IDENTIFICATION AND MOLECULAR CHARACTERISATION OF *Vibrio cholerae* IN MTU FISH POND WASTEWATER

By

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN STATE, NIGERIA

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DECLARATION

I hereby declare that this project report written under the supervision of Dr. O. I. OGUNSUYI and Dr. G. B. Akanni is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

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Date

CERTIFICATION

This is to certify that the content of this project entitled **IDENTIFICATION AND MOLECULAR CHARACTERISATION OF *Vibrio cholerae* IN MTU FISH POND WASTEWATER** was prepared and submitted by **ODOZI BLESSING** with matriculation number **18010101016,** in partial fulfilment of the requirement for the degree of Bachelor of Science in Microbiology, department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria. The original research work was carried out by her under my supervision and is hereby accepted.

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DEDICATION

I dedicate this project to God Almighty for giving me life, good health and all I needed to make this work a success and secondly to my dear irreplaceable Aunty Mrs. Babalola loveth for her guidance, understanding and sacrifice. I also dedicate this work to my course mates and friends for their support in the course of my four-year study of microbiology in Mountain Top University. May the Almighty God bless you all! Amen.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

ABBREVIATION	MEANING
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
MTU	Mountain Top University
TCBS	Thiosulphate-Citrate-Bile-Salt-Sucrose Agar
V.	*Vibrio*
TAE	Tris-Acetate-EDTA

ABSTRACT

Rearing of fish especially catfish is very common in many communities in Nigeria and the wastewater from these fish ponds are often discharged into the surrounding drains. Most of these wastewaters contains pathogenic organisms and the presence of these organisms may be an indication of contamination of the fish pond which may be pathogenic to the fishes and humans as well through the consumption of these fishes. Their presence in fish intended for human consumption may constitute a potential danger thus causing diseases. Assessment of the microbiological and physicochemical characteristics of wastewater fish ponds stocked with catfish was conducted in MTU. This study was designed to determine the presence of *Vibrio* cholerae in MTU fish pond wastewater. A total of 12 water samples were collected at the 3 different fishponds in MTU Nigeria. The results of physicochemical properties of the water samples showed the pH, 6.8 – 7.0, Temperature 28.6^0c - 28.8^0c, Salinity 094ppm - 120ppm, Conductivity (ms/cm) 0.12 - 0.17. *Vibrio* isolates were obtained on thiosulphate-citrate-bile-salt-sucrose agar with prior enrichment on alkaline peptone water. Presumptive isolates were identified and characterized using both conventional biochemical method and molecular characterization. *Vibrio* detection was possible using all two primer sets in a multiplexed, PCR strategy, *ompW (Vibrio cholera*)- 304*bp*, *flaE (Vibrio Parahaemolyticus)*- 897*bp.* Sample 2 was positive for *Vibrio cholerae.* The result showed that there was no fish pond water sample that was free from *Vibrio* contamination, an indication that the entire fish pond water samples were contaminated by microorganisms. It can be concluded from this study that there is need to monitor the quality of wastewater from fish ponds before being discharged into the environment since such wastewater harbours potential pathogens.

Keyword: Wastewater, *Vibrio*, PCR, Fish pond and Microorganisms.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Fish is a crucial component of the human diet since it provides inexpensive protein and a wealth of minerals and nutrients that are necessary for human survival (FAO, 2002). A significant portion of the animal protein consumed by more than 1 billion people around the world (at least 30% of their intakes) comes from fish (Omojowo and Omojasola, 2013). Fisheries and aquaculture are large agricultural industries and a major source of animal protein (Abo-Elela et al., 2005; El-Naggar et al., 2008). Fish are raised in a variety of culture media or under regulated conditions, including concrete or earthen ponds, wooden or fiber glass vats, and plastic tanks (Adebami et al., 2020). The concrete and earthen ponds are the most popular type of cultivation medium (Fakorede et al., 2020). In Nigeria, concrete pond culture systems have recently replaced the old earthen pond culture system as land becomes more expensive, limited, and unusable (Ifeonu et al., 2019). According to estimates, 73% of fish farmers in Nigeria utilize concrete ponds, compared to 27% who use earthen ponds (Njoku et al., 2015).

A sufficient, safe, and readily available quantity of water is essential for survival (WHO, 2000). Water sources that are unclean and contaminated with feces (from humans or animals) that contain pathogenic microorganisms have an effect on health (WHO, 2011). Water has typically been regarded as the primary means of cholera transmission (Mahapatra et al., 2014). Thankfully, water has the ability to naturally purify and replenish itself utilizing a variety of processes, including biological deterioration (decomposition), microbe predatory activity, aeration, dilution, sunlight, and others (Hanelore,2013). These actions are referred to as self-purging (Bitton,2005). Water contamination has long been a significant environmental issue since it affects the numerous uses of water and can also result in disease when consumed (Hanelore,2013). Another significant method of water pollution is the discharge of untreated fish pond waste water into other water bodies. Diseases like cholera can be brought on by contamination by drinking contaminated water. (Mahapatra *et al.*, 2014).

There are two main categories of ponds: concrete and earthen fish ponds. Typically, untreated surface water runoff from streams, rivers, or lakes serves as the water source for earthen ponds

(Emikpe et al., 2011). These ponds' fish feed, which incorporates organic cow dung components, has the potential to introduce a wide variety of microorganisms. Concrete ponds use subterranean resources (Onianwah et al., 2018). When the water in the ponds has to be replaced at intervals of around 3-5 days, it is pumped from subterranean sources, like wells, and kept in a storage tank (Adebami et al., 2020). The standard method of fish farming in Nigeria for a very long time was the earthen pond cultivation system (Osawe, 2004). The majority of the time, effluent from fish ponds is dumped into the environment without being treated, which can pollute and contaminate the surroundings. They frequently have an unpleasant odor and potentially dangerous microorganism. 2014 (Ajayi and Okoh).

The source of the water and the fish feed made from animal dung, which can serve as an ideal substrate for a wide variety of microbial activity in the pond, are two factors that can determine the quality of the water (Okpokwasili, 2014). However, the release of high concentrations of opportunistic and pathogenic bacteria into the ponds caused by organic manure is also dangerous to the public's health. (Adebami and others, 2020)

The usage of indicator bacteria, such as Escherichia coli, is extremely important for bacteria indices used to assess water quality. Fish health is influenced by a number of physical and chemical factors, including dissolved oxygen, pH, temperature, conductivity, total solids, total hardness, total alkalinity, nitrite nitrogen, sulphates, carbonates, and ammonia (Okpokwasili, 2014). Ten species of *Vibrio*, including *V.* cholerae, *V.* mimicus, *V.* fluvialis, *V.* parahaemolyticus, *V.* alginolyticus, *V.* cincinnatiensis, *V.* vulnificus, *V.* furnissii, *V.* metschnikovii, and *V.* harveyi (carchariae), are pathogenic to humans (Tarr et al., 2007).

1.2 Statement of problem:

Wastewater generated from fish ponds are mostly released into the environment without proper treatment and this can lead to contamination of the soil and other water bodies. Most of these wastewater contain pathogenic microorganisms such as *Vibrio spp, E.coli, shigella, salmonella* and when these organisms are released there are high chances of infecting humans because most people drink the water from the nearby river and by that, they end up being infected.

1.3 Aim and Objective of the study

The aim of this study was to assess the Mountain Top University fish pond wastewater for the presence of *Vibrio spp* using both biochemical and molecular approaches.

The objectives of this study are to:

1. Determine the physicochemical analysis of the water samples.
2. Morphologically characterize the *Vibrio* spp present.
3. Identify *Vibrio* spp using Biochemical tests.
4. Identify *Vibrio* spp at molecular level using Multiplex PCR and Agarose Gel Electrophoresis.

1.4 Significance of the Study

According to Hay (2021), there are evidences on the presences of pathogenic organisms in released wastewater from fish ponds and most of the wastewater from these fish ponds are frequently released into the environment and when these wastewaters come in contact with other water bodies, it tends to contaminate them. Subsequently, this study is led to determine the nature of this waste water in other to subject such water to proper treatment and prevent the outbreak of a preventable disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fish farming

Fish is significantly less expensive than other foods, is widely accepted and has little to no religious connotations. It has a great biological value for the body because to its high protein retention, low cholesterol content, and presence of important amino acids (Philips et al., 2004; Emikpe et al., 2011). Every farmer aims to create a fresh, disease-free fish that is of the highest quality, absolutely beautiful to the eye, and has a high yield in order to maximize profits (Ampofo and Clerk, 2010). Water is used by all types of fish ponds, and the majority of the water added to the ponds is expelled as effluent (Sato et al., 2017). Because wastewater effluents contain significant amounts of organic materials, nutrients, and organisms, dumping it into the environment without first treating it adds to environmental contamination (Huang et al., 2018). Fish excrement, dead organisms, and uneaten fish pellets are the sources of the majority of the organic elements in aquaculture wastewater (Erondu and Anyanwu, 2005). The high concentration of chemical oxygen demand (COD) would create a decrease within the dissolved oxygen concertation within the water and impair the life stability of the aquatic environment (sule et al., 2016). (sule et al., 2016). In addition to having a high COD concentration, wastewater also has a very high nutrient concentration (nitrogen and phosphorus) (Olukunle et al., 2017). When wastewater is dumped onto water bodies, high nutrient levels can also contribute to pollution. One of the main causes of eutrophication in the aquatic environment may be the high concentration of nutrients (Thomsen et al., 2020). The growth of floating macrophytes is encouraged by eutrophication, which reduces the amount of sunlight reaching the ocean (Lusiana et al., 2020). The reduction in underwater sunlight penetration interferes with phytoplankton's ability to photosynthesise, which lowers the amount of dissolved oxygen present in the aquatic environment (Karpowicz et al., 2020).

2.2 Fish pond

A fish pond is a small body of water, man-made lake, or reservoir that has been stocked with fish and is used for fishing, ornamentation, aquaculture, or fish farming. Ponds vary in size and are shallow (Magdy, 2021). The majority of ponds used for the culture of carps, tilapia, catfish, and sea bass are made of earth (Emmanuel, 2014). They are frequently artificial or have been enlarged past their initial depth. Runoff, groundwater, precipitation, or occasionally all three of these phenomena, can fill them (Francová, 2019).

2.2.1 Types of fish ponds

- Earthen pond

Similar to a river or stream, an earthen pond may serve as a nearly natural home for fish (such as catfish and tilapia) (Adebami et al., 2020). Although it is built according to the fish farmer's plans, it is primarily built in muddy or waterlogged areas to accommodate the fish and give them a natural sensation while they are being reared (Addo, 2021). Water quality (oxygen, ammonia, nitrite, etc.) is typically closely monitored, and fish farmers are typically highly skilled (Adebami et al., 2020). An artificial dam known as an earthen pond is created by excavating a hole that must be at least 1.5 meters deep. It is the most beneficial near-natural pond design for growing fish (Karpowicz et al., 2020). Compared to other types of ponds, fish prefer to eat organic food like worms. An earthen pond fosters rapid fish growth. An earthen pond's water system is simple to handle and has a low maintenance cost (Magdy, 2021). In this system, zooplankton that consumes pelagic algae or benthic organisms provide the food (Karpowicz et al., 2020). Fish species that hold diverse positions in the pond's ecology, such as tilapia (a filter algae feeder), carp or catfish (a benthic feeder), various carps (a zooplankton feeder), and grass carp (a submerged weed feeder), tap nearly all of the food sources that are present in the pond (Addo, 2021).

- Concrete pond

Blocks, sand, and cement are used to build a home for your fish in a concrete pond. The majority of these cultures are practiced in underdeveloped nations. One intriguing exception is the enclosure of bottom-dwelling scallops in mesh walls or fences (Magdy, 2021). In contrast to other types of ponds, concrete pond construction calls for professional expertise. This is possible since any flaw in the pond's construction could lead to leaks, and fixing them would be more expensive than building a new one. Concrete ponds often can be built anyplace in your home, which is one advantage they have over earthen ponds Production is centered on a compact site, which may be totally fenced in, making security considerably easier (Magdy, 2021).

2.3 Features of a fish pond

Although there are many kinds of fish ponds, the following are the main features and structures associated with them in general:

- pond walls or dikes, which hold in the water;
- pipes or channels, which carry water into or away from the ponds;
- water controls, which control the level of water, the flow of water through the pond, or both;
- tracks and roadways along the pond wall, for access to the pond;
- harvesting facilities and other equipment for the management of water and fish.

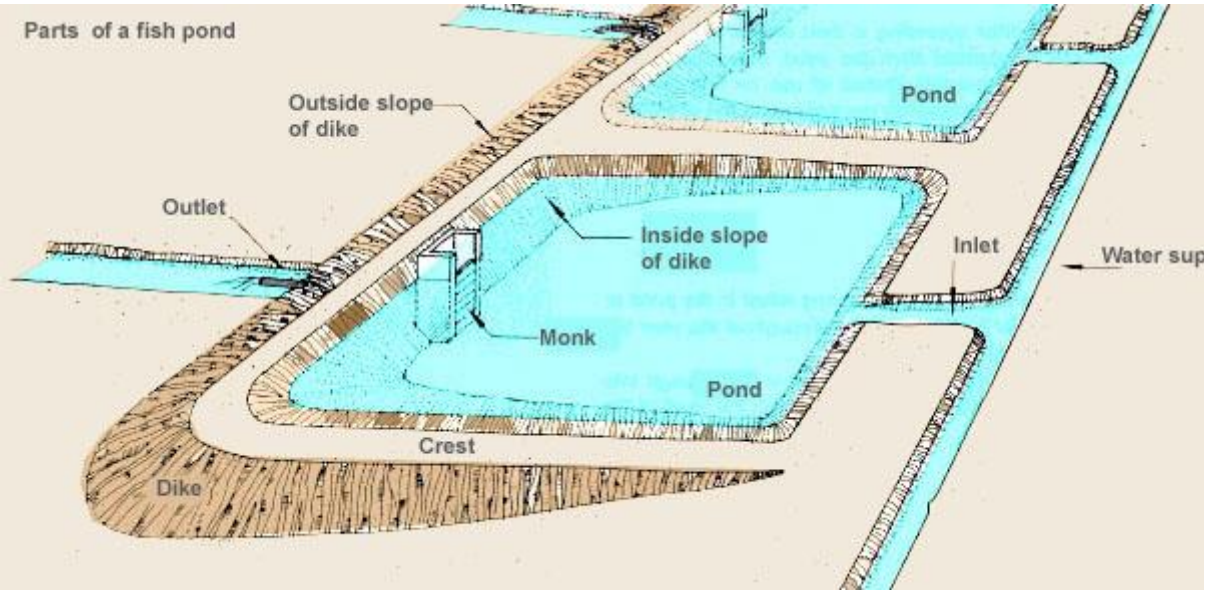

Figure 1 2.1 diagram of fish pond

Source: Ajadi *et al.*, (2016)

2.4 Sources of Waste in fish ponds

2.4.1 Feed

In aquaculture, feed is a crucial component of production, and its value varies depending on the type of culture method (Dauda et al., 2018). According to aquaculture frameworks, feed is a major source of waste (Martins et al., 2010). The amount of supplemental feed has an effect on how much trash is produced because of fish feed (Akinwole et al., 2021). The quantity of waste produced by feed depends on a vast array of factors, including its supplement, the method of production (expelled versus pelleted), the ratio of feed size to fish size, the amount of feed used per unit of time, the care strategy, and the capacity time (Miller ,2002).

2.4.2 Chemicals

The use of synthetic substances in fish farms is currently strictly limited by aquaculture practices, however some chemical substances are still used as sanitizers, antifoulants, and prescription medications (Read and Fernandes, 2003). The medications are used for chemotherapeutic purposes, which include the use of anti-infection agents for prophylaxis and corrective measures, sedatives, ectoparasitic ides, endoparasiticides, and immunizations for the treatment and control of parasites (internal and external), as well as microbial diseases (Ajadi et al., 2016). (Dauda et al., 2018). In essence, salts are used to lower fish pressure, lime is used to treat lake bottoms for acridity during lake planning, and other synthetic compounds thought to be safe for fish are also used. Although these synthetic substances are necessary for fish culture, they may also cause climate change (Boyd and Nevin, 2015). As the water is released from the lakes, it flows into common water sources. Depending on the concentration of synthetic chemicals used, the size of the ranch, and the size of the receiving water bodies, the influence of these synthetic waste upon these typical water frameworks will vary. (Adebami et al., 2020).

2.4.3 Pathogens

In aquaculture systems, this accumulation of trash is rarely observed, especially when the level below the surface affects the refined fish (Adebami et al., 2020). In any case, releasing bacteria together with wastewater could negatively affect oceanic bacteria in ordinary water bodies (Dauda et al., 2018). Oceanic organic organisms may experience strain or outright demise if fish culture frameworks add to the already-existing pathogenic burden of natural water systems (Read and Fernandes, 2003). In a semi-concentrated pond, lake effluent is released unrestrictedly, which is more common in Africa, where natural composts used in aquaculture have led to an increased level of bacteria. An increased level of waste streptococci is added by four natural composts (blood cow waste, cow dung, pig fertilizer, and poultry fertilizer) (Ampofo and Clerk,2003).

2.5 Factors Affecting the Productivity of Fish Farming

The water quality of a fish farm is determined by its physicochemical and biological properties (Read and Fernandes, 2003). This is because water as home of most edible aquatic foods has not been given adequate attention until recently when the effects on aquatic foods started being noticed. Ehiagbonare and Ogundiran (2010), stated that good water quality enhances optimal growth of aquatic organisms. Poor water quality is associated with heavy microbial load which consequently affect its microbial population and consequently the physio-chemical properties of the pond. Productivity, therefore, depends on the physicochemical characteristics of the farm's water body (Sandoval *et al.,* 2017).

2.5.1 Physicochemical Characteristics of wastewater

pH, dissolved oxygen (DO), oxygen demand (chemical and natural), solids (suspended and broken up), nitrogen (nitrite, nitrate, and ammonia), phosphate, and metals are the physicochemical characteristics of wastewater that are of extraordinary interest (Akpor and Muchie, 2011). Both regular and waste waters have a significant quality boundary that is the hydrogen-particle concentration. It is used to show the acidity or alkalinity of wastewater (Gray et al., 2002). Septic conditions are indicated by wastewater influent pH levels below 7, whereas pH levels between 5 and 10 indicate the presence of industrial wastes and incompatibility with biological processes. The pH concentration range for the existence of biological life is quite

narrow (typically 6-9). A sign of outrageous pH is known to harm natural cycles in organic treatment units (Akpor and Muchie, 2011)

Dissolved oxygen is another limit that significantly influences the characteristics of water (Gray et al., 2002). The solubility, temperature, halfway tension, and collection of pollutants like salinity and suspended particulates in water are all indicators of the actual amount of oxygen that can be present in an arrangement (Metcalf and Eddy, 2003). BOD or COD are two possible measures of the oxygen required by microbes when they consume natural particles in wastewater (Akpor and Muchie, 2011). The most frequently used natural contamination threshold for wastewater is the 5day BOD (BOD5) (FAO, 2007). The determination of the amount of dissolved oxygen used by microorganisms in the biochemical oxidation of natural matter is part of this process. The BOD only predicts the biodegradable organics and requests a fairly significant expense to obtain test results (Gray, 2002; Metcalf and Eddy, 2003).

The COD test essentially determines what may be compared to the natural substance in wastewater that can be chemically oxidized. Always, the COD will be greater than the BOD. This is due to the fact that the COD calculates the amount of chemicals that are oxidized both chemically and biologically (Gray, 2002).

2.5.2 Microbiological Characteristics of wastewater

Infections, bacteria, parasites, protozoa, and helminths are the major microorganisms present in wastewater influents (Oyewumi, 2017). While certain waterborne epidemics like pneumonia, diarrhea, meningitis, degenerative heart disease, and stomach ulcers are thought to be exacerbated by these microbes, they also play a number of beneficial roles in wastewater influents (Kris, 2007). Usually, dissolved organic matter is removed from wastewater by the possible use of microbes (Momba and Mfenyana, 2005). Depending on the type of treatment facility, the microorganisms are used in fixed film frameworks, suspended film frameworks, or tidal pond frameworks. Their presence during the various treatment phases can improve the solids' breakdown, reducing the production of slime (Ward-Paige et al., 2005a).

Infections, bacteria, parasites, protozoa, and helminths are the major microorganisms present in wastewater influents. waste and intestinal microbes like coliforms and Escherichia coli Streptococci are used to identify waste-related water source pollution (Akpor and Muchie, 2011; Momba and Mfenyana, 2005). Bacteriophages (physical and F-RNA coliphages) are used to demonstrate viral contamination. Similarly, Clostridium perfringens, a waste spore-

framing bacterium, is used as a marker for the presence of diseases, protozoa, or even helminth eggs because it is known to survive longer in the environment and has been proven to be resistant to chlorine (Akpor and Muchie, 2011). Additionally, diatoms are used to demonstrate the general nature of water with regard to complement enhancement, and they furnish significant translations regarding changes in water quality, like turbidity, conductivity, COD, BOD and chloride (Nimrat, 2008).

2.5.3 Effect of contaminated wastewater on the environment.

The arrival of harmful substances from wastewater into getting water bodies poisonously affects earthly plants and creatures (Momba and Mfenyana, 2005). The toxic effects might be acute. Acute effects from wastewater effluents are for the most part because of elevated degrees of ammonia and chlorine, high heaps of oxygen-requesting materials, or harmful centralizations of weighty metals and natural pollutants (Oyewumi, 2017). Combined influences are because of the progressive development of contaminations in getting water, which possibly become clear when a specific edge is surpassed (Akpor and Muchie, 2011). Nutrient prompted organism of sea-going plants in getting water bodies has the accompanying hindering outcomes: Algal bunches, scents and discoloration of the water, in this manner disrupting sporting and tasteful water use; broad development of established aquatic life impedes route, air circulation and channel limit; dead macrophytes and phytoplankton settle to the lower part of a water body, invigorating microbial breakdown processes that require oxygen, consequently causing oxygen exhaustion; outrageous oxygen consumption can prompt the demise of helpful amphibian life; siliceous diatoms and filamentous green growth might stop up water treatment plant channels and result in decreased discharging, and algal sprouts might conceal and lower amphibian vegetation, hence diminishing or dispensing with photosynthesis and efficiency (Akpor and Muchie, 2011).

Despite the fact that nitrogen and phosphorus are helpful to marine life in small amounts, they contribute to eutrophication when present in excess. Eutrophication stimulates the growth of plants and algae blooms in streams, lakes, reservoirs, estuaries, and along coastlines (Momba and Mfenyana, 2005). The passing of the large amounts of phytoplankton that make up the flowers may cover the lake bottom with natural material in lakes, waterways, streams, and waterfront waters where enormous algal sprouts are accessible. The decay of this material can consume most or all of the dissolved oxygen in the surrounding water, reducing the stamina of several fish and other sea life (Oyewumi, 2017).

2.5.4 Effect of contaminated water on fish

Adeleke (2020) contends that when fish are healthy and disease-free, aquaculture production may be done at a high level of efficiency. Fish disease management aims to stop the disease from spreading and takes precautions to decrease the effects of infection when it does. helps us understand certain common fish infections, their causes, and effective control measures. *Vibrio*sis is a bacterial disease that severely reduces the number of fish in marine fish farms; it accounts for 66% of illnesses seen in grouper species (Oyewumi, 2017). Extreme damage to fish's skin, muscles, blades, eyes, and internal organs is caused by *Vibrio*sis. High stocking density, unfavorable handling of fish, and a naturally contaminated culture climate are stressors that cause *Vibrio*sis events (Oyewumi, 2017).

2.6 *Vibrio* species

2.6.1 Vibrio cholerae

Domain: Bacteria

Kingdom: Bacteria

Phylum: proteobacteria

Class: Gammaproteobacteria

Order: *Vibrio*nales

Family: *Vibrio*nacaceae

Genus: *Vibrio*

Species: *Vibrio parahaemolyticus*

Cholera is typically thought to be caused by *Vibrio* cholerae, a gram-negative member of the *Vibrio*naceae family (Willey et al., 2008). *V.* cholerae has over 200 known serogroups, but only the O1 and O139 serogroups have been associated with the cholera infection that results in diarrhea (Gaffga et al., 2007). It is believed that any residual non-O1/non-O139 serogroups are the main causes of the sporadic and localized outbreaks of a disease similar to cholera (Elhadi, 2012). Water is essential to the transmission and epidemiology of cholera since *V.* cholerae is typically found in aquatic habitats (Tamrakar, 2009). Monitoring this microbe in water sources is essential (Choopun, 2002).

The *Vibrio* genus involves 74 species including ten species pathogenic to people: *V.* cholerae, *V. mimicus, V. fluvialis, V. parahaemolyticus, V. alginolyticus, V. cincinnatiensis, V.vulnificus, V.furnissii, V. metschnikovii, and V. harveyi (carchariae*) (Tarr *et al.,* 2007).

Vibrio are also common in aquatic settings (Susilo, 2021). There is Aeromonas spp., according to recent investigations. Since some species of this group of organisms can create a variety of virulence factors, drinking water could pose a risk (Mkali, 2014). Salt is necessary for *Vibrio,* however the concentration varies depending on the species. Mehdi, (2011) gives a method for separating pathogenic *Vibrio* into the salt-required halophilic species (*V.* cholerae and *V.* mimicus) and the non-halophilic species (*V.* cholerae and *V.* mimicus) that thrive on nutrient agar. Although most kinds of *Vibrio* will develop between pH 6.5 and 9.0, *Vibrio* may grow in a wide range of temperatures (20°C to >40°C) and will typically grow best under alkaline circumstances. Asymptomatic human carriers and sick individuals who excrete the bacteria in

their feces are the reservoirs of *V.* cholerae. Ingestion of tainted food or water triggers the onset of *V.* cholerae infection. In endemic regions, *Vibrio* cholerae has been discovered in surface and drinking water (Elhadi, 2012). Only in saline water can *Vibrio* develop more effectively. 2017 (Oyewumi).

2.6.2 *Vibrio* mimicus

V. mimicus is a bacterium that looks like *Vibrio* cholerae in numerous qualities, including its capacity to fill in low convergences of sodium and its antigenic design. It has been ensnared in individual cases and flare-ups of gastroenteritis and a few strains can likewise deliver cholera poison (Lee *et al.*, 2008).

2.6.3 *Vibrio* fluvialis

V. fluvialis has been identified as a resident of estuary and beachfront waters and has been linked to both isolated instances and outbreaks of gastroenteritis. Like many other *Vibrio*'s, illness is frequently linked to the consumption of raw or undercooked fish, especially raw clams (Lee et al., 2008). Halophilic microbe *Vibrio fluvialis* is frequently found in marine environments or marine-related objects. Due to the fact that *V. fluvialis'* clinical symptoms of gastroenteritis are nearly identical to those caused by *V.* cholerae, it is currently known to be a major disease-causer. After the new characterization of an enterotoxigenic El Tor-like haemolysin in *V. fluvialis*, which tackles one of the harmfulness parts of this, it turned out to be much more significant.

Normally, absence of supplement is the most widely recognized ecological pressure which microorganisms regularly experience in normal biological systems. Nonetheless, it was found that *Vibrio spp*. can get by for quite a while during starvation by consecutive changes in cell physiology and slow changes in morphology (Parada, 2016).

2.6.4 Vibrio alginolyticus

Extremely sensitive tissue contaminations, sepsis, and other extraintestinal illnesses are brought on by the halophilic bacteria *V. alginolyticus,* which has been found in marine and estuarine environments. People consume contaminated fish or come into contact with the microbes through open water (Kiratisin, 2012). One of the twelve Vibrio species that can be fatal and cause serious illness in humans is *V. alginolyticus* (Scallan, and Black, 2012). For a long time, *V. alginolyticus* was the third most common Vibrio species found in human disease, but starting in 2007, when the rate of Vibriosis as a whole increased, it moved up to the second most common Vibrio species (https://www.cdc.gov/Vibrio /surveillance.htmL).

2.6.5 Vibrio parahaemolyticus

V. parahaemolyticus is a Gram-negative halophilic bacterium that is widely disseminated in estuarine, marine and coastal surroundings (Nelapati et al., 2012; Ceccarelli et al., 2013; Zhang and Orth, 2013). *V. parahaemolyticus* is usually found in a free-swimming state; with its motility conferred by a single polar flagellum affixed to inert and animate surfaces including zooplankton, fish, shellfish or any suspended matter underwater (Gode-Potratz et al., 2011). In rare cases, *V.* p*arahaemolyticu*s causes wound infection, ear infection or septicaemia that may be life-threatening to individuals with pre-existing medical conditions (Zhang and Orth, 2013).

V. parahaemolyticus bacteria are extensively present in marine and estuarine environments but not all strains of this bacterium are considered pathogenic (Velazquez-Roman *et al.,* 2012). The strains isolated from environmental samples usually lack the pathogenic genes *tdh* and/or *trh* which cause illnesses to humans and marine animals (Deepanjali *et al.,* 2005; Canizalez-Roman *et al.,* 2011; Gutierrez West *et al.,* 2013)

various selective enrichment media have been utilized for the isolation and detection of *V. parahaemolyticus,* due to its natural presence in the marine environments with high tolerance and preference to alkaline pH condition, the selective media used for this pathogen is often prepared for pH 8.6–pH 9.4, alkaline with the additional 1–7% NaCl (Paydar *et al.,* 2013).

2.7 *Escherichia coli*

Escherichia coli, initially called "Bacterium coli community," was first isolated from the excrement of a little child in 1885 by the Austrian paediatrician Theodor Escherichia (Escherich, 2015). *E. coli* are Gram-negative, motile, non-spore shaping bacilli of the family Enterobacteriaceae. They are around 0.5 µm in measurement and 1.0-3.0 µm long. Most kinds of *E. coli* are innocuous; however, a little extent can cause clinical side effects in people and different well evolved creatures. (Mushtaq *et al.*,, 2011). The pathogenic types of *E. coli* that causes intestinal sicknesses are assembled into six classifications which incorporate; *enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), entero-intrusive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC),* and diffuse-follower *E. coli (DAEC)* Molokwu and Okpokwasili (2002) and Njoku *et al.*,. (2015a).

PATHOTYPES OF *E.COLI*

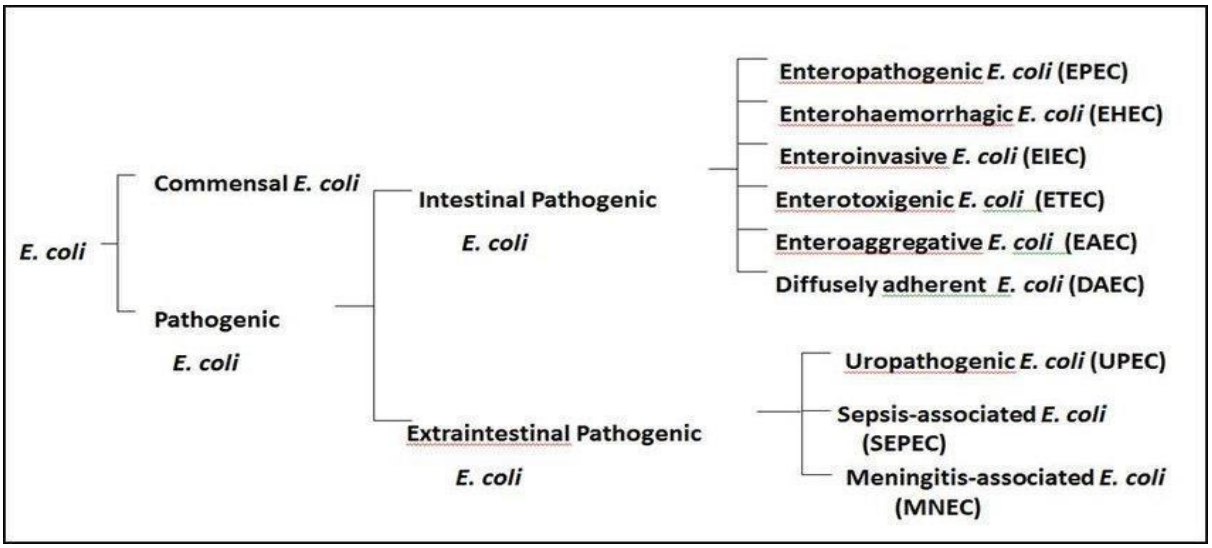

Figure 2.2 Escherichia coli species and its sub species classification.

Source: Wakeham, (2013)

2.7.1 Enterohaemorrhagic *E. Coli* (ehec)

This class' essential etiological specialist is *E. Coli* O157:H7, a general medical problem with numerous huge flare-ups contribution overall since it was first distinguished in 1983 (Riley, 2020). It was closed by Griffin and Tauxe (1991) that *E. coli* O157:H7 is an arising and new microbe, since they felt that such an unmistakable sickness which frequently has serious outcomes (haemolytic uremic condition) would certainly stand out at any period. Afterward, O26:H11, O45:H2 and three non-motile *E. coli* (O4, O111 and O145) were added to this gathering of life forms. *Escherichia coli* O157:H7 is a harmLess living being, which produces verotoxin as its essential destructiveness factor (Doyle, 2000) which are named for their cytotoxicity to African green monkey kidney cells called Vero cells (Meng *et al.,* 2001).

2.7.2 Enteroinvasive *E. Coli* (eiec)

This class addresses a little gathering of *E. coli*. Many secludes are non-motile (without the H antigen) and they are delayed to age lactose or are non-lactose maturing (Riley *et al.,* 2020). They are the reason for an illness like bacillary loose bowels brought about by *Shigella* and have substantial antigens that might cross-respond with those of *Shigella* (Dogan, 2018). Like *Shigella*, there are no known creature supplies; thus, the essential hotspot for EIEC is by all accounts tainted people (Dogan, 2018). Albeit the infective portion of Shigella is low and in the scope of 10 to few hundred cells, volunteer taking care of studies showed that something like 106 EIEC living beings are expected to cause sickness in sound grown-ups. EIEC can attack and duplicate in the cells of the gastrointestinal mucosa, particularly in the colon (Riley *et al.,* 2020). The intrusion aggregate of EIEC is encoded by a high sub-atomic weight plasmid, which can be recognized by intrusion measures utilizing HeLa or Hep-2 tissue culture cells Dogan, (2018) or by PCR and tests explicit for attack qualities. Sicknesses because of this microbe happens inside 8 to 24 hrs after ingestion of food or water containing this creature. Side effects because of Enteroinvasive E. coli incorporate stomach torment, fever, disquietude, myalgia, migraine and watery dung containing bodily fluid and blood (Dogan, 2018).

2.8 Review of methods

2.8.1 Biochemical test

The most common and useful staining procedure is the gram stain which separates bacteria into two groups according to the composition of their cell walls and were done as described by William *et al*., (2001). On microscopic examination the gram-positive organisms appeared purple and gram-negative organisms appeared pink (Ogeneogaga and Solomon, 2017).

Catalase enzyme protects bacteria from hydrogen peroxide (H_2O_2) accumulation, which can occur during aerobic metabolism. Catalase test was aimed at identifying organisms that produce the enzyme catalase, which converts hydrogen peroxide to water and oxygen bubbles. If bubbles become visible, this concludes that the organism produces catalase. Lack of bubbles indicates negative result (zym and Wondikom, 2018).

The oxidase test was based on detecting the production of enzyme cytochrome oxidase by Gram negative bacteria. Colour changed to purple or blue after 30s to 1 min was evidence that the result was positive (Ogeneogaga and Solomon, 2017).

The citrate utilization test was carried out to determine the ability of the isolates to use citrate as sole source of carbon and energy. A change in the medium from green to royal blue was recorded as a positive test (Abu and Wondikom, 2018).

Unhydrolyzed starch formed a blue-black colour, hydrolysed starch appeared as a clear zone and reddish-brown zones around the colony indicated partial hydrolysis of starch (Olutiola *et al*., 2000).

2.8.2 Polymerase Chain Reaction (PCR)

Molecular identification of isolates was performed using polymerase chain reaction (PCR) assay. Fragments of the bacterial 16S rRNA gene were amplified as an internal control for all presumptive isolates, using universal oligonucleotide primers 27F and 1492R (Stackebrand and Goodfellow 1991). While species specific primers were used to identify species, target genes included *sodB* (1) and *ompW* for *V. cholerae, rfb* specific for *V. cholerae.*

CHAPER THREE
MATERIALS AND METHODS

3.1 Materials used:

Petri-dishes, beakers, conical flasks, hockey stick, measuring cylinder, Eppendorf tubes, micro pipette (with their tips), test tubes (with their racks), spatula, filter paper, inoculating loop, wash bottles.

3.2 Reagent and Equipment Used:

Autoclave, incubator, weighing balance, thermal cycler, centrifuge, stomacher blender, distiller, Lamina air flow cabinet, Magnetic stirrer water bath (set at 50°C and 100°C), Bunsen burner. TCBS Agar, Nutrient Agar, Sorbitol MacConkey Agar.

3.2.1 Peptone Water

Peptone water is a microbial growth medium composed of peptic digest of animal tissue and sodium chloride. The pH of the medium is 7.2±0.2 at 25 ℃ and is rich in tryptophan. Peptone water is also a nonselective broth medium which can be used as a primary enrichment medium for the growth of bacteria.

Preparation

The dehydrated medium was dissolved in the appropriate volume of distilled water to make up 0.1% and 1% peptone water based on manufacturer's instruction's instructions in a conical flask and mixed thoroughly. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121℃ for 15mins. 9mL of the 0.1% was then dispensed into various test tubes for serial dilution. 225mL of the 1% was then dispensed into conical flask.

3.2.2 TCBS Agar

TCBS Agar is a differential medium used to culture *Vibriospp.*

Dehydrated TCBS agar of 26.7g was weighed and Poured in to a conical flask containing 100mL of distilled water. The flask opening was Sealed with an aluminum foil and Placed the TCBS solution on heating stirrer until it started boiling and completely dissolved. (autoclaving was not needed) the solution was allowed to cool down to 45°C. The TCBS solution was Poured into petri dish aseptically and allowed to solidify.

3.2.3 Sorbitol MacConkey Agar

Sorbitol MacConkey Agar is a selective medium used to isolate coliforms. It provides a color indicator distinguishing between organisms that ferment lactose (e.g., E. *coli*) and those that do not (e.g., *Salmonella*).

Preparation

The medium 36g was suspended in 1000mL distilled water and mixed thoroughly. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at $121^{O}C$ for 15minutes. The agar was allowed to cool to $45^{O}C$ and poured aseptically into sterile petri dishes and left to solidify.

3.2.4 Nutrient agar

Nutrient agar is a general-purpose nutrient medium used for cultivation of microbes supporting growth of a ride range of non-fastidious organisms.

Preparation

The medium 28g was suspended in 1000mL distilled water and mixed thoroughly. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at $121^{O}C$ for 15minutes. The agar was allowed to cool to $45^{O}C$ and poured aseptically into sterile petri dishes and left to solidify.

3.3 Sample collection

Wastewater was collected from Mountain Top University, Ogun state. At three different ponds (behind Girls hostel, behind CBAS and behind the ICT office). The wastewater was collected aseptically into a sterile 500 mL screwed plastic containers at about 10–15 cm depth at which the caps was opened and closed after filled and the well labelled containers were transported to the laboratory on ice for analyses within 8 hours (Abu and Wondikom, 2018).

3.4 Physicochemical analysis of water samples

Physical and chemical properties of the wastewater samples were determined. The properties included: Temperature, pH, total suspended solids, conductivity, salinity, were determined according to methods described by (Apha., 2005).

3.4.1 Determination of Temperature

Temperatures of pond water samples were determined at the sampling location using ordinary thermometer. This was done by fully inserting the tip of the thermometer into the water sample. The reading was immediately recorded.

3.4.2 Determination of pH

pH values of water samples were determined by means of electrometric pH meter by dipping the electrode into the water (pHep® HI 98107- Italy).

3.5 Isolation of *E. coli*, coliforms and total viable count

Fish pond wastewater sample 1mL was added into a sterile test tube containing 9mL of 1% buffered sterile peptone water. The mixture was diluted serially up to $10^{-4.}$ From the appropriate dilutions 0.1 mL was plated in duplicate onto Sorbitol MacConkey Agar, and Nutrient Agar for the isolation of enteropathogenic *E.coli*, coliforms and for the Total viable count using the spread plate technique. The plates were then incubated at 35^{O}C- 37^{O}C for 18- 24 hours.

3.6 Isolation of *Vibrio* spp.

3.6.1 Primary enrichment

Fish pond wastewater sample of 1mL was added into a sterile test tube containing 9mL of 1% buffered sterile peptone water at pH 8.0±0.2. The mixture was diluted serially up to $10^{-4.}$ From the appropriate dilutions and incubated for 8hours.

3.6.2 Secondary Enrichment

The thiosulphate-citrate-bile-salt-agar (TCBS) was prepared and poured onto sterilized petri-dishes. On solidification, 0.1mL of the pre-enrichment was transferred to the solidified agar in duplicate and spread evenly with a sterile hockey stick and it was incubated at 37^{O}C for 24-48 hours to allow selective enrichment for *Vibrio spp*. There were presences of *Vibrio* like yellow colony**,** 2 to 4mm of slightly flattened colonies with opaque centers was picked and streaked on another TCBS agar**, t**he plates were inverted and incubated at 37^{O}C for 24hours. The plates were examined for typical *Vibrio spp*.

3.6.3 Sub-Culturing

The plates were checked after the required duration for the growth a sub-culturing needs to be done. Sub culturing was done to purify the isolated bacterial colonies from a mixed culture to a new and single culture, the bacterial isolates transferred or sub-cultured were those differentiated on the basis of their colony morphology, shape, colour, elevation and other physical characteristics.

Presumptive colonies obtained after incubation were sub-cultured unto fresh nutrient agar plates using the streaking method procedure by taking a loopful of preferred isolate using the inoculating loop (the inoculating loop is heated using the Bunsen burner and allowed to cool for like 5 seconds before taking the loop from the original mixed culture and streaked onto the new petri-dish). The plates were inverted and incubated at 37^{O}C for 18- 24 hours.

3.6.4 Preservation of isolates

A loopful of each isolate was inoculated into a sterile test tube containing 5mL of brain heart infusion and incubated for 24hours. 1mL of the BHI was inoculated into an Eppendorf tube and 20 % sterile glycerol as cryoprotectant and it was stored in a -4^{0}c freezer until needed for further analysis.

3.7 Identification of the Isolates

The isolated bacteria were identified using morphological characteristics and biochemical tests.

3.7.1 Morphological Identification of the isolates

Morphological characterizations were done using their colonial, cellular and pigment appearances on culture plates.

3.7.2 Biochemical characterization of the isolates

Pure colonies of each bacteria were picked for identification using standard biochemical tests which included Gram's stain, catalase, citrate utilization, starch hydrolysis, oxidase tests. The test cultures for the biochemical tests were prepared by inoculating nutrient agar with each isolate from the stock culture. This was incubated for 18-24 hour at 37 ^{0}C.

3.7.2.1 Gram Staining procedure

A film was made on a clean slide by emulsifying part of a colony in loop full of distilled water. The film was then air dried and fixed by slight flaming and stained as with crystal violet solution for 1-2 minutes. The smear was rinsed rapidly with water and gram's iodine solution was added and left for 1-2 minutes. Iodine was poured off and the slide was washed with 70% ethanol for 5-15 sec. The smear was then washed with tap water and stained with safranin solution for 20 sec. The slide was washed with water and allowed to dry.

3.7.2.2 Catalase test

In this test small amount of the test organism was smeared from the petri-dish onto the head of a sterile slide using a sterile wire loop. Then a drop of hydrogen peroxide was added to the smear and mixed. Bubbles become visible and this concluded that the organism produced catalase.

3.7.2.3 Oxidase test

Some amount of the pure culture was swabbed into one of the ends of an oxidase dry slide using sterile wire loop. Colour changed to purple after 30s to 1 min which was evidence that the result was positive.

3.7.2.4 Citrate utilization test

Tubes of Simon's citrate agar were each inoculated with a test organism and incubated at 35 ^{0}C for 48 hrs.

3.7.2.5 Starch hydrolysis test

Molten starch agar 20mL was aseptically poured into each sterile petri dish, allowed to set and was inverted in an incubator at 37^0C. The organism was streaked across the surface of the plate and incubated at 37^0C for 24-48 hours. Afterwards, the plates were flooded with some quantity of Gram's Iodine.

3.8 Molecular identification

3.8.1 Activation of isolates

Pure Brain–heart–infusion broth (BHI) of 1mL was prepared into 2mL Eppendorf tubes and sterilized using the autoclave at 121°C for 15 minutes. 100µl of each thawed stock culture was added into the various Eppendorf tubes containing sterile BHI after it was allowed to cool, it was then incubated at 37°C for 48hours.

3.8.2 Prewashing

Each isolate was centrifuged in Eppendorf tubes at 5000rpm for 3 minutes. The BHI supernatant was discarded into a waste container, leaving the pellet in the tubes. 1.5mL of sterilized distilled water was added into the tubes, vortexed and then centrifuged at 5000rpm for 3 minutes. The supernatant was discarded and 200 µl of sterilized distilled water was added to the tubes and vortexed.

3.8.3 DNA extraction by boiling using heating block

The heating block was switched on and allowed to reach 100°C. The Eppendorf tubes containing the prewashes isolates were placed into the heating block and the lid was gently placed over it to prevent the tubes from popping open. It was allowed to boil for 15 minutes; the boiled DNA were then placed into ice to cool for 5 minutes. The already cooled DNA was centrifuged at 7000rpm for 6 minutes after which 150 µl of the DNA supernatant was carefully transferred into an already properly coded fresh Eppendorf tube.

3.9 Polymerase chain reaction (PCR)

The component of the PCR used for the characterization of *Vibrio* pathotypes is shown in table 3.1 below. After the PCR cocktail has been prepared it was placed in a thermocycler. The PCR products were confirmed by electrophoresis and visualized under UV light with a Gel

Table 3. 1 PCR Cycling conditions for *V. cholerae*

No of cycles	**Step**	**Temperature**	**Time**
1	Initial denaturation	94^0c	10 min
35	Denaturation	94^0c	1 min
35	Annealing	59^0c	1 min
35	Elongation	72^0c	2 min
1	Final elongation	72^0c	10 min

Source: (*Goel, 2007)*

Table 3. 2 PCR Cycling conditions for *V. parahaemolyticus*

No of cycles	**Step**	**Temperature**	**Time**
1	Initial denaturation	95^0c	10 min
35	Denaturation	92^0c	40 sec
35	Annealing	57^0c	1 min
35	Elongation	72^0c	1.5 min
1	Final elongation	72^0c	10 min

Source*: (Tarr, 2007)*

Table 3. 3 **Multiplex PCR reaction components**

TREATMENT 1

No.	**Reagents**	**Initial concentration**	**Final concentration**	**Volume per rxn(v/r)**	**n=20**
1	Master mix	5x	1x	2	20
2	OMPW F	20um	0.25	0.125	1.25
3	OMPW R	20um	0.25	0.125	1.25
4	FLAE F	20um	0.25	0.125	1.25
5	FLAE R	20um	0.25	0.125	1.25
6	700 F	20um	0.05	0.25	0.25
7	1325 R	20um	0.05	0.25	0.25
8	Mgcl2	25mm	0.5	0.2	2
9	dH_20			5.25	52.5
10	DNA			2	

Table 3. 4 Oligonucleotide primers used for PCR amplification of *Vibrio* species-specific gene fragments primer sequences16S rRNA amplification

	NAME: (TARGET GENE)	SEQUENCE	TARGET ORGANISM
1	700 F	CGGTGAAATGCGTAGAGAT	*Vibrio spp*
2	1325 R	TTACTAGCGATTCCGAGTTC	*Vibrio spp*
3	OMPW F	CACCAAGAAGGTGACTTTATTGTG	*V.cholerae*
4	OMPW R	GGTTTGTCGAATTAGCTTCACC	*V.cholerae*
5	FLAE F	GCAGCTGATCAAAACGTTGAGT	*V. parahaemolyticus*
6	FLAE R	ATTATCGATCGTGCCACTCAC	*V. parahaemolyticus*

3.10 Agarose Gel Electrophoresis

The agarose was prepared using dry agarose powder, 1g of the agarose powder was then dissolved in 50mL of Tris-Acetate-EDTA (TAE) buffer the mixture was then boiled until a clear solution was gotten 3ul of ethidium bromide was added to the mixture using a micropipette it was swirled and left to cool but not solidify, the content of the flask was then transferred into the gel cast with the combs in place, after, it was left to solidify and the gel was gently removed and placed in an electrophoresis tank containing TAE buffer. 4ul of the PCR products were then pipetted into each well that was formed after removing the comb. The tank was connected to the power pack and left to run till it gets to one-third of the gel and then it was turned off and the gel was viewed under the UV transilluminator.

CHAPTER FOUR
4.0 RESULTS AND DISCUSSION

4.1 Physicochemical parameters.

The physio-chemical Result.The physicochemical parameters of wastewater form the three fish pond samples are presented below and these parameters includes pH, Conductivity, Temperature, Salinity. The physio-chemical parameters of the water samples of the three ponds in different weeks are presented in Table 4.1. The parameters did not change significantly but week 4 had a higher parameter.

The results of this study showed that the temperature varied between (28.83 oC and 28.67 oC). Because Vibrio spp. are considered to be indigenous bacteria in aquatic environments and proliferate under favorable temperature conditions above 15oC, the existence of human pathogenic Vibrio isolated from the pond may be explained. The temperature of the pond promoted the development and spread of Vibrio spp. as a result, and the water's results are close to those recorded in Kumar's (2004) study, which noted temperature ranges of 20 to 30 oC, which were around the stated upper limits of WHO. For pond waters sampled from Lagos, Araromi, and Akure, Nigeria, separately, Ajayi and Okoh (2014), Omofunmi et al. (2016) observed lower temperature ranges of 26–27 oC, 27–31 oC, and 26–29 oC. Fish's digestion, physiology, and effectiveness are affected by temperature fluctuations (Olukunle and Oyewumi, 2017).

All of the ponds' pH readings fell within the range needed for aquaculture (6.8 -7.0). Since *Vibrio spp*. can only thrive in an alkaline environment, this is acceptable for fish production. Compared to pH 6.5 and 7.5, an alkaline pH of 8.5 was best for *V. cholerae* adhesion and growth. These pH readings fell within the desirable 6.5–9.0 range for uncontaminated fish productions (Njoku *et al.,* 2015). Low pH fish pond water is extremely acidic and can burn a fish's skin. Fish eggs will not hatch in water with a pH of 5, which is too acidic and kills fish. (Alexis, 2018).

4.2 Sample identification table

The Table 4.2 gives the full meaning of the isolate ID.

4.3 Colony count on TCBS Agar.

Figure 4.1 shows the total colony counts of the different ponds' wastewater. Wastewater sample $W3T3^{-1}$ had (3 colonies), $W2B2^{-4}$ had highest total colony count (23 colonies), $W2T2^{-3}$ (3 colonies), $W2G2^{-2}$ (5 colonies), $W2G2^{-1}$ (4 colonies), $W2B2^{-3}$ (2 colonies), $W2T2^{-1}$ (6 colonies), $W2T2^{-4}$ (4 colonies), $W2G2^{-3}$ had the least (1 colonies).

4.4.1 Morphological traits

The morphological parameters of bacterial isolates on TCBS Agar are displayed in Table 4.3 and Figure 4.2. All samples had opaque centers and were yellow, rounded, tiny, elevated, shiny, smooth, and colony sizes ranging from 2 to 4mm. Isolates were thought to be Vibrio spp. based on the selective media that were utilized for identification. Morphological characteristics of suspected *E.coli*. All samples had pink and white, convex, circular, smooth and shiny colonies (Table 4.4). This study demonstrates the prevalence of Escherichia coli and Vibrio spp. in the wastewater from fish ponds, since this could be harmful to the health of the fish and people who consume them. One of the major factors influencing the propagation of infection during unexpected illness outbreaks is the persistence of pathogens in the aquatic environment. Escherichia coli and Vibrio spp. found in the fish ponds imply that the water has been contaminated by feces. The defecation of fish into the ponds or fertilizing of the ponds with animal manure that is released directly into the fish ponds may be the cause of the faecal material. (Sule *et al.,* 2016).

4.5 Biochemical characteristics

Biochemical test was performed on all the purified isolates, all the isolates were positive (+) to catalase test, which was aimed at identifying organisms that produce the enzyme catalase, which converts hydrogen peroxide to water and oxygen bubbles. Bubbles become visible and the result was recorded as positive. The isolated organisms were Gram negative (-) as they appeared pink, retaining the colour of the counter stain- safranin. Oxidase test Colour changed to purple or blue after 30s to 1 min was evidence that the result was positive. Starch hydrolysis test came out positive (+). Citrate test had a change in the medium from green to royal blue and was recorded as a positive (+) result. The results are summarized in table 4.5

Infection of children and adults who swallow water from these water bodies while swimming is one way that these pathogenic organisms, when introduced to other water bodies, can cause diseases in both plants and animals. The majority of people in rural areas use these waters for domestic tasks like cooking and bathing. Some of our farmlands are connected to these water bodies to irrigate the crops, but when the crops come into contact with the harmful elements in the water, they risk dying. Additionally, consumers risk contracting the disease when they eat the plants without properly washing and preparing them. e.g. those who eat raw vegetables without proper washing have a high tendency of getting infected and when not properly treated can lead to death and an outbreak of cholerae cause the loss of many life.

4.6 Agarose Gel Electrophoresis

Figure 4.3 shows the result of Agarose gel electrophoresis. Amplicons of fragments of *flaE* (*V. parahaemolyticus*) and *ompW* (*V. cholerae*) 304bp genes were amplified during the study. Lane *L* = 300dp DNA marker, lane 2 = fragment of the *ompW* gene *V. cholerae* isolates was positive in the test.

Table 4. 1Physicochemical parameters

Samples	Isolate ID	**pH**	**Temperature (^{0}c)**	**Salinity**	**Conductivity (ms/cm)**
Week 1	G.H	6.8	28.7	094ppm	0.12
	ICT	7.0	28.8	120ppm	0.16
	CBAS	6.4	28.6	155ppm	0.17
Week 2	G.H	6.8	28.5	094ppm	0.13
	ICT	7.0	28.6	120ppm	0.16
	CBAS	6.4	28.4	140ppm	0.16
week 3	G.H	6.7	27.6	180ppm	0.13
	ICT	6.9	27.8	120ppm	0.17
	CBAS	6.6	26.7	155ppm	0.17
Week 4	G.H	6.8	29.2	196ppm	0.14
	ICT	7.0	28.2	135ppm	0.17
	CBAS	6.6	28.9	165ppm	0.18

Table 4. 2 Sample identification table

Samples	Isolate ID	
Sample 1 (water)	W1G1	Water sample, week one, girls' hostel, sample one
	W1T1	Water sample, week one, ICT, sample one
	W1B1	Water sample, week one, CBAS, sample one
Sample 2 (water)	W2G2	Water sample, week two, girls' hostel, sample two
	W2T2	Water sample, week two, ICT, sample two
	W2B2	Water sample, week two, CBAS, sample two
Sample 3 (water)	W3G3	Water sample, week three, girls' hostel, sample three
	W3T3	Water sample, week three, ICT, sample three
	W3B3	Water sample, week three, CBAS, sample three
Sample 4 (water)	W4G4	Water sample, week four, girls' hostel, sample four
	W4T4	Water sample, week four, ICT, sample four
	W4B4	Water sample, week four, CBAS, sample four

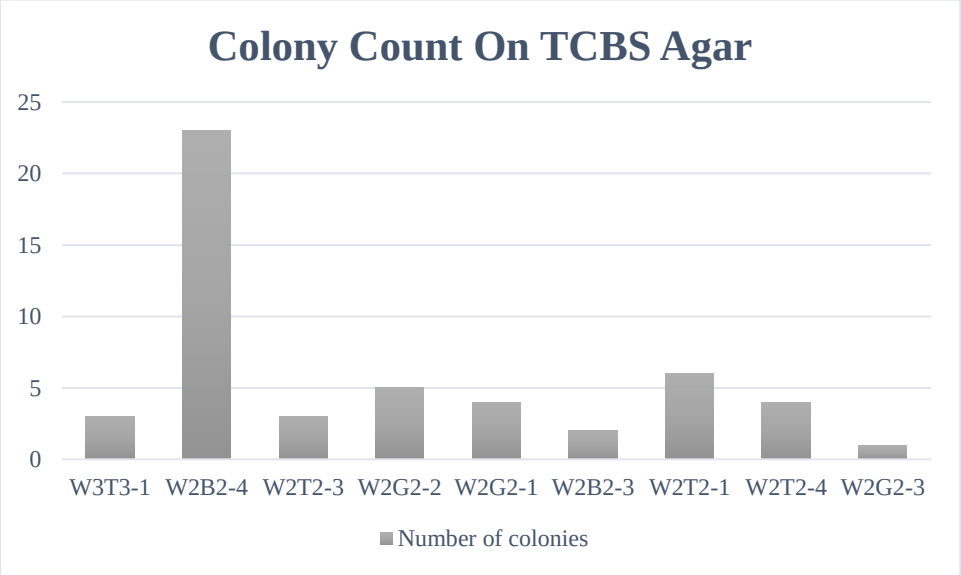

Figure 4. 1 Colony count on TCBS Agar

Table 4. 3 Morphological characteristics of bacterial isolates on TCBS Agar

Samples	Isolate id	Colour	Shape	Size	Elevation	Appearance	Texture	Opacity	Margin
Sample 1	W1G1	Yellow	Round	Small	Raised	shiny	smooth	Opaque	Entire
	W1T1	Yellow	Round	Small	Raised	shiny	smooth	Opaque	Entire
	W1B1	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
Sample 2	W2G2	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W2T2	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W2B2	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
Sample 3	W3G3	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W3T3	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W3B3	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
Sample 4	W4G4	Yellow	Round	Small	Raised	shiny	smooth	Opaque	Entire
	W4T4	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire
	W4B4	Yellow	Round	Small	Raised	Shiny	smooth	Opaque	Entire

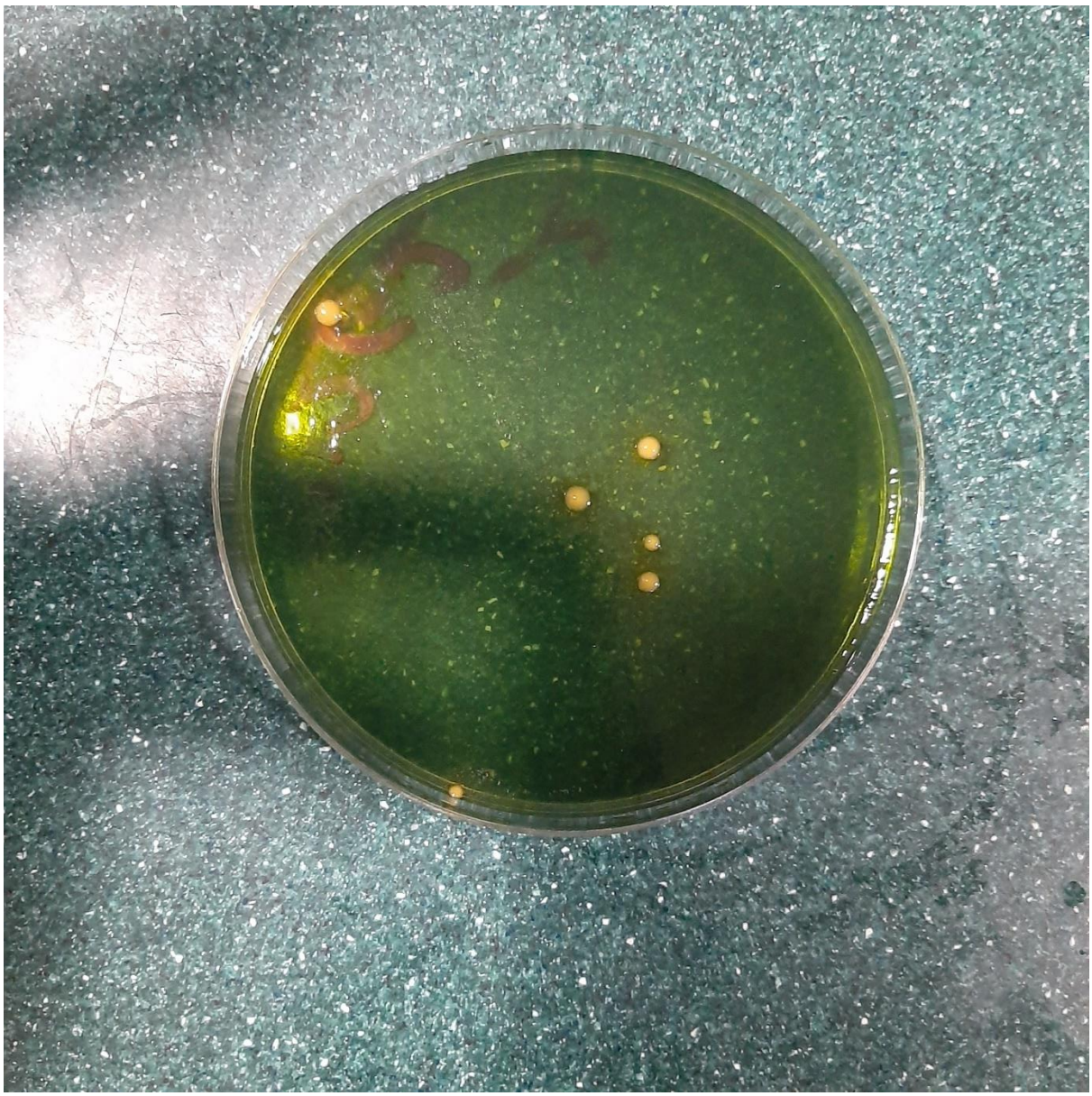

Plate 4. 1 *Vibrio* growth on TCBS plate

Table 4. 4 Morphological characteristics of bacterial isolates on Sorbitol MacConkey Agar

Samples	Isolate ID	Colour	shape	size	Elevation	appearance	texture	opacity	margin
Sampling 1	W1G1	White	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W1T1	Pink	Circular	Small	Raised	Butyrous	smooth	Opaque	Entire
	W1B1	White	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
Sampling 2	W2G2	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W2T2	White	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W2B2	Pink	Circular	Small	Convex	Butyrous	smooth	Opaque	Entire
Sampling 3	W3G3	White	Circular	Small	Convex	Butyrous	smooth	Opaque	Entire
	W3T3	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W3B3	White	Circular	Small	Convex	Butyrous	smooth	Opaque	Entire
Sampling 4	W4G4	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire
	W4T4	White	Circular	Small	Raised	Butyrous	smooth	Opaque	Entire
	W4B4	Pink	Circular	Small	Low convex	Butyrous	smooth	Opaque	Entire

Table 4. 5 Biochemical test results

Isolates	Isolate ID	Catalase	Gram-staining	Oxidase	Starch	Citrate
1ST samples	W1G1	+	Gram (-) rod	+	+	+
	W1T1	+	Gram (-) rod	+	+	+
	W1B1	+	Gram (-) rod	+	+	+
2nd Samples	W2G2	+	Gram (-) rod	+	+	+
	W2T2	+	Gram (-) rod	+	+	+
	W2B2	+	Gram (-) rod	+	+	+
3RD Samples	W3G3	+	Gram (-) rod	+	+	+
	W3T3	+	Gram (-) rod	+	+	+
	W3B3	+	Gram (-) rod	+	+	+
4TH Samples	W4G4	+	Gram (-) rod	+	+	+
	W4T4	+	Gram (-) rod	+	+	+
	W4B4	+	Gram (-) rod	+	+	+

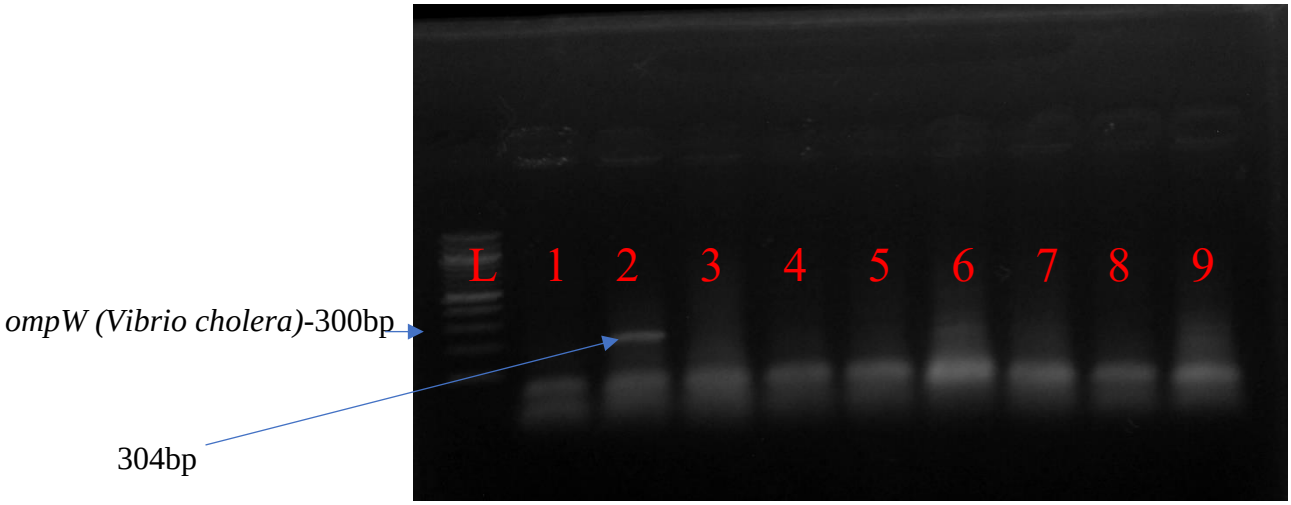

Plate 4. 2 Agarose gel electrophoresis image of multiplex-PCR product showing Amplicon of fragments *flaE* (*V. parahaemolyticus*) and *ompW* (*V. cholerae*) 304bp genes. Lane *L* = 300dp DNA marker, lane 2 = fragment of the *ompW* gene *V. cholerae* isolates were positiv

CHAPTER 5
5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In this study, it can be concluded that *Vibrio spp* and *E.coli* were present in MTU fish pond wastewater, and this was identified using both biochemical tests and molecular analysis. The study also revealed that most of the ponds were contaminated with pathogenic bacteria that could affect fish cultivation, since the microbial quality of the fish pond water reflects the fish itself and also, these organisms could cause an outbreak of cholera if not properly controlled.

5.2 RECOMMENDATIONS

It is therefore recommended that microbiological examination and physicochemical analysis of the discharged wastewaters from fish ponds be carried out regularly for proper monitoring so as to prevent avoidable epidemics in the country. Good quality waters such as well or borehole should be used in the fish pond rather than water from questionable sources such as river, stream, and surface runoff. The fish feeds should be sourced from reputable manufacturers. Water in the fish pond should be changed completely at regular intervals. Public awareness on the effects caused by the indiscriminate release of fish ponds' effluents into the surroundings should be organized for farmers especially those in the rural areas. Waste water should be treated either by physical methods which involve filtration through slow sand filters, rapid sand filters, sand beds or chemical methods such as addition of disinfectants e.g. chlorine before final disposal into surrounding drains. Oxygen and temperature should be measured at least twice daily to determine the influence of photosynthesis on concentrations. Salinity and pH should be measured daily.

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