Ghana Directorate of Fisheries estimated aquaculture fish production for human consumption at 950 tons (Aboagye, 2012). Particularly noteworthy are the sales from Kumasi, Ghana's second-largest city, where North African catfish and tilapia fetch one cedi and fifty pesewas (Ghc1.63) per kilogram. In the capital, In Accra, the cage culture farm charges 3 cedis and 50 pesewas (Ghc 3.80) per kilogram for tilapia at its sales outlets, while Clarias costs 5 cedis (Ghc 5.44) per kilogram. The average price per kilogram of farmed fish sold from the farm gate is

1 cedi and 50 pesewas (\$1.63). Most owners of small fish farms typically rely on these sales to support their families as well as to generate revenue (Yamamoto, 2010).

Thirty percent of the fish produced worldwide comes from inland fisheries and aquaculture. The fisheries in Ghana are vital to the development of the coastal poor population and contribute roughly 1.5% of the country's GDP annually, with a GDP progression rate of 5% (GSS, 2016). (Asiedu *et al.*, 2017), The economic potential of the sector is significant, with estimates suggesting that fishery revenue could increase by 30 to 60 percent, potentially yielding at least US\$ 300 million annually.

However, Ghana's capture fishing industry has reached a low-level equilibrium that offers limited opportunities and severely impedes socioeconomic development (Ghana Business News (GBN), 2016). Over the last five years, the amount produced domestically has increased from 444,000 tons in 2011 to 451,000 tons. Despite a slight increase in domestic fish production from 444,000 tons in 2011 to 451,000 tons over the past five years, the country faces a substantial demand exceeding a million tonnes annually, leading to a significant shortfall of over fifty percent (Rurangwa *et al.*, 2015). To augment its domestic consumption, Ghana imported 180,801 tonnes of fish worth US\$ 154,019,585 in 2015 (Asiedu *et al.*, 2017). This period also saw a 40% decline in revenue from the coastal fishery over the last decade (Ghana Business News (GBN), 2016).

In Ghana, as well as most other parts of the world, hunger and malnutrition continue to hinder human development. In Ghana, there is an exponential increase in the demand for food and nutrition. Malnutrition is a factor in about half of child fatalities in Ghana (Ghana Business News (GBN), 2016). Also, 17% of children in homes are severely underweight, and 29% of children are underweight overall (AmeriCares, 2014). Fisheries based on culture and aquaculture are

responsible for the recent rise in fish production. Strengthening these two systems without sacrificing environmental safety is an efficient solution. Sustainable development is a problem for aquaculture, though. Production of fish can rise as a result of sustainable aquaculture.

2.3 Fish farming systems and practices in Ghana

The majority of fish farming in the nation is done on a small scale for subsistence as well as for commercial purposes, primarily in freshwater environments. Small-scale subsistence farming employing a semi-intensive production technique is the most prevalent type of fish farming. Though it is also practiced extensively, extensive culture is typically connected to dams and small reservoirs. Contrarily, commercial fish farmers, who make up only 25% of the aquaculture industry, use intensive culture systems in cages of floating pens, primarily on rivers and lakes (Amenyogbe *et al.*, 2018b).

Pond culture systems are currently used extensively throughout the nation, particularly in the south. The most prevalent fish in this culture type are African catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*) (Amenyogbe *et al.*, 2018b). Rivers, reservoirs, and the production ponds of fellow farmers are common sources of fingerling tilapia and catfish. Additionally, a few fingerling producers, like Aquaculture Research, Water Research Institute and Development Centre (WRIARDEC) in Akosombo, now create and provide fish farmers with high-quality fingerlings. According to Antwi-Asare & Abbey (2011), most farmers raise their own fingerlings in concrete tanks for quality pond stocking. In agriculture, farmers can practice monoculture or polyculture, depending on the type of system they use.

2.4 Bacterial infections in aquaculture

One significant issue impeding fish farming's sustainability is fish disease (Walakira *et al.*, 2014). Numerous pathogens have been found to be the main culprits behind illness in aquaculture. In Ghana, bacterial diseases are the most common type of pathogen, despite the fact that many water bodies are home to viruses, bacteria, fungi, and parasites (Amenyogbe *et al.*, 2018b).

Weather that is conducive to the survival of pathogenic bacteria is partially responsible for the annual outbreaks of bacterial diseases observed in Ghanaian fish culture. Additional elements that contribute significantly to the growth of these infections are temperature, seasonal variations in pH, and dissolved oxygen (Mugwanya *et al.*, 2022).

Infections in cultured fish are caused by various genera of both Gram-positive and Gram-negative bacteria (Takyi *et al.*, 2012). Both inside or on the fish itself, as well as in its surroundings, are pathogens. Fish that have experienced stressors like malnutrition, contaminated water, or extreme temperatures are typically more vulnerable to bacterial infections. Fish that are under stress have weakened immune systems and are more vulnerable to illness. In fish, bacteria can spread through the mouth, gill damage, skin abrasions, and latent carriers. Ascites, ulcers, hemorrhages, fin and tail rot, “mouth fungus”, “saddle back lesions”, exophthalmia, and color changes are among the commonly seen signs of bacterial disease in cultured fish.

2.4.1 *Enterobacteriaceae*

The *Enterobacteriaceae* is a family of Gram-negative bacteria that are widely distributed in nature. This family includes several genera, such as *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, and others (Janda & Abbott, 2021). These bacteria are part of the microbiota in the gastrointestinal tract of both humans and animals but can also cause various infections, including urinary tract

infections, respiratory tract infections, bloodstream infections, and gastrointestinal infections (Flores-Mireles *et al.*, 2015).

One of the notable features of *Enterobacteriaceae* is their ability to acquire and transfer antimicrobial resistance genes. This family of bacteria has been associated with the emergence and spread of antimicrobial resistance, which is a major concern in healthcare settings (Reygaert, 2018). *Enterobacteriaceae* can acquire resistance genes through various mechanisms, including horizontal gene transfer, mutation, and selection pressure from antimicrobial use (Tao *et al.*, 2022). The increasing prevalence of antimicrobial resistance among *Enterobacteriaceae* poses a serious threat to the effective treatment of infections caused by these bacteria (Chinemerem Nwobodo *et al.*, 2022). The acquisition of resistance genes by these pathogens can render commonly used antibiotics ineffective, leading to a limited treatment arsenal and consequently higher rates of morbidity and mortality (Huemer *et al.*, 2020).

In recent years, specific *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae* have emerged as primary contributors to the global antimicrobial resistance crisis (Basak *et al.*, 2016; Farkas *et al.*, 2019). These bacteria have been associated with healthcare-associated infections, community-acquired infections, and infections in livestock and food-producing animals (Duin & Paterson, 2020). Surveillance of antimicrobial resistance in *Enterobacteriaceae* is crucial for understanding the prevalence, trends, and mechanisms of resistance. Monitoring the antimicrobial susceptibility patterns of these bacteria helps guide appropriate treatment choices and informs antimicrobial stewardship programs (Johnson, 2015; Majumder *et al.*, 2020).

2.4.2 Fecal coliform load in fish and aquaculture environment

Fecal coliform load in fish and the aquaculture environment is an important aspect to consider when assessing water quality, public health concerns, and the overall sustainability of aquaculture practices (Mrozik *et al.*, 2019). Fecal coliform bacteria, such as *Escherichia coli*, are used as common indicator of fecal contamination in water and food. They are present in the intestinal tracts of warm-blooded animals, including humans and animals raised in aquaculture (Duman *et al.*, 2019).

The presence of fecal coliforms in fish and the aquaculture environment suggests the potential presence of pathogens that can cause foodborne illnesses (Sanjee & Karim, 2016). The intensity of fecal coliform contamination from fish waste depends on factors such as fish density, feeding practices, and waste management strategies employed in the aquaculture operation (Pandey *et al.*, 2014). Monitoring the fecal coliform load provides valuable information about the overall sanitary conditions and potential risks associated with aquaculture operations (Leight *et al.*, 2018).

There are several methods for assessing the fecal coliform load in fish and the aquaculture environment. These methods typically involve collecting water samples, fish samples, or environmental samples such as sediment or biofilms (Abdallah-Ruiz *et al.*, 2022). The samples are processed in the laboratory using techniques such as membrane filtration or most probable number (MPN) estimation (MacIntyre *et al.*, 2019).

The recovered bacteria are then plated on selective media containing specific nutrients and indicators to facilitate the growth and identification of fecal coliforms. The colony-forming units (CFUs) are counted, and the results are reported as CFUs per unit volume or per unit weight of the sample (Cabral, 2010).

The fecal coliform load can provide insights into the potential presence of antibiotic-resistant bacteria in aquaculture settings. Fecal coliform bacteria can serve as reservoirs of antibiotic resistance genes and mobile genetic elements that can be transferred to other bacteria in the environment (Larsson & Flach, 2022). High fecal coliform loads indicate a higher likelihood of contamination with antibiotic-resistant bacteria that may pose a risk to fish health and public health (Mishra *et al.*, 2018).

Additionally, exposure to sublethal concentrations of antibiotics in aquaculture practices can select for antibiotic-resistant fecal coliform bacteria, thereby increasing the prevalence of resistance (Manyi-Loh *et al.*, 2018). By assessing the fecal coliform load and monitoring changes over time, aquaculture operators and researchers can gain a better understanding of the potential risks associated with antibiotic resistance in aquaculture environments (Adinortey *et al.*, 2020).

This information can guide the development and implementation of appropriate management strategies, such as improving water quality, implementing good farming practices, and reducing the use of antibiotics (Manyi-Loh *et al.*, 2018). Furthermore, tracking the fecal coliform load can contribute to early detection and intervention to prevent disease outbreaks and minimize the spread of antibiotic-resistant bacteria in aquaculture settings (Larsson & Flach, 2022).



2.4.2.1 *Salmonella* spp.

Salmonella, a common bacterial pathogen, has been identified in both freshwater and saltwater fish species (Porto *et al.*, 2022). Consumption of raw or undercooked contaminated fish can result in *salmonellosis*, a human illness characterized by diarrhea, abdominal pain, fever, and nausea (Teklemariam *et al.*, 2023). In severe cases, especially among vulnerable groups like children, the elderly, and immunocompromised individuals, medical intervention may be necessary (Ehuwa *et al.*, 2021).

2.4.2.2 *Vibrio* spp.

Vibrio bacteria are naturally present in marine environments and commonly inhabit fish and shellfish (Onohuean *et al.*, 2022). Among these, *Vibrio parahaemolyticus* and *Vibrio vulnificus* pose significant health risks to humans (Leighton *et al.*, 2023). Consuming raw or undercooked seafood contaminated with *Vibrio* can cause gastrointestinal illness, characterized by watery diarrhea, abdominal cramps, and vomiting (Iwamoto *et al.*, 2010). In severe cases, *Vibrio vulnificus* infections can enter the bloodstream, particularly affecting individuals with compromised health (Yun & Kim, 2018).

2.4.2.3 *Clostridium botulinum*

Clostridium botulinum is a bacterium producing a potent neurotoxin that causes botulism (Poulain & Popoff, 2019). While primarily associated with improperly canned foods, certain fish species, particularly smoked and vacuum-packed varieties, can harbor *C. botulinum* and its toxin (Min *et al.*, 2021). Consuming food contaminated with this toxin results in botulism, a severe illness characterized by muscle weakness, blurred vision, swallowing difficulties, and respiratory paralysis (Chaudhry, 2011).

2.4.2.4 *Listeria monocytogenes*

Listeria monocytogenes is a bacterium commonly found in the environment, including fish and seafood processing environments (Shamloo *et al.*, 2019). It can contaminate fish during processing and cause listeriosis in humans. *Listeriosis* poses a significant threat to pregnant women, increasing the risk of miscarriage, stillbirth, or severe illness in newborns (Shamloo *et al.*, 2019). Symptoms of listeriosis include fever, muscle aches, nausea, and diarrhea (Shamloo *et al.*, 2019).

2.4.2.5 *Aeromonas* spp.

Aeromonas species are bacteria commonly associated with aquatic environments, including fish (Pessoa *et al.*, 2022). While many *Aeromonas* strains are harmless, some can cause infections in humans. *Aeromonas* infections typically manifest as gastrointestinal illnesses, resulting in diarrhea, abdominal pain, and vomiting (Pessoa *et al.*, 2022). In rare cases, severe infections can develop, particularly in individuals with compromised immune systems.

2.4.2.6 *Escherichia coli*

Escherichia coli (*E. coli*) is a bacterium commonly found in the intestinal tracts of animals, including fish (Conway & Cohen, 2015). Though most strains are not harmful, certain pathogenic strains, such as Shiga toxin-producing *E. coli* (STEC), can cause severe foodborne illnesses (Bintsis, 2017). Consuming raw or undercooked fish contaminated with pathogenic *E. coli* can lead to gastrointestinal infections, with symptoms ranging from mild diarrhea to severe bloody diarrhea and kidney damage (Ziarati *et al.*, 2022).

It is important to note that the risk of bacterial infections from fish can be reduced by following proper handling, storage, and cooking practices (Ziarati *et al.*, 2022; Iwamoto *et al.*, 2010). Thorough cooking of fish to appropriate temperatures effectively eliminates bacteria, significantly

reducing infection risk (Iwamoto *et al.*, 2010; Abebe *et al.*, 2020). Complementing this with rigorous hygiene practices, such as consistent handwashing and utensil cleaning after handling raw fish, is crucial for preventing bacterial cross-contamination (Menini *et al.*, 2022).

2.5 Antibiotic use and antimicrobial resistance in aquaculture

Antimicrobial resistance, one of the key global health concerns of this era, is being caused by the increased use of antibiotics in people and food-producing animals. The sustainability of food animal production and animal welfare are threatened by the rising incidence of antibiotic-resistant pathogens that are significant for animal production. These pathogens also increase treatment failure rates (Prestinaci *et al.*, 2015).

Intensified aquaculture production and the increasing prevalence of pathogens of aquatic animal are driving factors in antimicrobial use and subsequent resistance, mirroring trends in terrestrial livestock (Henriksson *et al.*, 2018). Unlike terrestrial systems, aquaculture introduces a unique pathway for antimicrobial distribution through water, posing significant risks to ecosystem health. Antimicrobial residues in aquatic environments disrupt the environmental microbiome, impairing its crucial roles in biodiversity, nutrient cycling, carbon sequestration, and freshwater regulation (Mu *et al.*, 2023). Understanding the aquatic microbiome's role in disease control is equally vital.

Aquaculture settings, often reliant on antibiotics, can serve as reservoirs for antimicrobial-resistant bacteria, potentially exposing humans and other animals to these pathogens (Pepi & Focardi, 2021). The mobility of resistance genes encoded on genetic elements has facilitated their spread among bacteria from humans, aquatic animals, terrestrial, and the environment, though the exact pathways and directionality remain complex (Pepi & Focardi, 2021). The development and

dissemination of plasmid-mediated colistin resistance genes exemplify the potential for aquaculture to be a source of antimicrobial resistance.

The amount of antibiotics used in various production systems has been the subject of several geographically limited studies. Together with these, a strong body of qualitative reviews has produced insightful analysis on the use of antimicrobials in aquaculture and the factors that influence it. Unfortunately, there is still a lack of documentation regarding the levels and trends of antibiotic use in aquaculture worldwide, which makes it difficult to implement targeted interventions and policies that support responsible antibiotic stewardship in a rapidly expanding sector (Schar *et al.*, 2020).

2.5.1 Aquaculture-associated antimicrobial resistance in Ghana's aquatic systems

Pollution is acknowledged as the most common and serious issue in aquaculture, so despite the many opportunities for pisciculture in Ghana, its management faces certain difficulties (Liaqat *et al.*, 2017). Fishponds can be contaminated by biological, physical, or chemical pollutants. Fishpond biological pollution is primarily caused by infectious agents like fungi, bacteria, and parasites. These agents can also make fishery products unhealthy in addition to posing a threat to fish health. Coliform bacteria and *Escherichia coli* are two infectious bacterial agents that are known to be significant pathogens in fish populations (Winfield & Gerdeaux, 2015). Microbial assessment of both fish and their aquatic environment is crucial for accurately evaluating the hygienic conditions within aquaculture systems.

A crucial source of information regarding the hygienic state of aquaculture environments is the microbial assessment of fish and their aquatic environment (Costantini *et al.*, 2023). Fishponds have also been shown to have chemical pollution, primarily from pesticides and herbicides (Liaqat *et al.*, 2017), which has extremely unsettling effects on fish survival because fish are sensitive to

these substances (Costantini *et al.*, 2023). Antimicrobials, particularly antibiotic residues, have been found to be extensively present in aquaculture waters, products, and sediments in Ghana and other countries, in addition to pesticides and herbicides (Winfield & Gerdeaux, 2015). Studies have connected a number of factors, such as the ongoing use of antibiotics in farming and animal husbandry and improper disposal of different household, medical, and industrial wastes, to the presence of antibiotic residues and antibiotic-resistant bacteria in the natural environment.

Antibiotic-resistant bacterial strains emerge as a result of improper antibiotic use, which gradually reduces their effectiveness despite the fact that antibiotics are frequently used in agriculture with the primary goal of reducing economic losses associated with infectious diseases (Díaz-Cruz & Barceló, 2006). A major threat to global health, these resistant bacterial strains can spread to humans directly through the consumption of contaminated food (Smith, 2008) or indirectly through the transfer of their resistance genes to human or fish pathogens. Unfortunately, these resistant bacterial strains could seriously affect the treatment of diseases in fish and humans due to the high similarity between antibiotic classes prescribed in veterinary medicine and those used to treat human infections (Smith *et al.*, 1994).

2.6 Antibiotic resistance surveillance in aquaculture

Surveillance of antibiotic resistance in aquaculture plays a vital role in assessing the prevalence and trends of resistant bacteria in fish populations, farm environments, and associated ecosystems (Ferri *et al.*, 2022; Pepi & Focardi, 2021). By monitoring resistance patterns, it becomes possible to identify emerging resistance mechanisms, detect changes in resistance profiles over time, and evaluate the effectiveness of interventions aimed at reducing antibiotic use (Munita & Arias,

2016). Surveillance data also provide valuable information for risk assessment, policy development, and the implementation of targeted strategies to mitigate the spread of antibiotic resistance (Johnson, 2015).

Several countries and international organizations have established surveillance programs to monitor antibiotic resistance in aquaculture (Ferri *et al.*, 2020; Velazquez-Meza *et al.*, 2022). These programs often involve collecting samples from fish farms, hatcheries, and processing facilities, followed by laboratory analysis to identify and characterize resistant bacteria (Wamala *et al.*, 2018; Agbekoet *et al.*, 2022). However, these surveillance efforts may face challenges such as limited resources, lack of standardized methodologies, and variations in data collection and reporting practices (Gandra *et al.*, 2020).

Additionally, there may be gaps in surveillance coverage, particularly in regions with limited regulatory frameworks or inadequate infrastructure for monitoring antibiotic resistance (Kariuki *et al.*, 2022). Tracking the prevalence and spread of multidrug-resistant bacteria in aquaculture presents several challenges. Firstly, the diversity of aquaculture systems, including different species, production methods, and geographical locations, makes it difficult to generalize findings and establish comprehensive surveillance programs (Pepi & Focardi, 2021). Secondly, the complex dynamics of resistance development and transmission in aquaculture environments require a multidisciplinary approach, integrating epidemiology, microbiology, genetics, and environmental science (Lepper *et al.*, 2022).

Furthermore, the lack of standardized guidelines for sampling, laboratory testing, and data analysis hinders comparability and harmonization of surveillance efforts across different regions and countries (Ashley *et al.*, 2019).

2.7 Spread of AMR in fish production and its implications for human infections

The presence of antimicrobial resistance (AMR) in fish farming has significant implications for human infections (Watts *et al.*, 2017). The use of antibiotics in aquaculture, both for treating diseases and promoting growth, has contributed to the emergence and dissemination of bacteria in fish populations that are resistant to antibiotics (Pepi & Focardi, 2021). These bacteria, which are resistant to antibiotics, can be transmitted to humans through different pathways, increasing the risk of treatment failures and the spread of AMR (Huemer *et al.*, 2020).

Antibiotics are commonly used in aquaculture to prevent and treat bacterial diseases, and to enhance growth and feed efficiency (Pepi & Focardi, 2021). However, their overuse and misuse can drive the selection and proliferation of antibiotic-resistant bacteria (Manyi-Loh *et al.*, 2018). Continuous exposure to subtherapeutic antibiotic levels creates ideal conditions for the development and spread of antibiotic resistance (Pepi & Focardi, 2021; Manyi-Loh *et al.*, 2018).

Moreover, the use of critically important antibiotics, such as those used in human medicine, in aquaculture can further accelerate the emergence of resistance (Manyi-Loh *et al.*, 2018). The transmission of antibiotic-resistant bacteria in aquaculture environments can occur through several pathways. These include direct contact between fish and water, horizontal gene transfer among bacteria, and exposure to contaminated water sources (Pepi & Focardi, 2021). Antibiotic-resistant bacteria can be introduced into aquaculture systems through contaminated feed, water, or equipment (Watts *et al.*, 2017).

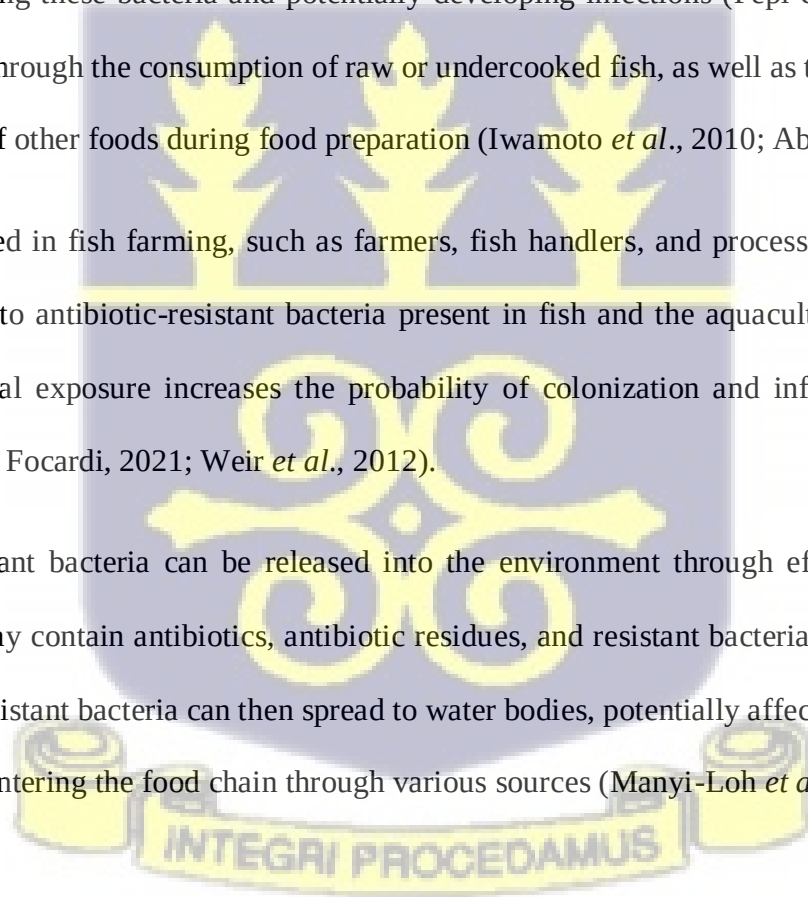
Besides, farm attendants, through their interactions with the fish and the environment, can act as carriers of antibiotic-resistant bacteria, facilitating their dissemination (Manyi-Loh *et al.*, 2018).

Antibiotic resistance in aquaculture can have significant economic implications for the industry. The emergence of antibiotic-resistant pathogens can lead to disease outbreaks in fish populations, resulting in decreased production, increased mortality rates, and financial losses for aquaculture farmers (Pepi & Focardi, 2021). In response to these challenges, farmers may resort to using higher doses or different types of antibiotics, which can further exacerbate antibiotic resistance issues. Additionally, the presence of antibiotic residues in aquaculture products can lead to trade restrictions and consumer concerns, affecting marketability and profitability (Hassan *et al.*, 2021).

Furthermore, when humans consume fish contaminated with antibiotic-resistant bacteria, there is a risk of acquiring these bacteria and potentially developing infections (Pepi & Focardi, 2021). This can occur through the consumption of raw or undercooked fish, as well as through the cross-contamination of other foods during food preparation (Iwamoto *et al.*, 2010; Abebe *et al.*, 2020).

Workers involved in fish farming, such as farmers, fish handlers, and processors, are at risk of direct exposure to antibiotic-resistant bacteria present in fish and the aquaculture environment. This occupational exposure increases the probability of colonization and infection with these bacteria (Pepi & Focardi, 2021; Weir *et al.*, 2012).

Antibiotic-resistant bacteria can be released into the environment through effluents from fish farms, which may contain antibiotics, antibiotic residues, and resistant bacteria (Pepi & Focardi, 2021). These resistant bacteria can then spread to water bodies, potentially affecting other aquatic organisms and entering the food chain through various sources (Manyi-Loh *et al.*, 2018).



2.8 Implications of multidrug-resistant bacteria in aquaculture

Infections caused by antibiotic-resistant bacteria acquired from fish can be challenging to treat. These bacteria possess resistance genes that reduce the effectiveness of commonly used antibiotics, limiting treatment options and increasing the risk of treatment failures (Manyi-Loh *et al.*, 2018; Pepi & Focardi, 2021).

Infections caused by antibiotic-resistant bacteria often lead to more severe illnesses, prolonged hospitalizations, escalating healthcare expenditures, and higher morbidity and mortality rates. The limited availability of effective antibiotics exacerbates the outcomes and complications associated with these infections (Llor & Bjerrum, 2014; Dadgostar, 2019).

Vulnerable populations, including children, the elderly, and immunocompromised individuals, are at heightened risk of severe infections. The transmission of antibiotic-resistant bacteria from fish to these groups can have severe health consequences (Davies & Davies, 2010; Stec *et al.*, 2022).

The presence of antibiotic-resistant bacteria in fish and their subsequent transmission to humans can contribute to the dissemination of resistance genes within the human microbiota (Argudín *et al.*, 2017). This dissemination has broader implications for the development of AMR in human pathogens, potentially compromising the efficiency of antibiotics in treating various infections (Mancuso *et al.*, 2021).

Addressing the spread of AMR in fish production requires a comprehensive approach. It is crucial to implement responsible antibiotic use practices in aquaculture, including proper disease prevention measures, accurate diagnosis, and targeted treatment (St-Hilaire *et al.*, 2023). Additionally, regulatory measures, surveillance programs, and good aquaculture management

practices can help minimize the occurrence and spread of antibiotic resistance in fish populations (Pepi & Focardi, 2021; Ferri *et al.*, 2022).

2.9 Prevalence of ESBL and carbapenem producers in aquaculture

Extended-spectrum beta-lactamase producers (ESBLs) are enzymes produced by bacteria that confer resistance to a broad range of beta-lactam antibiotics, including penicillins and cephalosporins. Carbapenems are a class of potent antibiotics often referred to as “last-resort” drugs, and resistance to carbapenems is particularly worrisome due to limited treatment options (Sawa *et al.*, 2020).

In recent years, studies have reported the presence of ESBL and carbapenem-producing bacteria in aquaculture environments, including fish farms and hatcheries. The introduction of antibiotics into aquaculture systems for disease prevention and treatment has contributed to the selection and dissemination of these antibiotic-resistant bacteria (Sivaraman *et al.*, 2021). Several studies have documented the presence of ESBL-producing bacteria in aquaculture environments worldwide. The prevalence of ESBL producers can vary depending on geographical location, aquaculture practices, and the specific bacterial species under investigation (Sivaraman *et al.*, 2021). Common ESBL producers in aquaculture include *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. (Sola *et al.*, 2022).

Although less extensively studied, carbapenem-resistant bacteria have also been reported in aquaculture settings. These bacteria can carry carbapenemase genes, which encode enzymes that inactivate carbapenem antibiotics (Caliskan-Aydogan & Alocilja, 2023). Carbapenem resistance

has been observed in various bacterial species, including members of the Enterobacteriaceae family (Dewi *et al.*, 2021).

Aquaculture workers and individuals in close contact with aquaculture environments can be exposed to ESBL and carbapenem-resistant bacteria (Hamza *et al.*, 2020). Direct contact with contaminated water, fish, or contaminated surfaces can pose a risk of colonization or infection by these bacteria. ESBL and carbapenem-resistant bacteria can be released into the environment through aquaculture effluents, potentially contaminating surrounding water bodies and aquatic organisms (Ramírez-Castillo *et al.*, 2023). This dissemination contributes to the spread of antibiotic resistance, including the potential transfer of resistance genes to other bacterial species (Tao *et al.*, 2022).

2.10 Studies on the prevalence of multidrug-resistant bacteria in aquaculture

Boulares *et al.* (2011) conducted a study on the microbial ecology of wild and aquacultured Tunisian fresh fish. They found that aquacultured fish had higher bacterial loads compared to wild fish. The dominant bacterial species identified in the aquacultured fish samples were *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Vibrio alginolyticus*. This suggests that farming practices can influence the microbial ecology of fish.

El Maksod *et al.* (2023) conducted a microbiological analysis of farmed fish sold in Sharkia Governorate, Egypt, identifying several bacterial pathogens including *Pseudomonas*, *Aeromonas*, and *Vibrio*. The study underscores the critical need for stringent hygiene and sanitation protocols throughout the aquaculture and marketing process to safeguard consumer health.

Kaktcham *et al.* (2017) conducted a quantitative analysis of the bacterial communities in earthen pond environments, tilapia, and carp. Their findings revealed that *Salmonella*, *Vibrio*,

Staphylococcus, and *Escherichia coli* were predominant in both pond water and fish intestines. Notably, the study identified lactic acid bacteria (LAB), particularly *Lactococcus lactis* subsp. *lactis*, exhibiting potent antimicrobial properties against fish spoilage and pathogenic bacteria. These LAB produce organic acids and bacteriocin-like compounds.

In another study, Babatunde *et al.* (2023) assessed the prevalence of bacterial pathogens associated with African catfish hatcheries in Nigeria. The study identified various pathogens, including species of *Aeromonas*, *Pseudomonas*, and *Vibrio*. The findings highlighted the importance of improved biosecurity measures and proper management practices in hatchery environments to mitigate the risk of pathogen transmission.

Afolabi *et al.* (2020) also assessed the bacterial loads of *Clarias gariepinus* obtained from cultured and natural habitats. They found higher bacterial contamination levels in fish from aquaculture systems compared to those from natural habitats. This underscores the importance of implementing proper management and hygiene practices in fish farming to minimize bacterial contamination and ensure food safety.

Furthermore, El Maksod *et al.* (2023) conducted a microbiological evaluation of farmed fish species marketed in Sharkia Governorate, Egypt. They identified several bacterial species, including potential pathogens such as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. This emphasizes the need for strict handling, storage, and processing protocols to ensure the safety of farmed fish and prevention of potential foodborne illnesses.

Moreover, Chitambo *et al.* (2023) focused on identifying bacteria with public health significance in farmed Nile tilapia in Zambia. They isolated and characterized bacteria such as *Vibrio cholerae*, *Shigella* spp., and *Salmonella* spp., which are known to cause gastrointestinal illnesses in humans.

The study highlights the importance of monitoring and controlling bacterial pathogens in aquaculture to mitigate potential health risks associated with the consumption of contaminated fish.

2.11 Studies on antibiotic resistance in aquaculture

Adinortey *et al.* (2020) investigated the presence of antibiotic-resistant coliform bacteria in fish farms in the Central Region of Ghana. The study revealed high levels of coliform bacteria in the examined fish farms, with a significant proportion exhibiting resistance to multiple antibiotics. This highlights the potential risk of antibiotic resistance in the aquaculture industry and its implications for public health.

In another study, Agbeko *et al.* (2022) focused on identifying molecular determinants of antibiotic resistance in fish farms in Ghana. They detected antibiotic resistance genes, including those conferring resistance to tetracycline, sulfonamides, and beta-lactams, in bacteria isolated from the fish farm environment. The study highlights the potential risk of antibiotic resistance transmission through fish farming practices and emphasizes the need for prudent antibiotic use in aquaculture.

Similarly, Ginovyan *et al.* (2017) conducted a study on antibiotic resistance in *Pseudomonas* species isolated from fish farms in Armenia. The research found that these bacteria exhibited a high level of resistance to commonly used antibiotics such as ampicillin, tetracycline, and chloramphenicol. The study emphasized the importance of responsible antibiotic use in fish farming to prevent the development and spread of antibiotic resistance.

In addition, Ruzauskas *et al.* (2018) investigated the composition and antimicrobial resistance profile of Gram-negative bacteria prevalent in aquacultured fish. The study identified a diverse range of bacteria, including species of *Pseudomonas*, *Aeromonas*, and *Enterobacter*. These bacteria displayed varying levels of resistance to antibiotics, highlighting the need for effective monitoring and management of antimicrobial use in aquaculture to mitigate the risk to food safety.

Moreover, Toh *et al.* (2018) screened and characterized *Pseudomonas aeruginosa* strains from aquaculture and water environments. The study revealed that these strains exhibited resistance to multiple antibiotics, such as cefotaxime, ciprofloxacin, and gentamicin. The findings suggest that aquaculture environments could serve as reservoirs for antibiotic-resistant bacteria, raising concerns about their potential impact on public health.

Shahrokhi *et al.* (2022) also investigated the prevalence, antibiotic resistance patterns, and virulence factors of *Pseudomonas aeruginosa* strains isolated from fish in Iran. The research demonstrated a significant prevalence of these strains, which displayed high resistance rates against antibiotics including ceftazidime, ciprofloxacin, and imipenem. The study underscored the importance of robust surveillance and control measures to prevent the spread of antibiotic-resistant *Pseudomonas* strains through fish products.

Furthermore, Ben Mhenni *et al.* (2023) examined the prevalence and antibiotic resistance phenotypes of *Pseudomonas* spp. In fresh fish fillets. The study found a relatively low prevalence of *Pseudomonas* spp. However, among the isolated strains, a notable proportion exhibited resistance to antibiotics commonly used in aquaculture, such as ampicillin, tetracycline, and chloramphenicol. The research emphasized the need for implementing good aquaculture practices to minimize the risk of antibiotic resistance in fish products.

In another study, Custodio *et al.* (2023) Investigated the diversity and antibiotic resistance of *Enterobacterales* in fish farm lagoon sediments. The study revealed a diverse range of *Enterobacterales* bacteria with varying degrees of resistance to antibiotics such as ampicillin, streptomycin, and tetracycline. The findings emphasized the potential persistence and spread of antibiotic-resistant bacteria in aquatic environments associated with fish farming.

2.12 Molecular epidemiology of fish-borne and pond-borne AMR pathogens

The determination of molecular determinants of antimicrobial resistance is crucial to understanding the mechanism by which antibiotic resistance is manifested. Additionally, it goes a long way to provide the genetic elements responsible for AMR. According to research conducted by Agbeko *et al.* (2022a) in Ghana, about 14 AMR genes were seen in the pond of containing fish. They included *blaTEM*, *blaTEM-1*, *blaEBC*, *blaCTX-M*, *blamecA*, *Sul1*, *Sul3*, *Cat1*, *cmIA*, and *grA*, among others. These genes code for specific proteins, enzymes, or chemicals, responsible for inhibiting the action of the antibiotics. In a study by Bell *et al.* (2023), metagenomic approach was used to screen for antimicrobial resistance genes present in a pool used in rearing of fish. From the study, it was seen that the majority of the genes encode resistance to aminoglycosides and sulphonamides and were present in about most of the fishes isolated. Consequently, the pathogens isolated were resistant to about 18 different antibiotics.

The ability to screen for the various AMR determinants using PCR, RT-PCT, and other advanced molecular techniques, helps to provide molecular or genetic evidence of the mechanisms employed by the pathogens present in the fishes or pond, withstand the actions of the antibiotics (Galhano *et al.*, 2021).

A study was conducted by Agbeko *et al.* (2022b) in which RT-PCR was used to detect AMR bacteria and their molecular determinants. The study focused on fish farms in Ghana's Central and Western Regions, examining management practices and antibiotic use through a questionnaire. Bacterial loads in catfish, tilapia, and pond water samples were analyzed, revealing elevated levels in pond water. Using polymerase chain reaction (PCR), molecular determinants of antibiotic resistance, including *bla*TEM, *cm*IA, *qnr*S, *tet*B, and *bla*CTX-M, were identified in multidrug-resistant bacteria among 145 bacterial isolates. Despite fish farm managers denying antibiotic use, the study highlights the need for awareness regarding antibiotic misuse and the potential spread of multi-drug resistant bacteria in the environment.

Similarly, another study conducted over 12 months across 32 aquaculture farms in states including Selangor, Negeri Sembilan, Melaka, and Perak (Dewi *et al.*, 2022) investigated the prevalence and antimicrobial susceptibility of *E. coli*, *Salmonella*, and *Vibrio* in farmed fishes along the west coast of Peninsular Malaysia. Notably, both *E. coli* and *Salmonella* exhibited high resistance to several antibiotics, and *Vibrio* showed significant resistance to specific drugs. Detection of multi-drug resistance (MDR) was accomplished using PCR. Findings revealed growing public health concerns regarding the resistance to critical antibiotics including tetracycline, erythromycin, ampicillin, and trimethoprim. The study emphasized high MDR levels in both *E. coli* and *Salmonella* among tilapia, as well as varying resistance rates in Asian seabass. Overall, the comprehensive methodology employed in this study provides essential insights into antimicrobial resistance (AMR) in aquaculture, offering a basis for effective strategies in antibiotic use at local and regional levels.

Jeamsripong *et al.* (2022) investigated the correlation between phenotypic and genotypic antimicrobial resistance (AMR) in waterborne *Escherichia coli* (*E. coli*), *Salmonella enterica*,

Vibrio parahaemolyticus, and *Vibrio cholerae* isolated from Thai coastal aquaculture areas. All *Salmonella* and over half of *V. cholerae* isolates carried virulence genes (*invA* and *ompW*, respectively). High rates of phenotypic AMR were observed across bacterial species, with nearly 9% of all coastal water isolates demonstrating multidrug resistance (MDR). These findings emphasize the need for comprehensive AMR monitoring in coastal waters to safeguard food safety and public health through recreational water activities.



CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design and site

This was a cross-sectional investigation in which samples were taken from a total of 35 newly farms reared fishes from (12 tilapia and 23 catfish) and water from 35 fish rearing ponds. Farmers raised fish in an intensive farming system. These include earthing pond, concrete tanks, plastic tank and tarpaulin system. These regions and fish ponds were discovered in Nii Ashley, Gbentaana, Mammomo, Adjringanor, Manhee, Amanfrom, Maledjor, Frafraha, and New Legon, all of which are situated in the Adenta District of Ghana. The Adenta Municipal Assembly, which also includes Adenta as its Central Business District, can be found 10 kilometers to the northeast of Accra. Its precise coordinates are 5 degrees 43 minutes north latitude and 0 degrees 09 minutes west longitude. Approximately 928.4 square kilometers of land make up the municipality. Borders Ashaiman and Kpong Akatamanso to the east and north, and La Nkwantanang to the west and south (Figure 1). In the Adentan Municipality, only 7.1% of families are involved in agricultural activities. There are 938 more agricultural households located in urban areas than there are agricultural households located in rural locations (515). The vast majority of the Municipality's agricultural households (88.9%) are dedicated to crop production. The majority of the animals kept as livestock in the municipality are poultry (chickens) and goats (Ghana Statistical Service, 2014).

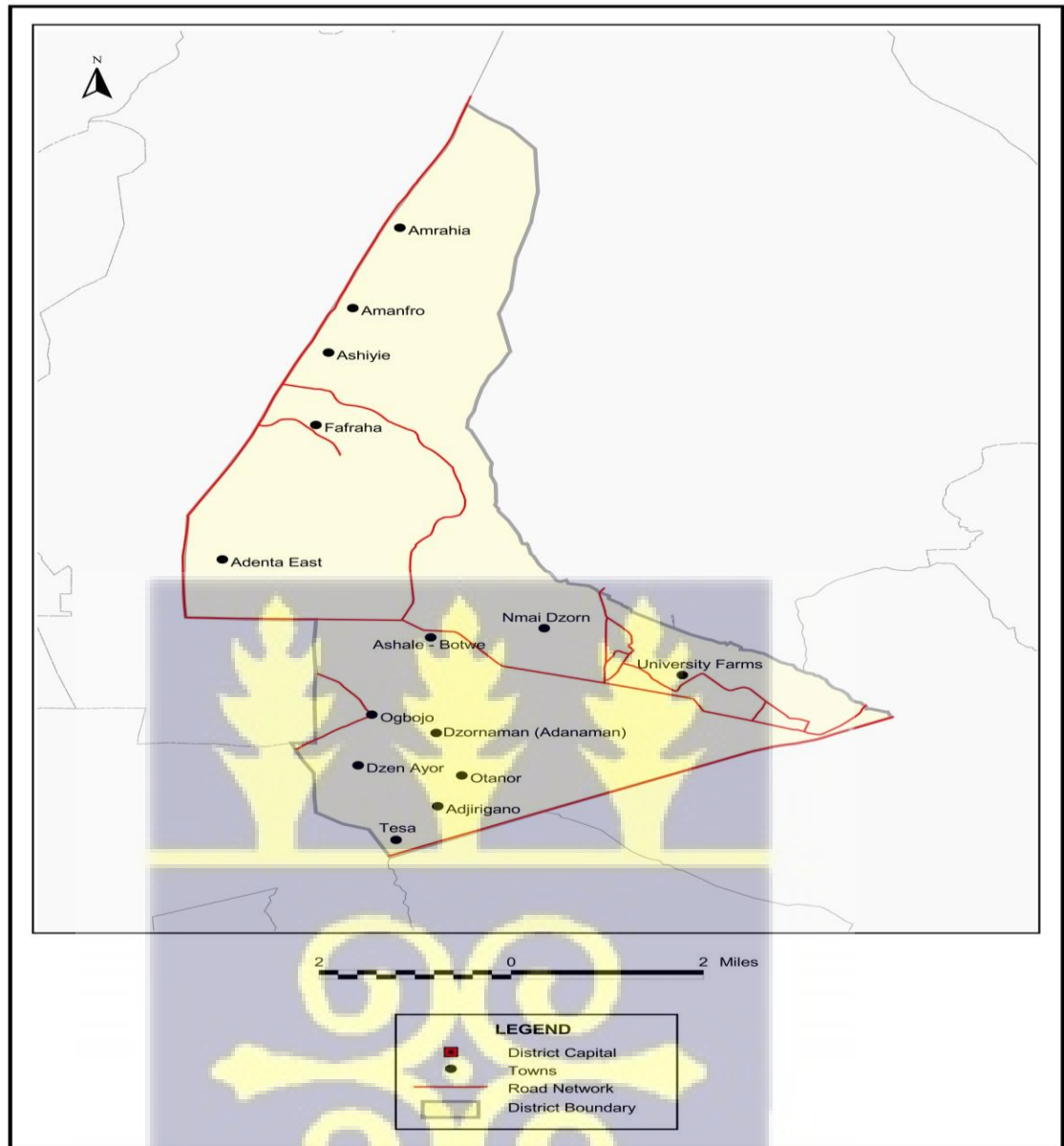


Figure 1: Map of Adenta Municipality (Source: Ghana Statistical Service, 2014)

3.2 Sample collection

All procedures performed on fish samples were conducted in strict accordance with the animal welfare policy established by the Veterinary Directorate of the Ministry of Food and Agriculture in Ghana, as well as relevant animal care and use guidelines (Hawkins *et al.*, 2011). The water and the species of farmed fish were both sampled with the cooperation of the farms' management, and

the sampling procedures were carried out in accordance with EPA (2007) recommendations. Sample collection, which involved administering interview guides and collecting water and cultured species, was conducted between 7:00 AM and 11:00 AM on each sampling day. First-hand information on farmers' management practices, with a focus on antibiotic use, was gathered. In total, 56 catfish and 24 tilapias were aseptically collected into uniquely labelled sterile zip-lock bags. Also, 500 ml water samples from the originating ponds were collected from each pond using sterile bottles, at a depth of 50 cm below the surface of the water. Fish samples were preserved by placing them on ice during transport to the research laboratory at the Department of Medical Microbiology, University of Ghana Medical School.

3.3 Media

The following media were used during the study; Blood agar, MacConkey agar, plate count agar, xylose lysine deoxycholate agar, nutrient agar, thiosulfate-citrate-bile salts-sucrose agar, modified semisolid Rappaport Vassiliadis agar, buffered peptone broth, normal saline, eosin methylene blue agar, Mueller Hinton agar and tryptone soya agar.

3.4 Laboratory analysis

Each of the containers containing the water was turned slowly and carefully about ten times, to ensure that the water in each of the samples gathered from the farms was completely mixed. Ten ml portion of each water was placed in a beaker and filled up to the 100 ml mark. The external surface of each fish sample was sterilized with 70% ethanol, and then an aseptic incision was made across the fish's peritoneal cavity using a sterile scalpel blade. The cut went from the pyloric valve all the way to the anus. After the intestines and internal organs had been removed, they were

completely homogenised. The combined fish (10%) content was added to 90% of buffered peptone water and at 37 °C incubated for 18–24 hours.

After incubation, a loopful of the neat sample was cultured or inoculated onto blood agar, MacConkey agar, and Thiosulfate-citrate-bile salts-sucrose (TCBS) agar. For modified semisolid Rappaport Vassiliadis agar, three drops of the neat sample were placed on the plate and incubated at 37 °C for 18–24 hours. From the mixed-growth culture, each group of isolates was obtained using aseptic technique with parallel overlapping strokes. This process was repeated for each distinct bacterial colony observed on the mixed growth culture plate, except for the modified semisolid Rappaport Vassiliadis agar, which was subsequently placed on Xylose Lysine Deoxycholate (XLD) agar and incubated for 18–24 hours at 37 °C. Isolates were Gram-stained and identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

3.5 Standard plate count or total viable count

Samples were thoroughly mixed (neat), after which 1 ml of the composite water and 1 ml of the composite cultured fish sample obtained from each farm were successively diluted in sterile normal saline solution and serially diluted up to the fifth ten-fold dilution factor. Using the spread-plate method, 1 ml of each diluted composite water sample and 1 ml of each composite cultured fish sample were individually pipetted onto the surface of sterile plate count agar plates using a sterile glass rod. Discrete colonies were counted using a colony counter (Stuart Scientific, UK) and expressed as colony-forming units per milliliter (CFU/ml).

3.6 Determination of coliform count (CC)

A 1 ml volume of the composite water and composite cultured fish sample obtained from each farm was successively diluted in sterile normal saline solution was serially diluted up to the fifth tenfold-dilution factor. With labelled plates, using the pour plate technique, 0.5 ml of each sample was added to 9.5 ml molten MacConkey and allowed to solidify on a flat bench. All distinct colonies were counted and expressed in CFU/ml using the colony counter.

3.7 Antibiotic susceptibility testing

The disk diffusion technique was employed to determine the susceptibility of the bacterial isolates to the following antibiotics: ampicillin (10 µg), gentamicin (10 µg), tetracycline (30 µg), cefuroxime (30 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), cefotaxime (30 µg), and ceftriaxone. The criteria provided by the Clinical and Laboratory Standards Institute (CLSI) (2023) were rigorously followed for conducting the test and interpreting the results [28]. A suspension of each bacterial isolate was prepared using sterile normal saline, and the turbidity was standardized to 0.5 McFarland. To obtain a bacterial lawn, a sterile cotton swab was used to inoculate the entire surface of a sterile Mueller-Hinton agar plate with the bacterial suspension. This was done in order to produce the bacterial lawn. After the plates had been inoculated, suspensions were allowed to be absorbed by media (Mueller-Hinton agar) for few minutes, and then antibiotic discs were carefully placed on the surfaces of the plates. The agar plates were incubated at 37 degrees Celsius for 16-20 hours. After that, diameter measurements of the zones of inhibition were recorded using a ruler and pair of dividers, and the results were written down.

3.8 Extended-spectrum B-Lactamase (ESBL)

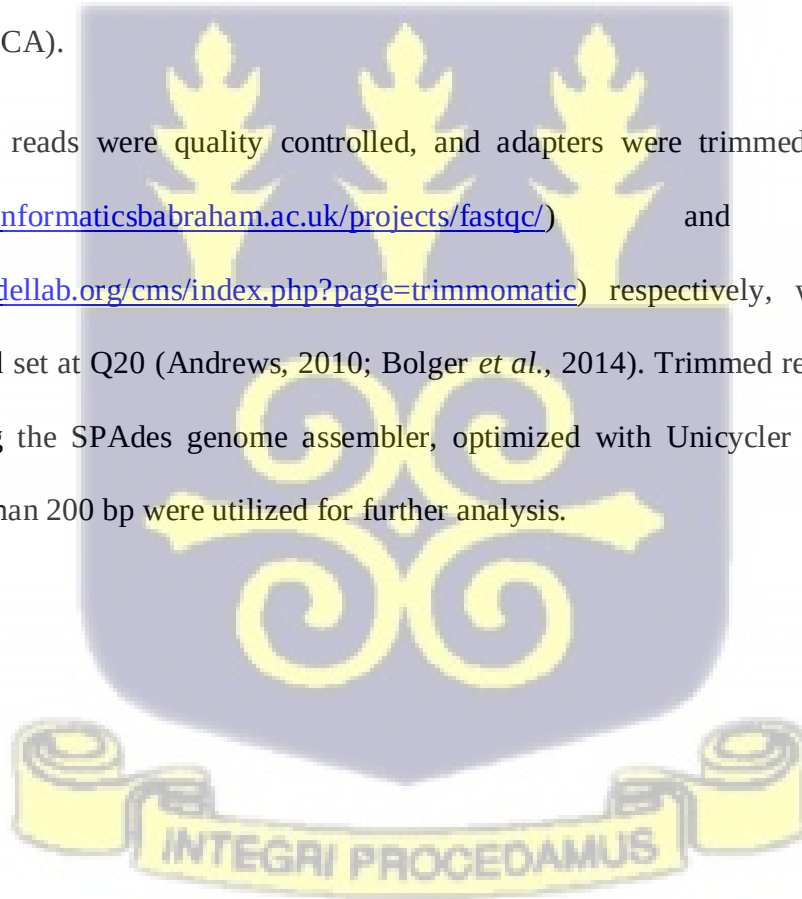
All bacterial isolates were sub-cultured onto tryptone bile X-glucuronide agar plus cefotaxime. At this stage, all non-ESBL bacteria did not grow. A suspension of each bacterial isolate was prepared using sterile normal saline, and the turbidity of the suspension was standardized to 0.5 McFarland. A sterile cotton swab was used to inoculate the entire surface of a sterile Mueller-Hinton agar plate with the bacterial suspension. This was done in order to produce the bacterial lawn. After the plates had been inoculated, suspensions were allowed to be absorbed by media (Mueller-Hinton agar) for few minutes, and then antibiotic discs were carefully placed on the surfaces of the plates. The agar plates were incubated at 37 degrees Celsius for 16-20 hours. After that, diameter measurements of the zones of inhibition were recorded using a ruler and pair of calipers or divider, and the results were written down. The measurements that were acquired were then converted into SI units using the CLSI (2023). All bacterial isolates were classified as either sensitive or resistant to each antibiotic. The following sensitivity discs were used: cefotaxime, cefotaxime/clavulanate, ceftazidime and ceftazidime/clavulanate.

3.9 Whole-genome sequencing and analysis

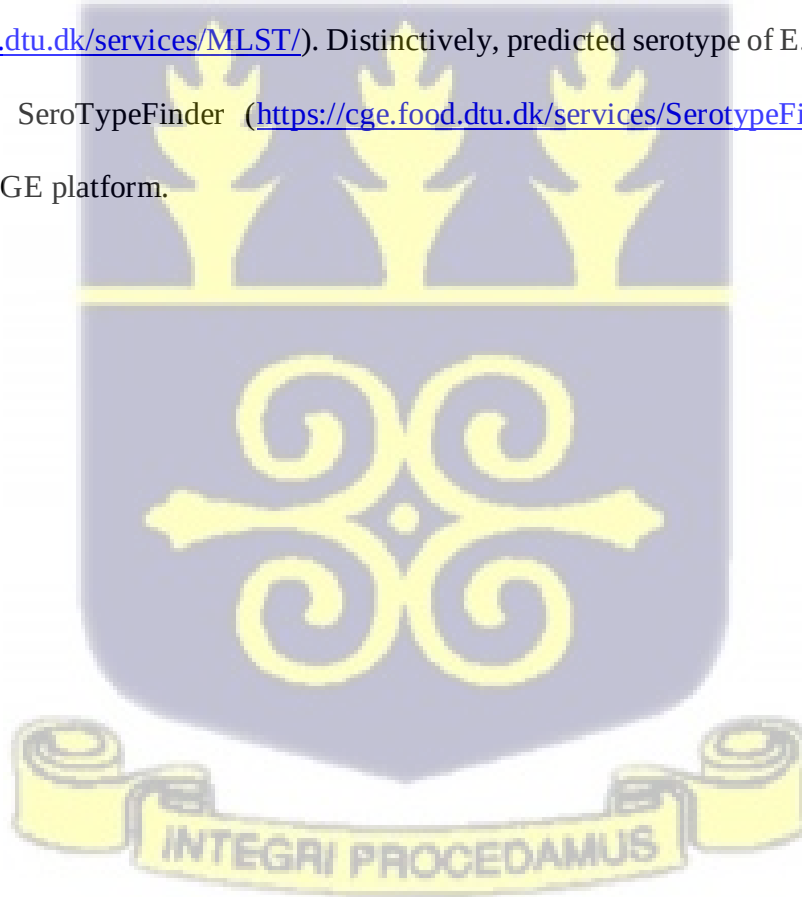
Whole genome sequencing (WGS) analysis was performed on ESBL-positive isolates at the Noguchi Memorial Institute for Medical Research, University of Ghana. DNA of freshly cultured isolates was extracted using Qiagen DNA MiniAmp kit following the manufacturer's instructions. Extracted DNA was quantified using a Qubit 4.0 Fluorometer assay kit (Thermo Fisher Scientific, MA), followed by library preparation with Illumina DNA prep following the manufacturer's instructions, DNA concentration between 100 to 500ng in 30µl was used as starting material for library preparation. The Beaded-Linked Transposomes (BLT), which included fragmenting and tagging the DNA with adapter sequences, were used to tag the DNA. Following this procedure, a

post-tagmentation clean-up was carried out to halt tagmentation and wash the adapter-tagged DNA prior to PCR amplification (PCR). The tagmented DNA was subsequently amplified by PCR, and paired index adapters and sequences needed for cluster formation on the flow-cell during sequencing were also added. The amplified libraries were then cleaned up using a post-PCR clean-up procedure to get rid of extra unbound index adapters. The quality and concentration of fragmented DNA were assessed with the 2100 bioanalyzer system (Agilent) and qPCR (Kapa Sybr Fast qPCR kit) respectively. Based on the fragment sizes obtained from the Bioanalyzer and the concentration of individual libraries from the qPCR, the libraries were then normalized, pooled, and loaded onto Illumina 2 x 300 cycle cartridges for sequencing on the Miseq platform (Illumina Inc., San Diego, CA).

Raw sequenced reads were quality controlled, and adapters were trimmed using FASTQC (<http://www.bioinformaticsbabraham.ac.uk/projects/fastqc/>) and Trimmomatic (<http://www.usadellab.org/cms/index.php?page=trimmomatic>) respectively, with a minimum quality threshold set at Q20 (Andrews, 2010; Bolger *et al.*, 2014). Trimmed reads were de novo assembled using the SPAdes genome assembler, optimized with Unicycler V0.4.9, and only contigs greater than 200 bp were utilized for further analysis.



Assembled files were uploaded to ResFinder (<https://cge.food.dtu.dk/services/ResFinder/>), a tool available on the Centre for Genomic Epidemiology (<http://www.genomicepidemiology.org/>) platform, to identify resistance genes present in the sequenced isolates using an identity threshold of 90% and a minimum length of 60%. Prediction of bacteria species and acquired virulence genes were done using KmerFinder (<https://cge.food.dtu.dk/services/KmerFinder/>) and VirulenceFinder (<https://cge.food.dtu.dk/services/VirulenceFinder/>) respectively. Plasmids and mobile genetic elements were predicted using Plasmidfinder (<https://cge.food.dtu.dk/services/PlasmidFinder/>) and MGEfinder (<https://cge.food.dtu.dk/services/MobileElementFinder/>) respectively. The sequence types of the isolates were predicted using MLSTFinder (<https://cge.food.dtu.dk/services/MLST/>). Distinctively, predicted serotype of *E. coli* isolates were done using the SeroTypeFinder (<https://cge.food.dtu.dk/services/SerotypeFinder/>) at default settings on the CGE platform.



CHAPTER FOUR

4.0 RESULTS

4.1 Fish/pond characteristics

Table 1 summarizes the fish species and pond types with their corresponding numbers and percentages. It shows that catfish represented 70.0% of the fishes sampled, while tilapia accounted for 30.0%. In terms of pond type, concrete ponds made up 47.5% of the total, followed by earthing, plastic tank, and tarpaulin ponds, each contributing 17.5% to the overall distribution.

Table 1: Fish/pond characteristics

Features	Number	%
Fish species		
Catfish	56	70.0
Tilapia	24	30.0
Pond type		
Concrete	38	47.5
Earthing	14	17.5
Plastic tank	14	17.5
Tarpaulin	14	17.5



4.2 Microbial loads in the fish and the ponds

Table 2 summarizes the microbial loads in terms of total viable count and total coliform count for fish and pond samples. The average total viable count was higher in fish samples (18.25 CFU/ml) compared to pond samples (6.55 CFU/ml), with an overall average of 12.4 CFU/ml. The average total coliform count is slightly higher in fish samples (0.35 CFU/ml) compared to pond samples (0.27 CFU/ml), with an overall average of 0.31 CFU/ml. This information provides insights into the microbial contamination levels in both fish and pond environments.

Table 2: Microbial loads in the fish and the ponds

Microbial Loads	Fish	Pond	Overall
Total viable count ($\times 10^5$ CFU/ml)	18.25	6.55	12.4
Total coliform count ($\times 10^5$ CFU/ml)	0.35	0.27	0.31

4.3 Distribution of the isolated bacteria in the fish and ponds

In total, 391 bacteria, that are of 39 different types were isolated from the fish and pond water. In the fish, a total of 201 bacteria from 38 different types, were isolated. Conversely, the bacterial isolates from the pond were 190 in total, from 34 species. As a composite, the top five bacteria were *Escherichia coli* (17%), *Aeromonas veronii* (11%), *Citrobacter freundii* (8%), *Bacillus cereus* (5%), and *Klebsiella pneumoniae* (5%). In the fish, the top five bacteria were *Escherichia coli* (15.4%), *Aeromonas veronii* (12.4%), *Citrobacter freundii* (9.5%), *Edwardsiella tarda* (5.0%), and *Klebsiella pneumoniae* (4.5%). In the pond water, the top five bacteria were *Escherichia coli* (19.5%), *Aeromonas veronii* (8.9%), *Bacillus cereus* (5.8%), *Citrobacter freundii*

(5.8%), and *Proteus mirabilis* (5.3%). Table 3 presents the details of the distribution of the bacteria in the fish and pond water.



Table 3: Distribution of the bacteria harboured in the fish and pond water

Organisms	Both Fish and Pond Water (N = 391)		Fish (N = 201)		Pond Water (N = 190)	
	N	%	n	%	n	%
<i>Escherichia coli</i>	68	17.0	31	15.4	37	19.5
<i>Aeromonas veronii</i>	42	11.0	25	12.4	17	8.9
<i>Citrobacter freundii</i>	30	8.0	19	9.5	11	5.8
<i>Bacillus cereus</i>	19	5.0	8	4.0	11	5.8
<i>Klebsiella pneumoniae</i>	18	5.0	9	4.5	9	4.7
<i>Kurthia gibsonii</i>	16	4.0	8	4.0	8	4.2
<i>Proteus mirabilis</i>	16	4.0	6	3.0	10	5.3
<i>Edwardsiella tarda</i>	15	4.0	10	5.0	5	2.6
<i>Plesiomonas shigelloides</i>	13	3.0	8	4.0	5	2.6
<i>Aeromonas hydrophila</i>	13	3.0	7	3.5	6	3.2
<i>Pseudomonas otitidis</i>	12	3.0	6	3.0	6	3.2
<i>Aeromonas caviae</i>	11	3.0	7	3.5	4	2.1
<i>Aeromonas jandaei</i>	10	3.0	5	2.5	5	2.6
<i>Vibrio albensis</i>	10	3.0	3	1.5	7	3.7
<i>Enterobacter bugandensis</i>	9	2.0	3	1.5	6	3.2
<i>Aeromonas enteropelogens</i>	8	2.0	4	2.0	4	2.1
<i>Lactococcus latis</i>	7	2.0	4	2.0	3	1.6
<i>Pseudomonas aeruginosa</i>	7	2.0	3	1.5	4	2.1
<i>Citrobacter braakii</i>	6	2.0	3	1.5	3	1.6
<i>Enterobacter kobei</i>	6	2.0	3	1.5	3	1.6
<i>Enterobacter cloacae</i>	6	2.0	2	1.0	4	2.1
<i>Pseudomonas putida</i>	6	2.0	2	1.0	4	2.1
<i>Klebsiella aerogenes</i>	5	1.0	3	1.5	2	1.1
<i>Aeromonas ichthiosmia</i>	5	1.0	3	1.5	2	1.1
<i>Enterobacter asburiae</i>	4	1.0	3	1.5	1	0.5
<i>Lysinibacillus sphaericus</i>	4	1.0	3	1.5	1	0.5
<i>Aeromonas jandaei</i>	3	1.0	2	1.0	1	0.5
<i>Comamonas aquatica</i>	3	1.0	1	0.5	2	1.1
<i>Salmonella sp.</i>	3	1.0	1	0.5	2	1.1
<i>Acinetobacter soli</i>	3	1.0	0	0.0	3	1.6
<i>Comamonas kerstersii</i>	2	1.0	1	0.5	1	0.5
<i>Klebsiella variicola</i>	2	1.0	2	1.0	0	0.0
<i>Vibrio fluvialis</i>	2	1.0	1	0.5	1	0.5
<i>Shewanella algae</i>	2	1.0	1	0.5	1	0.5
<i>Aeromonas caviae</i>	1	0.0	1	0.5	0	0.0
<i>Enterococcus hirae</i>	1	0.0	1	0.5	0	0.0
<i>Staphylococcus epidermidis</i>	1	0.0	1	0.5	0	0.0
<i>Providencia alcalifaciens</i>	1	0.0	1	0.5	0	0.0
<i>Citrobacter veronii</i>	1	0.0	0	0.0	1	0.5

4.4 AMR in the bacterial isolates

The antimicrobial resistance of the total bacteria isolated to tested antibiotics, stratified by sample type, are displayed in Figure 2, and in Tables 4–7 the distributions of the AMR rates across the individual bacteria are presented. Across each of the fish, pond water, and fish and pond water combined, the AMR rates were low to moderate, and were highest for tetracycline (31% to 42%), cefotaxime (29% to 35%), and chloramphenicol (19%). No resistance was recorded against either meropenem or ertapenem.

Regarding *E. coli*, the tetracycline resistance rate was 50% in the fish and pond water combined, 54.8% in the fish, and 45.9% in the pond water. Chloramphenicol resistance was 37.8% in the fish and pond water combined, 35.5% in the fish, and 37.8% in the pond water. Cefotaxime resistance was 38.2% in the fish and pond water combined, 41.9% in the fish, and 35.1% in the pond water.

In *Edwardsiella tarda*, the tetracycline resistance was as high as 80% in the fish and pond water combined, while chloramphenicol resistance was noted at 26.7%. In the fish exclusively, the resistance to tetracycline was 90%, accompanied by a chloramphenicol resistance rate of 30%. In the pond only, resistance rates were observed at 60% for tetracycline, 20% for chloramphenicol, and an additional 20% for cefotaxime.

For *Klebsiella pneumoniae*, in the combined fish and pond water, resistance rates were recorded at 66.7% for cefotaxime and 38.9% for tetracycline. In the fish alone, rates stood at 66.7% for cefotaxime and 33.3% for tetracycline. In the pond water only, resistance rates were 66.7% for cefotaxime and 44.4% for tetracycline.

Resistance rates in *Klebsiella variicola* were notable at 50% for chloramphenicol and 100% for tetracycline in combined fish and pond water. Similarly, in the fish alone, resistance persisted with rates of 50% for chloramphenicol and a maximum resistance of 100% for tetracycline.

Citrobacter freundii, in combined fish and pond water, showed resistance of 30% for cefotaxime, 43.3% for chloramphenicol, and 40% for tetracycline. In the fish alone, the resistance rates were 31.6% for cefotaxime, 42.1% for chloramphenicol, and 52.6% for tetracycline. In pond water only, the resistance was 27.3% for cefotaxime, 45.5% for chloramphenicol, and 18.2% for tetracycline.

Citrobacter braakii demonstrated tetracycline resistance of 33.3% in the combined fish and pond water and a higher resistance of 66.7% in the fish alone. In the case of *Kurthia gibsonii*, in the combined fish and pond water, it showed 25% resistance to ciprofloxacin, while in the fish alone, resistance rates were 37.5% for ciprofloxacin, 25% for gentamicin, and 50% for tetracycline.

For *Aeromonas veronii*, cefotaxime resistance was 21.4% in the combined fish and pond water, with a slightly elevated rate of 24% in the fish only. In the pond water only, resistance included 17.6% for cefotaxime and 23.5% for ciprofloxacin. *Aeromonas jandaei*, in the combined fish and pond water exhibited a tetracycline resistance of 30.8%, whereas in the fish only and pond water only, the resistance rates were 28.6% and 33.3%, respectively. Similarly, *Aeromonas caviae* had resistance in combined fish and pond water, with rates of 20% for cefotaxime and 50% for tetracycline, while in fish only, there was tetracycline resistance of 50%. In pond water only, cefotaxime, chloramphenicol, and tetracycline resistances are observed at 50%, 25%, and 50%, respectively.

Regarding *Enterobacter bugandensis*, in the combined fish and pond water, the resistance rates were 22.2% for cefotaxime, 44.4% for chloramphenicol, and 55.6% for tetracycline. In the fish

only, there was a high resistance to chloramphenicol (66.7%) and tetracycline (66.7%). Also, in the pond water alone, the resistance rate was 33.3% for cefotaxime, 33.3% for chloramphenicol, and 50% for tetracycline.

Enterobacter kobei displayed significant tetracycline resistance, with rates of 83.3% in the combined fish and pond water, 33.3% in fish only, and 66.7% in pond water. Likewise, *Enterobacter cloacae* exhibited a notable 50% cefotaxime resistance in the combined fish and pond water, while in pond water alone, cefotaxime resistance increased to 75%, accompanied by a chloramphenicol resistance of 25%.

In the combined fish and pond water, *Enterobacter asburiae* showed resistance rates of 25% for cefotaxime, 75% for chloramphenicol, and 50% for tetracycline. In the fish only, the resistance was 33.3% for cefotaxime, 100% for chloramphenicol, and 66.7% for tetracycline. For *Enterobacter hirae*, high resistance rates were found in both combined fish and pond water, as well as in the fish only, with 100% resistance to cefotaxime, chloramphenicol, and tetracycline.

Proteus mirabilis demonstrated resistance in combined fish and pond water at rates of 31.3% for cefotaxime and 100% for tetracycline. In the fish only, rates were 33.3% for cefotaxime and 100% for tetracycline. In the pond water only, the resistance was 30% for cefotaxime and 100% for tetracycline.

In the case of *Plesiomonas shigelloides*, in the combined fish and pond water, it exhibited resistance of 46.2% for cefotaxime and 38.5% for tetracycline. In the fish only, the resistance was 50% for cefotaxime and 37.5% for tetracycline, while in pond water only, the resistance was 40% for both cefotaxime and tetracycline.

Vibrio albensis displayed resistance in the combined fish and pond water environments, with rates of 30% for cefotaxime, 30% for ciprofloxacin, and 50% for chloramphenicol. In fish, the resistance rates were 33.3% each for cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline. In the pond water resistance included 28.6% each for cefotaxime and ciprofloxacin, and 57.1% for chloramphenicol.

Furthermore, in *Bacillus cereus*, in the combined fish and pond water, resistance rates were 63.2% for cefotaxime and 42.1% for tetracycline. In the fish alone, the resistance was 75% for cefotaxime and 37.5% for tetracycline. In the pond water, the resistance was 54.5% for cefotaxime and 45.5% for tetracycline.

Acinetobacter soli demonstrated 20% cefotaxime resistance in the combined fish and pond water, while in fish only, there was a higher cefotaxime resistance of 50%.

Moreover, *Lactococcus latis* displayed high cefotaxime resistance in combined fish water and pond environments (71.4%), with 50% resistance in fish only and 100% in pond water only. Similarly, *Pseudomonas putida* showed cefotaxime resistance of 83.3% in the combined fish and pond water, 100% in the fish only, and 75% in the pond water. In like manner, *Pseudomonas aeruginosa* exhibited 85.7% cefotaxime resistance in the combined fish and pond water, 100% in the fish only, and 75% in the pond water only.

Lysinibacillus sphaericus demonstrated complete resistance (100%) to chloramphenicol and tetracycline in the combined fish and pond water, as well as in the pond water alone, while in the fish, the only 100% resistance was to cefotaxime.

Comamonas aquatica, *Comamonas kerstersii*, and *Shewanella algae* all displayed cefotaxime resistance, with rates of 100% in all settings – combined fish water and pond, fish only, and pond water alone.

Salmonella sp. exhibited equal resistance rates of 33% to cefotaxime, chloramphenicol, and tetracycline in combined fish and pond water, while in pond water alone, the resistance increased to 50% for cefotaxime, chloramphenicol, and tetracycline.

Providencia alcalifaciens also displayed complete resistance to tetracycline in both combined fish and pond and fish only settings.

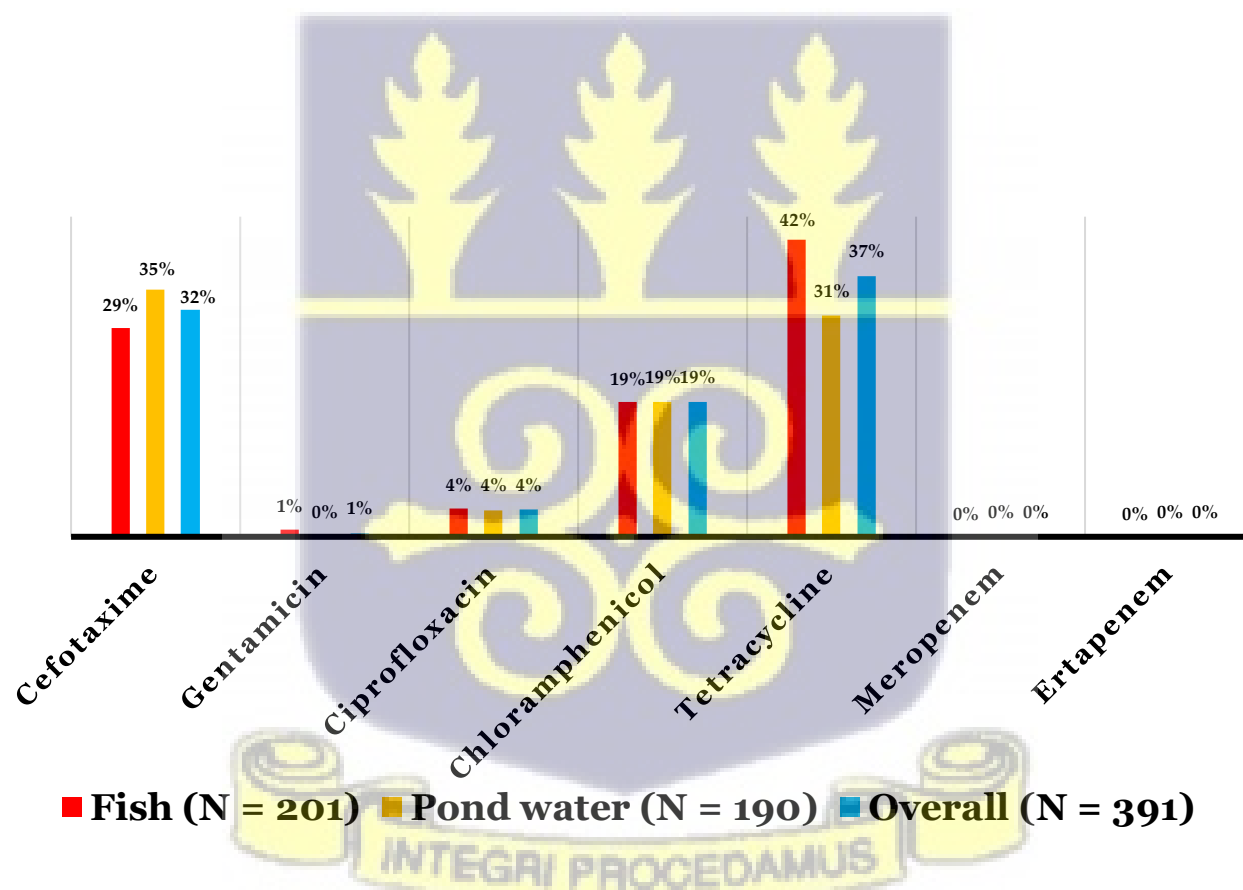


Figure 2. Antimicrobial resistance patterns of the bacterial isolates

Table 4: Antimicrobial resistance among the bacteria isolated from both the fish and pond water

Organisms/Antimicrobials	CTX	GM	CIP	C	TE	MEM	ERT
<i>Escherichia coli</i> (n = 68)	38.2%	0.0%	0.0%	36.8%	50.0%	0.0%	0.0%
<i>Edwardsiella tarda</i> (n = 15)	13.3%	0.0%	6.7%	26.7%	80.0%	0.0%	0.0%
<i>Klebsiella pneumoniae</i> (n = 18)	66.7%	0.0%	0.0%	0.0%	38.9%	0.0%	0.0%
<i>Klebsiella aerogenes</i> (n = 5)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Klebsiella variicola</i> (n = 2)	0.0%	0.0%	0.0%	50.0%	100.0%	0.0%	0.0%
<i>Citrobacter freundii</i> (n = 30)	30.0%	0.0%	0.0%	43.3%	40.0%	0.0%	0.0%
<i>Citrobacter braakii</i> (n = 6)	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%	0.0%
<i>Kurthia gibsonii</i> (n = 16)	0.0%	12.5%	25.0%	0.0%	31.3%	0.0%	0.0%
<i>Aeromonas veronii</i> (n = 42)	21.4%	0.0%	14.3%	7.1%	14.3%	0.0%	0.0%
<i>Aeromonas hydrophila</i> (n = 13)	7.7%	0.0%	7.7%	7.7%	15.4%	0.0%	0.0%
<i>Aeromonas jandaei</i> (n = 13)	7.7%	0.0%	0.0%	7.7%	30.8%	0.0%	0.0%
<i>Aeromonas caviae</i> (n = 10)	20.0%	0.0%	0.0%	10.0%	50.0%	0.0%	0.0%
<i>Aeromonas ichthiosmia</i> (n = 5)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Aeromonas enteropelogens</i> (n = 8)	0.0%	0.0%	0.0%	12.5%	12.5%	0.0%	0.0%
<i>Enterobacter bugandensis</i> (n = 9)	22.2%	0.0%	0.0%	44.4%	55.6%	0.0%	0.0%
<i>Enterobacter kobei</i> (n = 6)	0.0%	0.0%	0.0%	0.0%	83.3%	0.0%	0.0%
<i>Enterobacter cloacae</i> (n = 6)	50.0%	0.0%	0.0%	16.7%	0.0%	0.0%	0.0%
<i>Enterobacter asburiae</i> (n = 4)	25.0%	0.0%	0.0%	75.0%	50.0%	0.0%	0.0%
<i>Enterobacter hirae</i> (n = 1)	100.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Proteus mirabilis</i> (n = 16)	31.3%	0.0%	0.0%	6.3%	100.0%	0.0%	0.0%
<i>Plesiomonas shigelloides</i> (n = 13)	46.2%	0.0%	0.0%	0.0%	38.5%	0.0%	0.0%
<i>Vibrio albensis</i> (n = 10)	30.0%	0.0%	30.0%	50.0%	10.0%	0.0%	0.0%
<i>Vibrio fluvialis</i> (n = 2)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Bacillus cereus</i> (n = 19)	63.2%	0.0%	0.0%	5.3%	42.1%	0.0%	0.0%
<i>Acinetobacter soli</i> (n = 5)	20.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Lactococcus latis</i> (n = 7)	71.4%	0.0%	0.0%	14.3%	0.0%	0.0%	0.0%
<i>Pseudomonas otitidis</i> (n = 12)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Pseudomonas putida</i> (n = 6)	83.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Pseudomonas aeruginosa</i> (n = 7)	85.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Lysinibacillus sphaericus</i> (n = 4)	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Comamonas aquatica</i> (n = 3)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Comamonas kerstersii</i> (n = 2)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Shewanella algae</i> (n = 2)	100.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Salmonella</i> sp. (n = 3)	33.3%	0.0%	0.0%	33.3%	33.3%	0.0%	0.0%
<i>Staphylococcus epidermidis</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Providencia alcalifaciens</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%
<i>Citrobacter veronii</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

CTX = Cefotaxime; GM = Gentamicin; CIP = Ciprofloxacin; C = Chloramphenicol; TE = Tetracycline; MEM = Meropenem;

ERT = Ertapenem

Table 5: Antimicrobial resistance among the bacteria isolated from the fish

Organisms/Antimicrobials	CTX	GM	CIP	C	TE	MEM	ERT
<i>Escherichia coli</i> (n = 31)	41.9%	0.0%	0.0%	35.5%	54.8%	0.0%	0.0%
<i>Edwardsiella tarda</i> (n = 10)	10.0%	0.0%	10.0%	30.0%	90.0%	0.0%	0.0%
<i>Klebsiella pneumoniae</i> (n = 9)	66.7%	0.0%	0.0%	0.0%	33.3%	0.0%	0.0%
<i>Klebsiella aerogenes</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Klebsiella variicola</i> (n = 2)	0.0%	0.0%	0.0%	50.0%	100.0%	0.0%	0.0%
<i>Citrobacter freundii</i> (n = 19)	31.6%	0.0%	0.0%	42.1%	52.6%	0.0%	0.0%
<i>Citrobacter braakii</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	66.7	0.0%	0.0%
<i>Kurthia gibsonii</i> (n = 8)	0.0%	25.0%	37.5%	0.0%	50.0%	0.0%	0.0%
<i>Aeromonas veronii</i> (n = 25)	24.0%	0.0%	8.0%	4.0%	16.0%	0.0%	0.0%
<i>Aeromonas hydrophila</i> (n = 7)	14.3%	0.0%	14.3%	14.3%	14.3%	0.0%	0.0%
<i>Aeromonas jandaei</i> (n = 7)	0.0%	0.0%	0.0%	0.0%	28.6%	0.0%	0.0%
<i>Aeromonas caviae</i> (n = 6)	0.0%	0.0%	0.0%	0.0%	50.0%	0.0%	0.0%
<i>Aeromonas ichthiosmia</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Aeromonas enteropelogens</i> (n = 4)	0.0%	0.0%	0.0%	25.0%	25.0%	0.0%	0.0%
<i>Enterobacter bugandensis</i> (n = 3)	0.0%	0.0%	0.0%	66.7%	66.7%	0.0%	0.0%
<i>Enterobacter kobei</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%	0.0%
<i>Enterobacter cloacae</i> (n = 2)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Enterobacter asburiae</i> (n = 3)	33.3%	0.0%	0.0%	100.0%	66.7%	0.0%	0.0%
<i>Enterobacter hirae</i> (n = 1)	100.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Proteus mirabilis</i> (n = 6)	33.3%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%
<i>Plesiomonas shigelloides</i> (n = 8)	50.0%	0.0%	0.0%	0.0%	37.5%	0.0%	0.0%
<i>Vibrio albensis</i> (n = 3)	33.3%	0.0%	33.3%	33.3%	33.3%	0.0%	0.0%
<i>Vibrio fluvialis</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Bacillus cereus</i> (n = 8)	75.0%	0.0%	0.0%	0.0%	37.5%	0.0%	0.0%
<i>Acinetobacter soli</i> (n = 2)	50.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Lactococcus latis</i> (n = 4)	50.0%	0.0%	0.0%	25.0%	0.0%	0.0%	0.0%
<i>Pseudomonas otitidis</i> (n = 6)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Pseudomonas putida</i> (n = 2)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Pseudomonas aeruginosa</i> (n = 3)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Lysinibacillus sphaericus</i> (n = 3)	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Comamonas aquatica</i> (n = 1)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Comamonas kerstersii</i> (n = 1)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Shewanella algae</i> (n = 1)	100.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Salmonella</i> sp. (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Staphylococcus epidermidis</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Providencia alcalifaciens</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%

CTX = Cefotaxime; GM = Gentamicin; CIP = Ciprofloxacin; C = Chloramphenicol; TE = Tetracycline; MEM = Meropenem;

ERT = Ertapenem

Table 6: Antimicrobial resistance among the bacteria isolated from the pond water

Organisms/Antimicrobials	CTX	GM	CIP	C	TE	MEM	ERT
<i>Escherichia coli</i> (n = 37)	35.1%	0.0%	0.0%	37.8%	45.9%	0.0%	0.0%
<i>Edwardsiella tarda</i> (n = 5)	20.0%	0.0%	0.0%	20.0%	60.0%	0.0%	0.0%
<i>Klebsiella pneumoniae</i> (n = 9)	66.7%	0.0%	0.0%	0.0%	44.4%	0.0%	0.0%
<i>Klebsiella aerogenes</i> (n = 2)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Citrobacter freundii</i> (n = 11)	27.3%	0.0%	0.0%	45.5%	18.2%	0.0%	0.0%
<i>Citrobacter braakii</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Kurthia gibsonii</i> (n = 8)	0.0%	0.0%	12.5%	0.0%	12.5%	0.0%	0.0%
<i>Aeromonas veronii</i> (n = 17)	17.6%	0.0%	23.5%	11.8%	11.8%	0.0%	0.0%
<i>Aeromonas hydrophila</i> (n = 6)	0.0%	0.0%	0.0%	0.0%	16.7%	0.0%	0.0%
<i>Aeromonas jandaei</i> (n = 6)	16.7%	0.0%	0.0%	16.7%	33.3%	0.0%	0.0%
<i>Aeromonas caviae</i> (n = 4)	50.0%	0.0%	0.0%	25.0%	50.0%	0.0%	0.0%
<i>Aeromonas ichthiosmia</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Aeromonas enteropelogens</i> (n = 4)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Enterobacter bugandensis</i> (n = 6)	33.3%	0.0%	0.0%	33.3%	50.0%	0.0%	0.0%
<i>Enterobacter kobei</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	66.7%	0.0%	0.0%
<i>Enterobacter cloacae</i> (n = 4)	75.0%	0.0%	0.0%	25.0%	0.0%	0.0%	0.0%
<i>Enterobacter asburiae</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Proteus mirabilis</i> (n = 10)	30.0%	0.0%	0.0%	10.0%	100.0%	0.0%	0.0%
<i>Plesiomonas shigelloides</i> (n = 5)	40.0%	0.0%	0.0%	0.0%	40.0%	0.0%	0.0%
<i>Vibrio albensis</i> (n = 7)	28.6%	0.0%	28.6%	57.1%	0.0%	0.0%	0.0%
<i>Vibrio fluvialis</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Bacillus cereus</i> (n = 11)	54.5%	0.0%	0.0%	9.1%	45.5%	0.0%	0.0%
<i>Acinetobacter soli</i> (n = 3)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Lactococcus lactis</i> (n = 3)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Pseudomonas otitidis</i> (n = 6)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Pseudomonas putida</i> (n = 4)	75.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Pseudomonas aeruginosa</i> (n = 4)	75.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Lysinibacillus sphaericus</i> (n = 1)	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Comamonas aquatica</i> (n = 2)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Comamonas kerstersii</i> (n = 1)	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Shewanella algae</i> (n = 1)	100.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%
<i>Salmonella</i> sp. (n = 2)	50.0%	0.0%	0.0%	50.0%	50.0%	0.0%	0.0%
<i>Citrobacter veronii</i> (n = 1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

CTX = Cefotaxime; GM = Gentamicin; CIP = Ciprofloxacin; C = Chloramphenicol; TE = Tetracycline; MEM = Meropenem;
ERT = Ertapenem

In terms of the prevalence of multidrug resistant (MDR) and extended-spectrum beta-lactamase producing bacteria isolated, about 13.6% and 1.3% of the total bacteria were MDR and ESBL resistant, respectively.

Categorically, about 13.4% and 1.0% of bacteria isolated from fishes were MDR and ESBL resistant, respectively. Similarly, bacteria isolated from ponds recorded about 13.7% and 1.6% for MDR and ESBL, respectively. Among all the bacteria isolated, 20.6%, and 7.4% of *E. coli* were MDR and ESBL, with 19.6% and 6.5% isolated from fishes and ponds, respectively.

Table 7: MDR and ESBL prevalence among the bacteria

Bacteria/Source	MDR	ESBL
All Bacteria (from both fish and ponds) ($N = 391$)	13.6% ($n = 53$)	1.3% ($n = 5$)
All Bacteria from fish ($N = 201$)	13.4% ($n = 27$)	1.0% ($n = 2$)
All Bacteria from ponds ($N = 190$)	13.7% ($n = 26$)	1.6% ($n = 3$)
All <i>E. coli</i> ($N = 68$)	20.6% ($n = 14$)	7.4% ($n = 5$)
Fish <i>E. coli</i> ($N = 31$)	19.3% ($n = 6$)	6.5% ($n = 2$)
Pond <i>E. coli</i> ($N = 37$)	21.6% ($n = 8$)	8.1% ($n = 3$)
All <i>A. veronii</i> ($N = 42$)	11.9% ($n = 5$)	0.0% ($n = 0$)
Fish <i>A. veronii</i> ($N = 25$)	8.0% ($n = 2$)	0.0% ($n = 0$)
Pond <i>A. veronii</i> ($N = 17$)	17.6% ($n = 3$)	0.0% ($n = 0$)

The MDR proportion for *A. veronii* was about 11.9%, while none were ESBL-producing. The percentage of *A. veronii* found in fishes and ponds that are MDRs are 8.0% and 17.6%, respectively.

4.5 Molecular epidemiology (sequence types, serotypes, resistance genes, and virulence genes) of the ESBL isolates

Table 8 shows the molecular epidemiology of the ESBL isolates. The bacterial samples from fish and water (A1W, A5, A3, A3W, A4W) exhibited resistance to ampicillin, ciprofloxacin, cefuroxime, and cefotaxime, with specific genetic profiles including resistance genes *blaCTX-M-15*, *fosA7*, and *qnrS1*. Additionally, they carry virulence genes responsible for various functions, such as adhesive aggregative proteins (*aap*), aggregative adherence regulators (*aar*), type VI secretion system effectors (*aatA*), Dr fimbrial adhesins (*afaD*), aggregation proteins and regulators (*aggA*, *aggC*, *aggD*, *aggR*), anaerobic regulation proteins (*anr*), heat-stable enterotoxins (*astA*), curli subunit proteins (*csgA*), ferrous iron dicitrate transport proteins (*fdeC*), type 1 fimbrial proteins (*fimH*), glutamate decarboxylases (*gad*), hemolysin E proteins (*hlyE*), long polar fimbrial proteins (*lpfA*), lipoproteins (*nlpI*), outer membrane proteins (*shiB*, *yehA*, *yehB*, *yehC*, *yehD*), tellurite resistance proteins (*terC*), and serum resistance-associated proteins (*traT*). Notably, sample A3, originating from fish, lacks serotype information but exhibits a distinct sequence type (ST2005). Samples A3W and A4W, sourced from water, share the same sequence type (ST4684) and display similar genetic characteristics.

Table 8: Sequence types, resistance genes, and virulence genes of the ESBL isolates

Sample ID	Source	Antibiotype	Sequence types	Serotype	Resistance genes	Virulence genes	Plasmids
A1W	Fish	AMP+CIP+CX M+CTX	ST4684	H34:O9	<i>blaCTX-M-15</i> , <i>fosA7</i> , <i>qnrS1</i>	<i>ORF3</i> , <i>ORF4</i> , <i>aap</i> , <i>aar</i> , <i>aatA</i> , <i>afaD</i> , <i>aggA</i> , <i>aggC</i> , <i>aggD</i> , <i>aggR</i> , <i>anr</i> , <i>astA</i> , <i>csgA</i> , <i>fdeC</i> , <i>fimH</i> , <i>gad</i> , <i>hlyE</i> , <i>lpfA</i> , <i>nlpI</i> , <i>shiB</i> , <i>terC</i> , <i>traT</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , <i>yehD</i>	IncFIB (AP001918), IncFII (pRSB107), IncY
A5	Fish	AMP+CIP+CX M+CTX	ST4684	H34:O9	<i>blaCTX-M-15</i> , <i>fosA7</i> , <i>qnrS1</i>	<i>ORF3</i> , <i>ORF4</i> , <i>aap</i> , <i>aar</i> , <i>aatA</i> , <i>afaD</i> , <i>aggA</i> , <i>aggC</i> , <i>aggD</i> , <i>aggR</i> , <i>anr</i> , <i>astA</i> , <i>csgA</i> , <i>fdeC</i> , <i>fimH</i> , <i>gad</i> , <i>hlyE</i> , <i>lpfA</i> , <i>nlpI</i> , <i>shiB</i> , <i>terC</i> , <i>traT</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , <i>yehD</i>	IncFIB (AP001918), IncFII (pRSB107), IncY
A3	Fish	AMP+CIP+CX M+CTX	ST2005	H7	-	<i>csgA</i> , <i>fdeC</i> , <i>fimH</i> , <i>gad</i> , <i>hlyE</i> , <i>iss</i> , <i>lpfA</i> , <i>nlpI</i> , <i>ompT</i> , <i>terC</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , <i>yehD</i>	-
A3W	water	AMP+CIP+CX M+CTX	ST4684	H34:O9	<i>blaCTX-M-15</i> , <i>fosA7</i> , <i>qnrS1</i>	<i>ORF3</i> , <i>ORF4</i> , <i>aap</i> , <i>aar</i> , <i>aatA</i> , <i>afaD</i> , <i>aggA</i> , <i>aggC</i> , <i>aggD</i> , <i>aggR</i> , <i>anr</i> , <i>astA</i> , <i>csgA</i> , <i>fdeC</i> , <i>fimH</i> , <i>gad</i> , <i>hlyE</i> , <i>lpfA</i> , <i>nlpI</i> , <i>shiB</i> , <i>terC</i> , <i>traT</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , <i>yehD</i>	IncFIB (AP001918), IncFII (pRSB107), IncY
A4W	water	AMP+CIP+CX M+CTX	ST4684	H34:O9	<i>blaCTX-M-15</i> , <i>fosA7</i> , <i>qnrS1</i>	<i>ORF3</i> , <i>ORF4</i> , <i>aap</i> , <i>aar</i> , <i>aatA</i> , <i>afaD</i> , <i>aggA</i> , <i>aggC</i> , <i>aggD</i> , <i>aggR</i> , <i>anr</i> , <i>astA</i> , <i>csgA</i> , <i>fdeC</i> , <i>fimH</i> , <i>gad</i> , <i>hlyE</i> , <i>lpfA</i> , <i>nlpI</i> , <i>shiB</i> , <i>terC</i> , <i>traT</i> , <i>yehA</i> , <i>yehB</i> , <i>yehC</i> , <i>yehD</i>	IncFIB (AP001918), IncFII (pRSB107), IncY

CHAPTER FIVE

5.0 DISCUSSION

Aquaculture, the cultivation of aquatic life including fish, shellfish, and plants, is a rapidly growing sector vital for global food security. As wild fish populations decline and traditional fishing methods harm the environment, aquaculture presents a sustainable path to producing nutritious seafood. It serves as a catalyst for economic growth, job creation, and poverty reduction, especially in coastal and rural areas. Bacteria are integral components of aquatic ecosystems and play vital roles in nutrient cycling, water quality maintenance, and the overall health of aquacultured organisms. However, certain bacteria can also pose threats to fish health and food safety, necessitating effective monitoring and management strategies. This threat has been intensified by the pervasiveness of AMR, which is more problematic in western sub-Saharan Africa (Murray *et al.*, 2022). On a whole, the enormity of the AMR menace has necessitated the adoption of the One Health concept in AMR surveillance, to ensure a shift in AMR surveillance efforts from the traditional focus on human health to encompass animals and the food chain, the environment. This adjustment paradigm is due to the recognition of the viscerally intricate interconnection of human health, animal health, and the environment. However, most of the AMR surveillance studies, particularly those conducted in sub-Saharan Africa, have mainly focused on humans and rarely on animals and the environment (Eibach *et al.*, 2018; Adzitey *et al.*, 2019; Donkor *et al.*, 2019; Appiah *et al.*, 2020). Those few that have been conducted on animals have usually focused on poultry, etc., with very little attention given to the aquaculture sector (Adzitey *et al.*, 2019; Yafetto *et al.*, 2019; Adzitey *et al.*, 2020; Kunadu *et al.*, 2020). The goal of this study was, thus, to evaluate AMR in bacteria harboured in marketed reared fish in Accra and the pond water from which they originated, so as to help homogenize the data on AMR and overall, help to effectively resolve the

AMR menace. Specifically, the study focused on microbial loads (total viable and coliform counts), distribution of the harboured bacteria and their AMR patterns, as well as their ESBL phenotypes, genotypes, and sequence types.

The total viable count and total coliform count were each higher in the fish than in the pond water, recording 12.4×10^5 CFU/ml and 0.31×10^5 CFU/ml, respectively, albeit being within acceptable limits (Adinortey *et al.*, 2020). However, the prominence of *E. coli*, constituting 17% of the total isolates, signals faecal contamination (WHO Guideline Values for Drinking-Water Quality, 2018), raising concerns about water quality and hygiene practices in the farms. The higher total viable count and total coliform count in the fish compared to the pond water could be attributed to the bioaccumulation of microorganisms within the fish. This process allows for the concentration of bacteria and other microorganisms in the tissues of the fish, often in the gut, which can result in higher microbial counts in fish compared to the surrounding water. This bioaccumulation is particularly significant when considering the diet and feeding habits of the fish, which contribute to the diversity and concentration of bacteria in their gastrointestinal tract. Fish consume various materials, including plankton, known to harbour high levels of bacteria, thereby increasing the bacterial load within the fish beyond that of the surrounding water. Adinortey *et al.* (2020), in their report, pointed out that under stressful conditions, such as high faecal coliform levels in breeding ponds, bacteria may penetrate fish muscles, with the likelihood of muscle invasion increasing the longer fish are exposed to polluted water. This observation is critical, as it suggests that not only external, but also internal tissues of fish can harbour significant levels of pathogenic bacteria.

Essentially, the highlighted concerns about the microbial safety of aquacultured fish are especially validated by similar observations on frequent occurrence of *E. coli* in aquaculture systems (Boulares *et al.*, 2011; Abd El Maksod *et al.*, 2023; Afolabi *et al.*, 2020). The presence of *E. coli*

in both the fish and pond water samples underscores its wide distribution and potential for contamination throughout the aquaculture environment. Also, its high prevalence suggests the importance of implementing effective sanitation practices and water quality management to minimize the risk of fecal contamination and potential transmission of harmful strains. Enforcement of this recommendation is especially crucial, given the fact that these fishes (tilapia and catfish) are widely consumed in Ghana, and patronage of dishes from these grilled fishes is commonplace in the country. Thus, substandard cooking practices involving these fish could potentially endanger consumers.

To buttress the concerns about the microbial safety of the fishes and the potential risks they pose, *Aeromonas hydrophila* was the second most prevalent bacterium, accounting for 3% of the total isolates. It was primarily found in fish samples (3 %), indicating its association with the fish microbiota. This finding is consistent with previous studies that have identified *Aeromonas* spp. as common inhabitants of aquacultured fish (Boulares *et al.*, 2011; Babatunde *et al.*, 2023). The higher prevalence of *A. hydrophila* in the fish compared to the pond water samples suggests its potential transmission from the aquatic environment to the fish. The presence of *Aeromonas* spp. in the fish raises concerns about their pathogenicity and their potential to cause opportunistic infections, particularly in stressed or immunocompromised fish. Proper monitoring and management strategies, including water quality control and stress reduction, are essential to minimize the risk of *Aeromonas*-related infections in aquaculture systems, as well as potential transmission to humans.

Also, *Vibrio albensis* was detected in 5% and 4% of the isolates, for the fish and pond water samples, respectively. *Vibrio* species are commonly associated with aquatic environments and have been previously reported in aquaculture settings (Kaktcham *et al.*, 2017; Chitambo *et al.*,

2023). The presence of *Vibrio albensis* in this study highlights the importance of monitoring and controlling these pathogens to minimize potential health risks. However, it is crucial to note that the strains identified in this study may not necessarily be the toxigenic strains responsible for human cholera cases. Nonetheless, the presence of *Vibrio* species emphasizes the need for proper hygiene practices, water treatment, and appropriate management strategies to reduce the risk of *Vibrio*-related infections in both fish and humans.

Among the bacterial isolates, *Klebsiella pneumoniae* were predominantly found in the pond water samples rather than in the fish samples. This suggests that these bacteria may have a stronger association with the pond water environment. The higher prevalence of *Klebsiella pneumoniae* in pond samples aligns with the findings of Boulares *et al.* (2021), who reported the presence of *Klebsiella* spp. in aquaculture systems. These bacteria may interact with the pond water, sediment, or associated microorganisms, highlighting the complexity of the microbial community dynamics in aquaculture systems.

Other bacterial species, such as *Acinetobacter baumannii*, *Vibrio* spp., *Enterobacter cloacae*, and *Pseudomonas luteola*, showed varying distributions between fish and pond samples. This indicates their potential presence in both the fish and the surrounding environment. The variations in distribution patterns among these bacteria highlight the intricate interplay between aquacultured organisms and their environment, which can influence the microbial composition and potential transmission of bacterial pathogens.

It is evident that the distribution and prevalence of bacterial species in aquaculture systems can vary depending on the specific environment, culture practices, and geographical location. Factors such as water source, feeding regimes, water quality management, and biosecurity measures can

significantly influence the microbial ecology of aquaculture systems. Therefore, careful consideration of these variables is essential for accurately interpreting and generalizing findings across studies.

The presence of pathogenic bacteria, such as *Aeromonas hydrophila*, *Vibrio albensis*, and *Salmonella* sp, among the isolates emphasizes the importance of implementing appropriate management practices to mitigate the risk of bacterial contamination and potential transmission of antibiotic resistance genes. Aquaculture-associated bacterial pathogens can pose risks not only to fish health but also to human consumers through the consumption of contaminated seafood. Therefore, strategies such as regular monitoring of water quality, proper sanitation, adequate biosecurity measures, and responsible use of antibiotics are crucial for maintaining a safe and sustainable aquaculture industry.

The implications of antibiotic resistance within aquaculture are significant. This study's discovery of antibiotic-resistant bacteria, including *Acinetobacter baumannii* and *Klebsiella pneumoniae*, highlights the risk of these resistance genes transferring to human pathogens. The misuse or overuse of antibiotics in aquaculture contributes significantly to the global problem of antibiotic resistance by fostering the emergence and dissemination of resistant bacteria. It is important to implement prudent and responsible use of antibiotics in aquaculture practices, in line with regulatory guidelines and best management practices, to minimize the selection and spread of antibiotic resistance.

It is worth noting that this study focused on a specific aquaculture system and a limited set of bacterial species. Further research is needed to explore the microbial ecology of different aquaculture systems, including marine, brackish, and freshwater environments, and to investigate a broader range of bacterial species. Additionally, the application of advanced molecular

techniques, such as metagenomics and metatranscriptomics, can provide a more comprehensive understanding of the microbial community dynamics and functional interactions within aquaculture environments.

Generally, the AMR rates were low to moderate, and were highest for tetracycline (31% to 42%), cefotaxime (29% to 35%), and chloramphenicol (19%), while no resistance was recorded against either meropenem or ertapenem. These results align with the global concern of widespread resistance to these antibiotics in various bacterial species. The high levels of resistance to tetracycline are consistent with the findings reported by Adinortey *et al.* (2020) and Ginovyan *et al.* (2017) in fish farms in Ghana and Armenia, respectively. The substantial resistance to cefotaxime highlights the need for careful consideration when using these antibiotics in aquaculture.

The heightened prevalence of MDR (20.6%) and ESBL resistance (7.4%) in *E. coli*, a ubiquitous and indicator species, signifies the potential reservoir of AMR genes in aquatic environments. The prevalence variations between the fishes and pond water highlight the need for a more targeted investigation into the factors driving resistance in specific bacterial strains. This finding aligns with previous studies by Adinortey *et al.* (2020) and Ginovyan *et al.* (2017), which reported high levels of antibiotic resistance in coliform bacteria, including *E. coli*. Adinortey *et al.* (2020) found that a significant proportion of coliform bacteria in fish farms in Ghana exhibited resistance to multiple antibiotics, including ampicillin. Similarly, Ginovyan *et al.* (2017) highlighted the high resistance to ampicillin and other commonly used antibiotics among *E. coli* strains. The analysis of *A. veronii* revealed a moderate MDR prevalence (11.9%), with notable differences between the fishes (8.0%) and the pond water (17.6%). This emphasizes the niche-specific nature of antibiotic

resistance and underscores the importance of considering individual bacterial species when assessing resistance in aquatic environments.

In line with the observed AMR traits highlighted above, various AMR-conferring genes were observed in this study, as has been the case of aquatic sediments in previous reports, showcasing the intricate nature of the issue (Watts *et al.*, 2017). Notably, this study identified the resistance genes *bla_{CTX-M-15}*, *fosA7*, and *qnrS1*, in both fish and pond water samples. Among these, *bla_{CTX-M-15}* stood out as a widespread gene, encoding extended-spectrum β -lactamases with hydrolyzing activity against cephalosporins (Zhang *et al.*, 2013; Mazumder *et al.*, 2020). The *fosA7* gene, on the other hand, encodes an enzyme that catalyses a chemical modification of Fosfomycin (The Comprehensive Antibiotic Resistance Database, 2024). This modification typically involves the addition of a chemical group to the antibiotic molecule, rendering it unable to inhibit its target enzyme effectively. As a result, bacteria carrying the *fosA7* gene can neutralize the antibacterial effects of fosfomycin, leading to resistance.

Mechanisms of quinolone resistance frequently involve plasmid-encoded *qnr* proteins. In this study, the *qnrs1* gene, a plasmid-mediated quinolone resistance gene was identified (Le V *et al.*, 2015; Caputo *et al.*, 2023). Globally, plasmid-mediated quinolone resistance has been detected in bacteria associated with fish farms (Miranda *et al.*, 2013). For example, in Chile, Buschmann *et al.* (2012) reported the presence of *qnrS* in sediments impacted by fish farming. Studies conducted in China have revealed contrasting findings on *qnrS* genes in aquaculture. Jiang *et al.* (2012) observed a high prevalence of these genes in *Escherichia coli* from farmed fish, whereas Han *et al.* (2012) reported a low frequency of *qnrS1*-like genes in *Aeromonas spp.* from diseased fish and water. Further studies on the mutagenesis of *qnrS1* have shown that specific amino acid substitutions can affect its ability to confer resistance to antibiotics like ciprofloxacin (Tavio *et al.*,

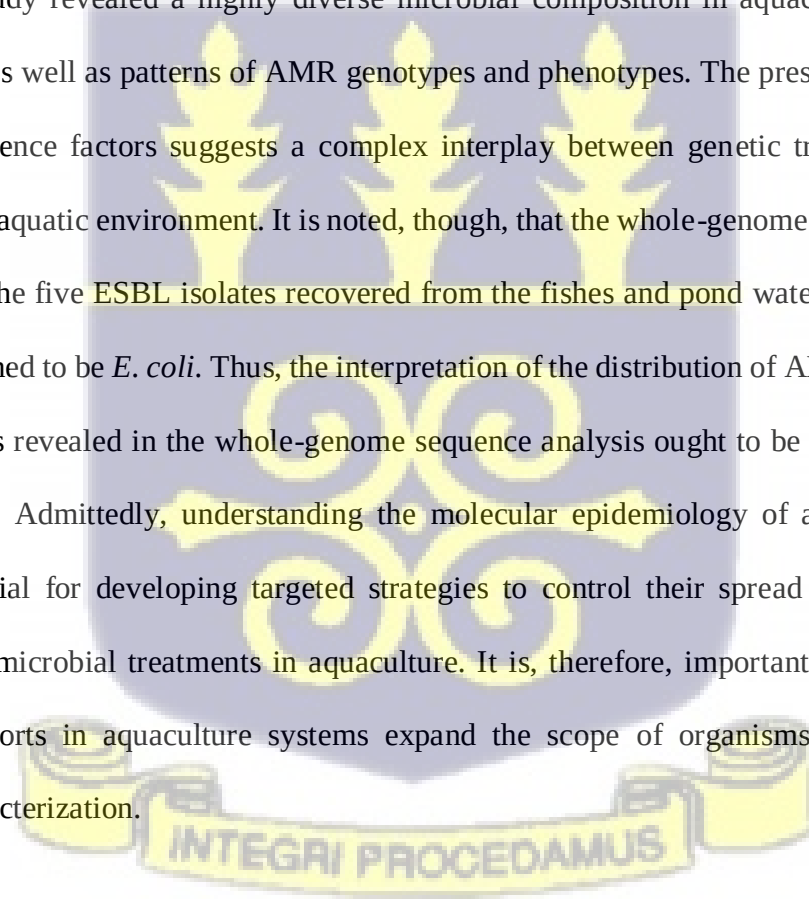
2014). The detection of these resistance genes in both fish and water samples points to a complex transmission network of AMR within aquaculture systems, aligning with existing literature that highlight water bodies as conduits for AMR spread, not only within aquaculture settings but also to surrounding environments (Ho *et al.*, 2021; Singh *et al.*, 2024).

Two distinct serotypes, H7 and H34:O9, were identified in this study, with both serotypes present in fish samples, while only H34:O9 was found in the water samples. Serotype differentiation, based on specific flagellar (H) and somatic (O) antigens, reveals the diversity of bacterial strains in these environments. Specifically, serotype H34:O9 expresses the 34th flagellar antigen and the 9th somatic antigen, whereas serotype H7 only expresses the 7th flagellar antigen (Suhaimi *et al.*, 2008). Although there is limited information on H34:O9 specifically, serogroup O9 was classified by Navarro *et al.* (2021) as commensal strains containing diarrheagenic *E. coli* genes. Also, Ghilardi *et al.* (2003) identified O142:H34 as one of the most frequent serogroups, also prevalent in diarrheagenic *E. coli*. Additionally, Onmaz *et al.* (2020) reported the presence of *E. coli* O157:H7 in fish samples from various fish farms and retail outlets. These findings, however, contradict those of Fernandes *et al.* (1997), who did not identify *E. coli* O157:H7 in 240 catfish fillet samples despite immunological testing with the 3M Petri film HEC test kit and subsequent culture on 3M Petri film *E. coli* plates.

The identified prevalence rates of antibiotic-resistant bacteria in aquatic environments have potential implications for public health. Also, the transfer of resistance genes from aquatic bacteria to human pathogens, through the food chain or waterborne routes, could contribute to the dissemination of antibiotic resistance in clinical settings. Furthermore, the discernible differences in resistance patterns between the fishes and pond water suggest that the aquatic habitat plays a role in shaping the antibiotic resistance landscape. Moreover, the slightly elevated prevalence of

MDR in fishes compared to the overall sample underscores the potential influence of ecological factors on resistance dynamics within specific niches. In addition, the observed patterns of AMR in the fish and pond water bacteria underscore the need for prudent antibiotic use in aquaculture. Plus, the resistance of bacteria to certain antibiotics, such as cefotaxime, highlights the potential challenges in disease management within this aquatic ecosystem. Besides, the effectiveness of gentamicin against a high percentage of bacteria isolated from both the fishes and pond water is promising and emphasizes the importance of selecting antibiotics carefully in aquaculture practices.

Overall, this study revealed a highly diverse microbial composition in aquaculture systems in Accra, Ghana, as well as patterns of AMR genotypes and phenotypes. The presence of resistance genes and virulence factors suggests a complex interplay between genetic traits and selective pressures in the aquatic environment. It is noted, though, that the whole-genome sequence analysis was limited to the five ESBL isolates recovered from the fishes and pond water in this study, all of which happened to be *E. coli*. Thus, the interpretation of the distribution of AMR genes, as well as other insights revealed in the whole-genome sequence analysis ought to be interpreted in that limited context. Admittedly, understanding the molecular epidemiology of antibiotic-resistant bacteria is crucial for developing targeted strategies to control their spread and maintain the efficacy of antimicrobial treatments in aquaculture. It is, therefore, important that future AMR surveillance efforts in aquaculture systems expand the scope of organisms subjected to the molecular characterization.



6.0 CONCLUSION, RECOMMENDATIONS, AND LIMITATIONS

6.1 Conclusion

The fish and the pond water had high microbial contamination, mainly accounted for by *Escherichia coli* and *Aeromonas veronii*. The AMR, MDR, and ESBL rates were low to moderate. Moreover, the main sequence type and serotype of the ESBL isolates were ST4684 and H34:09, respectively, and the AMR genes were *bla*CTX-M-15, *fosA7*, and *qnrS1*.

6.2 Recommendations

It is recommended that regulatory bodies enforce microbial food safety policies in the fish production chain and educate consumers about the importance of thorough cooking to eliminate harmful bacteria. Additionally, in-depth research is necessary to explore the microbial ecology of different aquaculture systems, including marine, brackish, and freshwater environments, and to investigate a broader range of bacterial species. Moreover, It is crucial to establish a robust surveillance system to track antibiotic resistance trends among fish populations and the surrounding environment. Furthermore, regularly testing of water samples and fish for the presence of antibiotic-resistant bacteria and resistance genes are recommended, as this information will aid in early detection, prompt intervention, and the development of targeted interventions to mitigate the spread of antibiotic resistance. Additionally, the application of advanced molecular techniques, such as metagenomics and metatranscriptomics, can provide a more comprehensive understanding of the microbial community dynamics and functional interactions within aquaculture environments.

6.3 Limitations

The pond water and fish samples were not evaluated for the presence of antibiotic residues, and hence the farmers' claims about not using antibiotics in their aquaculture practice could not be verified.



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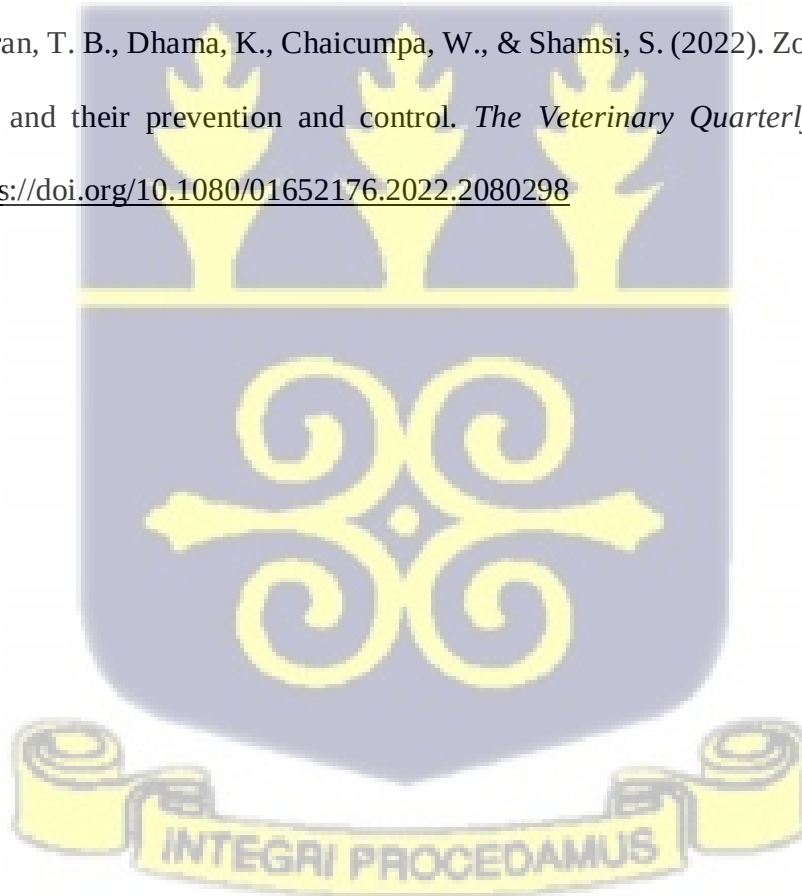
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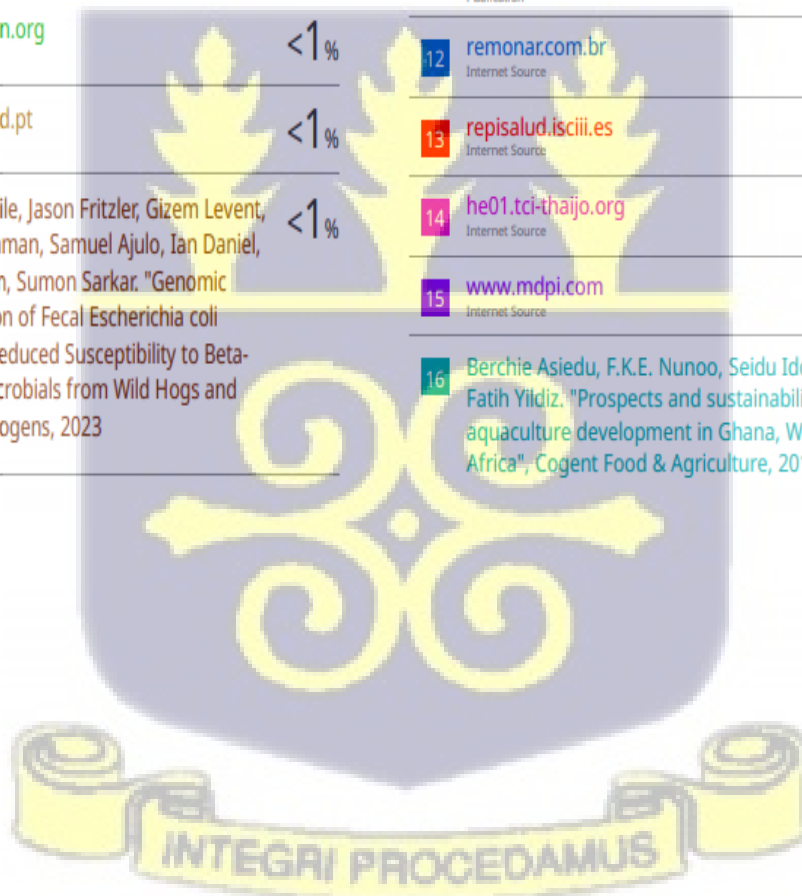
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