MOLECULAR CHARACTERISATION OF *VIBRIO* SPECIES FROM UNILAG LAGOON AND MTU POND

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE, COLLEGE OF BASIC AND APPLIED SCIENCES,

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY.

AUGUST, 2021.

DECLARATION

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of reference.

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ADEYEMO TOLUWANI OPEYEMI

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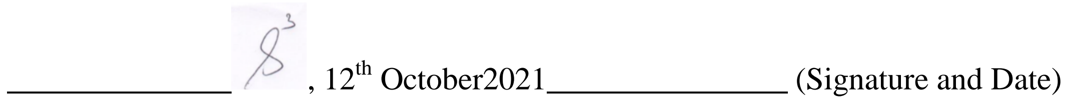
Date

CERTIFICATION

This is to certify that the content of this project entitled **'Molecular Characterisation of *Vibrio* Species from UNILAG Lagoon and MTU Pond'** was prepared and submitted by **ADEYEMO TOLUWANI OPEYEMI**, in partial fulfilment of the requirements for the degree of **BACHELOR OF SCIENCE IN MICROBIOLOGY**. 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 parents, Mr. Adeyemo Olusegun and Mrs. Adeyemo Bamidele, for their guidance, understanding, love and sacrifice. I also dedicate this work to my siblings Tobi, Gideon, Ogo-oluwa and Immanuella for their care and always checking up on me, in the course of my four-year study of Microbiology in Mountain Top University.

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ABBREVATIONS

TCBS (Thiosulphate citrate bile salt sucrose)

MTU (Mountain Top University)

UNILAG (University of Lagos)

PCR (Polymerase chain reaction)

BHI (Brain Heart Infusion)

ABSTRACT

Water being a necessity in our everyday lives and the major habitat of *Vibrio* species, *Vibrio* spp are the most common genera associated with water, human and even crustaceans often causing significant economic losses. Vibrios are ubiquitous in the aquatic environment and are commonly present in or on shellfish and other seafood or other environmentally related food or water sources. In this study two water samples were collected from two different locations (MTU pond and UNILAG lagoon) and plated on 50 different plates this was examined for the presence and absence of *Vibrio* species. The water samples were enriched into alkaline peptone water then inoculated into Thiosulfate citrate bile salt sucrose (TCBS) agar medium. After incubation of the 50 TCBS plates for 24hrs at 37^0C, the colonies were screened out for biochemical identification and confirmed using 16S rRNA PCRs. To the best of my knowledge, this is the first study done on determining the presence of potential *Vibrio* species in MTU pond but research has already been done on the UNILAG lagoon. This study has found that *Vibrio* species are present in this study areas, as four of our isolates tested positive after the 16S rRNA was done. The highly conserved 16S rRNA (rrs) gene is generally used for bacterial identification. In conclusion, this study has helped to create awareness of the presence of *Vibrio* species in MTU pond and to discourage MTU communities and inhabitants about the risk of using water as a source of water or using the fishes as a source of food.

Keywords: *Vibrio* spp, Water source, Identification, Isolates.

CHAPTER ONE

1.1 INTRODUCTION

Water is essential for life, and having an appropriate, safe, and accessible supply is critical. The presence of water on Earth makes life possible.

Drinking water quality is directly linked to human health, and ensuring safe drinking water is a top public health issue (Okeyo *et al.,* 2018).

In most communities with little or no access to drinkable safe water, freshwater bodies serve as the primary source of water for drinking, cooking, and irrigation for agriculture.

Vibrio cholerae is a common bacterium found in surface water. Human illnesses are caused by ingesting contaminated food or drinking contaminated water, or by direct wound contact with contaminated food or water (Cho *et al*., 2008).

There are at least twelve *Vibrio* spp that are clinically important to humans, with *V. cholerae, V. parahaemolyticus, and V. vulnificus* being the three most common (Gomez-Gil *et al.,* 2014).

Several studies have focused on the severity of *V. cholerae* related disorders through the years, leaving aside relatively small *Vibrio* spp of medical significance, some of which are regarded as emerging pathogens capable of causing mild to severe human disorders (Forsythe., 2000).

Liquefaction, purification, and DNA recovery are all frequent procedures in DNA extraction. These procedures are designed to successfully lyse cells, denature protein complexes, remove biological and chemical impurities, and recover DNA. Only the lysis step is used in the simplest approaches.

Gram positive bacteria are identified using biochemical tests such as the catalase test, coagulase test, starch hydrolysis test, and nitrate test, while Gram negative bacteria are identified using biochemical tests such as the oxidase test, urease test, indole test, sulfur test, and methyl red / voges-proskauer test (Muhammad *et al.,* 2020). However, because *Vibrio* spp are Gram negative bacteria, we will be focusing more on several of the Gram-negative tests.

1.2 OmpW Protein

OmpW (Outer membrane protein) non-O1/non-O139 serogroup, is one that is highly conserved among *V. cholerae* strains which serves as a good species-specific marker of *V. cholerae*, and this has led to the development of primers targeting the gene for rapid identification of the microorganism (Nandi *et al.,* 2005).

The presence of these serogroup in water is of public health importance and suggest that environmental conditions are suitable for the survival of epidemic *V. cholerae* O1 in the water source.

OMP plays an important role in the pathogenesis of many gram-negative bacteria and they are involved in bacterial adaptive responses like iron uptake, antimicrobial peptide resistance, serum resistance, multidrug resistance and bile salt resistance (Lin *et al.,* 2002). OmpW protein is well characterized in *V. cholerae* (Nandi *et al.,* 2005).

Expression of OmpW in *V. cholerae* has been associated with cultural conditions such as salinity, temperature, nutrient and oxygen availability and this has led to the idea that the expression is linked to adaptive responses (Nandi *et al.,* 2005).

Biochemical test may not be able to differentiate what kind of *Vibrio* spp we are dealing with as a result of sharing serological markers and phenotypic characters among *Vibrio* spp.

1.3 Statement of Problem

Vibrio species are bacteria that can be found in aquatic environments all over the world, and water is an aquatic habitat that may be utilized for bathing, washing, and cooking. Humans also consume some aquatic species as a food source.

Mountain Top University and the University of Lagos lagoon is an aquatic environment and since *Vibrio* species can the found in aquatic environment, it is a suitable place and can promote the growth and prevalence of *Vibrio* spp in these environments.

Drinking water quality is directly linked to human health, and ensuring safe drinking water is a top public health issue. It's the dispersion medium for everyone who eats seafood (Parveen *et al.,* 2008).

The aim of this study is to detect the presence of *Vibrio* spp in MTU pond and UNILAG lagoon as possible source of transmission of *Vibrio* spp, see how suitable the Mountain Top University Pond and the University of Lagos lagoon are, whether they are safe for aquatic life, and what kind of *Vibrio* species are present in the water.

1.4 Objective of the Study:

The aim of this study is to detect the presence of *Vibrio* spp in MTU pond and UNILAG lagoon as possible sources of transmission of *Vibrio* spp, and this can be achieved by the following objectives:

1. To identify *Vibrio* spp using biochemical tests.
2. To carry out DNA extraction and confirm the presence of *Vibrio* spp by PCR analysis using 16S rRNA.

1.5 Research Question

1. Are *Vibrio* spp really present in MTU pond and UNILAG lagoon?
2. What are the types of *Vibrio* species commonly found in those regions?

1.6 Significance of Study

Vibrio species have been implicated in diarrhoeic infections with high mortality if not properly managed. Water bodies remain the main reservoirs of these bacteria and serves as a link in the transmission of the bacteria. Although, pathogenic *Vibrio* spp. have been isolated from UNILAG lagoon no such data is available for MTU pond. It is therefore imperative to isolate and characterize *Vibrio* spp. in MTU pond and compare data with those isolated from UNILAG lagoon. Data from this study will provide information that will help MTU community and inhabitants in their hygiene and safety conducts.

1.7 OPERATIONAL DEFINATION OF TERMS

Characteristics: Being a distinguishing feature of a person or a thing.

Molecular Characteristics: Molecular characterization refers to characterizing an organism at the molecular level without regard for the organism's surroundings, development, or physiological state. Because of this, DNA-based markers are referred to as molecular markers, and characterization based on them is referred to as molecular characterization.

***Vibrio* spp:** Pathogens that cause gastrointestinal disease, such as cholera, belong to the *Vibrio* genus of short stiff motile bacteria that are straight or curved rods.

Pond: A pond is a small body of fresh water that is motionless. It differs from a river or a stream in that it does not have flowing water, and it differs from a lake in that it has a tiny surface area and is only around 1.8 meters deep. Some ponds are naturally produced, filled by an underwater spring or rainwater (often referred to as "dewponds"), while others are man-made.

Lagoon: A lagoon is a body of water with a natural barrier separating it from larger bodies of water. Atoll and coastal lagoons are the two types of lagoons.

When an island totally submerges beneath the ocean, a ring of coral grows upwards, forming an atoll lagoon. Coastal lagoons are shallower than atoll lagoons and form along gently sloping beaches.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Vibrio* Bacteria

Coastal waterways are home to *Vibrio* bacteria. *Vibrio* spp can cause gastrointestinal sickness (gastroenteritis) in humans in about 12 different ways.

They are seen in higher densities between May and October, when water temperatures are warmer. The term "vibriosis" refers to infections caused by *Vibrio* spp. There are about a dozen *Vibrio* spp that can cause human sickness.

Vibriosis is most commonly contracted by consuming tainted food, such as raw or undercooked shellfish from tainted water (Michael *et al*., 2015).

When an open wound is exposed to contaminated water, certain *Vibrio* spp can induce a skin infection.

Watery diarrhoeic stomach cramping, nausea, vomiting, fever, and chills are symptoms and indications that are similar to those of food poisoning. One approach for preventing vibriosis is to avoid raw seafood.

In most situations, no specific treatment is required, although it is critical to drink enough of fluids to replace fluids lost due to diarrhoeic or vomiting. Most persons with a moderate case of vibriosis recover in three days without lasting symptoms.

Vibriosis is more common in people with weakened immune systems, particularly those with chronic liver illness (Michael *et al*., 2015).

Vibriosis can be contracted by eating raw shellfish, particularly oysters, and by exposing exposed wounds to polluted water (Allisen *et al.,* 2018).

Inadequate hygiene practices might also result in the contamination of safe water after it has left the water source making it unsafe to drink.

Mainly in low-income areas of cities and towns within developing countries, a large proportion of waste water is discharged directly into the closet channel, sometimes with very little or no treatment at all. (Bulus *et al.,* 2015).

Vibrio spp are gram-negative bacteria that live in freshwater and estuarine habitats with a wide range of salinity and temperature, where they are primarily found in both culturable and non-culturable states.

Vibrio parahaemolyticus, V. cholerae, V. vulnificus, V. tubiashi, and *V. fluvial* are pathogenic *Vibrio* spp that are commonly spread through water and sea foods.

2.2 Brief History of Cholera and *Vibrio* Bacteria

Cholera is a bacterial illness of the small intestine caused by *Vibrio* cholerae strains. Epidemic cholera is an abrupt, painful, and frequently fatal disease that afflicted practically the whole world during many severe outbreaks throughout the nineteenth century.

This diarrhoeic disease can kill an untreated patient within a few hours due to dehydration, and it is particularly contagious in areas where sanitation is lacking (Lippi and Gotuzzo., 2013).

Severe cholera outbreaks were documented in various parts of the world between the early and mid-nineteenth centuries, from India in 1817 through sections of Europe and America in the 1850s.

It was widely assumed at the time that the outbreak was caused by poisonous vapour mixed with particles from rotting materials.

Pacini noted that all of the people who died of the disease were contaminated by these materials after analyzing their feces and intestinal mucosa, leading him to believe that it was these vibrions, not harmful vapour, that caused the ailment.

However, because he had not undertaken any experiments to demonstrate a link between the vibrion and the disease, his ideas were dismissed by the scientific community, which believed that the disease was caused by poor air quality.

Based on Louis Pasteur's work, a German scientist named Robert Koch developed numerous ways for cultivating and analyzing the features of various microbes in the 1880s.

He was able to extract and culture the organism from the internal mucosa of patients who died of the condition, as well as define its overall morphology, using these procedures.

Koch believed that this particular microbe was responsible for the disease since he only isolated it from the bodies of patients who died of the condition.

Koch discovered that the bacterium multiplied in moist, filthy linen as well as damp soils in later studies, but was unable to duplicate the sickness in animals. Despite giving substantial data demonstrating a link between the organism and the sickness, his results were widely dismissed across Europe (Lippi and Gotuzzo., 2013).

2.3 Treatment of *Vibrio* Infection

Antibiotics used to treat vibriosis infections are generally drugs of choice, such as amoxicillin, ampicillin, chloramphenicol, cotrimoxazole, ciprofloxacin, doxycycline, erythromycin, fluoroquinolone, furazolidone gentamicin, kanamycin, nalidixic acid, neomycin, norfloxacin, polymyxin B, quinolone, streptomycin, sulfamethoxazole-trimethoprim, sulphonamides, tetracycline, trimethoprim and vancomycin (CDC., 2017), (Lima., 2001), and (Kitaoka *et al.,* 2011).

Antibiotic resistance genes discovered in *Vibrio* spp include *pen* A, *bla* TEM-I and Beta-lactam, chloramphenicol resistant genes, and tetracycline resistant genes (Srinivasan *et al.,* 2005) and (Zhang *et al.,* 2009).

Amoxicilin, ampicillin, tetracycline, chloramphenicol, doxycycline, all have multidrug resistance in *V. cholerae* (Akoachere *et al.,* 2013).

2.4 Outbreaks Involved with *Vibrio* spp

The majority of vibriosis cases reported to the CDC are not part of an outbreak, although some are. However, larger outbreaks across multiple states are possible. A multistate outbreak of gastrointestinal ailments attributed to oysters imported from Mexico occurred in 2019, however as of June 21, 2019, the incident appeared to be resolved.

Sixteen people had reported ill from five states, with illnesses beginning on December 16, 2018, and ending on April 17, 2019. Two people had been hospitalized and there were no deaths reported.

• Multiple microorganisms causing illnesses were found by laboratory examination of patient samples. Some persons were afflicted with multiple pathogens. Some of which were caused

by *Vibrio parahaemolyticus* and one instance of *Vibrio albensis* infection, also one case of *Vibrio* of unknown species infection (CDC., 2019).

• In a multistate outbreak in 2018, Vibrio parahaemolyticus diseases were related to fresh crab meat imported from Venezuela. The disease was linked to the consumption of fresh crab meat imported from Venezuela, which was labeled as "fresh" or "pre-cooked" and offered as a ready-to-eat product.

As of September 18, 2018, *Vibrio parahaemolyticus* diseases had been documented in Colorado, Delaware, DC, Louisiana, Maryland, New York City, Pennsylvania, and Virginia. Nine persons were sent to the hospital with no deaths reported. This was traced to crab meat's origin from restaurants and grocery stores traced to various Venezuelan sources.

• *Vibrio* infection usually starts 24 hours after the germ is swallowed. Watery diarrhoea, stomach cramps, nausea, vomiting, fever, and chills are all signs of infection.

• Symptoms persist around 3 days on average, and most patients recover without therapy. Drink plenty of drinks if you have a *Vibrio* infection to restore fluids lost through diarrhoea.

2.5 Empirical Review

In a study report by **Mohammad *et al*., (2014),** vibrionaceae were isolated from Venetian lagoon shellfish and studied in depth to determine its structure and dispersion in the environment. The research was based on a simultaneous study of core genetic data and a set of aetiological parameters (season, host species, risk level of the area, water temperature, sampling area, and depth), with the goal of determining whether they were linked.

Yael *et al.,* (2020), employed sequencing data to conduct a report on two frequent fish pathogenic organisms from the Eastern Mediterranean Sea, Mycobacterium species and *Vibrio* species. Molecular identification and characterization revealed distinct strains of Mycobacterium species and Vibrio species using PCR with 16SrRNA primers.

Azwwai *et al*., in **(2016)**, reported that out of 93 sea foods, meat and meat products from various geographical locations in Libya 48% were positive for *Vibrio* spp by culture and molecular methods.

Glenn *et al*., (2003), studied sporadic sea food associated infections in which epidemic *V. cholerae* served as a trigger for cholera epidemic in the underdeveloped globe. People who have immunocompromised immune system, cirrhosis or have characteristics that predispose them to greater iron saturation of transferrin are at the greater risk. People in this group were advised to avoid raw oysters.

Thomson *et al.,* (1998), used non-01 and non-0139 *V. cholerae* isolated from three different environmental sites and drinking water in Vellore South India. No culturable 01 and 0139 strains were detected in the environment but when environmental isolate florescent antibody staining was used it detected both serotype in all areas although it is not clear if the organism detected were in a viable form.

Finally, **Bisweswar *et al.,* (2000),** employed PCR primers targeted to the gene of the outer membrane protein ompW to identify *Vibrio cholerae* species. The findings of the amplification revealed that all (100%) of the 254 *V. cholerae* strains tested were positive for ompW, and 229 (98%) of the 233 were positive for ToxR. Using OmpW and ToxR specific probes in a DNA dot blot experiment, this was extended to a representative number of strains.

CHAPTER THREE

3.0 Material and Methods

3.1 Sample Collection

Samples were collected from Mountain Top University Pond and University of Lagos lagoon, on the first of July 2021. Water samples were collected in sterile one litre bottles and transported to the laboratory immediately.

3.2 Isolation and Characterization of *Vibrio* spp.

One milliliter of each water sample was inoculated into sterile alkaline peptone water (APW) with pH 8.6 containing sodium chloride and incubated for 6 hours. After incubation, 100 µL of APW culture was plated onto Thio-sulphate Citrate Bile Salt Sucrose Agar (TCBS) and incubated overnight. Large yellowish colonies were subcultured on nutrient agar and biochemical tests including Gram staining, oxidase, catalase, glucose fermentation, gas production and string test were performed (Milon Kumar Das Sarker *et al*., 2019).

3.3 Biochemical Tests

Different metabolic activities displayed by different species of bacteria were employed in biochemical tests for microbiological identification.

TESTS

Catalase Test

Bacteria produce the catalase enzyme, and this test is performed to detect if they produce it. The catalase enzyme produced by these bacteria will neutralize the hydrogen peroxide, and bubbles will appear, indicating a positive test. Catalase enzyme is produced mostly by obligatory aerobes and facultative anaerobic bacteria. The test involves mixing a colony of bacteria with a few drops of 3 percent hydrogen peroxide (H2O2) in a tube or on a slide and looking for bubble formation within 10 seconds. (Facklam and Elliott, 1995).

Oxidase Test

The oxidase test is useful in identifying microorganisms that may manufacture cytochrome oxidase enzyme. Cytochrome oxidase works on the basis of electron transfer from the donor (electron transport chain) to the final acceptor (oxygen), with water as the final acceptor. The electron donor will be oxidized by cytochrome oxidase, and the color will change to dark purple. This test is carried out by impregnating a filter paper with 1 percent tetra-methyl-p-phenylenediamine dihydrochloride, which acts as an artificial electron donor, and drying it. The bacteria colonies were smeared on a paper strip and the color change was observed after 10 seconds.

Motility Test

Broth culture of 18-24 hours old were used, using hanging drop technique, a little immersion oil was placed round the edge of the depression in the slide. Then using a wire loop, a small loopful of the culture was added to a clean dry coverslip laid on the bench. The cavity slide was inverted over the so that the drop was at the center of the cavity and the slide was press down gently but firmly so that the oil could seal the coverslip in position. This made the culture to be in form of a hanging drop, and this was examined immediately using a high power (×40) dry objective in position.

Gram staining

Heat fixed smears from an 18-24 hours culture was prepared, this was stained with crystal violet solution for 1minute, Gram iodine solution was also added and left for one minute then the iodine was poured off and blotted dry and washed using 70% of ethanol for 30 seconds and the counter stain safranin was added for another 30 seconds and then washed off with water and blotted dry.

3.4 DNA Extraction

The DNA extraction method includes both breaking cells open to release the DNA and verifying the DNA's existence and quality. (Caio *et al.,* 2014).

DNA extraction is a technique for isolating DNA from cell membranes, proteins, and other biological components from a sample using physical and/or chemical processes. (Nalini Gupta., 2019).

Boiling method of DNA extraction

- Firstly, the isolates were resuscitated using BHI (Brain heart infusion) and incubated till the next day.
- Then the isolates were centrifuged at 5000×g for 3min.
- The supernatant was eliminated, and the pellet was resuspended using distilled water
- Distilled water was added to the tubes containing the pellet and then vortexed.
- It was then centrifuged for another 3 minutes
- The supernatant was eliminated, and the pellet was resuspended using 200uL of nucleic free water and then centrifuged at 5000×g for 3min.
- After which it was vortexed and placed the Eppendorf tubes into the heating block for 15 minutes and placed the Eppendorf tubes inside ice for 5 minutes.
- It was finally centrifuged again at 7000×g for 6 minutes.
- And then 150uL of DNA was transferred into a fresh Eppendorf tube while the pellet was discarded.

3.5 Agarose Gel Electrophoresis

The agarose was prepared using dry agarose powder, 1g of the agarose powder was dissolved in 50ml of TAE buffer and the mixture was boiled until a clear solution was gotten, while 3μL of ethidium bromide was added to the mixture using a micropipette. It is 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 put in an electrophoresis tank containing TAE buffer. Four uL of the PCR products are 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 is turned off and the gel was viewed under the UV transilluminator.

3.6 Verification of the Integrity of the DNA of the Extracted *Vibrio* DNA

- After the preparation of the agarose gel
- Four microliters of DNA and two microliters of Quick-load purple 100bp DNA ladder was mixed in a 0.2 mL tube.
- This mixture was poured into the gel wells and subjected to a voltage of 80 volts for 20 min in a horizontal electrophoresis system.

- Following that, the system did DNA visualization and photo documentation.
- This procedure was performed on 20 randomly selected samples.

3.7 PCR (Polymerase Chain Reaction)

Polymerase chain reaction (PCR) is a reliable technique for amplifying a specific section of DNA *in vitro*.

Target sequence is the sequence within the DNA template, which will be amplified by PCR.

PCR primers are single-stranded DNA primers (typically 18–25 nucleotides long) that match the sequences at the ends of or within the target DNA are required to start DNA synthesis in PCR. (Nalini Gupta., 2019).

3.8 PCR Protocol

16S rRNA amplification

Partial 16S rRNA gene amplification using forward primer 27f (5´-AGA GTT TGA TCC TGG CTC AG-3´) and reverse primer 16sr (5´-CTA CGG CTA CCT TGT TAC GA-3´) (Weisburg *et al*., 1991).

The components of the PCR and constituent mixes were summarized in Table 3.1 below. The PCR was carried with initial denaturation at 95°C for 12 min; 35 cycles of 95°C for 30 sec; 61°C for 30 sec and 72°C for 1 min; and a final elongation step at 72°C for 10 min. The PCR products were confirmed by electrophoresis and visualized under UV light with a Gel.

Table 3.1: PCR reaction components used for 16S rRNA amplification for *Vibrio* species

No.	Component	Initial concentration	Final concentration	Volume/1 rxn
1	Master mix	5x	1x	2ul
2	16sf	20um	0.25um	0.125ul
3	16sr	20um	0.25um	0.125ul
4	dH_2O			5.75ul
5	DNA			2ul
6	Total			10ul

Table 3.2: Procedure for Thermal Cycler

Analysis	Step	Temperature	Time
1x	Initial denaturation	95℃	12 min
35x	Denaturation Annealing Extension	95℃ 61℃ 72℃	30sec 40 sec 1 min
1x	Final Extension	72℃	10 min
1x	Hold	10℃	∞

Table 3.3 Oligonucleotide primers and PCR reactions used in this study

Primer	Oligonucleotide sequence (5′–3′)	Composition of 50-μL reaction1	Target (product size)	Reference
27f 1495R	AGAGTTTGATCCTGGCTCAG CTACGGCTACCTTGTTACGA	5.0 μL of 10× buffer, 2 μL of dNTP mix, 2 μL of each primer, 200 ng of DNA, 2.5 U of Taq	16S rRNA gene (1,500 bp)	Weisburg *et al.*, 1991

3.9 Precautions

• Aseptic techniques were observed at every stage of work.

• Personal protective technique was also observed, such as wearing of covered shoe, nose cover, gloves, lab coat, etc.

• Ensured that the inoculating loop cooled before picking the organism when sub-culturing in order not to kill organism of interest.

• Ensured that the petri-dish was incubated inverted.

• Ensured proper timing, most especially during autoclaving.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

The microbial analysis of the water samples gotten from MTU pond and UNILAG lagoon were reported. The morphological characteristics of the isolates are shown in Table 4.1, 4.2, 4.3 and 4.4 for total viable counts, general *Vibrio* species respectively.

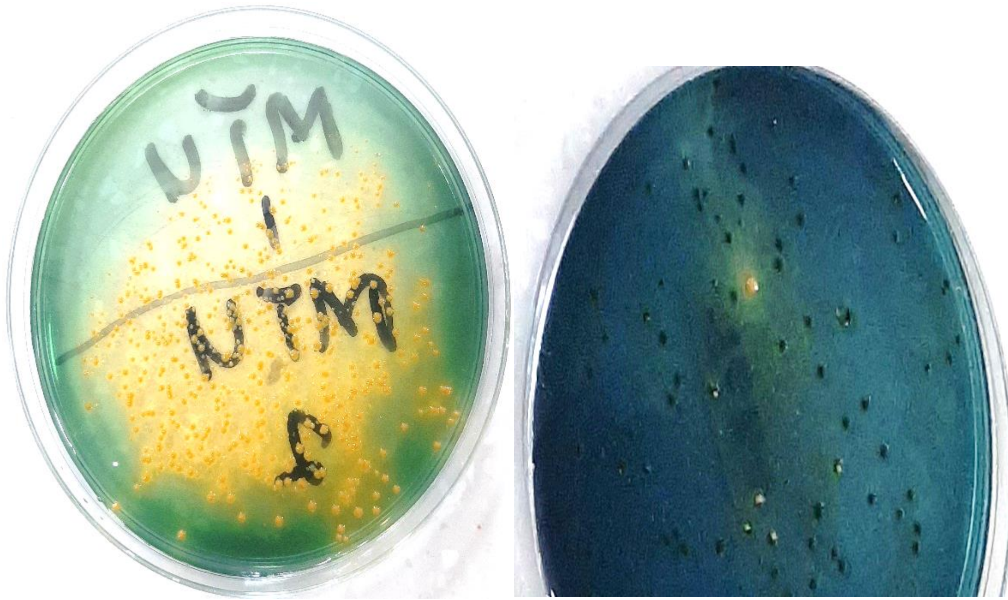
Table 4.1: Morphological Characteristics of bacterial isolates on Thio- sulphate Citrate Bile Salt Sucrose Agar (TCBS)

Isolate ID	Colour	Size	Shape
SMV_1 (1)	Yellow	Large	Circular
SMV_1 (2)	Yellow	Large	Circular
SMV_1 (3)	Yellow	Small	Circular
SMV_1 (4)	Yellow-Greenish	Small	Circular
SMV_1 (5)	Yellow	Large	Circular
SMV_1 (6)	Green colonies	Small	Circular
SMV_1 (7)	Yellow	Large	Circular
SMV_1 (8)	Yellow	Large	Circular
SMV_1 (9)	Yellow	Large	Circular
SMV_1 (10)	Blue colonies with green centres	Small	Circular
SMV_1 (11)	Yellow	Large	Circular
SMV_1 (12)	Yellow	Large	Circular

Table 4.2 showing total number of growths

Location	Total no of isolates	No that yielded colonies
MTU	25	13
UNILAG	25	18

Out of **25 plates** that was incubated containing samples from the **MTU pond** only **13 of the plates** yielded growth while **12 of the plates** did not yield any growth, and out of the **25 plates** that was incubated containing **UNILAG lagoon** only **18 of the plates** yielded growth and **seven of the plates** did not yield growth.



Plates 4.1 and 4.2: Showing growth of *Vibrio* species on TCBS agar

Table 4.3 showing the results of biochemical test

	1	**2**	**3**	**4**	**5**	**6**
Motility	+	+	+	+	+	+
Lysine	+	+	-	-	+	+
Catalase	-	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Ornithine	-	+	-	+	-	+
Moller's decarboxylase	-	+	-	-	-	+
Growth with 0% NaCl	+	+	-	-	-	+

Table 4.4 showing the numbers of the 16s rRNA DNA band codes.

Numbers	Codes
1	A (UNILAG)
2	3 (UNILAG)
3	4 SMV_2 (MTU)
4	5 SMV_1 (MTU)
5	6 SMV_1 (MTU)
6	7 (UNILAG)
7	8 (UNILAG)
8	8 SMV_1 (MTU)
9	9 (UNILAG)
10	10 (UNILAG)
11	11 (UNILAG)
12	11 SMV_1 (MTU)
13	14 SMV_1 (MTU)

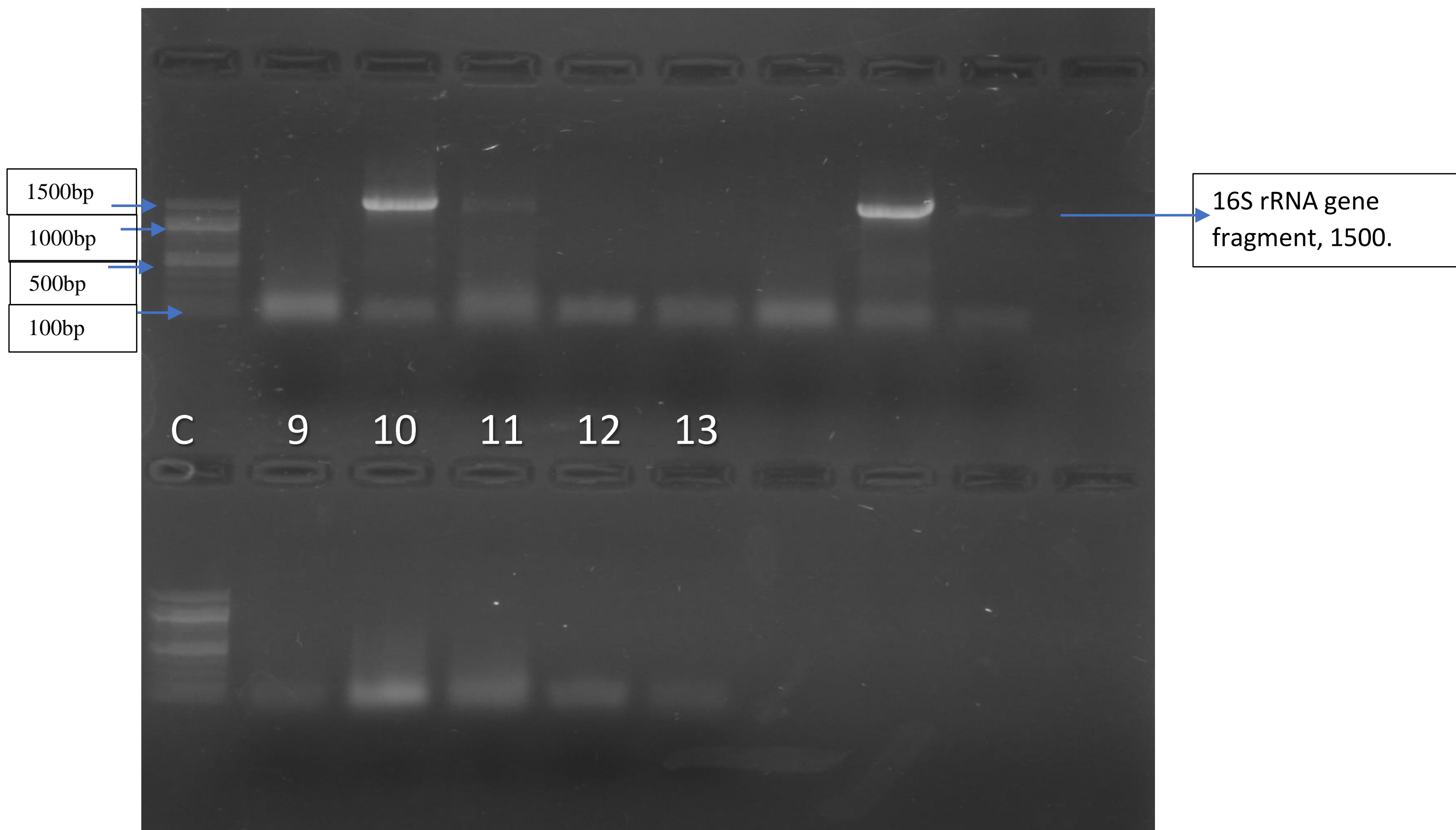


Fig 4.1: Agarose gel electrophoresis showing 16S rRNA amplification of *Vibrio* spp isolated from MTU ponds and UNILAG lagoon. Lane C = 100bp ladder, lanes 1,2,6,7,9,10,11: samples from UNILAG, lanes 3,4,5,8,12,13: samples from MTU. Lanes 2,3,7,8 are fragment of 16S rRNA gene of positive isolates of *Vibrio* spp.

4.2 Discussion

Vibrio spp are widespread in nature, especially in the marine environment, Fish infected by these bacteria could be a source of zoonotic risk for human health (Ceoccarelli *et al*., 2014) and are known to cause infections in humans with different degrees of severity, especially in immunocompromised individuals (Bercovier *et al*., 2001)

Vibrio species are found in aquatic settings all over the world and are omnipresent (Rivera *et al.,* 2006).

Over the last few decades, the 16S rRNA gene has emerged as a good standard for determining phylogenetic relations of bacteria (Woese *et al*., 1987), by using PCR amplification and direct sequencing of 16S rRNA products (Knibb *et al*., 1993).

Despite the fact that this gene is still considered a major standard for bacterial identification (Gillman et al., 2001), (Turenne et al., 2001) as more sequence information has accumulated over time, it has become clear that the resolving capability of 16S rRNA sequences alone is often insufficient when closely related organisms are examined (Palys et al., 1997). The results of the study show amplification in the 16S rRNA region which is a confirmation of the presence of *Vibrio* spp in our MTU ponds for the first time as well as Unilag lagoon that has been previously reported.

In many countries, especially in the developing world, fresh water resources are severely contaminated with pathogens leading to various waterborne disease outbreak (Pruzzo *et al*.,2005). *Vibrio* species occur naturally typically surviving in fresh water and marine environments.

Bacterial infections are the most significant barrier to aquaculture productivity and sustainability. Vibriosis is the most frequent bacterial disease that affects mariculture fisheries and aquatic life around the world, resulting in significant economic losses (Carmen Balebona et al., 1998). An increase in the populations of pathogenic *Vibrio* spp. is likely to cause vibriosis, though this does not necessarily imply an increase in the whole Vibrio population (Sung et al., 2001). As a result, the current investigation proved beneficial in determining the pathogenic vibrios.

Water sources can serve as a possible route for the transfer of *Vibrio* species to consumers, posing serious public health risks when consumed.

This study however aimed at detecting the presence of *Vibrio* spp in MTU pond and UNILAG lagoon as a possible source of transmission of *Vibrio* spp, see how suitable the MTU pond and UNILAG lagoon are, whether they are safe for aquatic life and what kind of *Vibrio* spp are present in the water, this was done by biochemical test as shown in table 4.3 and DNA extraction and later confirmed by PCR analysis using 16S rRNA and genes of *Vibrio* spp also shown in table 4.4.

Since *Vibrio* spp has been implicated in diarrhoeic infections with high mortality if not properly managed, and since there is no existing data yet on the isolation of *Vibrio* spp in MTU pond, this work has provided baseline information that will help MTU community and inhabitants in their hygiene and safety conducts and also to discourage anybody who might want to even think of using it as a source of water or food.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The water samples from both MTU pond and UNILAG lagoon were found to be contaminated with *Vibrio* spp. Therefore, there is a possible risk to humans and even animals from consumption of these water which can lead to other illnesses like gastrointestinal infection.

5.2 Recommendations

It is recommended that fish or water gotten from this area should be boiled and cooked thoroughly before consumption.

It is recommended that there should be adequate data collection and dissemination of information about infections or outbreak of pathogenic microorganisms in local government areas.

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